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STRATEGY FOR ENSURING BIOSECURITY OF NATURAL AND ECONOMIC SYSTEMS AS AN EXAMPLE OF THE CULTURE OF RATIONAL NATURE MANAGEMENT

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ABSTRACT

The article presents an example of the implementation of the bioprotection strategy for cultivated phytocenoses. Vegetable crop losses due to pests are currently increasing. The mass infestation of the diamondback moth in vegetable agrophytocenoses in the Caucasus region poses a serious agricultural problem. Therefore, control measures against this phytophage become critically necessary. Our work reveals the importance of bacterial strains of the genus *Bacillus* from local ecosystems as microbiological pest control in vegetable agrophytocenoses. Within the integrated pest management system, unlike chemical insecticides, significant attention is now given to the application of bacterial insecticides of the Bt type, which are safe for humans and the environment. The isolation of bacteria of the Bt-type from dead larvae on nutrient media was performed under laboratory conditions, according to the methodological manual [21,35]. The detection of insecticidal crystalline inclusions in the bacterial smear was carried out according to the recommendation [15]. The sizing of vegetative cells, formed colonies, endospores, and crystalline particles of the isolated strains of the Bt was performed using an ocular micrometer. The biological efficacy of the insecticides was calculated using the Abbott's formula. The results of the experiments were subjected to statistical analysis [2,6]. The three strains of the Bt (BtHK-22, BtMS-49, BtAS-87) isolated from the biocoenosis differed from each other in the morphometric indicators of vegetative cells, formed colonies, endospores, and crystalline particles, as well as in the characteristics of carbon source utilization. The culture fluids produced individually based on the aforementioned strains, with the titer of 300 million spores/ml, demonstrated high biological efficacy (92.6–97.8% on average) against low-instar (I–II) larvae of the diamondback moth under laboratory conditions and 7 days after spraying in cabbage plantations. Statistical data confirmed that the results of the experiments are accurate and there is no significant difference between the biological efficacy indicators demonstrated by the local Bt-type culture fluids and the standard reference commercial *Lipocid*. Culture fluids with the titer of 300

million spores/ml will be recommended for application in farm enterprises as effective control measures against diamondback moth larvae. The culture fluids produced based on the Bt-type bacterial strains isolated from the biocoenosis, which differ from each other in certain morphological and physiological characteristics, with the titer of 300 million spores/ml, demonstrate high biological efficacy against low-instar diamondback moth larvae under laboratory conditions and in cabbage plantations. The density of diamondback moth larvae in the cabbage plantations was within the economic threshold of harmfulness for this pest. Statistical indicators confirm that the results of the experiments are accurate and there is no significant difference between the biological efficacy indicators demonstrated by the bacterial culture fluids of the Bt-type and the standard reference Lipocid against diamondback moth larvae. The bacterial strains used for bioprotection of agrophytocenoses are environmentally friendly and safe for human health. The results and conclusions of the study demonstrate the high effectiveness of biological pest control measures for vegetable plants. Furthermore, the work aims to rationalize (biologize) the management of biological resources to reduce dependence on commercial pest control products for cultivated phytocenoses.

KEYWORDS: Ecological Culture; Rational Use Of Resources, Biologization Of Nature Management, Cultural Phytocenoses, Vegetable Crops, Cabbage Plantations, Bacterial Strains, Bacillus Thuringiensis (Bt), Laboratory And Field Experiments, Biosecurity, Effectiveness Of Measures; Environmental Safety, Sanitary And Hygienic Safety.

1. INTRODUCTION

Issues of resource security and ensuring a culture of rational use of natural resources are particularly pressing now. Ecological culture, as applied to modern nature management, should be oriented towards the strategy and possibilities of biologization.

Plant growing is a complex area of nature management and largely depends on the supporting influence of society [1,2,3]. Modern agrophytocenoses [4,5] are designed to provide the growing population with high-quality, environmentally friendly agro-food products. This is, among other things, a question of agricultural resource availability and food security [6]. Unfortunately, many cultural ecosystems are subject to modern limiting and even degrading impacts of natural [7,8] and anthropogenic [9,10,11,12] origin. In this regard, vegetable products represent food raw materials that possess the biochemical and bioecological properties necessary for normal life, that is, for maintaining the normal life and health of the population.

Among vegetable crops, cabbage is of universal agricultural, food, feed, and other (ornamental, etc.) importance. This crop is widespread throughout almost the entire world [13,14,15]. White cabbage (*Brassica oleracea* convar. *capitata* L.), due to its valuable chemical composition, is classified among essential food crops. It is consumed fresh, pickled, and used in the production of canned goods. It is also a valuable medicinal raw material and can be processed into various uses. More than 71000000 tons of cabbage are produced annually worldwide [13]. Interest in this vegetable crop is growing globally [13-17]. In Russia, cabbage crops occupy more than 70000 hectares. In the North Caucasus, cabbage crops occupy 23% of agricultural land [13]. In the South Caucasus, the share of agricultural land occupied by vegetable crops exceeds 18-35% (depending on the crops grown and the region). In Azerbaijan (South Caucasus), cabbage crops account for less than 6000 hectares [14,16].

