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STUDY OF THE CONDITION OF THE SEED MATERIAL OF SUGAR BEET IN THE REPUBLIC OF KAZAKHSTAN AND THE DEVELOPMENT OF A BIOPREPARATION TO INCREASE THE DURATION OF ITS PRESERVATION

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Abstract

In modern conditions, beet-sowing farms in Kazakhstan suffer great losses from widespread diseases of sugar beet, leading to the death of plants in the field.

In this case, up to 30-35% of the sugar beet obtained is lost in the storage stage due to non-compliance with various technological conditions, the development of microbiological diseases and other factors. From the above, the problems of studying the condition of seed material of zoned varieties of sugar beet and the development of an effective biopreparation for its safe and long-term storage with the aim of introducing into the practice of sugar production in Kazakhstan become very important.

Aim of the research: to study the condition of the seed material of sugar beet in the Republic of Kazakhstan and the development of a biopreparation to increase the duration of its preservation.

Research methods: the isolation and study of microorganisms from the seed material of sugar beet, were carried out by taking samples from specialized farms and studying them using conventional methods for microbiological research, and then the development of a biopreparation in order to increase the shelf life of the seed material of sugar beet was conducted using an isolated effective strain of a wide

spectrum microorganism actions, in particular, of the selected strain "Trichoderma asperillum - KazNIIPPP-19" on the basis of generally accepted and our improved methods.

The results obtained: a scientifically grounded patent search was conducted. By studying the seed material of sugar beet, a strain of the microorganism "Trichoderma asperillum - KazNIIPPP-19" was selected to create a biopreparation in order to increase the shelf life of seed material of sugar beet. Based on the results obtained under experimental conditions, the developed biopreparation is recommended for the use of sugar beet seed treatment for long-term storage (within 6 months - observation period), as well as effective drug combinations: "Maxim" + "Extrasol" and "Celestop" + "Fitosporin - M" (approval certificate received). One patent application was filed for the elements of novelty and methodological recommendations for implementation into practice were developed.

Scientific novelty: for the first time in Kazakhstan, the condition of the seed material of sugar beet was studied and an effective biopreparation was developed to increase the duration of its preservation.

Implementation of the results: from the implementation of this project, ultimately, the yield will increase, the terms of preservation of sugar beets and products - sugar, and as a result of which there will be an increase in the economic state, food security in Kazakhstan.

Applications: food and processing industry.

Practical significance: the obtained research results in scientific and practical terms are significant for increasing the profitability of production, a strategic product, like sugar products in Kazakhstan.

Keywords: sugar beet, seeds, microorganisms, bactrenia, fungi, biopreparation.

1. Introduction

In the modern conditions of the development of the market economy of the Republic of Kazakhstan, especially as part of the World Trade Organization, for the domestic food and processing industry, the most urgent issues are to improve the quality and safety of products (Kusainova, 2019).

At the same time, it is necessary to take into account that it is impossible to obtain high-quality products if the raw materials used do not meet regulatory requirements (Spiridonov, 2000). This directly applies to such a strategic food product as sugar, which is mainly obtained in the Republic of Kazakhstan from sugar beet.

The world sown area for sugar beet is about 9 million ha (80% - in Europe), of which more than 40% of the sown area is concentrated in the CIS countries, the main crops are located in Ukraine, and small areas are available in Kyrgyzstan, Kazakhstan, Georgia, Armenia, Lithuania, Latvia and Belarus (Shamin and Stognienko, 2017).

Sugar producers in Kazakhstan are sugar corporations. Today, sugar factories are mainly located in the south-east of Kazakhstan, where the main areas of sugar beet and sugar factories are concentrated, since the soil and climatic conditions are quite consistent with the biological requirements for growth, development, and accumulation of sugar beet yield (Golyshin, 1988; Sanin, 2003).

In 2019, 14.4 thousand ha were sown with sugar beet in the Almaty region, the gross harvest amounted to 385.3 thousand tons. This season, the sown area is not going to change, but it is planned to increase the gross harvest to 388.7 thousand by increasing the yield.

At the same time, up to 30-35% of the sugar beet obtained is lost during the storage stage due to non-compliance with various technological conditions, the development of microbiological diseases and other factors.

In this case, first of all, the question arises: what technics and methods can be used to minimize the weight loss of root crops and sugar associated with the temporary storage of beets.

Equally important, one of the reasons for the loss of beets during storage is due to the fact that sugar beets can become susceptible to diseases, especially during storage. The causative agents of sugar beet disease are microorganisms that are found on root crops, especially with unwashed soil. All diseases of the beet ultimately lead to its rotting. Rotting of beets in clumps does not occur under the influence of any one pathogen. Many types of fungi and bacteria are involved in this process (Maui, 2009). Diseases of the roots of beets during storage are characterized by the common name - clump rot. Losses from clump rot can be very significant: in some sugar factories, losses were up to 30% or more. When the amount of a mixture of rotten mass is 8-10% or more, factories often do not receive crystalline sugar at all (Ludilov et al., 2001).

For the technology of safe long-term storage of zoned varieties of sugar beet, it is necessary to provide beet-sowing farms with high-quality seeds of domestic and foreign selection and with a low cost, and to study the condition of seed material, as well as to study microorganisms that spoil products and offer the most acceptable effective ways to increase the safety and growth characteristics of sugar beet seeds, and on the basis of the results obtained, the development of effective proposals and biopreparations for the safe and long-term preservation of sugar beet seeds, with their subsequent use in industrial conditions, is relevant for sugar production in Kazakhstan.

2. Methods

2.1 Research objects

Research objects: seed materials of sugar beet of domestic and foreign selection, grown in Kazakhstan, obtained from the southern and northern regions of growing sugar beet and microorganisms.

2.2 Research methodology

2.2.1 Study of seed material of domestic and foreign selection of sugar beet grown in Kazakhstan

Studied on the basis of the monitoring analysis of enterprises for the cultivation of sugar beet and the sale of seeds in the republic, as well as information obtained from literary and computer sources, and we also analyzed the works on seed materials of the main research institutes, in particular, the Kazakh Scientific Research Institute of Agriculture and Plant Growing and Kazakh Research Institute of Horticulture of the Ministry of Agriculture of the Republic of Kazakhstan.

