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Analyzing the Counteractive Impact of *Bacillus thuringiensis* on Oriental Fruit Moth, *Grapholita molesta* in Peach Orchards of Armavir, Armenia

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ABSTRACT

Background: The present investigation, conducted during 2020-23 under both laboratory and field conditions, was aimed at isolation and evaluating *Bacillus thuringiensis* (*Bt*), specifically Bt $^{MB-23}$ and Bt $^{Tv-97}$, against larvae of the oriental fruit moth, *Grapholita molesta*. The oriental fruit moth is a major pest in peach cultivation in the Republic of Armenia, which causes substantial damage to fruit and leaves.

Methods: The *Bt* strains isolated from naturally deceased larvae were evaluated at a concentration of 600 million spores/ml, both individually and in combination with commercial insecticide at sublethal concentrations of 3x and 10x dilutions. Damage assessment in terms of the number of affected leaves and fruits per tree was recorded and analyzed.

Result: All tested treatments with *Bt* strains showed high biological efficiency, recording 86.8-97.0% damage reduction as compared to the control. The combination treatments of *Bt* and chemical insecticide demonstrated the effectiveness of the treatments in pest suppression. Statistical analysis validated the experimental outcomes, showing that overall fruit yield and damage metrics were comparable between treatments and controls, while biocontrol treatments achieved these results with reduced chemical use, confirming the reliability of the findings.

Key words: *Bacillus thuringiensis*, Biological control, *Grapholita molesta*, Peach orchards.

INTRODUCTION

The Oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae), is a significant quarantine pest affecting a variety of fruit crops including peach, apple and pear (Bellerose *et al.*, 2007; Şahin, 2021. This moth has now become widespread across the globe, particularly in countries with relatively temperate climates (Clayton *et al.,* 2007; Amat *et al.,* 2021), causing extensive agricultural damage due to its larval feeding habits. The larvae bore into shoots and fruits, leading to direct damage and facilitating secondary infections by pathogens (Hodges *et al.*, 2015). In the Republic of Armenia, it also causes damage to Greek walnut fruits (Terlemezyan and Tevosyan, 2009). The damage caused by this insect can reach up to 100% in peach and quince fruit and up to 85-90% for apples (Terlemezyan and Tevosyan, 2009). Even at a lower pest density of 380 (mean total catches (± SE), crop loss can still reach up to 50% (Tiring *et al.*, 2023). The damage caused by this insect results in slow tree growth, significantly reduces fruiting capacity and leads to low-quality fruit yields (Rothschild and Vickers, 1991; Najar-Rodriguez *et al.*, 2013; Tiring *et al.*, 2023). Hence, effective management of *G. molesta* is crucial for maintaining the health and productivity of orchards as a whole (CABI, 2020).

In integrated pest management systems, emphasis has been given on use of biopesticides including *Bacillus thuringiensis* (*Bt*), which are safe for humans and the environment (Brar *et al.*, 2006; Bravo *et al.*, 2011; Castañeda and Sánchez, 2018; Berretta *et al.*, 2020; Kumar *et al.*, 2021; Terlemezyan *et al.*, 2022 and Vamshi *et al.* 2024). Research into the efficacy of *Bt* and other biological agents continues to be a critical area of focus in sustainable agricultural practices.

In the Republic of Armenia, efforts are underway to establish the production of an ecologically safe and highly

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effective commercial bacterial preparation. This process begins with isolating local *Bacillus thuringiensis* (*Bt*) strains from larvae that have naturally died in the ecosystem and developing culture fluids from these strains. The next step is to determine their lethal effective concentration (titer) at low cost. Building on this foundation, combining sublethal concentrations of these bacterial strains with chemical insecticides makes it possible to create economically viable solutions with high biological efficiency, which can then be introduced to horticultural farms.

MATERIALS AND METHODS

The experimental site

The experiment on evaluating bioefficacy of *Bt* strains against *Grapholita molesta* was conducted during 2020-23 under laboratory as well as peach orchards located in the Nalbandyan community of Armavir Province (40.063635, 43.998720), Republic of Armenia (Fig 1).

