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Research Article

Efficacy of native strains of *Bacillus thuringiensis* against apple ermine moth and their impact on soil biological activity in the Republic of Armenia

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ABSTRACT: The apple ermine moth *Yponomeuta malinellus* (Zeller, 1838) is a major pest in apple orchards, causing severe defoliation and significant yield losses in the Republic of Armenia. While chemical insecticides are effective, their environmental and health risks necessitate the development of safer alternatives. This study evaluates the efficacy of locally produced microbiological insecticides based on *Bacillus thuringiensis* (Bt) strains (Bt JT-21, Bt AS-50, Bt SS-112) isolated from naturally deceased insect larvae during 2021-2024. The results demonstrated high biological effectiveness, with mortality rates reaching 90.2-98.2% in field trials and 100% in controlled settings. Statistical analysis confirmed the reliability of the findings, showing no significant differences between the tested culture liquids and the commercial Bt insecticide standard (BTB). Additionally, soil fertility indicators, including ammonifier populations and extracellular catalase activity, exhibited dynamic seasonal variations but were not negatively affected by Bt treatments. These findings highlight the potential of locally developed Bt-based insecticides as environmentally friendly alternatives for controlling defoliating pests in Armenian apple orchards.

KEYWORDS: *Bacillus thuringiensis,* efficacy, insecticide, *Yponomeuta malinellus*

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INTRODUCTION

The apple ermine moth, *Yponomeuta malinellus* (Zeller, 1838), is a monophagous insect pest with a widespread distribution across Asia, Europe, and North America and is recognised as a significant threat to apple orchards in the Republic of Armenia (Menken, 1992). During mass outbreaks, overwintered larvae emerge from their mines and actively feed on the foliage of host trees, often leading to complete defoliation. Consequently, by mid-summer, affected trees are left devoid of leaves. Although partial foliar regeneration may occur, the trees experience a significant reduction in cold resistance, rendering them highly susceptible to environmental stress and overall physiological decline (Aleksidze & Abashidze, 1983; Sarantseva & Bobreshova, 2006; Turekhanova & Tanabekova, 2018).

The feeding activity of phytophagous larvae disrupts the physiological processes of host trees, inhibiting flower bud development. As a result, affected trees fail to produce fruit in the following season, or, if fruiting occurs, the yield is significantly reduced in both quantity and quality. During severe *Y. malinellus* outbreaks, crop losses can reach 60-70% (Jashenko *et al*., 2023).

Following emergence, larvae begin spinning protective silk webs, binding leaves together in pairs while continuing to feed. The damaged foliage undergoes chlorotic and necrotic changes, turning brown, curling, and often detaching from the tree. As defoliation progresses, larvae consume the leaf tissue down to the primary veins before migrating to adjacent branches, where they construct dense webbed nests. Given the extensive damage associated with *Y. malinellus* infestations, implementing timely and effective control strategies in outbreak-prone areas is critical for mitigating economic losses and preserving orchard health (Sinev, 1994; Roessingh *et al*., 2000).

Traditional reliance on chemical insecticides for its management has raised concerns regarding human health and environmental safety. Consequently, biological control strategies have been explored and implemented globally as sustainable alternatives. A recent study by Tosun *et al*. (2022) evaluated indigenous isolates of Entomopathogenic Fungi (EPFs) against *Y. malinellus* larvae under laboratory

conditions. Among the 14 EPF isolates tested, three *Beauveria bassiana* isolates (BbDm-1, BbDs-2, and BbKm-3) exhibited high pathogenicity, causing 96.7% to 100% larval mortality at the highest concentration nine days post-treatment. These findings suggest that certain *B. bassiana* isolates hold significant potential as biocontrol agents against *Y. malinellus.*

In the Pacific Northwest of the United States, classical biological control efforts have been undertaken to manage *Y. malinellus* populations. Between 1988 and 1991, four parasitoid species were introduced from Eurasia: *Ageniaspis fuscicollis, Herpestomus brunnicornis, Diadegma armillata,* and *Eurystheae scutellaris*. Among these, *A. fuscicollis* established successfully, leading to a four- to five-fold increase in parasitism rates and a consistent decline in moth populations from 1989 to 1995. This parasitoid also parasitised the cherry ermine moth (*Yponomeuta padellus*), indicating its broader ecological impact (Hopper *et al*., 2003).

Extensive studies have demonstrated that *Bacillus thuringiensis* (Bt) is a widely utilised biological control agent too, which synthesises crystalline inclusions and is renowned for its specificity in targeting certain insect pests while exhibiting minimal adverse effects on humans, warm-blooded animals, beneficial entomofauna, and fish (Batashova, 1986; Korol, 1993; Ivantsova, 2004; Zhou *et al*., 2014; Cossentine & Kuhlmann, 2021).