2.2.1.1 Isolation and study of microorganisms from seed material of sugar beet

Isolation and study of microorganisms from soil from the southern and northern regions of cultivation and seed material of sugar beet, for the presence of pathogens, will be carried out by sampling soil and seed material of sugar beet from specialized farms and then studying them using conventional methods for microbiological research

and analysis (Buga, 2017).

In this case, microbiological studies were carried out using our developed and proposed in practice a more modern one, which does not require cooking culture media for microbiological analyzes, based on membrane filtration of samples using sterile culture media - dry nutrient cardboard substrates (NCS) (an innovative patent "Method for monitoring microbiological contamination of vegetables at the stages of growing and storage" No. 23667 was received). Identification of bacterial, yeast, and fungal microflora to the genus, isolated from the surface of seeds, soil, etc., was carried out in accordance with the identifiers of bacteria, yeast, and filamentous fungi (Golyshin, 1990; Nadykta, 2014; Naumova, 1970; Hoult, 1980; Hoult, 1997; Krasilniko, 1949).

However, in the absence of the above apparatus, it is possible to use the generally accepted classical method for microbiological analysis of samples. In this case, the identification of mesophilic aerobic and facultative anaerobic microorganisms was carried out according to GOST 10444.3-75, yeast - GOST 10444.12-75, the identification of viable molds according to GOST 10444.13-75, the total number of microorganisms was carried out by counting growing colonies on Petri dishes, according to GOST 10444.15-75. When isolating a pure culture of aerobic microorganisms, the enrichment culture is sown on the surface of a dense medium.

To identify microorganisms to the genus, it is sufficient to determine the morphological characteristics. The morphological characteristics of microorganisms include the shape of cells, their combination and size, mobility, the ability to form spores and capsules, as well as the presence of some inclusions in the cells. The study of morphological features was carried out using microscopic methods and various methods of staining cells. When describing the morphology of cells, the age of the culture, the composition of the medium, and the cultivation conditions were taken into account.

The agricultural technology of cultivation of sugar beet was studied in those farms where samples were taken together with specialists on the basis of the collection of agricultural materials from farms and their analysis. As a basis for studying the methodology of the field experiment, we used the methodology of research on sugar beet of the All-Russian Research Institute of Sugar Beet (ARISB, 2018).

2.2.2 Selection of a strain of microorganisms for work with the aim of creating a biopreparation to increase the shelf life of sugar beet seed

On the basis of the isolated and studied microorganisms of the investigated materials (from samples of seed material of sugar beet and soil), a strain of a microorganism was selected - an antagonist against pathogens of sugar beet diseases, and on their basis experimental batches of culture liquid for obtaining a biopreparation were developed. The object of our research was the micromycete strain: Trichoderma asperellum. The strain was proposed to protect sugar beet seeds from pathogenic fungi - causative agents of root and foot rot Botrytis cinerea Pers., and bacteria Pseudomonas syringae. The material for the research was sugar beet seeds and soil samples taken in the area of the rhizosphere and the root layer of soil (5–10 cm).

2.2.3 Conducting experimental studies to study the selected strain of microorganisms to obtain a control sample of a biopreparation for treatment seed material of sugar beet of domestic and foreign selection, in order to increase the duration of their storage

After that, the optimal dose of dilution of the biological product "Trichoderma asperillum - KazNIIPPP-19", with an activity of $1x10^8$, used to suppress seed infection of sugar beet during storage, was determined by determining the number of colonies of microorganisms on the seeds of various zoned varieties of sugar beet ("Aisholpan" , "Taraz" and "Kyrgyz single-seeded") after their treatment with different doses (0.5; 1.0; 1.5; 2.0 and 2.5%) and microbiological studies (after 15 days).

In order to study the degree of suppression of a control sample of a biopreparation developed on the basis of the isolated strain of the fungus "Trichoderma asperillum - KazNIIPPP-19", in the laboratory "Biotechnology, quality and food safety" of "Kazakh Research Institute of Processing and Food Industry" LLP of the Ministry of Agriculture of the Republic of Kazakhstan (KazRIPFI), seed infection in samples of sugar beet seeds, during the period of their long-term storage, we tested various variants of drugs in laboratory conditions, in comparison with controls with biopreparations, in continuation of studies for 6 months (observation period).

For the experiments, preparations were taken that were approved for use in the territory of Kazakhstan against sugar beet diseases (Shamin and Stognienko, 2017; Golyshin, 1988; Sanin, 2003). For setting experimentally taken 2 zoned (varieties: "Taraz" and "Aisholpan") and 1 foreign (variety "Kyrgyz single-seeded") varieties of sugar beet seed.

Experiments to study the degree of biopreparation influence, based on the isolated strain "Trichoderma asperillum - KazNIIPPP-19" (with activity: 1×10^8), on the storage duration of sugar beet seeds, were carried out according to the following scheme:

1. Treatment of 500 seeds of the specified varieties of sugar beet with the following preparations: “Celestop” (treatment dose: 0.01/100 ml of distilled water) + “Fitosporin – M” (in a treatment dose: 1.0 ml/100 ml of distilled water) (basic control);

2. Treatment of 500 seeds of the specified varieties of sugar beet with preparations: "Maxim" (treatment dose: 1.0 ml/100 ml of distilled water) + "Extrasol" (treatment dose: 5.0 ml/100 ml of distilled water) (comparative control);

3. Treatment of 500 seeds of the indicated varieties of sugar beet with a biopreparation, based on the culture liquid of the isolated strain of the fungus "Trichoderma asperillum - KazNIIPPP-19", (with activity: 1.0×10^8) (treatment dose: 2.0 ml of the drug /100 ml distilled water) (control sample);

4. Control: treatment of 500 seeds of the specified varieties of sugar beet with water, without treatment with drugs.

In this case, 3 variants of seed treatment were assessed and the indicated seeds without treatment served as control. Subsequently, these variants of the samples were monthly examined for the presence of obligate

microflora, in a comparative aspect of the results obtained in the control sample in comparison with the control ones.

