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Use of Entomopathogenic Bacteria *Bacillus thuringiensis* in the Republic of Uzbekistan: Problems and Prospects

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A B S T R A C T

The Republic of Uzbekistan is a country specializing in agriculture, with favorable climatic conditions that allow high yields of almost all types of crops.At the moment, in our republic, 1 million 387 thousand hectares of land is used for growing cotton, about 1 million 700 thousand hectares of grain products (including 1 million 200 thousand hectares of irrigated areas), more than 560 thousand hectares of fruits and vegetables and products horticulture, and 967.8 1000 hectares are specialized in growing fodder plants. In the absence of large losses in the cultivation and storage of agricultural products, this amount of land would be enough to freely provide the population of the republic with food and technical raw materials and export some of the products. This article provides analytical information about the work carried out on the basis of the entomopathogenic bacterium *Bacillus thuringiensis* in the protection of agricultural crops from harmful insects. Information is also provided on the production of β-exotoxin, *L*-exotoxin, *α*-exotoxin, Y-exotoxin, crystalline δ-endotoxin and their classification.

Introduction

Considering that according to statistics, the population of the Republic of Uzbekistan is increasing by 650-670 thousand people per year, it will reach 47 million by 2026. This shows that the population's demand for agricultural products will increase several hundred times. In particular, the day by day increasing demand for environmentally friendly products requires reducing the amount of

chemical preparations during the cultivation of agricultural products and instead using preparations obtained by environmentally friendly biological methods.

During the cultivation of agricultural products in our country, 20-30% of productivity is lost as a result of various harmful insects and microbiological diseases. Practical experience shows that the productivity of agricultural plants is lost from 10% to 80% due to the development of some extremely harmful organisms. (Turabekova *et al.,* 2022). Pests such as locusts, bollworms, and spiders can be cited as extremely harmful organisms. In 2002, the 1st quarantine objects, that is, 131 species that are not found in the territory of Uzbekistan, and 12 species that are partially distributed in the 2nd territory of Uzbekistan were registered as quarantine objects in our country. More than 150 harmful organisms have been recorded during the cultivation of spiky plants. In particular, it has been proven in many studies that the yield of one grain is lost from 5% to 50% due to the influence of harmful organisms during its storage. The fact that more than 220 types of pests and diseases have been reported to damage cotton during its growth indicates the urgency of combating these harmful organisms

At this point, a natural question arises, what is the status of the use of microbiological preparations in the fight against harmful insects in our country? It seems that the answer to this question is not difficult. Because the fight against pests is mainly based on chemical preparations. Because a number of microbiological preparations have been approved by the Republican Committee for Testing and Authorization of Chemical and Biological Preparations and included in the register for 2021-2026: *lepidocide, dendrobacillin, navodor*, etc. So, it is necessary to find an answer to another legitimate question. Why microbiological preparations available in the registry are not widely used.

Because they have a number of negative indicators, such as the low level of impact and not being compatible with our climate. This makes it necessary to carry out in-depth scientific research on this direction in our Republic. Because when we analyzed the scientific research conducted before 1970-1988, it was proved by a number of our scientists that microbiological preparations are very effective. The conclusions of the scientific research conducted in this direction and the fact that these preparations are not used in agriculture at the moment caused us to conduct scientific research on this problem.

First, I will talk about some important features of the entomopathogenic bacteria *Bacillus thuringiensis* (Bt). *Btentomopathogenic* bacteria are mainly soil microorganisms and were discovered by the Japanese scientist (Ishiwata, 1901). Since then, its effect on insects has been widely studied in agriculture. It has been widely used in agricultural practice since the 50s of the last century.

During growth, the Bt bacterium secretes poisonous crystalline protein compounds (toxins) that differ from each other in terms of chemical composition. These toxins can be divided into several groups.

β-exotoxin

β-exotoxin - released into the liquid nutrient medium of the culture during cell growth. β-exotoxin, by its nature, is a compound formed by the combination of adenine nucleoside and phosphoric acid. Under its influence, ATF biosynthesis slows down and eventually stops completely, the relationship between nucleases and DNA-RNA-polymerases is broken, and RNA synthesis stops as a result. The toxin affects a wide range of insects and has a very active effect on their early years.

When we compare the toxins produced by Bt bacteria with other toxins, its effect is slow, based on a certain pattern, it acts during the transition of the insect from one stage of development to another. Sublethal doses cause stomach upset in most insect full-stage: egg-larva-beetle-puppet butterflies. When we observe the developmental stages of insects, β-exotoxin is a mutagen for them, that is, it causes

changes in their genetic apparatus. All kinds of negative situations are observed in their next generations.

