doi:10.14720/aas.2023.119.3.14714 Original research article / izvirni znanstveni članek

Results of testing of the efficacy of sublethal concentrations of bacterial-chemical insecticides combinations against cabbage moth larvae

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Received June 26, 2023; accepted September 14, 2023.
Delo je prispelo 26. junija 2023, sprejeto 14. septembra 2023

The experiments of sublethal concentrations of bacterial-chemical insecticides against cabbage moth larvae

Abstract: Using chemical pesticides has adverse effects on the environment and humans. Bacterial preparations may provide an alternative to chemical pesticides. The study aims to test different combinations of sublethal concentrations of bacterial and chemical preparations against cabbage moth larvae.

During 2020-2022 different combinations of sublethal concentrations of bacterial (Lepidocide) and chemical (Arrivo, Voliam Flexi, Proclaim Fit) preparations were tested in laboratory and field conditions, against cabbage moth young larvae (stage I-II).

The combinations of insecticides with bacterial and chemical sublethal concentrations show high biological efficiency against the cabbage moth larvae. No statistical difference was found between the efficiency indicators of the combined and standard chemical (Arrivo, Voliam Flexi, Proclaim Fit) options and the significance level was generally between 2.0 and 5.9 %, showing that the results of the scientific experiments are reliable.

Key words: biological effectiveness, cabbage moth larvae, insecticides, laboratory and field tests, statistical analysis

Poskusi s subletalnimi koncentracijami bakterijsko-kemijskih insekticidov na gosenice kapusnega molja

Izvleček: Uporaba kemijskih insekticidov ima škodljive učinke na okolje in ljudi. Pripravki iz bakterij so lahko alternativa kemijskim pesticidom. Namen raziskave je bil preiskusiti različne kombinacije subletalnih koncentracij bakterijskih in kemijskih pripravkov proti gosenicam kapusnega molja.

V letih 2020-2022 so bile v laboratoriju in poljskih razmerah preiskušene različne kombinacije subletalnih koncentracij bakterijskih (Lepidocide) in kemijskih (Arrivo, Voliam Flexi, Proclaim Fit) pripravkov za zatiranje mladih gosenic kapusnega molja (razvojni štadij I-II).

Kombinacije bakterijskih in kemijskih insekticidov v subletalnih koncentracijah so pokazale veliko biološko učinkovitost na tretiranih gosenicah. V kazalnikih učinkovitosti ni bilo statistično značilne razlike med kombiniranimi pripravki in standardnimi kemijskimi insekticidi (Arrivo, Voliam Flexi, Proclaim Fit). Raven značilnosti je bila nasplošno med 2,0 in 5,9 %, kar kaže, da so izsledki poskusov zanesljivi.

Ključne besede: biološka učinkovitost, gosenica kapusnega molja, insekticidi, laboratorijski in poljski poskusi, statistična analiza

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1 INTRODUCTION

The soil and climatic conditions of Armavir region of the Republic of Armenia are favorable for the cultivation of white-head cabbage (*Brassica oleracea* L. ssp. *oleracea* convar. *capitata* (L.) Alef f. *alba*). The increase of the yield of this plant is often hindered by the cabbage moth *Plutella maculipennis* (Curtis, 1832) which belongs to the Plutellidae family of the insect order Lepidoptera. The damage caused by its larvae reduces the yield and lowers the quality of the crop.

Hatched larvae eat the parenchyma of the leaves, leaving the epidermis intact, resulting in the formation of areas covered with a thin membrane, called "windows" (Avetyan & Marjanyan, 1976), and the more mature larvae open through holes on the leaves. The damage becomes more dangerous when they feed on the young leaves forming the head of the plant (Safaryan, 1968; Terlemezyan, 1996; Philips et al., 2014; Andreeva et al., 2021).

Besides the white-head cabbage, phytophagous larvae also damage other economic importance cruciferous plants, for example cauliflower, broccoli, rapeseed, etc. (Tsedeler, 1931; Harcourt, 1957; Terlemezyan, 1996; Churikova & Silaev, 2010; Shpanev, 2015; Kholod & Korenyuk, 2016; Tuleeva & Sarmanova, 2019). Therefore, it is extremely important to implement effective, environmentally safe control measures against harmful larvae.

