

Impact of rhizobacteria (*Bacillus subtilis* RT-3, *Bacillus subtilis* RT-6) and Entomopathogenic nematodes (*Steinernema feltiae*) on soil agrochemical and biological properties in potato cultivation

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Abstract

The article presents data on the study of the effect of effective potato rhizobacteria with biofungicidal properties of *Bacillus subtilis* RT-3 and *Bacillus subtilis* RT-6 and entomopathogenic nematodes *Steinernema feltiae* on the background of the use of mineral fertilizers (NP) on microflora, agrochemical composition of soils, and nematode content in potato cultivation. As a result of their application at the beginning of the growing season, before sowing potatoes and by the end of the growing season, positive results were obtained on changing the microbiological, agrochemical, and entomological composition of soils and increasing the number of tubers by 5 pieces on average per 1 plant.

Keywords: Mineral fertilizers, soil microorganisms, agrochemical parameters of soils, K-Gumat leaf treatment

Introduction

Potatoes are an indispensable food product for the population, they are widely used for livestock feed and as raw materials for various products, starch and alcohol. One of the main factors in obtaining high potato yields is the use of organic and mineral fertilizers. Potato plants are demanding of soil nutrients, which must be in it in an accessible form and sufficient quantity. This is largely due to the biological characteristics of potatoes and, first of all, the underdeveloped root system, mainly located in the upper layer of the soil. To ensure high potato productivity, it is necessary to comply with and improve the technology of cultivation, one of the elements of which is a scientifically based fertilizer system (Bedding and Akhurst, 1975; Miller, 1989; 2017). Fertilizers play one of the most important roles in the system of

agricultural technologies that ensure high potato yields. Usually, quite large doses of mineral tucks are used for these purposes, but the trend observed in recent years towards the transition to biological farming methods makes it urgent to search for new ways to grow this tuberous crop (Usmonkulova et al., 2022; Usmonkulova et al., 2024; Liu et al., 2024). The systematic use of high doses of mineral fertilizers leads to a decrease in the biological component of the soil. As a result, humus is destroyed, the structure of the site deteriorates and the arable layer is compacted. The latter becomes less provided with available air, moisture and nutrients, and therefore labor costs for its mechanical processing increase. Reducing the volume of mineral fertilizers by 50% in combination with pre-planting treatment of potato tubers with bacterial additives restored the biological activity of soils.

Of all the factors determining the productivity of the soil-plant system, microorganisms play a dominant role. The introduction of high doses of mineral fertilizers and pesticides helps to reduce the total number of bacteria and actinomycetes, and also leads to an increase in the number of fungi. In addition, the number of beneficial insects and earthworms decreases in the arable layer against the background of a weakening of enzymatic activity. In this regard, when growing potatoes, it becomes relevant to use special microbiological additives based on *Bacillus subtilis* bacteria of the H-13 strain, for example, Extrasol, BisolbiSan, BisolbiFit, and others. The interaction of these microorganisms with the plant is based on their colonization of the rhizosphere zone of the roots, which makes it difficult for pathogens to access food sources and prevents infection of crops.

When using them, there is a normalization of nutrition due to the transfer of soil reserves of nitrogen, phosphorus, and potassium into a form accessible to plants, stimulation of the development of root hairs, and an increase in their absorption capacity. The use of conventional fertilizers without bacterial components in this link did not always create positive indicators of the balance of nutrients (Gaffke et al., 2022). In the fight against insect pests, an important place is given to the use of their natural enemies - parasites, predators, and pathogens. In this regard, in the last two decades, there has been an increased interest in entomopathogenic nematodes, which can be used to regulate the number of harmful insect species. The development of technology for the use of environmentally friendly nematode-bacterial complexes in the integrated protection of potatoes is extremely relevant. Biopreparation based on entomopathogenic nematodes, *Steinernema feltiae*, is approved for use in the territory of the Russian Federation for processing potatoes against wireworms in the budding phase (Danilov, 2001)

In recent years, a high invasive activity of a new subspecies of entomopathogenic nematodes *Steinernema feltiae* protense subsp. N. (Nematoda: Steinernematidae) has been established against wireworm larvae of beetles of the family Click beetles (Elateridae), *R. Agriotes*.