In Armenia, biologically highly effective and environmentally safe strains have been adopted as alternatives to chemical insecticides. While these biological agents demonstrate high efficacy against phytophagous pests, the application of chemical insecticides continues to pose potential risks to human health and environmental safety (Terlemezyan & Harutyunyan, 2013; Terlemezyan *et al*., 2023a, b; 2025a, b).

In plant protection research, the economic efficiency of applied formulations is often prioritised, while their effects on soil fertility—particularly the abundance of soil-dwelling microbes and extracellular enzyme activity—are frequently overlooked. This gap in assessment may lead to the misinterpretation of long-term soil health and biological activity trends. Given these considerations, this study aims to isolate biologically highly effective and environmentally safe *B. thuringiensis* strains against apple ermine moth larvae using microbiological methods from specific elements of the biocenosis in the Republic of Armenia. Following the characterisation of culture liquids derived from these strains, the most promising candidates will be selected for the development of commercially viable biopesticides.

MATERIALS AND METHODS

The study utilised bacterial insecticides that were isolated from naturally deceased larvae of the cabbage white butterfly *Pieris brassicae* L., the black-veined white butterfly *Aporia crataegi* L., and the gypsy moth *Ocneria dispar* L. within the biocenosis. Specifically, the crystal-forming *B. thuringiensis* (Bt) strains Bt JT-21, Bt AS-50, and Bt SS-112 were identified and designated by the Scientific Centre. Additionally, the commercially available *B. thuringiensis*-based insecticide Bitoxibacillin (BTB) was included in the study as standard check. This preparation, formulated as a powder with 1500 IU/mg of active ingredient, is officially approved for use against harmful insects under the regulatory framework of the Republic of Armenia (Permitted Plant Protection Products for, 2025).

The study also incorporated *Malus domestica* (cultivar 'Renet Simirenko') apple trees, brown soils from apple orchards, soil-dwelling ammonifying microorganisms, and extracellular catalase enzyme activity as key experimental components.

Experiments were conducted under laboratory conditions and in apple orchards within farmer enterprises in the Karbi community, Aragatsotn Province, Republic of Armenia, from 2021 to 2024.

The isolation of *B. thuringiensis* (Bt) bacterial strains from naturally deceased larvae was performed in 2021 under laboratory conditions, following established scientific guidelines and microbiological practical manuals (Netrusov *et al*., 2005; Brar *et al*., 2006; Mamajanyan, 2018). The production of culture liquids from the isolated bacterial insecticides was carried out using a Biof Fermenter, Flexem Labfrez brand (with a yeast fermenter). The titer of the culture liquids was determined at the Armenian Biotechnology Scientific-Production Centre. The inoculation of *B. thuringiensis* spores, proteinaceous crystalline inclusions, and vegetative cells was conducted following the specified guidelines (Ivanov & Gukasian, 1966). The biological effectiveness of the insecticides was assessed in apple orchards through both plot-based (small-scale) and production (large-scale) trials, following methodological guidelines (Arisov & Arkhipov, 2018). Biological effectiveness calculations were performed using Abbott's formula (Avagyan, 2012).

In plot-based trials, insecticide application was carried out using a back-mounted Ozdesan sprayer, while large-scale production trials employed OVT-1A model tractor-mounted sprayers.

In the plot-based trials, insecticide application was conducted on model trees with heights ranging from 1.9

to 2.7 m, with the working liquid applied at a rate of 1.2 to 1.8 l/tree, depending on tree height. In the production trials, the application rate was standardised at 1000 l/ hectare. Each bacterial culture liquid was tested in a 0.3-hectare experimental plot within the apple orchard. The working culture liquids contained *B. thuringiensis* spores at a concentration of 600 million viable spores/ml, with an application rate of 3 L of culture liquid concentrate per 1000 L of water. The optimal concentration for field efficacy was determined through plot-based trials by testing culture liquids at varying spore concentrations (400, 500, 600, 700, 800, and 900 million spores/ml). Spraying was conducted at different developmental stages of the *Y. malinellus* larvae. The first application occurred one week before apple tree flowering, coinciding with the emergence of overwintered larvae from shield-shaped scales on 1- to 2-year-old branches, as they began feeding on young leaf tissue. The second application was carried out in early May, targeting larvae emerging from the damaged leaves.

