RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 59 Issue 3: 410-416 (March 2025)

Evaluation of *Bacillus thuringiensis* for the Control of *Euproctis chrysorrhoea* (L.) (Lepidoptera: Erebidae)

Hrant Terlemezyan[1], Harutyun Harutyunyan[1], Sona Sargsyan[1], Gabriel Karapetyan[1], Hapetnak Mkrtchyan[1], Noushig Zarikian[2], Masis Sargsyan[1]

10.18805/IJARe.AF-896

Abstract

Backround: The strains of *Bacillus thuringiensis* bacteria (Bt-type) are utilized for controlling the brown-tail moth pest, *Euproctis chrysorrhoea* (Linnaeus, 1758), in apple orchards in Armenia from 2020 to 2022.

Methods: The study focused on the brown-tail moth larvae in all developmental stages (I-VII) on the *Renet Simirenko* apple variety. Culture fluids, based on crystal-forming insecticidal strains Bt_{EM-54} and Bt_{EM-82} were employed.

Result: Research has demonstrated that local Bt_{EM-54} and Bt_{EM-82} culture fluids exhibit significant biological efficiency against brown-tail moth larvae across various stages (I-II, III-IV, and V-VII) in both laboratory settings and apple orchards. Furthermore, no statistical differences were observed when comparing the biological efficiency factors of these scientific experiments with the standard lepidocide.

Key words: Biological control, Biological efficiency, Brown-tail moth, Bt-type, Pest control.

Introduction

The brown-tail moth *Euproctis chrysorrhoea* (Linnaeus, 1758), (Lepidoptera: Erebidae) poses a significant threat to orchards and forests in the Republic of Armenia, leading to defoliation, interrupted photosynthesis and subsequent weakening or drying up of trees (Fifth National Report of Republic of Armenia, 2014). Therefore, it necessitates integrated pest control strategies. The economic implications of *E. chrysorrhoea* infestations in orchards are profound, with studies by the University of Maine research team led by Crosby (2023), indicating substantial losses of crop yield and economic productivity. Furthermore, the potential long-term effects on orchard ecosystems demand a holistic examination of the ecological interactions between *E. chrysorrhoea* and other key components of the orchard environment. Recent work by Zaller *et al.* (2023) highlights the cascading effects of pest-induced stress on orchard biodiversity and the delicate balance that exists between pest management practices and the preservation of beneficial insects.

The global applicability of bacterial biocontrol strategies for leaf-eating caterpillars is underscored by studies conducted in diverse regions [Vimala Devi and Sudhakar, (2006); Veeranna *et al*. (2021); Harpal *et al*., 2024]. Research by Longkumer *et al*. (2018) showcases successful implementations of Bt-based formulations in Asia, providing insights into the adaptability and effectiveness of bacterial interventions in combating lepidopteran pests in rice fields and vegetable crops. Similarly, investigations by Dively *et al*. (2021) in America emphasize the potential of Bt formulations for the management of Lepidopteran pests infesting maize crops. In combating leaf-eating insects, our integrated approach favors the use of *Bacillus thuringiensis* (Bt)-based bacterial preparations. Unlike chemical insecticides, these Bt-based solutions are environmentally safe for humans and warm-blooded animals and beneficial for the entomofauna and fish [Ghazaryan *et al*. (2006); Krushev and Mashnina, 1977, Avagyan *et al*., 2012, Sujayanand *et al*., 2020]. Demonstrating high biological efficiency against susceptible phytophages, they avoid phytotoxic effects, preserve the taste characteristics of plant products and can be applied at various stages of plant growth (Likhovidov and Kolchevsky, 1989; Avagyan, 2012).

However, the reliance on imported Bt-type bacterial preparations significantly escalates production costs threefold, rendering it economically impractical (Chapanyan and Sargsyan, 2018a). Therefore, there is an imperative to establish conditions conducive to developing

[1]Department of Plant Protection, Food Safety Risk Analysis and Assessment Research Center, 107/2 Masis Highway, Yerevan, Armenia.

[2]Invertebrate Ecology Laboratory, Scientific Center of Zoology and Hydroecology of National Academy of Sciences of Republic of Armenia, Yerevan, Armenia.

