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BACILLUS THURINGIENSIS SSP. *ISRAELENSIS* AND CONTROL OF *AEDES AEGYPTI* INVASIVE MOSQUITOES SPECIES IN ECOSYSTEMS

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The review presents materials on microbiocontrol of vector mosquitoes using entomopathogenic bacteria Bacillus thuringiensis ssp. israelensis (Bti). Control of invasive ectoparasites is a major health issue, as mosquito species are capable of transmitting diseases, including extremely dangerous human and animal infections (malaria, tularemia, yellow fever, hemorrhagic fevers, dengue fever, taiga (or tick-borne) encephalitis, filariasis, Ku fever, cattle anaplasmosis and many other infections and invasions). Bti is considered worldwide as a promising microbial agent that combines targeted efficacy in protective measures and environmental safety. The study of the effect of Bti δ-endotoxins on the intestinal epithelium of Aedes genus mosquitoes, search and characterization of specific receptors are extremely important for understanding the mechanism of action and activity of entomocidal proteins, the basis of the pathogenic effect of polytypic B. thuringiensis. Synergism, a combination of selective larvicidal action of natural Bti strains in combination with strategies of resistance emergence preventing demonstrate a wide range of possibilities of their use and unique evolutionary features of this endospore-forming bacterium as a modern larvicidal agent against Aedes aegypti population.

Keywords: Bacillus thuringiensis ssp. israelensis, Aedes aegypti, larvicidal properties, δ-endotoxins, entomospecificity.

Ecosystems are a source of natural resources. The current scale of change and degradation of the upper «living» cover of the planet forces us to choose the unconditional preservation of natural biological systems and their diversity as a priority. The existence of biological diversity and its complexity is based primarily not on the totality of genomes, species or ecosystems, but on the diversity of the formation of terrestrial resources on the principle of distribution of functional orientation [1–3]. Total disruption and even destruction of natural systems, primarily due to climate change, «undermines» species diversity and the hierarchical structure of groups – a necessary condition for stability (buffering) and homeostasis of the biosphere. Minor and short-term climate change is compatible with the resilience of ecosystems and its functions, but long-term – negatively affect biodiversity. It has already been proven that climate change will lead to an irreversible reduction in biodiversity, and many species will not be able to adapt to new living conditions. Therefore, the changes and disturbances in the flora and fauna are inevitable, which will also affect the diversity and complexity of biochemical processes of the main habitat of biome – soil [4, 5].

A significant range of species has spread in the pole direction and in the vertical direction and this trend will continue. The consequence is a gradual redistribution of dominant and edificatory species, a change in the number and structure of realized ecological niches in biocenoses and, finally, the displacement of aboriginal species and the formation of new complexes that will be different from those that have evolved. Phenological changes in populations, including shifts in reproduction cycles or delays in growth periods, affect species interactions. Insects belong to poikilothermic animals – their body temperature depends on the temperature of the environment, and the ability of thermoregulation is limited. Therefore, among the abiotic factors of the ecological niche of insects, temperature is one of the main factors that affects them not only directly (due to the state of physiological and biochemical systems of the body), but also indirectly [5, 6]. For example, because of the state of vegetation. Significant number and complexity of other measurements

of an ecological niche depend on temperature. Such as humidity of soil, mobility and number of parasites and predators and the rate of development of entomopathogens and the like. Populations of different species of insects that are part of the ecological group are characterized by specific temperature preferences. Therefore, temperature also significantly affects the ecological and taxonomic structure of the entomological grouping, levels of dominance, etc.