In addition, in order to determine the degree of influence of various compositions of drugs on the sowing qualities of seeds of various varieties of sugar beet during the storage period, also monthly for 6 months (observation period), samples of seeds treated with the above drugs were taken and the indicators of their germination energy on the 3rd and viability on the 7th day in laboratory conditions.

Based on the experimental data obtained, in a comparative aspect, the degree of influence of the proposed biopreparation, on the basis of the isolated strain "Trichoderma asperillum - KazNIIPPP-19", (with an activity of 1.0 x 10^8), on the storage duration of sugar beet seeds was clarified.

2.2.4 Statistical analyzes

The experiments were carried out in triplicate. For all measurements, the values are specified ± standard deviation. Differences in measurements between experimental and control groups were calculated using analysis of variation (one-way ANOVA) using the Tukey test. The measurement value $P<0.05$ was taken into account as significant.

3. Results

3.1 Isolation and study of microorganisms from seed material of sugar beet

The studies were conducted in laboratory conditions at “Kazakh Scientific Research Institute of Processing and Food Industry” LLP. For research, we have selected two regions on the basis of monitoring the beet-sowing farms of the Republic of Kazakhstan, in particular, the farms of Zhambyl and Almaty regions, since there are large sugar beet processing plants in these regions.

At the same time, we examined the seeds of sugar beet, varieties "Aisholpan", taken in the Almaty region, Taldukorgan city of the Eskildi district in the farm "Segizbay" and seeds of the variety "Taraz" in the Zhambyl region of the Merken district in the farm "Dakhir".

As a result of the conducted studies, it was found that the maximum number of isolated microorganisms was represented by fungal flora - 53±3.0%, then bacteria - 23±1.0%, actinomycetes - 16±1.0% and the least amount of yeast - 8±0.3%.

Identification of the selected strains of microorganisms using classical microbiological methods showed that representatives of the fungal flora were assigned to the genera: Alternaria alternata, Cladosporium sp., Mucor sp., Fusarium sp., Penicillium sp., Aspergillus sp.

It has been established that the greatest danger is represented by the fungi Alternaria alternata, Fusarium sp. These phytopathogenic microscopic fungi are the causative agents of the sugar beet root borer.

Table 1 - Frequency of occurrence of microorganisms of fungal flora on the surface of sugar beet seeds, %

Types of microorganisms	Frequency of occurrence,%	
	Pericarp	Internal infection
Alternaria alternata	36,6±2,0	37,0±2,0
Aspergilus flavus	10,5±1,0	
Botrytis cinerea	6,3±1,0	5,3±1,0
Cladosporium sp.	5,8±1,0	
Fusarium oxisporum	15,8±1,0	5,3±1,0
Fusarium sp.	5,3±1,0	
Penicillium claucum	11,1±1,0	5,3±1,0
Mucor sp.	10,6±1,0	

Values stated ± are standard deviation calculated from three parallel measurements

As can be seen from the data in Table 1, the most common microorganisms are fungi of the genus Alternaria (on the pericarp 36.6±2.0%, internal infection 37±2.0%) and Fusarium (on the pericarp from 5.3±1.0% up to 15.8±1.0%, internal infection 6%). Almost all seeds contained the fungus Penicillium claucum (pericarp 11.1±1.0%, internal infection 5.3±1.0%).

At the same time, the pathogenic fungal microflora isolated from the surface of sugar beet seeds were the following genera: Alternaria alternata, Fusarium oxisporum, Penicillium claucum, Aspergilus flavus, Mucor sp., Botrytis cinerea, Cladosporium sp., and the bacterial flora is represented by the genera - Pacseudomonas, Bacillus, Paenibacillus.

Yeasts and yeast-like microorganisms (2 isolates) were represented by the genera: Cryptococcus, Saccharomyces. Actinomycetes are represented by the genus Streptosporangium.

It can be said that the genus Alternaria dominates in the complex of seed microscopic fungi in the beet-sowing regions of Kazakhstan. They were also often found in the microflora of Fusarium and Penicillium seeds, both in number and occurrence, which, under certain conditions (high humidity and optimal temperature), are the causative agents of the root disease.

Consequently, when developing protective measures for sugar beet seeds, the indicated research results, accordingly, drugs which are active to equip these microorganisms should be taken into account and selected.

3.2 Selection of a strain of microorganisms for work with the purpose of creating a biopreparation to increase the shelf life of sugar beet seed.

In the course of our earlier screening studies to study the microflora of soil samples taken from the rhizosphere of sugar beet and the surface of seeds, a fungus of the genus Trichoderma was isolated. It is known

from the literature data that this fungus is one of the natural phytopathogenic microorganisms that exhibits cosmopolitanism and is found in all types of soils (Golyshin, 1990; Nadykta, 2014; Naumova, 1970; Hoult, 1980; Hoult, 1997; Krasilniko, 1949; Pidoplichko, 1977; Popova and Sadykova, 2014; Yemtsev and Mishustin, 2005; Sutton et al., 2001; Kornienko et al., 2002; Andreyeva and Ryabykh, 2002). Populations of fungi of the genus Trichoderma are a natural reservoir for the search for strains of producers of biologically active compounds effective for controlling a wide group of organisms: opportunistic and pathogenic bacteria, phytopathogenic (Likhachev and Sadykova, 2007; Gneusheva et al., 2013; Aspite et al., 1981). However, the potential possibilities of using representatives of the Trichoderma species in the biological control of pathogens of plant seed infection have not been studied enough. Therefore, we made an attempt to find out whether the Trichoderma fungus isolated by us has antagonistic activity against the main pathogens of sugar beet seeds (Sadykova, 2012; Chet, 2017; Reino et al., 2018).

To determine the species belonging of the antagonist fungus, we studied its cultural and morphological features. It was found that on the 3rd day of growth of the fungus Trichoderma on Czapek's medium at 25°C, the colony has a diameter of 55-65 mm. Colonies form several concentric rings, on the surface of which intense sporulation is noticeable. In the central part, the colony is darker, earlier acquires a green color, as the distance from the center forms an aerial mycelium (Fig. 1).