All treatments considered the economic damage threshold for apple ermine moth infestations, which is defined as one nest per tree (Soroka, 2018). In the plot-based trials, the number of living and dead larvae was assessed on three infested trees per treatment variant, whereas in the production trials, evaluations were conducted on five infested trees, with each infested tree serving as a single replicate. The control group consisted of apple trees infested with *Y. malinellus* larvae but left untreated. A 0.3% aqueous suspension of Bitoxibacillin (BTB) was used as the standard treatment, applied at a rate of 3 kg/ha.

The total population of soil-dwelling ammonifiers, including bacteria, actinomycetes, and microscopic fungi, in apple orchards treated with bacterial insecticides and in the untreated control plots was quantified using Meat Peptone Agar (MPA) as an artificial medium, following the serial dilution method (Netrusov *et al*., 2005; Tóth *et al*., 2013). Catalase enzyme activity in the soils of the experimental plots was measured according to established methodological guidelines (Chabot *et al*., 2020). The activity was expressed as the volume of oxygen released (cm^3 O_2) per gram of soil per minute.

The experimental results were analysed statistically following established methodologies (Avagyan, 2012). According to the authors' criteria, research findings were considered reliable if the calculated error (P) was below 6% and the variation factor (V) did not exceed 15%. Additionally, the Student's t-test was applied to compare the mean values of biological effectiveness, the abundance of ammonifying microorganisms in the brown soils of apple orchards treated with insecticides and in the untreated control plots, as well as extracellular catalase activity. These statistical evaluations were conducted separately for each experimental and control variant.

RESULTS AND DISCUSSION

The results of the 2021 experiments confirmed that the *B. thuringiensis* (Bt) bacterial insecticides isolated from various elements of the biocenosis—Bt JT-21, Bt AS-50, and Bt SS-112—formed colonies of varying sizes, as well as distinct vegetative cells, endospores, and endotoxin crystalline inclusions when cultured on MPA artificial medium. These strains also exhibited differences in sugar assimilation characteristics. The plot-based trials conducted in 2022 further demonstrated that both first-instar (I instar) larvae emerging from the shield and second-instar (II instar) larvae emerging from the mine of the apple ermine moth were effectively controlled by culture liquids with a titer of 600 million spores/ml or higher, confirming their high biological efficacy. Subsequent research focused on evaluating the effectiveness of culture liquids with this titer, given their promising results. Notably, in plot-based trials, the culture liquids of Bt JT-21, Bt AS-50, and Bt SS-112 at a titer of 600 million spores/ml exhibited high biological effectiveness against *Y. malinellus* larvae emerging from both the shield and the mine, achieving overall effectiveness rates of 100% and 97.5%-98.2%, respectively. The strong biological efficacy observed in the plot-based trials justified further testing of the culture liquids at a titer of 600 million spores/ml under production-scale conditions, as outlined in Table 1.

The data presented in Table 1 indicate that the culture liquids of Bt JT-21, Bt AS-50, and Bt SS-112, each with a titer of 600 million spores/ml, demonstrated high efficacy against apple ermine moth larvae under production-scale conditions. Specifically, in the 2023-2024 trials, the biological effectiveness of the tested culture liquids remained consistently high, with effectiveness rates ranging from 90.2% to 94.9% seven days post-application.

The biological effectiveness of the control sample (BTB) treatment was 95.2% and 94.5% for the respective years under study. In the two-year production trials, the biological effectiveness values for both the experimental and control treatments were significantly lower at 3- and 5-days post-application compared to day 7 (Table 1). This trend is likely attributed to the specific mode of action of *B. thuringiensis* bacterial insecticides, which require time to exert their full effect on target larvae. In the orchard plots treated with bacterial insecticides, a marked reduction in the movement of ermine larvae was observed compared to the control (unsprayed) plots. Larvae exhibited a progressive

Table 1. Bio-efficacy of *B. thuringiensis* (Bt) against apple ermine moth larvae 2023-2024

Treatment	Culture liquid (million spores/ml) and the concentration of the commercial preparation (%)	Average number of larvae per treatment (individuals)	Bio-efficacy by days of assessment (based on larvae mortality) (%)		
			3	5	7
2023					
Bt $_{JT}$-21	600	52	76.9	84.6	94.2
Bt $_{AS}$-50	600	46	67.4	73.9	91.3
Bt $_{SS}$-112	600	59	71.1	81.4	94.9
BTB (control sample)	0.3	63	80.9	87.3	95.2
2024					
Bt $_{JT}$-21	600	44	72.7	86.4	93.2
Bt $_{AS}$-50	600	61	60.6	68.8	90.2
Bt $_{SS}$-112	600	51	64.7	78.4	92.1
BTB (control sample)	0.3	55	76.4	80.0	94.5