Corresponding Author: Noushig Zarikian, Invertebrate ecology laboratory, Scientific Center of Zoology and Hydroecology of National Academy of Sciences of Republic of Armenia, Yerevan, Armenia. Email: nzarikian@gmail.com
ORCID: 0000-0003-0726-2639.

How to cite this article: Terlemezyan, H., Harutyunyan, H., Sargsyan, S., Karapetyan, G., Mkrtchyan, H., Zarikian, N. and Sargsyan, M. (2025). Evaluation of *Bacillus thuringiensis* for the Control of *Euproctis chrysorrhoea* (L.) (Lepidoptera: Erebidae). Indian Journal of Agricultural Research. 59(3): 410-416. doi: 10.18805/IJARe.AF-896.

Submitted: 04-07-2024 **Accepted:** 10-10-2024 **Online:** 21-11-2024

a cost-effective commercial preparation based on Bt-type bacterial insecticides. This involves isolating ecologically safe bacterial strains exhibiting high biological efficiency against harmful insects from specific elements of the biocenosis.

In Armenia, such research has been conducted by Terlemezyan *et al*. (2023a) where the effectiveness of the BT_{TER-55} + Coragen and BT_{TER-94} + Coragen analyzed against European Grapevine Moth, *Lobesia botrana.* Also, combinations of sublethal concentrations of bacterial (Lepidocide) and chemical (Arrivo, Voliam Flexi, Proclaim Fit) preparations were tested against cabbage moths (Terlemezyan *et al*., 2023b).

This research addresses the current environmental considerations in the Republic of Armenia toward the insecticides' use. By creating a foundation for the release of a local commercial preparation and isolating eco-friendly bacterial strains, we aim to overcome the economic challenges associated with imported Bt solutions. This work not only proposes a practical solution but also emphasizes the importance of ecological research in predicting and preventing adverse consequences associated with insecticide use.

MATERIALS AND METHODS

The evaluation of Bt was conducted from 2020 to 2022, both in laboratory settings at the "ARMBIOTECHNOLOGY" Scientific and Production Centre (SPC) and also at the apple orchards located in Ujan village, near Ashtarak City (Aragatsotn province, 1200-1320 m.a.s.l. N 40°172 483 E 44°122 183). The study was focused on the seven larval stages of brown-tail moth on the Renet Simirenko apple variety. Culture fluids, produced by the "ARMBIOTECHNOLOGY" SPC based on crystal-forming insecticidal strains Bt_{EM-54} and Bt_{EM-82}, (named by the staff of the scientific centre) were employed. These strains were derived from larvae of the mottled umber *Erannis defoliaria* L. and mountain tent caterpillar *Malacosoma parallela* Stgr., obtained through microbiological methods after their natural death. The commercial bacterial lepidocide preparation used exhibited a biological activity of 3000 units/mg and was authorized for use in the Republic of Armenia (Terlemezyan *et al*., 2015). Therefore, the apple orchards' light-brown soils and soil-dwelling ammonifying bacteria were utilized as soil indicators of biological materials to implement this research. Detailed information about the microbiological isolation of Bt strains from naturally deceased larvae is available in a previously mentioned scientific paper (Terlemezyan *et al*., 2015). To evaluate the biological effectiveness of Bt-type culture liquids and the aqueous suspension of the standard lepidocide, the apple orchards in the Ujan village near Ashtarak city were chosen as test sites, where the orchards exhibited severe economic damage due to brown-tail moth larvae, averaging 1-3 larvae per linear meter (tree branch) (Rogacheva, 1968). The body length of brown-tail moths in the orchards was determined by measuring the size of the head capsule of the larvae (Calvo and Molina, 2008). The biological efficiency of the bacterial insecticides Bt_{EM-54} and Bt_{EM-82} in both laboratory and apple orchard was determined following the methodology outlined in the manual (Armstrong and Hilton, 2010). The standard (control) for the study was the number of brown-tail moth larvae naturally present in the foliage of unsprayed apple orchards, which were subsequently treated with an aqueous suspension of lepidocide (0.2%). In the small-scale experiments, we utilized an Ozdesan brand sprayer for spraying. The amount of working fluid sprayed onto model trees varied based on their height, ranging from 1.5 to 2.0 liters per tree for trees with heights between 1.9 to 2.6 meters. The titter (density) of viable spores in the culture fluids used for spraying was 600 million spores per ml. Bt suspensions were sprayed under the field conditions 20 ± 0.5°C and 45-50% humidity. Each of the variants included in the experiments had three replicates. The biological efficiency was determined using Abbott's formula (Abbott, 1925). To assess the quantities of Bt insecticides and soil-dwelling ammonifying bacteria in light-brown soil following spraying throughout the growing season, the meat-peptone agar (MPA) method on Petri dishes (a nutrient medium for them), utilizing the serial dilution technique was adopted (Netrusov *et al*., 2005). In the samples sprayed with bacterial insecticides, the presence of insecticidal spore-crystal components in the bodies of dead larvae, as well as in the soil was identified during the vegetation period according to the recommendation (Ahern, 2018). Each of the variants included in the microbiological studies had 5 replicates. Statistical analysis of the survey results was conducted following the methodology outlined in the manual (Ilstrup, 1990) and references (Bernstein, 1968).

