Inoculation of seed with strains of nitrogen-fixing bacteria (exampled by oats and millet)

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Abstract. As a result of the conducted researches the expediency and effectiveness of pre-sowing inoculation of seeds of field crops with strains of nitrogen-fixing bacteria has been proved, which allows to increase fodder productivity of oat and pearl millet crops up to 36,8 and 18,8 %, respectively. The process of ammoniation and mineralization of organic substances is accelerated. Application of biological preparations such as Sahabaktisubtil and Mamontenok stimulates the activity of microorganisms using mineral nitrogen and accelerates the process of mineralization of organic substances in the soil.Application of mobile inoculator provides an increase in the quality of pre-sowing seed treatment in field conditions due to uniformity of seed treatment with the preparation in the form of "aerosol mist" formed by a vibrating centrifugal disc. Thus, the completeness and uniformity of treatment reaches 90% (compared to 65-75% for analogues), the speed of treatment at the same time increases.

1 Introduction

The most important problem in crop production is biological nitrogen fixation, which is one of the priority scientific problems. Biological fixation is a global process that ensures the possible existence of life on Earth (Schott, 2007) [1]. In poor permafrost soils of Yakutia, nitrogen supply is a limiting factor for plant growth and development; therefore, additional introduction of nitrogen-fixing bacteria with seed or in the soil is an important step in solving the problem of restoration of old plowed abandoned lands.

Until recently, cereals and other non-legumes were thought to be unable to provide even a small amount of biological nitrogen. The discovery of nitrogen-fixing bacteria in the rhizosphere of non-legumes has changed this view. These discoveries are associated with the name of Brazilian professor D. Dobereiner, who discovered associations of bacteria (azospirillum) with some tropical cereals (Chumakov, 1988) [2]. To increase the level of

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biological nitrogen fixation of legume crops, plant treatment with selected bacterial strains capable of associative nitrogen fixation can be used.

Currently, the important role of microorganisms in soil fertility and plant nutrition is generally recognized. Root nutrition of plants is realized as a result of complex interaction between soil and soil microflora inhabiting it (Karyagina, 1983) [3]. The quantitative and qualitative composition of microorganisms in soil is conditioned by the content of organic matter, moisture, pH, temperature, climatic conditions, method of treatment, etc. As the amount of organic matter in soil increases, as a rule, the number of microorganisms also increases.

In soil, under the influence of living organisms, organic matter is continuously formed (mainly by plants) and destroyed (mainly by microbes) (Muromtseva, 1976) [4, 5]. Microbial communities, being ecosystems' reducers, play a huge role in the destruction of soil organic matter, transforming it into nutrient elements available to the producers, thus closing the cycle of substances. High concentrations of heavy metals and other toxicants cause various changes in microbiological and biochemical indicators of soils. Thus, as a result of anthropogenic pollution there is a decrease in the total number, narrowing of species diversity of microorganisms, decrease in the intensity of basic microbiological processes and activity of soil enzymes, appearance of phytotoxic forms, etc. (Nekrasova, 1978). (Nekrasova, 1978) [6].

In permafrost soils of Yakutia, the process of mineralization of organic matter is slow, the response of microflora is observed only after a year, and the aftereffect persists for 2 -3 years (Popov, Nikolaeva, 2013) [7].

Thus, it is important to studying the effectiveness of inoculation with strains of nitrogen-fixing bacteria of the seed material of field crops cultivated on permafrost floodplain meadow soils of old plowed abandoned lands; the use of effective devices for pre-sowing treatment of agricultural seeds; and microbiological evaluation of the effect of organic fertilizers on permafrost floodplain meadow soils.

2 Materials and Methods

Studying the effectiveness of inoculation with strains of nitrogen-fixing bacteria of seed material of field crops cultivated on permafrost floodplain meadow soils of old plowed abandoned lands. Experiment scheme: 1) control - without inoculation; 2) seed inoculation with Rizoagrin; 3) seed inoculation with Sahabaktisubtil; 4) seed inoculation with Mamontenok; 5) seed inoculation with Bioprofit Start.