Isolation and laboratory culture of Bt

To collect the oriental fruit moths, we used the “Izomate-M” synthetic pheromone traps introduced in the middle-aged peach *Prunus persica* (L.) Batsch 1801 orchards. The bacterial insecticidal strains BtMb-23 and pigment-synthesizing BtTv-97 (strain name by the scientific center) were used to produce experimental liquid culture versions, developed by the Scientific and Production Center “Armbiotechnology. The strains were acquired from the larvae of cabbage Moth (*Mamestra brassicae* L.) and green oak tortrix (*Tortrix viridana* L.) that died naturally on meat peptone agar (MPA) artificial nutrient medium.

The commercial *Bt* bacterial preparation, Lepidocide (powder, with a biological activity of 3000 activity units/mg), served as the standard version.

The preparation of bioformulation

The chemical preparation Ampligo (15% microencapsulated suspension, Active ingredients are Chlorantraniliprole and Lambda-cyhalothrin) was used in the form of water-diluted (x8-x12) diluted at sublethal concentrations for experimental variants. The study also examined damaged and healthy peach shoots, fruits and pulp.

Fermentation of the strains Bt^{-23} and Bt^{-23} was performed in a laboratory Bioreactor ”Labfreez BIOF-10L” (China) with a 7.0 L working capacity. Fermentation was carried out at an oxygen dissolution rate of 30°C, pH 7.0-7.2, for 48 hours. Òhe following medium composition was used: yeast autolysate 50 L per 1000 ml, $CaCl_2$ - 0.008%, $MgSO_4$-0.02%.

To identify the effective density of the bacterium against oriental fruit moth larvae, different concentrations (titers) of the liquid culture (ranging from 200 to 800 million viable spores/ml with an increase by 50 million spores/ml in a sequential manner) were one-time sprayed tested in 2021 through partial sprays. The titer of the culture liquid at 200 million spores/ml was increased stepwise by 50 million spores/ml, resulting in a total of 13 culture liquid variants with different concentrations being included in the experiments. The titers of the culture liquids were determined at the “Armbiotechnology” Scientific-Production Center.

In 2022, field experiments were conducted on fruiting peach trees to evaluate the effectiveness of various treatments against larvae of the oriental fruit moth. The orchard covered 1.8 ha and was divided into plots of 0.2 ha each, with experimental plots selected randomly. The treatments consisted of culture fluids with an initial titer of 600 million viable spores/ml, diluted 2-6 times to obtain sublethal concentrations of 100, 120, 150, 200 and 300 million spores/ml. These diluted suspensions were combined with a lethal concentration of the chemical insecticide Ampligo (0.3 L/ha). In addition, sublethal suspensions of Ampligo (0.0027%, 0.0030%, 0.0033% and 0.0037%), corresponding to 8-11-fold dilutions with water, were tested separately.

In 2023, supplementary trials were performed to assess the effectiveness of culture fluids and low-cost insecticide concentrations in combination with sublethal



(a) (b)

Fig 1: The experimental site. a. map, b. view of the orchard.

doses of bacterial and chemical preparations. These experiments were carried out under production conditions using large-scale spraying against phytophagous larvae.

In the partial experiments, Ozdesan conducted insecticide spraying against oriental fruit caterpillars before they entered the shoots or fruit. This was done using OVT - 1A tractor sprayers.

In the partial experiments, when spraying the model trees, the working liquid consumption was 2.0-2.4 liters per tree, depending on their height (2.5-3.0 meters). In production experiments, the consumption was 1000 liters per hectare. Optimal density culture fluids and insecticides combined with sublethal bacterial and chemical concentrations (experimental versions), as well as a standard lepidocide version, were sprayed on 0.2 hectares in the experimental peach plots with three replicates. The unsprayed control plot covered 0.05 hectares of the peach orchard inhabited by the pest.

The biological efficiency of the insecticides used in the experiments was determined according to Abbott's formula, which is expressed as:

$$E\ (\%) = \frac{M_t - M_c}{M_t} \times 100$$

Where,

E (%) = Corrected efficacy (efficiency).

M_t = Mortality, infestation, or damage (%) in the control (untreated).

M_c = Mortality, infestation, or damage (%) in the treatment.

Also, according to the relevant methodological instructions by Arisov and Arkhipov, (2018).

Each version of plot experiments included 5 repetitions, while in the production experiments 10 repetitions. Replicates were selected diagonally across the plot, with each pest-infested model peach tree considered as one replicate.