Miller (2017) investigated the effectiveness of *Steinernema feltiae* against *Agrotis segetum* larvae under Japanese conditions, finding that the pest population was reduced by 65-85%. This study demonstrates

the potential of using nematodes to significantly reduce the need for chemical control measures. Frias et al (2022) explored the long-term persistence of *Steinernema feltiae* in German agricultural fields, revealing that the isolate's population remained at 50-60% even after harvest, confirming its effectiveness and long-term protective impact in the soil environment. Wang et al. examined the efficacy of *Steinernema feltiae* against local pests in China, reporting a reduction in pest numbers by up to 75% (Wang, 2016). This study highlights the global potential of nematodes as a biological control agent. Kaya and Gaugler assessed the environmental safety and applicability of entomopathogenic nematodes in pest control, emphasizing their efficacy as well as their safety for the environment (Kaya & Gaugler 1993). Grewal et al. focused on the benefits of using entomopathogenic nematodes for biological control of plant pests, noting their ability to effectively manage pests without harming beneficial entomofauna or disrupting soil and plant microbiota. This is crucial for their widespread application (Grewal, 2015). Kurbonova documented the first record of the local *Steinernema feltiae* isolate in Uzbekistan and found that it reduced potato pest populations by 75-80% (Kurbanova, 2023a). Furthermore, she emphasized the potential of using entomopathogenic nematodes as an alternative to chemical pesticides for producing eco-friendly agricultural products. The protective effect of the nematode-bacterial complex on potatoes has been established. The high efficiency of the use of entomopathogenic nematodes against wireworms has been shown. The use of metabolic products of symbiotic bacteria and entomopathogenic nematodes increased productivity and suppressed the development of late blight. An assessment of the biological effectiveness on potatoes has shown that the nematode-bacterial complex can be used when planting tubers and in the budding phase (Agansonova et al., 2013). In connection with the above, the purpose of our research was to study the effect of the association of soil bacteria. *Bacillus* and entomopathogenic nematodes, *Steinernema feltiae*, on the agrochemical, microbiological, and entomological composition of soils during potato cultivation.

Materials and methods

Experimental Place and Soil Conditions

The experiment was conducted at the Research Institute of Vegetable and Potato Crops, Ministry of Agriculture of the Republic of Uzbekistan. The experimental site was chosen based on its typical soil composition and agroecological conditions suitable for potato cultivation. Soil samples were collected at the beginning of the study to determine initial soil properties, including pH, organic matter content, and nutrient composition (nitrogen, phosphorus, potassium). The experimental plot was prepared using standard pre-sowing tillage methods to ensure uniform planting conditions.

Research Materials

Potato Rhizobacteria

Bacillus subtilis RT-3 and *Bacillus subtilis* RT-6 strains were used as biofungicides for pre-sowing

treatment of potato tubers. These bacterial strains are known for their antagonistic properties against several pathogens, including *Phytophthora infestans*, *Fusarium* spp., *Rhizoctonia solani*, and *Alternaria solani*. The strains were applied to the potato tubers before planting, following the investigated dosage and application methods.

Entomopathogenic Nematodes

Steinernema feltiae nematodes were applied to the soil during pre-sowing tillage. This entomopathogenic nematode was selected due to its effectiveness in controlling soil-dwelling insect pests that can adversely affect potato growth. Nematode application was performed using an irrigation system to ensure even distribution across the plot at the recommended rate.

Leaf Treatment with K-Gumat

K-Gumat, a potassium-based humic substance, was applied to the potato plants twice during the growing season. The treatment was performed during the vegetative phase to enhance plant growth, improve disease resistance, and increase stress tolerance. The K-Gumat solution was sprayed directly onto the leaves of the plants following the recommended dosage.

Potato Variety

The potato variety used in this study was Belarus, known for its adaptability and resistance to common potato diseases. The tubers were treated with the rhizobacteria and subsequently planted in the experimental plot.

Experimental Design

The study followed a randomized block design with multiple treatment groups, including control and treatment plots. The treatments were as follows:

1. Control, N200P200 kg/ha +K-Gumat (by the leaves)
2. Etalon, N200P200 kg/ha - Entonem (*Steinernema feltiae*)+K-Gumat (by the leaves)
3. Combination experience, bacterial association (*Bacillus subtilis* PT-3 + *Bacillus subtilis* PT-6) +*Steinernema feltiae*+K-Gumat (by the leaves)

Each treatment was applied to three replicates to ensure statistical robustness.

Statistical Analysis

The collected data were analyzed using analysis of variance (ANOVA) to determine significant differences between treatment groups. Post-hoc comparisons were performed using Tukey's test for pairwise comparisons with a significance level set at $p < 0.05$. All analyses were performed using appropriate statistical software.

