Use of pathogenic microorganisms in the control of the Colorado potato beetle (*Leptinotarsa decemlineata* Say.)

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Abstract. The focus of this article is the comprehensive study of the Colorado potato beetle population within potato agro-biocenosis. The research delves into the intricate processes that contribute to the formation and bioecological characteristics of this beetle population. The article also delves into a specific strain known as *Beauveria bassiana* VTq-28, which was isolated from the Colorado potato beetle. This strain was subjected to testing both in laboratory settings and in the field, targeting various developmental stages of the Colorado potato beetle. The objective was to evaluate the effectiveness of *Beauveria bassiana* VTq-28 as a biocontrol agent against the beetle. Additionally, the research assessed the insecticidal activity of locally sourced strains of B. thuringiensis against the Colorado potato beetle. This analysis provides insights into the potential of *B. thuringiensis* strains as another avenue for biocontrol. By thoroughly examining the population dynamics, bioecological characteristics, and the potential of specific microbial agents for controlling the Colorado potato beetle, this study contributes to the understanding of pest management strategies within potato agro-biocenosis. The findings have implications for sustainable agricultural practices and the effective control of this economically significant pest.
Keywords. *Beauveria bassiana*, *B. thuringiensis*, biocontrol, microorganisms, Colorado potato beetle.

1 Introduction

To study infectious diseases of insects and determine their significance, it is necessary to have sufficient knowledge about microorganisms [1]. Therefore, for practical use in the fight against these pests of microorganisms that cause infectious diseases of insects, scientists in this field have proven the development of microbiological preparations based on them [2]. Several microbiological practices are being developed based on entomopathogenic viruses, bacteria, fungi, microsporidia and nematodes.

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The world is constantly expanding the use of microbiological preparations to protect plants from harmful organisms. According to experts, the market share of biopesticides was determined at 20% in 2020 and amounted to $8 billion. Regarding the scale of use, preparations based on *Bacillus thuringiensis* Berliner (Bt) were the first in the world. In 2009, practices based on *Bacillus thuringiensis* Berliner (Bt) were used on 50 million hectares worldwide, and 33 million hectares were found in the United States [3, 4].

The microorganism *Galleria mellonella* has been found to be pathogenic for a number of insect species after oral infection by Hurst et al. [5]. The combined use of entomopathogenic microorganisms *B. bassiana* and *B. thuringiensis* against the Colorado potato beetle showed that the synergism coefficient was high at low temperatures and led to a 100% death rate.

In the experiments of Wojda et al. [6], the entomopathogens B. bassiana and B. thuringiensis were isolated from insects that died from various infectious diseases, and the competitive relationship between the fungus and bacteria was also studied. Both pathogens have been found to coexist in dead insects. With a mixed infection, it was found that both fungi and bacteria are isolated from dead insects. Still, in most cases, entomopathogenic fungi are isolated at low temperatures. And at high temperatures - bacteria. As a result, an intensive development of bacteria at high temperatures was established.

Experiments were carried out [7-9] to determine the biological activity of the fungus *Beauveria bassiana* in relation to the larvae of the Colorado potato beetle. The experiment used natural pest populations in potato plantations of the Almaty region (Kazakhstan) [10]. Infection was carried out through an aqueous suspension of fungal conidia. When used in a titer of 1x107 ml per larvae, it was found that the death of larvae on the 7th day of registration was 32.0-47.0%, and on the 13th day - 85.0-100.0% [11-13].

In the fight against agricultural pests in various climatic conditions of Uzbekistan by the microbiological method

The relevance of biopreparations based on *B. thuringiensis* bacteria (dendrobacillin, bitoxibacillin, dipel, turicide, lepidocide, Veta Pro, Atibak-Uz, etc.) for the protection of crops from pests was noted [6-8]. Also, these microorganisms were studied and extensive scientific resources were created.

2 Materials and methods

Analysis was carried out in 2022 at the Research Institute of Quarantine and Plant Protection, the Laboratory for Pest Control of Cereals and Legumes, the Laboratory of Molecular Biology of the Institute of Microbiology of UzRF, and the Turkiston Shernazarova farm. Nargiza, Kybray district, Tashkent region in Uzbekistan. When performing scientific research, methods widely used in microbiology, mycology and entomology were used.