RESULTS AND DISCUSSION

Based on the findings from scientific experiments conducted in laboratory settings in 2021, it was confirmed that the insecticidal crystal-forming bacterial culture fluids Bt_{EM-54} and Bt_{EM-82}, which were microbiologically isolated from deceased larvae of the brown-tail moth and mountain tent caterpillar in (2020) under natural conditions, exhibited high biological efficiency ranging from 95.0% to 98.3% against brown-tail moth larvae at various stages (I-II (early), III-IV (middle), V-VII (adult)) within 10 days after spraying. The notable levels of biological effectiveness observed in laboratory conditions warranted further evaluation of these bacterial culture fluids against brown-tail moth larvae at different stages in apple orchards located in the Ujan village, through both small-scale and production experiments (Table 1 and 2). In the orchards selected for our study, the average number of brown-tail moth larvae in the I-II stages (with larval head capsule sizes of 0.4 and 0.5 mm, respectively), III-IV stages (0.7 and 0.9 mm) and V-VII stages (1.3 - 2.5 mm) per linear meter of branch reached the economic damage threshold. Overall, the average number of larvae per linear meter of branch ranged between 2.8 and 3.4. Spraying against larvae in the I-II, III-IV and V-VII stages was conducted in forests on 07.25.2021, 05.12.2022 and 05.26.2022, respectively. Results from small-scale scientific experiments (Fig 1) confirmed that local culture fluids Bt_{EM-54} and Bt_{EM-82} exhibited high biological efficiency of 95.2% and 96.4%, 93.5% and 95.2%, 92.8% and 93.1% against the larvae in the I-II, III-IV and V-VII stages, respectively, within 10 days after spraying. The biological efficiency indicators of standard lepidocide variants used against the same stages of these phytophagous larvae (I-II, III-IV and V-VII) during the same observation period were 95.4%, 94.2% and 93.5%, respectively. As shown in the table (Table 1), the biological efficiency indicators recorded in both experimental (Bt_{EM-54}, Bt_{EM-82}) and standard (lepidocide) treatments, measured at 3 and 7 days after spraying (ranging from 41.0% to 69.7% and 82.0% to 90.6%, respectively), were lower compared to those observed on the 10th day after spraying. This difference can be attributed to the specific mechanism of action of Bt-type bacterial insecticides. It is noteworthy, that the indicators of biological efficiency recorded on the 10th day in both experimental (Bt_{EM-54}, Bt_{EM-82}) and standard (lepidocide) versions, were constant until the larva reached the pupal stage.

In the days following the spray, larvae in the experimental groups treated with bacterial culture fluids gradually ceased feeding, became unresponsive to mechanical stimuli, gradually reduced in size, softened, discolored and exhibited other negative symptoms, ultimately resulting in mortality.

Microbiological examination of the deceased larvae from the experimental groups sprayed with bacterial insecticides confirmed the presence of insecticidal spore-crystal components within the partially decomposed tissues and intestinal cavities of the larvae. In some cases, bacterial vegetative cells were also detected. These findings validate that the mortality of phytophagous larvae resulted from exposure to Bt-type bacterial insecticides.

