Annals of Agrarian Science

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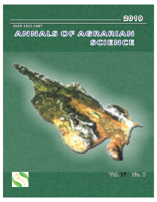

Development and laboratory and field testing of new combined insecticides against the resistant and non-resistant populations of the Brown Marmorated Stink Bug (Halyomorpha Halys)

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Received: 10 September 2021; Accepted: 19 October 2021

ABSTRACT

The Brown Marmorated Stink Bug (BMSB) is among the most serious pests for agriculture. It can cause a significant social nuisance. Development and laboratory testing of new synergistic insecticidal mixtures highly effective against this invasive pest and less toxic to the living environment (especially humans, mammals, birds, pollinators and other useful insects, aquatic organisms) is an extremely urgent challenge for the modern applied enthomology. Synergy of pyrethroidal (bifenthrin, gamma-cyhalothrin) and organophosphorous (malathion, dimethoate) active substances was investigated and the contribution of additional components (mineral dusts, essential oils, emulsifiers, dispersers and hydroxiethilcellulose) to the total joint biological effectiveness of the mixtures was assessed. It was found that the both studied mixtures (with byfenthrin + malathion and gamma cyhalothrin + dymethoate) showed a clearly expressed synergy and increase of biological effectiveness in comparison to the separate components. At the same time, the observed synergistic effect of malathion- and bifenthrin- based mixture against the insect populations was obviously lower in Abasha and Senaki municipalities, than in Kobuleti and Khelvachauri municipalities of Georgia. Difference in biological effectiveness of gamma-cyhalothrin- and dimethoate-based mixtures for the insect groups was significantly lower. The most reliable explanation of this shift could be the resistance developed by Abasha and Senaki populations due to the five-year long enhanced treatment with bifenthrin based formulations. The acute toxicity of the newly developed combinations to mammals and bird embryos was found to be 3-5 times lower, than of the widely applied insecticidal modalities. The data of laboratory testing of the biological effectiveness of insecticidal combinations were supported by the results of field trials of the five insecticidal formulations in a corn field and a hazelnut plantation held with the technical support of the National Food Agency of Georgia of the Ministry of Environment of Georgia.

Keywords: Brown Marmorated Stink Bug, Synergy, Biological effectiveness, Combined insecticides, Resistance, Acute toxicity, Nanoinsecticides.

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Introduction

Importance and necessity of BMSB management in Georgia. Development and study of a wide range of new highly effective pesticides having low toxicity against the living environment (primarily - humans, mammals, bees and other beneficial insects, aquatic organisms, reptiles, etc.) is of a paramount importance for agriculture and environmental safety. One of the most acute current task in Georgia and globally is to combat the polifague invasive pest called the Brown Marmorated Stink Bug (BMSB, Halyomorpha halys) [1-14].Management of BMSB in Georgia is provided by the Ministry of Environment and Agriculture of Georgia with an active support of the Food and Agriculture Organization of the United Nations (UNFAO), the United Nations Development Program (UNDP) and the United States Agency for International Development (USAID) within the framework of the programs implemented by the National Food Agency of Georgia [15, 16].

Relevance and the main aim of the study. In recent years, special attention has been paid to the development and testing of synergistic combinations of active substances of different chemical class and types of action, which should be highly toxic against the nymphs, adult and mature instars and, at the same time, provide effective inhibition of the development of pest resistance to insecticides and acaricides [3, 5-8]. This approach gives us the possibility of making a significant reduction of the applied doses of the active substances and minimizes their negative impact on human, pollinators and other useful insects, farming animals, cattle, poultry and wildlife.

For taking into account the possible low effectiveness of the developed combinations against drug resistant pest populations and achieving the high environmental and economic effect, it is necessary to conduct laboratory and field testing of the combined drugs on the both resistant and non-resistant population [9-14].One of the most important characteristics of insecticides is their acute toxicity to warm-blooded animals. At the same time, the requirements for the standards of humane treatment of test animals (which should exclude killing of animals, causing them severe pain, significant deterioration of health, prolonged immobilization and anesthesia, etc.) are becoming increasingly stringent [5-7, 17-20].

One of the most important aims of this study is to determine the biological effectiveness and validity terms of the developed insecticides against BMSB populations, spread in the Abasha-Senaki and Kobuleti-Khelvachauri municipalities, and their acute toxicity to mammals (white rats) using new methods developed and tested at the Georgian Technical University and I. Beritashvili Center for Experimental Biomedicine and adhering to all modern 4 R principles of humane treatment of test animals.

An especially important part of the study is the field testing of the developed combinations with the aim of demonstrating applicability of the developed and used methods for the accurate and reliable determination of the main characteristics of the pesticides.

Evaluation of the acute toxicity involved the long-term continuous monitoring of cognitive, behavioral, physiological and rheology characteristics of the exposed groups of test animals, with the aim of excluding any kind of testing contradicting to the 4R principles of human treatment of laboratory animals. Preliminary testing of nano-based combinations containing nanostructured alumina was carried out to examine the prospective of nanoparticles and nanobased complexes as active components of insecticidal combinations with increased biological effectiveness and safety.

Materials and methods

Test insects

The main subjects of the three-year laboratory study were as follows: 3900 adult pests of both sexes (Group A), provided by the Georgian National Food Agency and collected from the farmers in Abasha and Senaki municipalities; 3900 adult pests of both sexes collected in the Autonomous Republic of Adjara from the farmers in Kobuleti and Khelvachauri municipalities (Group B); 900 adult pests of both sexes **collected** from the farmers in Abasha and Senaki municipalities (control group C); 900 adult pests of both sexes collected from the farmers in Kobuleti and Khelvachauri municipalities (control group D. The purpose of dividing into groups was to determine whether there was a noticeable prevalence in resistance to the widely used insecticide bifenthrin in the populations of Abasha and Senaki municipalities compared to populations of Kobuleti and Khelvachauri municipalities. The reported study showed that the biological effectiveness of combinations and synergy of components depends significantly on the ratio of synthetic components of the combination.

Components of the developed insecticidal combinations

Synthetic and natural components for the insecticidal combinations used in this study were selected according to the following criteria: registration in the US, Australia, EU or Georgia; widespread and free availability to farmers; the low prescribed concentrations of the active substances and low recommended rates of application, high lethality to mature BMSB adults. The choice between essential oils with the approximately equal effectiveness [21–23] was made in favor of the less harmful rosemary oil. Among the pyrethroid insecticides, bifenthrin (widely used and well studied in Georgia [24, 25]) and gamma-cihalotrin (which has a sufficiently high biological effectiveness against the target pest[26, 27]) have been selected. Dichlorvos was initially excluded from the list of potentially applicable organophosphorus insecticides, as its use is banned or severely restricted in many parts of the world [28, 29]. Among other organophosphorus insecticides, malathion [30, 31] and dimethoate [32, 33] were selected, as they showed noticeably higher synergies with bifenthrin and gamma-cyhalothrin than the other eight widely used pyrethride insecticides probed during the pre-testing period. We also selected Lansperse-BIO868 [34] (a well-known bio-based 100 % biodegradable surfactant) from “Lankem” (UK) and Hydroxyethilcellulose (HEC) [35] (widely used in medicine and pharmacology) from “Sakshi Chem Sciences Private Limited” (India), which allowed us to provide highly stable disperse insecticidal combinations.

One of the selected mineral components DE (amorphous diatomaceous earth or diatomite) [36-40] is practically non-toxic to mammals and warm-blooded animals, and is widely used in agriculture and veterinary medicine (as a pesticide, anti-parasitic drug and animal food additive), in cosmetics, hygien products and food industry (for composition of food additives, various types of filtration, food protection, soil cultivation, in hydroponic mixtures, etc.). The insecticidal-acaricidal action of amorphous diatomaceous earth (diatomite) is mainly due to the effect of the mineral on the cuticle and exoskeleton causing dehydration of constituent substances and absorption of fatty compounds. Micro-powder of the DE mined in Georgia (Kisatibi mine), dried at a temperature of 150 ^{0}C and ground to a size of tens of micrometers, is noticeably less toxic to bees than to target pests due to several differences in the structure of the protective covering layer and lifestyle of insects. The chemical content of the utilized DE was practically the same as the typical composition given in [40]. The second mineral component used by us was kaolin (aluminum hydrosilicate) [41, 42] with the chemical formula $Al_2Si_2O_5(OH)_4$. Kaolin is a widely used phylosilicate clay compound which consists of silicate sheets (Si_2O_5) bonded with layers of aluminum oxide/hydroxide ($Al_2(OH)_4$) and is widely used for manufacture of medicines, textiles, petroleum and building materials. It has high thermal stability, porosity and a large area of the capillary surface, which (among other properties) provides its sufficiently high insecticidal properties (including the ability to dehydrate insects’ exoskeleton). Due to its lower strength and toughness, damaging effect of kaolin on the outer layer of pollinators covered with body-hairs is insignificant, and it is practically harmless or less harmful for different species of bees. In all insecticidal combinations we utilized the food grade product from “Neelkanth Finechem LLP” (Boranada, Jodhpur, Rajastan, India). The chemical content of kaolin micro powder dried at 200°C was practically the same as the typical composition given in paper [42]. Pyrethroidal and organophosphorus synthetic drugs were selected primarily considering the higher lethality index and low recommended doses of application [43-46].

