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Deciphering the role of a biological fungal formulation to stimulate the growth and improving the storage of sugar beet seeds

Short/Running Title: Role of a fungal formulation to improve shelf life of sugar beet seeds

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Abstract

Sugar beet (*Beta vulgaris* L.) is a highly beneficial and profitable crop, accounts for 30% of the world's sugar production. In order to secure the food security of Kazakhstan, it is necessary to strive to find a safe eco-friendly method for long time storage of sugar beet. In the current study, soil samples from the sugar beet cultivating regions of the southern and northern Kazakhstan has been studied for the presence of microbial diversity. The domestically and foreign-produced varieties of sugar beet which preferred to grow in Kazakhstan were screened to find out suitable candidate for bio-preparation. An isolate of the fungi *Trichoderma asperellum* has been found and used to formulate a bio-product to improve the storage life of the sugar beet material. It has been stipulated that the bio-preparation can be used to increase the storage time for sugar beet seeds based on the findings in the laboratory and field testing. While it was observed that the mixture of drugs (Maxim with Extrasol; and Celestop with Phytosporin-M) also enhance the growth processes of seeds. It was found out that a bio-preparation based on an isolated strain of fungus: "*Trichoderma asperellum* - KazNIIPPP-19" is quite acceptable to improve the long-term storage of sugar beet seeds. Additionally, it also improves the treated seeds' growth activities and promotes high-quality products in economic conditions.

Keywords: Sugar beet, Bio-preparation, Trichoderma, Microbiota, Germination

1.0 Introduction

About 25% of the world's sugar needs are met by sugar beets, an essential crop that is especially grown in humid areas to make beet sugar. Major producers of sugar beet, a high-yielding crop cultivated in temperate climates across the world, including Russia, Africa, Europe, and the United States. The major sugar beet producers in the world include France, Germany, Turkey, the USA, the Russian Federation, Poland, Ukraine, China, and Italy (Tayyab et al., 2023). The share of domestic sugar from sugar beet in Kazakhstan is 7%. The domestic sugar sector should strive for a substantial decrease in reliance on imports and a shift to self-sufficiency in order to guarantee Kazakhstan's food security (Khusnitdinova et al., 2023). In Kazakhstan, 14.5 thousand hectares of sugar beet fields are located in the Almaty, Zhetysu, and Zhambyl areas; these fields make up 99 percent of all sugar beet planting fields in the nation. It is one of the most important industrial crops in terms of economic impact in south and southeast region of Kazakhstan. Sugar beet is vulnerable to several diseases which result in significant yield losses and impact grower profitability. Depending on the severity of the disease and the frequency of the infection, sugar beet has experienced severe crop losses ranging from 30% to 60% or occasionally totals failure (Misra et al., 2023). Additionally, when beetroots are stored for an extended period of time, an important amount of recoverable sugar may be lost (Van Eerd et al., 2012). Due to a variety of technological issues, the emergence of microbiological illnesses, and other variables, 30–35% sugar beet harvest is destroyed when it is in the storage phase. There are large losses of sugar beet in the republic caused by a complex of pests and diseases. Among the pathogenic microflora of sugar beet, seed infection occupies a special place. It is well known that more than 60% of all pathogens of beet diseases (*Fusarium*, *Alternariasis*, root rot, etc.) are spread with seeds. Currently, there is a wide spread of fungal, bacterial and viral diseases on sugar beet plantations. All this complex of diseases cause significant harm to beet farming worsens the quality of products and their shelf life during storage (Lin et al., 2023). It also required to study of microorganisms that cause spoilage of the sugar beet seeds. Numerous microbial genera have already been identified as helping sugar beet plants growth, and their efficacy as biocontrol agents has also been investigated (Sacristán-Pérez-Minayo et al., 2020, Wolfgang et al., 2023). In this case, it is equally important to study the condition of the seeds and soil for the development of technology and eco-friendly methods for improving the storage of sugar beet. So it is need of the time to develop most effective strategies to improve the growth and shelf life of sugar beet using some biological sustainable methods (Wöber et al., 2025). To date, Tetramethyl thiuram disulfide (TMTD) protectants, 80% pp and fundazol, 50% pp, are recommended against seed infection on beet plantations (Gorshkov et al., 2020). However, they do not suppress infections of bacterial and fungal etiology. Microbiological preparations are effectively used against these phytopathogens. The effectiveness of most of them is associated not only with the action on the pathogen, but also with the growth-stimulating effect and the ability to activate the immune system of plants (Wöber et al., 2025). During current research, after conducting studies of the phytosanitary conditions of soil in beet-growing farms in the southern and

northern regions of Kazakhstan before planting, complexes of dominant species of soil microscopic fungi were identified in the agrocenosis of sugar beet in the Akmola region and in individual farms of the Almaty and Zhambyl regions. Therefore, to developing measures for preservation of sugar beet seeds, the specified research results are taken into account and, accordingly, active preparations for the suppression of pathogens have been formulated. The bioformulation prepared by using selected fungal strain, *Trichoderma asperellum*, showed its potential to improve the showing qualities of seeds as well as helped to improve the storage time of seeds.