L-exotoxin

In some literature it is also called L-exotoxin. Phospholipase C is an α-exogenous bacterial enzyme. In addition, this enzyme is called lecithinase and "phospholipase-3". So named because it cleaves the glycero-3-phosphate bond with natural phospholipids. It is also known in the literature as lecithinase or S-phospholipase. Biochemical and immunochemical analyzes confirmed that phosphodilinositol is identical to specific S-phospholipase. At the same time, attention is paid to lecithinase-producing cultures of *Bac.thuringiensis*. It has an active toxic effect on some insects (lepidoptera).

α-exotoxin

α-exotoxin is a product of growing cells of bacteria. The enzyme disrupts the exchange of phospholipids in the insect tissue, resulting in death (Bykov *et al.*, 1987).

Ivanov and Kuzmanova (1990) studied the relationship between virulence and enzyme activity in entomopathogenic bacteria and found that the S-phosphalipase enzyme plays a special role in the strain's high virulence. The authors studied the relationship between specific phospholipase S of *Bac.thuringiensis* and pathogenicity and concluded that S-phospholipase is the main factor in the entomocidal effect of Bt bacteria. They also provided information on the release of S-phospholipase of Bt bacteria, its specific nature and hydrolysis of n-nitrophenylphosphoryl-choline, and its entomopathogenic properties.

Y-exotoxin

The nature of this toxin has not yet been fully elucidated (Bykov *et al.*, 1987). This toxin is found in entomocidus culture (Bt serotype VI).

Crystalline δ-endotoxin

Or double spore crystalline endotoxin is produced during the sporulation process of a bacterium after the spore is formed in one part of the cell, the resulting crystal has a regular octagonal appearance.

Crystal synthesis takes place in the stationary phase of the culture for about three hours. Several crystals of various shapes can be formed in the cell (correctly bipyramidal, rhombic, cubic to oval).

Their sizes can be reduced from 0.5×1.3 to 1×3.5 mkm and even submicroscopic. They are insoluble in organic solutions, but can be separated from the spore, they dissolve well in highly alkaline conditions (pH above 11.5) and their solubility increases in the presence of a reductive alkaline buffer (pH 7.9-9.5). Crystals lose their toxicity when heated at a temperature of 100ºC for 30-40 minutes (De Barjac, 1978; Azizbekyan, 1988).

Its chemical composition includes 19 elements, including carbon, nitrogen, hydrogen, oxygen and amino acids. In addition to these, there are significant amounts of calcium, magnesium, silicon, iron and small amounts of elements such as nickel, titanium, zinc, aluminum, chromium, copper and manganese. Protein crystals in different strains are very close to each other in amino acid composition.

Crystal proteins are closely related to the protein of spore shells according to their chemical nature. There are theories in the literature that spores are formed from an overproduced protein in the cell envelope. These protein crystal endotoxins are gaining importance due to their extreme toxicity to insects.

Strains of Bt bacteria differ from each other in terms of virulence and specific characteristics of the effect on insects. Cultures of *Bacillus thuringiensis* bacteria are grouped into several serotypes according to their N-antigenicity (Khujamshukurov *et al.*, 2001; Khujamshukurov and Davranov, 2003; Khujamshukurov *et al.*, 2001).

Serotype 1 (N_1)

Bacillus thuringiensis. var. Thuringiensis produces acetylmethylcorbinol and lecithinase. Does not form pigment. Digests sucrose, cellobiose and mannose. Hydrolyzes starch. Several strains produce heat-resistant toxins.

Serotype 2(N_2)

Certain microorganisms that have not yet been used in the fight against agricultural pests are considered.

Serotype3a (N_{3a})

Bacillus thuringiensis. var. alesti produces acetylmethylcorbinol and lecithinase. Ferments sucrose and mannose. Forms acid from cellobiose. Hydrolyzes starch. Forms pigment. Does not form toxins resistant to high temperatures.

Serotype 4a 4b (N_{4a}, N_{4b})

Bacillus thuringiensis. var. dendrolimus. It produces acetylmethylcarbinyl and lecithinase. It does not form acid from sucrose and mannose. Hydrolyzes starch. Does not produce urease and pigment. Several strains produce heat-resistant exotoxins.

Serotype 5 (N_5)

Bacillus thuringiensis. var. galleriae. Forms acetylmethylcarbinyl. Digests salicin and cellobiose. Hydrolyzes starch. Does not absorb sucrose and mannose. It does not form a toxin resistant to high temperatures.

Serotype 6(N_6)

Bacillus thuringiensis. var. entomocidus. It forms acetylmethylcorbinol. Absorbs sucrose, mannose and starch. It does not form a toxin resistant to high temperatures.

Serotype 7 (N_7)

*Bacillus thuringiensis. var. aizavai.*Forms acetylmethylcorbinol. Absorbs calicin and cellobiose. Does not absorb sucrose and mannose. It does not form a toxin resistant to high temperatures. Some strains hydrolyze starch.