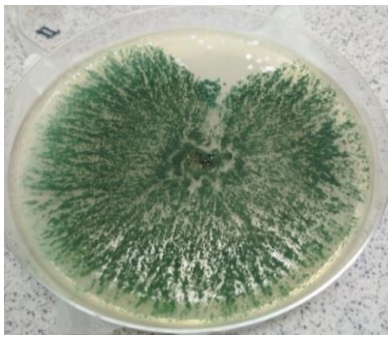

Figure 1. Culture of Trichoderma asperellum on Czapek's medium on day 3

When cultured at 25°C in the absence of light, conidia are formed after 35-45 hours. Diffusion of pigment in agar does not occur; old colonies have a weak specific smell. Paired branches are formed below the apex of the conidiogenous and are located at an angle of about 90° with respect to the main axis. The width of the conidiogenous is 2.1-5.0 µm. Phialids are formed at the ends of branches of the first and second order, clusters of 2-4 phialids are formed. Conidia are dark green in color, slightly ovoid, often closer to spherical, with a diameter of 3.5-4 µm.

Thus, according to morphological and cultural characteristics, the fungus was previously assigned to the species Trichoderma asperellum. To confirm its species, we carried out a polymerase chain reaction (PCR). DNA study using specific ITS fragments of nuclear ribosomal DNA isolates of Trichoderma confirmed the identification results determined by morphological properties. A special strain number and designation were assigned: «Trichoderma asperellum KazNIIPPP-19".

Fragment: 16SrRNA gene of Trichoderma isolate has the following nucleotide sequence:

CCAAACTGTTGCCTCGGCGGGGTCACGCCCCGGGTGCGTCGCAGCCCCGGAACCAGGCGCCCGCCGGAGGAACCAA
CCAAACTCTTTCTGTAGTCCCCTCGCGGACGTATTTCTTTACAGCTCTGAGCAAAAATTCAAAATGAATCAAAACTTTCAACAAC
GGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGG
ATCGGCGTTGGGGATCGGGACCCCTCACACGGGTGCCGGCCCCTAAATACAGTGGCGGTCTCGCCGCAGCCTCTCCTGCGCAG
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TAGGAATACCCGCTGAACTTAAGCATATCA

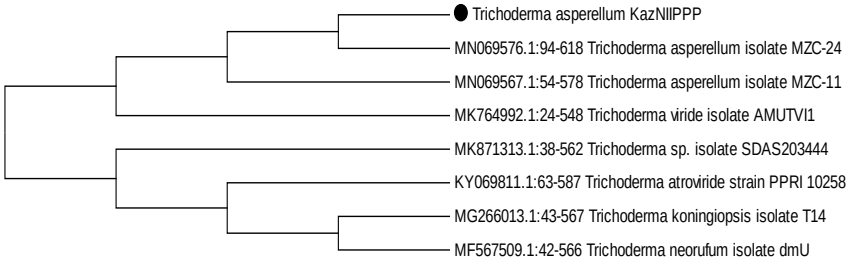


Figure 2. Phylogenetic position of the natural isolate “Trichoderma asperellum – KazNIIPPP-19”, based on the analysis of nucleotide sequences of the 16SrRNA gene fragment

The next stage of our research was to determine the inhibitory activity of the selected antagonist «Trichoderma asperellum KazNIIPPP-19" in suppressing pathogens of sugar beet seeds during storage. To identify antagonism of the studied strain to phytopathogenic microorganisms, the agar block method was used. A suspension of spores and mycelium fragments of phytopathogens was sown on Petri dishes with potato dextrose agar. At the same time, "Trichoderma asperellum KazNIIPPP-19" lawn was sown in a separate Petri dish with the same medium. All microorganisms were placed for 3-5 hours in thermostats at a temperature of 27°C in order for conidia and spores to germinate. After the specified time elapsed, agar blocks were cut out from the medium on which the culture "Trichoderma asperellum KazNIIPPP-19" was inoculated with a sterile cork drill and, using a microbiological needle, they were placed into Petri dishes with phytopathogens so that the side of the block on which the culture "Trichoderma asperellum KazNIIPPP-19", turned out to be directly adjacent to the surface of the environment with a developing culture of the phytopathogen. The control was lawns of phytopathogens, sown simultaneously with the experimental ones, but to which no agar blocks with trichoderma were placed. Control and

experimental plates with cultures were placed in a thermostat at 27°C. Observations were carried out on days 2-5, depending on the growth rate of the phytopathogen.

The main fungi of the genera Fusarium, Alternaria, Botryti, Sclerotinia were taken as test cultures.

To describe the types of relationships between fungi of the genus Trichoderma and phytopathogens of the genera Fusarium, Alternaria, Botrytis, and Sclerotinia, the Johnson and Karl scale was used, modified and supplemented by F.K. Alimova.

The antagonistic effect of the fungus of the genus "Trichoderma asperellum KazNIIPPP-19", pathogens causing damage to sugar beet seeds, is presented in Tables 2, 3 and in Figure 3.

Table 2 - Antagonism index of Trichoderma asperellum KazNIIPPP-19, in relation to pathogens

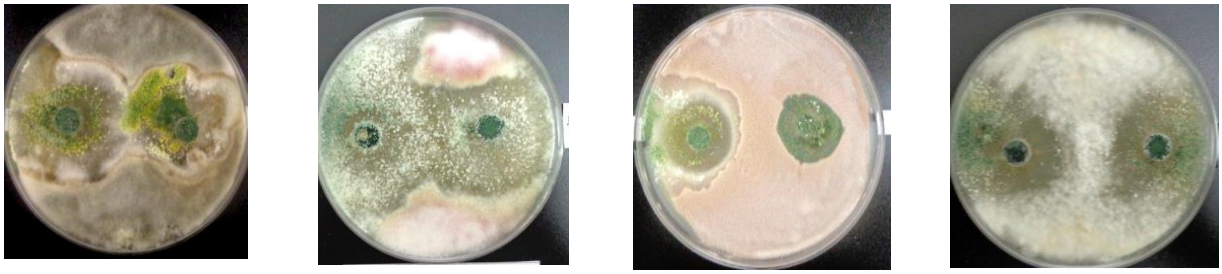
No.	Pathogenic fungal flora	Score
1	Fusarium sp.	4
2	Alternaria sp.	4
3	Botrytis sp.	3
4	Sclerotinia sp.	1

Table 3 - Antagonistic activity of fungi of the genus Trichoderma asperellum - KazNIIPPP-19, against the main causative agents of sugar beet diseases isolated in 2020

Growth suppression zone (mm)				
	Fusariumsp.	Fusariumsp.	Botrytis sp.	Sclerotiniasp.
«Trichoderma asperellum KazNIIPPP-19"	26±1,0	25±1,0	30±1,0	20±1,0

Values stated ± are standard deviation calculated from three parallel measurements.