But temperature is not the only factor that affects the dynamics of the number of species. Environmental factors affect ecological systems collectively through direct and reverse pathways. In this regard, it should be noted the significant role of trophic relationships, which indirectly modify the number of individuals in the population at the stations. Thus, the most important parameters of climate change, i.e. temperature, precipitation, CO_2 emissions and concentrations and the dynamics of water regime and resources, affect all levels of biodiversity organization – genes, species and habitats. At the basic level of biological diversity, climate change can increase genome tension and thus reduce the genetic diversity of populations through changes in the direction of selection, genetic drift, population differentiation and their rapid migration. As a result, the ability of living organisms to adapt to new environmental conditions is reduced, which means that the risk, including extinction is growing. In addition, changes in species interactions directly affect the functioning and homeostasis of ecosystems [7, 8]. At a higher level of biodiversity, climate change is causing changes in plant and microbial communities, which will have a greater impact on the integrity of the biosphere as a whole. Estimation of changes in ecosystems over the millennium shows that they have been exposed to about 5–20 % of the Earth's ecosystems [9, 10]. The issues of ecosystem resilience thresholds, which lead to irreversible changes in biomes, are of particular concern. Such thresholds are actually possible due to the ecological understanding of the parameter of sustainability or changes in environmental factors as alternative states of ecosystems. As the potential for hysteresis shows, groups and ecosystems can be in such configurations that they are poorly restored after change. There are examples of the consequences of the invasion of exotic species, and undesirable changes in vegetation in terrestrial ecosystems. Once the ecosystem enters the danger zone, one of its parts is in danger of crossing the threshold, the other is not. Measures to increase the resilience of ecosystems, i.e. the conservation of biodiversity, are becoming crucial.

Thus, ecosystems can be considered as a source of natural resources, generating flows of intermediate and final goods and services of ecosystems, which will be affected by the above factors and climate change. Natural resources include renewable (primarily due to natural conditions and living organisms) and non-renewable resources (for example, biotic, geological, water, atmosphere and land resources).

Circulation of entomopathogenic microorganisms in the natural environment (soils, plants, water bodies, insect populations and their habitats) allows to regulate the number of skin creatures, limiting their reproduction. The potential of *B. thuringiensis* bacteria as a working pure insect method grew high using insects, which was the main source of their screening and specialized environment, using the "moderator" of functional activity responsibilities of employees in natural ecosystems. *B. thuringiensis* strains have been isolated worldwide from many habitats, including soil, insects, stored-product dust, and deciduous and coniferous leaves, all of which have a limited host range, however together span a wide range of insects orders which include: *Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Phthiraptera, Orthoptera, Acari*, and *Mallophaga* and other organisms such as nematodes, mites, and protozoa [11, 12]. The search for new varieties of crystallogenic bacilli strains with entomotoxic properties (*B. thuringiensis* ssp. *israelensis (Bti)*, *B. sphaericus, B. subtilis, Peanibacillus macerans* and others) in different climatic zones of the world is actively continuing and due to their biodiversity the range of target insects is expanding [13–15]. Exploratory studies of entomopathogenic bacteria are carried out in places of mass reproduction and number of insects with foci of spontaneous epizootics. When collecting material in such places, dead insects are preferred, from which infectious agents are isolated. In other cases (if the pathogen was not isolated from the carcasses of insects) live material is collected in different phases of development, placed in high-density insect conditions (to create a stressful situation). If a population is infected with an entomopathogen, it should manifest under these conditions and will be easily isolated.

Diversity of species composition of invasive mosquito species and the need to develop targeted programs to control their numbers. The role of blood-sucking mosquitoes (*Culicidae*)

in different ecosystems is extremely diverse and therefore quite difficult to form human interactions with them (protection, regulation of their populations, etc.). The number of blood-sucking mosquitoes species is more than 3,500, which are distributed on all continents (except Antarctic Continent). There are about 90 species of mosquitoes in Europe, which belong mainly to the genera *Anopheles, Aedes, Culex, Culiseta, Mansonia*. Being ectoparasites, attacking people and animals en masse, they deplete their owners (parasite-host), reducing the efficiency of humans and animal productivity. In the historical literature, «mosquitoes» are known for torturing people (by immobilizing a person in places of mass mosquito attack). In addition, the role of mosquitoes as carriers of a significant number of transmissible infections, including particularly dangerous for humans and animals (malaria, tularemia, yellow fever, hemorrhagic fevers, dengue fever, taiga (or tick-borne) encephalitis, filariasis, Ku fever, cattle anaplasmosis and many other infections and invasions) is significant. Invasion of vectors creates conditions for the emergence of new natural foci of these infections and determines the main feature of possible outbreaks – their territorial unpredictability and extremely active development during the introduction of the pathogen [16, 17]. Since the 1990s, there has been an increase in the prevalence of invasive mosquito species (*Aedes albopictus, Aedes aegypti*), which are carriers of arboviruses, in the WHO European Region. [18-20]. Among the most invasive species are Asian tiger mosquitoes *Aedes albopictus*, yellow fever *Ae. aegypti*, and Asian shrub *Ae. japonicus* [21].

