INTRODUCTION OF *Bacillus thuringiensis* IN THE POPULATIONS OF PHYTOPHAGES *Pieris brassicae* L. AND *Archips crataegana* HB. (*LEPIDOPTERA*) TO CREATE ARTIFICIAL EPIZOOTY

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Abstract. The dominant position among the complex of entomopathogenic microorganisms known in the world and used in agricultural biotechnologies, is occupied by *Bacillus thuringiensis* bacteria. The introduction of *B. thuringiensis* strains into the population of sensitive species of phytophagous insects is an effective method of correcting the microbiome and the trophic structure of the metabolism of multicomponent biological systems "soil-plant-phytophagus". A high entomocidal activity of technological strains of *B. thuringiensis* CryI (BtH_1 $87/_3$, BtH_1 $20/_3$) with a spore titer of 2.0-4.0 billion/mL of culture liquid for phytophages *Pieris brassicae* L. and *Archips crataegana* Hb. (*Lepidoptera*) was confirmed. The features of the occurrence of microepizootic outbreaks under the influence of *B. thuringiensis* and high sensitivity of populations to exotoxin-containing BtH_1 strains (level of exotoxinogenity according to LC_{50} in the range of 2.6-3.1 μL/g of feed for the *Musca domestica* L. biotest). The feasibility of creating artificial epizootics of *B. thuringiensis* in places of phytophagous reservation and the effectiveness of microbiological control of *Lepidoptera* population has been proven.

Key words: *Bacillus thuringiensis*; microbiological control; strain; artificial epizootic; number.

INTRODUCTION

Microbiological control of target insect populations in modern agricultural and ecological systems should be considered as an applied part of agricultural microbiology and animal diseases [24, 25]. Identifying the influence of "microbe-pathogen-macromicrobe-host" interactions on the occurrence, intensity, and spread of complex natural disease processes in animals, and understanding the factors that drive them, is an important part of modern microbial management and insect it is important to perform a number of controls [13, 29].

Microbial metabolites of entomopathogenic bacteria *Bacillus thuringiensis* and their structural diversity of compounds with biological activities (antimicrobial, antiviral, immunosuppressive, antitumor) play the most important ecological and evolutionary role: they contribute to the development of high adaptive capacity, the survival of subspecies of bacteria in various environments, biocenoses and the implementation of the corresponding role in the pathogenesis of insects, the expansion of the spectrum of sensitive species [7, 8, 26, 31].

The development and stability of the epizootic process are significantly influenced by abiotic and biotic factors: 1) virulence, contagiousness of the pathogen, which is unstable and can change; 2) the sensitivity of the host insect to the pathogen, which is determined by the physiological and immunobiological state of the organism and the population as a whole, the so-called species and population sensitivity; 3) external conditions that directly or indirectly affect the pathogen, host and their interaction; 4) conditions of preservation of the pathogen in the environment and ways of its transmission from an infected (diseased) organism to a healthy (susceptible) one. The effectiveness of the epizootic introduction of the entomopathogen *B. thuringiensis* in the population of phytophages depends on compatibility of the host target and the pathogen, persistence, and genetic features of the bioagent [15, 17]. To date, the genetic basis underlying the ability of *B. thuringiensis* strains to cause epizootics remains poorly understood. Epizootics of the bacterial pathogen *B. thuringiensis* may depend both on its biological activity with the optimal concentration of the spore-crystal complex, and on multiple infection, the latent period, the physiological status and stage of the insects, as well as the host plant.