The experiment was conducted on the seed material of oats and pearl millet when cultivated for fodder productivity. Seed inoculation before sowing was carried out at the installation developed at the Department of Technological Systems of Agroindustrial Complex (Arctic State Technical University). The mobile inoculator is shown in Fig. 1.

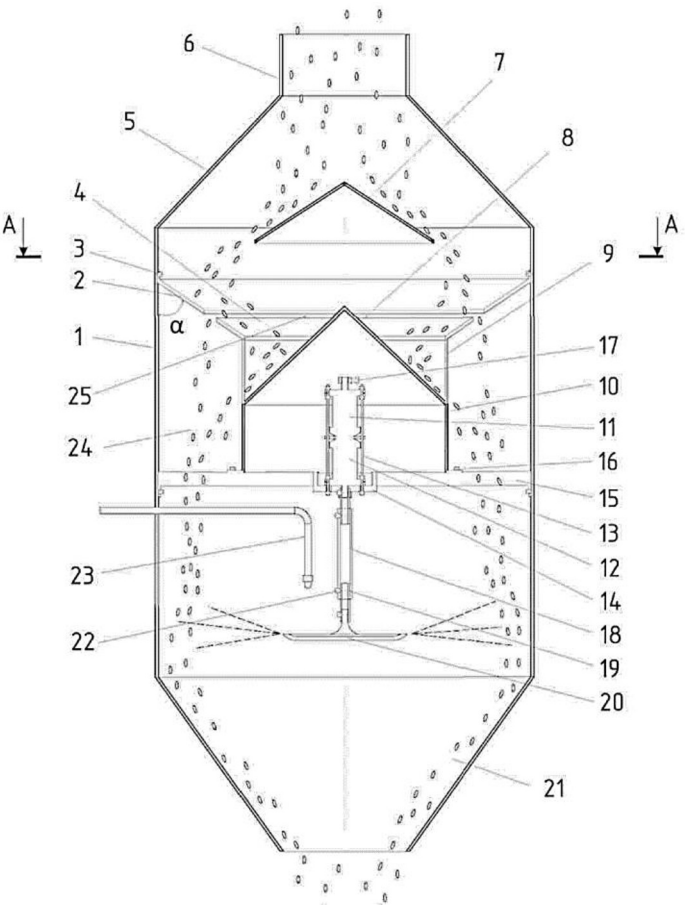

Fig. 1. Schematic diagram of the inoculator device.

The device for pre-sowing treatment of seeds in the field with liquid preparations includes an external vertical hollow cylinder 1 with a ring plate 2 installed inside by means of a bolt connection 3. The angle between the formers of the cylinder 1 and the plate 2 is $\alpha \leq 45^{o}$. An additional ring plate 4 is installed coaxially with the ring plate 3 with a gap. Above the annular plates 3 and 4 concentrically with them is installed loading hopper 5 with a receiving spigot 6, under which coaxially with them is made cone-shaped bottom 7. Under it is installed distribution cone 8. Ring plate 4 is installed on vertical rods 9 on the inner vertical hollow cylinder 10. The distribution cone 8 is fixed on the cylinder 10. Vibration drive motor 11 is connected to the spreading centrifugal disk motor 12 by means of studs 13 through flange 14, as well as to radial flexible bars 15 by means of bolt connection 16. An unbalanced vibrator 17 is installed on the output shaft of the motor 11. A flexible tubular coupling 18 is installed on the output shaft of the motor 12. It is connected to the shaft 19 of the spreading centrifugal disk 20, under which a funnel 21 is installed for discharging the treated seed. The coupling 18 and shaft 19 of disk 20 are fastened with bolts 22, which make it possible to adjust the height position of disk 20. Above the disk 20 is an underwater tube 23 of liquid preparation. Cylinder 1 and cylinder 10 form an annular cylindrical channel 24. An annular gap 25 is made between the annular plates 2 and 4. The arrangement of plates 2 and 4 with an annular gap increases the effectiveness of the vibration effect on the seeds coming to the distribution cone 8, as in this design vibration is not transmitted to all the seed material in the feed hopper.

Motors 11 and 12 are driven from the 12-volt on-board system of mobile units, with which the device for seed pre-sowing treatment in the field is coupled.