Pheromone traps (4 traps per hectare) were placed in the peach foliage at a height of 1.5 meters above the soil surface during the flowering period to determine the optimal timing for controlling oriental fruit moth larvae. Daily counts of the moths caught in the traps were recorded. The planned spraying against the larvae was carried out in the test plots 2-3 days after the peak moth count began to decline, following mass spawning.

Pheromone pods were replaced every four weeks and glue pads were changed as they became soiled. Damage caused by the oriental fruit moth was assessed by examining the damaged petals and fruits of the peach trees. In batch experiments, 500 fruits and 250 twigs were examined, while 1000 fruits and 500 twigs were analyzed in production experiments.

Studies were conducted on 10% of the trees' twigs, fruits and the collected fruits. Assessments were made every 10-14 days and just before harvest. The experiment results were subjected to statistical analysis according to Bernstein, (1968) and Ilstrup, (1990).

RESULTS AND DISCUSSION

Data from pheromone traps installed in a uniform peach orchard confirmed that the flight of wintering generation oriental fruit moths began on April 24, May 5 and April 29 in 2021, 2022 and 2023, respectively, under average air temperatures of 12.9-14.8°C. The flights of the first, second, third and fourth-generation moths occurred from early June to the first ten days of October.

Research confirmed that female moths of the wintering generation laid their eggs on peach blossoms in the spring, while moths of the summer generation laid eggs on both the fruits and blossoms. The larvae hatched 3-5 days after the eggs were laid and burrowed into the leaves and fruits. After 19-23 days of feeding, the larvae emerged from the hollowed-out buds or damaged fruit to mate. It was found that while feeding on the peach tree pulp, the larvae created tunnels 13-16 cm long, causing the leaves on the damaged parts of the shoot to initially wither and eventually dry up.

Observations from 2021-2023 confirmed that each larva damages 2-3 larvae during its development. The larvae primarily entered the peach tree stem through the growth cone, accounting for 95.1-96.7% of the damaged stems. Entry through the smooth surface of the lips and leaf folds accounted for 2.2-3.1% and 1.1-1.8%, respectively.

For fruit infestation, larvae of the oriental fruit borer mainly (67.4-69.6%) entered through the fruit funnel and made their way to the seed. Entry through other parts of the fruit accounted for 30.4-32.6%.

Observations also confirmed that one peach fruit typically hosts one larva, although in some cases, 2-3 larvae may be present.

In 2021, culture fluids with titers ranging from 200 to 800 million spores/ml (in increments of 50 million) were tested against oriental fruit moth larvae before they entered the peach pit or fruit (partial experiments). The results of these scientific experiments and statistical analyses confirmed that the biological efficiency of the culture fluids Bt^{Mb-23}, Bt^{Tv-97}, with a titer of 600 million spores/ml, was significantly higher than that of culture fluids with titers of 550 million spores/ml or lower. This was determined because, with a confidence level of 0.95 and n = 5, the calculated Student's t-values (2.682-2.921) exceeded the critical value (2.571), indicating a significant difference.

However, there was no significant difference in biological efficiency between culture fluids with titers of 600 million spores/ml and higher, as the calculated Student's t-values (1.053-2.140) were less than the critical value of 2.571 at the same confidence level and sample size.

Therefore, the recorded data confirmed that individual culture fluids Bt^{Mb-23}, Bt^{Tv-97}, with a density of 600 million spores/ml, exhibited high biological efficacy against the phytophage.

The biological efficacy of individual insecticides Bt^{Mb-23}, Bt^{Tv-97} (experimental) and standard lepidocide with a titer of 600 million spores/ml is presented in Table 1 (partial experiments). The data indicate that both the experimental

and standard insecticides significantly reduced fruit damage at harvest. The reduction in fruit damage compared to the control was notably high across these versions, ranging from 95.0% to 97.0%.

Similar results were observed for leaf damage. Compared to 24.6% in the unsprayed (control) version, the damage to leaves in versions sprayed with Bt^{Mb-23}, Bt^{Tv-97} and standard lepidocide insecticides was significantly lower, at 2.4%, 1.8% and 1.6% respectively. This confirms a substantial reduction in lip damage compared to the control, ranging from 90.2% to 93.5%.