When creating an artificial epizootic of *B. thuringiensis*, long-term foci of infection are observed in places of phytophagous reservation [14]. Undoubtedly, environmental conditions, stresses and pesticide load on agrocenoses play an important role in the effective interaction of the host and the pathogen, affecting the intensity and final result of the epizootic, the degree of contact between healthy individuals and the sick (including contact with a sufficient dose of the inoculant that will cause the disease). *B. thuringiensis* strains in natural conditions show resistance, they are ecologically safe and able to actively penetrate the host's body, reducing its protective mechanisms [16, 28]. It should be taken into account that the epizootological regularities found in populations of different insect species under the same or similar conditions of existence will not proceed in the same way, since each insect species has its biological features, which can largely determine the possibility of the development of epizootics in general. Research by scientists show that the same variety of *B. thuringiensis* can be isolated from different econiches and soil-climatic conditions (regardless of the presence and distribution of the host insect of this entomopathogen). So, *B. thuringiensis* CryI (*berliner*, *thuringiensis*) are allocated in the USA, Germany, Ukraine, the Czech Republic, Romania, France, Italy and other countries; *B. thuringiensis* CryII *(kurstaki)* – isolated from

biotopes of Canada, Poland, Ukraine, France; *B. thuringiensis* CryIII (*tenebrionis*) – Denmark, Czech Republic, Belgium; *B. thuringiensis* CryIV (*morrisoni*, *israelensis*) was removed in Israel, Nigeria, the Czech Republic, France, but it was proved that the bacteria *B. thuringiensis* differ from each other not by varieties, but mainly by the characteristics of a competitive strain [1, 2, 27]. Thus, the entomopathogenic bacteria *B. thuringiensis* is an important factor in the population dybanics of phytophages. The use of these pathogens in plant protection is promising in monitoring studies on the spontaneous development of epizootics, for the formation and strengthening of signaling factors that contribute to this development. The creation of artificial epizootics (introduction of microbial preparations based on strains producing biologically active substances) in places of mass accumulation of phytophagous species is relevant. Introducing the most contagious, resistant entomopathogens into the insect population to accelerate and intensify the intensity of epizootics. Integrated technological methods in plant protection regarding the gradual alternation of new generation pesticides with such entomopathogens that effectively reduce the relative adaptability of resistant individuals in a wider range of conditions, economically feasible in insect control, are not excluded.

The purpose of this work was to study the influence of exotoxinogenic strains of *B. thuringiensis* CryI on natural populations of *Pieris brassicae* L. and *Archips crataegana* Hb. (*Lepidoptera*) and reveal the features of microepizootics in sensitive insect species.

MATERIALS AND METHODS

The work used strains of spore-forming bacteria *B. thuringiensis* CryI (*BtH*$_1$ 87/$_3$, *BtH*$_1$ 20/$_3$ and *BtH*$_1$ 800 standard), which contain a thermostable ß-exotoxin with a wide spectrum of action against target insects (working collection of non-pathogenic microorganisms of the Department of Phytopathology named after Academician V. F. Peresypkin of the National University of Life and Environmental Science of Ukraine). Microbiological analyzes (maintenance of pure cultures, preparation of serial dilutions of bacterial suspensions of strains to 10^{-7} and 10^{-8}, cultivation in 1.0-1.5% agarized nutrient media MPA, LB, calculation of the titer of viable colony-forming units, titer of spores) was carried out by generally accepted methods [11, 30, 32]. The studies used light microscopy (Sigeta MV-130, China, magnification 40× and 100×), a digital eyepiece-free cell imaging system EVOS FL Imaging System, (Thermo Fisher Scientific, USA). Determination of the level of exotoxinogenity of *B. thuringiensis* strains after a long period of storage and selection procedures was carried out in bioassay using *Musca domestica* L. population [14]. LC_{50} (lethal concentrations, which cause the death of 50% of affected individuals), was calculated according to Kerber's formula:

$$\text{lgЛК} = \text{lgC}_{\text{м}} - \delta \times \left(\sum n/n_0 - 0.5\right)$$

where $C_м$ – is the maximum test concentration; σ – the logarithm of the ratio of each previous concentration to the next (logarithm of the multiplicity of dilutions), for a twofold dilution 2 = 0.3;

$$\sum n/n_0$$

where $\sum^{n/n_0}$ – the sum of the ratios of the number of insects that died from a given concentration to the total number of insects that were exposed to this concentration [14].