The device works as follows. Seed material from the feeding spigot 6 enters the feeding hopper 5. By cone-shaped bottom 7 is directed to the ring plate 2. Part of the seed (smaller) falls through the annular gap 25 and enters the cylindrical channel 24, and the other part of the seed enters the distribution cone 8 and is also directed into the channel 24. The seed stream at the exit of the channel 24 is treated with drops of the preparation, atomized disk 20. At the same time seeds are enveloped by the pre-preparation and fall into the funnel 21, from where they are removed from the device.

Field experiments are presented on a plot of 0.52 ha in 4-fold repetition, the placement of variants randomized. The accounting area of plots is 100 m2 (Dospekhov, 1985) [8]. The method of sowing oats is continuous row-by-row, millet - wide-row with a row spacing of 30 cm. Soils of the site are permafrost floodplain meadow loamy sandy loam. Agrochemical composition of soil featured low content of humus 2,0 %, mobile phosphorus 259 mg/kg, mobile potassium 220 mg/kg, pH 8,5.

Counting and observations were carried out in accordance with generally accepted methods (Methodology of field experiments of B.A. Dospekhov, V.R. Williams, 1985, statistical program SNEDECOR, Ecxel).

For microbiological analysis of soil, soil samples were collected from the following sites at a depth of 10-20 cm using a soil borer, sterile bouquets, spatulas and ethyl alcohol. The soil borer is sterilized with ethyl alcohol after sampling from each layer. The collected samples are placed into sterile bouquets using a sterile spatula.

Microbiological analyses on the isolation of microorganisms from soil were carried out by sowing on dense nutrient media in three repetitions. The total number of microorganisms utilizing organic nitrogen was counted on meat-peptone agar (MPA). Bacteria using mineral nitrogen and actinomycetes - on starch-ammonia agar (KAA), microscopic fungi - on agarized acidified medium-Chapek (Zvyagintsev, 1991; Ezhov, 1974) [9, 10].

3 Results

The data of biometric surveys showed that the height of oat grass stand on the variants with seed inoculation significantly exceeded the control - by 14-16 cm or 14-16%. Foliage of oat plants in the variants with seed inoculation before sowing increased by 4.2-6.2 cm or 14.0-20.7% (Table 1).

Table 1. Biometric indices of oat grass stands depending on inoculation of seed material with strains of nitrogen-fixing bacteria.

No.	Variant	Plant height		Plant Foliage	
		cm	in % kK	%	in % kK
1	Without inoculation, control	98	0	30.0	0
2	Seed inoculation with Rizoagrin preparation	112	114.3	36.2	120.7
3	Seed inoculation with Sahabaktisubtil preparation	114	116.3	35.8	119.3
4	Seed inoculation with Mamontenok preparation	114	116.3	36.0	120.7
5	Seed inoculation with Bioprofit Start preparation	113	115.3	34.2	114.0
	X±	0.90	-	1.02	-

Table 2 shows that the plant height of pearl millet plants did not depend on seed inoculation, whereas the percentage of foliage increased significantly (by 9.8 and 10.5 %).

Table 2. Biometric parameters of pearl millet grass stands depending on the inoculation of seed material with strains of nitrogen-fixing bacteria.

no.	Variant	Plant height		Plant Foliage	
		cm	in % of kK	%	in % of kK
1	Without inoculation, control	176	0	42.0	0
2	Seed inoculation with Rizoagrin preparation	175	99.4	46.2	110.0
3	Seed inoculation with Sahabaktisubtil preparation	177	100.6	46.4	110.5
4	Seed inoculation with Mamontenok preparation	177	100.6	46.1	109.8
5	Seed inoculation with Bioprofit Start preparation	175	99.4	46.3	110.2
	X±	1.10	-	0.90	-

Consequently, pre-sowing inoculation of oat seeds stimulated plant growth up to 16.3 %, leaf area growth up to 20.7 %; seed inoculation with biopreparations had no noticeable effect on the height of millet herbage, but the percentage of foliage increased up to 9.8-10.5 %.