Results

The agrochemical composition and the number of microorganisms of various physiological groups of the initial soil were studied before sowing potato tubers and at the end of the growing season, during the

potato ripening phase (Table 1-4, Fig.1-3).

From the data presented in Table 1, it can be seen that in the experimental variant, the use of rhizobacteria *Bacillus subtilis* RT-3, *Bacillus subtilis* RT-6 for pre-sowing treatment of potato tubers and entomopathogenic nematodes *Steinernema fultiae* for soil treatment before planting potatoes against the background of mineral fertilizers showed that the humus content in the soil by the end of the growing season decreased by 0.12% compared to compared with the control, compared with the reference - by 0.06%, compared with the initial soil - by 0.44% (Table 1, Fig.1).

Table 1. The content of humus and the degree of salinity of the soils of the field site, Research Institute of Vegetable Crops and Potatoes

Experience variants	Humus %	Carbon of humus, %	Degree of salinity ECe, dS/m	Assessment of the degree of salinity ECe, type of salinity	pH
The initial soil (May, 2024r)	1,31	0,76	0,68	unsalted chloride-sulfate type soils	8,7
The end of the growing season (July, 2024r)					
1. Control, N ₂₀₀ P ₂₀₀ kg/ha + K-Gumat (by the leaves)	0,99	0,58	0,84		8,6
2. Etalon, N ₂₀₀ P ₂₀₀ kg/ha + Koppert Entonem (<i>Steinernema filtiae</i>)+ K-Gumat (by the leaves)	0,93	0,54	0,64		8,5
3. Experience, bacterial associa-tion (<i>Bacillus subtilis</i> PT-3 + <i>Bacillus subtilis</i> PT-6) + <i>Steinernema filtiae</i> + K-Gumat (bv the leaves)	0,87	0,50	0,56	unsalted sulfate-type soils	8,0

The degree of salinity (ECe) in the experimental variant in the soil by the end of the growing season decreased by 0.28 dS/m compared with the control, by 0.08 dS/m compared with the etalon, and by 0.12 dS/m compared with the initial soil. In addition, the studied initial soils, soils of the control and etalon variants by the end of the growing season were unsalted and of the chloride-sulfate type. By the end of the growing season, the soils of the experimental variant also remained unsalted, but of the sulfate type. The pH level in the experimental variant decreased by 0.6 units by the end of the growing season compared to the control, by 0.5 units compared to the etalon, and by 0.7 units compared to the initial soil. By the end of the growing season, the content of nutrients was the best in the experimental variant compared with the control and the etalon due to improved nutrition of potato plants as a result of a decrease in gross and mobile forms of nitrogen, phosphorus, and potassium.