The entomotoxic effect of exotoxinogenic strains of *B. thuringiensis* CryI and the features of the formation and functioning of microepizootic foci (microepizootics) were investigated in the field and in laboratory experiments, where intact and contact populations of *Lepidoptera* insects – cabbage butter fly (*P. brassicae*) and hawthorn leaf roller (*A. crataegana*). Infection of phytophages with suspensions of *B. thuringiensis* CryI strains with the appropriate spore titer was carried out by the per os method in various concentrations, including sublethal ones: dilution 1:2 (0.05 mL/1 g of feed), 1:4 (0.025 mL/1 g of feed). The titer of viable spores of experimental strains of *B. thuringiensis* CryI ranged from 2.7 to 3.2 billion in 1 mL of culture liquid. The number of individuals in each version of the experiment is 25, the repetition is threefold, the control is sterile water and the results were recorded on the 3rd, 5th, 10th, and 15th day. The biological activity of *BtH*$_1$ 87/$_3$ and *BtH*$_1$ 20/$_3$ strains to different stages of insect development (according to the instars of individuals) was determined by LC_{50}, and the entomopathogenic effect was determined by the Franz formula:

$$M = 100 \times \left(1 - \frac{K_1}{K_2} \times \frac{P_2}{P_1}\right)$$

where M – is mortality, %; K_1, K_2 – the number of live individuals before and after treatment in the control; P_1 – is the number of live individuals before and after treatment in the experiment [14].

The overall assessment of the entomotoxic effect of *B. thuringiensis* CryI strains on the microbiome of the *Lepidoptera* population was carried out taking into account the primary lethal effect and the aftereffect. Statistical analysis was carried out using the Statistica 8.0 program, data were calculated using MS Excel.

RESULTS

The ability of *B. thuringiensis* bacteria to persist in various substrates of the insect habitat (soil, water, plant remains, insect corpses) and, under appropriate conditions, to be a source of spontaneous infections and, therefore, epizootics for subsequent generations of phytophages, has been experimentally confirmed [10, 12].

As a result of studies of the viability and residual number of *B. thuringiensis* spores on plants, it was

established that *B. thuringiensis* CryI strains (BtH_1 87/$_3$ and BtH_1 20/$_3$) persist on the leaf surface of hawthorn and apricot, while a slight decrease of the entomopathogen in the crown of infected trees is observed after precipitation (Table 1).

In laboratory conditions, the technological effectiveness of strains BtH_1 87/$_3$ and BtH_1 20/$_3$ was confirmed after two years of storage on agarized nutrient meat-peptone medium (MPA) at a temperature of $+1^0$C and after stages of multi-stage selection in comparison with the level of exotoxinogenity of cultures (production of thermostable β-exotoxin). The results are shown in Table 2.

Positive dynamics of practically valuable biological properties of experimental strains are observed, in particular, their productivity after long-term storage is not lower than 2.0 billion/mL of culture liquid, and selection makes it possible to stabilize and increase the spore titer of strains to the initial level and higher (up to 3.89-4.15 billion/mL).

The entomocidal activity of strains BtH_1 87/$_3$ and BtH_1 20/$_3$ in model conditions against the caterpillars of *Pieris brassicae* L. and *Archips crataegana* Hb. was established, the physiological state of individuals after their infection with entomopathogens was analyzed (Table 3).

Already on the third day of the experiment, 48.4 to 69.8% of mortality of experimental individuals was recorded.

With single (incomplete) processing of hawthorn feed branches in natural conditions, the death of 65.0% of caterpillars was recorded *Archips crataegana* Hb. and the rest of the younger individuals that remained on the plant had slow development, which indicates the occurrence of microepizootic foci under the influence of the entomopathogen BtH_1 on the population.

The antifeedant effect of BtH_1 was observed, which is characteristic of contact infection and is carried out initially through the taste receptors of the insect (Table 4).

After a part of the culture fluid of the strains enters the body, trophic functions are disturbed due to suppression of the activity of the central nervous system, disturbances of trophic rhythms, suppression of the secretion of digestive enzymes. Against young caterpillars *Archips crataegana* Hb. strains of *B. thuringiensis* BtH_1 87/$_3$ and BtH_1 20/$_3$ with full processing are effective on the tenth day of the experiment – 96.0%.