Laboratory studies conducted in 2021 confirmed that sublethal concentrations of the chemical preparation Ampligo (specifically, a lethal 0.03% suspension diluted 9-12 times with water) are compatible with bacterial insecticides. Sublethal concentrations of Ampligo do not exhibit bactericidal or bacteriostatic effects, allowing for the testing of combined sublethal concentrations of bacterial and chemical insecticides against oriental fruit borer larvae in peach orchard conditions.

In 2022, confirmation was obtained through particle experiments and Student's t-test that an effective low dose of Ampligo is a sublethal concentration of 0.003% (resulting from diluting the lethal concentration 10 times with water). This concentration was combined with a 3 times lethal dilution of bacterial culture fluid (with a titer of 200 million spores/ml).

It's worth noting that sublethal concentrations of both Bt culture fluids and Ampligo generally showed low efficacy (4-7%) when tested against individual phytophages. However, combinations of bacterial culture fluid (with a titer of 200 million spores/ml) + Ampligo (0.003%) at sublethal concentrations demonstrated high biological efficiency compared to the unsprayed control, effectively reducing the number of damaged fruits and leaves.

Partial scientific experiments (Fig 2) confirmed that combinations with sublethal concentrations of Bt^{Mb-23} + Ampligo and Bt^{Tv-97} + Ampligo reduced fruit and leaf damage by 94.2% and 89.6% and 95.9% and 91.9%, respectively. Similar results were observed with standard lepidocide, with reductions in fruit and leaf damage at 96.4% and 93.0%, respectively.

The significant biological efficiency indicators recorded in batch experiments facilitated the testing of insecticides included in these experiments (both individually and in

Table 1: Biological effectiveness of tested insecticides against the eastern codling moth (plot experiments).

Variations	Concentrations of culture fluid (million spores/ml) and chemical preparation (%)	Fruit damage in the harvested crop (%)	Reduction of fruit damage compared to the control version (%)	Reducing damage to shoots (%)	Reduction of shoot damage compared to control (%)
2021 year					
B^{tMb-23}	600	2.8	95.0	2.4	90.2
Bt^{Tv-97}	600	1.7	97.0	1.8	92.7
Lepidocide (standard)	600	1.8	96.8	1.6	93.5
Control (Without spraying)	-	55.2	-	24.6	-
2022 year					
Bt^{Mb-23} + Ampligo	200+0.003	3.6	94.2	2.8	89.6
Bt_{Tv-97} + Ampligo	200+0.003	2.5	95.9	2.2	91.9
Lepidocide (standard)	600	2.2	96.4	1.9	93.0
Control (Without spraying)	-	61.5	-	27.0	-

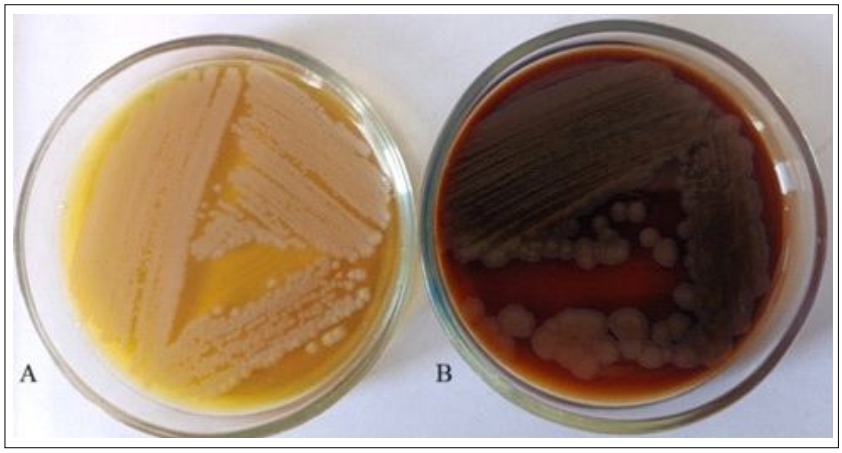


Fig 2: Morphology of colonies of Bt^{Mb-23} (A) strains and pigment-forming mutant Bt^{Tv-97} pig (B).

Table 2: Biological and economic effectiveness of insecticides against eastern codling moth (production tests, 2023).