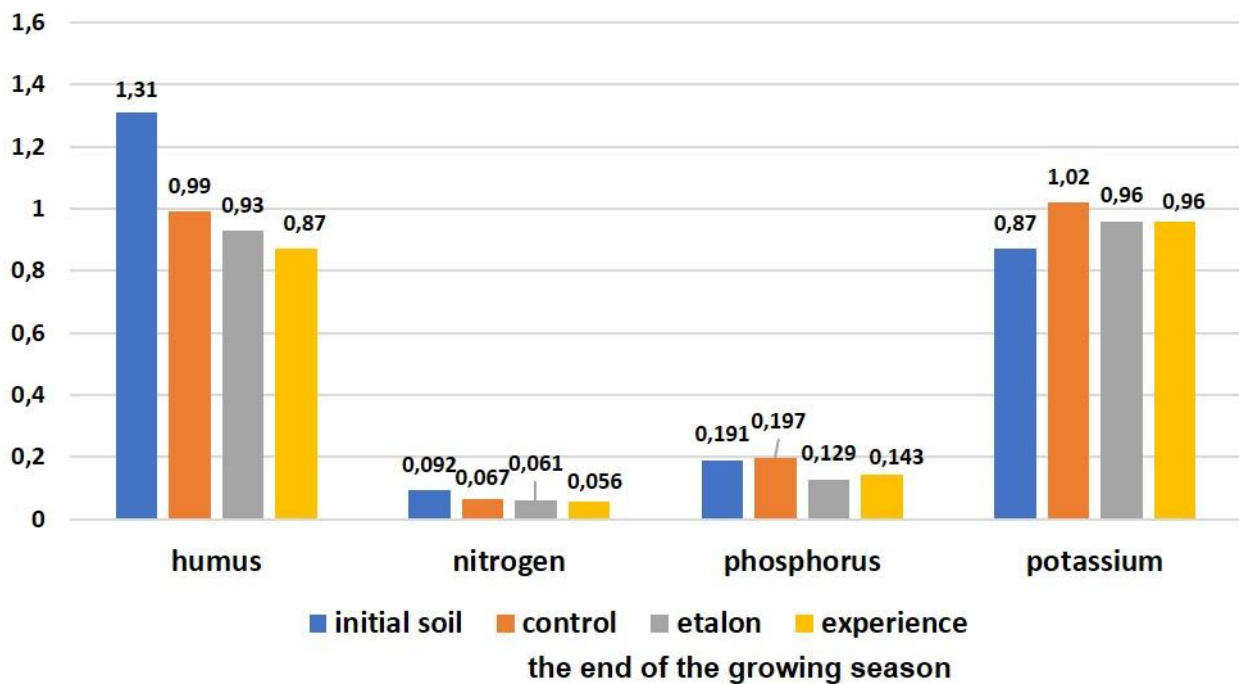


Figure 1. The content of humus and biogenic elements in the soils of the field site of the Research Institute of vegetable crops and potatoes, %

In the experimental variant, by the end of the vegetation period, the total nitrogen content in the soil decreased by 0.011% compared with the control, by 0.005% compared with the etalon, and by 0.036% compared with the initial soil. The content of mobile nitrogen in the soil decreased by 33.1mg/kg compared with the control, by 1.2 mg/kg compared with the etalon, and by 26.2 mg/kg compared with the initial soil. In the experimental version, by the end of the vegetation period, the total phosphorus content in the soil decreased by 0.054% compared to the control, increased by 0.014% compared to the reference, and decreased by 0.048% compared to the initial soil. The content of mobile phosphorus in the soil decreased by 15.5 mg/kg compared to the control, increased by 16 mg/kg compared to the reference, and decreased by 26.2 mg/kg compared to the initial soil. In the experimental version, by the end of the vegetation period, the content of total potassium in the soil decreased by 0.06% compared to the control; compared to the reference, it did not change, compared to the initial soil, it increased by 0.09%. The content of mobile potassium in the soil increased by 24.0 mg/kg compared with the control, increased by 73.0 mg/kg compared with the reference, and decreased by 36.0 mg/kg compared with the initial soil (Table 2, Fig.1,2).

Table 2. The content of biogenic elements in the soils of the field site, Research Institute of Vegetable Crops and Potatoes

Experience variants	Nitrogen		Phosphorus		Potassium	
	N, %	N-NO ₃ , мг/кг	P, %	P ₂ O ₅ , мг/кг	K, %	K ₂ O, мг/кг

The initial soil (May, 2024r.)	0,092	66,0	0,191	84,0	0,87	349
The end of the growing season (July, 2024r.)						
1. Control, N ₂₀₀ P ₂₀₀ kg/ha +K-Gumat (by the leaves)	0,067	72,9	0,197	54,5	1,02	289,0
2. Etalon, N ₂₀₀ P ₂₀₀ kg/ha +Koppert Entonem (<i>Steinernema filtiae</i>)+ K-Gumat (by the leaves)	0,061	41,0	0,129	24,0	0,96	240,8
3. Experience, bacterial association (<i>Bacillus subtilis</i> PT-3 + <i>Bacillus subtilis</i> PT-6) + <i>Steinernema filtiae</i> +K-Gumat (by the leaves)	0,056	39,8	0,143	40,0	0,96	313,0