Field studiesit is shown that to control the population and prevent the harmful activity of *Pieris brassicae* L. in economically acceptable terms, during which the phytophagous does not have time to cause serious damage to plants, the most appropriate is the use of entomopathogenic agents of *B. thuringiensis*. Caterpillars of all instars of development of *Pieris brassicae* L. were found to be highly sensitive to BtH_1 (Table 5).

Table 1. Dynamics of the number of *B. thuringiensis* on leaves of hawthorn plants

Initial number of bacteria (per 5 cm^2)	**The number of bacteria after processing (by days of assessment)**					
	10	**20**	**30**	**40**	**50**	**60**
55.0x10^4	41.0x10^4	26.0x10^4	88.0x10^3	74.0x10^3	49.0x10^3	42.0x10^3
71.0x10^4	59.0x10^4	34.0x10^4	29.0x10^4	68.0x10^3	61.0x10^3	22.0x10^3

Table 2. Characteristics of BtH_1 87/$_3$ and BtH_1 20/$_3$ after two years of storage on meat-peptone nutrient medium

Strain	**Spore titer, billion/mL of culture liquid**			**Exotoxinogenicity according to LC_{50}, µL/g of feed for biotest *Musca domestica* L., L_2**		
	output indicators	after storage	after selection	output indicators	after storage	after selection
$BtH_1$87/$_3$	3.26	2.43	3.89	2.69	4.38	2.94
$BtH_1$20/$_3$	3.48	2.60	4.15	2.58	5,10	3.15

Note*:* the lower the LC_{50} value (µL of culture liquid suspension per 1 gram of feed for the *Musca domestica* L. biotest), the higher the rate of exotoxin formation.

Table 3. Entomicidal activity of *B. thuringiensis* CryI against the caterpillars of *Pieris brassicae* L., *Archips crataegana* Hb. (laboratory model experiments)

Research option	**Spore titer, billion/mL of culture liquid**	**Mortality of individuals by days of assessment, %**			
		3	**5**	**10**	**15**
		Pieris brassicae L.			
No treatment *B. thuringiensis* (control)	–	0	0	1.1 (1SD: 0.3)	1.2 (1SD: 0.3)
$BtH_1$87/$_3$	2.7 (1SD: 0.11)	64.3 (1SD: 1.3)	71.3 (1SD: 1.7)	79.2 (1SD: 1.8)	93.4 (1SD: 2.3)
$BtH_1$20/$_3$	3.2 (1SD: 0.15)	69.8 (1SD: 1.4)	80.6 (1SD: 1.4)	95.2 (1SD: 2.3)	96.8 (1SD: 2.0)
		Archips crataegana Hb.			
No treatment *B. thuringiensis* (control)	–	0	1.0+0.3	1.3+0.7	1.3+0.7
$BtH_1$87/$_3$	2.7 (1SD: 0.11)	48.4 (1SD: 1.4)	60.2 (1SD: 2.7)	83.8 (1SD: 1.3)	90.5 (1SD: 2.3)
$BtH_1$20/$_3$	3.2 (1SD: 0.15)	59.0 (1SD: 2.0)	65.8 (1SD: 1.4)	90.4 (1SD: 2.3)	97.3 (1SD: 1.4)

Table 4. The effectiveness of *B. thuringiensis* CryI in the treatment of hawthorn plants inhabited by *Archips crataegana* Hb. caterpillars younger instar

Research option	The number of caterpillars	Death of individuals by days of assessment, %				
		5	7	10	15	20
No treatment (control)	300	0	0.8 (1SD: 0.3)	1.6 (1SD: 0.7)	2.0 (1SD: 0.3)	2.4 (1SD: 0.3)
Partial processing		20.3 (1SD: 2.1)	42.4 (1SD: 1.7)	57.7 (1SD: 2.4)	62.0 (1SD: 1.4)	65.0 (1SD: 1.4)
Full processing		26.0 (1SD: 2.8)	74.6 (1SD: 5.2)	96.3 (1SD: 3.1)	100.0	–