2.0 Methodology

2.1 Sample collection and isolation of the microbes from soil

The top layer of the soil was removed by 2-3 cm, since extraneous microflora may be present in it. After that, samples were taken from the studied area of the soil. The length of each of them corresponded to the thickness of the layer from where the sample was taken. Five to seven samples were taken on a plot of 100-200 m² and the weight of each sample was 0.5 kg. After that, an average sample was taken, weighing approximately 0.5 kg. It was placed in a sterile bag enclosed in a cloth bag with the number of the plot indicated. Before the analysis, the samples were stored in the refrigerator (Gaete et al., 2020; Li et al., 2019). Based on the study of isolated microbes from the selected materials (sugar beet seeds and soil samples), an antagonistic fungal strain against the pathogens of sugar beet diseases was selected for further study.

2.2 Isolation and identification of the Fungal strain

The fungus was grown in 9 cm Petri dishes on potato dextrose agar (PDA) at 23 °C in the dark conditions. Following the colony's growth to 8 cm diameter, the plates were used for inoculation. The selected fungal strain *Trichoderma asperellum* was used in all experiments. The strain was analyzed by microscopic and molecular method for identification. The strain was deposited in the Republican Collection of Microorganisms (RCM) in Kazakhstan (Jiang et al., 2022).

2.2 Antagonistic activities of the selected strain

Trichoderma asperellum-Kaz NIIPPP-19, has been considered as a suitable candidate to prepare bioformulation on the basis of its potential to inhibit the pathogens of seeds during storage. The main pathogenic fungi (*Fusarium*, *Alternaria*, *Botryti*, *Sclerotinia*) of the sugar beet were taken to confirm the inhibitory potential of the isolated antagonist. To determine the antagonistic potential of the fungal strain to selected phytopathogenic fungi, the generally accepted procedure of the dual-culture plate antagonism was used (Zhang et al., 2014a). Potato dextrose agar media has been used to grow fungal strains at 25°C for 7 days. The 5mm plugs of fungi were placed symmetrically on each side of the Petri dishes, 2 cm apart from each other. Antagonistic strains were screened according to the size of the zone of inhibition (Liu et al., 2023).

2.3 Development of bio-preparation from *Trichoderma asperellum*

The biopreparation have been developed by using the selected strain of fungi (*Trichoderma asperellum*- KazNIIPP-19) to inhibit the infections in seeds of sugar beet. The optimal dose of the biopreparation i.e. 1×10^8 spores/ml, was prepared by observing the number of fungal colonies from the sugar beet seeds of different selected varieties (Aisholpan, Taraz and single-seeded Kyrgyz) (Zhang et al., 2016; Zhang et al., 2014b). Seeds were treated by using the different concentrations (0.5; 1.0; 1.5; 2.0 and 2.5%) of the prepared formulation upto 15 days. Simultaneously, the trials were conducted three times, and the average study outcomes were used as an initial basis.

2.4 Application of the bio-preparation

Two zonal sugar beet seed varieties including Taraz and Aisholpan; and one foreign single-seeded Kyrgyz variety were selected for the experimentation. Aisholpan" (2015), are approved for use in the main beet growing area (Almaty, Zhambyl regions), and the hybrid "Taraz" has been allowed in the Zhamybla region since 2017. The main criteria for making a decision on the pre-sowing treatment of sugar beet seeds are their germination, germination energy and phytosanitary condition of seedlings.

2.4.1 Influence on the storage duration of seeds

To check the potential of biopreparation on the seeds storage time of sugar beet, three different fungicides including Celest Top, Phytosporin-M and Maxim were selected to make the comparison. In addition to these, one bacterial based inoculant, Extrasol, was also chosen which contains two *Bacillus subtilis* strains with a minimum 1×10^8 viable cells/ml. For this purpose, five hundred seeds of the sugar beet varieties had been treated with the selected fungicides. Following treatments had been utilized to check their influence on the seeds storage duration.

1. Treatment I (main control) - Celest Top (dose: 0.01/100ml distilled water) + Phytosporin-M (dose: 1.0 ml/100ml distilled water);
2. Treatment II (comparative control) - Maxim" (1.0 ml/100ml) + Extrasol (5.0 ml /100 ml) in distilled water
3. Treatment III (prototype) – fungal biopreparation based on the isolated strain "*Trichoderma asperellum* - KazNIIPP-19", (1.0×10^8 spores/ml) (2.0 ml fungal inoculant / 100 ml)
4. Treatment IV (Control) - water, without using any preparations or fungicides.

Afterwards, these sugar beet seeds were tested monthly for the presence of microflora and germination parameters, in the experimental samples in comparison to the control.