The investigated strain Trichoderma asperellum - KazNIIPPP-19 had the same effect on the pathogens Fusarium, Alternaria. The index on the Johnson and Karl scale was 4 points, i.e. suppression of one organism by another by direct contact, when the antagonist overgrows the colony of the pathogenic organism and at the same time forms a sterile zone of suppression of the growth of the pathogen (25-26 mm). The fungi Trichoderma asperellum Kaz NIIPPP-19, acted on the pathogens Botrytis sp. and Sclerotinia sp. (zone of inhibition 20±1.0 mm and 30±1.0 mm), the antagonism index was 3 and 1 points.



a b c d

a - strain of the fungus of the genus «Trichoderma asperellum KazNIIPPP-19" against fungi of the genus Alternaria, b - against fungi of the genus Fusarium, c - against fungi of the genus Botrytis, d - against fungi of the genus Sclerotinia.

Figure 3. Antagonistic activity of fungi of the genus "Trichoderma asperellum KazNIIPPP-19", against pathogenic fungi infecting sugar beet seeds

Under laboratory conditions, the selected antagonist "Trichoderma asperellum KazNIIPPP-19" was cultivated for 10-14 days in Czapek's liquid nutrient medium on a shaker (120-140 rpm).

As a result of the studies, an antagonist fungus was selected, on the basis of which a control sample of a biopreparation was developed.

A new strain of the fungus of the genus Trichoderma asperellum KazNIIPPP-19, will in the future become the basis for creating a drug that will be used in the agricultural industry to protect sugar beet seeds during storage. The strain of the fungus Trichoderma asperellum KazNIIPPP-19 will be used in the form of a spore-mycelial preparation to suppress more phytopathogens and to work with the aim of creating a biological product to increase the shelf life of sugar beet seed.

3.3 Experimental studies on the study of a selected strain of microorganisms to obtain a control sample of a biological product for the treatment of seed material of sugar beet of domestic and foreign selection, in order to increase the duration of their storage.

Recently, attention has increased to the development of biological means of combating pests and diseases of agricultural and industrial crops.

On the basis of preliminary studies, we have selected a strain of the fungus Trichoderma asperillum - an antagonist against pathogens of sugar beet diseases, to determine the species identity of the antagonist fungus, we studied its cultural and morphological characteristics and to confirm its species identity, we carried out PCR analysis in the laboratory of chemical and molecular genetic research and analysis methods of the “Scientific and Production Center for Microbiology and Virology” LLP (Conclusion of molecular genetic examination of March 29, 2020, No. 01-03-04/32, Protocol No. 4).

The strain is a strict aerobic, saprotrophic. DNA study using specific ITS fragments of nuclear ribosomal DNA isolates of Trichoderma confirmed the identification results determined by morphological properties. After that, a special strain number and designation were assigned to the isolated culture: «Trichoderma asperellum KazNIIPPP-19". Then, on the basis of the accredited testing laboratory of the Academy of Nutrition "NUTRITES" LLP, the virulence of the isolated strain was studied using standard methods on white mice, at concentrations from 10^3 to 10^{11} CFU/cm^3. At the same time, it was found that according to the existing classification of strains, the culture "Trichoderma asperellum KazNIIPPP-19" belongs to the 3rd hazard class (There is a conclusion of 301 K from March 12, 2020).

On the basis of the results obtained, the Passport of the culture strain of the microorganism "Trichoderma asperellum KazNIIPPP-19" was compiled and this strain was submitted for deposition in the RSE on the REM "Republican Collection of Microorganisms" of MES RK and a Certificate of deposition of the microorganism strain "Trichoderma asperellum" (Registration number No. 09/12-April 2020).

Subsequently, under laboratory conditions, on the basis of a microorganism strain "Trichoderma asperellum KazNIIPPP-19", experimental batches of a biopreparation were developed, which were used for experiments, according to the research schedule (Figure 4).

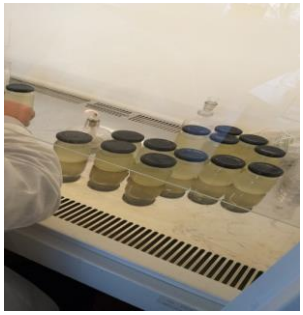
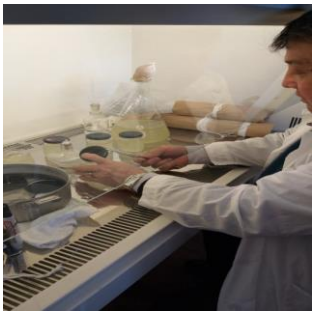



Figure 4. On the basis of the microorganism strain "Trichoderma asperellum KazNIIPPP-19", experimental batches of the biopreparation have been developed

At the same time, we took into account the fact that the strains of the fungus Trichoderma are among the recommended producer strains for creating biological products, since they are capable of suppressing a number of phytopathogenic fungi and microorganisms and are technologically advanced (Hoult, 1997). At the same time, preparations obtained on the basis of the strains of the fungus Trichoderma are used to protect sugar beet seeds from pathogenic fungi and other microorganisms, in particular against the causative agents of root and foot rot - Botrytis cinerea Pers. and the bacteria Pseudomonas syringae.

