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COMPATIBILITY OF ENTOMOPHAGES WITH BIOLOGICAL AND BIORATIONAL PESTICIDES

I.S. AGAS'EVA, M.V. NEFEDOVA, E.V. FEDORENKO, A.O. MKRTCHYAN, A.S. NASTASII, V.Ya. ISMAILOV

All-Russian Research Institute of Biological Plant Protection, PO box 39, Krasnodar, 350039 Russia, e-mail agasieva5@yandex.ru, dollkaSneba@yandex.ru, ms.fedor1960@mail.ru, mialusker22@gmail.com, nastasy.anton@yandex.ru, vlyaism@yandex.ru (✉ corresponding author)
ORCID:
Agas'eva I.S. orcid.org/0000-0002-1216-1106
Nefedova M.V. orcid.org/0000-0001-5390-233X
Fedorenko E.V. orcid.org/0000-0003-4411-626X
Mkrtchyan A.O. orcid.org/0000-0002-3252-9780
Nastasii A.S. orcid.org/0000-0001-8324-0058
Ismailov V.Ya. orcid.org/0000-0002-6713-0059
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Abstract

Severe adverse effects of chemical pesticides have driven demand for ecologically friendly technologies of plant growing with alternative pest control tactics. Traditional insecticides cause massive death of predatory ground beetles, bedbugs, coccinellids, lizards, flies and tachinid flies, parasitic trichogrammatids, ichneumonids, braconids and other useful species. Harmonized biological and chemical controls are becoming more relevant, which should include the use of beneficial entomofauna. This necessitates more data on sensitivity of entomophages to biologicals, biorational pesticides (i.e. natural substances and their synthetic analogues) and other selective chemistries. In this work, for the first time, we determined laboratory and field toxicity of several Russian and foreign conventional biologicals and chemicals for beneficial entomofauna of corn, potato and apple-tree agrocenoses. The originality of this study lies in its focus on searching commonly used biopesticides which can be integrated with entomophages in organic farming technology. The obtained data indicate that biorational insecticides Fitoverm® EC (emulsion concentrate) (Pharmbiomed, Russia, 1.3 l/ha), Vertimek® EC (Syngenta AG, Switzerland, 1.0 l/ha) and Atabron® SC (suspension concentrate) (ISK Biosciences, Belgium, 0.75 l/ha) are highly effective against harmful lepidopterans and aphids on corn, soy and pea crops without toxic effect on the massively used entomophagous *Habrobracon hebetor* Say and *Aphidius matricaria* Hal. Our findings also indicate effectiveness of combination of predatory bugs podisus (*Podisus maculiventris* Say) and perillus (*Perillus bioculatus* Fabr.) with biologicals against Colorado beetle on solanaceous crops. In using Bitoxybacillin® P (powder) (Sibbiopharm, Russia, 4 kg/ha) and Fitoverm® EC (1.3 l/ha), the survival rates of *P. masculentris* imagoes were 88% and 82%, respectively, with 64% for older larvae. When using the same pesticides, the survival rates of *P. bioculatus* imagoes were 97% and 91%, respectively, with 58% and 52% for fourth- to fifth-instar larvae. Fitoverm® at 1 l/ha rate recommended against aphids does not affect the viability of the aphidophages *Cycloneda sangvinea* Mul. and *Harmonia axyridis* Pallas on maize, vegetable pea and apple, and allows for survival of 85% adult beetles *C. sangvinea* and of 88% Asian ladybeetles *H. axyridis.* These data can be used in protocols for co-application of biologicals, biorational preparations and entomophages in organic and ecological farming to effectively control pests of maize (cotton moth, corn stalk moth, corn and cereal aphids), potatoes (Colorado potato beetle, potato aphids), peas (leguminous aphis), and apple trees (apple moth, Apple green aphid).

Keywords: biological preparations, entomopathogenic, insect sensitivity to pesticides, *Habrobracon hebetor* Say, *Aphidius matricaria* Hal., *Perillus bioculatus* Fabr., *Podisus maculiventris* Say, *Cycloneda sangvinea* Mul., *Harmonia axyridis* Pallas

Over the last 16 years, organic farming has been on the rise worldwide, with the total area use quadrupling to about 1% of all the farming land; more than 2 million organic producers have been certified, of which 75% are based in emerging economies [1-3]. The desire to advance organic production is overdue in more than 170 countries, with even more joining the team as such products are in increasing demand [4-7].