2.4.2 Influence on the germination parameters of seeds

The germination energy percentage and germination rate of seed samples treated with the aforementioned preparation were examined in a laboratory setting to ascertain the extent to which the fungal preparation affected the sowing characteristics of seeds of different sugar beet varieties during storage. The extent of influence of the biological product, prepared by using the fungal strain

"*Trichoderma asperellum* - KazNIIPPP-19", on the duration of storage of sugar beet seeds was found out in a comparative aspect.

Results

4.1 Study of microbial composition in soil samples

By comparing the results of studied soil samples to find out the total microbial load, it was noted that the number of colonies per one gram of soil in all samples were almost at the same level (Figure 1 & 2). In general, the taxonomic groups of microorganisms in the samples are in the appropriate orders: bacteria- 1.0×10^4 CFU/g, fungi- 1.0×10^2 CFU/g, actinomycete - 1.0×10^4 CFU/g. In addition, the most numerous groups of microorganisms in soil samples obtained from Almaty, Zhambyl, and North Kazakhstan regions are bacteria and actinomycetes. In the course of conducted research, diverse dominant species of soil microscopic fungi were identified in the agroecosystem of sugar beet from the Akmola region (North Kazakhstan) and the individual farms of the Almaty and Zhambyl regions (Table 1). At the same time, the intensity of microbial transformation of organic substances in soils increases in the direction from the northern to the southern regions. In the soil of the southern regions, the relative content of cellulose-destroying bacteria increases as compared to fungi. The soils of the northern regions are significantly poorer in the content of spore producing bacteria and actinomycetes compared to southern soils. These microorganisms multiply in the late stages of decomposition of plant residues. Fungi belonging to the genus *Penicillium* are more prevalent in the soils of the northern areas, where mineralization processes are slower. In the south, there is an increase in the content of representatives of the genus *Aspergillus*. Fungi of two genera make up more than 70% of the micromycetes of a number of soil types. In soils where intensive mineralization processes occur, spore bacteria are widespread, capable of assimilating both organic and mineral nitrogen. On the contrary, in soils where the processes of mineralization of organic compounds are slow, spore-forming bacteria that consume organic forms of nitrogen prevail.

Table 1 – Detail about the beet-growing farms of Almaty and Zhambyl regions

Sample No.	Area	District	Farm	Area, (ha)
1	Almaty	Koksy	Mukry	10
2		Koksy	Balpyk bi Enbekshi	10
3		Jambyl	Beslildyk	8
4		Eskeldi	Segizbai	5
1	Zhambyl	Baizak	Talap	6
2		Merken	Kart-Jup	7
3		Merken	Transfer	11
4		Kordai	Samgai	12

The analyses of the conducted results, it was discovered that bacterial flora accounted for the greatest number of identified microorganisms. So in soil samples (No. 1-No. 4) of the Almaty region, the observed bacterial load was 34.0 ± 1.0 - 45.0 ± 2.4 CFU/g. In soil samples (No. 1-No.4) collected from the Zhambyl region, the bacterial count was between 36.0 ± 1.5 to 38.0 ± 1.2 CFU/1g (Figure 1&2).

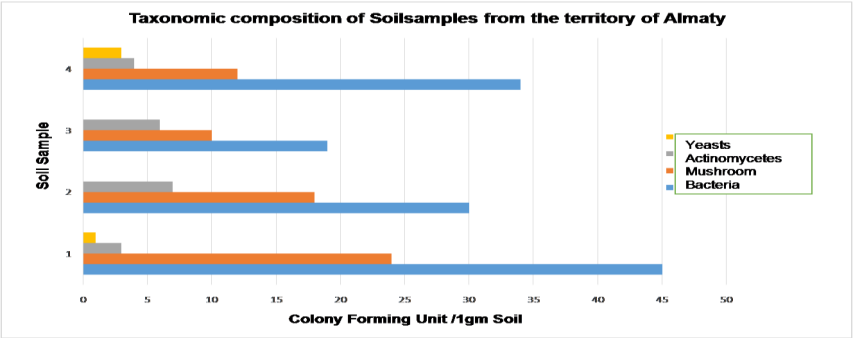


Figure 1 – Graphical Representation of Taxonomic composition of microbes in soil samples of Almaty region

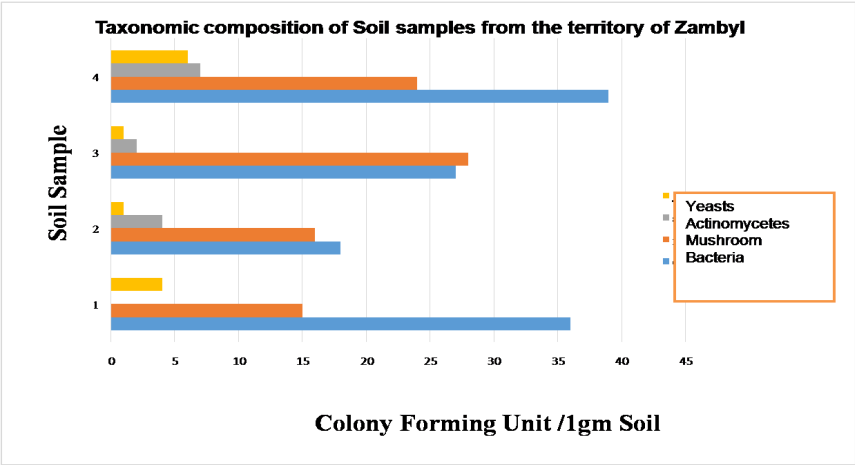


Figure 2 – Graphical Representation of Taxonomic composition of microbes in soil samples of Zhambyl region