The term «vector ability of the mosquito population» indicates the degree of risk of transmission of pathogens in specific conditions, i.e., in the presence of local sources of infection, the appropriate climate and the presence of appropriate target hosts (so-called «caregivers host»). The probability of transmissible infections transmission increases with the number of vectors. Transmission of diseases is also possible at low or medium density of vectors, if they have a high degree of competence (high percentage of infected females).

The diversity of species composition of invasive mosquito species in Ukraine and the countries of independent states, their phenology, biotope distribution, ecological features – depending on external factors and vital features of *Diptera* – necessitate the development of targeted comprehensive regional programs to control their numbers, which include careful entomological monitoring, distribution the number of mosquitoes, clarification of their species composition, carrying out preventive, protective measures against the most epidemically significant species of insects [21]. Identification of *Culicidae* vectors and understanding of the genetic diversity of invasive insect populations allow scientists to develop appropriate preventive measures [22].

Pesticides (larvicides) are among the most common methods of mosquito control, which are used in the habitats of larvae, against adults at open stations, as well as residual insecticides, which are used to treat the premises and so on. In the last decade there has been a gradual increase in the use of pyrethroids (deltamethrin, permethrin). International scientific papers are increasingly reporting the emergence and development of insect resistance to various classes of pesticides (carbamates, organochlorine, organophosphorus compounds and pyrethroids). [23, 24], and there is a growing evidence that the problem of pesticide resistance threatens the success of pesticide protection measures [25, 26].

Today it is important to raise awareness of modern methods of ectoparasite control, the most important aspects of invasive mosquitoes and new communicable diseases, as well as to promote the development of analytical skills needed for strategic planning and implementation of preventive and environmentally safe (selective) protection measures. Thus, mosquito control is a difficult task, as these *Diptera* have enormous reproductive potential, short generation time, high environmental plasticity and high ability to spread, including by passive transfer in air currents. For successful practical use of larvicidal biological (microbial) preparations, it is advisable to take into account many factors and circumstances, including the characteristics of habitats – reservoirs, their area to be treated, depth, water supply, flow, overgrowth and organosalt indicators of water. The species composition of mosquitoes and their numerical ratio must be taken into account when choosing the dose of the drug. The current main requirement for insecticides used in practice is a combination of targeted efficacy and safety. In this regard, it is difficult to overestimate the role of biological agents, in particular microbial preparations based on entomopathogenic bacteria *B. thuringiensis*, which show a significant degree of effectiveness against targets and environmental safety.

According to long-term studies, the spectrum of *Bti* strains larvicidal activity includes *Diptera* of the genera *Anopheles, Aedes, Culex, Culiseta, Limatus, Uranotaenia, Psorophora, Mansonia, Armigeres, Trichoprospon, Coduellettidida, Cricotopus, Simulium, Cnepia, Austimulium, Prosimulium, Prosimulium*, and the number of sensitive species is increasing (there are already more than 95) [17, 27]. The efficiency of *Bti* microbial preparations on the larvae of *Aedes* mosquitoes (*Ae. communis, Ae. aegypti*) after three days exceeds 90.0 %. Studies have established the rapid death of mosquito larvae. So, the death of insects began from the 6th min after application of 4 μg/ml of the pathogen and reached a maximum 27 min later. When the lethal concentration (LC_{50}) (10 ng/ml) was applied, the death of insects began after 37 min and reached a maximum 120 min later [28]. The toxicity of the crystal-spore mixtures obtained from the studied *Bti* isolates indicated that the 50% LC_{50} varied from 0.19 to 4.5 μg/l^{-1} [29]. Infectious load of *Bti* spore forms from $5x10^4$ to $5x10^7$ spores/ml causes complete death of *Culex pipiens molestus* mosquito larvae within 1-4 hours. Strains retain activity for up to 11 days in the laboratory at a dose of $1x10^5$-$4x10^5$ spores/ml in water. Larvicidal activity, which is determined by LC_{50}, is $1.8x10^3$ after 48 hours for *Aedes communis* mosquito larvae (L_4), and $4.2x10^3$ for *Culex pipiens* (L_2). To completely destroy the larvae of mosquitoes of the genera *Aedes*, *Culex*, and *Anopheles*, a dose of $2x10^4$-$3x10^5$ spores and *Bti* endotoxin crystals is sufficient. The metatoxic effect provides high efficiency of microbial preparations on the basis of *Bti* in natural conditions and is shown not only on the processed generation of insects, but also on the subsequent generations [27, 30].

