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ENTOMOPATHOGENIC BACILLUS THURINGIENSIS TO ISOLATE BACTERIAL STRAINS AND APPLY THEM AGAINST GREENHOUSE MITES

To'xtamishyeva Nilufar Alisher kizi

Master of Tashkent State Agrarian University

Jumanazarov G'ayrat Xusanovich

Associate professor of Tashkent State Agrarian University

Abstract: The article presents experimental data on methods for isolating Bacillus thuringiensis bacterial strains from dead greenhouse aphids and soil, and on the use of different concentrations against greenhouse aphids that damage tomato plants.
Keywords: Bacillus thuringiensis, bacteria, strain, culture medium, tomato, pest, greenhouse aphid, nutrient medium, entomopathogen, soil.

Introduction. The eve of the 21st century is characterized by an unprecedented scale of conscious use of microorganisms in agricultural production. In all areas of the agro-industrial complex, the need for the use of microorganisms and their metabolites was recognized, and, accordingly, the development and use of biopreparations for the needs of Plant Science, Livestock, Veterinary and feed production is being established.

There is also an increasing need for microbial preparations in the field of protecting plants and agricultural animals from pests and parasites. As a competitor to the chemical means of controlling insects, rodents and phytopathogens, biopesticide is becoming an attribute of advanced agricultural systems. The need for them is associated with the size of man-made loads on the environment, the inefficiency of chemical pesticides, the risk of toxic, allergic and even genetic adverse effects with their widespread use and the development of resistance in pests, the elimination of natural mechanisms for regulating their number and, finally, the pollution of landscapes, water resources and food products.

Scientific sources know that in later times the microbiological method of combating insects is widely used in the agrarian industry. Therefore, the production of environmentally friendly microbial pesticide biopreparations is of great scientific theoretical and practical importance.

Research methods. The distribution of the main pests of tomato and cucumber plants in the greenhouse was examined by taking samples diagonally or chess. In the course of the survey, samples of pest insects that had died were collected in sterilized paper bags, which were prepared in advance. Each sample was stored in refrigerators until it was examined in the laboratory.

In order to extract entomopathogenic fungi from pests of tomato and cucumber plants, insect specimens that died due to the disease were cleaned of microorganisms, that is, their outer part was sterilized. The pest samples that were being tested were soaked in a 0.5-1% sodium hypochloride (NaOCl) solution for 30 seconds and thoroughly washed out of the solution and rinsed once again in another container of sterilized water, then the pest samples were picked up in front of an alcohol lamp flame on the surface of a solid food medium placed on sterilized Petri saucers. Each specimen was placed in a separate petri saucer with a pest sample of 10-15 pieces. Petri was stored in thermostats with a temperature of 24-260 C. The growth of bacteia in petri lycobchas was observed for 15 days from the third day. Then the fungal mycelium grown from the specimens planted in the artificial food environment in Petri's saucers was planted in test tubes with a sterilized microbiological loop in front of an alcohol lamp flame. In order to extract disease-causing bacteria from insects, the composition was used from different food environments (Agar Chapek, agaric beer suslosi, agaric potatoes). The pure culture of the bacteias was assigned to the solid food environments in the test tubes.

To isolate the isolates of Bacillus thuringiensis, extinct effluent samples collected from the field were used. Bacterial extraction was conducted according to the method described by Gonzalez et al (2011). The collected effluents were previously crushed in an 80 oC water bath for 10 minutes to kill vegetative cells and non-spore-forming bacteria. This ground oocyanote was then grown in a GPA nutrient medium. Colonies similar to those of Bacillus have been isolated and examined for the presence of papasporal crystals. Then B.thuringiensis colonies were identified and isolated. Here are 3 B.isolated thuringiensis isolates (BTE-1, bte-2, bte-3). They were found to differ from each other in crystal shape and morphology of their colony.