In the integrated pest control system, the preference is given to the use of bacterial preparations based on *Bacillus thuringiensis* Berliner, 1915 (Bt) species, which have high biological efficiency against leaf-eating harmful insects and, unlike chemical preparations, are safe for humans, warm-blooded animals, entomophages and fish. (Talekar & Shelton, 1993; Belyaev & Nozdrenko, 2004; Ivantsova, 2004; Sarantseva & Bobreshova, 2006; Sargsyan, 2013; Fathipour & Mirhosseini, 2017; Semerenko, 2019; Zakharova et al, 2022).

Currently, the implementation of economically justified control of phytophagous larvae through combinations of sublethal concentrations (used in small quantities) of bacterial and chemical insecticides is also emphasized (Mesropyan, 2011; Avagyan, 2012; Chapanyan, 2022).

Based on the above, we aimed to test different combinations of sublethal concentrations of bacterial and chemical preparations against cabbage moth larvae in laboratory and field conditions.

2 MATERIALS AND METHODS

The scientific experiments were carried out during 2020-2022, in the laboratory conditions at the Scientific Center for Risk Assessment and Analysis of Food Safety and cabbage plantations of Nalbandian community of Armavir region.

The research materials were: the young cabbage moth larvae (stage I-II), the cabbage plant (variety: Slava), the commercial bacterial lepidocide preparations KA 3000 IU mg^{-1} in the powder for liquid suspension: the usage rate is 1.0 kg ha^{-1} (Russian Federation), chemical preparations: 25 % concentrated emulsion Arrivo: the usage rate is 0.3 l ha^{-1} (FMC, USA), 30 % concentrated suspension Voliam Flexi: the usage rate is 0.3 l ha^{-1}, and 45 % water-soluble granules of Proclaim fit: the usage rate is 0.1 l ha^{-1} (Syngenta, Switzerland).

All the above-mentioned preparations are allowed to be used against harmful insects in the Republic of Armenia.

Cabbage plantations, where the number of moth larvae was at the threshold of economic damage of a specified pest (that is: 2-5 larvae per plant), when 10 % or more of the plants in the experimental site are occupied by them (Polyakov, 1984), were selected as experimental sites.

The biological effectiveness of insecticides combined with sublethal concentrations (Lepidocide + Arrivo, Lepidocide + Voliam Flexi, Lepidocide + Proclaim Fit) was determined according to the methodological manual (Methodological guidelines for testing biological products for plant protection from pests' diseases and weeds, 1973). The lethal concentrations of 3 (in case of lepiocide: 0.33 kg ha^{-1}) and 10 dilutions (in case of Arrivo and Voliam Flexi: 0.03 l ha^{-1}, in case of Proclaim Fit: 0.01 l ha^{-1}) of bacterial (lepidocide) and chemical (Arrivo, Voliam Flexi and Proclaim Fit) insecticides, respectively, were combined.

The samples sprayed with different solvents (Lepidocide: the usage rate is 1.0 kg ha^{-1}, Arrivo: the usage rate is 0.3 l ha^{-1}, Voliam Flexi: the usage rate is 0.3 l ha^{-1}, Proclaim Fit: the usage rate is 0.1 l ha^{-1}) were taken from the plantations naturally inhabited by moth larvae.

During small-scale and production experiments, cabbage plants grown under laboratory conditions (in camps) and artificially inhabited by moths were sprayed with a hand-held sprayer full of working fluid, using backpack AO - 2 and motorized K-14 sprayers. The working fluid consumption was 400 l ha^{-1}. In small-scale experiments, the size of the experimental area for each (sampled separately and experimentally combined) option was 100 m^2, as for large-scale spraying, it was 0.2 ha.

Each option included in the experiments had 3 replicates.

In laboratory conditions, 30 larvae were included in each option (10 larvae in each replicate), and

in two-year small-scale and production experiments, the number of phytophagous larvae was generally between 51 and 70, and 55 and 77, in certain cases.

The numbers of alive and dead larvae in the experimental plots were counted before spraying (baseline), and 3, 5 and 7 days after spraying, also before the mating phase.