The material for the research was the seeds of sugar beet, selected in the area of the rhizosphere and the root layer of the soil (5–10 cm). In this case, the antifungal properties of the fungus Trichoderma lignorum 3M in relation to the pathogens Botrytis cinerea Pers and Pseudomonas syringae were studied by the block method on solid potato-sucrose nutrient medium. At the same time, they were cultivated at a temperature of 25^0C. The obtained pure substance was tested against Botrytis cinerea Pers and Pseudomonas syringae by the method of cylinders and paper disks. In this case, the bacterial flora isolated from the surface of the sugar beet seeds was from the genus: Pseudomonas syringae.

The positive effect of the saprophytic fungus Thrichoderma asperillum on the growth of higher plants due to the release of physiologically active substances has been proven. The greatest effect was observed at dilutions of 1:10 and 1:50. This strain was further used by us to obtain a highly effective drug with a wide spectrum of antagonistic and growth-stimulating activity.

Recently, information has appeared that fungi of the genus Thrichoderma are also capable of producing phytohormones, in addition to protective properties, a direct stimulating effect on plant growth. This is due to the fact that microorganisms are capable of producing growth substances and antibiotics, immunizing plants, and increasing the absorption activity of roots (Pidoplichko, 1977). Fungi of the genus Trichoderma are capable of synthesizing a powerful complex of hydrolytic enzymes that break down not only insoluble polysaccharides - pectin, cellulose, and hemicelluloses, but also lignin (Pidoplichko, 1977).

Such effects of fungi of the genus Trichoderma on plant development are very important for their application in agriculture and forestry, as well as for understanding the role of these fungi in natural and artificial ecosystems (Khramtsov, 2008).

After that, we laid down experiments to study the dynamics of the species composition and the number of microorganisms on sugar beet seeds during long-term storage. The obtained research results were processed according to the biometric method of G.F. Lakin (Lakin, 2015).

Initially, in laboratory conditions, a phytoexamination of sugar beet seeds of 2 regional (varieties: "Taraz" and "Aisholpan") and 1 foreign ("Kyrgyz single-seeded") varieties of sugar beet seeds, single-seeded to identify the

initial population of their various microflora, was conducted. In the course of the analyzes, a number of accompanying fungi from the genera Mucor, Penicillium, Aspergillus and pathogenic fungi from the genera Alternaria, Fusarium were identified. Microorganisms were isolated from the surface and inside the seeds. The bacterial flora is represented by the genus Paenibacillus. Bacillus, Lactobaccilus, Pseudomonas. It was found that the genus Alternaria dominates in the complex of seed microscopic fungi in the beet-sowing regions of Kazakhstan.

In this case, both in terms of number and frequency of occurrence in the microflora of seeds, there were genera of fungi Fusarium and Penicillium, which, under certain conditions (high humidity and optimal temperature), are the causative agents of the root borer disease.

After that, the optimal dose of dilution of the biopreparation "Trichoderma asperillum KazNIIPPP-19", with an activity of $1x10^8$, used to suppress seed infection of sugar beet during storage, was determined by determining the number of colonies of microorganisms on the seeds of various zoned varieties of sugar beet ("Aisholpan", "Taraz" and "Kyrgyz single-seeded") after their treatment with various doses (0.5; 1.0; 1.5; 2.0 and 2.5%) and microbiological studies (after 15 days). At the same time, the experiments were carried out with 3-fold repetition and the obtained average research results were taken as the basis. The research results are presented in Table 4.

Table 4 - Determination of the optimal dose of cultivation of the biological product "Trichoderma asperillum KazNIIPPP-19", used to suppress seed infection of sugar beet during storage, by determining the number of colonies of microorganisms on the seeds of various zoned varieties of sugar beet after treatment

Experience variants		Number of colonies of microorganisms, pcs.					
	Doses of dilution of the drug ml per 100.0 ml, in distilled water, per 75.0 g of seeds	Seeds of the variety "Aisholpan"		Seeds of the variety "Taraz"		Seeds of the variety "Kyrgyz single-seeded"	
		bacteria	fungi	bacteria	fungi	bacteria	fungi
Trichoderma asperillum KazNIIPPP-19", with an activity of $1x10^8$	0,5	42±1,0	1,0	47±1,0	2±1,0	49±1,0	2±1,0
	1,0	38±1,0	1,0	39±1,0	1,0	38±1,0	1,0
	1,5	35±1,0	0	35±2,0	0	35±2,0	0
	2,0	32±2,5	0	30±2,0	0	32±1,0	0
	2,5	32±2,5	0	31±2,0	0	32±1,0	0
Control	Treatment with distilled water	95±2,0	4±1,0	90±2,0	4±1,0	98±1,0	3±1,0
Significance	-	*	*	*	*	*	*

Values stated ± are standard deviation calculated from three parallel measurements. * P<0.05, significant. NS, not significant (P>0.05)

According to Table 4, it can be seen that the most optimal dose of dilution of the biopreparation "Trichoderma asperillum KAZNIIPP-19", with an activity of $1x10^8$, used to suppress seed infection of sugar beet during storage, by determining the number of colonies of microorganisms on the seeds of various zoned varieties of sugar beet after their treatment, the treatment dose is: 2ml/100.0ml, in distilled water, based on 75 g of seeds.

In further experiments on the study of the biological product "Trichoderma asperillum KAZNIIPPP-19", with an activity of $1x10^8$, used to suppress the seed infection of sugar beet during the storage period, the specified worked out optimal dose of treatment will be used, i.e., 2 ml of the drug /100.0 ml, in distilled water, at the rate of 75.0 g seeds.

In order to suppress the seed infection of sugar beet during the period of long-term storage, we tested various variants for the use of drugs in laboratory conditions, in combination with growth regulators and separately with a biopreparation, in continuation of studies for 6 months (observation period). In this report, the results of these studies are presented within 3 months (observation period).

For the experiments, drugs were taken in combination with a growth regulator, approved for use in Kazakhstan, against sugar beet diseases (Shamin and Stognienko, 2017). As a comparative biopreparation, a control sample of a biopreparation was tested, developed on the basis of the isolated fungus strain "Trichoderma asperillum KazNIIPPP-19", in the laboratory "Biotechnology, quality and food safety" of "Kazakh Research Institute of Processing and Food Industry" LLP of MA RK.