Natural entomopathogens of *B. thuringiensis* ssp. *israelensis* as producers of larvicidal entomotoxins. Screening of *B. thuringiensis* strains and study of their variability with entomospecific toxins continues worldwide [31, 32]. The selective specificity of pathogens of this group in relation to the circle of insects is directly related to the functional characteristics of the endotoxin and a certain biochemical structure of both the toxin itself and the infected insect. The genetic diversity of *B. thuringiensis* toxins plays a crucial ecological and evolutionary role: it promotes the development of high adaptability and survival of subspecies of entomopathogenic bacteria in different biocenoses. Science already knows about 500 types of δ-endotoxins produced by *B. thuringiensis* [32]. The first information about entomopathogenic *Bti* H_{14} and H_{13} strains with larvicidal properties appeared in the 70s of the twentieth century in Israel and Pakistan, which were successfully used against blood-sucking mosquitoes and midges. On the basis of natural *Bti* H_{14} strains in the former USSR and abroad a series of biological products of long-acting larvicidal action (Bactoculicid, Teknar, Baktimos, Vektobak, Mosquitur and others) was created. These drugs, despite the homogeneity of the producer of *Bti* H_{14}, differed significantly in effectiveness [27]. In California, E. Davidson [33] isolated an insecticidal strain, a potential mosquito larvicide *B. sphaericus*, from the larvae of *Culiceta incidens*. Ecologically, this bacterium is considered a plastic species [34]. *B. sphaericus*, like *B. thuringiens* H_{14}, is the best source of highly effective larvicidal drugs for microbiological control of mosquitoes and gnats. In parallel with scientific research there was a development of technology of production of biologicals on the basis of *B. thuringiensis* entomopathogens of various serological options and increase of volumes of their production and application [27, 35].

Entomopathogenic bacteria *Bti* H_{14} do not produce a thermostable water-soluble exotoxin – its effect on the target is due to crystalline δ-endotoxin. Thus, the biological effect of entomotoxins of *Bti* spore bacteria is based on the cytopathological action of δ-endotoxins on the intestinal epithelial cells of insects. Endotoxin, which is found in the shell of spores and vegetative cells, causes destructive changes in the intestinal wall of mosquito larvae, especially its middle part. Therefore, the study of the effect of δ-endotoxins on the intestinal epithelium, search and characterization of relevant receptors are extremely important for understanding the mechanism of action and specificity of entomocidal proteins, the basis of the pathogenic effect of *B. thuringiensis* strains. At least three genes for insecticidal proteins are encoded using the 128 kB pBtoxis conjugative plasmid containing the toxin genes: Cry10Aa, Cyt2Ba, and Cyt1Ca [36], but the degree of representation of each protein is still being actively studied worldwide and is likely to vary depending on the strain and prescription form of commercial biotechnology products. To construct *Bti* strains with more efficient synthesis of larvicidal, entomocidal components and a wide range of susceptible host insects, protoxin identification and genetic characterization should be done, and its localization in the plasmid or in chromosomal DNA should be defined. The main tool of phylogenetic analysis of *B. thuringiensis*