Table 5. Effectiveness of *B. thuringiensis* strains CryI against *Pieris brassicae* L. at different temperatures (field experiment)

Research option		Efficiency, % death of individuals		
		5 days	10 days	20 days
		The temperature is 15-17.5^{0}C		
$BtH_I87/_3$	dilution 1:2	54.0 (1SD: 2.3)	78.6 (1SD: 1.8)	100.0
	dilution 1:4	63.1 (1SD: 2.7)	88.3 (1SD: 1.4)	100.0
$BtH_I20/_3$	dilution 1:2	62.4 (1SD: 0.9)	100.0	–
	dilution 1:4	59.8 (1SD: 1.7)	84.3 (1SD: 1.3)	100.0
Strain 800 (reference)		95.3 (1SD: 2.3)	100.0	–
		Temperature 22-24^{0}C		
$BtH_I87/_3$	dilution 1:2	76.3 (1SD: 3.8)	98.8 (1SD: 2.8)	100.0
	dilution 1:4	74.2 (1SD: 2.2)	91.8 (1SD: 2.2)	100.0
$BtH_I20/_3$	dilution 1:2	79.6 (1SD: 1.8)	96.5 (1SD: 1.4)	100.0
	dilution 1:4	77.4 (1SD: 2.8)	92.3 (1SD: 2.3)	100.0
Strain 800 (reference)		98.6 (1SD: 1.4)	100.0	–

Fluctuations in the temperature regime of the environment hurt the effectiveness of microbial cultures *B. thuringiensis* do not lead Yes, the death of caterpillars *Pieris brassicae* L. from dilutions of bacterial suspensions BtH_I 87/3 and BtH_I $20/_3$ was 98.8 and 96.5%, respectively, at an ambient temperature of 22-24^{0}C. At a temperature of 15-17.5^{0}C, the death rates of this phytophage were observed within 78.6–88.3 and 84.3–100% of the experimental BtH_I strains.

It was established that the microbiome of *B. thuringiensis* CryI, having an antifeedant effect, actively affects the caterpillars of *Archips crataegana* Hb. and *Pieris brassicae* L. After the entomopathogen enters the alimentary canal, individuals leave the infected environment, thereby contributing to the spread of epizootic micro-focuses.

DISCUSSION

Entomopathogenic microorganisms with different levels of virulence constantly persist in host insect populations, have diverse relationships and play a leading role in the management of phytophages. According to scientists, the main strategies for using microorganisms in the biomethod are the introduction of pathogens to the insect population, as well as the use of microbial preparations with new properties to reduce the poipulations of target phytophages [19, 24]. The obtained experimental data proved the high functional activity of strains BtH_I $87/_3$ and BtH_I $20/_3$ against the insect line of insects *Musca domestica* L., as well as natural populations of *Pieris brassicae* L. and *Archips crataegana* Hb. The presence of a thermostable exotoxin and stable manufacturability indicators during long-term storage and selective stages of culture support significantly expands the spectrum of entomocidal action of *B. thuringiensis* bacteria.

The results of many years of scientific fundamental and applied research indicate that a competitive, active and technological entomopathogenic strain of bacteria and quality indicators of the inoculant (high titer, functional activity of bacterial cells during their storage, etc.) increase the effectiveness of microbiological control of the number of phytophages [14]. According to scientific reports of recent years, the priority of search research for screening new producer strains of *B. thuringiensis* according to the criteria for determining the biochemical, molecular biological, genetic profile of biologically active substances that play a relevant role in the pathogenesis of insects (for example, δ-endotoxins, cytolytic Cyt parasporal toxins, α-, β-, γ- exotoxins and other enzyme complexes, lipopeptide cyclic antibiotics, other metabolites) [3, 6, 21, 22, 23, 33]. Therefore, the expansion of knowledge about the synthesis and production of entomotoxins by *B. thuringiensis* bacteria, pathogenicity in relation to harmful organisms allows them to be effectively used as a model system for studying the interaction and ecology of bacterial virulence. The selective specificity of *B. thuringiensis* for the circle of insects is directly related to the functional characteristics of insecticidal toxins and a certain biochemical structure of both the toxin itself and the infected insect. The sensitivity of insect varieties depends on a number of factors, namely, firstly, on the properties of the pathogen itself, which determine its aggressiveness, the production of