For example, in Europe [15], Russia [13], Asia [17,18] and Africa [18] serious efforts are being made to cultivate this crop. The need to find environmentally and hygienically safe biological methods of pest control of economically valuable plants is growing.

In general, for the Caucasus Region, vegetable growing is a traditional type of agricultural plant production [13,16,19,20]. In total, more than 30000 hectares of land are allocated for vegetable agrocenoses in Armenia [20]. Of these, the area under

crops of all types of cabbage occupies 3376 hectares, of which 393 hectares (or 11.6% of the total area under cabbage cultivation) are located in the Lori Region / Lori Marz (the South Caucasus) [14]. This is explained by the warm climate and the generally low weather variability in the flat landscapes of the Caucasus Region [20,22,23,24].

However, a major constraint to achieving high yields and quality product is the damage caused by harmful insects, particularly the cabbage moth – *Plutella xylostella* L. (= *Plutella maculipennis* Curt.) [17] belonging to the order Lepidoptera. This insect is cosmopolitan, as it has a wide range of ecological valences to many limiting environmental factors [17,25]. For example, for this pest of agricultural crops of the Brassicaceae family, the amount of heat required to complete the full development cycle of one generation is highly variable: from 180 to just over 400 °C. Moreover, the ecological optimum for survival, development cycle implementation and high fertility of the cabbage moth is a positive temperature of 20 °C. This largely determines the wide distribution of this pest in the world across regions (America, Australia, Hawaii, Europe, Asia, Africa, etc.) with relatively close or similar weather and temperature conditions during the growing seasons [26].

The larvae of this pest create characteristic feeding holes (commonly referred to as "windows") on the leaf blades, and often damage the growing points (growth cone/apical meristems), which leads to underdeveloped or malformed cabbage heads that fail to grow properly [27]. According to professional literature [28], maximum yield losses for early- and late-maturing cabbage varieties under Armenian conditions can reach up to 80 % and 52 %, respectively. For comparison, in the Southeast Asia, yield declines can reach 50-80% [29] and even 90% [30]. A similar decline in cabbage yields of up to 90% has been recorded on the island of Taiwan [31] and in Africa [32]. In this regard, pest control in areas of mass reproduction of phytophages (and even more so in the context of a wider territorial coverage) becomes relevant. Given the cosmopolitan nature of the distribution of many pests, including *P. xylostella*, this is a global agricultural and economic-ecological challenge in modern times.

Globally, annual expenditures by crop producers to combat of the *P. maculipennis* Curt. amount to approximately \$5 billion [25].

In the integrated pest management system for agricultural crops, chemical preparations have been the primary control agents used to date. Although these compounds exhibit high biological efficacy

[18,33], they simultaneously pose risks to the environment [34]. The clear risk of chemical insecticides (essentially poisons) from the *P. xylostella* (and other insect pests) to human health is obvious [18,33,34]. In addition, resistance to traditional chemical insecticides has been demonstrated in the *P. xylostella* populations. Moreover, increasing resistance to pesticides is genetically fixed in this insect species [31].

There is an opinion that microbiological measures to combat pests of vegetable crops and, in particular, cabbage, are not widespread in crop production complexes, but are environmentally and hygienically safe and have agricultural prospects [18,33]. Representatives of the genus *Bacillus* possess many bioecological properties beneficial to society. Some representatives of this genus are used as feed additives [35], primarily in medicine [36], and even in bio-space and pharmacological testing [37]. There is fragmentary information about experiments on representatives of the genus *Bacillus* as plant biostimulants [38]. The ecological role of some bacteria of the genus *Bacillus* in the bioremediation of heavy metals, the most important environmental pollutants in modern times, has been determined [39]. In the context of the subject of our work, the most important is the bioinsecticidal property of bacteria of the genus *Bacillus* on insect pests of vegetable crops: tomatoes [40], peppers and eggplants [41], radishes, pumpkins and other vegetable plants, as well as grain and leguminous crops [42].

Separate experiments were conducted to establish the bioinsecticidal effect on pests (*Hyposidra* spp., *Spodoptera litura*, *Helicoverpa armigera*, *Plutella xylostella*, etc.) of cabbage, cauliflower, and organic tea using the example of the local Bangladesh strain of the Bt JSc1 [43]. Similar experiments have been conducted in Turkey on forestry and agricultural plants of the cruciferous family [44]. In South America (in Brazil), the bioprotective role of bacterial strains of the *Bacillus* from local ecosystems has been identified in relation to agrophytocenoses based on coffee, citrus, soybean, corn, wheat, as well as tomatoes and a number of other crops [45].