The promising results obtained from small-scale experiments paved the way for large-scale testing of local bacterial culture fluids of Bt type against brown-tail moth

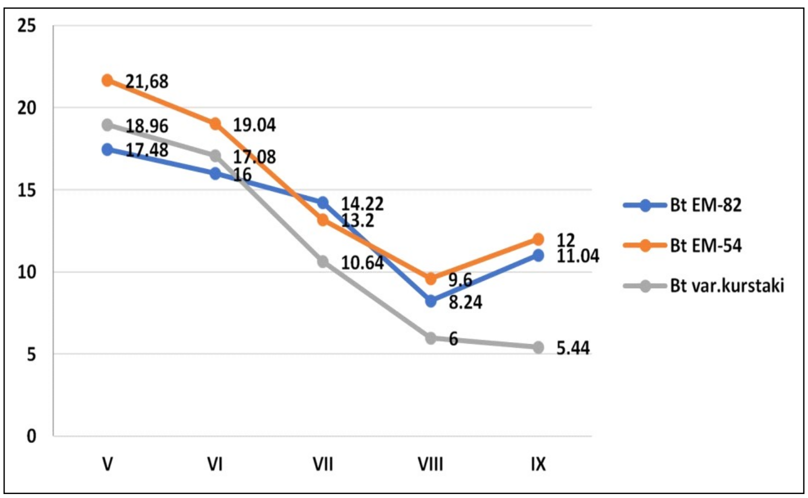

Fig 1: Bacteria spore density in the soils of apple orchard after spraying Bt (2022) Bt EM-82 (blue), Bt EM-54 (orange) and Bt var. kurstaki (grey).

Table 1: Efficacy of different Bt-strains against brown-tail moth larvae spread in apple orchards (2021).

Types of Bt strain	Spore count millions per ml	No of larvae / sample unit	% efficacy 3rd after spray	% efficacy 7th after spray	% efficacy 10th after spray
Against I and II instar larvae					
Bt_{EM}-54	600	186	61.3	89.8	95.2
Bt_{EM}-82	600	192	65.1	90.6	96.4
Lepidocide (standard)	0.2	175	69.7	90.3	95.4
Against III and IV instar					
Bt_{EM}-54	600	170	42.9	85.3	93.5
Bt_{EM}-82	600	167	49.1	88.0	95.2
Lepidocide (standard)	0.2	190	56.3	89.5	94.2
Against V and VII instar					
Bt_{EM}-54	600	183	41.0	82.0	92.8
Bt_{EM}-82	600	189	48.7	84.7	93.1
Lepidocide (standard)	0.2	201	55.2	87.1	93.5

larvae at different stages under production conditions through large-scale spraying. Each bacterial insecticide was tested on a separate experimental site, covering an area of 2000 m^2 (0.2 hectares).

The results obtained from scientific experiments conducted under production conditions are summarized in Table 2. and that revealed the highest levels of biological efficiency for Bt-type bacterial culture fluids against brown-tail moth larvae in stages I-II, III-IV and V-VII were observed 10 days after spraying with rates of 94.1% and 95.0%, 92.4% and 94.5%, 92.1% and 93.4%, respectively. In the case of standard lepidocide, the indicators for the same groups and observation period were 95.7%, 93.1% and 92.9%, respectively.

No larval mortality was observed in the control groups of both small-scale and production experiments. The biological efficiency indicators remained consistent until the larva reached the pupal stage on the 10th day in both experimental and standard groups. Furthermore, the biological efficiency indicators recorded on the 3rd and 7th days of spraying were also relatively low compared to those on the 10th day, as outlined in the table (Table 2). In the literature, there is conflicting data regarding the viability of Bt bacterial insecticides in the soil after spraying. Some researchers stated that depending on the type of bacterial insecticide and soil composition, the crystal-forming bacteria can persist in the soil for 2 days (Akiba, 1986). Conversely, others stated that these bacteria remain active for 1-5 months (Ghazaryan ***et al***., 2006; Tetreau ***et al***., 2021) or even 1-10 years (Li ***et al***., 2022; Metze ***et al***., 2023). Given these varying reports, our objective was to assess the viability of Bt_{EM-54} and Bt_{EM-82} isolated from the individual elements of the biocenosis, as well as Bt var. *kurstaki* bacterial insecticide, which is the basis for the release of the commercial lepidocide bacterial preparation.