Table 1. *Basic synthetic components of insecticidal combinations*

Active substance	Name of the drug	Registration in Georgia, EU, Australia, USA	Content of the active substance, mass %	Lethality index to BMSB, % [41-42]
Bifenthrin	Insakar	Georgia, 1/8/2015, 137	10	91.5
Gamma-cyhalothrin	Trojan 150 CS	EU, 11/07/2009	15	64.2
Malathion	Malafos	Georgia 12/2/2015, 144	50	83.8
Dimethoate	Dimevit	Georgia 9/25/2017, 288	40	93.3

In all insecticidal combinations (see Table 1) we used the widely applied insecticides registered in EU or Georgia: "Insacar" ("Parijat Industries PVT. Ltd", India) with 100 ml of bifenthrin per liter; "Trojan 150 CS" (FMC, Australia) with 150 ml/l of gamma-cyhalotrin per liter; "Malafos" ("Parijat Industries PVT. Ltd", India), with 500 ml of malathion per liter; and "Dimevit" ("Ukravit Ltd.", Ukraine - Georgia), with 500 ml of dimethoate per liter.

Emulsifier-dispersant (surfactant) "Lansperse BIO-868" has entirely natural and 100%-biodegradable components, does not contain any volatile organic compounds (VOCs), does not cause flocculation (coagulation), provides a balanced homogenous distribution of micro-particles, does not cause skin irritation, is slightly eco-toxic (in terms of used concentrations and dosages is practically not-eco-toxic). Hydroxyethylcellulose (HEC) is widely used as lubricant, water-binder and thickening agents in many industry applications, that is, personal care products, pharmaceutical formulations, building materials, adhesives, etc., and as stabilizer for liquid soaps, provides high stability of dispersions and is practically non-toxic.

Methodology

Insecticidal formulation for control (relative) measurements

One of the main difficulties of the study was the fact that (due to various objective reasons) the number of test insects was limited and, consequently, the number of replications in the laboratory testing process was reduced. Therefore, in order to increase the accuracy and reliability of testing, it became necessary to make relative measurements in comparison to a combined "reference" insecticidal formulation comprehensively described in the literature, widely used in practice and characterized by a high biological effectiveness and a low persistence in processed plants. ProStore 420 EC supplied by FMC was studied and chosen as the most appropriate insecticidal formulation [47]. This combined drug contains malathion (up to 45%), bifenthrin (up to 5%), calcium dodecylbenzenesulfonate (up to 5%), 2-ethylhexane-1-ol (up to 5%), ethoxylated polyarylphenol (up to 5%), petroleum and heavy aromatic hydrocarbons (up to 50%), 1,2,4-trimethylbenzene (up to 2%). In order to compare the biological effectiveness and toxicity of the studied insecticidal combinations of the warm-blooded mammals, the following composition was chosen: malathion 400 g/l and bfenthrin 20 g/l, which is characterized by especially high biological effectiveness against various harmful pests in cereals, flour and groats.

Preparing of disperse insecticidal combinations

Food grade freshwater diatomite of the Kisatibi (Southern Georgia) deposit was immersed in water for 30 minutes, filtered, and after 45 minutes of heating in a microwave oven at 135 °C ground into a 10-50 μm particle micro-powder. All other components were dispersed in water using a standard homogenizer at a frequency of 15000 rpm for 8–10 min. Diatomaceous earth micro-powder was added to the dispersion under constant mixing conditions and treated in an ultrasonic homogenizer for 25–30 min to obtain a stable dispersion. Food grade kaolin supplied by Neelkanth Finechem LLP (Jodhpur, Rajasthan, India) was thermally processed for 10 minutes in a microwave oven at 155 °C. All other components were dispersed using a standard homogenizer at 12000 rpm, followed by a continuous stirring for 4-5 minutes in a microwave oven at 65-70 °C. The yielded mixture was dispersed using an ultrasonic homogenizer for 15-20 minutes to obtain a stable dispersion.

Methodology of the biological effectiveness study

The essence of the synergistic approach is the determining of optimal ratio of the amounts of active components, solvents and emulsifier-dispersers and of the biological effectiveness and terms of action of the corresponding combinations. Optimization of the ratio of each of the active insecticidal synthetic and natural substances should ensure maximum synergistic effect, while the optimal ratio of natural materials (diatomite, kaolin), emulsifier-disperser, Hec (Hydroxyethyl –cellulose) and water content should ensure the production of the long-lasting disperse mixtures. So, the following sequence of the relevant operation stages was identified: dispersing of diatomite or kaolin, rosemary, and hydroxyethyl-cellulose to obtain the most stable dispersions in water and emulsifier; dispersing of appropriate ratios of synthetic pyrethroid and organophosphorus insecticides in the manufactured stable dispersions; determining the biological effectiveness of the developed and tested combinations; evaluation of the total biological effect and synergy of active components (compared to the reference insecticidal formulation). Based on the obtained results, it became possible to assess the acquired resistance of pests, evaluate the total share of the combined mineral and essential oil components and assess the acute toxicity of the tested drugs to warm-blooded mammals and avian embryos.

Experimental: laboratory research and field trials

Composition of the tested insecticidal combinations

Formulations made from the components selected on the basis of literal data and the results of the preliminary research must meet the following major requirements relevant to the main purposes of the study: concentration of synthetic components in combinations should not exceed the values recommended by the relevant regulations; their concentration and the average total biological effectiveness must be relatively low to allow an accurate estimation of the “strong” synergistic peaks; their average biological effectiveness must be high enough for observing and accurate estimation of the relatively “weak” peaks and “non-synergistic” (slowly varying) contributions; the active component ratio step should be small enough to detect and evaluate all noticeable peaks of synergy.

Considering these requirements, 37 combinations with the conditional name “Dibifmal” (combinations D-1 – D-37) and 37 combinations with the conditional name “Kagcidim” (combinations K-1 – K-37) were prepared. In combinations D-1 – D-37 the mass content of active synthetic components varied in a constant step as follows: “Insakar - from 0 to 10 %, “Malafos” - from 10 % to 0. The mass content of active synthetic components in K-1 – K-37combinations varied in a constant step as follows: “Trojan 150 EC - from 0 to 10%, Dimevit - from10% to 0. Content of the active synthetic components in the tested insecticidal combinations and their expected total relative biological effectiveness are given in Tables 2, 3 and Figures 1, 2.The expected total biological effectiveness of the tested combinations (ETBE) characterizing the summarized effectiveness of components in absence of any synergistic (super-additive or antagonistic) interaction can be easily calculated using literary data (e. g. [24-27, 30-33, 43]).

Table 2. *Composition of the tested malathion-, bifenthrin- and diatomite-based insecticidal combinations D1- D7 (water - 73.3 %, HEC - 1.2 %, BIO-868- 3.3 %, essential oil – 0.2 %, diatomaceous earth –12 %).*

No	Malafos, mass %	Insakar, mass %	ETBE, %	No	Malafos, mass %	Insakar, mass %	ETBE, %
D1	10	0	100	D20	4.72	5.18	104.88
D2	9.7	0.28	100.25	D21	4.45	5.55	105.14
D3	9.4	0.56	100.51	D22	4.19	5.81	105.4
D4	9.16	0.84	100.77	D23	3.92	6.08	105. 66
D5	8.88	1.12	101.03	D24	3.64	6.36	105.91
D6	8.6	1.4	101.29	D25	3.36	6.64	106.17
D7	8.32	1.68	101.55	D26	3.08	6.92	106.43
D8	8.04	1.96	101.8	D27	2.8	7.2	106.69
D9	7.78	2.22	102.05	D28	2.52	7.48	106.94
D10	7.51	2.49	102.30	D29	2.24	7.76	107.2
D11	7.24	2.76	102.56	D30	1.96	8.04	107.46
D12	6.96	3.02	102.81	D31	1.68	8.32	107.71
D13	6.7	3.30	103.06	D32	1.4	8.6	107.97
D14	6.42	3.58	103.32	D33	1.12	8.88	108. 23
D15	6.16	3.84	103.58	D34	0.84	9.16	108.48
D16	5.9	4.10	103.84	D35	0.56	9.44	108.74
D17	5.63	4.37	104.11	D36	0.28	9.72	109
D18	5.36	4.64	104.36	D37	0	10	109.24
D19	5.09	4.91	104.62				

Table 3. *Composition of the tested dimethoate-, gamma-cyhalothrin- and kaolin-based insecticidal combinations K1-K37 (water - 73.3 %, HEC - 1.2 %, BIO-868 - 3.3 %, essential oil – 0.2 %, diatomaceous earth –12 %).*