Organic farming uses special formulations for pest control based on entomopathogenes, as well as predatory and parasitic laboratory-reproduced insects [8-11]. An early introduction of artificially grown entomophages helps improve the efficiency of biological pest control [12-14]. However, entomophages are often unable to restrict the pest population in the field to an economically imperceptible level. This can be caused by the asynchronous phenology of phytophages and entomophages, or by the very low post-winter population of the latter, which stimulates uncontrolled reproduction of the pests; however, it is the conventional chemical treatment that is believed to kill the beneficial entomofauna while not affecting the phytophagous populations which are resistant to many insecticides [15-18]. This makes it imperative to use biologicals and biorational products that will not affect the beneficial arthropods and additionally introduced entomophages and acariphages [19]. The former group comprises biologicals to combat pests, plant pathogens, and weeds; these are derived from living microorganisms or their metabolic products. The latter group comprises chemical compounds or natural substances that are low- or non-toxic to warm-blooded organisms (pheromones, essential and vegetable oils, growth regulators, etc.). The formulations of both groups feature quicker and eco-safer degradation compared to other preparations; besides, they are not accumulated in the food chain.

In green farming, pesticide load must be reduced by 50-75% (i.e. brought to the levels allowed for integrated protection); it is preferable to use biorational formulations (insect growth and development regulators, pesticides not affecting the beneficial entomofauna, etc.). Organic farming uses only biologicals and biorational products; conventional chemical insecticides are completely banned [14, 20, 21].

When choosing the protective agents, their toxicity for the beneficial organisms must be evaluated [22, 23]. The death of beneficial arthropods is most pronounced in perennial plantations (orchards, vineyards), as these cenoses contain numerous species of phytophages and their respective entomophage complexes that are important for controlling the population of the former. Using insecticides has been found to cause mass death of predatory ground beetles (*Carabidae*), *Pentatomoidea*, *Nabidae*, and *Anthocoridae* bugs, coccinellids (*Coccinellidae*), lacewings (*Chrysopidae*), flower flies (*Syrphidae*) and tachinids (*Tachinidae*), trichogrammatids (*Trichogrammatidae*), ichneumonids (*Ichneumonidae*), braconids (*Braconidae*), and sundry beneficial species. Annual crops also contain a significant number of entomophagous species (up to 200 in winter wheat, and up to 300 in peas). A one-hectare potato field contains 2,000 to 3,400 flower flies, more than 720 predatory spiders, and 2,400 to 2,800 ground beetles; conventional insecticides kill nearly all of them [24]. Treatment of wheat with insecticides targeted against corn bug (*Eurygaster integriceps* Put.) will negatively affect *Carabidae*, *Coccinellidae*, and *Scelionidae* entomophages. Fungicides and herbicides usually have a far lesser effect on entomophages than insecticides. It is preferable to use such biological and biorational protective agents that are safe for entomophages and acariphages [25]. Researchers have identified that biorational formulations based on thiamethoxam, chlorantraniliprole, tefluthrin, difenoconazole, fludioxonil, thiacloprid, imidacloprid, or their combinations (low-toxic, low- or moderately-hazardous) differ in the toxicity to the beneficial entomofauna [26-28]. Thus, Herold® WSC (water-suspension concentrate), a biorational diflubenzuron (240 g/l) preparation (ZAO Firma August, Russia), Proclaim® WSG (water-soluble granules) a biorational emamectin benzoate (50 g/kg) product (Syngenta AG, Switzerland), and Lepidocide® P (powder), a biological preparation based on the spore-crystal complex *Bacillus thuringiensis* var. *kurstaki*, biological activity (BA) = 3,000 EA/mg (PO Sibbiofarm, Russia) do not inhibit the natural orchard entomophages: lacewings, *Nabidae* predatory bugs, and *Coccinellidae* beetles [29].

This paper is the first to demonstrate the compatibility of entomophages targeting Colorado potato beetles *Perillus bioculatus* Fabr. and spined soldier bugs *Podisus maculiventris* Say, the ectoparasites of cotton bollworm and sundry Lepidoptera species *Habrobracon hebetor* Say, aphid parasite *Aphidius colemani* Vier, predatory coccinellids *Cycloneda sangvinea* Mul. and *Harmonia axyridis* Pallas, with various biological and biorational insecticides.

The study objective is to determine the sensitivity of entomophages to biologicals and biorational agents while developing a system for the biological protection of crops against pests.