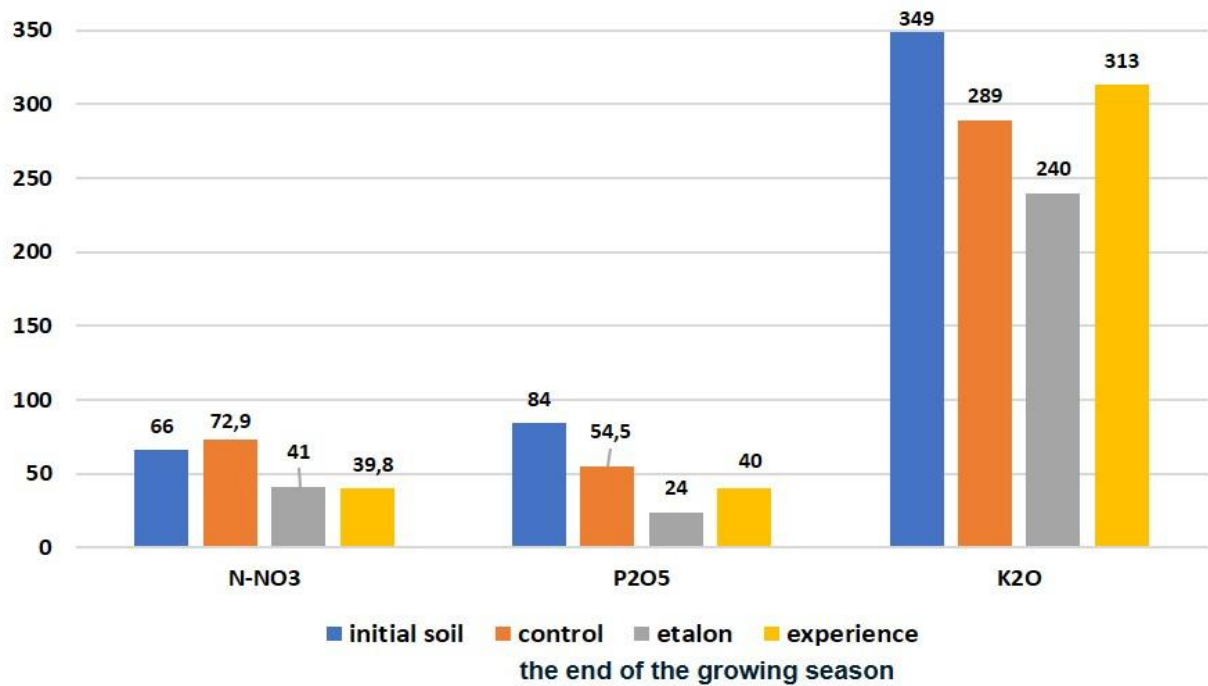


Figure 2. The content of mobile forms of biogenic elements in the soils of the field site of the Research Institute of vegetable crops and potatoes, mg/kg

We have studied the content of toxic and non-toxic salts in the initial soil and at the end of the potato growing season. As a result of the research, it was revealed that the largest amount of salts - 0.093% was in the control variant at the end of the potato growing season and the least in the experimental variant using *Bacillus subtilis* RT-3, *Bacillus subtilis* RT-6 and *Steinernema filtiae* - 0.067% (Table 3, Fig.3).

Table 3. The salt content in the soils of the field site of the Research Institute of vegetable crops and potatoes, %

Experience variants	Ca(HCO ₃) ₂	CaSO ₄	MgSO ₄	Na ₂ SO ₄	NaCl	The amount of salts
The initial soil (May, 2024r.)	0,023	0,015	0,018	0,020	0,012	0,088
The end of the growing season (July, 2024r.)						
1. Control, N ₂₀₀ P ₂₀₀	0,021	0,020	0,015	0,025	0,012	0,093

kg/ha +K-Gumat (by the leaves)						
2. Etalon, N ₂₀₀ P ₂₀₀ kg/ha Entonem (<i>Steinernema filtiae</i>)+K-Gumat (by the leaves)	0,024	0,014	0,012	0,021	0,012	0,083
3. Experience, bacterial association (<i>Bacillus subtilis</i> <i>PT-3</i> + <i>Bacillus</i> <i>subtilis PT-6</i>) + <i>Steinernema</i> <i>filtiae</i> +K-Gumat (by the leaves)	0,020	0,009	0,011	0,018	0,009	0,067

