#### Human and Animal Physiology

## Development and Testing of New Combined Insecticides against the Brown Marmorated Stink Bug (BMSB, Halyomorpha Halys) in Laboratory Conditions

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Thirty-six insecticidal combinations with various ratio of bifenthrin and malathion were prepared and the dependence of biological effectiveness of combinations on the ratio of bifenthrin to malathion amount was investigated. Contribution of other components (mineral materials, essential oil, emulsifier-dispersant, hydroxyethyl cellulose, etc.) to the total biological effectiveness of the combined insecticides was estimated. It has been found that synergy of components and biological effectiveness of combinations against the target pest noticeably differs in Abasha-Senaki and in Kobuleti-Khelvachauri populations. This fact can be explained by a noticeable resistance to bifenthrin developed by the populations of Senaki and Abasha municipalities of Georgia. The acute toxicity of combinations to mammals (white rats) has been investigated, which has been found to be several (3-5) times lower than that of the widely used combined insecticides currently available. This circumstance can be explained by the significantly reduced total content of synthetic insecticidal components in synergystic combinations. © 2021 Bull. Georg. Natl. Acad. Sci.

Brown Marmorated Stink Bug, synergy, combined insecticides, acute toxicity, resistance

The present study aims to determine the biological efficacy of the developed insecticidal combinations against BMSB populations and their acute toxicity to white rats using new methods developed and tested at the Georgian Technical University and

Ivane Beritashvili Center of Experimental Biomedicine according to all modern principles of laboratory animal testing.

The object of the laboratory study were 1800 adult BMSBs of both sexes (group A) obtained

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Active ingredient	Name of formulation	Place of registration	Bifenthrin content, ml/l	Lethality index [7], %
Bifenthrin	"Insakar"	Georgia	100	91.5
Malathion	"Malafos"	Georgia	500	92.5

Table. The main synthetic components of the studied insecticidal combinations

from farmers of Abasha and Senaki municipalities; 1800 adult BMSBs of both sexes obtained from farmers of Kobuleti and Khelvachauri municipalities of the Autonomous Republic of Adjara (Group B); 600 adult BMSBS of both sexes from Abasha and Senaki (control group C) and 600 adult BSMBs of both sexes from Kobuleti and Khelvachauri (control group D).

# Components for Insecticidal Combinations

The components of the insecticides used for the study were selected according to simple criteria such as registration in Georgia, easy access to farmers, low content of active ingredients and low recommended rate of use, high mortality rate (lethality index) against BMSB.

Finally, the choice was made and rosemary oil [1], bifenthrin-containing preparation Zonder [2], malathion-containing preparation Malafos [3], biodegradable emulsifier-dispersant Lansperse-BIO868 [4], hydroxyethyl (CEC) [5] and freshwater diatomite [6], a natural powder material containing silicon dioxide of biological origin (which, if properly processed and treated, is much less harmful to bees and other pollinators than all synthetic and the most of natural insecticidal formulations) were chosen. Two main synthetic components of the studied insecticidal combinations are listed in Table.

#### Methodology

In order to increase the accuracy and reliability of both biological efficacy determination and toxicity testing, it became necessary to carry out relative measurements. Biological efficacy was measured in relation to the efficacy of water solution containing only Malafos, while the toxicity of combinations was determined in comparison with a widely used combined insecticidal formulation ProStore 420 EC [8].

The following sequence of works was selected: Dispersion of synthetic pyrethroid organophosphorus insecticides (in variable ratios) in prefabricated stable dispersions containing diatomaceous earth, rosemary oil, emulsifierdiperger and HEC; Determining the total biological effectiveness of tested combinations; Evaluation of the total biological effectiveness of the synthetic components of tested preparations and assessment of the synergy of the components (by comparing to aqueous solution of "Malafos with the same concentrations); Determining of acute toxicity of tested combinations to mammals.

Based on the obtained results, it will also be possible to assess the possible resistance acquired by pests and to evaluate the joint share of diatomaceous earth, rosemary oil, emulsifier-dispersant and HEC to the total biological effectiveness of the combined preparation.

36 combinations with the conditional name "Dibifmal" (combinations D1 - D36) have been prepared from the selected components, in which the volume content of active synthetic components changed by a fixed step as follows: "Insacar" – For a better interpretation of the results of the experimental research, the total expected biological efficiencies of all combinations (as a sum of the biological effectiveness of each component) were calculated based on the literature data (Fig. 1). Clearly, any deviation from the expected value indicates synergy (super- effectiveness or antagonism) of the components of the tested combinations.

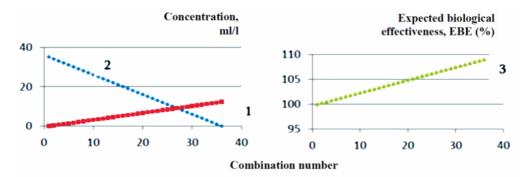
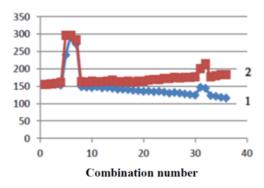


Fig. 1. Concentration of bifenthrin (1) and malathion (2) and the expected biological effectiveness (3) of the tested insecticidal combinations, %.