In this context, increased attention is now being given to the use of bacterial insecticides derived from the *Bacillus thuringiensis* (Bt), which are considered safe for humans, warm-blooded animals, and beneficial entomofauna [46,47]. In this regard, increasing attention should be paid to the use of bacterial insecticides derived from the *Bacillus thuringiensis* (Bt). In addition, they are considered safe for humans, warm-blooded animals and

beneficial entomofauna [46,47].

However, it should be noted that the cost of imported commercial bacterial preparations of the Bt-type increases in two to three times due to importation expenses, making them economically inefficient [48].

Given these considerations, and the need to establish preconditions for the production of locally manufactured bacterial insecticidal formulations, an objective has been set up to isolated strains of the *Bacillus thuringiensis* from individual components of the biocenosis through microbiological methods, and to test the culture-based liquid preparations derived from these strains both under laboratory conditions and in cabbage plantations against early-instar larvae of the cabbage moth.

After determining the spectrum of activity against various insect pests, bacterial strains with high biological efficiency will be selected for the development of a locally produced commercial preparation.

2. MATERIALS AND METHODS

2.1. Research Objects and Materials

The research materials used in this study included bacterial insecticides of the BtHK-22, BtMS-49, and BtAS-87 (the strain names were entitled by the co-authors), which were isolated under microbiological conditions from naturally deceased larvae of the gypsy moth (*Oncideria dispar* L.), the apple ermine moth (*Yponomeuta malinellus* Zell.), and the mountain ringed silkworm (*Malacosoma parallela* Stgr.) in natural environments. The isolations were performed on meat peptone agar (MPA) artificial nutrient medium. Liquid cultures produced based on these bacterial strains, as well as the commercial bacterial insecticide preparation Lepidocide (the preparation powder based on strain of the *Bacillus thuringiensis* var. *kurstaki*, with the biological activity of 3000 IU/mg in the formulation powder, permitted for use against harmful insects in the Republic of Armenia) have been also used [49]. Other study materials included the cabbage moth (*Plutella xylostella* L.) and the mid-season cabbage variety Slava 1305 (*Brassica oleracea* convar. *capitata* L.), approved for cultivation in Armenia [50].

2.2. Isolation of Local Strains Of *Bacillus Thuringiensis* (Bt) And Establishment Of Liquid Cultures

The study was conducted between 2021 and 2024 under laboratory conditions and in cabbage plantations of the Fioletovo community of the Lori

Region / Lori Marz (the Caucasus, Republic of Armenia).

The isolation of local bacterial strains of the Bt from naturally deceased larvae in the biocenosis was carried out in 2021 in accordance with methodological guidelines [51,52]. Liquid cultures based on the isolated bacterial insecticides were produced using the BioFlo Fermenter (Flexem LabFreez). The titers (concentration) of the liquid cultures were determined at the Armbiotechnology Scientific and Production Center.

2.3. Bioinsecticidal Activity Of Liquid Cultures Of Bacteria Of *Bacillus Thuringiensis* (Bt)

In 2022, under laboratory conditions, the biological efficiency of liquid cultures with varying titers (100, 200, 300, 400, 500, 600, and 700 million spores/ml) against phytophagous larvae was assessed. The culture with the low titer (300 million spores/ml), which showed high biological efficacy under laboratory conditions, was tested in cabbage plantations against diamondback/cabbage moth larvae according to the methodological guideline [53,54].

2.4. Experimental Infestation Of Cabbage Crops With A Pest

In the test plots of cabbage plantations, the larval population of the cabbage/diamondback moth was at the economic threshold of harmfulness [55,56]. Due to the high infestation rate with the pest – 26–45 % of the crop at the cabbage leaf formation stage being colonized by two or more larvae – the cabbage plots in Fioletovo were selected as experimental plots/sites.

Under laboratory conditions, cabbage plants grown in 2-liter clay pots filled with soil were artificially infested with early instar (I–II) larvae of the cabbage/diamondback moth. Each test variant included 3 clay pots. One pot with infested plants and larvae was considered one replication. Each variant involved 45 larvae.

In both experimental and reference (control/standard) variants, under laboratory conditions cabbage plants were sprayed using a hand-held sprayer. In field experiments, spraying was carried out using the knapsack sprayer from the Ozdesan. The planting area per variant was 90 m², with 30 m² allocated per replication. The working solution consumption was 4.5 l per 90 m².

2.5. Scheme of Spraying Experimental Crops with Solutions of Liquid Bacterial Cultures

In production/farm scale experiments/trials,

each variant covered the area of 0.3 hectares (0.1 hectare per replication). Spraying was carried out using the RTR MAX motorized sprayer. The working solution consumption rate was 500 l/ha.

The control group consisted of infested but unsprayed plants, while the reference standard was the 0.2 % aqueous suspension of the Lepidocide.

Each variant in the laboratory, field plot, and production experiments was replicated three times.