Serotype 8 (N_8)

Bacillus thuringiensis. var. anagastae. Forms acetylmethylcorbinol. Does not absorb salicin and mannose. Absorbs sucrose and cellobiose. Some strains hydrolyze starch. Produces a toxin resistant to high temperatures.

Serotype 9 (N_9)

Bacillus thuringiensis. var. tolvorthi. Forms acetylmethylcorbinol. Absorbs calicin, cellobiose, starch and sucrose. Does not absorb mannose. Produces a toxin resistant to high temperatures.

Serotype 10 (N_{10})

Bacillus thuringiensis. var.darmstadiensis. It forms acetylmethylcorbinol. It does not form acid from sucrose, silicin and mannose. It forms acid from cellobiose. Hydrolyzes starch. Does not produce urease. Produces an exotoxin.

Serotype 11 (N_{11})

Bacillus thuringiensis.var.toumanoffii. It forms acetylmethylcorbinol. Forms acid from mannose and cellobiose. Hydrolyzes starch. Produces urease. It does not form a toxin resistant to high temperatures.

Serotype 12 (N_{12})

Bacillus thuringiensis. var. thompsoni. Acetylmethylcorbinol does not form. Hydrolyzes starch. Forms acid from mannose. Produces urease. Produces high temperature resistant exotoxin.

Serotype 13 *(N_{13})*

Bacillus thuringiensis. var. pakistani. Acetylmethylcorbinol does not produce lecithinase or phospholipase C. It forms acid from salicin and

sucrose. Hydrolyzes starch and cellobiose. Does not produce urease and high temperature resistant exotoxin.

Serotype14 (N_{14})

Bacillus thuringiensis.var.israelensis. Forms acetylmethylcorbinol. It ferments mannose. Hydrolyzes starch. Does not produce urease and high temperature resistant toxin. It forms a light pink pigment. It is highly toxic to the larvae of Culexpipiens, *Aedes aegypti* and some other mosquitoes.

It is known from scientific sources that the ability of Bt bacteria to form spore-crystals depends on its serotype and strains (Smirnova *et al.*, 1977).

Based on these characteristics, it is important to identify the types of *Bacillus thuringiensis* entomopathogenic bacteria found in the conditions of Uzbekistan, to classify them, to develop technological indicators for practical processes based on researching their agriculturally important biologically active properties, including insecticidal, larvicidal, antifungal, antibacterial properties. It is appropriate to analyze the scientific research carried out in the conditions of Uzbekistan up to that time.

Materials and Methods

Methods of studying the bacterium *Bacillus thuringiensis*. *Bacillus thuringiensis* entomopathogenic bacterium was isolated with some modifications to the generally accepted methods (Skvortsova, 1977), and the generally accepted method was used for sterilization of insects (Kamenyok, 1998) with some modifications. This modification was applied to larvae that were too small to clean the upper part. Further work was carried out in three stages: first, it was kept in 70% alcohol for 2 seconds, in a 5% sodium hypochlorate solution for 3 minutes, and in a 10% sodium thiosulfate solution for 5 minutes. Then the samples were washed 3 times in distilled water. Two different methods were used to isolate *B. thuringiensis* samples. In both cases, the larvae were aseptically placed in sterile flasks. Then they were diluted by adding 10 ml of distilled water.

In the first method, the suspension was placed in a tubular test tube and kept at a temperature of 65ºC for 12 minutes, then it was diluted from 1×10-2 to 1×10-4 and planted in a solid nutrient medium with agar. Cultures were grown at 28-32°C for 24 hours. In the second method, separation was done using the method recommended by Faust *et al.*, (1974), in which heating was carried out as mentioned above. The difference between these methods is that in the second one, only spores of entomopathogenic bacteria *B.thuringiensis* can be selected. Sodium acetate from 0.25M to 0.5M added to the feed in this separation stops the growth of spores of entomopathogenic bacteria. The first method can be used in cases where the amount of materials is not large.

Identification of specimens

Bacterial cultures were grown on agar medium, washed in 0.7% NaCl solution, and studied under a microscope for the production of crystal toxin characteristic of the entomopathogenic bacterium *B.thuringiensis*.

Biochemical and physiological properties were determined based on Bergi's instructions (Holt *et al.*, 1997), and biochemical properties of cultures were studied according to the scheme for identification of *Bacillus thuringiensis* species (De Barjac, 1978).

Standard nutrient media

In the cultivation and maintenance of cultures, a standard nutrient medium with peptone ((%) Peptone-1.0; NaCl-0.05; K2HPO4-0.05; MgSO4-0.02; pN 7.0) was used. We carried out the investigations mainly in the MBI-3 light microscope (LOMO, Russia). Fuchsin dye was used to stain the cultures. Bacterial cultures were planted in a liquid nutrient medium and grown at a temperature of 28-300C for 36-48 hours using a microbiological shaker

at 180-200 revolutions per minute (Khujamshukurov, 2003).