4.2 Diversity of microbes in Southern Region of Kazakhstan

The soil samples of the southern regions including Almaty and Zhambyl, had been searched to study the mycobiota of sugar beet area. In the conducted studies of the taxonomic composition of microorganisms in soil samples of these regions, it was found that bacterial microflora prevailed in all soil samples on the second day. The highest frequency of occurrence is observed in bacteria *B. subtilis* (5.4 ± 0.3 CFU/g of soil), *Paenibacillus* (3.3 ± 1.5 CFU/g of soil) and *Pseudomonas* (2.6 ± 1.9 CFU/1 g of soil). There were also individual colonies of the following bacteria observed related with genera *Corynebacterium*, *Mycobacterium*, *Micrococcus*, *Pseudobacterium*, *Sarcina*, *Promyobacterium*, *Azotomonas*, *Lactobacterium* (Table 2).

Mushrooms were the second most abundant and common in the soil mycobiota of the beetroot agrocenosis of the South and South-East of Kazakhstan. According to the results reported in the table (Table 2), it can be seen that in the frequency of occurrence of soil microorganisms in various samples selected in sugar beet plantings, the species composition of the fungal flora varies and the most common species are: *Fusarium oxysporium*, *Botrytis stegea*, *Penicillium notatum*, *Mucor* sp., *Trichoderma viride*, *Mortierella* sp., as well as known species like *Aspergillus flavus*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Different species of *Penicillium* and *Alternaria* dominated the mycobiota in the both the selected regions. The rare mycobiota with less than 10% frequency of occurrence includes *Sclerotinia sclerotiorum*, *Mortierella* sp., *Trichoderma viride*, *Acremonium* sp. and *A. candidus*.

In all soil samples, *Fusarium*, *Alternaria* and *Penicillium* are dominated genera of microorganisms in terms of frequency and abundance of species. Fungi of the genus *Fusarium* are the most harmful pathogens of the sugar beet root diseases. They are resistant to drought and persist in the soil or on plant residues in the form of chlamydospores and mycelium for several years. The genus *Fusarium* in the soil is represented by species (*Fusarium* sp., *F. oxysporum*) that cause root rot, tracheomycosis, as well as infection in sugar beet seeds. Their number ranged from 23.4 ± 1.9 to 35.0 ± 1.9 CFU/ per 1g (frequency of occurrence was 76.7%). *Alternaria alternata* occurred in the soil samples with the frequency of occurrence 44.0% and this fungus is the causative agent of the sugar beet root diseases, leaf spotting, black mold of root crops in kagats. Before sowing seeds in the soil, it was observed in the range (from 24.0 ± 1.9 to 30.0 ± 0.7 CFU/g of soil). The number of *Penicillium* species in the soil under sugar beet ranged from 19.1 ± 1.1 to 27.0 ± 1.1 CFU/g of soil with the frequency of their occurrence was 51.2%.

From the data in Table 2, it can be seen that in soil samples No. 1 - No. 4 of the Almaty region, *Fusarium oxysporium* is the dominant species of fungal flora, and in samples No. 1 - No. 4 of the Zhambyl region, *Botrytis cinerea*. Other types of fungi were found to varying degrees in all samples.

Table 2 The frequency of occurrence of the fungal flora for Almaty and Zhambyl regions