2.6. Biological Efficacy of the Tested Bacterial Insecticides

The biological efficiency of the tested bacterial insecticides against the cabbage moth was calculated using the Abbott's formula, incorporating the number of live and dead larvae in each treatment and control variant (unsprayed plants) [57,58].

Staining of vegetative cells, bacterial spores, and crystalline bodies of the Bt insecticides, which are the active components affecting the pest, was performed according to established protocols [59], and measurements were taken using the ocular micrometer [60,61].

The biological efficiency indicators observed in production experiments were subjected to statistical analysis [62,63]. The main statistical data are presented in Table 2.

3. RESULTS

3.1. Basic Biological Characteristics of Isolated Strains of *Bacillus* from Individual Components of Local Ecosystems

Results from experiments conducted under laboratory conditions in 2021 confirmed that the bacterial insecticides of the BtHK-22, BtMS-49, and BtAS-87, which we isolated by microbiological methods from larvae that died of natural causes, differed from each other in the following characteristics.

The sizes of vegetative cells (average longitudinal and transverse sizes of vegetative cells) of the indicated strains were 3.60 × 1.32, 4.12 × 1.44, 3.68 × 1.38 μm, respectively.

Features of the use of mineral nitrogen and sugars: strains of the BtHK-22, BtMS-49, and BtAS-87 do not use the compounds Ca(NO₃)₂, Fe(NO₃)₃ and Cu(NO₃)₂ as sources of mineral nitrogen, respectively; from sugars, they use xylose, arabinose and the sugar alcohol mannitol, respectively.

Size and appearance of spores and insecticidal crystal particles formed inside the cell: the average longitudinal and transverse dimensions of the spores formed inside the vegetative cell for the

aforementioned strains were 1.58×0.78 , 2.32×0.96 , and 1.64×0.80 micrometers, respectively; for the crystal particles, the dimensions were 1.32×0.66 , 2.20×0.84 , and 1.52×0.78 micrometers, respectively. In the case of the BtMS-49 strain, the crystals were of the regular shape, whereas in the other two strains, the crystals had elongations of varying sizes.

Size and color intensity of colonies formed on meat-peptone agar medium: the colony sizes formed by strains of the BtHK-22, BtMS-49, and BtAS-87 on solid medium were 9.8 mm, 11.2 mm, and 10.5 mm, respectively. The colony color of the BtAS-87 strain was dark cream, while that of the other two was light cream.

Nature of colony surface granulation: the colonies formed by strains of the BtMS-49 and BtAS-87 had the coarse-grained structure, while in the case of the BtHK-22 it was fine-grained.

Based on the results of laboratory experiments conducted in 2021, it was confirmed that the bacterial insecticidal strains of the BtHK-22, BtMS-49, and BtAS-87, isolated through microbiological methods from naturally deceased larvae, exhibited distinct differences.

These included variations in the size of vegetative (actively growing) cells, their ability to assimilate mineral nitrogen and sugars, the size and morphology of spores and insecticidal crystal inclusions formed within the cells, the intensity of colony pigmentation on the MPA nutrient medium, the nature of colony surface granulation, and other

specific characteristics.

3.2. Bio-efficacy of Bacterial Strains of *Bacillus* in Killing Cabbage Moth Larvae in Laboratory Conditions and Through Small-Plot Trials

In 2022, laboratory trials were conducted using culture fluids derived from the aforementioned strains of the Bt at varying concentrations (100–700 million spores/ml) against first- and second-instar larvae of the cabbage moth.

The trials confirmed that the most effective low-dose treatment was a culture fluid with a concentration of 300 million spores/ml. Seven days after application, this formulation demonstrated high biological efficiency against phytophagous larvae, ranging from 95.6 % (BtMS-49) to 97.8 % (BtAS-87). These efficiency levels were maintained until the larvae reached pupation.

The high biological efficacy observed under laboratory conditions allowed for the 300 million spores/ml culture fluids, developed from individual strains, to be tested further under field-like conditions in cabbage plantations (small-plot trials) against first- and second-instar cabbage moth larvae.

Results from the small-plot trials confirmed that seven days after spraying, the individual culture fluids at the concentration of 300 million spores/ml continued to exhibit high biological efficiency (94.9–96.0 %) against early-instar cabbage moth larvae in the cabbage fields/plantations (Table 1).

Table 1: Biological Efficiency of Bt-Type Bacterial Insecticides against I–II Instar Cabbage Moth Larvae in Cabbage Plantations of the Caucasus (Small-Plot Trials/Experiments, the Lori Region, 2023)

| Variants | Total number of larvae counted on 30 plants, <i>n</i> | Biological efficacy per observation days, % | | |
|--------------------------------|---|---|------|------|
| | | 3 | 5 | 7 |
| <i>BtHK-22</i> | 69 | 58,0 | 78,3 | 95,7 |
| <i>BtMS-49</i> | 78 | 52,6 | 69,2 | 94,9 |
| <i>BtAS-87</i> | 75 | 61,3 | 80,0 | 96,0 |
| Lepidocide (Reference/control) | 84 | 65,5 | 81,0 | 97,6 |

In the Lepidocide control variant, the biological efficiency on the same observation day reached 97.6 %. In both the experimental and standard variants, the biological efficacy observed on the 7th day after spraying remained consistent in the cabbage plantations until larval pupation.