Microscopic examination of insects and microorganisms

A dead insect or a certain part of it (depending on the size) was placed on the objective glass and studied in the light through a small eyepiece. And the difference of organs can be observed very well in one or several drops of water. In this case, using a bacteriological hook or a pointed needle, a drop of water is taken and dripped onto the glass of the object, and a microscopic analysis is carried out. The infected organ or tissue of the insect was carried out according to the following method and as a disinfectant, an aqueous solution of iodine (0.1%-5 min), lysol (3%-3 min), formalin (5%-15 min) and corbalic acids (2% -2-3 min) was used and then dipped in insect ether or peroxide. After the top part of the insect egg was sterilized, the shell part was removed and examined under a microscope in a drop of water.

Results and Discussion

Professor E. N. Troitskaya has conducted extensive scientific research on the study of various properties of the entomopathogenic bacterium Bt in the conditions of Uzbekistan. In particular, the species of Bt bacteria found in various agricultural pest insects were classified and their levels of insecticidal activity were determined. Troitskaya (1982) carried out monitoring of changes in their biological activity during long-term storage and storage of *Bacillus thuringiensis* strains, and it was noted that the most effective storage method is storage in a dried pest insect organism.

Professor A. Khamroev of the Research Institute of Zoology of the RFA and his students (Juginisov *et al.*, 2009) have comprehensively studied the activity of Bt bacteria against termites, which is one of the least studied properties, and wooden columns that are architectural monuments of ancient cities of Uzbekistan, including Khiva the degree of damage by termites was studied, the biological activity of *Btentomopathogenic* bacteria was shown in combating them, and a collection of Bt bacteria was created (Khamraev *et al.*, 2010).

The employees of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan identified the types of Bt-entomopathogenic bacteria that are common by insect species in the city of Tashkent and the Tashkent region, when classifying them, the serological properties of dissociation are shown, and for the normal growth and development of *Bacillus thuringiensis* cultures, nutrition is recommended based on residual products formed during cooking cocoons, composition of the environment (Magay, 2007). In addition, scientific information was obtained on the molecular properties of β-exotoxins produced by the entomopathogenic bacterium *Bacillus thuringiensis*, the level of its formation, and the factors affecting its biological activity (Khujamshukurov, 2016).

In recent years, the scientists of the Scientific Research Institute of Plant Protection of Uzbekistan have been studying the biological effectiveness of insecticides produced on the basis of Btentomopathogenic bacteria in Uzbekistan against various harmful insects. According to the obtained results, almost 85 percent of the studied preparations were recommended for use. These include Russian Biotoxibacillin, Lepidotsid, Beta-pro drugs produced in South America.

The specific effect of the Btentomopathogenic bacterium on various types of insects is being extensively studied by the staff of our scientific laboratory (Khujamshukurov, 2004). According to the obtained scientific results, crystals in insects are activated only after they are divided into small toxic fragments under various effects.

Under the influence of the molecular mechanism of these toxic fragments, it has been shown that insects stop feeding, paralyze, cause symptoms such as epithelial breakdown, disrupt the ability of

endotoxin to selectively pass through the intestine of insects (Khujamshukurov *et al.*, 2013).

It is known from scientific sources that blood-sucking insects, especially malariogenic species, pose a very high risk to human health. They are characterized by massive blood-sucking and spreading of a number of dangerous and extremely dangerous diseases.

According to the World Health Organization, 300 million to 500 million people are infected with malaria every year, including 2.5 million. patients die without treatment.

Currently, more than 2 billion people worldwide live in dangerous epidemic areas where blood-sucking insects that cause malaria, yellow fever, dengue fever, encephalitis and other such transmissible diseases are widespread. There are more than 3000 species of these insects, of which more than 400 species cause dangerous diseases in humans. Culex and Aedes species, which are considered to be vectors of malaria, are known to have spread to areas other than Karel, Sindbis, and South Nile, where arboviral, transmissible fever is widespread.

Dangerous and extremely dangerous diseases spread on a large scale by blood-sucking insects have also been recorded in neighboring CIS countries. In particular, in neighboring countries such as Russia, Tajikistan, Kyrgyzstan, Turkmenistan, Kazakhstan, the blood-sucking insect species Anopheles is important and plays the main role as a carrier of dangerous diseases (Makadzhanova, 1992).

Malaria is an acute transmissible infectious disease, the main source of which is malaria patients. The disease is mainly transmitted to a healthy person by the bites of malarial mosquitoes. Malaria patients have symptoms such as high body temperature, chills, anemic liver and enlarged spleen.