enzymes, toxins and other metabolites, and secondly, on the quantitative characteristics of the initial infectious agent, which are necessary for guaranteed infection of insects. These factors affect the process of pathogenesis (from slow to rapidly developed with maximum death during the first days after infection). Thus, it was experimentally proven that the most sensitive to the action of the bacterial complex *B. thuringiensis* are the pre-imaginal stages of development (especially young caterpillars) of insects, and the direct dependence of the death of infected bioassays on the dose of the pathogen (infectious load) was revealed. In the conducted research, we did not stop at the exclusive assessment of the percentage of death of insects (caterpillars) for a specific period. In subsequent studies, attention was focused on taking into account the death of insects at other phases of development, that is, specific age sensitivity in subsequent generations, as well as on changes that activate the decrease in the number of insects. Exotoxin causes a metatoxic effect to the greatest extent, thus including in the processes of growth and metamorphosis of the host insect (prolonged action of the entomopathogen). The versatile effect of microbial preparations based on *B. thuringiensis* bacteria consists of various parameters determined by the "pathogen-host" relationship. Therefore, the metatoxic effect of *B. thuringiensis* affects not only the treated generation, but also the following generations, which is confirmed by the studies of various scientists [18, 24, 25]. Microbiome *B. thuringiensis* CryI has an antifeedant effect. After intoxication, caterpillars use the fodder plant less actively, and individuals that have received sublethal doses of the entomopathogen cannot develop normally and are prone to various morphological and physiological abnormalities (retardation of growth, development and metamorphosis, teratogenesis, dereproductive effect etc.). After contact with an entomopathogen, individuals leave the infected environment, thereby contributing to the spread of epizootic micro-focuses. The action of the exotoxin of *B. thuringiensis*, introduced into the body orally, has the character of chronic intoxication of the insect. In terms of the pathological effect of action on phytophages, it is important that the exotoxin of *B. thuringiensis* can act by contact (through the integument of the insect), and in combination with the spore-crystal complex, it exhibits synergistic properties [4, 5, 20].

Thus, for the rational use of microbial preparations based on *B. thuringiensis* bacteria, the development and scientific substantiation of the parameters of their use, the analysis of the main factors that determine the effectiveness of the introduction of bioagents into the agrocenosis becomes extremely important. The specificity of the mechanism of action of *B. thuringiensis* allows the preservation of non-target objects, guarantees the safety of humans and the environment [9, 15, 30]. Accumulated data show that after the treatment of pest populations of insects with microbial agents *B. thuringiensis*, a longer period of insect death is observed, which leads to their stable depression. Due to this phenomenon, the tactics of using phytoprotective entomopathogenic drugs also change, that is, there is no need for multiple treatments of the same phytophagous population. A one-time introduction of bioagents against each generation and at specified times, when the larval phases are most sensitive to their pathogenic action, is sufficient. Considering the cyclical nature of epizootics and their correlation with the dynamics of the number of host insects, their role in regulating the number of bedbugs in natural populations is difficult to overestimate, while the influence of abiotic factors acquires secondary importance.

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REFERENCES

[1] Abo-Bakr, A., Fahmy, E.M., Badawy, F., Abd El-latif, A.O., Moussa, S., (2020): Isolation and characterization of the local entomopathogenic bacterium, *Bacillus thuringiensis* isolates from different Egyptian soils. Egyptian Journal of Biological Pest Control, 30: 54.

[2] Abulreesh, H.H., Osman, G.E., Assaeedi, A.S., (2012): Characterization of insecticidal genes of *Bacillus thuringiensis* strains isolated from arid environments. Indian Journal of Microbiology, 52(3): 500-503.

[3] Adang, M.J., Crickmore, N., Jurat-Fuentes, J.L., (2014): Chapter Two – Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action. Advances in Insect Physiology, 47: 39-87.