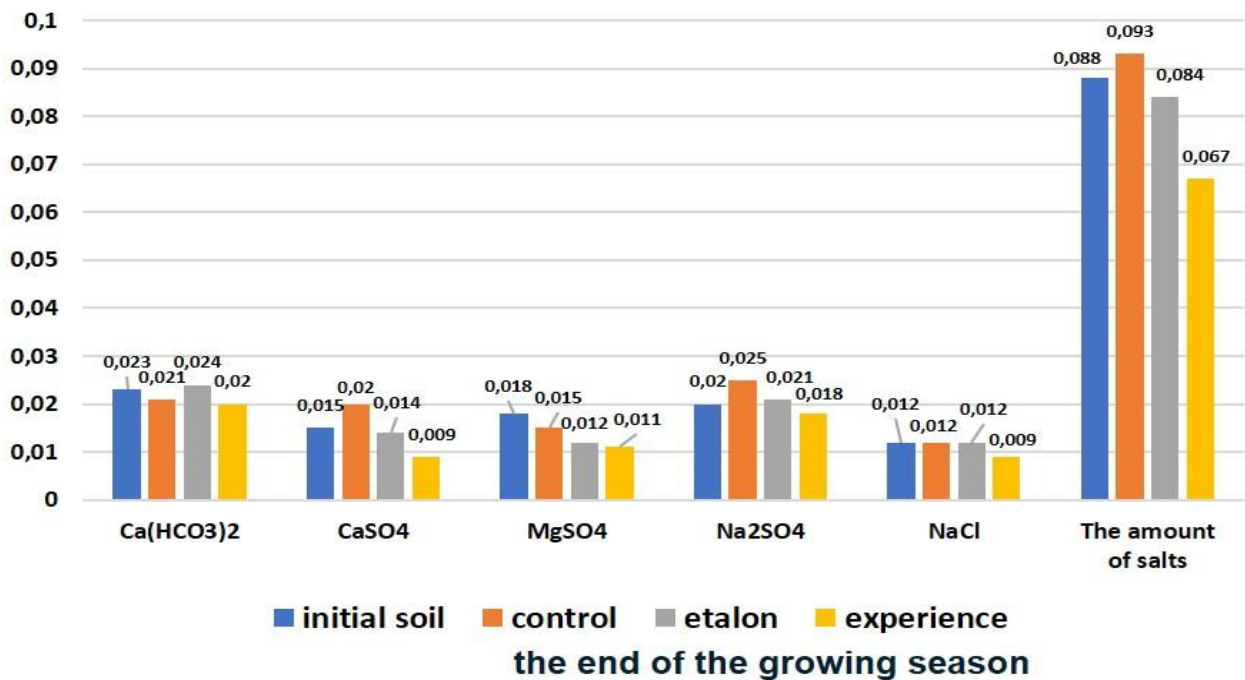


Figure 3. Salt content in the soils of the field site of the Research Institute of Vegetable Crops and Potatoes, %

The number of the main physiological groups of soil microorganisms in the initial soil and at the end of the growing season was studied according to the experimental variants. In the initial soil, only ammonifiers in the amount of 9.0×10^4 cfu/g of soil and mold fungi in the amount of 4.0×10^3 cfu/g of soil were found among the microorganisms. Nitrogen-fixing, phosphorus and potassium-mobilizing bacteria and actinomycetes were not detected (Table 4).

Table 4. The number of microorganisms in the soils of the field site of the Research Institute of vegetable crops and potatoes (CFU/1 g. soil)

Experience variants	Ammo nifters	Nitrogen fixators	Phosphorus- mobilizing bacteria	Potassium- mobilizing bacteria	Micro micets	Actinomy cetes
The initial soil (May, 2024r)	9,0x10 ⁴	not detected	not detected	not detected	4,0x10 ³	not de tected
The end of the growing season (July, 2024r)						
1. Control, N ₂₀₀ P ₂₀₀ kg/ha +K- Gumat (by the leaves)	3,0x10 ⁷	not detected	not detected	not detected	not detected	not detected
2. Etalon, N ₂₀₀ P ₂₀₀ kg/ha Entonem (<i>Steinernema filtiae</i>)+K-Gumat	4,0x10 ⁷	not detected	not detected	not detected	not detec ted	not detec ted

(by the leaves)						
3. Experience, bacterial association (<i>Bacillus subtilis</i> PT-3 + <i>Bacillus subtilis</i> PT-6) + <i>Steinernema feltiae</i> + K-Gumat (by the leaves)	2,4x10 ⁸	1,3x10 ²	3,3x10 ⁴	4,1x10 ⁴	not detected	not detected

By the end of the growing season, ammonifiers were found in the amount of 3.0×10^7 cfu/g of soil in the control and 4.0×10^7 cfu/g of soil in the Etalon variant and an order of magnitude higher in the amount of 2.4×10^8 cfu/g of soil in the experimental variant. As a result of the use of *Steinernema feltiae* isolate, the number of potato pests (for example, *Agrotis segetum*) has significantly decreased by 70-80%. It also reduced the degree of damage to the plants. After harvesting, the soils in the areas treated with *Steinernema feltiae* contained a large number of beneficial nematodes, the average amount of which was 50-70 ml per 100 g of soil. These results showed that the *Steinernema feltiae* isolate can persist in the soil environment for a long time. In the experimental version, where entomopathogenic nematodes were used, a significant reduction in the number of pests was observed. This confirms the effectiveness of the *Steinernema feltiae* isolate as a biological control agent. According to the ANOVA results, a significant difference ($p < 0.05$) in the number of pests was found between the sites where the isolate was used and the control sites.