Techniques. The studied preparations were biologicals Bitoxybacyllin® P, Lepidocide® SC (suspension concentrate, PO Sibbiofarm, Russia), Helicovex® SC (Andermatt Biocontrol AG, Switzerland), the biorational products Vertimec® EC (emulsion concentrate) and Actara® WDG (water-dispersible granules) (Syngenta AG, Switzerland), Phytoverm® EC (Farmbiomed, Russia), Insegar® WDG and Atabron® SC (suspension concentrate, ISK Biosciences, Belgium), and the chemical insecticides Decis® Expert EC (Bayer AG, Germany), and Coragen® SC (DuPont, United States).

The compatibility of the biologicals Bitoxybacyllin® P (application rate of 4 kg/ha against lepidoptera, 3 kg/ha against aphids and Colorado potato beetle), Lepidocide® SC (2 l/ha against codling moth and aphids), Helicovex® SC (200 l/ha against Lepidoptera) and the biorational products Vertimec® EC (1 l/ha against Lepidoptera), Phytoverm® SC (1.3 l/ha against aphids and 0.2 l/ha against Lepidoptera), Coragen® SC (0.1 l/ha against Lepidoptera), Insegar® WDG (0.6 kg/ha against Lepidoptera), Atabron® SC (0.7 l/ha against Lepidoptera) with entomophages was compared in a laboratory tests using *Habrobracon hebetor* Say cocoons and cereal aphids infested with *Aphidius colemani* Vier., as well as in the field 40-m^2 potato plots in 4 repetitions (for the Colorado potato beetle entomophages *Perillus bioculatus* Fabr. and *Podisus maculiventris* Say), in a 4-ha apple orchard containing 10 model trees, in 50-m^2 maize and pea plots in 4 repetitions (for the ladybirds *Cycloneda sangvinea* Mul. and *Harmonia axyridis* Pallas).

For the laboratory culturing of the *Habrobracon*, the hosts were the larvae of middle-aged wax moth (*Galleria mellonela* L.). The larvae were placed in 0.5 l glass jars and infested with the parasite. The jars were closed with a calico cloth and a cotton swab soaked in 20% sugar solution to feed the entomophage and then placed in a thermostat (28 to 30 °C). Cocoons formed 7 or 8 days after the infestation; those were treated thrice with agents recommended for protecting maize against Lepidopteran pests. We used Bitoxybacyllin® P (BA = 1,500 EA/mg, with a titer of 20 billion/g, application rate of 4 kg/ha), Lepidocide® SC (BA = 2,000 EA/mg, with titer of 10 billion/g, application rate of 2 l/ha); Vertimec® EC (ai = 18 g/l, application rate of 1 l/ha), Helicovex® SC (7.5×10^{12} NPV/l, application rate of 200 ml/ha), Insegar® WDG (application rate of 0.6 kg/ha), Atabron® SC (application rate of 0.75 l/ha), Coragen® SC (application rate of 0.1 l/g), Decis® Expert EC (application rate of 0.1 l/ha). The controls were treated with distilled water.

Aphidius reproduction involved cereal aphids (*Schizaphis graminum* Ron.) cultured on wheat seedlings. On post-inoculation day 3 or day 4, *Aphidius* (*A. colemani*) were placed on the plants, the resultant mummies were treated with Phytoverm® EC (application rate of 1.3 l/ha), Lepidocide® SC (application rate 2 l/ha); Bitoxybacyllin® P (application rate 3 kg/ha), Actara® WDG (250 r/kg, application rate of 0.2 kg/ha).

The toxicity of the protective agent for predatory bugs and coccinellids in the field was determined for the products recommended for use against Colorado potato beetle and aphids: Phytoverm® EC (2 g/l, application rate of 0.2 and 1.3 l/ha, respectively) and Bitoxybacyllin® P (BA = 1,500 EA/mg, application rate of 3 kg/ha). Experimental plots with entomophages, Colorado potato beetle and aphids were treated with a stock solution of products using a Pulverex hydraulic knapsack sprayer, Switzerland. The population of predatory bugs and coccinellids was counted before and after spraying.

Statistical processing followed the standard procedure [30]. The tables present the mean (*M*) and standard deviation (±SD). Statistics were run in Statistica 12.6 software (StatSoft, Inc., United States). Statistical significance was evaluated by Duncan's test at P = 95%.

Results. Table 1 presents the main characteristics of the compared biological and chemical products.