[4] Crickmore, N., Berry, C., Panneerselvam, S., Mishra, R., Connor, T.R., Bonning, B.C., (2021): A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. Journal of Invertebrate Pathology, 186: 107438.

[5] Chandrasekaran, R., Revathi, K., Senthil-Nathan, S., Kalaivani, K., Hunter, W.B., Duraipandiyan, V., Esmail, G.A., (2018): Eco-friendly formulation of wild *Bacillus thuringiensis* secondary metabolites through molecular characterization against the lepidopteran pest. Physiological and Molecular Plant Pathology, 101: 93-104.

[6] Chen, D., Moar, W.J., Jerga, A., Gowda, A., Milligan, J.S., Bretsynder, E.C., Rydel, T.J., Baum, J.A., Semeao, A., Fu, X., Guzov, V., Gabbert, K., Head, G.P., Haas, J.A., (2021): *Bacillus thuringiensis* chimeric proteins Cry1A.2 and Cry1B.2 to control soybean lepidopteran pests: New domain combinations enhance insecticidal spectrum of activity and novel receptor contributions. PLOS One, 16(6): e0249150.

[7] Chakroun, M., Banyuls, N., Bel, Y., Escriche, B., Ferré, J., (2016): Bacterial vegetative insecticidal proteins (Vip) from entomopathogenic bacteria. Microbiology and Molecular Biology Reviews, 80(2): 329-350.

[8] De Bock, T., Zhao, X.C., Jacxsens, L., Devlieghere, F., Rajkovic, A., Spanoghe, P., Höfte, M.I., Uyttendaele, M., (2021): Evaluation of *B. thuringiensis* – based biopesticides in the primary production of fresh produce as a food safety hazard and risk. Food Control, 130: 108390.

[9] EFSA Panel on Biological Hazards (BIOHAZ), (2016): Scientific opinion: Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. EFSA Journal, 14(7): 4524, 93 p.

[10] Federici, B.A., (1999): *Bacillus thuringiensis* in biological control. pp. 575–593. In Fisher, T.W., Bellows, T.S., Caltagirone, L.E., Dahlsten, D.L., Huffaker, C., Gordh, G. (eds.): Handbook of biological control. Academic Press, San Diego, California, USA.

[11] Goldman, E., Green, L.H., (2015): Practical handbook of microbiology. 3rd Edition. CRC Press, Boca Raton, 1055 p.

[12] Glare, T.R., O'Callaghan, M., (2000): *Bacillus thuringiensis*: biology, ecology and safety. John Wiley & Sons Ltd, Chichester, UK, 350 p.

[13] Irsad, Shahid, M., Haq, E., Mohamed, A., Rizvi, P.Q., Kolanthasamy, E., (2023): Entomopathogen-based biopesticides: insights into unraveling their potential in insect pest management. Frontiers in Microbiology, 14: 10.3389/fmicb.2023.1208237.

[14] Kandybin, N.V., Patyka, T.I., Ermolova, V.P., Patyka, V.F., (2009): Microbiocontrol of the number of insects and its dominant *Bacillus thuringiensis.* Innovation center for plant protection, St. Petersburg-Pushkin, 254 p.

[15] Khorramnejad, A., Gomis-Cebolla, J., Talaei-Hassanloui, R., Bel, Y., Escriche, B., (2020): Genomics and proteomics analyzes revealed novel candidate pesticidal proteins in a lepidopteran –toxic *Bacillus thuringiensis* strain. Toxins, 12(11): 673.

[16] Khorramnejad, A., Talaei-Hassanloui, R., Hosseininaveh, V., Bel, Y., (2018): Characterization of new *Bacillus thuringiensis* strains from Iran, based on cytocidal and insecticidal activity, proteomic analysis and gene content. BioControl, 63(8): 807–818.

[17] Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S., (2015): Insect pathogens as biological control agents: back to the future. Journal of Invertebrate Pathology, 132: 1–41.

[18] Liu, X., Ruan, L., Peng, D., Li, L., Sun, M., Yu, Z., (2014): Thuringiensin: a thermostable secondary metabolite from *Bacillus thuringiensis* with insecticidal activity against a wide range of insects. Toxins, 6: 2229-2238.