For the experiment, 2 zoned (varieties: "Taraz" and "Aisholpan") and 1 foreign (variety "Kyrgyz single-seeded") varieties of sugar beet were taken. Experiments to study the degree of influence of a biopreparation, based on the isolated strain "Trichoderma asperillum KazNIIPPP-19" (with an activity of $1x10^8$), on the storage duration of sugar beet seeds, was carried out according to the scheme specified in the research methods. In this case, 3 variants of seed treatment were evaluated and the indicated seeds without treatment served as a control. Subsequently, these variants of the samples were monthly examined for the presence of obligate microflora, in a comparative aspect of the experimental samples in comparison with the controls.

In this case, it was also found that bacterial colonies prevail in all variants of the experiment. They were assigned to the genera: Bacillus, Paenibacillus, Pseudomonas and Mycococcus. In the context of varieties in terms of the number of microorganisms, no significant differences were observed. They were almost on the same level. The number of bacterial colonies at the beginning of laying seeds for storage in the variety "Aisholpan" was at the level of: 22-33±1.0 pcs, at the 4^{th} month of storage it reached the level: 34-45±2.0 pcs, and by 6^{th} month were detected at the level: 38-49±2.0 pcs, in the variety "Taraz" - 33±1.0 pcs, 35-44±1.0 pcs and 39-47 ± 1.0 pcs and in the variety "Kyrgyz single-seeded" - 24±1.0 pcs, 36-49±2.0 pcs and 42-53±1.0 pcs, respectively. On the control, this indicator at the beginning of laying seeds for storage, depending on the variety, ranged from 99-111±2.0 and in the

4th month of storage it reached the level: 145-162±2.0 pcs, and by 6th month they were detected at the level of 168-183±2.0 pcs.

From the data obtained during the study, it was found that, as at the beginning of the experiment in all experimental groups, the number of colonies of microbes was at an insignificant level and 3-4 times lower (22-±1.0-53±1.0) compared to with the control group (99±2.0-183±2.0). Surely, this is due to the use of drugs that inhibit the growth of microorganisms. At the same time, it was also found that in all experimental and control groups, compared with the first months, by the 4th and 6th months, there was an approximately equal increase in the number of detected microbes by 5-10%.

In this case, only in the control group single colonies of fungi of the genus Aspergillus and Penicillium were detected in the variants of the experiment already in the 3rd decade of April and their isolation continued for the 4th-6th months of observation.

Thus, it can be argued that the composition of the drug: Celestop (0.01/100) + Fitosporin M (1 ml/100) and Maxim (1 ml/100) + Extrasol (5 ml/100), as well as a biopreparation based on the strain fungus Trichoderma asperillum KazNIIPPP-19 (with an activity of $1x10^8$) (2ml/100) for 6 months (observation period) inhibit the growth of fungal microflora and reduce the number of bacteria.

In order to determine the degree of influence of various compositions of drugs on the sowing qualities of seeds of various varieties of sugar beet during the storage period, they continued to study them in laboratory conditions for 6 months (observation period). At the same time, also monthly, and for 4th-6th months, samples of seeds treated with the above drugs were taken and tested in a comparative aspect for germination energy and laboratory viability (Table 5).

Table 5 - Study of the degree of influence of various compositions of drugs on the sowing qualities of seeds of the tested varieties of sugar beet, during 6 months of storage

Variant	Seeds of the variety "Aisholpan"		Seeds of the variety "Taraz"		Seeds of the variety "Kyrgyz single-seeded"	
	Germination energy, %	Laboratory viality, %	Germination energy, %	Laboratory viality, %	Germination energy, %	Laboratory viality, %
1st month of research						
Control	51±2,0	78±2,0	52±1,0	54±1,0	51±1,0	65±2,0
Celestop + Fitosporin-M	75±1,0	85±1,0	70±2,0	81±2,0	74±1,0	83±1,0
Maxim + Extrasol	72±2,0	82±1,0	68±1,0	77±2,0	73±1,0	79±2,0
Trichoderma	70±1,0	80±2,0	65±2,0	75±1,0	71±2,0	81±1,0

asperillum KazNIIPPP-19						
3rd month of research						
Control	65±1,0	75±1,0	68±1,0	76±1,0	64±2,0	75±2,0
Celestop + Fitosporin-M	89±1,0	96±1,0	85±1,0	92±2,0	88±1,0	90±1,0
Maxim + Extrasol	83±1,0	94±2,0	79±1,0	90±2,0	84±2,0	89±2,0
Trichoderma asperillum KazNIIPPP-19	81±2,0	96±1,0	80±1,0	88±2,0	82±1,0	86±1,0
4th month of research						
Control	67±1,0	73±1,0	69±1,0	75±1,0	68±2,0	75±2,0
Celestop + Fitosporin-M	90±1,0	97±1,0	86±1,0	93±2,0	86±1,0	91±1,0
Maxim + Extrasol	85±1,0	95±2,0	82±1,0	91±2,0	85±2,0	90±2,0
Trichoderma asperillum KazNIIPPP-19	84±2,0	95±1,0	81±1,0	90±2,0	84±1,0	90±1,0
6th month of research						
Control	64±1,0	75±1,0	64±1,0	76±1,0	71±2,0	75±2,0
Celestop + Fitosporin-M	93±1,0	96±1,0	89±1,0	94±2,0	86±1,0	96±1,0
Maxim + Extrasol	88±1,0	93±2,0	86±1,0	92±2,0	85±2,0	93±2,0
Trichoderma asperillum KazNIIPPP-19	87±2,0	92±1,0	85±1,0	90±2,0	85±1,0	92±1,0
Significance	*	*	*	*	*	*

Values stated ± are standard deviation calculated from three parallel measurements. * P<0.05, significant. NS, not significant (P>0.05)

At the same time, it was found that, in general, the indices of the germination energy of the seeds of sugar beet taken for the experiment were comparatively weaker in comparison with the first month, which were most likely associated due to their intensive damage by microorganisms during the period of long-term storage. In the

following months, the compositions of the drugs effectively influenced the development of fungal diseases and bacteria, and in subsequent experiments show that all the compositions of the drugs had a positive effect on the sowing qualities of seeds. At the same time, the indicators of germination energy in the experimental variants were higher, on average by 15.0-23.0±1.0%, and in germination - by 13.0-24.0±1.0%, than in the control groups that were not treated with drugs.