During the maturation phase, potato tubers from 3 studied experimental variants were selected from the experimental site, and it was determined that in the control with N₂₀₀P₂₀₀ kg/ha and K-Gumat (by leaf), 4 potato tubers were found on average per 1 plant. In the 2nd variant of the experiment (Etalon) using N₂₀₀P₂₀₀kg/ha, Koppert Entomem (*Steinernema feltiae*) and K-Gumat (according to the leaf) - 6 potato tubers and in the 3rd experimental variant with N₂₀₀P₂₀₀kg/ha, an association of bacteria (*Bacillus subtilis* RT-3 and *Bacillus subtilis* RT-6) and entomopathogenic nematodes *Steinernema feltiae* + K-Gumat (by leaf) - on average, 9 potato tubers were found on 1 bush.

Discussion

The application of *Bacillus subtilis* RT-3, *Bacillus subtilis* RT-6, and *Steinernema feltiae* in potato cultivation has demonstrated significant effects on soil properties and plant growth. Our findings align with previous studies that highlight the role of rhizobacteria and entomopathogenic nematodes in improving soil microbiota, reducing soil salinity, and enhancing plant resistance to pathogens (Kaya & Gaugler, 1993; Gaffke et al., 2022). The study results confirm that the combined use of *Bacillus subtilis* strains and *Steinernema feltiae* significantly improves soil fertility by enhancing the availability of nitrogen, phosphorus, and potassium (Liu et al., 2024). The decrease in salinity levels observed in the experimental plots can be attributed to the beneficial microbial activity that promotes soil structure stabilization and nutrient mobilization (Frias et al., 2022). Moreover, the suppression of plant pathogens by *Bacillus subtilis* has been well documented in previous research, which suggests that these bacterial

strains produce antifungal compounds that inhibit the growth of phytopathogens such as *Phytophthora infestans* and *Rhizoctonia solani* (Grewal et al., 2005; Usmonkulova et al., 2022). Additionally, the application of *Steinernema feltiae* has proven effective in controlling soil-borne insect pests, leading to reduced crop damage and increased yield (Miller, 2017; Kurbonova, 2023a).

The increased number of tubers per plant in the experimental plots supports the hypothesis that bioinoculants can significantly enhance potato productivity by improving nutrient uptake and plant health (Bedding and Akhurst, 1975; Wang et al., 2016). These findings suggest that integrating biological control agents into conventional fertilization strategies can contribute to sustainable agricultural practices while reducing reliance on chemical inputs (Danilov, 2001). Our study also observed a notable increase in beneficial soil microorganisms, particularly nitrogen-fixing and phosphorus-mobilizing bacteria, in plots treated with bacterial and nematode bioagents. This is consistent with findings from Kudrin & Sushchuk (2022), who reported that microbial diversity plays a crucial role in maintaining soil fertility and plant health. The persistence of *Steinernema feltiae* in the soil further underscores its potential as a long-term biological control agent (Frias et al., 2022). The results of this study highlight the potential benefits of integrating bio-fungicides and entomopathogenic nematodes into potato cultivation systems. Future research should focus on optimizing application methods and evaluating the long-term effects of these bioagents on soil health and crop productivity.

Conclusion

Based on the conducted studies, it can be concluded that the combined use of mineral fertilizers, active strains of potato rhizobacteria as biofungicides against potato phytopathogens and entomopathogenic nematodes significantly improves the soil microflora, agrochemical composition, and a decrease in the salt content in the arable soil layer of only the experimental site of the Research Institute of Vegetable crops and potatoes. Ultimately, as a result, the normalization of potato plant nutrition due to the transfer by rhizobacteria with the property of biofungicides *Bacillus subtilis* RT-3 and *Bacillus subtilis* RT-6 of soil reserves of humus, nitrogen, phosphorus and potassium and fertilizers to a form accessible to plants, as well as through the use of entomopathogenic nematodes, the number of tubers increased by 75% compared to the etalon and 125% compared to the control.

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