S. N o.	Mycobiota in sugar beet soil samples			
	Dominant (>60%)	Frequent (30-60%)	Rare (10-30%)	Random <10%
Almaty Region				
1	<i>Alternaria alternata</i>	<i>Mortierella</i> sp	<i>Cladosporium herbarum</i>	<i>Sclerotinia sclerotiorum</i>
	<i>Penicillium</i> sp.	<i>Aspergillus candidus</i>	<i>Trichoderma viride</i>	<i>Botrytis cinerea</i>
	<i>Fusarium oxysporum</i>	<i>Mucor himalis</i>		<i>Rhizoctonia solani</i>
2	<i>A. alternata</i>	<i>S. sclerotiorum</i>	<i>T. viride</i>	<i>C. herbarum</i>
	<i>P. claucum.</i>	<i>A. candidus</i>		<i>Mortierella</i> sp
	<i>F. oxysporum</i>			
3	<i>F. oxysporum</i>	<i>Mucor</i> sp.	<i>A. niger</i>	<i>R. solani</i>
	<i>A. alternata</i>	<i>R.stolonifer</i>		<i>A. candidus</i>
	<i>P. claucum.</i>			<i>Acremonium</i> sp
4	<i>Penicillium</i> sp.	<i>A. alternata</i>	<i>Botrytis cinerea</i>	<i>T. viride</i>
	<i>F. oxysporum</i>	<i>A. candidus</i>	<i>A. niger</i>	<i>Acremonium</i> sp
	<i>Mucor</i> sp.			<i>R. solani</i>
Zhambyl Region				
1	<i>B. cinerea</i>	<i>C. herbarum</i>	<i>S. sclerotiorum</i>	<i>Mortierella</i> sp.
	<i>Penicillium</i> sp	<i>Mucor himalis.</i>	<i>T. viride</i>	<i>Aspergillus</i>
	<i>A. alternata,</i>	<i>Aspergillus</i> sp.		<i>S. sclerotiorum</i>
	<i>Fusarium</i> sp.			
2	<i>B. cinerea</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>T. viride</i>
	<i>A. alternata</i>		<i>T. candidum</i>	<i>Mortierella</i> sp.
	<i>P. claucum</i>			
3	<i>B. cinerea</i>	<i>T. viride</i>	<i>C. herbarum</i>	<i>R. solani</i>
	<i>F. oxysporum</i>		<i>A. flavus</i>	<i>A. candidus</i>
	<i>Alternaria</i> sp.			
4	<i>B. cinerea</i>	<i>Mucor</i> sp.	<i>Mortierella</i> sp	<i>C. herbarum</i>
	<i>P. claucum</i>	<i>A. niger</i>	<i>S. sclerotiorum</i>	<i>Acremonium</i> sp
	<i>A. alternata</i>			

4.3 Selection of a fungal strain for bio-preparation

A strain of an antagonist fungus against sugar beet disease pathogens has been chosen based on the materials' gathered and examined microorganisms (sugar beet seeds and soil samples). *Trichoderma asperellum*, a fungal isolate of micromycete, was chosen to shield sugar beet seedlings from harmful bacteria and fungus.

4.3.2 Antagonistic effect on pathogenic fungi

The fungus genus "*Trichoderma asperellum*-KazNIIPPP-19" has an antagonistic effect on pathogens that harm sugar beet seeds, as shown in the tables. The studied strains of *Trichoderma asperellum* have showed the inhibitory impact against the tested four phytopathogens. The index reported as 4 points on the Johnson and Karl scale in case of *Alternaria* sp. and *Fusarium* sp., i.e. inhibition of one organism by second one growing in close proximity, wherein the antagonist simultaneously creates a sterile zone (25-26mm) of suppression (inhibition) of pathogen growth and outgrows a colony of a harmful organism (Figure 4). The selected fungal strain also acted against the phytopathogens *Botrytis* sp. and *Sclerotinia* sp. with the inhibition zone of 20 mm and 25 mm, respectively, and the antagonism index was 2 and 3 points respectively (Table 3).

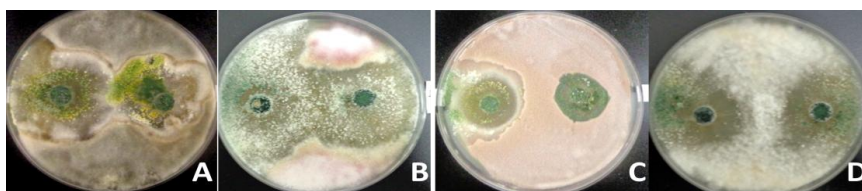


Figure 4. Antagonism of representative strain of *Trichoderma asperellum* against various phytopathogens: *Alternaria* sp.(A); *Fusarium* sp. (B); *Botrytis* sp. (C); *Sclerotinia* sp. (D)

The inhibition effect of antagonistic fungi had shown antibiosis against all pathogens. Antibiosis can be determined through metabolic products secreted by antagonistic fungal strains to inhibit the growth of selected pathogenic fungi, and results in the emergence of inhibition zones around their biomass

Table 3. Antagonism activity (Zone of inhibition) reported against the phytopathogens

Name of Fungi	Zone of Inhibition (mm)			
	<i>Fusarium</i> sp.	<i>Alternaria</i> sp.	<i>Sclerotinia</i> sp.	<i>Botrytis</i> sp.
<i>T. asperellum</i>	30	26	25	20

4.4 Determination of optimal dose

To improve the storage of seeds of sugar beet by using fungal bio-preparation, optimal dose of formulation (with an activity of 1×10^8) has been determined after testing with different dose percentage (0.5; 1.0; 1.5; 2.0 and 2.5%). The number of colonies of microbes after giving treatment with different doses on seeds of different varieties of sugar beet was reported (Table 4). It was observed that 2 ml of the preparation in 100 ml of distilled water has given satisfactory results as compare with other doses.