The results of the experiments (Fig 1) confirm that the quantities of Bt_{EM-54}, $Bt_{EM-82\ and}$ Bt var. *kurstaki* bacterial insecticides that fell into the light-brown soil of the apple orchard as a result of spraying in May (initial amounts were 21.68, 18.96 and 17.48 million/g, respectively), has decreased during the following months of vegetation (June, July, August). Furthermore, the viability of these insecticides in this soil subtype was found to be 5 months after a single spraying during the vegetation period. Findings of the present work also suggest that soil-dwelling antagonists such as *Bacillus mesentericus*, *Bacillus sp., Sarcina sp. and Penicillium puberulum* (Chapanyan and Sargsyan, 2018b) contribute to the reduction in the quantity of Bt-type bacterial insecticides. This phenomenon appears to influence our scientific experiment as well. Notably, the amounts of Bt_{EM-54} and Bt_{EM-82} insecticides recorded in September increased by 20.0% and 25.4%, respectively, compared to August.

This phenomenon can likely be attributed to the gradual adaptation of Bt_{EM-54} and Bt_{EM-82} insecticides to the environment, alongside the autumnal replenishment of soil

Table 2: Efficacy of different Bt-strains against brown-tail moth larvae spread in apple orchards (2022).

Types of Bt strain	Spore count millions per ml	No of larvae / sample unit	% efficacy 3rd after spray	% efficacy 7th after spray	% efficacy 10th after spray
Against I and II instar larvae					
Bt_{EM}-54	600	205	59.0	88.3	94.1 / 0.916
Bt_{EM}-82	600	179	64.8	89.4	95.0 / 0.772
Lepidocide (standard)	0.2	188	68.1	90.4	95.7 / -
Against III and IV instar					
Bt_{EM}-54	600	185	41.1	83.2	92.4 / 0.903
Bt_{EM}-82	600	164	47.0	86.0	94.5 / 0.513
Lepidocide (standard)	0.2	173	53.2	87.3	93.1 / -
Against V and VII instar					
Bt_{EM}-54	600	177	39.0	79.7	92.1 / 1.461
Bt_{EM}-82	600	181	45.3	82.9	93.4 / 1.138
Lepidocide (standard)	0.2	198	52.0	86.4	92.9 / -

Note. *) - in the numerator we have the indicator of biological efficiency, in the denominator: Student's ι-criterion.

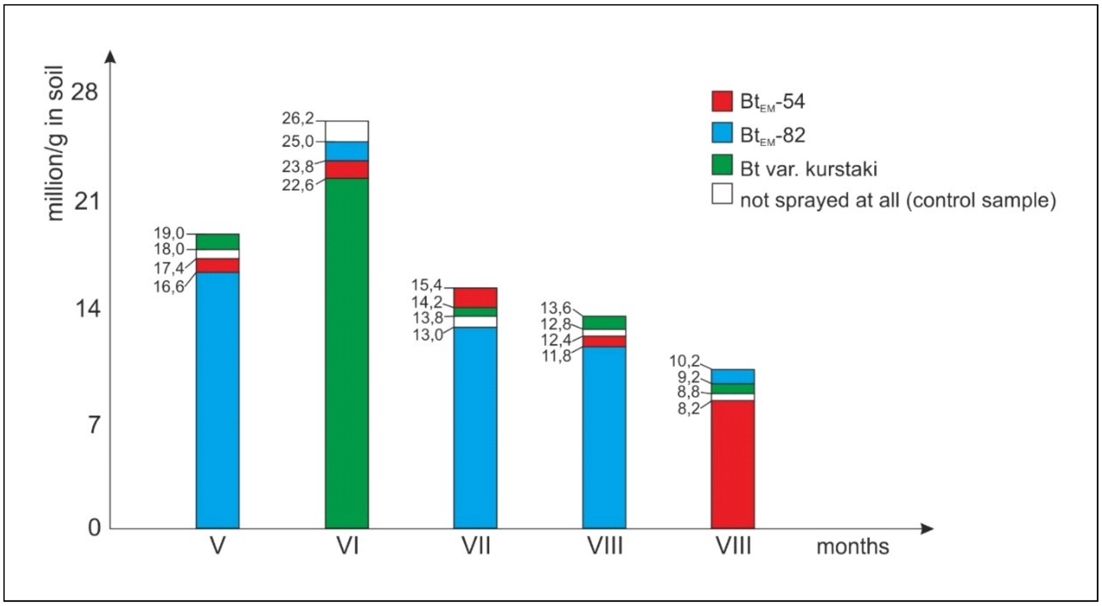

Fig 2: Spore density of ammonifying bacteria in the soils of apple orchard after sprayed with Bt insecticide.