In laboratory conditions when experimenting the options with the sub-threshold concentrations, the microbiological isolation of *Bacillus thuringinsis* var. *kurstaki* Bulla et al. 1979 pathogens which are the basis for the production of the sprayed commercial lepidocide bacterial preparations, were isolated according to the methodological manual (Netrusov et al., 2005).

The statistical analysis of the results of the scientific experiments was carried out according to the protocol presented by (Ashmarin and Vorobyev, 1962; Bernstein, 1968).

3 RESULTS AND DISCUSSION

According to the results of scientific experiments carried out in laboratory conditions in 2020, it was proved that the combinations of standard lepidocide bacterial (3 dilutions of the lethal concentration) and diluted lethal chemical (Arrivo, Voliam Flexi and Proclaim Fit) concentrations (10 dilutions of the lethal concentration) have shown a high biological efficiency against phytophagous larvae (stage I-II) just in 7 days after spraying, generally ranging from 93.3 % to 96.7 %. The indicators of biological efficiency of the sample options for the same recording period were also high, ranging from 93.3 to 100 %.

No mortality of phytophagous larvae was observed on the sprayed sample, during the observation period.

The high rates of biological efficiency recorded in laboratory conditions made it possible, as well, to test the insecticides individually (standard/sample options) and in combination with sublethal concentrations (experimental options) against the cabbage moth larvae under field conditions (field and production experiments).

According to the results of the partial (small-scale) research, it was demonstrated that even 7 days after spraying, the indicators of biological efficiency of combined options, such as Lepidocide + Arrivo, Lepidocide + Voliam Flexi, Lepidocide + Proclaim Fit, were still high, generally ranging from 91.5 % to 94.3 % (Table 1).

As it is presented in Table 1, Lepidocide (sample), Arrivo (sample), Voliam Flexi (sample) and Proclaim Fit (sample) options have also demonstrated high biological efficacy (overall 85.7 % - 96.1 %).

The pattern of high biological efficiency in small-scale experiments demonstrated by individual and sublethal concentrations of insecticides against the cabbage moth larvae was maintained during production experiments conducted in 2021-2022 (Table 2).

According to the two-year data from Table 2, the indicators of biological efficiency (7 days after spraying) of the standard lepidocide were 82.4 % and 85.4 %. As for the combined options of sublethal concentrations, such as Lepidocide + Arrivo, Lepidocide + Voliam Flexi, and Lepidocide + Proclaim Fit, those were 89.1 % and 90.1 %, 92.2% and 93.0 %, and 91.3 % and 91.5 %, respectively.

The indicators of biological efficiency recorded for all standard (sample) chemical insecticides were between 86.3 % and 95.2 % for the same period of observation. Moreover, the above-mentioned indicators recorded on the 7th day were constant for all tested options before the mating period.

From the data in Tables 1 and 2, it is clear that the indicators of biological efficiency recorded in the experiments conducted 3 and 5 days after spraying were relatively low compared to those recorded on the 7th day,

Table 1: The indicators of biological effectiveness of standard (sample) and combined insecticides against cabbage moth larvae (stage I-II) (small-scale experiments, Nalbandyan, 2020)

Options	The number of larvae on the plant 20 option, quantity	Biological efficiency according to accounting days, %		
		3	5	7
Lepidocide + Arrivo	59	57.6	78.0	91.5
Lepidocide +Voliam Flexi	53	62.3	84.9	94.3
Lepidocide + Proclaim Fit	67	56.7	80.6	92.5
Lepidocide (Sample)	70	51.4	77.1	85.7
Arrivo (Sample)	58	67.2	84.5	89.7
Voliam Flexi (Sample)	51	78.4	88.2	96.1
Proclaim Fit (Sample)	62	62.9	87.1	93.5

Table 2: The indicators of biological effectiveness of standard (sample) and combined insecticides against cabbage moth larvae (stage I-II) by years (production experiments, Nalbandian, 2020)