3.3. Bioefficacy of Bacterial Strains of *Bacillus* in the Destruction of Cabbage Moth Larvae under Industrial Conditions

Given the high levels of biological efficiency recorded in the small-plot trials, the testing of

individual culture fluids at the concentration of 300 million spores/ml against cabbage moth larvae was continued under production (field-scale) conditions (Fig. 1.).

According to the data presented in the diagram, the highest levels of biological efficiency in the experimental variants were recorded on the 7th day after spraying, reaching 92.6 %–95.7 %, compared to 96.1 % observed in the Lepidocide standard variant for the same indicator [64, 65]. No larval mortality of the cabbage moth was observed in the untreated control variant.

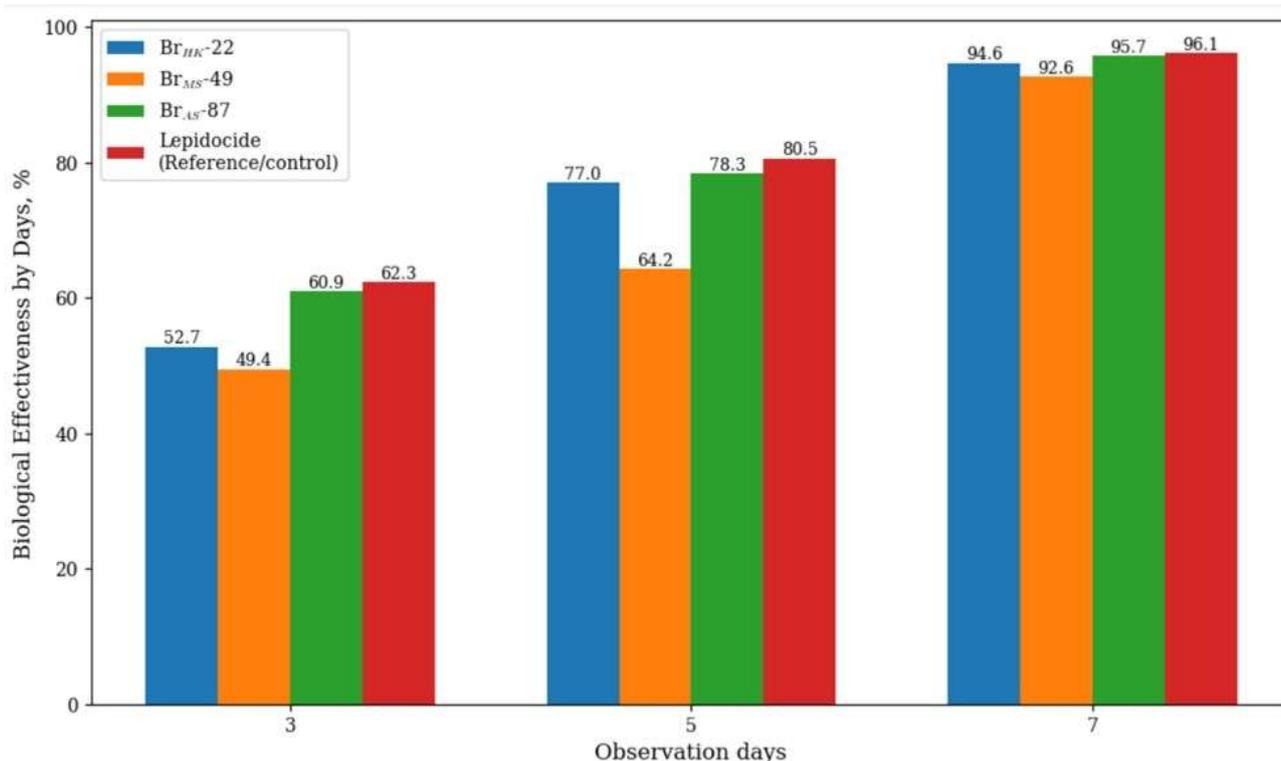


Figure 1: The Biological Efficiency of Bt-Type Bacterial Insecticides against I-II Instar Larvae of the Cabbage Moth in Cabbage Plantations of The Caucasus According To Observation Days (Production Trials/Experiments, The Lori Region, Fioletovo, 2024).

The experimental results confirmed that in laboratory, small-plot, and production/field-scale trials, the biological efficacy of treatments against cabbage moth larvae was significantly lower on the 3rd and 5th days following spraying compared to the 7th day.