nutrients from decomposing plant matter. In the case of Bt var. *kurstaki*, the quantitative reduction continued in September. Ammonification, a crucial process shaping nitrogen balance in biocenosis, involves ammonia-producing microbes such as bacteria, actinomycetes and microscopic fungi. This process converts organic nitrogen into NH_3 or NH_4, which is readily absorbed by plants (Harutyunyan, 2010). Karyagina, Kazaryan and Wang have highlighted that ammonification is a key determinant of soil fertility, with a strong positive correlation (r=0.89) observed between humus levels and soil-dwelling ammonifiers in the soil (Karyagina, 1983; Kazaryan, 2007; Wang, 2017). Along with the dynamics of change in the quantity of Bt-type bacterial insecticides that fell into the soil as a result of spraying during the vegetation period, we have studied the dynamics of change in the quantity of ammonifying bacteria in the light-brown soil of apple orchards, which were either sprayed with Bt_{EM-54} and Bt_{EM-82} and Bt var. *kurstaki* bacterial insecticides separately or not sprayed at all (control sample), based on which the impact of Bt bacterial insecticides on soil fertility has been assessed. Microbiological analyses (Fig 2) demonstrated fluctuations in the total quantity of ammonifying bacteria during the vegetation period both in the experimental and control plots, with peak levels in June (22.6-26.2 million/g of soil) and a decline level in September (typically 8.2-10.2 million/g of soil). The intensity of ammonification has been correlated with soil moisture, temperature, air saturation and the composition of decomposing organic matter (Beeckman *et al*., 2018) as observed in our experiments. Statistical analysis of larval quantities affected by Bt insecticides, along with those found in soil due to spraying and soil-dwelling ammonifiers, revealed a consistent range of statistical error (2.7 to 5.5) and coefficient of variation (4.75% to 12.41%). These findings validate the accuracy of our scientific experiments.

The calculated values of the Student's ι test when P < 0.95 and n=5 generally ranged from 0.513 to 1.461. These values were smaller than the tabulated value of 2.571, indicating no significant statistical difference between the quantitative indices of soil-dwelling ammonifiers observed in soil samples treated with Bt bacterial insecticides compared to untreated samples.

Similarly, for all larval stages, the calculated values of Student's ι test when P0.95 and n=3 generally ranged from 0.513 to 1.461 (Fig 2) and were smaller than its tabulated value of 3.182, This confirms no significant statistical difference between the biological efficiency indicators calculated for samples treated with Bt_{EM-54} and Bt_{EM-82} culture fluids compared to those treated with an aqueous suspension of lepidocide (standard).

Conclusion

Based on our scientific experiments, we concluded that Bt_{EM-54} and Bt_{EM-82} culture liquids, with a spore density of 600 million spores/ml, exhibit significantly high biological efficiency against all the larval stages of *Euproctis chrysorrhoea* both in the laboratory and apple orchard settings. Additionally, there wasn't any statistical difference between the biological efficiency indicators of locally sourced Bt_{EM-54} and Bt_{EM-82} culture liquids and the standard aqueous suspension of lepidocide. The quantities of Bt insecticides in the light-brown soil of the apple orchard decreased over the vegetation period following spraying, with no statistical difference noted between the quantitative indicators of ammonifying bacteria both in the Bt treated and untreated control soil. Statistical indicators affirm the accuracy of the results obtained from various scientific studies. Bt_{EM-54} and Bt_{EM-82} culture fluids have been identified as environmentally safe insecticides.

Author contribution

Conceptualization HT and MS; methodology HH; formal analysis SS; investigation GK and HM; writing MS; review and editing, NZ; visualization SS. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Conflict of interest

No potential conflict of interest was reported by the authors.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 18: 265-267.

Ahern, H. (2018). Microbiology: A Laboratory Experience Open SUNY Textbooks 3, Milne Library State University of New York at Geneseo. 138 pages.