Table 4. Optimum dose determination for the use of fungal biopreparation

Experience Options	Doses of dilution of the formulation per 100.0 ml, in distilled water, per 75.0 g of seeds	Number of colonies of microorganisms, pcs.					
		Seeds of the variety "Aisholpan"		Seeds of the variety "Taraz"		Seeds of the variety "Kyrgyz single-seeded"	
		Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
<i>Trichoderma asperellum</i> КАЗНИИПП-19", activity 1×10^8	0.5	42±1,0	1,0	47±1,0	2±1,0	49±1,0	2±1,0
	1.0	27±1,0	1,0	29±1,0	1,0	28±1,0	1,0
	1.5	26±1,0	0	27±2,0	0	25±2,0	0
	2.0	18±2,5	0	19±2,0	0	18±1,0	0
	2.5	18±2,5	0	18±2,0	0	18±1,0	0
Control	Distilled processing water	65±2,0	4±1,0	64±2,0	4±1,0	68±1,0	3±1,0

4.5 Influence on the sowing qualities of seeds

Seed samples treated with the aforementioned preparations were collected monthly and compared for laboratory germination percentage and germination rate in order to ascertain the extent to which different pesticide compositions affected the sowing characteristics of seeds of different sugar beet varieties during storage for three months (the observation period). Thus, it can be seen from the presented results that for 3 months, a fungal preparation of the isolate "*Trichoderma asperellum*-KazNIIPPP-19" has an efficient preservative property against pathogenic microorganisms present on the seeds and has a beneficial impact on their sowing qualities. In the laboratory conditions, the indicators of germination energy and laboratory germination percentage of various varieties of sugar beet seeds were studied.

After three months, seeds of the Aisholpan variety (treatment 1) treated with the chemical preparation Celestop + Phytosporin exhibited high germination, with an $89.0 \pm 1.0\%$ germination rate. The Aisholpan variety seeds in the control (treatment-4), which was left untreated, had the lowest germination rate, measuring $65.0 \pm 1.0\%$ (Figure 5). It also exhibited good seed germination in experimental treatment 2 and 3, which were treated with a chemical preparation

Maxim + Extrasol ($83.0 \pm 1.0\%$ germination) and a biopreparation based on prepared fungal formulation ($81.0 \pm 2.0\%$ germination). In the conducted experiments, significant results were observed on the Aisholpan sugar beet variety with the Celestop-Phytosporin combined variant. The germination energy indicators for 4-6 months at approximately on the same level were $84-93 \pm 1.0\%$ for the Aisholpan variety, $81-89 \pm 1.0\%$ for the Taraz variety and $84-88 \pm 1.0\%$ for the Kyrgyz single-seeded variety, laboratory germination on these varieties were $92-97 \pm 1.0\%$, $90-94 \pm 2.0\%$ and $90-96 \pm 1.0\%$, respectively (Figure 6).

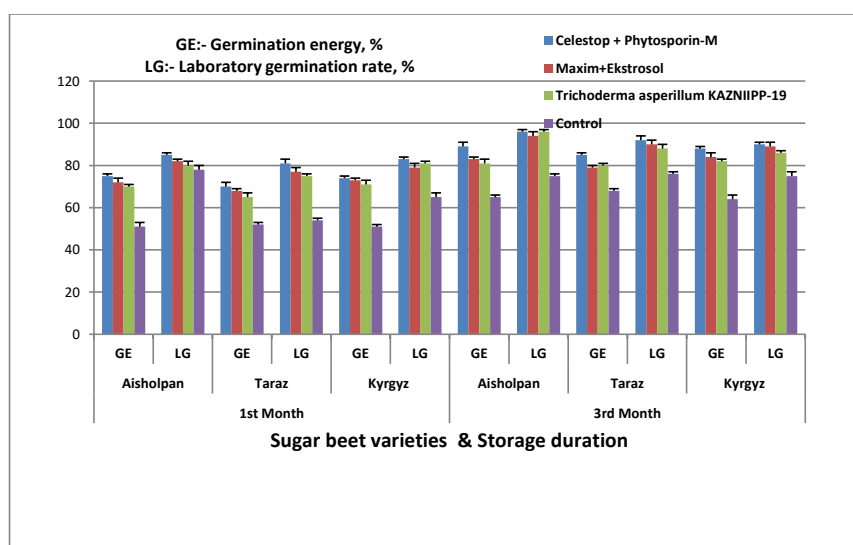


Figure 5. Effect of biological preparation on the germination energy percentage and germination rate percentage in different varieties of sugar beet after 1 and 3 months

Simultaneously, it came to light that a six-month seed treatment that included a fungicide and biological preparation improved the seeds' ability to germinate. The biological preparation based on the fungus "Trichoderma asperillum-KazNIIPP-19" was found to be as effective as the drug "Maxima" + "Extrosol" version in this instance, and somewhat less effective than the drug "Celestop" + "Phytosporin - M" variant.