Under laboratory conditions, the combined efficacy indicators for the experimental and standard/reference variants ranged from 57.8 % to 64.4 % on day 3, and from 77.8 % to 84.4 % on day 5. In small-plot trials, the efficacy ranged between 52.6 % and 65.5 % on the 3rd day, and between 69.2 % and 81.0 % on the 5th day. In production/field-scale trials, the efficiency values ranged from 49.4 % to 62.3 % on day 3, and from 64.2 % to 80.5 % on day 5.

The relatively lower efficiency observed on days 3 and 5 is attributed to the specific impact mode of bacterial insecticides of the Bt-type.

Visual observation confirmed that, compared to the untreated control plots, larvae in the treated cabbage plantations gradually ceased feeding, showed reduced body size and greying, all of which contributed to their death.

Furthermore, microbiological analyses of the bodies of deceased larvae in the treated variants confirmed that their internal cavities and tissues

were filled with vegetative cells of bacteria of the Bt-type, predominantly spore-crystal complexes, which are known to affect larvae. The presence of these structures validated that larval mortality resulted from the impact of bacteria of the Bt.

3.4. Statistical Reliability Of The Bioefficiency Of Isolated Bacterial Strains As Bioprotection Against Pests Of Vegetable Agrophytocenoses

The indicators for experimental error and coefficient of variations presented in Table 2 ranged respectively from 3.3 % to 5.3 % (not exceeding the 5.5 % threshold) and from 5.64 % to 9.09 % (not exceeding the 15 % threshold), confirming the reliability of the research experimental results.

The calculated values of the Student's t-test ranged from 0.192 to 1.550 (Table 2), and under the parameters $P_{\square, \square}$ and $n=3$, these values were lower than the tabulated t value of the Student's of 3.182. This confirmed that there is no statistically significant difference in biological efficacy between the experimental variants (using methods of variation statistical analysis [62,63]) treated with bacterial insecticides and the Lepidocide standard on the 7th day after spraying.

Table 2: Statistical Indicators Of The Average Number Of Dead Cabbage Moth Larvae (Instars I-II) Recorded 7 Days After Spraying (Production/Field-Scale Trials, 2024).

| Variants | Total number of dead larvae per variant, n | Average number of dead larvae per replicates, n | Statistical indicators | | | | |
|--------------------------------|--|---|--|--|--------------------------------------|--|--|
| | | | Square deviation | Variation coefficient, % | Mean error | Experimental error, % | Calculated Student's t-test indicators |
| | | $\bar{X} = \frac{\sum X_i}{n}$ | $\sigma = \frac{\sqrt{\sum (X_i - \bar{X})^2}}{n-1}$ | $V = \frac{\sigma}{\bar{X}} \cdot 100$ | $\sigma_x = \frac{\sigma}{\sqrt{n}}$ | $P = \frac{\sigma_x}{\bar{X}} \cdot 100$ | |
| BtHK-22 | 70 | 23.33 | 1.711 | 7.33 | 0.988 | 4.2 | 0.859 |
| BtMS-49 | 75 | 25.00 | 2.000 | 8.00 | 1.155 | 4.6 | 0.192 |
| BtAS-87 | 66 | 22.00 | 2.000 | 9.09 | 1.155 | 5.3 | 1.550 |
| Lepidocide (Reference/control) | 74 | 24.67 | 1.391 | 5.64 | 0.803 | 3,3 | - |

Note: Under conditions of the P0.95 and n=3, the tabulated index of the Student's t-test is equal to 3.182.

4. DISCUSSION

Recently, there has been an understanding of the unique bioecological and biochemical qualities and potential capabilities of microbiological objects [40,41,66]. They can be used as natural plant protection products [40,41] and for bioimprovement (biocomposting, ecological optimization of the biogeochemical cycle between components of soil-plant systems, increasing the biodiversity of beneficial soil microbiota) of natural and economic ecosystems, especially agrophytocenoses (most dependent on the means of maintaining and protecting cultivated plants) [67]. In terms of content and in relation to agricultural crops (and to the health of consumers of agro-food products), these are universal, nature-like methods and technologies of agricultural bioresource use.

Today, it is known that bacteria of the genus *Bacillus* have wide practical applications worldwide. First of all, this concerns medical and industrial-pharmaceutical purposes [36,68], various biotechnological tasks [68] and the processing of organic waste and the closed-loop economy [67], feed and agro-food production [67], bioprotection and biorehabilitation (microbioremediation) from pesticides [69], heavy metals [39,70] and petrochemical pollutants [70] of economically disturbed and degraded territories and natural objects.

The results of experiments on biostimulation of cultivated plants using bacteria of the genus *Bacillus* are briefly reflected [71]. Assumptions and forecast estimates are given regarding the potential of bacteria of the *B. thuringiensis* as biostimulants for the growth and development of economically valuable plants, including vegetable plants [72]. Overall, the potential of biostimulation should be further explored in detail. Furthermore, the bioecological properties of *B. thuringiensis* bacteria allow them to be used as natural microbiological

agents not only for biostimulation and biocorrection of growth and development. Most importantly, such microorganisms hold promise for the biological protection of crops. The fact is that up to half or more percent of crop yields are damaged by pests [29, 30, 31].