Table 3 presents the main criterion of effectiveness of seed inoculation of field crops - yield of fodder mass. As can be seen from this table, productivity of oat and pearl millet cenoses under cultivation for fodder significantly increased with inoculation with bacterial preparations. The highest yield of green mass of oats is achieved with inoculation with Rizoagrin and Sahabaktisubtil - 12.8 and 13.0 t/ha, respectively, which is 34.7 and 36.8 % higher than the control. The highest yield of fodder mass of pearl millet is achieved when seeds are inoculated with Sahabaktisubtil - 23.4 tons/ha, which is 18.8% higher than the control variant.

Table 3. Yield of green mass of oats and sown millet depending on of different doses and combinations of biohumus.

no.	Variant	Oats			Sown millet		
		t/ha	in t of K	in % kK	t/ha	in t of K	in % kK
1	Without inoculation, control	9.50	0	0	19.7	0	0
2	Seed inoculation with Rizoagrin preparation	12.8	3.3	134.7	22.0	2.3	111.7
3	Seed inoculation with Sahabaktisubtil preparation	13.0	3.5	136.8	23.4	3.7	118.8
4	Seed inoculation with Mamontenok preparation	11.7	2.2	123.2	22.5	2.8	114.2
5	Seed inoculation with Bioprofit Start preparation	12.0	2.5	126.3	22.1	2.4	112.2
	HCP_{05}	0.95	-	-	1.86	-	-

Phenological observations of growth and development of oat and pearl millet plants (Table 4) made it possible to note that the onset of the duration of the period of full sprouting-full flowering slightly depended on the inoculation of seeds before sowing with strains of nitrogen-fixing bacteria (48-49 days in oat and 47-48 days in pearl millet). However, the onset of plant development phases of these crops varied from 1 to 4 days. It should be noted that bacterial preparations Rizoagrin and Mamontenok accelerated the development of oat and pearl millet plants by 3-4 days.

Table 4. Phenological observations of growth and development of oats and sown millet, sowing 08.06 2023.

no.	Variant	Sprouts		tillering		Output to the tube		sweeping		Blooming		Growing season (from full sprouting to flowering)	
		Oats	Sown millet	Oats	Sown millet	Oats	Sown millet	Oats	Sown millet	Oats	Sown millet	Oats	Sown millet
1	Without inoculation, control	21.06	26.06	30.07	03.07	23.07	28.07	31.07	08.08	09.08	14.08	48	48
2	Seed inoculation with Rizoagrin preparation	18.06	23.06	27.07	02.06	21.07	25.07	28.07	06.08	06.08	10.08	48	47
3	Seed inoculation with Sahabakt isubtil preparation	19.06	24.06	29.07	02.07	22.07	26.07	29.07	07.08	08.08	11.08	49	47
4	Seed inoculation with Mamontenok preparation	18.06	23.06	27.07	01.07	21.07	25.07	29.07	07.08	06.08	10.08	48	47
5	Seed inoculation with Bioprofit Start preparation	18.06	23.06	27.07	02.06	21.07	25.07	28.07	06.08	06.08	10.08	48	47

The total number of microorganisms inhabiting the soil in the studied plots averaged from 212 to 612.5 million CFU/g soil depending on the experiment variants.

Bacteria prevail in the soil, making about 78-89% of the total number of microorganisms, fungi - 2-8% and actinomycetes - 8-15%. This ratio of microorganism groups is stable.

Bacteria are represented by a group of spore bacteria, such as Bacillus agglomeratus, Bac. idosus, Bac. mycoides and Bac. subtilus, as well as non-spore bacteria of the genus Pseudomonas, which includes fluorescent (Pseudomonas fluorescens) and yellow-pigmented (Pseudomonas herbicola). Actinomycetes are represented by Actinomucesalbum species. Fungi are represented by several genera, among which Penicil-lium, Trichoderma, Fusarium, Mucor, less frequently Aspergillus, Alternaria dominate.