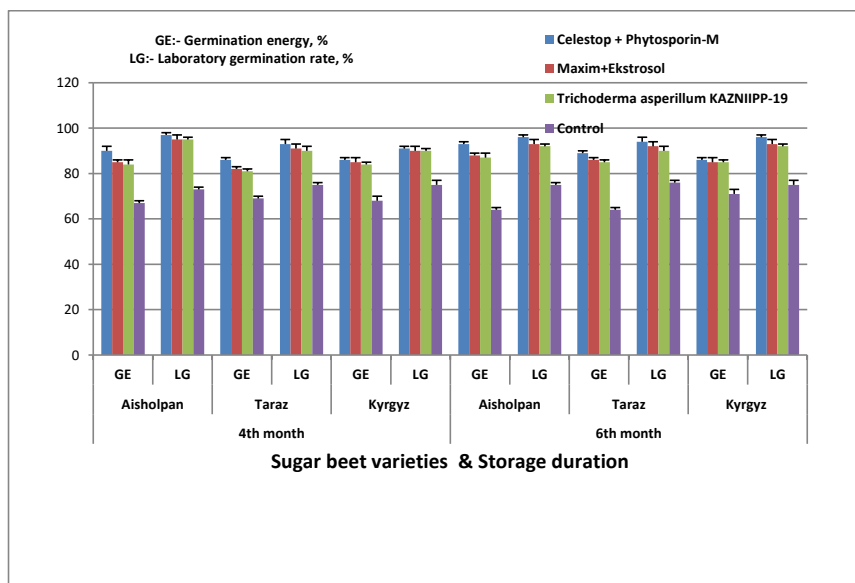


Figure 6. Effect of biological preparation on the germination energy percentage and germination rate percentage in different varieties of sugar beet after 4 and 6 months

In case of germination energy percentage, the experiments conducted after the 4th-6th months, by using a formulation prepared from "*Trichoderma asperillum* - KazNIIPP-19" the results observed were more significant as compared with the control. In case of the variety "Aisholpan" it is $17.0-29.0 \pm 1.0$, on the variety "Taraz" - $12.0-25.0 \pm 1.0\%$ and in the variety "Kyrgyz single-seeded", $14.0-19.0 \pm 1.0\%$ higher.

At the specified time of the experiment, the germination rate results in different variants with a biological preparation based on the above fungal isolate were also better than observed in the control. It was $17.0-24.0 \pm 1.0\%$ on the variety "Aisholpan", $15.0-19.0 \pm 1.0\%$ on the variety "Taraz" and $15.0-21.0 \pm 1.0\%$ for the variety "Kyrgyz single-seeded".

4.5 Influence on the storage duration of seeds

The number of bacterial colonies after one month of storage in the Aisholpan variety was 22.0 ± 1.0 pcs, and it reached 43.0 ± 1.0 pcs after third month, in the Taraz variety from 33.0 ± 1.0 pcs to 42.0 ± 2.0 pcs, in the Kyrgyz single-seeded variety from 24.0 ± 1.0 pcs to 48 ± 2.0 pcs, respectively. In case of control, the microbial load depends on the variety, ranged from 99.0 ± 2.0 to 123.0 ± 2.0 . From the data obtained during the study, it can be seen that in all conducted experimental setups, the microbial colonies were at an insignificant number and 3 to 4 times lower ($22.0 \pm 1.0-48.0 \pm 1.0$) compared with the control group ($90.0 \pm 2.0-$

124,0±1,0) (Figure 7&8). There were no significant differences in the number of microorganisms in the context of varieties. They were almost on the same level.

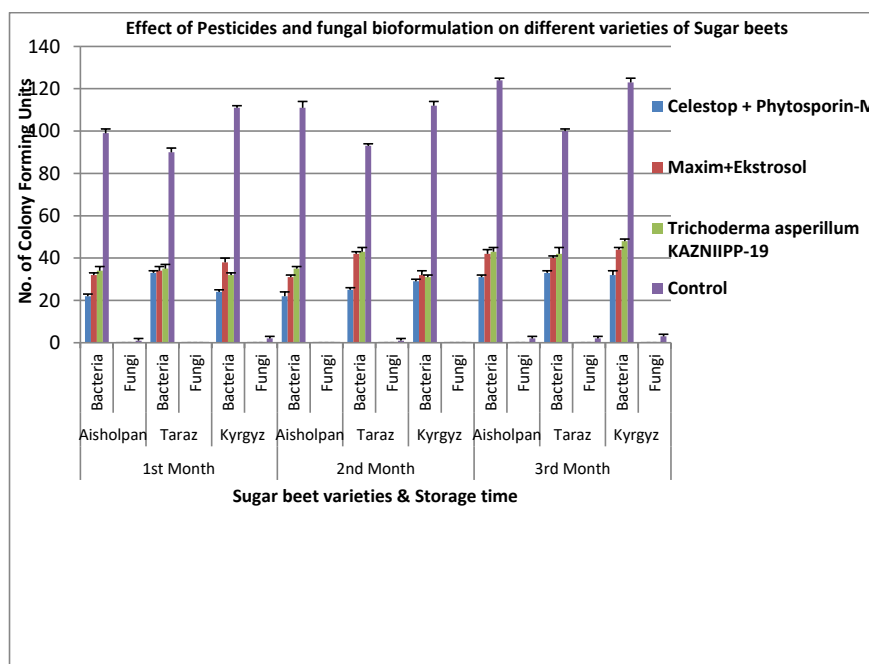


Figure 7. Effect of various treatments on the composition of microorganisms observed on different varieties of sugar beet seeds after storage of 1 to 3 months

