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Biotechnological Potential of Bacterial Agents in Modern Conditions of Cultivation of Agricultural Crops

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***Abstract*—The paper highlights the problem of nitrogen fixation in a biological manner and considers prospects for the use of bacterial agents of an associative nature as an element of biotechnology in practice. Studies of promising domestic bacterial agents, based on associative nitrogen fixers, were carried out on zonal soils (chernozems) in the Rostov Region. The presented materials of scientific studies on preparative forms based on nitrogen-fixing microorganisms showed high performance, which was manifested in the progress of crop yields, and therefore these data can be used in agricultural production. Application of bacterial agents with nitrogen-fixing species is profitable, since their cost is not high and the increase in additional crop production fully covers the costs and it cuts down the introduction of mineral nitrogen fertilizers. Nevertheless, it should be noted that the environmental aspect of bacterial agents' application consists in obtaining a clean source of nitrogen by cultivated plants and reduces pollution of the soil, atmosphere and water bodies.**

Keywords—biological nitrogen, bacterial agents, crop yield, efficiency.

I. Introduction

The fixation of molecular nitrogen determines the planet's biological productivity and is the main research task in modern biology. It is an integral part of complex substances – proteins, as well as other molecules that form the structural basis of the organizational nature of all animal organisms. Animals and humans use it as proteins of different animal and vegetable origin; plants use it in the form of ammonia or ammonium ions and salts of nitric acid (nitrate forms) [1].

The feasibility of using bacteria in agricultural practice located in the direct rhizosphere of the root system of cultivated plants is dictated by crises that are of an economic and environmental nature, and a decrease in the quality of crop production. All this leads to a drop in the level of natural soil fertility, which determines the increasing interest in the so-called biological farming. This is manifested by replacement of the so-called "gray" technologies in agriculture, which are traditional, with non-traditional technologies (nature-like, environmental, "green") [2].

According to the ideas of contemporary scientists, the basis of microbial community regulation is plants – they are located both in spatial and in functional unity [3]. Study of their relationships and interactions is an actively developing direction in modern biology [4].

Knowledge in this field, which has been accumulated by entire generations of scientists, makes it possible to raise the question of the purposeful design of phyto-microbial systems and increase the properties associated with adaptation to a new living environment in order to reproduce soil fertility, increase crop yields, increase resistance to risk factors and stress under various energy consumption levels [5].

Companies (research institutes) that create bacterial agents based on nitrogen-fixing bacteria are primarily guided by the tasks of maintaining the species activity in a certain preparative form (nutrient medium or substrate) with the aim of their widespread practical use.

The positive aspects of nitrogen fixation by associative free-living rhizospheric bacteria are manifested in nitrogen fixation in atmospheric air, the appearance of biologically active substances in the environment, increased stress resistance to the action of factors, and suppression of the development of harmful microorganisms [6-8].

Since the living principle in bacterial agents is the living organisms, subjected to various environmental influences, the following issue arises: how can we create the most favorable conditions that can fully manifest their beneficial properties? The first condition is the parallel provision of mineral fertilizers for both the bacteria agents and the growing culture. Under favorable conditions, bacteria in the rhizosphere of cultivated plants begin to multiply and actively perform; the soil is getting enriched with nutrients in this process, interacting with native microorganisms, which ultimately contributes to the intensive mineralization of the organic matter of the soil.

Bacterial nitrogen is more accessible, unlike the nitrogenous substances that make up the humus. Enrichment of plants with nitrogen will occur more intensively under favorable conditions for the performance of the microorganisms' biomass. Stimulation of the microbiological ability of the soil, as well as an increase in the specific weight of microorganisms, forms effective soil fertility. So, in the works of T.A. Gil, M.G. Sokolova, T.F. Kazarinova [9] the introduction of "Complex microbiological fertilizer" (CMF) contributed to the formation of microbial coenosis with higher mineralization ability in fertile soil with long-term introduction of fertilizer N60P40K60 kg a.s./ha, and the introduction of a mixture of biological products Azotobakterina, Fosfobakterina , Kremnebakterina – in deprived not fertilized soil, which was expressed in an increase in the proportion of spore bacteria, an increase in the ratio of bacteria using ammonia nitrogen to the number of bacteria using organic nitrogen (mineralization coefficients).

A set of measures aimed at increasing these indicators in the cultivation of crops will ensure the production of environmentally friendly products by reducing the chemical effect of fertilizers [10].

The largest developer of biological products in Russia has been the All-Russian Scientific Research Institute of Microbiology (St. Petersburg) for many years. The assortment of bacterial agents of microbiological nature, developed by the employees of this organization, is competitive in modern conditions of the world market and is not inferior to the development of foreign companies [11].