Adults and larvae of the Colorado potato beetle were collected, and those who died from the disease were subjected to microbiological analysis in the laboratory. The Colorado potato beetle with signs of a fungal disease had the upper part of its body covered with fungal mycelium. Colorado beetles with symptoms of bacterial infection were observed to be discoloured, runny and smelly. The upper body layer of the Colorado potato beetle was sterilised with 96.0% alcohol. Colorado beetles infected with fungal and bacterial diseases were placed in Petri dishes with fixed wet special paper (wet chamber) and grown at room temperature. To isolate microorganisms from diseased and dead Colorado potato beetles, they were cultivated on a nutrient medium and pure samples were taken. The necessary solutions were prepared from the biological material, and fungal species were identified using an NLCD-307B binocular microscope.

3 Results and discussion

To study the formation and bioecological features of the Colorado potato beetle population in the agro biocenosis of potatoes in 2022, studies were carried out on 2.0 hectares of potato plantations of the Turkiston Shernazarov Nargiza farm, Kibray district, Tashkent region in Uzbekistan. Research is carried out on potatoes “Santa” and “Romano” fields in the morning and evening planting.

During our observations, it was found that the Colorado potato beetle hibernates as adults at a depth of 15-50 cm. However, it was noted that when the soil warms up to 11-14 °C and relative humidity of 80%, the beetles begin to come out gradually. The beetles that emerged from the village were first fed with weeds from the sedge family, then planted on March 15.

On April 10, he began to feed in the field with fully sprouted potatoes. After feeding, the beetles lay up to 20–30 eggs in clusters under the potato leaves on which they were provided (Figure 1).

Figure 1. Monitoring work was carried out on 2.0 hectares of potato sown area of the farm "Turkiston Shernazarova Nargiza".

The embryonic development of the Colorado potato beetle depends on air temperature and relative humidity. At an air temperature of 22 °C and relative humidity of 70%, hatching larvae from eggs takes 3-5 days. The hatched larvae are the most harmful in potato crops and pupate in 15-20 days at an air temperature of 25°C and relative humidity of 60%. The larvae turned into cones at a depth of 1.5 to 15 cm. In some cases, they were observed to turn into cones on the soil surface at high soil moisture. The fungus development required 10–15 days at an air temperature of 30°C and relative humidity of 50%, during which a new generation of beetles develops (Figure 2).

According to the given data, Colorado potato beetles begin to leave the village in the second decade of April in the Kibray district of the Tashkent region. The development of beetles continued until the second decade of April. In the second decade of April, the beetles began to lay eggs, which continued until the first decade of May. The larvae started to emerge from the eggs in the first decade of May; the larval stage continued until the third decade of May. The transformation of larvae into cocoons coincided with the first decade of June.

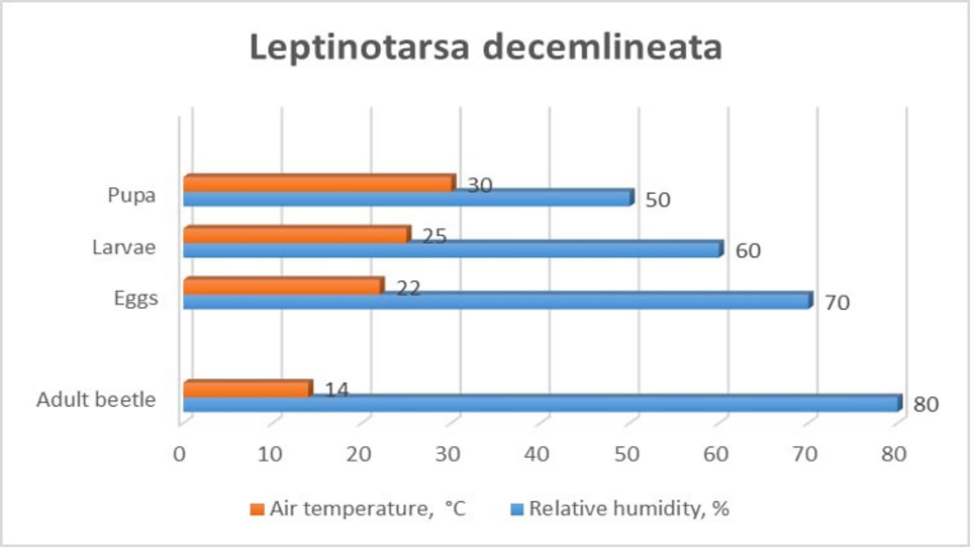

Figure 2. Influence of temperature and relative air humidity on the development of the Colorado potato beetle population in potato agro biocenosis

The hatching of larvae of the second generation started in the third decade of June, and the larval period continued until the second decade of July. The development of nymphs into cocoons corresponded to the second decade of July, and the emergence of beetles from cocoons of this generation corresponded to the third decade of July. The beetle period continued until the first decade of August (Table 1).