Options	The number of larvae on the plant 20 option, quantity	Biological efficiency according to accounting days, %		
		3	5	7
During 2021				
Lepidocide + Arrivo	55	56.4	76.4	89.1
Lepidocide + Voliam Flexi	64	60.9	84.4	92.2
Lepidocide + Proclaim Fit	59	50.8	78.0	91.5
Lepidocide (Sample)	55	49.1	76.4	85.4
Arrivo (Sample)	73	64.4	83.6	86.3
Voliam Flexi (Sample)	61	77.0	85.2	95.1
Proclaim Fit (Sample)	62	61.3	85.5	93.5
During 2022				
Lepidocide + Arrivo	71	54.9	77.5	90.1
Lepidocide + Voliam Flexi	57	61.4	82.4	93.0
Lepidocide + Proclaim Fit	69	53.6	79.7	91.3
Lepidocide (Sample)	74	50.0	75.7	82.4
Arrivo (Sample)	77	66.2	81.8	88.3
Voliam Flexi (Sample)	63	76.2	87.3	95.2
Proclaim Fit (Sample)	70	60.0	84.3	91.4

which is apparently due to the specificity of the mechanism of action of insecticides on larvae.

Compared to the water-spraying practices, when the experimental options, i.e., the combined bacterial and chemical sublethal concentrations, were applied, the cabbage larvae gradually refused to feed on plants. Moreover, no response to contact or any other mechanical stimuli was observed, which, eventually, contributed and led to larval death. The bodies of dead larvae, compared to healthy larvae, have become grey and have reduced in size.

Microbiological studies have confirmed that the gut cavity and decayed tissues of dead moth larvae were full of vegetative cells of the *Bacillus thuringiensis* pathogen, as well as insecticidal spore-crystal components.

Using the Student's t-test criteria, it was proved (Table 3) that the two-year indicators of biological efficiency of the experimental options, when the lepidocide was combined with sublethal concentrations of insecticides, significantly exceeded those of the standard lepidocide samples, because in the first case with $p = 0.95$ and n = 3, the student's t-test scores, generally ranging from 3.601 to 7.095, were higher than the tabulated Student's t-test score of 3.182. It was also statistically confirmed that there was no significant difference between the indicators of biological effectiveness recorded in the combined versions, on the one hand, and in the standard individual options of Arrivo, Voliam Flexi and Proclaim Fit, on the other hand (with $p = 0.95$ and n = 3, the calculated two-year average scores of the student's t-tests were between 0.056 and 1.756, which were less than its table (3.182) index).

In the two-year production studies, the statistical error was generally ranging from 2.0 % to 5.9 %, confirming that the results of the scientific experiments are reliable (Table 4).

4 CONCLUSIONS

Based on the results of the experiments, we came to a conclusion that the combinations of insecticides with bacterial and chemical sublethal concentrations show high biological efficiency against the cabbage moth larvae. The efficiency indicators for the latter are statistically different from those of the standard bacterial lepidocide. However, no statistical difference was found between the efficiency indicators of the combined and standard chemical (Arrivo, Voliam Flexi, Proclaim Fit) options.

The statistical error indicators prove that the results of the scientific experiments are accurate.

Table 3: The comparative assessment of indicators of biological efficiency recorded in experimental and standard (sample) options during production experiments verified by Student's t test criteria (by years)

Options	Indicators of biological efficiency 7 days after spraying, %	Student's t test scores	Indicators of biological efficiency 7 days after spraying, %	Student's t test scores
	During 2021		During 2022	
Lepidocide + Arrivo	89.1	3.601*1.756	90.1	4.424*1.208
Lepidocide + Voliam Flexi	92.2	4.687**1.756	93.0	7.095**1.535
Lepidocide + Proclaim Fit	91.5	4.643***1.628	91.3	4.537***0.056
Lepidocide (Sample)	85.4	-	82.4	-
Arrivo (Sample)	86.3	-	88.3	-
Voliam Flexi (Sample)	95.1	-	95.2	-
Proclaim Fit (Sample)	93.5	-	91.4	-

Note. * in the numerator: the combined experimental and lepidocide (sample) options, in the denominator: the comparative indicators of biological efficiency recorded in the experimental and Arrivo (sample) options, ** in the numerator: experimental and lepidocide (sample) options, in the denominator: the comparative indicators of the biological efficiency recorded in the experimental and Voliam Flexi (sample) options and *** in the numerator: the comparative indicators of biological efficiency recorded in the experimental and lepidocide (sample) options, in the denominator: the experimental and Proclaim Fit (sample) options