No	Dimevit, Mass%	Trojan 150 CS, mas %	ETBE, %	No	Dimevit, mass%	Trojan 150 CS, mas %	ETBE, %
K1	10	0	100	K20	4.72	5.18	101.9
K2	9.72	0.28	100.1	K21	4.45	5.55	102.
K3	9.44	0.56	100.2	K22	4.19	5.81	102.1
K4	9.16	0.84	100.3	K23	3.92	6.08	102.2
K5	8.88	1.12	100.4	K24	3.64	6.36	102.3
K6	8.6	1.4	100.5	K25	3.36	6.64	102.4
K7	8.32	1.68	100.6	K26	3.08	6.92	102.5
K8	8.04	1.96	100.7	K27	2.8	7.2	102.6
K9	7.78	2.22	100.8	K28	2.52	7.48	102.7
K10	7.51	2.49	100.9	K29	2.24	7.76	102.8
K11	7.24	2.76	101	K30	1.96	8.04	102.9
K12	6.96	3.02	101.1	K31	1.68	8.32	103
K13	6.7	3.30	101.2	K32	1.4	8.6	103.1
K14	6.42	3.58	101.3	K33	1.12	8.88	103.2
K15	6.16	3.84	101.4	K34	0.84	9.16	103.3
K16	5.9	4.10	101.5	K35	0.56	9.44	103.4
K17	5.63	4.37	101.6	K36	0.28	9.72	103.5
K18	5.36	4.64	101.7	K37	0	10	103.6.
K19	5.09	4.91	101.8				

To check the liability of the calculated data on the expected biological effectiveness, we prepared 6 water solutions corresponding to formulations D-1, D-18, D-37, K-1, K-18, K-37 containing only the active synthetic insecticidal components (without diatomite, kaolin, hydroxyethyl- cellulose, rosemary essential oil and emulsifier-disperser Lansperse-BIO8) and determined their biological effectiveness against the test insects of groups E and F. The comparison with the formulations D-1, D-18, D-37, K-1, K-18, K-37 allowed us to estimate the joint contribution of the mineral components, hydroxyethyl-cellulose, rosemary essential oil and emulsifier-disperser in the integral biological effectiveness of the mixtures. In principle, in such a way it is possible to evaluate the contribution of each component to the integral (total) biological effectiveness.

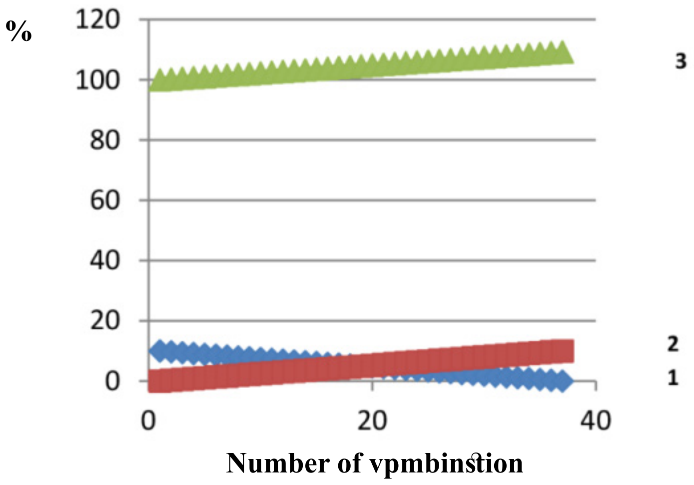

Figure 1. Content of “Malafos”(1, mass %) and “” “Insakar” (2, mass %) in insecticidal combinations D1-D37, and the dependence of expected relative biological effectiveness% (3, %) on the number of combination

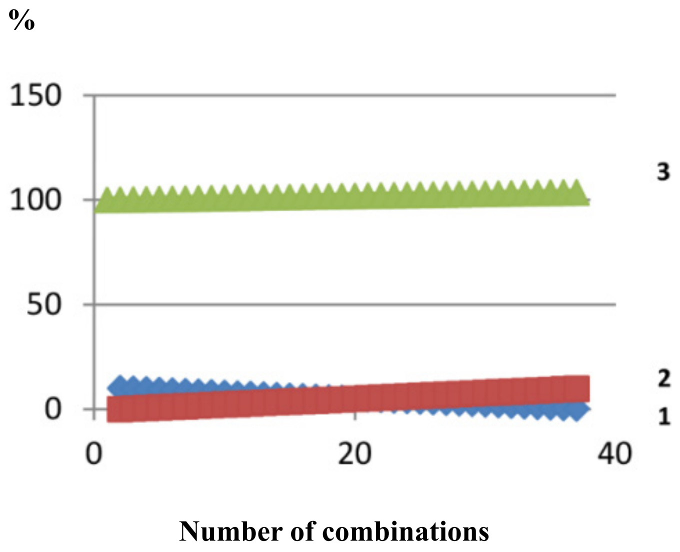

Figure 2. Content of "Dimevit" (1, mass %) and "Trojan" (2, mass %) in insecticidal combinations D1- D37, and the dependence of expected relative biological effectiveness% (3, %) on the number of combination

Determination of the relative biological effectiveness of developed insecticidal combinations

The biological effectiveness of all the above insecticidal combinations was compared to the biological effectiveness of D-1 and K-1 combinations respectively to determine their relative biological effectiveness utilizing the experimental method and design given in [48]. The only difference was that the test insects were disposed in 5 l volume transparent containers (25 insects in each) with sprouted wheat. The provided and controlled testing conditions were as follows: air temperature T = 25 ± 2 ^{0}C, relative humidity RH= 55-65 %, bright time - 16 hours, dark time - 8 hours. An important advantage of the developed experimental design was the possibility of the direct evaluation of the change of residual biological effectiveness over the testing time. All containers were sprayed just before the first group of insects was placed in the containers. During the week all insects in the test and control groups were observed and the dead insects were daily removed and counted. On the last day of the week all the dead and survived insects were removed and counted. The food layovers were daily carefully removed. The described procedure was repeated for two times. Biological effectiveness was determined daily and weekly, summarized and averaged during the whole period of testing, providing the data on daily, weekly and total (averaged) biological effectiveness of the tested combinations and its dynamics. According to [4] and [43] we expected to have moribund insects when applying the bifenthrin containing combinations, but no-one moribund case was fixed during our 3-week study. Probably, the insecticidal impact of diatomite, kaolin and rosemary oil did not allow the moribund insects to recover. All insects were exposed to the equal dose of the tested combinations (50 ml per container) which was approximately 5 times lower than the recommended application dose of malathion formulations [43]. The results of testing are given in Figures 4-7. The biological effectiveness (BE) of the combinations was determined using the well-known Abbott formula ("modified" by us to account for the insect mortality in the control group):

$$BE\ (\%) = [1 - (n_1 + k \times n_2)/n_3] \times 100 \quad (1)$$

where, BE is the biological effectiveness of the insecticide, n_1 is the number of insecticide-treated surviving insects in the test group (in our case, groups A and B), n_2 is the number of insects killed in the control group, and n_3 is the number of surviving insects in the control group, k is the empirical coefficient close to 1 (we used the value k = 1).

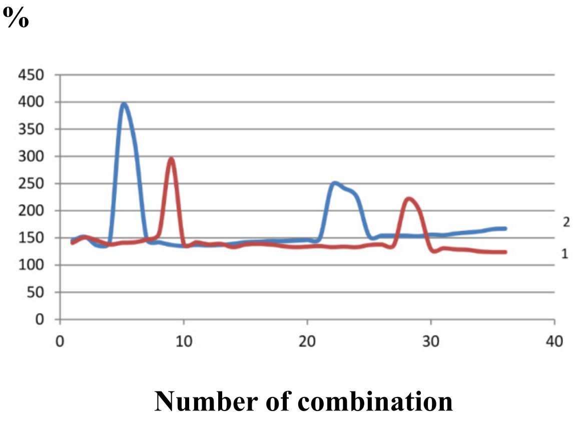

Figure 3. Dependence of the relative biological effectiveness of malathion-, bifenthrin- and diatomite-based insecticidal combinations against group A (1, %) and B (2, %) insects on the number of combination.

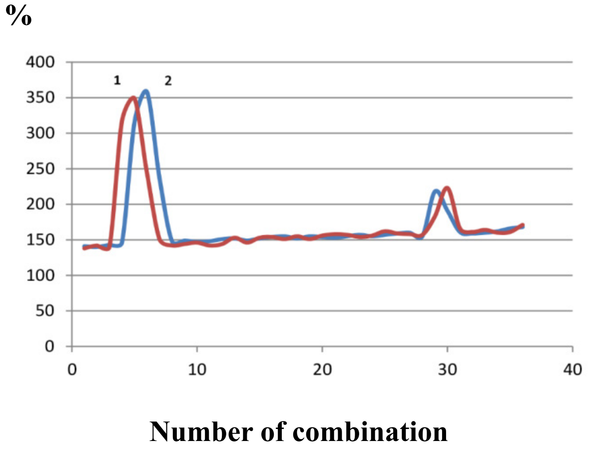

Figure 4. Dependence of the relative biological effectiveness (RBE) of gamma-cyhalothrin-, dimethoate- and kaolin-based insecticidal combinations against the group A (1,%) and B (2, %) insectson the number of combination.

It should be noted that the modified formula can be easily applied both to the laboratory and field trial data. The determined total (averaged) relative biological effectiveness of the malathion–bifenthrin-diatomite based and the dimethoate-gammma-cyhalothrin-kaolin based insecticidal combinations against group A and B insects is given in Figures 3 and 4.