Table 1. Phenological table of the development of the Colorado potato beetle in the Kibray district of the Toshnetsky district (2022).

Month	**April**			**May**			**June**			**July**			**August**			**Sep**			**October**		
in the decade	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
1st generation		◙																			
		■																			
		●	●	●																	
				∞	∞	∞	○														
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2nd generation								■													
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(◙) – A beetle from the village; ● – Eggs; ∞ - Larva; ○ - Sponge; ■ - Beetle; (□) – A beetle that went to the village.

The beetles of the third generation corresponded to the first ten days of August and began to lay eggs in the first ten days of this month; the oviposition period continued until the second ten days of August. The larvae hatched from the eggs in the second decade of August, and the larval period continued until the first decade of September. In the second decade of September, the larvae began to turn into cocoons; in the second decade of this month, beetles began to emerge from the cocoons, and the beetle period continued until the third decade of September. Wintering of the Colorado potato beetle was observed in the first decade of October.

In our experiment, we studied the pathogenicity of the B. bassiana VTq-28 strain grown on different nutrient media in a titer of 2×108 ml against the Colorado potato beetle. To this end, the strain B. bassiana VTq-28 isolated from the Colorado potato beetle was tested against various young Colorado potato beetles. First, multiple juveniles of the Colorado potato beetle collected from the field were reared in the laboratory. Then, after wrapping a bunch of 1 and 2 branches of the potato crop for food, each of them was treated with different titers of 1×108 and 2×108 ml to determine the effect of B. bassiana VTq-28 strain on small and large young larvae of the Colorado potato beetle in laboratory conditions. Pests became infected mainly when pest bodies and feed were used. In the control variant, it was treated with water.

Under laboratory conditions, the B. bassiana VTq-28 strain was used in liquid form. The results of experiments on the sensitivity of the strain B. bassiana VTq-28 to tiny young larvae of the Colorado potato beetle are given in the table. 4.7. According to the results of the experiment, when the B. bassiana VTq-28 strain was treated with a 1 × 108 ml titration solution of 35.0%, 48.3%, 73.3% when used in a 1 × 108 ml titer against adult larvae, it was 25.0%, 36 .6%, 65.0% on the 3rd, 7th and 14th days of accounting, and the above accounting days 13.3, 33.3% against its adults and the efficiency of 50.0%. According to the results of the experiment, when the strain B. bassiana VTq-28 was treated with 2 × 108 ml of a titer solution, on the 3rd day 41.6%, on the 7th day 63.3%, on the 14th day 85.0%, and in adult larvae versus 30.0%, 46.6%, 78.3% on the above reference days; and adults - 18.3%, 41.6% and 56.6%.

As a result of the application of the B. bassiana VTq-28 strain, the fungal action begins with the penetration of the spore through the skin (cuticle) of the insect into the body cavity. Once in the body, the spore turns into hyphae, the mycelium grows, and conidia are released. Conidia in the body pass into the hemolymph. At this stage, due to some strains' significant release of toxins, insects can cause harm, primarily affecting muscle tissue. The growth of the fungus continues until all tissues are destroyed. Then conidiophores appear, which break the cuticle and surround the dead larva. The infected insect looks like white cotton wool. Then the spores mature, and mass sporulation occurs. The insect dies 12-14 days after infection (Figure 3).

In 2018-2020, our research was carried out on the potato fields of the Turkestan Shernazarova Nargiza farm in the Kibray district of the Tashkent region to test the B. bassiana VTq-28 strain isolated from the Colorado potato beetle in the field. Field experiments were carried out with different juveniles of the Colorado potato beetle. The working liquid was applied at 300 l/ha using an OVKh-600 tractor sprayer against the Colorado potato beetle. The experiment was carried out during the mass development of Colorado potato beetle larvae on the Santa variety of potato culture. Before treatment, Colorado potato beetles in control and experimental fields ranged from 3.7 to 4.5 specimens. Beetles per plant, and the number of larvae is 23.4 to 30.7 ind.