Note: No larval mortality was observed in the control (unsprayed) treatment. The number of larvae recorded in the control groups in 2023 and 2024 was 49 and 54 individuals, respectively, and these values were incorporated into the calculations using Abbott's formula.

decline in feeding activity, followed by a lack of response to tactile and mechanical stimuli, ultimately leading to mortality. The bodies of deceased larvae became grayish in colouration and showed noticeable shrinkage compared to healthy individuals. Statistical analysis of the biological effectiveness indices recorded in the 2023 production trials confirmed the accuracy of the results. The experimental error values for the insecticide-treated variants ranged between 2.4% and 4.5%, while the variation factor fluctuated between 5.30% and 10.10%. For parameters p = 0.95 and n = 5, the calculated Student's t-test values ranged from 0.093 to 1.094. As these values were lower than the critical threshold of 2.571, it was further confirmed that there was no statistically significant difference in the biological effectiveness between the tested culture liquids and the control (standard) BTB treatment. According to the literature, only a small fraction of sprayed insecticidal formulations directly affects phytophagous insects, while the majority—approximately 60% to 99%—eventually reaches the soil through various pathways, including drift during application, rainfall, leaf drop, and the decomposition of infected larvae (Ivantsova, 2004; Terlemezyan *et al*., 2023a). Furthermore, *B. thuringiensis* bacterial insecticides that enter the soil as a result of spraying tend to persist for 3 to 5 months or longer, gradually decreasing in concentration over time.

The ecological studies conducted in 2023 examined the impact of *B. thuringiensis* (Bt) bacterial insecticides—Bt JT-21, Bt AS-50, and Bt SS-112—on soil fertility indicators, specifically the abundance of soil-dwelling ammonifying microorganisms and the activity of extracellular catalase. These insecticides entered the soil as a result of foliar spraying. Data presented in the Figure 1 demonstrate that the population of ammonifying bacteria in the brown

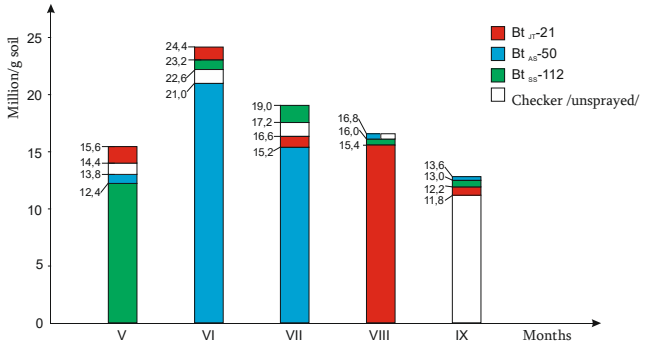


Figure 1. The number of ammonificators in the Bt bacterial insecticide-treated and untreated (control) brown soils of an apple orchard (2023).

soils of apple orchards treated with locally produced Bt culture liquids exhibited dynamic fluctuations throughout the vegetation period (May to September). The highest bacterial counts were recorded in June (21.0-24.4 million/g of soil), while the lowest was observed in September (11.8-13.6 million/g of soil). According to existing literature (Ivantsova, 2004), seasonal variations in the abundance of soil-dwelling ammonifying microorganisms are influenced by factors such as soil moisture, temperature, the availability of mobile nitrogen and phosphorus forms, and aeration. These factors were likely also present in the conditions of this study. The composition of ammonifying microorganisms in both the Bt-treated and untreated (control) soils included diverse physiological groups, including spore-forming and non-spore-forming bacteria, actinomycetes, and microscopic fungi.

According to the data presented in Table 2, the error and variation factor values for the studied variants between May and September ranged from 2.9% to 5.7% and 6.48%

Table 2. Statistical indicators of the number of ammonifying microorganisms in the brown soils of the apple orchard treated with Bt insecticides and untreated (control) plots (2023)