Determination of the synergy (co-toxicity) factor. The relative synergy (co-toxicity) coefficient C_o is usually applied in laboratory research [21, 43-46] to characterize synergy of components (see formula 2):

$$C_o\ (\%) = 100 \times [(\text{Observed Mortality} - \text{Expected Mortality})/\text{Expected Mortality}]\ (2)$$

We introduced and used a modified version (formula 2') which is more precise and simple in use and can be used for an arbitrary number of components of mixture and takes into account the natural mortality (mortality of insects in the control group):

$$CT\ (BE, \%) = 100 \times (BEM - BEE) / BEE \qquad (2')$$

where, BEM and BEE respectively are the experimentally measured and predicted (expected) values of the relative biological effectiveness.

The above formula does not require the data on lethal doses and can be easily used both in the laboratory and field trials. In this case, the measured relative biological effectiveness (BEMR) is determined using the data of field trials in the test sites after applying the tested insecticidal combinations, while the expected relative BE (BETR) will be a sum of BEMRs of all components determined using the data of field trials conducted in the control sites.

Taking into account that almost all data on the lethality and biological effectiveness of insecticides are determined by testing of non-resistant populations of insects (e. g. [43-45]) and that the non-synergistic part of the biological effectiveness of bifenthrin-based combinations against insects collected in Abasha-Senaki sites decreased with the increase of content of bifenthrin, indicating a significant resistance to bifenthrin, we decided to apply the formula (2'), where the expected biological effectiveness(BEE) is supposed to be equal to the linearized non-synergistic (lowly varying) part of the measured biological effectiveness. This approach allows several times to reduce the amount of time and labor expended to quantify the synergy of the components and assess the possible practical benefit from the use of insecticidal “cocktails” against both resistant and non-resistant pest populations. The accuracy of the results obtained using this approach can easily be verified by measuring the biological effectiveness of several specific formulations of the studied combinations and determining the average correction factor. The obtained data on the relative synergy (co-toxicity) factor values are given in Figures 5 and 6. It is especially important that both combinations show a pronounced synergy of components, but the synergy peaks of the malathion-bifenthrin-diatomite based combinations in case of group A insects are noticeably “weaker” than in case of group B insects, while the synergy peaks of the dimethoate-gamma-cichlothrin-kaolinbased combination are practically the same for the both groups of insects. Another non-trivial result is the observed shift of the synergy peaks towards the higher content of bifenthrin in case of A group insects. In our opinion, this shift can be explained by the increased amount of bifenthrin required to neutralize the additional mechanism(s) of protection against bifenthrin, which is (are) the main cause of resistance development.

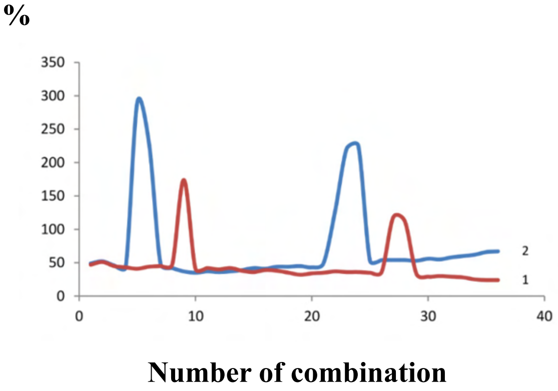

Figure 5. Dependence of the synergy (co-toxicity) factor CT of malathion-, bifenthrin- and diatomite-based insecticidal combinations against group A (1, %) and group B (2, %) insects on the number of combination.

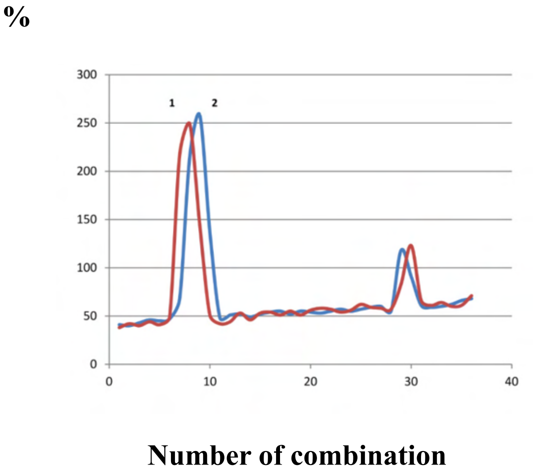

Figure 6. Dependence of the synergy (co-toxicity) factor CT of dimethoate-, gamma-cyhalothrin- and kaolin-based insecticidal combinations against group A (1, %) and group B (2, %) insects on the number of combination.

Field trials in the cornfield located in Senaki municipality

The field testing of the developed insecticidal combinations was held in the cornfield in village Ettseri of Senaki municipality located in the subtropical clay podzole zone which is typical to many districts of the Western Georgia (mainly in Imereti, Guria, Samrgrelo and Ajara regions) which is strongly infested by BMSB. The trials were carried out by the researchers of the Institute for Problems of Engineering Physics of the Georgian Technical University (GTU) and agro-technical group of the National Food Agency (NFA) of Georgia, with the help and under supervision of NFA. The corn field was divided horizontally and vertically into six “columns” and five conditionally “rows”, each with 18 observation plants located at equal distances. The distance between first five “columns” was 20 m. The sixth (control) “column” was separated from all other “columns” by a 50 m wide fruit garden with trapping host plants, which were assumed to prevent migration of insects to the test site. The number of BMSB imago insects on all selected trees was counted and averaged. In all cases their number ranged from 44 to 71. Finally, the average number of insects per plant was estimated as 59 ± 2.On the first day, from 8 to 9 o’clock p.m., six “columns” were sprayed with the most effective developed combinations (K5, D9, K24 and D28 respectively) and the insecticide Prostore 420 EC using backpack-type “Solo” engine. Thereafter, during the next 9 days from 5 to 6 o’clock p.m., the survived insects in all six “columns”[2] were daily counted by the local volunteers.1On the 10-th day the BMSB two-part pheromone lures supplied by the „Evergreen Growers Supply“ (Clackamas, Oregon, US) were fixed to the observation trees to attract insects, and on the 11-th day field trials were continued.The data on the biological effectiveness of the four tested and one control insecticidal formulation are given in Figures 7 (days 1 to 9) and 8 (days 12-17). Systemized data of 18 days of the field trial are given in table 4.

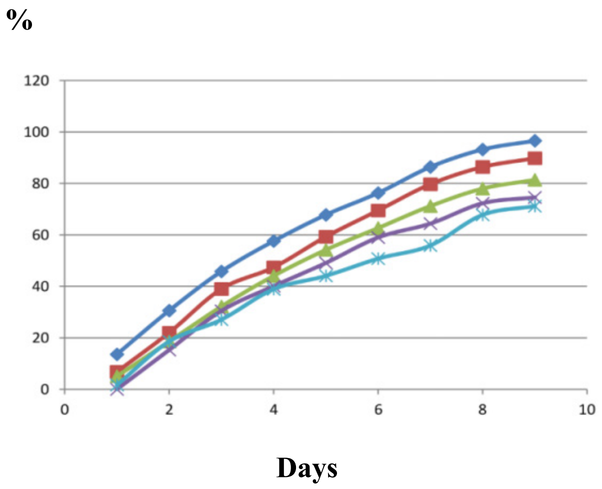

Figure 7. Biological effectiveness of the field tested insecticides (from up to down) K5 (%), D9 (%), K30 (%), D28 (%) and control insecticide ProStore 420 EC (%), days 1-9).

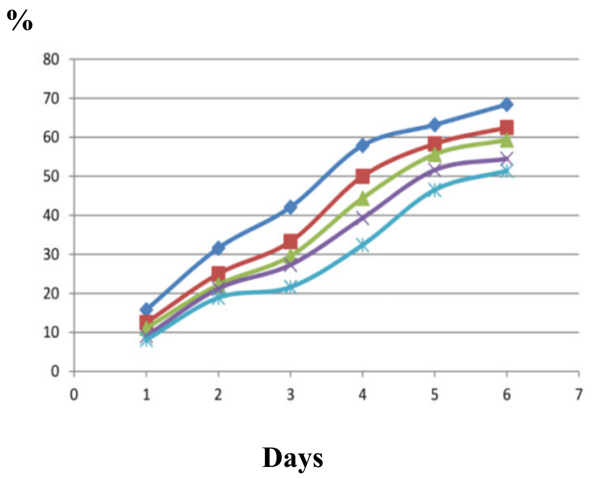

Figure 8. Biological effectiveness of the field tested insecticides (from up to down) K5 (%), D9 (%), K30 (3, %), D28 (%) and control insecticide ProStore 420 (%), days (12-18)

The average number of insects N_1 counted on the test plants of the corresponding five “columns” was taken as the number of insects N_1 survived in the test groups. The averaged reduce of the insects on the plants of the sixths (control) “column” was taken as the number of insects N_2 killed in the control group. The averaged number of insects N_3counted in “column” 6 was taken as the number of insects survived in the control group. Calculation of the biological effectiveness was made using formula (3):

$$BE (\%) = [1 – (N_1 + k \times N_2)/N_3] \times 100 \qquad (3)$$

where, BE is the biological effectiveness of the insecticide, N_1 is the number of surviving insecticide-treated insects on the plants (“colimns” 1-5), N_2 is the decrease of the average number of insects in the control group (“column 6”), and N_3 is the average number of surviving insects in the control group (“column” 6), k is the empirical coefficient close to 1 (here we used the value k = 1).