Figure 3. *B. bassiana* VTq-28 strain isolated from the Colorado potato beetle.

Our research obtained the following results when testing the strain B. bassiana VTq-28 against adults and larvae of the Colorado potato beetle. When using the strain B. bassiana VTq-28 against adults of the Colorado potato beetle in a titer of 1×108 ml on the 3rd, 7th and 14th day of counting, respectively, 15.4; 28.4; An efficiency of 41.0% and 17.3 was achieved on days 3, 7, 14 when used in a titer of 2×108 ml; 35.6; An efficiency of 47.3% has been achieved.

35.4 days in the above calculations in 1×108 ml titer against larvae; 46.3; An efficiency of 68.6% was observed, and when used in a titer of 2×108 ml, on the 3rd day of calculation 39.2; On the 7th day, an efficiency of 57.2% was achieved and 78.1% on the 14th day (Table 2).

Table 2. Biological effectiveness of the strain B. bassiana VTq-28 against the Colorado potato beetle (Field experience, Turkestan Shernazarova Nargiza farm, 2022).

#	Options	Titre	Average number of insects in 1 plant, pcs				Efficiency, % by days		
			# of insects before processing	Days after processing, pcs					
				3	7	14	3	7	14
Mature breed (beetle)									
1	Control (idle)	-	4.0	4.3	4.7	5.4	-	-	-
2	Bioslip BV (template) 3.0 l/ha	1×10^8	3.7	3.1	2.9	2.8	22.0	33.3	43.9
3	*B. bassiana* VTq-28	1×10^8	4.4	4.0	3.7	3.5	15.4	28.4	41.0
4	*B. bassiana* VTq-28	2×10^8	4.5	4.0	3.4	3.2	17.3	35.6	47.3
To the larvae									
1	Control (idle)	-	28.3	28.5	29.3	35.9	-	-	-

2	Bioslip BV (template) 3.0 l/ha	1×10^8	30.7	19.5	15.2	11.0	36.9	47.8	71.7
3	*B. bassiana* VTq-28	1×10^8	23.4	15.2	13.0	9.3	35.4	46.3	68.6
4	*B. bassiana* VTq-28	2×10^8	27.1	16.6	12.0	7.5	39.2	57.2	78.1

The obtained results conclude that the use of the strain B. bassiana VTq-28 in a titer of 2×10^8 ml showed a high efficiency of 78.1% against Colorado potato beetle larvae. Currently, in cooperation with the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan and the Research Institute of Quarantine and Plant Protection, scientific research is being carried out to determine the insecticidal activity of local strains of bacteria B. thuringiensis against the Colorado potato beetle and in the future to create bioinsecticide preparations based on these strains.

For this purpose, the bacteria B. thuringiensis 1, B. thuringiensis 3, B. thuringiensis 5, B. thuringiensis 9fo, B. thuringiensis 18fo, B. thuringiensis are stored in the collection of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan to determine the insecticidal activity against Colorado potato beetle larvae. strains thuringiensis 31, B.thuringiensis 26, B.thuringiensis 54, B.thuringiensis 84, B.thuringiensis 91 and B.thuringiensis 94. Strains B. thuringiensis 1, B. thuringiensis 3, B. thuringiensis 5, B. thuringiensis 9fo, B. thuringiensis 18fo, B. thuringiensis 31, and B. thuringiensis 26 exhibited different insecticidal activity against the wax moth and a heat-resistant β-exotoxin. The formation is noted. It is known from the literature that the activity of strains of bacteria B. thuringiensis synthesising β-exotoxin against the Colorado potato beetle was studied. The strains of bacteria B. thuringiensis used by us, synthesising β-exotoxin, exhibited different levels of insecticidal activity. In addition, these strains were found to synthesise β-exotoxin and produce crystalline d-endotoxin (Figure 4).