1. Sensitivity of ectoparasite *Habrobracon hebetor* Say to biological and chemical insecticides in a lab tests (*M*±SD)

Insecticides, ai	Dosage, l/ha, kg/ha	Cocoons before treatment, pcs.	Imagoes				
			day-specific number			total	% of initial number
			day 3	day 5	day 7		
Biological insecticides							
Lepidocide® SC (*Bacillus thuringiensis* var.*kurstaki*)	2.0	69.2	10.2±2.1	37.6±1.6	7.4±1.8	55.2[ab]	79.8
Bitoxybacyllin® P (*Bacillus thuringiensis* var. *thuringiensis*)	4.0	46.6	6.8±1.5	19.3±3.4	3.1±2.3	29.2[a]	62.7
Helicovex® SC (nuclear polyhedrosis virus of cotton bollworm)	200	83.4	22.5±3.7	39.6±2.1	21.3±3.4	83.4[ab]	100
Biorational chemical insecticides							
Vertimec® EC (abamectin)	1.0	64.0	9.6±2.4	32.5±1.8	4.5±1.1	46.6[bc]	72.8
Insegar® WDG (fenoxycarb)	0.6	80.3	14.7±1.5	51.9±3.3	12.4±1.1	79.0[c]	98.4
Atabron® SC (chlor fluazuron)	0.75	76.2	24.8±3.2	44.3±1.3	9.9±1.6	76.2[c]	100
Chemicall insecticides							
Decis® Expert EC (deltametrin)	0.1	87.5	0	0	0	0[a]	0
Coragen® SC (chlorantraniliprole)	0.1	91.0	22.5±2.2	54.0±1.9	14.5±3.1	91.0[ab]	100
Control		93.0	20.9±1.6	56.2±4.2	13.9±2.3	93.0[c]	100

Note. The samples marked with the same letter index have no statistically significant difference by Duncan's test at P = 95% within the same column.

In the laboratory test (Table 1), application rates were as recommended for protecting maize, soybean, and sunflower against cotton bollworm and sundry Lepidopteran pests. The survival rates of the *Habrobracon* ectoparasite exposed to the biorational insecticide Vertimec® EC, biologicals based on *Bacillus thuringiensis* (Bitoxybacyllin® P) and Lepidocide® SC was 72.8, 62.7, and 79.8%, respectively. Coragen® SC data are interesting, as the *Habrobracon* imago release rate was 100% in the test. The biological Helicovex® SC based on nuclear polyhedrosis virus of the cotton bollworm was non-toxic for *H. hebetor* (100% post-treatment imago release indicates the complete entomophage compatibility with this product when used for the bioprotection of maize, soybeans, and tomatoes against cotton bollworm). Decis® Expert had the strongest suppressive effect, as the ectoparasite death rate was 100%.

American researchers found that insecticides based on cyantraniliprole, chlorantraniliprole, spinetoram, which are deemed eco-friendlier than carbamate-based products, had negative effects on the development of *Chrysoperla carnea* Stephens (*Neuroptera Chrysopidae*) and *Trioxys pallidus* Haliday (Hymenoptera: *Braconidae*) [31].

Evaluation of the sensitivity of the aphid parasite *A. colemani* to the biologicals and chemicals recommended for protecting winter wheat, fruits, and vegetables against aphids shows good insect viability when treated with Phytoverm® EC or Bitoxybacyllin® P, see Table 2. When using Actara® WDG, only 12 *Aphidius* species were released from 180 mummies. Russian researchers also found the biologicals Bitoxybacyllin® P and Lepidocide® SC to be non-toxic for such aphidophages as lacewings, coccinellids, and mirid bugs [29].

2. Sensitivity of *Aphidius colemani* Vier. to biological and chemical insecticides in a laboratory test (*M*±SD)

Insecticides	Dosage, l/ha, kg/ha	Mummies before treatment, pcs.	Parasites flied				
			day-specific number			total	% of initial number
			3-и сут	5-е сут	7-е сут		
Biological insecticides							
Lepidocide® SC	2.0	178.0±2.6	28.0±2.4	21.0±3.4	1.9±1.3	68[bc]	38.2
Bitoxybacyllin® P	3.0	186.0±3.4	39.0±4.7	79.0±2.3	55.0±1.8	173[ab]	93.0
Biorational chemical insecticides							
Phytoverm® EC	1.3	198.0±4.5	48.0±2.3	96.0±3.5	17.0±2.3	161b	81.3
Chemical insecticides							
Actara® WDG	0.2	180.0±3.8	4.0±1.6	8.0±1.7	0.0±0.0	12[c]	6.6
Control		194.0±4.4	65.0±3.5	105.0±4.5	17.0±2.5	187[a]	96.3

N o t e. The samples marked with the same letter index have no statistically significant difference by Duncan's test at P = 95% within the same column.