The following is indicated for a number of regions of the world. In general, bacteria of the *B. thuringiensis* can be a serious and at the same time independent biological (natural) means of controlling pests of economically valuable plants in agriculture [43,46,47,65], in forestry [44], in urban plant growing (in urban gardening, in backyard plant growing, in street landscaping, etc.) and in other types of plant growing [42]. It is important that such eco-friendly work, both in its objectives and content, utilize strains of the *B. thuringiensis* from local ecosystem components. This is because the most rational, nature-friendly – and ecosystem-based – principle in nature management, including in crop production, is essential. This approach should be a leading one in the horticultural management of modern cultivated landscapes and be scaled up more widely in modern crop production areas.

We have isolated the first *B. thuringiensis* strains for the Caucasus: the BtHK-22, BtMS-49, and BtAS-87. They have demonstrated the necessary biological and ecological characteristics for increasing the productivity of vegetable agrophytocenoses. Our data were obtained in three series of experimental studies: laboratory, small-plot field, and field production (training, pilot, and production) conditions. They confirmed the hypothesis of the usefulness of these microorganisms as bioinsecticides.

Our studies confirmed high biological efficacy in controlling diamondback moth larvae using isolated strains of the BtHK-22, BtMS-49, and BtAS-87 (*B. thuringiensis*) from local ecosystems typical of the Caucasus Region. High efficacy in biocontrol against

the ecologically cosmopolitan insect pest of the *P. xylostella* was achieved in cabbage phytocenoses even when scaling up the experiments to production conditions (that is, up to full-scale field scales). This means that the isolated bacteria of the *B. thuringiensis* are useful for microbiological pest control in agricultural fields and gardens in the Caucasus and adjacent areas with similar soil and climate conditions and the agricultural orientation of plant growers.

Unfortunately, microbiological methods of bioprotection of vegetable crops, including cabbage plantations, are not yet widespread in the world due to isolated or fragmentary scientific information about the microorganisms themselves as biologically and environmentally safe bioinsecticides [18,33,42].

It is obvious that it is necessary to take into account the available (albeit sparse) data on the results of experiments on microbiological pest control of vegetable crops using a number of strains of the *Bacillus* in the number of regions of the world [40,67], including Russia [42] and its Caucasian regions [74], Europe [44], large regions of Asia [18,41], Africa [18,75] and the Latin America [76]. For example, separate experiments were conducted to establish the bioinsecticidal effect on pests (*Hyposidra* spp., *Spodoptera litura*, *Helicoverpa armigera*, *Plutella xylostella*, etc.) of cabbage, cauliflower, and organic tea using the example of the local Bangladesh strain Bt JSc1 [43].

The use of local isolates of the *B. thuringiensis* (and other species of the *Bacillus*) can be recommended for detailed research and targeted practical application. This is currently in high demand for large-scale and highly effective bioprotection of agrophytocenoses.

Having established climate change [24,77,78,79,80,81], one can predict increased pressure from limiting biotic factors [5,7,80,81,82,83,84], primarily pests [20,26,29,80,82,84,85], on agrophytocenoses. This is an additional factor reducing the resilience and bioproductivity of agrophytocenoses, leading to increased pest (and disease) pressure on plants. Reorienting modern crop production, including vegetable growing, toward effective microbiological protection against pests will meet the needs of rational bioresource management and, most importantly, the growing (strategic) role of agro-food and agro-ecological farming in the economy.

Furthermore, the results of our microbiological and agroecological research (provided that local isolates of the genus *Bacillus* are used for the relevant territories) can be used as a basis for developing and

scaling up comprehensive programs to improve the effectiveness of biological control of cosmopolitan pests of vegetable and other agricultural (and forestry) crops. This is one of the most important implications of our research and its analysis.

The results and conclusions of our microbiological research as a scientific and industrial test of bacterial strains of the *B. thuringiensis* common in various types of natural and man-made ecosystems can be applied universally. The practical use of local strains of the *B. thuringiensis* as those best adapted to local limiting environmental conditions by plant-growing complexes for the effective bioprotection of vegetable agrophytocenoses can be introduced and scaled up in various areas of the subtropical and temperate climate of Russia, in the Caucasus and adjacent regions (Turkey, Iran, Iraq), in vast areas with vegetable and other agrophytocenoses in the Central, East and Southeast Asia, as well as in Africa, the South and Central America.