Variations	Concentra-tions of culture fluid and chemical preparation, %	Fruit damage in the		Reduction of fruit damage compared to the control,%	Reducing damage to shoots %	Reduction of shoot damage compared to control, %	The harvest (quintal/hectare)
		Fallen fruits,%	Harves-ted crop,%				
Bt^{MB-23}	600 (million spores/ml)	5.3	4.2	93.4	4.0	88.0	171
$Bt^{Tv\ 97}$	600 (million spores/ml)	4.6	3.1	95.1	3.6	89.2	180
Bt^{MB-23} + Ampligo	200+0.003 (million spores/ml)	5.2	5.2	91.8	4.4	86.8	163
$Bt^{Tv\ -97}$ + Ampligo	200+0.003 (million spores/ml)	4.9	4.4	93.1	3.6	89.2	171
Lepidocide (standard)	600 (million spores/ml)	5.7	4.9	92.3	2.5	92.5	169
Control (without spraying)	-	71.4	63.8	-	33.3	-	120

combination at sublethal concentrations) against oriental fruit moth larvae under production conditions.

Analysis of the data presented in Table 2 reveals that the patterns of biological performance observed in the batch experiments were consistent with those observed in the production experiments.

Consequently, based on the results of scientific experiments, it was confirmed that separately spraying Bt $^{Mb-23}$ and Bt^{Tv-97} culture fluids with a titer of 600 million spores/ml resulted in reductions of peach fruit and leaf damage by 93.4% and 88.0%, respectively, compared to the unsprayed control under production conditions; with reductions of 95.1% and 89.2%, respectively.

High levels of effectiveness were also observed when spraying with insecticides combined with sublethal concentrations.

According to the data in Table 2, when Bt^{Mb-23} + Ampligo and Bt^{Tv-97}were sprayed, the reduction in damage compared to the unsprayed control was 86.8% and 89.2%, respectively. For twigs, the reduction in damage was 92.3% and 92.5%, respectively, when standard lepidocide was used.

Furthermore, it is evident from the data in Table 2 that the damage to peach fruits exceeded that of the harvested yield in the field.

The results of the production test confirm that in the variants sprayed with Bt insecticides, the yield per hectare ranged between 163-180 cents, while it was 120 cents per hectare in the control group.

For parameters P0.95 and n=10, the calculated indicators of Student's t-test generally ranged from 1.236 to 2.018, which were smaller than the tabulated Student's t-criterion indicator of 2.230. This confirmed that, both in the experimental and standard versions, there were no significant differences between the quantitative indices of damaged fruits and leaves.

The experimental error (P) and coefficient of variation (V) indicators ranged from 2.5% to 5.3% and from 8.27% to 11.36%, respectively. These results confirm the accuracy of the scientific experiments.

CONCLUSION

The present study demonstrated that Bacillus thuringiensis (*Bt*) strains Bt^{Mb-23} and Bt^{Tv-97}, isolated from naturally deceased larvae in the local biocenosis, exhibit high biological efficacy against the oriental fruit moth (*Grapholita molesta*) under both laboratory and field conditions. Culture fluids with a titer of 600 million spores/ml significantly reduced fruit and shoot damage, achieving control efficiencies ranging from 86.8% to 97.0% compared with the untreated control.

Furthermore, combinations of sublethal concentrations of *Bt* culture fluids with the chemical insecticide Ampligo enhanced control efficacy while reducing chemical input, thus demonstrating the potential of integrated biocontrol strategies. These treatments not only minimized crop damage but also increased fruit yield by 43-0 quintals per hectare compared with the control plots.

Statistical analyses confirmed the robustness of the experimental results, showing no significant differences in biological effectiveness between the experimental *Bt* strains and the commercial standard (Lepidocide). Importantly, no negative effects on soil microbial communities or extracellular catalase activity were observed, indicating that the use of these *Bt*-based bioinsecticides does not compromise soil fertility.

Overall, the results highlight that local *Bt* strains can serve as an ecologically safe and economically viable alternative to synthetic insecticides in peach orchards of Armenia. Their integration into pest management programs offers a sustainable solution for controlling *G. molesta* while safeguarding environmental and human health.

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Informed consent

All animal procedures for experiments were approved by the Committee of Animal Care and Techniques were approved by the Scientific Committee of the Scientific Center of Zoology and Hydroecology.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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