We considered it necessary to make a special note about it as a study area, since the incidence of malaria is mainly recorded in the Surkhandarya region. This region is mainly located in the south of our republic, borders on the neighboring republics of Afghanistan and Tajikistan, the climate here is continental. In total, there are 14 districts and 1 city in the region with a population of 1,896,857 people. District farms are mainly specialized in cotton growing, grain growing, cocoon growing, animal husbandry and gardening, and law enforcement. In total, there are 10,325 reservoirs in the region, the total area of which is 10,836 hectares, of which 3,379 hectares are the water area where fly larvae were found (Khujamshukurov, 2016).

2,028 hectares of identified water bodies are swamps and reservoirs, 5,237 hectares are streams and tributaries, and 2,784 hectares are collectors and abandoned water bodies.

Malaria is a disease transmitted mainly by mosquitoes of the genus Anopheles, and in the Surkhandarya region there are 5 species of malarial mosquitoes: *An.superpictus*, *An.gerkanus*, *An.pulkherimus*, *An.martenus*, *An.clovigir*. Malaria mosquitoes breed mainly in ponds, swamps and rice fields. There are 4 types of malaria: three-day malaria, four-day malaria, three-day malaria and tropical malaria. In Surkhandarya region, which is the southern gate of our country, a three-day type of malaria is mainly recorded. Surkhandarya region is recognized as the region with the largest number of cases of malaria in the country.

The reason for this is the prevalence of malaria in the neighboring republics of Afghanistan, Tajikistan and Kyrgyzstan. Including 1 million in Afghanistan in 2002. more than 11,500 people from Tajikistan and more than 3,000 people from Kyrgyzstan (Khujamshukurov, 2016).

As a result of such factors as the economic and social ties of our republic with these countries, the migration of the population from neighboring republics, the risk of spreading malaria remains in our country. In addition, there are cases of widespread malarial insects due to the settlement of sholi-poyas in restricted areas, as well as the lack of

processing them with the appropriate biological and chemical method. In particular, in the Muzrobot district, Anopheles flies breed mainly in ponds and ponds. Only on the example of this region during 2002 it was recorded that rice was sown on a total of 76.5 hectares in the yards of the collective farms Beshkoton, Attermizy, Navbakhor, A.Ikromov, such situations cause problematic situations.

Therefore, we set ourselves the goal of studying the possibilities of using environmentally friendly, microbial preparations in the fight against blood-sucking insects. As a result of extensive screening, various strains of Bacillus thuringiensis bacteria were studied according to their morphocultural, physiological and biochemical characteristics and conditionally divided into four groups: *Bac.th.var.galleriae,* II-*Bac.th.var.thuringiensis*, III-*Bac.th. var.dendrolimus,* IV-*Bac.th.var.kurstaki.*

For the first time, the analysis of malaria zones in the Surkhandarya region was carried out, the larvae of Anopheles superpictis, *An.hyrcanus* and *An.pulcherrimis* mosquitoes belonging to endogenous and exogenous species were isolated, compared with them in laboratory conditions in short-term (24 hours) and long-lived (48-72 hours).

Entomopathogenic bacteria *Bacillus sphaericus* and *Bacillus thuringiensis*, moderate conditions were chosen to determine the larvicidal activity. It was established that strains of *Bacillus thuringiensis var.thuringiensis-45t, Bacillus sphaericus-Bsph47, Bacillus thuringiensis var.israelensis-Bti-30a* under these conditions exhibit high larvicidal activity against the larvae of the studied insects and were chosen as objects for further research. For the first time, a technology has been developed for obtaining a biopreparation "Biolyarva", which is highly effective in the fight against blood-sucking insects.

It is known that in recent years the range of application of insecticidal, larvicidal, fungicidal and bactericidal preparations created on the basis of entomopathogenic bacteria *Bacillus thuringiensis* has been expanding. However, there are some problems in the implementation of these drugs. This limits the range of applications of these drugs, which are environmentally friendly, inexpensive and relatively easy to use.

In particular, a number of shortcomings can be listed, such as low biological activity of the preparations being created compared to chemical preparations, limited range of use, insufficient effectiveness in wide field conditions, lack of phage-resistant producers, intolerance of crystalline toxins based on the preparation in dry and hot climates.

If we look at the mentioned shortcomings from a fundamental point of view, we can see a number of neglected issues that still need to be solved. In particular, in the process of spraying the drug on plants, the level of adhesion of vegetative cells, spores and crystal toxins to plant stems and leaves and the possibilities of natural preservation have not been studied. However, isolated strains of the entomopathogenic bacterium *Bacillus thuringiensis* have been found to be isolated from soil and dead insect organisms in relatively equal amounts from plant leaves and stems.

In addition, when using preparations based on these bacteria, with various additives (talc, kaolin, etc.), for sticking to the leaves and branches of plants, as well as protection from various influences (wind, rain, sunlight, various mechanical vibrations, etc.) biofilms and adhesives are used for this.