strains is the comparison of similar in structure genes or proteins, and above all, the comparison of their primary sequences. Using a combination of data from the study of 16S rRNA, it is possible to confirm the species affiliation of strains and phylogenetic relationships within the species [37, 38]. Thus, using the reference strain *Bti* H_{14} 7-1/23 (ARRIAM, St. Petersburg, Russia) in phylogenetic studies allowed to confirm the existence of a strain polymorphism for *B. thuringiensis* ssp. *thuringiensis*, *darmstadiensis* and *israelensis* [37], associated with their entomocidal activity. Comprehensive analysis of crystallogenic (virulent) and acrystallogenic (avirulent) *Bti* strains 404, 87, 7–1/23 using the method of polymerase chain reaction in the early stages of development of cultures of the studied microorganisms (not more than 18 hours) revealed genes encoding target insecticides Cry4 and Cry11, while acrystallogenic strains of these genes do not have this genes [39]. It was proved that crystallogenic and acrystallogenic variants of *Bti* did not differ in basic biochemical properties, morphology of vegetative culture and colonial diversity on nutrient media, including in terms of technological productivity (in the range of 3.36–4.02 x 10^9 and 3.74–4.13 x 10^9 CFU/ml). High larvicidal activity according to LC_{50} in relation to larvae of the 4th age of the insect line *Ae. aegypti* (0.12–0.15 x 10^{-3}%) was recorded after 15 minutes of laboratory experiment for active strains producing δ-endotoxin (1.0% suspension of culture fluid).

Some *Bti* strains after selection and storage may lose the ability to produce toxins, as confirmed by microscopic analysis. This may be a manifestation of the dissociation of the culture into different populations, which are characterized by significant differences in their biological properties. Any microbiological reseeding or rehydration of *Bti* spore cultures certainly affects the manifestation of their variability. In this context, the original genetically determined heterogeneity of culture properties should be considered. With the help of modern molecular biological methods it is possible to obtain positive results of toxin formation after amplification with appropriate primers and to confirm the biogenesis of δ-endotoxin (or to prove the violation of the expression of target genes) [40, 41]. To control the processes of dissociation, including in the aspect of genetic determination of toxin formation and to avoid its negative consequences for practical tasks, scientists are actively developing selection criteria aimed at linking the physiological properties of culture with different (critical) characteristics. It is important that *B. thuringiensis* is a polytypic species, many serotypes of which have significant differences. Therefore, specific *Bti* strains will have correlated almost valuable properties with some biochemical characteristics of the culture, others – with some other indicators of physiological and biochemical properties of entomopathogenic bacteria [42]. When discovering new knowledge about the spectrum of species-specific action of *B. thuringiensis* ssp. bacteria it is necessary to «reset» application technologies, problems of persistence of these entomopathogens in the natural environment, to make new requirements concerning the preparative forms which differ from biological preparations on the basis of *Bti* of terrestrial application. To control the qualitative and functional characteristics of strains, their molecular genetic certification (creation of a strain-specific passport) can be carried out using various methods: analysis of polymorphism of lengths of amplified DNA fragments, ALFP fingerprinting; pulse electrophoresis; of highly productive full-genomic sequencing or analysis not of the whole genome, but of individual taxonomically and functionally significant genetic loci, reference complexes [43–46]. The comparative analysis of nucleotide sequences of *Bti* strains reveals unique genes (housekeeping genes), the expression of which is necessary to maintain vital functions of bacteria, as well as virulence genes, sequencing of which is essential for authentication of commercial strains, confirmation of their technological quality in entomotoxins [47].

For model laboratory studies it is advisable to use the larval stage of development of *Ae. aegypti* insect populations of third age, which are reborn from eggs of a homogeneous population of the species during the first two hours. The larvae are maintained at a temperature of 28^{0}C and the frequency of lighting for at least 12 hours. A mixture of dry non-fat yeast can serve as food for larvae up to the stage of pupae. In further work, as a rule, bioassays are placed in insect conditions, with metamorphosis of insects to the stage of imago and the laying of new generation eggs on the prepared filters. Larvae revived from eggs within 2–3 hours are caught and placed in a cuvette with water and after reaching third stage of age (after 4 days) are used in further experiments. Depending on the purpose and objectives of research, it is possible to use a population of two-winged insects, which are collected from natural reservoirs of different natural and climatic zones. A solution of an aqueous

suspension of cultures of *Bti* entomopathogenic bacteria in various concentrations (1.0; 0.5; 0.25; 0.125; 0.06 mg/l), provides death of 90.0-96.0 % of the test object [27]. The criterion for the activity of these entomopathogenic bacteria is the value of their concentration in water, which provides death of 50.0 % of the studied insects (LC_{50}) when freely absorbed by the larvae of the sporo-crystalline complex from an aqueous suspension of the drug (this is in line with WHO guidelines for the standardization of larvicides).