Table 4. *Data on the biological effectiveness of insecticidal formulations during the field trial*

Day of the trial	The average number of insects on the plants in the test and control “columns” 1 to 6						Decrease of the average number of insects on the plants in the test and control “columns” 1 to 6						Biological effectiveness of insecticidal combinations in the test “columns” 1 to 5, %				
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5
0	59	59	59	59	59	59	-	-	-	-	-	-	-	-	-	-	-
1	48	52	53	54	55	56	11	7	6	5	4	3	14	7	5	3	1.7
2	38	43	45	47	48	56	21	16	14	12	11	13	31	22	19	15	12
3	32	36	40	41	43	59	27	23	19	18	16	0	46	39	32	31	27
4	27	33	35	37	38	61	32	26	24	22	21	-2	58	47	44	40	39
5	18	23	26	30	32	58	41	36	33	29	27	1	68	59	54	49	44
6	14	19	22	24	29	59	45	41	37	35	30	0	76	70	62	59	51
7	10	14	19	23	28	61	49	45	40	36	31	-2	86	79	71	65	56
8	7	11	16	19	22	62	52	48	43	40	37	-3	93	86	78	72	68
9	4	8	13	17	19	61	55	53	48	42	42	-2	97	90	81	75	71
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

12	19	24	27	33	37	59	-	-	-	-	-	-	-	-	-	-	-
13	18	23	26	32	36	61	1	1	1	1	1	-2	16	13	11	9	8
14	12	19	22	27	31	60	7	5	5	6	6	-1	32	25	22	21	19
15	10	15	18	23	28	58	9	9	9	10	9	1	42	33	29	27	22
16	9	13	16	21	26	60	10	11	11	12	11	-1	58	50	44	39	32
17	8	11	13	17	21	60	11	13	24	14	16	-1	63	58.3	57	51.6	47
18	6	9	11	15	18	60	13	16	16	18	19	0	68	62	59	54.5	51

Testing of the acute toxicity of the developed compositions to mammals

One of the most important characteristics of insecticides is their acute toxicity to the warm-blooded mammals. On the other hand, modern requirements for the standards of humane treatment of test animals (which should exclude the death of animals, causing them severe pain, significant deterioration of health, prolonged forced immobility, whole body anesthesia, etc.) are becoming increasingly stringent, and the modern methods of animal testing must be fitted to all modern 4 R (replace, refine, reduce, responsibility) principles of humane treatment of laboratory animals. The acute toxicity of developed drugs was tested using an original methodology developed and tested at the Georgian Technical University and Ivane Beritashvili Center for Experimental Biomedicine [49-51]. Methodology is based on the observation of the behavioral and physiological parameters of white rats in a standard branched training maze consisting of lighted and darkened sections.

In addition to behavioral and mental characteristics, the four indicators, measured by means of the veterinary system of non-invasive blood pressure measurement of rodents "Systola", the non-contact infrared thermometer for animal research BIO-IRB153, the Free Radical Analytical System FRAS5 for measuring oxidation stress caused by reactive species (RS) and reactive oxygen species (ROS), and the pulse oximeter transflectance sensor NONIN 2000T were used for characterization of the general acute toxicity of insecticidal formulations to the exposed animals. The acute toxicity induced by injection of 30 mg/kg of ProStore 420 EC was taken as a 100% value. The acute toxicity was characterized by the Combined Toxicity Index (CTI) calculated according to formula (3):

$$CTI = [(N_1/N) \bullet (T_e + T_l) /T)] \bullet (\Delta B_p \bullet \Delta B_t \bullet \Delta ROS/S)^2 \quad (4)$$

where CTI (dimensionless quantity) is the combined toxicity index, N_1 is the number of errors made during passage through the maze, N is the total number of decisions made during passing through the maze, T is the total time of passing through the branched maze,T_l is the time spent in the lighted sections of the maze, T_d is the time spent in the darkened sections of the maze, ΔB_p is a relative change of blood systolic pressure, ΔB_t is a relative change of body temperature, ΔROS is a relative change of reactive oxygen species in blood, whereas S is a relative change in blood oxygen saturation as a result of passing through the maze [18–20]. During the first 3 days of observation, 96 white rats divided into 8 sub-groups were injected intramuscularly with equal doses 30 (10 + 10 +10) mg / kg of solution of tested formulations (D18 or K18), one reference insecticidal formulation(ProStore 420 EC) and standard saline solution total dose. 3-day exposure was followed by standard maze tests for 10 days, under monitoring of all parameters given in formula (3) using the above listed devices. With aim to estimate the possible influence of other abiotic and biotic stresses (high temperature, dehydration, low humidity, long-term immobilization, restriction of mobility, lack of oxygen, etc., another group of 96 white rats was 3-times exposed to the Whole Body Hyperthermia (WBH) in a special camera at 42 ± 1 ^{O}C for 10 minutes per day and then tested during 10 days according to the above scheme. The averaged results of the carried out research of hyper-thermally untreated and hyper-thermally treated animals are given in Figures 9 and 10.

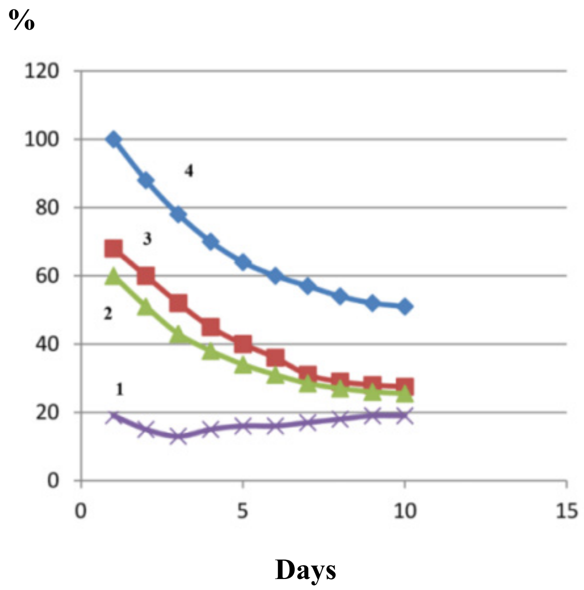

Figure 9. Acute Toxicity (CTI) of the standard physiological solution (1, %), formulation D18 (2, %), formulation K18 (3, %), and insecticide ProStore 420 EC (4, %) against the group A insect.

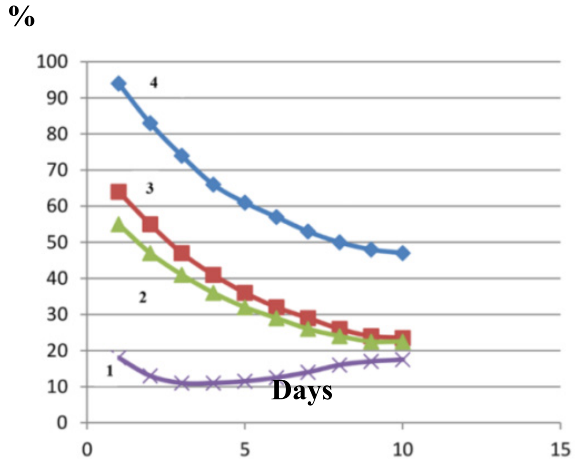

Figure 10. Acute toxicity (CTI) of the standard physiological solution (1, %), formulation D-18 (2, %), formulation K-18 (3, %), and insecticide ProStore 420 EC (4, %) against group A insects previously treated in WHB camera.

However, the WBH exposure has noticeably improved all registered parameters and reduced the CTI for all tested combinations, which can be the result of a strong immune response of the organism to the hyperthermia stress providing (due to the Hormesis phenomenon) a higher resistance to the inhibitory impact of toxicants on the endocrinal metabolic activity of the affected organism [49].

Testing of the acute toxicity of the developed compositions to bird embryos

In order to support or refute the reliability of the results of above experimental studies, we decided to prove their reliability using an alternative method of the nondestructive diagnostics of bird embrios. Among the appropriate modern methods of the visible, infrared and ultrasonic ovoscopy [52-55] widely applied in the medical, veterinary and agricultural research, we have chosen the most easily available, simple and low cost method of the visible light ovoscopy. The results of our study based on the visible range ovoscopy of eggs of chicken, duck and goose eggs exposed to various kinds of potential toxicants (combined nanofluids, radiomimetics, anticancer drugs, radiation, short-term mild hyperthermia, etc.) will be published in the nearest future.

The main criteria for the evaluation of toxicity was the rate of mortality of duck and chicken embryos, mortality of hatched ducklings chicks and disruption of the normal development of embryos during their development within 21 and 24 days, depending on the bird species. The useful additional criteria are as follows.