Akiba, Y. (1986). Microbial ecology of *Bacillus thuringiensis*. 1. Germination of Bacillus thuringiensis spores in the soil. Applied Entomology and Zoology. 21: 76-80.

Armstrong, R.A., Hilton, A.C. (2010). Statistical Analysis in Microbiology: Statnotes. School of Life and Health Sciences, Aston University, United Kingdom. 170 pages. doi: 10.1002/9780470905173.

Avagyan, A.M. (2012). Enzymatic activity of typical black soils planted with cabbage after spraying with bacterial insecticides, Agricultural Science. 5-6: 319-323 (in Armenian).

Avagyan, A.M., Sargsyan, M.A., Movsesyan, H.S. (2012). The membership of insecticidal crystal-forming bacteria in typical black soils planted with cabbage, Scientific Bulletin (Chemistry and Biology). 2: 55-60 (in Armenian).

Beeckman, F., Motte, H., Beeckman, T. (2018). Nitrification in agricultural soils: Impact, actors and mitigation. Current Opinion in Biotechnology. 50: 166-173.

Bernstein, A. (1968). Reference Book of Statistical Solutions. M: Statistics. 162 pages.

Calvo, D., Molina, J.M. (2008). Head Capsule Width and Instar Determination for Larvae of *Streblote panda* (Lepidoptera: Lasiocampidae), Annals of the Entomological Society of America. 101(5): 881-886.

Chapanyan, E., Sargsyan, M. (2018a). The biological efficiency of the local bacterial insecticide of *Bacillus thuringiensis* species against the caterpillars of the brown-tail moth, Bulletin of National Agrarian University of Armenia. 3: 5-8.

Chapanyan, Y.N., Sargsyan, M. (2018b). Biological effectiveness of local bacterial insecticides based on *Bacillus thuringiensis* species against winter moth larvae, Biological Journal of Armenia, LXX, 4: 43-49 (in Armenian).

Crosby, S. (2023). Browntail Moth (*Euproctis chrysorrhoea*) Integrated Pest Management Program: Evaluation of Monitoring Traps and Biopesticides. Electronic Theses and Dissertations. 3778. https://digitalcommons.library.umaine.edu/etd/3778.

Dively, G.P., Kuhar, T.P., Taylor, S., Doughty, H.B., Holmstrom, K., Gilrein, D., Nault, B.A. *et al.* (2021). Sweet corn sentinel monitoring for lepidopteran Field-evolved resistance to Bt toxins. Journal of Economic Entomology. 114(1): 307319.

Ghazaryan, N.P., Grigoryan, K.V., Sargsyan, M.A., Kharyan, Sh. S. (2006). The quantity of ammonium creating bacterium of light brown and gray semi-desert soils of apple orchards after the introduction of entomo pathogenic bacterium creating crystals. Scientific Bulletin of Yerevan State University. 3: 71-77.

Harpal, K.K., Shukla, A.K., Dhawan (2024). Effects of *Bacillus thuringiensis* ∆-Endotoxin on Life History Parameters of Rice Leaf Folder *Cnaphalocrocis Medinalis* (Lepidoptera). Indian Journal of Agricultural Research. 42(3): 183 -188.

Harutyunyan, V. (2010). Environmental monitoring, National Agrarian University of Armenia, Yerevan. 451 pages.

Ilstrup, D.M. (1990). Statistical methods in microbiology. Clinical Microbiology Reviews. 3(3): 219-26.

Karyagina, L.A. (1983). Microbiological principles to improve the soil fertility. Science and Technology, Minsk, 181 pages.

Kazaryan, N.P. (2007). The influence of entomopathogens of the Bacillus thuringiensis species on the biological activity of the soils of apple orchards in the Aragatsot region. Dissertation for PhD of Life Sciences, 128 pages, Yerevan, RA.

Krushev, L.T., Mashnina, T.I. (1977). Application of bacterial means to protect the forest from harmful insects. Overview: Series: Forest protection. M: CBSTR of Leshoz, 52 pages.

Li, Y., Wang, C., Ge, L., Hu, C., Wu, G., Sun, Y., Song, L., Wu, X., Pan, A., Xu, Q., Shi, J., Liang, J., Li, P. (2022). Environmental Behaviors of *Bacillus thuringiensis* (Bt) Insecticidal Proteins and Their Effects on Microbial Ecology. Plants (Basel). 11(9): 1212. doi: 10.3390/plants11091212.