Preparations based on bacteria *Bacillus thuringiensis* have two different effects: firstly, crystal-toxins, once in the body of an insect, decompose under the action of alkali, poison the body and cause the death of the insect. The next effect occurs as a result of the synthesis of spores and crystallotoxins during the development of spores that have entered the insect's body in a favorable environment. At the same time, vegetative cells and crystal toxins, which have not yet entered the body of Bt bacteria and soil, stuck to the plant body and leaves, and crystal toxins have a very high probability of death in a short period of time.

Fig.1 Diagram of isolation and purification of *Bacillus thuringiensis* entomocidal crystal proteins (Azizbekyan, 1988)

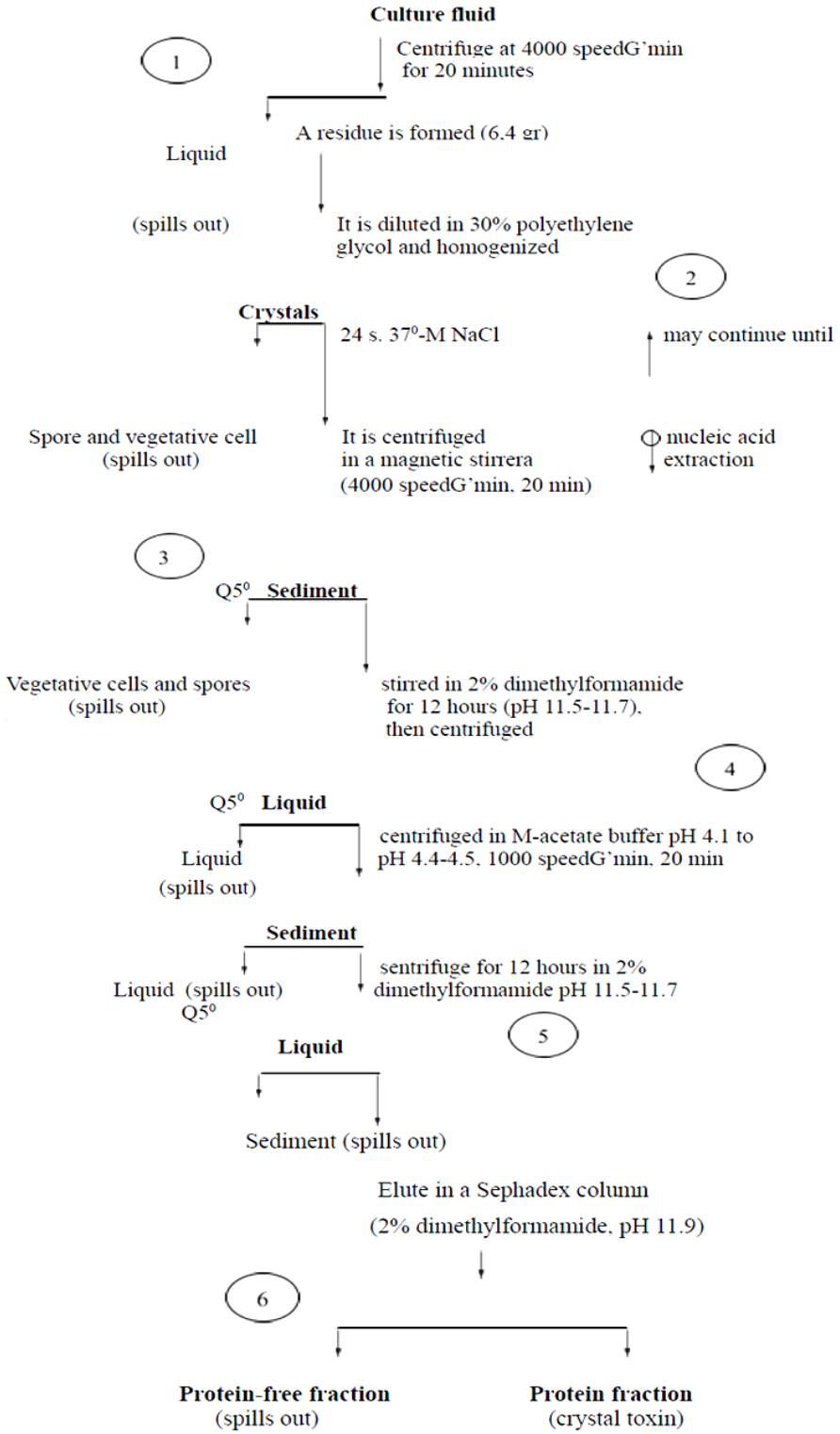
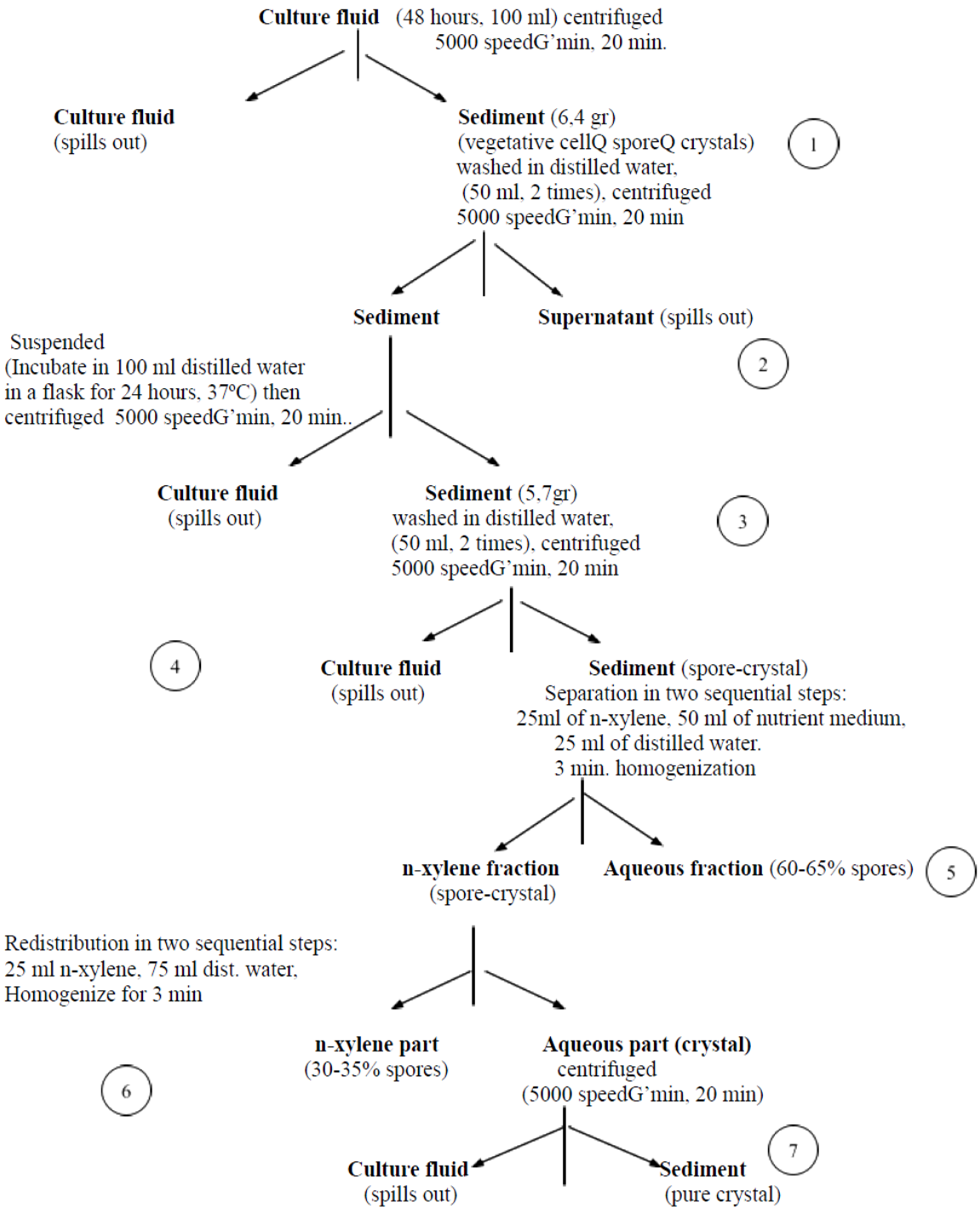