There is evidence [84] that bacteria of the genus *Bacillus* are resistant to climate aridity factors. In addition, bacteria of the genus *Bacillus* stimulate the development and ensure the bioproductivity of cultivated plants under the aggressive influence of environmental factors due to drought [84]. This additional beneficial property of such bacteria for cultivated phytocenoses, given the observed general trend toward climate transformation in many regions of the world and globally. Strains of the *Bacillus* from local ecosystem components can and should be used to ensure sustainable and highly productive crop production in the Caucasus and surrounding regions, in southwest, south, and southeast Russia, in Asia, Oceania, Africa, and other regions of the world with documented aridization processes.

Due to their unique ecosystem, biostimulating, and bioprotective functions, these microorganisms are essential for creating biosecurity and increasing the yield of agrophytocenoses. We believe that the introduction of cost-effective microbiological protection of agrophytocenoses will also prevent the alienation of land from crop production and promote more rational bioresource use (on lands of various target categories).

We consider microbiological pest control in agricultural fields to be one of the most effective and economically and environmentally sound measures for the agricultural maintenance of agrophytocenoses. Microbiological pest control in vegetable gardens (following the example of our materials on Caucasian isolates of the genus *Bacillus*), as well as in vegetable gardens, summer cottages, urban horticultural land management, and

throughout rural areas, has obvious agricultural and environmental advantages.

These advantages are determined by the environmental safety of culture solutions containing of bacteria of the *B. thuringiensis* as bioinsecticides for treating vegetable plants. Furthermore, working solutions containing of bacteria of the *B. thuringiensis* are safe for the plants themselves and for the health of agricultural workers. The bioinsecticides we've studied and analyzed, using local isolates of the *Bacillus* as examples, are safe for the health of agricultural and food consumers. These products are environmentally friendly and healthy. This creates additional conditions for ensuring the sustainable operation of vegetable farms, as well as food and hygiene security for the population.

5. CONCLUSION

The results demonstrate that locally isolated strains of the *Bacillus thuringiensis* BtHK-22, BtMS-49, and BtAS-87, derived from naturally deceased lepidopteran larvae within the biocenosis, possess high entomopathogenic activity against the early pest *Plutella xylostella* L. Culture fluids containing 300 million spores ml⁻¹ consistently achieved > 92 % larval mortality under laboratory, small-plot, and full-scale production conditions, values statistically equivalent to the commercial reference *Lepidocide*.

The delayed peak of efficacy (day 7) agrees with the known mode of action of the Cry protoxins: ingestion, solubilisation in the alkaline mid-gut, receptor binding, pore formation, septicaemia, and final larval death. The transient "greying" and feeding cessation we observed are typical symptoms of the Bt intoxication, while recovery of vegetative cells and spore-crystal complexes from cadavers provides direct evidence of infection.

Strain-level differences in colony pigmentation, crystal size, and carbon-source utilisation did not translate into significant variation in final mortality, indicating that any of the three isolates could be selected for scale-up. The low coefficients of experimental error (< 5.5 %) and variation (< 9.1 %) confirm robustness of the spray protocol, tank-mix preparation, and larval counting procedure.

From a practical standpoint, the domestically produced 300 million spores ml⁻¹ formulation eliminates import mark-up, reduces foreign-

currency expenditure, and fits existing spray schedules (500 L ha⁻¹) used by Armenian growers. Integration into the IPM programmes could lower synthetic insecticide applications, thereby preserving natural enemies and reducing pesticide-residue risks.

1. Three strains of the *B. thuringiensis* (BtHK-22, BtMS-49, BtAS-87) isolated from biocenosis cadavers differ morphologically and physiologically but all yield high insecticidal activity.
2. Culture fluids at 300 million spores ml⁻¹ cause 92.6–97.8 % mortality of I–II instar of the *Plutella xylostella* larvae within 7 days under laboratory, small-plot, and production conditions, statistically on par with the *Lepidocide*.
3. No phytotoxicity or operational adjustments are required; the concentration is directly compatible with standard 500 l/ha⁻¹ hydraulic sprayers.
4. Three strains of the *B. thuringiensis* (BtHK-22, BtMS-49, and BtAS-87), isolated from animal carcasses in local ecosystems, differ morphologically and physiologically. All exhibit high bioinsecticidal activity.

The final choice will be based on the broad spectrum of action against additional pests and fermentation productivity. The bioecological properties of the selected local strains contribute to this.

We have tested and proposed, for the first time in the Caucasus Region, an environmentally friendly and hygienically safe method for controlling pests in vegetable agrophytocenoses using local strains of the *Bacillus*. We analyzed the application rates of culture broth solutions containing of strains of the BtHK-22, BtMS-49, and BtAS-87 for the bioprotection of cabbage plantations. The results of our studies, using the strains we isolated as examples, demonstrate their potential as microbiological insecticides, providing a biologically effective and environmentally sound method for bioprotection of vegetable agrophytocenoses, thus stimulating widespread use in modern agriculture. The results and material of the work demonstrate a strategy for the culture of rationalization of nature management in food crop production based on plant bioprotection.

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