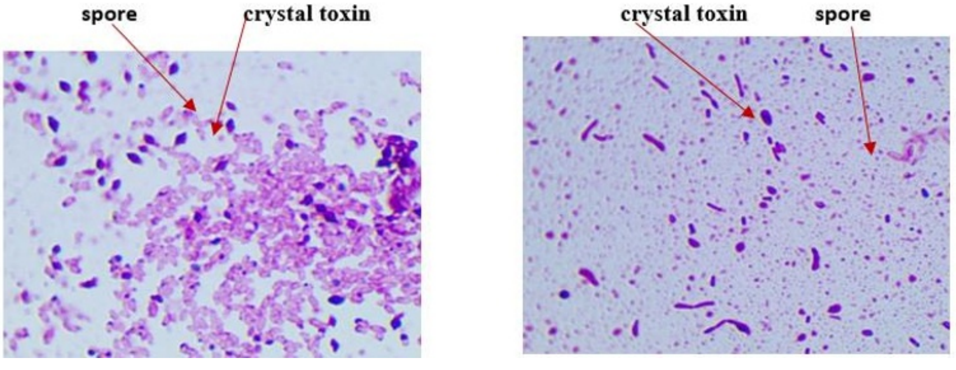

Figure 4. Microscopic view (1000x magnification) of bacterial strains B. thuringiensis 18fo and B. thuringiensis 26.

The strains B. thuringiensis 54, B. thuringiensis 84, B. thuringiensis 91 and B. thuringiensis 94 used in the experiment were not tested for the synthesis of β-exotoxin. Still, their d-endotoxin activity was studied on honey wax (Figure 5).

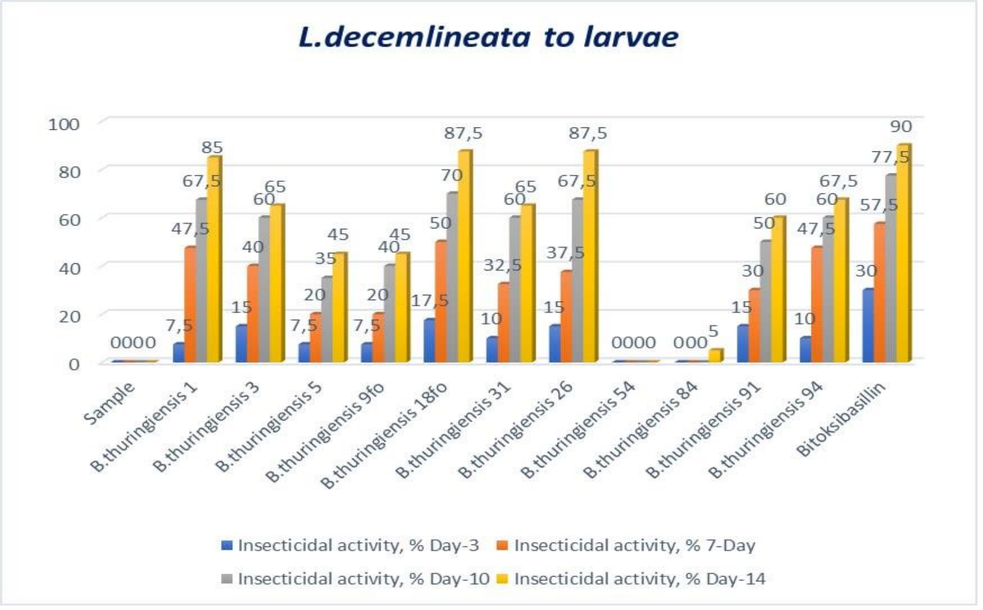

Figure 5. Insecticidal activity of indigenous Bacillus thuringiensis strains against the Colorado potato beetle.

As shown in Figure 5, during the 14-day experiment, B. thuringiensis 18 fo, B. thuringiensis 26, and B. thuringiensis 1 showed the highest insecticidal activity and killed 87.5%, 87.5%, and 85.0% of the insects, respectively. y it was determined that he had died (Figure 6). In addition, when strains of Bacillus thuringiensis were applied to 2-3-year-old larvae of the Colorado potato beetle, it was found that the external morphology of the beetle, which passed into the mushroom phase in subsequent generations without dying from bacterial deformation, and then to the state of emago, was deformed. Reproduction (laying eggs) in beetles affected by bacteria was not observed. It is important to note that the time of manifestation of the most excellent insecticidal activity of these studied strains is 7- and 10-days, where 60% of the total estimated insecticidal activity was recorded on these days (Figure 6).

The average insecticidal activity of B.thuringiensis 3, B.thuringiensis 5, B.thuringiensis 9fo, B.thuringiensis 91 and B.thuringiensis 94 strains was 65.0%, 45.0%, 60.0% and 67.5%, respectively. The lowest level of insecticidal activity was noted in the B. thuringiensis 84 strain (5%), and the B. thuringiensis strain 54 did not show insecticidal activity at all.