Likhovidov, V.E., Kolchevsky, A.G. (1989). Application of bacterial preparations against pests of agricultural crops (recommendations). M.: Agropromizdat, pages 3 and 6.

Longkumer, I.Y., Patil, V.K., Singh, L., Kh. A. (2018). *Bacillus thuringiensisas* a sustainable approach toward integrated pest management. Journal of Zoology and Entomology Studies. 6(2): 2959-2963.

Metze, D., Schnecker, J., Canarini, A., Fuchslueger, L., Koch, B., Stone, B., Hungate, B., Hausmann, B., Schmidt, H., Schaumberger, A., Bahn, M., Kaiser, C., Richter, A. (2023). Microbial growth under drought is confined to distinct taxa and modified by potential future climate conditions. Nature Communications. 14: 5895. doi: 10.1038/s41467-023-41524-y.

Ministry of Nature Protection (2014). Fifth National Report of the Republic of Armenia to the Convention on Biological Diversity RA Ministry of Nature Protection, Yerevan, 136 p.

Netrusov, A.I., Egorov, M.A., Zakharchuk, L.M. (2005). Workshop on microbiology. Ed. A.I. Netrusov. M: Academy, 608 p. (In Russian)].

Rogacheva, A.Ya. (1968). Economic thresholds of the harmfulness of the most harmful species of insects and mites. M: Agroprom izdat, page 20.

Sujayanand, G.K., Pandey, S., Bandi, S.M. (2020). Efficacy of Coragen 20 SC against Lepidopteran Pest in Greengram and Its Compatibility with Bacillus thuringiensis Isolates.Legume Research. 44(12): 1521-1528. doi: 10.18805/LR-4481.

Terlemezyan, H., Aharonyan, A., Ter-grigoryan, A., Grigoryan, A. (2015). Directory of chemical and biological means of plant protection allowed for use in the Republic of Armenia, Yerevan, RA. (in Armenian). page 242.

Terlemezyan, H., Sargsyan, M., Harutyunyan, H., Sargsyan, S., Zarikian, N. (2023). Environmentally friendly strategies for controlling the European Grapevine Moth, *Lobesia botrana* (Denis and Schiffermüller) (Lepidoptera: Tortricidae). Arab Journal of Plant Protection. 41(3): 272-277.

Terlemezyan, H., Sargsyan, M., Harutyunyan, H., Zarikian, N., Sargsyan, S., Karapetyan, G., Mkrtchyan, H. (2023). Results of testing of the efficacy of sublethal concentrations of bacterial-chemical insecticides combinations against cabbage moth larvae. Acta Agriculturae Slovenica. 119(3): 1-6.

Tetreau, G. andreeva, E.A., Banneville, A.S., De Zitter, E., Colletier, J. (2021). Can (We Make) *Bacillus thuringiensis* Crystallize More Than Its Toxins? Toxins. 13(7): 441. https://doi.org/10.3390/toxins13070441.

Veeranna, D., Mittal, A., Rajashekhar, M., Kalia, V.K. (2021). Characterization of native *Bacillus thuringiensis* trains against storage pest *Tribolium castaneum* (Coleoptera: Tenebrionidae). Indian Journal of Agricultural Sciences. 91(8): 1230-5.

Vimala, Devi, P.S. and Sudhakar, R. (2006). “Effectiveness of a Local Strain of *Bacillus thuringiensis* in the Management of Castor Semilooper Achaea janata on Castor (*Ricinus communis*). Indian Journal of Agricultural Science. 76(7): 447-449.

Wang, C., Wang, N., Zhu, J., Liu, Y., Xu, X., Niu, Sh., Yu, G., Han, Xi., He, N. (2017). Soil gross N ammonification and nitrification from tropical to temperate forests in eastern China. Functional Ecology. 32: 83-94. https://doi.org/10.1111/1365-2435.13024.

Zaller, J.G., Oswald, A., Wildenberg, M., Burtscher-Schaden, H., Nadeem, I., Formayer, H., Paredes, D. (2023). Potential to reduce pesticides in intensive apple production through management practices could be challenged by climatic extremes. Science of The Total Environment. 872: 162237. https://doi.org/10.1016/j.scitotenv.2023.162237.