Therefore, it is evident from the results that the fungal preparation based on the isolate "*Trichoderma asperillum* - KAZNIIPP-19" has a positive impact on the germination rates of sugar beet seeds and effectively protects them from harmful microorganisms for six months of storage (the observation period). It can restrained the growth of various fungal flora and reduced the number of bacteria

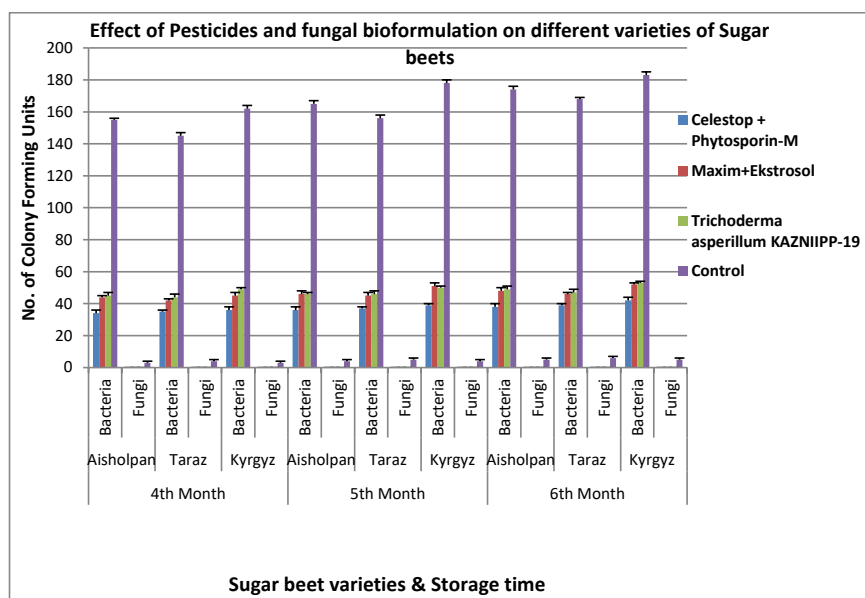


Figure 8. Effect of various treatments on the composition of microorganisms observed on different varieties of sugar beet seeds after storage of 4 to 6 months

Discussion

Interest in microbiome-assisted biofertilization, and biocontrol of plant diseases and pests is growing as efforts to make sugar beet agriculture more sustainable intensify (Wolfgang et al., 2023). Microbiological preparations are effectively used against phytopathogens of sugar beet. The effectiveness of these bio-preparations is associated not only with the action on the pathogens, but also with the growth-stimulating effect and the ability to activate the immune system of plants. Various bacterial endophytes (*Acinetobacter*, *Achromobacter*, *Bacillus*, *Burkholderia*, *Chryseobacterium* and *Pseudomonas*) were successfully tested as plant growth promoter for sugar beet and reflected positive outcomes in the germination rate, shortened germination time, and plant height etc. (Piernik et al., 2017). Unlike all other microbiome research linked to sugar beets, post-harvest microbiome studies concentrate on fungal populations because they are the primary cause of post-harvest sucrose losses.

Notably, this study represents the pioneering investigation into the microbial diversity of the sugar beet growing regions of Kazakhstan including Zhambyl and Almaty. The highest bacteria frequency of occurrence is observed for *B. subtilis* (5.4 ± 0.3 CFU/g of soil), *Paenibacillus* (3.3 ± 1.5 CFU/g of soil) and *Pseudomonas* (2.6 ± 1.9 CFU/1 g of soil) in comparison to other genera. In the current study, fungal isolate has been used to prepare the bio-formulation, after studying the

microflora of the sugar beet seeds and soil from sugar beet growing regions. The results showed that a biological preparation based on the fungus "*Trichoderma asperellum*-KazNIIPPP-19" has a positive impact on the germination rates of sugar beet seeds and effectively protects them from harmful microorganisms for six months of storage (the observation period). Based on the investigated activities, it has been shown that given fungal strain exhibits a broad spectrum of activities and offer a high potential for the development of plant growth promoter and biological control agents. Based on the results, strategies have been developed for studying the state of seed material and soil for the technology of safe long-term storage of zoned varieties of sugar beet. It also suggested the most acceptable and effective ways to increase the safety and growth rates of sugar beet seeds. Utilizing microorganisms that promote plant development and biocontrol has a lot of promise, particularly for high-yielding sensitive cultivars. A large number of possible BCAs in sugar beet are accessible and have been studied scientifically, but they have not yet been included into conventional farming methods.

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