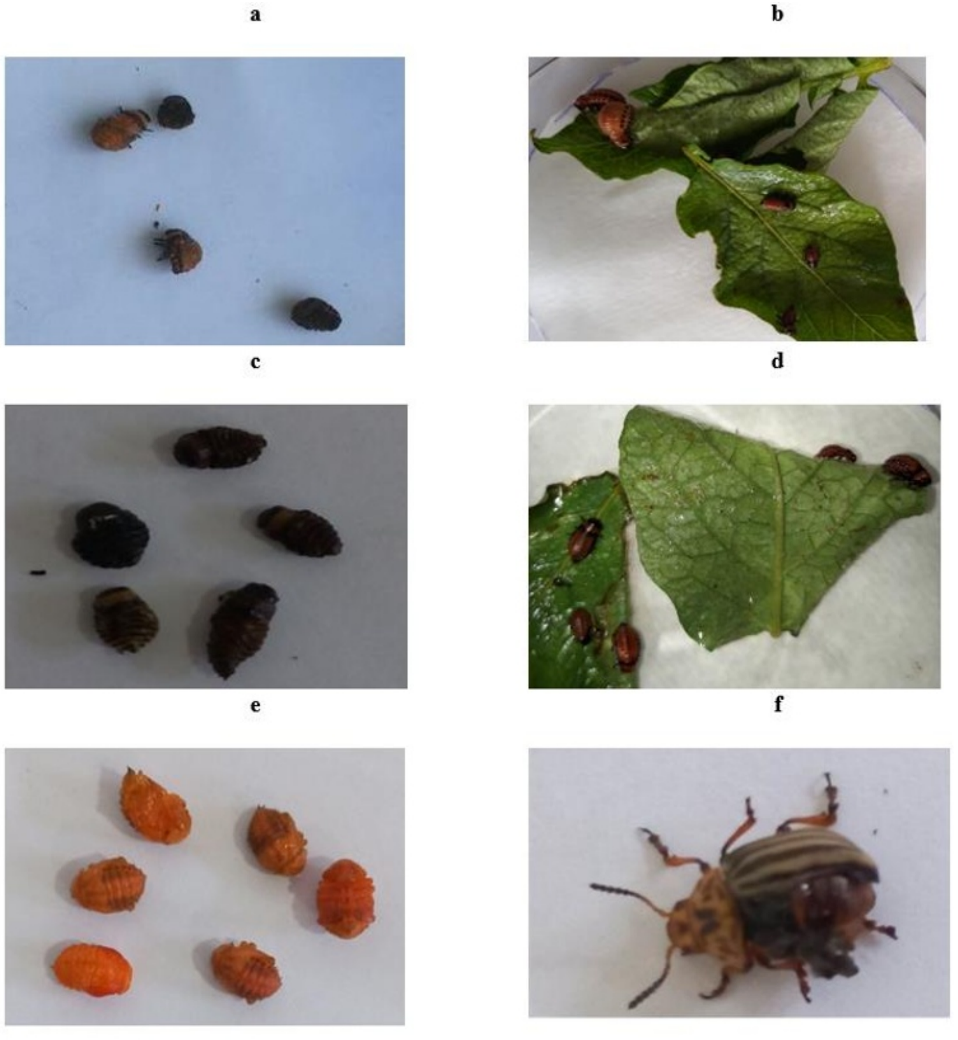

Figure 6. A – treatment of the 2nd and 3rd generations of the Colorado beetle with B. thuringiensis 18 fo strain; B – dead insects; C – the 2nd and 3rd generations of the Colorado beetle were treated with B. thuringiensis strain 26; D – dead insects; E – the state of the fungus after treatment of the 2nd and 3rd generations of the Colorado beetle with B. thuringiensis strain 26; and, F – View of the Colorado beetle in a deformed, mutated state of the beetle emerging from the cocoon.

4 Conclusions

Thus, the results of the studies showed that the strains of B. thuringiensis bacteria used in the experiment exhibited different insecticidal activity against the Colorado potato beetle. The strains B. thuringiensis 18 fo, B. thuringiensis 26, and B. thuringiensis 1 showed the highest insecticidal activity. Their external morphology became disabled. This means that although bacterial toxins do not affect some first-generation Colorado potato beetles, they can affect the structure of the insect's genome, mutate it and cause damage to insects emerging from adults. It is important to note that the mutated insects were not observed to lay eggs and produce offspring.

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