Also isolated from the soil were the isolates Bacillus thuringiensis. To do this, 2-3 cm of the top layer was removed. Then a 20 gr soil sample was placed in special bags. The special bags were kept at 4 oC until laboratory work began. To extract bacteria from soil samples, Song et al. (2008) through the method described. 1 g of soil sample 10 ml 0.85% NaCl was suspended. Then heated in a vibrating thermostat at 70 °C for 10 minutes. The nutrient agar (0.5% pepton, 0.3% beef extract, 0.5% NaCl and 1.5% agar) was suspended for 100 µl. This used 50 petri dishes, all incubated at 30±2 °C for 48 hours. Bacterial colonies showing a phenotype similar to Bacillus thuringiensis were isolated and moved to a new food environment. These colonies were reared a second time. The cultures were stained with fuchsin and examined under a standard light microscope for initial identification. From The Soil B.2 isolates similar to thuringiensis were isolated (BTE-4, BTE-5).

Figure 1. Isolated B.colonies of thuringiensis isolate and its appearance under a microscope

Research results. The fact that the greenhouse leech, which is considered the most dangerous pest insect in our God at the next time, has increased, causes enormous problems in growing pamidors and cucumbers in the greenhouse. As a result of this, 25-30% of the crop extracted from pamidor and cucumbers in the cultivated areas of the Republic is destroyed under the influence of harmful organisms.

In solving this problem, in order to obtain biopreparation in our work, Bacillus thuringiensis was tested in various concentrations against the most economically damaging greenhouse leachate in the greenhouse industry by preparing a cultured liquid as a starting material from bacterial strains.

To study the entomopathogenic activity of the resulting strains, it was studied in relation to ethanol in small field conditions. Our experiment was carried out as part of the study of the entomopathogenic effects of Bacillus thuringiensis bacterium 5-endotoxin on greenhouse effluents.

Table 1 below records the data obtained from the study of the effects of greenhouse effluents of 0.3% and 0.5% solution of isolated Bacillus thuringiensis bacterial cultures. From bacterial strains Bacillus thuringiensis, a cultured liquid was prepared as the starting material. The experiments were carried out in a greenhouse that harms the pamidor plant planted in the experimental Agricultural Greenhouse of the Tashkent State Agrarian University in the Qibray District of the Tashkent region. Before processing with a cultured liquid made from bacterial strains, the damage to the greenhouse plant was monitored, that is, the number of pests was calculated. In the experiment, 10 pamidor plants were selected in one row for each option, and the number of pests on the plant was taken into account (Table 1).

Table 1.

Bacillus thuringiensis application of different concentrations of cultural fluidity of bacterial strains against greenhouse fluidity

№	Types	Concentration of cultural fluid, %	Number of living greenhouse leaks until processing, donadona	Number of pests that die, %			
				Days of receipt of the chisobi after processing			
				3-day	6-day	9-day	12-day
1	BTE-1	0,3	82,7	9	20	36	49
		0,5	47,4	14	27	41	54
2	BTE-2	0,3	65,3	11	23	39	51
		0,5	72,5	17	32	53	68
3	BTE-3	0,3	49,7	-	-	15	27
		0,5	56,4	-	9	18	31
4	BTE-4	0,3	67,2	-	-	-	-
		0,5	58,6	-	-	-	17
5	BTE-5	0,3	77,8	-	-	13	26
		0,5	81,1	-	7	16	29
6	Control (water)		48,9	-	-	-	-

During the experiment, the number of pests killed on 3, 6, 9 and 12 days after treatment with a cultured liquid made from bacterial strains was calculated. The best in the experiment showed the result at a concentration of 0.5% of the BTE-2 strain. According to this, when the account of the 3rd day after processing was taken, 17% recorded the death of the pest, by the 12th it was found that 68% of greenhouse leeches had died. In our experiment, no pests that died in our variant, which used a concentration of 0.3% of the BTE-4 strain, were observed.

Conclusion. Based on the experience carried out, it is possible to draw conclusions below. The entomopathogenic activity of isolated BTE-1, bte-2, bte-3, bte-4, and bte-5 isolates against greenhouse leaching has been studied. In doing so, isolated BTE-2 and bte-1 isolates from the effluent itself were found to be most active against greenhouse effluents. And a 0.5% solution of the cultured liquid obtained from them was recommended for use against greenhouse leaks.

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