However, before introducing a specific strain of bacteria into agricultural production, it is necessary to test it in various soil and weather and climatic conditions.

The following is an analysis of the use of nitrogen-fixing biological products with associative microflora in the conditions of the Rostov region by employees of the Don State Agrarian University.

II. Research methodology

Field experiment of studying the effect of mineral fertilizers and bacterial agents on contrasting agricultural crops was made in the Rostov Region on ordinary celandine-carbonate chernozem. Corn cultivation was carried out in dryland farming conditions and watermelon cultivation was irrigated, in compliance with zonal recommendations of growing technology.

Watermelon culture was studied in 2007-2009 in the Semikarakorsky district. Later, in 2015-2017, corn was studied in the Don State Agrarian University on the basis of the Educational Scientific Industrial Complex in the Oktyabrsky District.

During research on the effectiveness of biological products on watermelon crops, we used agents based on associative nitrogen fixers of the following trade marks: Azorizin-8 and Agrofil, on corn – 204; Mizorin; KL-10; 2P-9; 2P-7. The study of these bacterial agents was carried out under a cooperation agreement with the All-Russian Research Institute of Agricultural Microbiology (St. Petersburg).

The method of introducing biological products was confined in mixing them with dry structured soil during sowing in accordance with the patent [12]. Application of mineral fertilizers for watermelon was carried out for cultivation before sowing, for corn – during the spring cultivation.

III. Research results

The introduction of associative nitrogen fixers for the watermelon was effective, which was reflected in the yield increase up to 42.3 t/ha, due to Azorizin-8 strain inoculation, or 10.4% more than in the option without the use of fertilizers.

The watermelon yield increase in nitrogen in the experimental plots using only mineral fertilizers occurred up to 120 kg/ha, phosphorus and potassium – up to 160 kg/ha. When summarizing the results of a three-year field experiment, the highest crop yield was achieved with option $N_{120}P_{160}K_{160}$ with a yield level of 50.3 t/ha, as well as an increase in control version within 12.0 t/ha and 31.3% in absolute and relative values, respectively. Further strengthening of the complete mineral nutrition of plants did not have a positive effect.

Combined application of mineral fertilizers in a dose of $N_{80}P_{160}K_{160}$ and the bacterial agent Azorizin significantly increased watermelon yield to 60.0 t/ha and was not inferior in efficiency to the best option in combination with mineral fertilizer – the $N_{120}P_{160}K_{160}$; the advantage was 25.4%. Combination of Azorizine with a reduced dose of complete mineral nutrition ($N_{60}P_{120}K_{120}$) was more effective compared to the $N_{120}P_{160}K_{160}$ option. Thus, the introduction of bacterial agents leads to nitrogen savings of up to 60 kg/ha; 40 kg/ha of P_2O_5 and K_2O and an increase in the yield up to 6 t/ha. When comparing both agents, the Agrofil was clearly inferior in effect on the watermelon yield.

The increase in the corn crop yield during the research period was led by an improvement in potassium nutrition, with a low supply of mobile forms of phosphorus in soil.

The doses of fertilizers $N_{60}P_{40}K_{40}$ and $N_{60}P_{80}K_{40}$ ensured the maximum yield increase on average during the experiment (61.3-62.3%) for three years. The use of biological products Mizorin, 2P-7 and 2P-9 in its pure form (without combination with mineral fertilizers) with the level of natural soil fertility increased the yield of corn in relation to control version within 38.7-40.1%. When mineral strains were added to the abovementioned strains by applying mineral fertilizers in a dose of $N_{30}P_{40}$, the efficiency increased by another 14.9-15.9%.

We made a graph in order to illustrate the yield dependence on the mineral fertilizers application in the interval where an increase in the dose of nitrogen led to positive changes (Fig. 1). Visually, this phenomenon is presented in the form of a curve with a very low slope. When referring to this graph, it should be noted that a yield increase of 4.0 t/ha can be achieved by applying nitrogen fertilizers within 37 kg/ha, and yield increase 3.3 t/ha – by 28 kg/ha, respectively.