Table 4: The statistical indicators of the average number of dead cabbage moth larvae (stages I-II) per replicate, 7 days after spraying, by years (production experiments)

Options	The average number of dead larvae per replicate, quantity	Statistical indicators			
		The squared deviation	The coefficient of variation, %	The average error	The statistical error, %
During 2021					
Lepidocide + Arrivo	16.33	0.575	3.52	0.332	2.0
Lepidocide + Voliam Flexi	19.67	1.661	8.44	0.959	4.9
Lepidocide + Proclaim Fit	18.00	1.414	7.85	0.816	4.5
Lepidocide (Sample)	15.67	1.205	7.69	0.696	4.4
Arrivo (Sample)	21.00	1.633	7.78	0.943	4.5
Voliam Flexi (Sample)	19.33	1.737	8.99	1.003	5.2
Proclaim Fit (Sample)	19.33	1.009	5.22	0.583	3.0
During 2022					
Lepidocide + Arrivo	21.33	1.741	8.16	1.005	4.7
Lepidocide + Voliam Flexi	17.67	1.199	6.79	0.692	3.9
Lepidocide + Proclaim Fit	21.00	2.160	10.29	1.247	5.9
Lepidocide (Sample)	20.33	1.739	8.55	1.004	4.9
Arrivo (Sample)	22.67	1.185	5.23	0.684	3.0
Voliam Flexi (Sample)	20.00	1.633	8.17	0.943	4.7
Proclaim Fit (Sample)	21.33	1.303	6.11	0.752	3.5

5 ACKNOWLEDGEMENTS

The work of was supported by the Science Committee of the Republic of Armenia within the frames of the research projects 23SCZHE-I-1F.

6 REFERENCES

Andreeva, I.A., Shatalova, E.I. and Khodakova, A.V. (2021). Cabbage moth *Plutella xylostella*: ecological and biological aspects, harmfulness, population control. *Bulletin of Plant Protection, 104*(1), 28–39. [In Russian]. https://doi.org/10.31993/2308-6459-2021-104-1-14947

Ashmarin, I.P., &d Vorobyev, A.A. (1962). *Statistical methods in microbiological research*. Medgiz, 180 p. [In Russian].

Avagyan, A.M. (2012). *Development of integrated control on ecological basis against main pests – Horse radish leaf beetle in conditions of Lori marz*. Abstr., PhD. Biol. Thesis. Erevan. 23 p. [In Armenian].

Avetyan, H.S. and G.M. Marjanyan (1976). *Pests of agricultural crops, forests and warehouses of Armenia*. Publishing House of the Academy of Sciences of the Armenian SSR, Yerevan. 832 pp. [In Armenian].

Belyaev, O.V., & Nozdrenko, Ya.V. (2004). Lepidocide against the unpaired silkworm, *Protection and Quarantine of Plants, 6*, 42-43. [In Russian].

Bernstein, A. (1968). *Handbook of Statistical solutions*. M.: Statistics, 162 p. [In Russian].

Chapanyan, E.N. (2022). *Developing integrated pest management measures against the main forest leaf beetles with the ecologically-based crystal-forming local bacterial insecticides of Aragatsotn region*. Abstr. PhD. Biol. Thesis. Erevan. 27 p. [In Armenian].

Churikova, V.G., & Silaev, A.I. (2010). Pests of spring rape in the Lower Volga region, *Agro, XXI*, (4-6), 24–27. [In Russian]. https://doi.org/10.28983/asj.y2020i11pp71-77

Fathipour, Y., & Mirhosseini, M.A. (2017). Diamondback moth (Plutella xylostella) management. In: G.V.P. Reddy (ed.). *Integrated Management of Insect Pests on Canola and Other Brassica Oilseed Crops* (pp.13–43). Oxford; Boston, MA: CAB International. https://doi.org/10.1079/9781780648200.0013

Harcourt, D.G. (1957). Biology of the diamondback Moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae), in Eastern Ontario. *Canadian Entomologist, 89*(12), 554–564. https://doi.org/10.4039/Ent89554-12