The content of actinomycetes in the studied soils is 40 million CFU/g of soil. The most widespread are white, gray actinomycetes and brown actinomycetes with pronounced pigmentation on the nutrient medium. The number of actinomycetes reaches 8-15%, while the number of fungi is 8%. Fungi constitute a smaller part of soil microorganisms. They are represented by several genera, among which Penicillium, Trichoderma, Fusarium, Mucor, less frequently Aspergillus, Alternaria dominate. The number of fungi ranges from 3.2 to 7.2 thousand CFU/g soil. The qualitative composition of this soil has a number of characteristic features similar to permafrost soils of Eastern Siberia (Nekrasova, Gukasyan, 1978) [6]. One of these features is the predominance of fungal flora of the genus Penicillium, which accounts for about 80% of the total number of fungi. Fig. 2 shows the number of microorganisms before inoculation.

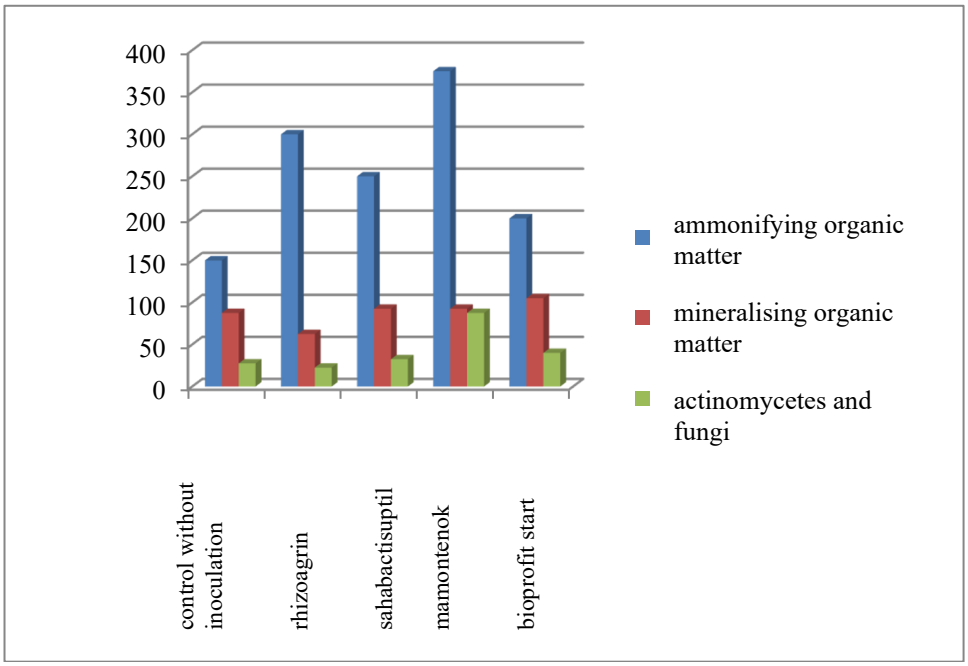

Fig. 2. Numbers of microorganisms in soil in plots before sowing inoculated seeds, million CFU/g in soil.

Ammonifiers are microorganisms that convert nitrogen of high-molecular organic compounds into mineral nitrogen as a result of their vital activity, mainly represented by aerobic bacteria. Their number on the studied variants varies on average from 150 to 550mln CFU/g soil, the highest content is found on the variants with the application of the bacterial preparation Bioprofit Start. The number of microorganisms participating in the process of mineralization of organic matter in soil ranged from 22.5 to 92.5 million CFU/g soil (Fig.3).

The ratio of the number of microorganisms that use mineral nitrogen to the number of microorganisms that use organic nitrogen is an important indicator of the intensity of organic matter mineralization in the soil. This indicator shows how quickly organic matter in the soil is decomposed and transformed into mineral nitrogen forms.

On the studied plots low values of mineralization coefficient are observed, which vary from 1.8 to 24.4. This means that the process of mineralization of organic matter in the soil is relatively slow. However, it should be noted that on the plots where preparations of Sahabaktisubtil and Mamontenok were applied, the mineralization process is actively accelerated. This may be due to the fact that these preparations contribute to the increase in the number of microorganisms that use mineral nitrogen, which in turn accelerates the process of decomposition of organic matter [11, 12].