The visible delamination of the shell membrane is manifested by the displacement of the air chamber in the lateral direction or towards the sharp side of the egg; an abnormally large air chamber indicates that the egg is old and stale, which means it is not suitable for hatching; if the translucent egg looks completely orange or orange-red, it means that the yolk has burst and mixed with the protein; if the grains are torn off, then the yolk will dangle freely throughout the intranasal space; If the yolk sticks to one wall of the shell, it also means that the egg is either old or stale. The appearance of blood rings foreshadows the death of embryos in the early stages of incubation, when the yolk is overgrown with blastoderm. A dark embryo adhering to one side of the shell indicates the absence or insufficient development of the allantois blood vessels (in later stages, the embryo is immobile and the circulatory system is underdeveloped); with insufficient heating of the eggs, the growth of embryos slows down, the development of embryos is delayed in time and the number of suffocations increases; overheating causes uneven development, resulting in ducklings and chickens hatching with an unstretched yolk sac or unused protein; with a lack of moisture, eggs lose much in weight, heat exchange increases, which leads to an increase in temperature inside the incubation material and causes various developmental disorders, ducklings and chickens hatch early, the shell becomes dense, shrinks and it is much more difficult to pierce it; with an excess of moisture, an increase in amniotic fluid occurs, which is detrimental to the chicks (when they try to break through the shell, they swallow this liquid and suffocate).

Typical images derived using the visible light ovoscopy of the duck and chicken eggs with the normally developing embryos are given in Figures 11 and 12.

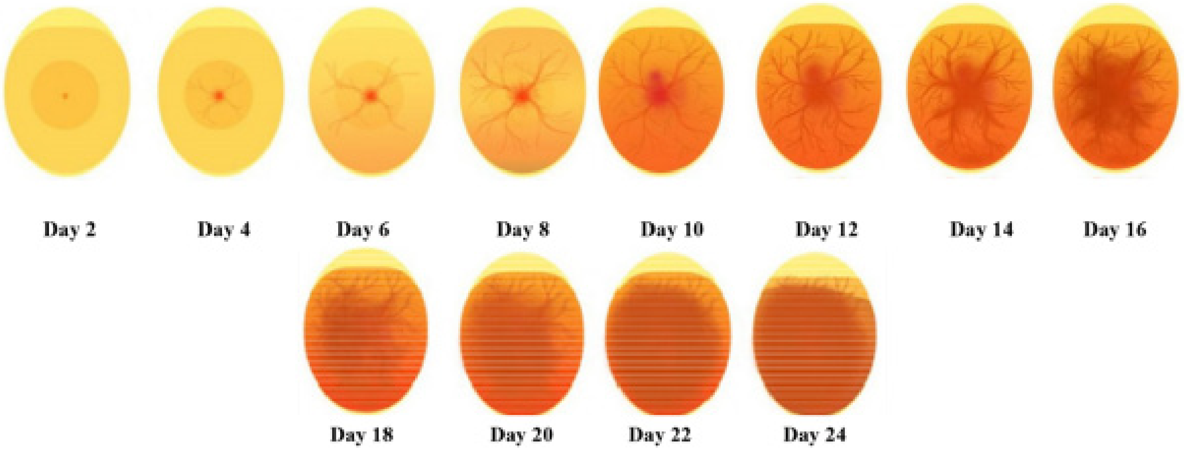

Figure 11. Typical images derived using the visible light ovoscopy of the duck eggs with normally developing embryos.

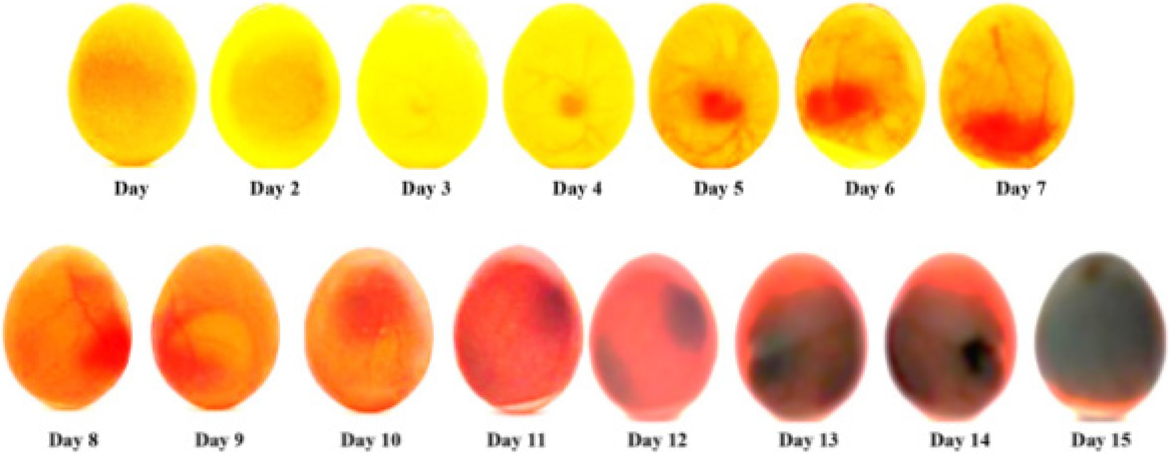

Figure 12. Typical images derived using the visible light ovoscopy of the chick eggs with the normally developing embryos.

The test and control groups of 128 eggs in each were incubated and monitored in the 80 Wt and 120 V AC / 12 V DC computer controlled mini-incubators with red light emission diodes (LEDs) and self-assembled detection systems. The incubation temperature was (38± 0.5) C^0 and the relative humidity (RH) was (68 ±1) %. About 1900 images were made, transferred to a computer program and evaluated.

Results and discussions

Biological effectiveness and observed synergy of the components of developed insecticidal combinations

The results of the reported research gave us the possibility of experimental evaluation of the following significant characteristics of the developed insecticidal combinations, such as: a) relative biological effectiveness against the BMSB and its dependence on the ratio of active synthetic components; b) relative synergy against the BMSB and its dependence on the ratio of active synthetic components; c) acute toxicity of tested and control drugs to the warm-blooded mammals and bird embryos. It should be noted that the step of changing the ratio of the active synthetic components appeared to be small enough to make it possible to fix and evaluate the synergistic effect. At the same time, the use of smaller steps and the sequential removal of the active components can enable a more accurate estimation of the contribution of each component to biological effectiveness and synergy of the whole combination.

Biological effectiveness of the developed and tested bifenthrin-based insecticidal combinations against the insects collected in Abasha and Senaki municipalities was found to be significantly lower than against the insects collected in Kobuleti and Khelvachauri municipalities. At the same time, the indicated difference for gamma-cyhalothrin based formulations was many times smaller.

In contradiction to the results of calculations based on the data from publications [43-46], the non-synergistic part of the biological effectiveness of bifenthrin-based combinations against insects collected in Abasha-Senaki sites decreased with increase of content of bifenthrin. Meanwhile, the non-synergistic part of the biological efficacy of the combination against test insects supplied from the Kobuleti and Khelvachauri regions increased with increasing of the bifenthrin content. It is also noteworthy, that the height of synergy peaks of bifenthrin based formulations in case of insects supplied from Kobuleti and Khelvachauri municipalities was significantly more than the height of synergy peaks in case of the insects supplied from Abasha and Senaki municipalities.

The results of our study clearly show that the non-synergistic relative biological effectiveness of the insecticidal combinations based on dimethoate, gamma-cyhalotrin and kaolin increases with increasing of gamma-cyhalothrin content in both studies, while the peaks of synergy have a practically equal height.

In our opinion, all these facts can be explained by the fact that significantly more intensive spraying with bifenthrin was carried out in the Abasha-Senaki region than in the Kobuleti and Khelvachauri municipalities, which led to the development of high resistance to bifenthrin in BMSB population being widespread there.

Importantly, in addition to strong synergy peaks, all of the investigated combinations have significant non-synergistic (or less synergistic) components due to mineral (diatomite, kaolin) and essential oils (rosemary essential oil), accounting for about one-third of the total non-synergistic part. This component can become important in case of strong resistance to the synthetic components of the pesticides. In addition, it is highly probable that the synergy of synthetic insecticides, diatomite, kaolin and essential oils against the Brown Marmorated Stink Bug and other agricultural pests can be used for long-term storage and transportation of cereals and other food products, which is becoming increasingly relevant because of tightening of international regulations for the synthetic pesticides (e. g. [56]).

The acute toxicity of the developed compositions to mammals

The results of testing in white rats showed that: a) the negative effect caused by the effect of the tested combinations on the first day of observation is almost twice less than the negative effect of the control drug; b) the tested animals within 10 days after exposure practically completely recovered their behavioral and physiological functions (activity, memory and learning skulls, blood oxygen saturation, systolic pressure and body temperature stability, oxidative stress level, etc.). Further prolonged (30 days) observation showed that their general state of health is practically unchanged).

The above results in more than 93 % of cases were supported by the data of the computer assisted visible light ovoscopy of the duck and chick embryos, which can be regarded as a highly prospective tool for replacing of a considerable part of the living animal testing. Ultrasound sonography can be an important step to a more informative non-invasive study of the acute toxicity of the developed formulations to bird embryos.