The larvicidal properties of natural *Bti* strains demonstrate a wide range of capabilities in mosquito protection systems. High efficiency of dry and liquid forms of microbial preparation on the basis of *Bti* (Bactoculicid) within 24–72 hours is revealed. Since the biological product of this type has a mechanism of intestinal action, its biological activity is manifested during the period of active feeding of young insects. The death of mosquitoes is observed during the first three days after treatment. When the entomopathogen enters the digestive tract of larvae, toxicosis occurs (the body of individuals becomes vitreous with a noticeable rigidity), infected larvae do not respond to touch, accumulate in groups and then sink to the bottom. In the complete absence of mobility, the larvae remain alive for some time, and then their death occurs.

At various fluctuations of a temperature mode of water larvicidal activity of *Bti* remains at a high level. In the technological aspects of biolarvicides use it is necessary to take into account the peculiarities of water supply, flow, the degree of vegetation growth and organosalt parameters. When choosing the dose of the microbial drug, it is necessary to study the species composition of mosquitoes and the numerical ratio of species. It is established that the final larvicidal action of a biological product based on *Bti* depending on the chemical composition of water; the content of organic impurities, lighting and temperature of water and the type of larvae can vary within 5–10 days [48]. Today, there are more than 19 new types of toxins with larvicidal properties in addition to the known ones Cry16Aa, Cry17Aa, Cry19Aa, Cry19Ba, Cry20Aa, Cry20Ba, Cry24Aa, Cry24Ba, Cry24Ca, Cry25Aa, Cry27Aa, Cry29Aa, Cry30Aa, Cry30Ba, Cry30Ca, Cry30Da, Cry30Fa, Cry30Ga, Cry39Aa, Cry40Aa, Cry40Ba, Cry44Aa, Cry48Aa, Cry49Aa, Cry52Ba, Cry54Aa, Cry56Aa, Cry60Aa, Cry60Ba, belonging to different serotypes of *B. thuringiensis*. Thus, the synergism, the combination of selective entomotoxic action and the anti-resistant effect of *Bti* strains are the unique evolutionary features of this bacterium, which makes it an ideal biocontrol agent for the *Aedes* genus mosquito populations.

Existing microbial preparations based on *B. thuringiensis, B. sphaericus* actively block the spectrum of sensitive pests. Knowledge about other entomopathogenic species, in particular *Brevibacillus laterosporus* (formerly *Bacillus laterosporus*), which produce crystalline endotoxins, is deepening. The larvicidal activity of native culture fluid or sediment of strains of these bacteria against mosquitoes *An. stephensi, Ae. aegypti* has been proved [49]. High level of larvicidal activity of *B. laterosporus* (LAT006) strain crystals for mosquitoes *Ae. aegypti, An. stephensi* (LC_{50} 3.0 ng/ml and 5.0 ng/ml, respectively), which can be compared with the activity of highly toxic *B. thuringiensis* and *B. sphaericus* strains, allows us to consider *B. laterosporus* as producer of biological insecticides.

Information on the selectivity, hygiene and environmental safety of *Bti* is the rationale for the widespread introduction of biological products based on these bacteria. In terms of virulence, toxicity and toxigenicity, *Bti* strains are not pathogenic for warm-blooded animals, non-target aquatic organisms and belong to class IV toxicity (low toxicity), meet the requirements for industrial microorganisms. *Bti* cultures and formulations with a spore titer of at least 100 billion spores/g in experiments on vertebrates at different routes of entry into the body in high doses do not have pathogenic or toxic effects, do not have skin irritants, allergens and sensitizing properties. LD_{50} for *Bti* (Bactoculicid) is 10 g/kg of body weight, and *Bti* H_1 (Bitoxybacillin) – 6 g/kg of body weight of warm-blooded animals [50]. The maximum permissible concentration (MPC) of the biological agent *Bti* in water is 5 mg/l. These values correspond to the safest hazard class for warm-blooded animals, the value of which exceeds 1000 mg per 1 kg of body weight. Thus, the results of *Bti* comprehensive tests, tests for toxicological characteristics and hazards of biological preparations are consistent with the world practice of widespread use of bioinsecticides based on entomopathogenic *B. thuringiensis*.