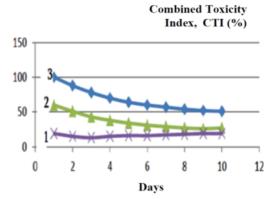
### **Experimental Research**

**Determination of the relative biological efficacy of developed insecticidal combinations.** The relative biological effectiveness of the above insecticidal combinations was determined by the laboratory experiment method described in papers [9-12]. Each observation on test insects lasted for 14 days, under the same dose of all combinations (50 ml per container) and at concentrations approximately 5-fold lower than the recommended dose for malathion field use against BMSB [7]. The data obtained during this study are given in Fig. 2. Biological effectiveness (BE) of combinations was determined using the "modified" Abbott formula [9, 13].

## Experimental value of biological effectiveness, BE (%)



**Fig. 2.** Relative biological effectiveness BE (%) of insecticidal combinations (BE, %) against BMSBs in groups A (1) and B (2).



**Fig. 3.** The Combined Toxicity Index CTI (%) of the standard saline solution (1), combination D-18 (2) and formulation ProStore 420 EC against BMSB.

For the acute toxicity testing of the studied combinations a novel methodology [10-12] developed at the Georgian Technical University and Ivane Beritashvili Center for Experimental Biomedicine and based on the continuous observations of behavioral and physiological characteristics of the test animals passing a standard branched maze was used. During the first 3 days of observation, 50 white rats divided into 10 groups were injected intramuscularly with the same dose (30 (10 + 10) + 10) mg/kg) and concentration of D-18 combination, of the control drug (ProStore 420 EC) or of the standard saline solution, followed by a standard maze test for 10 days under control of all parameters given in [10-12]. Averaged results of the study are given in Fig. 3.

#### **Results and Discussion**

The reported study made it possible to experimentally determine and evaluate the most important characteristics of the developed insecticidal combinations, such as the dependence of relative biological effectiveness against BMSB populations against the volume ratio of active synthetic components (see Fig. 2) and the total contribution of diatomite, rosemary oil, emulsifier-dispersant, hydroxyethylcellulose and water to the total biological effectiveness of the combinations. A valuable result is the fixation of synergy areas and of the noticeable difference in the impact on BMSB populations from Abasha-Senaki and

Kobuleti-Khelvachauri populations possibly caused by a higher resistivity of higher resistance of Abasha-Senaki population. The methodology used will also allow us in the nearest future to determine with high accuracy the contribution of each component of insecticidal combinations to the total biological effectiveness and the degree of their synergy (co-toxicity) against BMSB. It was also found that the acute toxicity of the studied combination is (3-5)-times lower than that of the control drug PROSTORE 420 EC. This circumstance can be easily explained by the significantly reduced total content of synthetic insecticidal components in the tested combinations.

ადამიანისა და ცხოველთა ფიზიოლოგია

აზიური ფაროსანას (Halyomorpha Halys) წინააღმდეგ ახალი კომბინირებული ინსექტიციდების შემუშავება და ტესტირება ლაბორატორიულ პირობებში

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დამზადდა ბიფენტრინის და მალათიონის შემცველი პრეპარატების, მტკანარი წყლის დიატომიტის, როზმარინის ეთერზეთის, ბიოდეგრდირებადი ემულგატორ-დისპერგანტის და ჰიდროქსიეთილცელულოზის 36 კომბინაცია ბიფენტრინის და მალათიონის ცვლადი თანაფარდობით. განსაზღვრულია მათი ბიოლოგიური ეფექტურობის დამოკიდებულება პირეთროიდული და ორგანოფოსფორული კომპონენტების თანაფარდობაზე. შეფასებულია დანარჩენი კომპონენტების (მინერალური მასალები, ეთერზეთი, ემულგატორ-დისპერგანტი, ჰიდროქსიეთილცელულოზა და სხვა) წვლილი კომბინირებული პრეპარატების ჯამურ ბიოლოგიურ ეფექტიანობაში. დადგენილია, რომ კომბინირებული პრეპარატის კომპონენტების ბიოლოგიური ეფექტიანობა აბაშისა და სენაკის მუნიციპალიტეტებში გავრცელებული პოპულაციის მიმართ შესამჩნევად განსხვავდება ქობულეთისა და ხელვაჩაურის მუნიციპალიტეტებში გავრცელებული პოპულაციების მიმართ მათი ბიოლოგიური ეფექტიანობისგან. ეს ფაქტი შეიძლება აიხსნას სენაკისა და აბაშის მუნიციპალიტეტებში გავრცელებული პოპულაციების მიერ ბიფენტრინის მიმართ გამომუშავებული შესამჩნევი რეზისტენტობით. გამოკვლეულია კომბინირებული პრეპარატების მწვავე ტოქსიკურობა ძუძუმწოვარა ცხოველების (თეთრი ვირთაგვა) მიმართ, რომელიც აღმოჩნდა რამდენჯერმე (3-5-ჯერ) უფრო ნაკლები, ვიდრე სადღეისოდ ფართოდ გამოყენებული კომბინირებული ინსექტიციდური პრეპარატეზის. ეს გარემოება შეიძლება მარტივად ავხსნათ კომბინაციებში სინთეზური ინსექტიციდური კომპონენტების მნიშვნელოვნად შემცირებული ჯამური შემცველობით.

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