[19] Mazid, S., Kalita J.C., Rajkhowa R.C., (2011): A review on the use of biopesticides in insect pest management. International Journal of Advanced Science and Technology, 1: 169−178.

[20] Mac Innes, T.C., Bouwer, G., (2009): An improved bioassay for the detection of *Bacillus thuringiensis* β-exotoxin. Journal of Invertebrate Pathology, 101(2): 137-139.

[21] Mendoza-Almanza, G., Esparza-Ibarra, E.L., Ayala-Luján, J.L., Mercado-Reyes, M., Godina-González, S., Hernández-Barrales, M., Olmos-Soto, J., (2020): The cytocidal spectrum of *Bacillus thuringiensis* toxins: from insects to human cancer cells. Toxins, 12: 301.

[22] Melo, A.L.D.A., Soccol, V.T., Soccol, C.R., (2016): *Bacillus thuringiensis*: mechanism of action, resistance, and new applications: a review. Critical Reviews in Biotechnology, 36: 317–326.

[23] Mohan, M., Rangeshwaran, R., Sivakumar, G., Verghese, A., (2014): Relative toxicity of subspecies of *Bacillus thuringiensis* against lepidopterous insect pests of agricultural importance. Biological Control, 28(4): 197–203.

[24] Patyka, N.V., Patyka T.I., (2020): Symbiotic microbial communities of insects: functioning and entomopathogenic action potential initiation on the example of *Bacillus thuringiensis*. Microbiological Journal, 82(1): 52–63.

[25] Patyka, T.I., Lesovoy, N.M., Patyka, N.V., Kolodjazhnyi, A.Yu., (2016): Biocenotical approaches using entomopathogenic bacteria of *Bacillus thuringiensis* the season growing potatoes and storage. Microbiological Journal, 78(3): 69–77.

[26] Patyka, T.I., Patyka, N.V., (2020): *Bacillus thuringiensis* ssp. *israelensis* and control of aedes aegypti invasive mosquito species in ecosystems. Microbiological Journal, 82(5): 88–97.

[27] Pujiastuti, Y., Astuti, D.T., Afriyani, S.R., Suparman, S., Irsan, C., Sembiring, E.R., Nugraha, S., Mulawarman, Damiri, N., (2018): Characterization of *Bacillus thuringiensis* Berl. *indigenous* from soil and its potency as biological agents of *Spodoptera* litura (*Lepidoptera*: *Noctuidae*). IOP Conference Series: Earth and Environmental Science, 102: 012064.

[28] Pujiastuti, Y., Arsi, A., Sandi, S., (2020): Characteristics of *Bacillus thuringiensis* isolates indigenous soil of South Sumatra (Indonesia) and their pathogenicity against oil palm pests *Oryctes rhinoceros* (*Coleoptera*: *Scarabaeidae*). Biodiversitas, 21(4): 1287–1294.

[29] Raymond, B., Federici, B.A., (2017): In defense of *Bacillus thuringiensis*, the safest and most successful microbial insecticide available to humanity – a response to EFSA. FEMS Microbiology Ecology, 93(7): fix084.

[30] Sánchez–Yáñez, J.M., Rico, J.L., Ulíbarri, G., (2022): *Bacillus thuringiensis* (*Bt*) is more than a special agent for biological control of pests. Journal of Applied Biotechnology & Bioengineering, 9(2): 33–39.

[31] Sufyan, M., Abbasi, A., Wakil, W., Gogi, M.D., Arshad, M., Nawaz, A., Shabbir, Z., (2019): Efficacy of *Beauveria bassiana* and *Bacillus thuringiensis* against maize stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). Gesunde Pflanzen, 71: 197–204.

[32] Tepper, Ye.Z., Shil'nikova, V.K., Pereverzeva, G.I., (1979): Microbiology manual. M.: Kolos, 138 p.

[33] Vachon, V., Laprade, R., Schwartz, J.L., (2012): Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: a critical review. Journal of Invertebrate Pathology, 111(1): 1–12.

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