In experiments, good results were obtained on the variety "Aisholpan" with the variant Celestop + Fitosporin. The indicators of germination energy for 4^{th}-6^{th} months at approximately the same level on the 3^{rd} day were 84-93±1.0% on the variety "Aisholpan", 81-89±1.0% on the variety "Taraz" and variety "Kyrgyz single-seeded" - 84-88±1.0%, laboratory viability on these varieties on the 7^{th} day was: 92-97±1.0%, 90-94±2.0% and 90-96±1.0%, respectively.

It was found that the treatment of seeds in a combination of the composition of the fungicide and biostimulant, as well as the biopreparation for 6 months, had a positive effect on the sowing quality of seeds. Thus, it was found that the biological preparation based on the fungus "Trichoderma asperillum KazNIIPPP-19" showed its effectiveness at the level of the variant Maxim + Extrosol and slightly lower than the variant Celestop + Fitosporin - M.

In this case, for 4-6 months of the experiments, the indicators of the results of germination energy in the experimental variants with a biopreparation based on the fungus "Trichoderma asperillum - KazNIIPPP-19" were higher than in the control, on the variety "Aisholpan" by 17-29±1.0%, on the variety "Taraz" - 12-25±1.0% and on the variety "Kyrgyz single-seeded" - 14-19±1.0%.

In the indicated terms of the experiment, the germination results in experimental variants with a biopreparation based on the fungus "Trichoderma asperillum KazNIIPP-19" were higher than in the control, on the variety "Aisholpan" by 17-24±1.0%, on the variety "Taraz" - 15-19±1.0% and on the variety "Kyrgyz single-seeded" - 15-21±1.0%.

Thus, according to the presented results, it can be seen that a biopreparation based on the fungus "Trichoderma asperillum KazNIIPP-19", within 6 months of storage (observation period), has an effective protective property against pathogens of sugar beet seeds and has a charitable effect on their germination indicators.

Conclusion

Thus, as a result of the studies conducted, it was established:

- Microorganisms from the seed material of zoned varieties of sugar beet were studied by washing and it was found that the maximum number of isolated microorganisms was represented by fungal flora - 53%, then bacteria - 23%, actinomycetes - 16% and the least amount of yeast - 8%.

- The identification of strains showed that the seeds were mainly affected by representatives of the fungal flora Alternaria alternata, Cladosporium sp., Mucor sp., Fusarium sp., Penicillium sp., Aspergillus sp. The

greatest danger is represented by Alternaria alternata, Fusarium sp., these phytopathogenic microscopic fungi are the causative agents of the sugar beet root borer.

- On the basis of the isolated and studied microorganisms of the investigated materials (from the seed material of sugar beet), a strain of microorganisms was selected to work with the purpose of creating a biopreparation to increase the shelf life of seed material of sugar beet. At the same time, in the course of earlier screening studies to study the microflora of soil samples taken from the rhizosphere of sugar beet and the surface of seeds, a microscopic fungus of the genus Trichoderma was isolated, which is one of the natural phytopathogenic microorganisms that is found in all types of soils. The selected fungus was assigned to the species Trichoderma asperellum by morphological and cultural characteristics and by PCR.

- To suppress fungal and bacterial infections in sugar beet seeds, a biopreparation has been developed based on a new strain of the fungus Trichoderma asperellum KazNIIPPP-19, and has been comparatively studied with the drugs: Celestop, Maxim, etc., included in the list of approved drugs that will be used for seed treatment at the stage of storage and pre-sowing treatment.

- Subsequently, on the basis of the accredited testing laboratory of the Academy of Nutrition "NUTRITES" LLP, the virulence of the isolated strain was studied according to standard methods on white mice, at concentrations from 10^3 to 10^{11} CFU/cm^3. At the same time, it was found that according to the existing classification of strains, the culture of Trichoderma asperellum KazNIIPPP-19 belongs to the 3rd hazard class (There is a conclusion of 301 K from March 12, 2020).

- Based on the results obtained, the Passport of the culture strain of the microorganism "Trichoderma asperellum KazNIIPPP-19" was compiled and this strain was submitted for deposition in the RSE on the REM "Republican Collection of Microorganisms" of MES RK and a Certificate of deposition of the microorganism strain "Trichoderma asperellum" (Registration number No. 09/12-April 2020).

- The optimal dose of cultivation of the biopreparation "Trichoderma asperillum KazNIIPPP-19", used to suppress the seed infection of sugar beet during storage, by determining the number of colonies of microorganisms on the seeds of various zoned varieties of sugar beet after treatment, has been determined. In further experiments on the study of the biopreparation "Trichoderma asperillum KazNIIPPP-19", with an activity of 1x10^8, applicable to suppress the seed infection of sugar beet during storage, will be used in the worked out optimal dose of treatment, i.e., 2 ml of the drug/100.0 ml , in distilled water, based on 75 g seeds.

- It was found that a biopreparation based on the fungus "Trichoderma asperillum KazNIIPPP-19", within 6 months of storage (observation period), has an effective protective property against pathogens of sugar beet seeds and has a charitable effect on their germination rates.

Based on the results obtained, a biological product based on a strain of a microscopic fungus: "Trichoderma asperillum KazNIIPPP-19" is recommended for the use of sugar beet seed treatment for long-term storage (within 6

months - observation period), while the drug also enhances growth processes of treated seeds and contributes to the production of high-quality products in economic conditions.

In addition, we recommend for the use of treatment of sugar beet seeds for their long-term storage in the republic, selected by experimental tests effective combinations of specific drugs: "Maxim" + "Extrasol" and "Celestop" + "Fitosporin-M", which are produced in production conditions and included in the list of approved drugs in Kazakhstan.

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