To summarize, it should be noted that nature has all the mechanisms to control the most important biosphere processes — the synthesis of biologically active substances that significantly affect the

physiological state of the object and its immune status, intermicrobial interactions, entomotoxic effects on target insect populations, etc. The system of biological control of ecocenoses plays a leading role in solving problems that arise as a result of pesticide exposure, disturbance of homeostasis. The concept of such protection is based on classical models of bioregulation (microbiomethod), i.e. the use of living organisms or products of their metabolism against living components of the ecocenoses, as well as methods aimed at managing primarily natural (biotic) environmental factors that can limit the number and harm validity of organisms. The development of a new generation of biopesticides based on *Bti* bacteria with increased resistance to targets significantly expands the range of use of specific bacterial cultures and allows to optimize fermentation processes. The specificity of the mechanism of *Bti* bacteria action allows to store non-target objects, guarantees safety of the person and environment (transformation of protoxin into active toxin occurs only in the conditions of alkaline pH and in the presence of certain proteinases). Success in improving insecticidal activity through the genetic evolution of Cry toxins will depend on knowing the steps of limiting Cry toxicity in different target insects, reflecting the specificity of region binding in Cry toxins, and improving mutagenesis strategies and selection procedures. The presence of toxin genes creates a platform for the formation of new combinations in the simultaneous infection of the target insect with two or more *Bti* strains. The system of coding on plasmids of characteristic features that are essential for the survival of microorganisms under appropriate conditions, determines the widespread use of *Bti* in various ecological niches. The widespread distribution of toxigenic bacteria in nature and the «variegation» of substances with toxic properties indicate the great importance of toxins for the ecology of microorganisms. Selection of naturally occurring beneficial producer strains that combine virtually valuable properties, in particular high initial manufacturability, stability of the titer of sporo-crystalline complex and toxigenicity of lauricidal metabolites is important, as well as further research to identify optimal conditions for their cultivation to obtain formulations of biopesticides.

BACILLUS THURINGIENSIS SSP. *ISRAELENSIS* І КОНТРОЛЬ ІНВАЗИВНИХ ВИДІВ КОМАРІВ *AEDES AEGYPTI* В ЕКОСИСТЕМАХ

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Резюме

В огляді представлені матеріали відносно мікробіоконтролю комарів-векторів за допомогою ентомопатогенних бактерій *Bacillus thuringiensis* ssp. *israelensis* (*Bti*). Контроль інвазивних ектопаразитів є головним питанням охорони здоров`я, оскільки різні види комарів здатні до передачі трансмісивних, у тому числі особливо небезпечних інфекцій людини і тварин (малярія, туляремія, жовта лихоманка, геморагічні лихоманки, лихоманка Денге, тайговий (або кліщовий) енцефаліт, філяріоз, лихоманка Ку, анаплазмоз великої рогатої худоби і немало інших інфекцій та інвазій). *Bti* розглядають у всьому світі як перспективний мікробний агент, котрий поєднує в собі цільову ефективність у захисних заходах і екологічну безпеку. Вивчення впливу *δ*-ендотоксинів *Bti* на кишковий епітелій комарів роду *Aedes*, пошук і характеристика специфічних рецепторів надзвичайно важливі для розуміння механізму дії і активності ентомоцидних білків, основ патогенного ефекту політипового виду *B. thuringiensis*. Синергізм, комбінація селективної ларвицидної дії природних штамів *Bti* у поєднанні з антирезистентними стратегіями захисту демонструють широкий діапазон можливостей їх використання і унікальні еволюційні особливості цієї ендоспорової бактерії в якості сучасного ларвицидного агента по відношенню до популяції *Aedes aegypti*.

Ключові слова: Bacillus thuringiensis ssp. *israelensis, Aedes aegypti,* ларвицидні властивості, *δ*-ендотоксини, ентомоспецифічність.

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