Prospective of the farther development of insecticidal combinations with increased biological efeectiveness and implementation of the findings of the reported research

Testing of the acute toxicity of the developed compositions to pollinators and aquatic organisms

The studies carried out are a part of the comprehensive research of the developed insecticidal formulations necessary for their registration and introduction in Georgia and other countries. This process will be continued with the help and guidance of the National Food Agency of Georgia. An important part of the further research will be the study of acute toxicity in relation to pollinators (honey bees) and aquatic organisms (freshwater fish Cyprinus Carpyo and other freshwater fish species) which should be performed in the Institute for Problems of Engineering Physics of the Georgian Technical University, in the Science Research Center of the Ministry of Environment Protection and Agriculture of Georgia, in the laboratory of the technological and special measures for the prevention of bee diseases of the National Research Center of P. Prokopovych Institute of Beekeeping of the National Academy of Agrarian Sciences of Ukraine, in the company Ukrainian Bee LLC, and in the Department of pond aquaculture and hydrobiont ecology of the Institute of Fisheries of National Academy of Agrarian Sciences of Ukraine.

Honey bee is among the five most important pollinators over the world and the main pollinator of the agricultural plants. Synthetic insecticides and their residues (together with Varroa destructor mite), due to their high lethality to living organisms and extremely wide spread in the environment, form the main hazard threatening this insect, which is vitally necessary for humans and all living nature. The widely used lethal doses of the acute toxicity (LD_{10}, LD_{25}, LD_{50}, LD_{75}, etc.), cannot directly reflect the short- and long-term impact of insecticides on the vital characteristics of honey bees like the moving and foraging activities. At the same time, in our opinion, applying of the lethality doses approach to the honey bees and other important pollinators is in a direct conflict with the modern principles of scientific ethics and ecological thinking. Moreover, even the use of sub-lethal doses close to lethal, in our opinion, is meaningless, since in the overwhelming majority of cases it does not provide the necessary information about the real magnitude of the impact during the massive and long-term use of insecticides in food and ornamental plant growing, forestry, vector deisinsection, etc. Therefore, in the last two decades, in laboratory and semi-field studies more and more often are used the doses at least an order of magnitude less than the lowest lethal dose (LD0) measured ad that time for the tested kind of insects. So, foraging behavior and colony conditions become the main indicators for assessing the toxicity of pesticides and their combinations. The typical identified cases of the impact of insecticides on honey bees are the following (e. g. [57]) : precocious foraging; no hypopharangeal gland development; inhibited hypopharangeal gland development; regression of hypopharangeal glands; significantly suppressed hypopharangeal gland development; early degeneration of hypopharangel gland; decreased acquisition and persistence in conditioned response test; decreased house-cleaning; decreased learning ability, abnormal behavior (antennae leaning, rubbing together of hind legs, decreased flight activity and olfactory discrimination performance; increase in number of trials to abolish or induce habituation; slower learning of odor-mediated response; incorrect direction (angle of dance) on vertical surface; incorrect distance on horizontal surface; failure to return to colony; increased self-cleaning; abdomen tucking; rotating and cleaning of abdomen plus rubbing hind legs together; decreased walking; body insertion in cells; decreased brood, pollen patty consumption and foraging; hypothermia; differences in queen egg laying cycle, and numbers of larvae and pupae; less eggs laid; reduction in surviving brood; lower numbers of larvae ejected and increase brood production; elimination of brood production; decrease of sucrose intake; arrested egg development and larval mortality; queens ceased laying; increased queen supersedure; stimulated growth and maturation, failure to spin cocoon, hypersensitivity in simulation; adults emerge with wing malformation, stunted bodies, crippled legs and crippled or shortened wings; transient inhibition of activity; reduced honeybee visitation. Taking into account the modern principles of scientific ethics and ecological thinking we, like a number of researchers [e.g., 63] have chosen the following two Behavioral Sublethal Effects bio assays for determination of significantly subletal toxicity of the developed and control insecticidal formulations against the species of the Ukrainian honey bee (Apis mellifera sossimai Engel): the Forager Free Choice test and the Walking Activity Bioassay of Adult Workers.

Toxicity to aquatic organisms is also one of the main characteristics of insecticidal preparations (e. g. [58]). According to the generally accepted classification, impacts on aquatic organisms should be distinguished by their duration (long-term and short-term) and by the degree of threat to the life of living organisms (lethal and sub-lethal). Long-term exposures are considered to last more than 96 hours, and lethal ones lead to irreversible consequences and, during or shortly after exposure, bring to the death of the tested organism. For the above ethical reasons, we give up the use of lethal doses and concentrations from our studies and give preference to sub-lethal exposures excluding death as the endpoint of testing. In accordance with a preliminary agreement with the Department of pond aquaculture and hydrobiont ecology of Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine, the long-term monitoring of social behavior (locomotion, anxiety, and startle responses), other behavioral and respiratory disfunctions and alterations in respiratory rate and food consumption in various several fish species will be carried out during the sub-lethal testing.

Development and testing of the nano based insecticidal combinations

Along with the rapid increase of the world population the need for a substantially more productive agriculture is also growing. Weathering, erosion and desertification of fertile soils put on the order of the day the real danger of hunger not only in underdeveloped, but also in developing and developed countries on all continents of the globe. Irreparable climate change, and weathering, erosion and desertification of fertile soils put on the order of the day the real danger of hunger not only in underdeveloped, but also developing and developed countries on all continents of the globe. Even before the COVID-19 pandemic, around 690 million people worldwide suffered from hunger, which is 8.9 percent of the world's population, according to a UN report. Moreover, over the past year, this number has increased by 10 million, and over five years - by almost 60 million. The number of those experiencing food shortages is also growing. Last year, their number was about 750 million - this is almost every tenth inhabitant of the planet. Today, almost two thirds of severely food insecure people live in just 8 countries: Afghanistan, Democratic Republic of Congo, Ethiopia, Nigeria, South Sudan, Sudan, Syria and Yemen.

Last year, 21.3 percent of children under 5 years of age were stunted due to malnutrition, and 6.9 percent suffered from wasting; another 5.6 percent of children in this age group were overweight, which, as experts have repeatedly noted, is also associated with inadequate and irregular nutrition. The Global Food Crisis Network, which includes 15 major international partners, including UNICEF and the World Food Program (WFP), estimates that the number of people facing severe food insecurity will grow extra-linearly and could have disastrous consequences. 20 countries around the world are in urgent need of food aid, otherwise they face full-scale famine. This warning was issued by two UN food agencies. Residents of Yemen, South Sudan and Northern Nigeria are already on the verge of starvation, warn the report prepared by the World Food Program (WFP) and the Food and Agriculture Organization of the United Nations (FAO). While the vast majority of countries facing hunger are located in Africa, the problem also applies to other regions. Afghanistan in Asia, Syria and Lebanon in the Middle East, Haiti in Latin America - these and other states will not do without the help of the international community, since 34 million people are experiencing “the fourth degree of acute food shortage”, that is, they will be one step away from starvation.

Despite the huge efforts of the most important international organizations (UN, UNICEF, WFP, FAO, etc.) the problem cannot be solved without increasing of the fertility of soils and saving more of the harvested food. The existing and widely used fertilizers and pesticides are unlikely to be able to provide the necessary increase in the yield and safety of food, since a further increase in their use will certainly lead to an irreversible impact on the soil, surface and underground waters, wildlife and human health. It seems that synergistic combinations, including the use of nano-scale particles (e. g. [59]) will help humanity to significantly mitigate the terrible threat of mass starvation. Every year, about a third of all food produced in the world is either lost or wasted. In developing countries, 40 percent of food is lost during the harvest or processing phase - this is called food waste.

To increase the yields of crops it becomes necessary to increase continuously the frequency and volume of fertilizer and pesticide applications, while the development of resistance in pest populations makes the problem even more acute. Pollution with various agricultural chemicals causes a growing severe environmental impacts on soil quality, purity of water resources, safety of useful soil microorganisms, non-targeted insects including pollinators, reptiles, birds (including poultry), mammals (including cattle and other home animals) and human health and life. Moreover, the uncontrolled use of pesticides can, in principle, bring to a dramatic decrease of populations of pollinators and other useful insects and, therefore, cause even lower yields, The attempt to replace the hazardous synthetic compounds with the organic natural substances has a considerable potential, but seems to be useless against the most hazardous pests like BMSB and locusts.

Use of nano-materials (especially of natural mineral origin) as synergistic components of the fertilizers and pesticides can likely provide a novel approach to come closer to a more sustainable balance between the sharp increase in agricultural production and conservation of nature. However, due to the peculiarity of the nano-scale objects (which exhibit many properties that are completely different from those of bulk materials) toxicity of nanoparticles and their suspensions should be studied with special care. From general considerations, the use of nanofertilizers and nanopesticides should be allowed only in cases where the ecological effect of reducing the applied doses is several (at least 2-3) times higher than the negative effect of their measured or estimated toxicity. At the same time the so called nano-insecticides can significantly slow down the degradation of "usual" pesticides, enhance their persistence and increase their acute and chronic toxicity to the living nature (e. g. [60]). That is why, beginning from 2015 the Organization of Economic Cooperation and Development (OECD) established, developed and published the risk-assessment guidelines and various regulatory documents regarding manufactured nano-materials (e. g. [61]).