Variants	The sampling period	Total number of bacteria (pieces)	Statistical indicators				
			Standard deviation	Coefficient of variation (%)	Mean error	Experimental error (%)	Calculated value of the Student's t-statistic
		$\bar{x}$	σ	V	$\sigma_{\bar{x}}$	P	
Bt_{JT} - 21	May	15.6	1.486	9.53	0.665	4.3	1.230
	June	24.4	1.635	6.70	0.731	3.0	1.327
	July	16.6	1.428	8.60	0.639	3.8	0.524
	August	15.4	1.855	12.04	0.830	5.4	1.004
	September	12.2	0.800	6.56	0.258	2.9	0.617
Bt_{AS} - 50	May	13.8	0.894	6.48	0.400	2.9	0.775
	June	21.0	2.028	9.66	0.907	4.3	1.079
	July	15.2	1.951	12.83	0.873	5.7	1.511
	August	16.8	1.612	9.60	0.721	4.3	0
	September	13.6	1.265	9.30	0.566	4.2	2.216
Bt_{SS} - 112	May	12.4	1.265	10.20	0.566	4.6	2.236
	June	23.2	1.985	8.56	0.888	3.8	0.409
	July	19.0	2.176	11.45	0.973	5.1	1.278
	August	16.0	1.173	7.33	0.525	3.3	0.669
	September	13.0	1.200	9.23	0.537	4.1	1.524
Control (untreated)	May	14.4	1.265	8.78	0.566	3.9	-
	June	22.6	2.165	9.58	0.968	4.3	-
	July	17.2	1.789	10.40	0.800	4.7	-
	August	16.8	2.084	12.40	0.932	5.5	-
	September	11.8	1.020	8.64	0.456	3.9	-

Note: When performing the calculations of "-", the control data were included in the calculation formula of the Student's t-test.

to 12.83%, respectively, confirming the accuracy of the experimental results. The calculated Student's t-test values (Table 2) ranged from 0 to 2.236. Given the parameters p = 0.95 and n = 5, these values were lower than the critical threshold of 2.571 from the Student's t-distribution table, indicating no statistically significant difference in the abundance of ammonifying microorganisms between Bt bacterial insecticide-treated and untreated (control) brown soils. Similar trends were also observed in the extracellular catalase activity of the soil.

Catalase is not synthesised within the soil but is introduced by living organisms, including soil-dwelling bacteria, plant root systems, and invertebrates, both during their lifetime and post-mortem (Terlemezyan *et al*., 2025a). This enzyme plays a crucial role in decomposing hydrogen peroxide (H_2O_2), a potentially toxic compound for microorganisms and plants, into water and oxygen. Hydrogen peroxide accumulates in the soil due to the oxidation of organic materials such as proteins and lipids (Ivantsova, 2004). Experimental data (Figure 2) confirm that catalase activity in soils treated with bacterial insecticides (Bt JT-21, Bt AS-50, Bt SS-112)

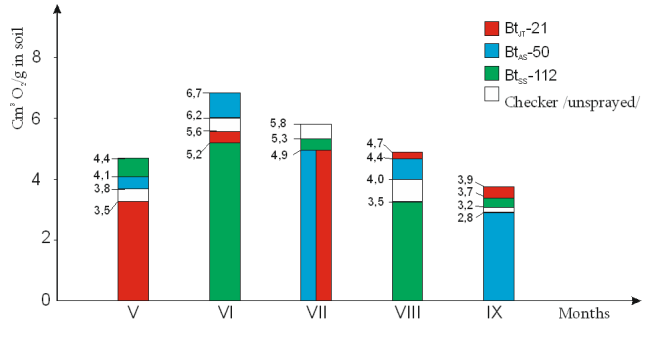


Figure 2. Catalase activity in the Bt bacterial insecticide-treated and untreated (control) brown soils of the apple orchard (2023).

and in untreated control soils exhibited dynamic variations throughout the vegetation period. The highest enzymatic activity was recorded in June, with average values ranging from 5.2 to 6.7 cm³ O_2/g of soil, while the lowest activity was observed in September, with values between 2.8 and 3.9 cm³ O_2/g of soil.

According to literature sources (Ivantsova, 2024; Terlemezyan *et al*., 2025b), fluctuations in extracellular

catalase activity are influenced by several factors, including soil organic matter content, biogenic composition, temperature, and moisture levels. These factors likely contributed to the observed seasonal variations in catalase activity in both Bt-treated and untreated brown soils of the apple orchard.

CONCLUSION

The study demonstrated that the native *B. thuringiensis* isolates Bt JT-21, Bt AS-50, and Bt SS-112 exhibited high bioefficacy against *Y. malinellus* larvae under orchard conditions, achieving 90.2-94.9% control comparable to the commercial standard (BTB). Statistical analyses confirmed the reliability of these results, showing no significant difference in mortality rates among tested treatments. Evaluation of soil biological parameters revealed that Bt-based treatments did not adversely affect soil fertility: populations of ammonifying microorganisms and extracellular catalase activity which showed only natural seasonal variations without statistical significant differences from untreated controls. These findings demonstrated that application of Bt bacterial insecticides does not negatively affect soil fertility parameters, supporting their potential as environmentally sustainable biocontrol agents for managing defoliating insect pests in apple orchards with brown soil types.

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