Ivantsova, E.A. (2004). Biopreparation against mustard pests, *Protection and Quarantine of Plants, 6*, 36. [In Russian]. DOI: https://dx.doi.org/10.15688/jvolsiu 11.2015.2.6

Kholod, A.S., & Korenyuk, E.F. (2016). Cabbage moth – a threat to rapeseed crops in the Omsk region, *Protection and Quarantine of Plants, 5*, 32–33. [In Russian]. https://doi.org/10.28983/asj.y2020i11pp71-77

Mesropyan, H.R. (2011). *Effect of BTB and Lepidocide on the microflora and enzymatic activity of afforestation typical chernozem*. Abstr. PhD. Biol. Thesis. Erevan. 24 p. [In Armenian].

Methodological guidelines for testing biological products for plant protection from pests, diseases and weeds. 1973. Moscow, Kolos, 112 p. [In Russian].

Netrusov, A.I., Egorov, M. A., & Zakharchuk L.M. (2005). *Workshop on microbiology*. Moscow: IC Academy, 608 p. [In Russian].

Philips, C.R., Fu, Z., Kuhar, T.P., Shelton, A.M., & Corder,o R.J. (2014). Natural history, ecology, and management of diamondback moth (Lepidoptera: Plutellidae), with emphasis on the United States. *Journal of Integrated Pest Management, 5*(3), D1–D11. https://doi.org/10.1603/IPM14012

Polyakov, I.Ya. (1984). *Forecast of the development of pests and diseases of agricultural crops (with a workshop)*. L.: "Ear", Leningrad branch, 318 p. [In Russian].

Safaryan, S.E. (1968). *The harmful fauna of cabbage in Armenia and the biological basis for the development of control measures*. Abstract of the dis. candidate of agr. sciences. Yerevan, 29 p. [In Russian].

Sarantseva, N.A., & Bobreshova, I. Yu. (2006). Biopreparations against the Colorado potato beetle, *Protection and Quarantine of Plants, 7*, 27–28. [In Russian].

Sargsyan, A.M. (2013). *The biological efficiency of bacterial insecticides isolated from biocenosis against the major leaf beetle pests of the forest and their influence on biological activity of forest brown soils*. Abstract of the thesis candidate of biol. sciences. Yerevan, 26 p. [In Armenian].

Semerenko, S.A. (2019). Pheromonitoring of cabbage moth in rapeseed crops and a search of effective chemicals for pest control in the conditions of the Western Ciscaucasia. Oil Crops. *Scientific and Technical Bulletin of VNIIMK, 4*(180), 143–151. [In Russian]. https://doi.org/10.25230/2412-608X-2019-4-180-143-151

Shpanev, A.M. (2015). Mass reproduction of cabbage moth, *Protection and Quarantine of Plants, 9,* 40-42. (In Russian). DOI: 10.31857/S0002188122080130.

Talekar, N.S., & Shelton, A.M. (1993). Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology, 38*(1), 275–301. https://doi.org/10.1146/annurev.en.38.010193.001423

Terlemezyan, H.L. (1996). *Harmful fauna of vegetable crops of the Ararat Plain: bioecological features of the main species and integrated control against them*. Ph. D. Thesis, Yerevan University, Armenia. 301 pp. [In Russian].

Tsedeler, O.E. (1931). Cabbage moth (*Plutella maculipennis* Curt.) in connection with cultural mustard, *Journal of Experimental Agrochemistry of the South-East, 9*(2), 165–195. [In Russian]. https://doi.org/10.28983/asj.y2020i11pp71-77

Tuleeva, A.K., & Sarmanova, R.S. (2019). Pests of spring rape in Akmola region. *Protection and Quarantine of Plants, 12*, 20–23. [In Russian]. https://doi.org/10.28983/asj.y2020i11pp71-77

Zakharova, Yu.A., Frolov, A.N., & Artemyeva, A.M. (2022). Monitoring of the diamondback moth (*Plutella xylostella* L.) on the *Brassica oleracea* L. collection in the vicinity of St. Petersburg. *Proceedings on Applied Botany, Genetics and Breeding*, *183*(4), 219–228. https://doi.org/10.30901/2227-8834-2022-4-219-228