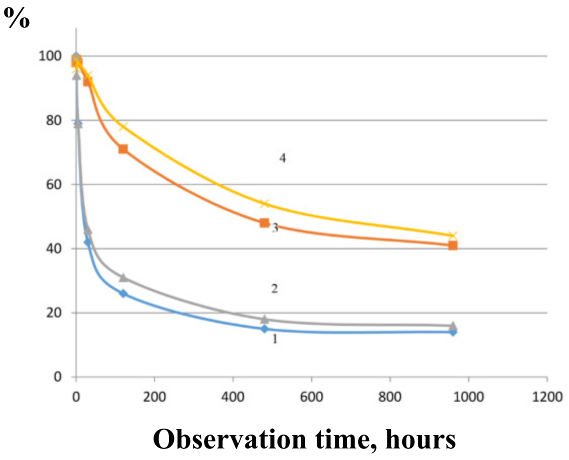

Figure 13. Typical dependence of the sedimentation rate of 50 nm alumina at room temperature in combinations: D18A before sonification (1, %), K18A before sonification (2, %), K18 A after sonification(3, %) and K18A (4, %) after sonification.

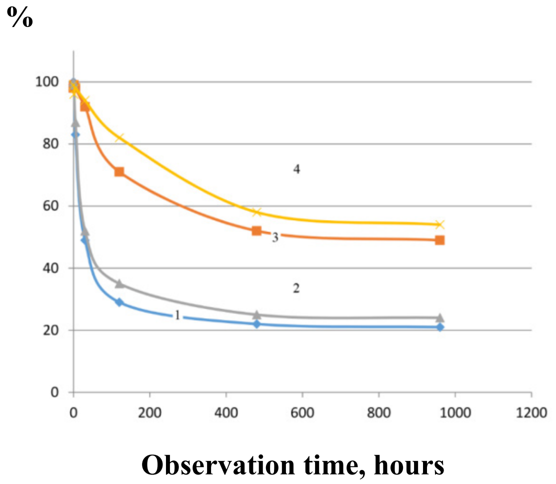

Figure 14. Typical dependence of sedimentation process of 40 nm alumina at room temperature in combinations: D18A before sonification (1, %), K18A before sonification (2, %), K18 A after sonification (3, %) and K18A (4, %) after sonification.

Taking into account the above given considerations, nano based insecticidal combinations D1A - D37A and K1A – K37A (similar to combinations D1 – D37 and K1 – K37) were developed, with the only difference that instead of diatomite and kaolin powders the nano structured alumina with average particle sizes 40 nm, 50 nm and 100 nm supplied by MK Nano and SS Nano was added in the same proportions and processed according to paper [62] using mechanical mixing and ultrasound agitation. Preliminary study of the stability and agglomeration of the prepared dispersions were studied also according to [62].

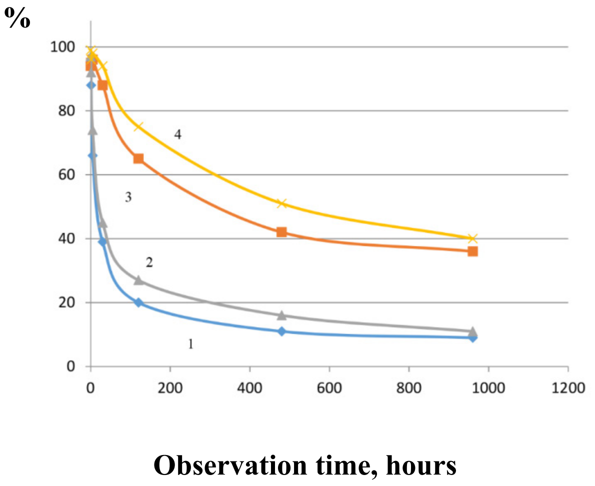

Figure 15. Typical sedimentation process of 100 nm alumina at room temperature in combinations: D18A before sonification (1, %), K18A before sonification (2, %), K18 A after sonification (3, %) and K18A (4, %) after sonification.

Sedimentation rates in alumina containing insecticidal combinations were measured and compared to sedimentation rates in alumina-water-ethanol mixtures. The sedimentation measured rates of 50 nm nano alumina in the insecticidal combinations were about 40 - 60-times lower than in ethanol-water (25 wt%) mixture. Ultrasonic agitaton increased the stability of dispersions about 1.8 – 2.8 times. Stability of dispersion, determined considering the height of the white colored columns in liquids, significantly depended on the concentration and the averaged size of alumina nanoparticles (see figures 13 – 15). In the nearest future we are going to carry out a comprehensive laboratory and field study of the toxicity of developed nano based combinations against BMSB, Italian locust, honey bee, fish and mammals (white rat) using all the methods given above. Special attention will be paid to pesticide exposure safety issues and risk assessment indicators (e. g. [63]), prospective of use of nanoparticles for environmental clean-up (e. g. [64]) and to market analysis of the nanoparticle based insecticidal combinations (e. g. [65]).

Conclusions

The reported study showed that it is necessary to perform a large number of laboratory experiments and field tests clarifying the further details on the basis of systematization and reliable statistical analysis of the accumulated data about the optimal composition and concentration of the newly developed combined formulations, and taking into account the specific field conditions and climatic factors. At the same time, even today it is possible to conclude with a high probability that in certain rural areas invaded by resistant populations of insects it will be preferable to use insecticidal combinations based on dimethoate and gamma-cyhalothrin, while in other areas the combinations based on malathion and bifenthrin will still be more effective.

The results of the field trials are surely affected by a number of natural factors (weather and humidity, migration activity of insects, counting errors, etc.). Many of the above factors can attenuate the difference between the measured effectiveness of the tested insecticidal combinations. Anyway, the results of the field trials are in good coincidence with the data of laboratory testing, although more studies are required to increase the accuracy and reliability of both the laboratory and field trial data. Analyzing the data given in Figures 7 and 8 we concluded that the biological effectiveness of the developed and control combinations keep sufficiently high during all the period of the field trial, although the effectiveness during the last six days was for 15-20 % lower than during the first six days of the field trials. In our opinion, the relatively low biological effectiveness during the first day can by explained as the result of the prolonged release of insecticidal solution “trapped” in the diatomaceous earth and kaolin.

Considering that the relative biological effectiveness of the developed combinations in the area of maximum synergy is about 1.4-1.5 times higher, while the relative acute toxicity to warm-blooded animals (rats) is about twice less than that of the control insecticide (“ProStore 420 EC), we assume, that it is about 2 times safer for humans and the living environment than all widely used analogues. The results of the non-invasive testing of acute toxicity to white rats and bird embryos are in a good coincidence and show that insecticide “cocktails” can be used successfully against the agricultural pests, as a highly effective insecticide-acaricide agent, less harmful for the living environment. Grounding on the research conducted, we can also conclude that the algorithm and method developed and used by us for the study of synergy and acute toxicity can become a basis for standardized methods of the objective quantitative testing for the biological efficacy and safety of insecticides. To enhance the accuracy and reliability of the main results and findings of the reported experimental study, improve the efficiency and safety of the insecticidal combinations and provide the successful implementation of the findings of reported study a long list of experimental works should be executed in the nearest future. A special interest should be paid to the recent publications on the acute and chronic toxicity of nano insecticides to soil microorganisms, plants, invertebrates, non-targeted and useful insects (especially –pollinators), reptiles, aquatic organisms and fish, birds, mammals and human biological effectiveness against a broad spectrum of pests.

Numerous authors have published articles with the aim of predicting the toxicity and assess the possible negative impact using analysis of various direct and indirect indicators for the reliable estimation of environmental and human health risks. A big variety of physical and biological methods has been used to provide enough information for the predictive models based on the qualitative and semi quantities data on the morphology (size, specific areaЪ, hydrophobic and mechanical properties, solubility, stability of dispersions, tribo-charging, ability to damage DNA and discompose protein expression, capacity of dehydration of tissues and generate reactive oxygen species, cause cell membrane depolarization and cell death. Several authors also made attempts to analyze more complex mechanisms of affecting living organisms through provoking proteolysis and methabolic disorders, pro-inflammatory factors, carcinogenicity and immune toxicity, etc. of the nanostructured nano-alumina and other nanoparticles.

Acknowledgement

The Authors thank Professor Greg Kravczyk from the Pensilvania University, Professor Sabu Thomas from the Mahatma Gandhi University, Professor Giorgi Pichkhaia from the Alexandre Natishvili Institute of Morphology of Ivane Javakhishvili Tbilisi State University, Professor Giorgi Kvartskhava from the Georgian Technical University, Professor Nelly Sapojnikova from the Ivane Javakhishvili Tbilisi State University for their kind help in experimental design and interpretation of the obtained results. We also would like to thank the students of the Faculty of Agrarian Sciences and Bio systems Engineering of the Georgian Technical University for their help in laboratory testing and field trials.

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