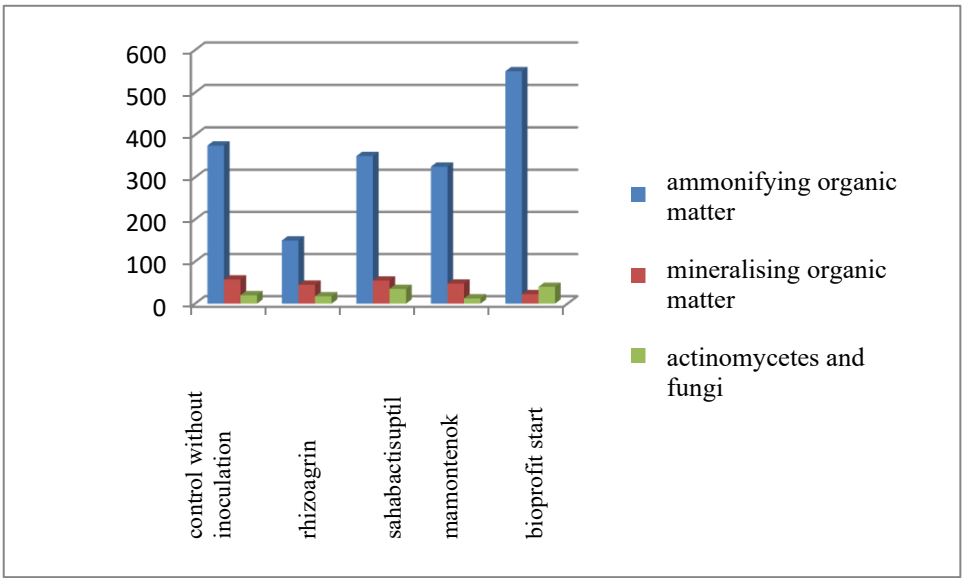

Fig. 3. Numbers of microorganisms in soil in plots before harvesting, grown from seeds inoculated with different preparations, million CFU/g in soil.

On control plots of soil, where measurements were carried out before sowing inoculated seeds, the decomposition of mineralization products of organic matter is slower. This indicates the important role of biological preparations in stimulation of mineralization and enrichment of soil with mineral forms of nitrogen (Table 5).

Table 5. Intensity of microbiological processes.

	Total number of microorganisms, mln CFU/g soil	**Surface module/CAA micro-organism ratio**	**Ratio of actinomycetes to fungi**
Variant			
Before sowing	380	5.2	2.5
Without inoculation, control	375	2.7	1.8
Seed inoculation with Rizoagrin preparation	555	4.1	1.0
Seed inoculation with Sahabaktisubtil preparation	330	2.2	1.4

Seed inoculation with Mamontenok preparation	335	5.4	1.5
Seed inoculation with Bioprofit Start preparation			
After sowing before harvesting	212.5	3.3	3.2
Without inoculation, control	440	6.4	1.6
Seed inoculation with Rizoagrin preparation	385	6.8	4.5
Seed inoculation with Sahabaktisubtil preparation	612.5	24.4	1.4
Seed inoculation with Mamontenok preparation	356	6,5	1.6

4 Conclusions

As a result of research, the feasibility and effectiveness of presowing inoculation of field crop seeds with strains of nitrogen-fixing bacteria was proved, which allows increasing the fodder productivity of oat and pearl millet crops up to 36.8 and 18.8% respectively.

Data obtained indicate that microbiological processes work quite vigorously when biological preparations are applied, which accelerate the process of ammonification and mineralization of organic substances [15]. Application of biological preparations, such as Sahabaktisubtil and Mamontenok, stimulates the activity of microorganisms using mineral nitrogen and accelerates the process of mineralization of organic matter in the soil.

The use of mobile inoculator [16] provides an increase in the quality of seed pre-sowing preparation in field conditions by increasing the uniformity of seed coverage with atomized droplets by increasing the uniformity of the seed layer in the annular cylindrical channel, as well as increasing the monodispersity and expansion of the droplet atomization torch by giving vibrations to the spreading centrifugal disk. Thus the completeness and uniformity of processing reach 90% (compared to 65-75% for analogs), and the processing speed increases.

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