Fig.2 Diagram of separation and purification of spore-crystals of *Bacillus thuringiensis* bacteria from culture fluid



However, the resistance of spores to various influences has been studied in detail in the literature and persists in nature for a long time. Therefore, using its resistance to various influences, it becomes possible to further increase and expand the scope of these drugs. To this end, one of the urgent fundamental tasks is to study the nature of spore adhesion to the surface of bodies and sources that determine this property.

Due to the fact that there are very few scientific studies on this topic in scientific sources, in the course of our research, spores of Bt bacteria strains with different biological activity, which are stored in our collection, were studied.

To do this, first of all, the problem arises of isolating spore crystals of Bt bacteria in a pure form. Therefore, we first studied several methods for isolating spore crystals of Bt bacteria in the culture liquid.

The nature of δ-endotoxin of Bt-bacteria, its chemical composition and mechanism of action on insects are widely covered in scientific sources. One of the distinguishing features of this group of bacteria is the formation of protein-like crystaltoxins along with the formation of spores. Therefore, the separation of spore and crystalline toxins and the study of their properties requires the creation of separate methods.

These methods can be divided into four groups according to the nature of the spores and crystals: solubility, surface area, spore density and growth. Since the insecticidal property of these bacteria is associated with the activity of crystalline toxins, the main attention is focused on it. And since our main goal is the study of spores, we focused on spores when choosing suitable methods. Although no specific methods have been developed in this regard, the fact that spores are also separated during crystal separation is widely reported in the literature.

While familiarizing with scientific sources, one can see that there are many such methods as well as their incompatibility. This can be explained by the fact that the possibility of choosing a specific method is limited due to the fact that different modifications are used for different studies.

One of them is the method of isolating insecticidal crystaltoxins recommended by Afrikyan (1973), and it is recommended to dissolve the crystals in a slightly more slightly alkaline medium with 2% dimethylformamide. Our research process with this method is shown in Figure 1. As can be seen from Figure 1, it is not possible to separate individual spores in our experiments. In particular, the amount of added mixture of vegetative cells and spores increases as the possibility of isolation of crystals in the 2nd, 3rd, 4th and 5th stages of the process increases. In this case, the possibilities for studying the features of spores are limited. As a result of the research, it was noted that this method is not possible to study disputes. Therefore, we used one of the most modern methods - the method of cell disintegration using high-speed ultrasound in the process of spore formation. In this method, spore-forming cells are destroyed by high-voltage ultrasound. After cell destruction, spores and crystals are separated by centrifugation.

The present study carried out in this way can be seen in microscopic studies that the crystal-proteins are not fragmented. Difficulty centrifuging the lysed cells in this method was noted. Because the density of spore crystals is 1.30-1.25 g/sm3. However, the potential for spore outgrowth or distortion may limit the applicability of this method.

Therefore, we tried to separate spores and crystals using this n-xylene. It was noted that the spores can be isolated cleanly and harmlessly in the following manner (Figure 2).

The advantage of this method is that the composition of the crystalline proteins is not disturbed when separated using n-xylene. Also, spores are not damaged because they are surrounded by a protein shell. The sequence of the implemented process is shown in Figure 2. As can be seen from

the diagram 2, in the 5th step of the process, it is observed that the spores pass to the aqueous part up to 60-65%, in this case, it is possible to extract the spores cleanly, but it does not reach 100%.

In order to be accurate in research, 90-95% of spores are required to be isolated. Therefore, we tried to continue the process carried out in step 5. As a result, in the 6th stage, crystals remained in the aqueous phase, and only spores remained in the xylene phase (25-30%). So, spores can be separated from vegetative cells and crystals by this method. In addition, it is possible to separate the crystals themselves in a very clean state (95-96%) at the next stage.

The resulting spores can be precipitated from the xylene phase using distilled water and studied. The fact that the spores and crystals extracted by this method can be stored in a refrigerator for long-term study with drops of immersion oil is also useful for our further research. This method is also suitable for studying the effects of purified spores and crystals on various insects. Through this method, it is also possible to dry the isolated spores and store them for a long time as a source of reproduction. In theory, their biological activity will not be lost. Therefore, on the basis of the obtained scientific results, a method was developed for the pure isolation of crystal proteins and spores of Bt entomopathogenic bacteria, which differ according to their specific characteristics, using n-xylene, using a two-stage sequential processing method. In addition, on the basis of research conducted on the basis of 52 strains, it was noted that the location and size of the tumors in them were different and they were conditionally divided into 4 groups: three groups were subject to the peritrichous and 1 lophothric organ of movement (Turabekova *et al.*, 2023).

It was found that the spores of the studied cultures contain tubular, filamentous, cylindrical and villi-shaped outgrowths. Also, it was found that all the studied strains have different follic agglutinating activity, among which the high activity was found to be shown by spore growths in tubular form. It was concluded that these growths are actively involved in the adhesive activity of spores of the entomopathogenic bacterium Bt. This, in turn, can serve to increase the adhesive activity of spore outgrowths in insecticidal preparations sprayed on the surface of plant leaves.

Another example of this problem is that in mountainous areas, ponds formed by rainwater, rivers and tributaries, and the failure of water bodies to support appropriate biological and chemical treatments also create problematic situations. For example, there is a risk of the disease spreading due to the lack of gambuzization in mountain and sub-mountain areas, as a result of the consumption of children, shepherds, especially livestock from the resulting ponds and water bodies.

Currently, blood-sucking insects are mainly controlled by chemical insecticides. The development of resistance in insects to these insecticides requires an increase in the dosage of this insecticide or its replacement. Analyzing the example of Surkhandarya, the following larvicides are currently used against blood-sucking insects: methoprene (altozid), dimilin (diphtorbenzuron), diphos (abbat, 50% k.e., 10% granules), VTI vectobac, spherolarvicide, kerosene, gasoline (non-ethylated), baytex (fenthion, sulfidiphos, lebaitzid, 50% k.e.) actellik (pirimiphos, 50% k.e.), metathion (folithion, sumithion, fenitrothiol, 50% k.e.), oil, oil and etc.

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