3. Sensitivity of *Asopinae* bugs to biological and chemical insecticides (*M*±SD, VNIIBZR test plot, Krasnodar, 2015)

Dosage, l/ha, kg/ha	Insect development	Insect survival rate on day 7, %	
		Podisus maculiventris Say	*Perillus bioculatus* Fabr.
Phytoverm® EC, 2 g/l			
0.2	Imago	81.8±0.10[cd*]	90.9±0.10[b]
	Older larvae (III-IV)	63.6±0.10[c]	51.5±0.12[a]
	Younger larvae (I-II)	24.2±0.06[a]	0.0±0.00[a]
Bitoxybacyllin® P, biological activity 1,500 EA/mg			
3.0	Imago	87.9±0.12[bd]	97.0±0.06[b]
	Older larvae (III-IV)	63.6±0.10[c]	57.6±0.16[ac]
	Younger larvae (I-II)	27.3±0.10[a]	3.0±0.65[a]
Actara® WDG, 250 g/kg			
0.2	Imago	0±0.0[a]	0±0.0[a]
	Older larvae (III-IV)	0±0.0[a]	0±0.0[a]
	Younger larvae (I-II)	0±0.0[a]	0±0.0[a]
Control			
0	Imago	97.0±0.06[b]	93.9±0.06[b]
	Older larvae (III-IV)	93.9±0.12[b]	87.9±0.12[bc]
	Younger larvae (I-II)	97.0±0.06[b]	97.0±0.06[b]

П р и м е ч а н и е. Между вариантами, обозначенными одинаковыми буквенными индексами, при сравнении в пределах столбца нет статистически значимых различий по критерию Дункана при уровне вероятности P = 95 %.

Field trials with potato plants estimated the sensitivity of the predatory bugs *P. maculiventris* and *P. bioculatus* to Phytoverm® EC and Bitoxybacyllin® P, see Table 3. The products were tested at the application rates recommended for use against Colorado potato beetle. Actara® WDG was the chemical reference. Experiments carried out on the potato-field plots showed that Phytoverm® EC dosed at 0.4 l/ha was toxic for the predatory bugs' larvae; thus, day 7 survival rate of *P. maculiventris* I-II larvae was reduced drastically while that of *P. bioculatus* was 0%. Imagoes of predatory bugs were less sensitive to the product: the *Podisus* survival rate reached 81.8%, the *Perillus* survival rate was 90.9%. Bitoxybacyllin® P did not have toxic effects on the imagoes: *Podisus* had a survival rate of 87.9%, *Perillus* had a survival rate of 97.0%, see Table 3.

In an apple orchard, as well as in maize and peas, the biorational product Phytoverm® EC dosed at 1.3 l/ha did not affect the viability of *C. sangvinea* and *H. axyridis*. Imago survival rate was 85.0% for *Cycloneda* and 87.7% for *Harmonia*. Older larvae of both species were resistant to the product; younger *C. sangvinea* larvae had been wiped out by day 7. Egg treatment caused hatching and 100% death of larvae, while the survival rate of the control sample was

100%. Vertimec® EC was non-toxic for aphidophages. For reference, Actara® WDG was used, which killed 100% of insects. Phytoverm® EC was toxic for the younger larvae of predatory bugs and coccinellids, while Bitoxybacyllin® P and Ver-timec® EC kept the stock of entomophages alive. Similar results were obtained when testing the biological pesticide АқКөbelek™ (based on *Bacillus thuringiensis* var. *kurstaki*) against alfalfa, soybean, and maize pests in South East Kazakhstan; this product was not toxic for braconids, coccinellids, or *Nabidae* and *Miridae* predatory bugs [32].

Thus, as this research has shown, that Helicovex® SC, Bitoxybacyllin® P, Lepidocide® SC, and biorational pesticides such as Vertimec® EC or Phytoverm EC combined with entomophages *Habrobracon*, *Aphidius*, and ladybirds can protect maize against cotton bollworm, corn worm, and corn leaf aphids, as well as apple trees and peas against aphids. Coragen® SC, Insegar® WDG, and Atabron® SC are recommendable for ecologic farming, as they do not have negative effects on agrocenosis and can be combined with entomophages or used on their own. The biorational products Phytoverm® EC and Vertimec® EC recommended for protecting potatoes against Colorado potato beetle are compatible with predatory bugs and coccinellids. The results of these studies are usable for combining biologicals, bioactive products, and entomophages for effective pest control in organic and ecological farming.

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