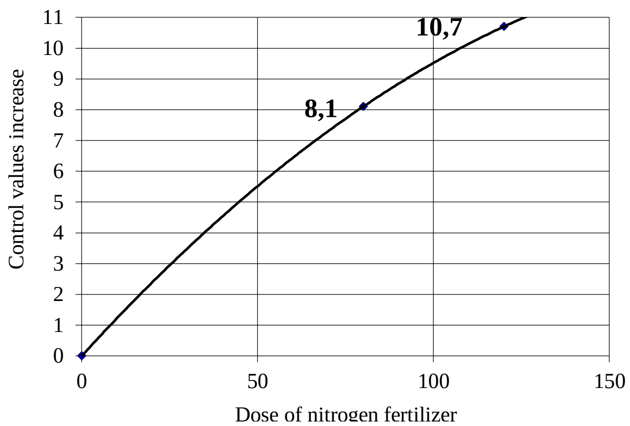

Fig. 1. Connection of watermelon yield increase and doses of nitrogen fertilizers (in the range of positive changes)

Thereby the effectiveness of introducing Azorizin-8 in “pure form” for watermelon crops can conditionally be equated by its action to 40 kg/ha of mineral nitrogen, and Agrofill – by 30 kg/ha. This situation can be caused by two reasons: firstly, application of microorganisms capable of fixing molecular nitrogen not only leads to an improvement in the nitrogen nutrition of watermelon, but also stimulates the assimilation of other nutritional elements; secondly, the total effect of the nitrogen, phosphorus and potassium action of mineral fertilizers is superimposed.

Bacterial agents were less effective as separate method in comparison with their joint use against the background of mineral fertilizers. In particular, the introduction of $N_{80}P_{120}K_{120}$ ensured an increase in watermelon yield for an average of 8.1 t/ha over three years, and in the option with Azorizin-8 – half as much. The total effect of increases due to their application is 12.1 t/ha, and in combination – 18.0 t/ha, which is 1.49 times more. If we assume that the increase in the action of each element in this combination occurred equally, then the biological product Azorizin-8 effect can be conditionally equated to 60 kg/ha, and Agrofil – to 45 kg/ha.

Analyzing the data in Table I, we can conclude that the share of the influence on the watermelon yield from the biological product is 38.6%, and for mineral fertilizer is 61.4% with the combined application of the biological product A-8 and mineral fertilizers in a dose of $N_{80}P_{120}K_{120}$. When combined with a full blend of minerals (nitrogen, phosphorus and potassium) in the composition of mineral fertilizers, the proportion of Agrofil is less and amounted to 32.0%.

The greatest effect in the combination of biological products and mineral fertilizers on the watermelon yield was achieved by the mineral component $N_{80}P_{160}K_{160}$. Due to the fact that there is no such dose in the experiment scheme, we applied a fertilizer that was similar in composition, which has the maximum effect on the watermelon yield – $N_{120}P_{160}K_{160}$.

TABLE I. COMPARISON OF THE EFFECT OF BACTERIAL AGENTS AND MINERAL FERTILIZERS ON THE WATERMELON YIELD

Combined action of biological agents and mineral fertilizers		**Fertilizer system elements' effect on yield**				
bacterial strain and mineral fertilizer dose	*yield increase compared to control, t/ha*	*fertilizer system element*	*separate yield increase due to action of each element of fertilizer system, t/ha*	*estimated share of each element effect during combined application minus action of another element "in pure form"*	*average value of an element action of fertilizer system* *t/ha*	*%*
A-8 $N_{80}P_{120}K_{120}$	18	bacteria min. fertilizer	4.0 8.1	9.9 14.0	6.95 11.05	38.6 61.4
АФ $N_{80}P_{120}K_{120}$	13.3	bacteria min. fertilizer	3.3 8.1	5.2 10.0	4.25 9.05	32.0 68.0
A-8 $N_{80}P_{160}K_{160}$	21.7	bacteria min. fertilizer	4.0 10.0*	11.7 17.7	7.85 13.85	36.2 63.8
АФ $N_{80}P_{160}K_{160}$	15.8	bacteria min. fertilizer	3.3 10.0*	5.8 12.5	4.55 11.25	28.8 71.2

** Data was taken from the optimal option in the block with mineral fertilizers (N120P160K160) with the similar fertilizers' dose*

In the combinations of bacterial agents and mineral fertilizers which were optimal and ensured an increase in watermelon productivity by 21.7 and 15.8 t/ha, the share is somewhat less than with $N_{80}P_{120}K_{120}$ – which was 36.2% with Azorizin-8 and 28, 8% with Agrofil. Mineral fertilizers had a more significant effect.

The situation is somewhat different with the proportion of nitrogen of bacterial agents for corn.

The proportion of strains of the studied nitrogen fixers in ensuring the level of corn grain productivity with the joint application of nitrogen-phosphorus fertilizers varied in the range from 34.4% to 64.5% is shown in Figure 2. Biological products 2P-9, 2P-7 and Mizorin provided the greatest contribution to the increase in the corn yield (Fig. 2).

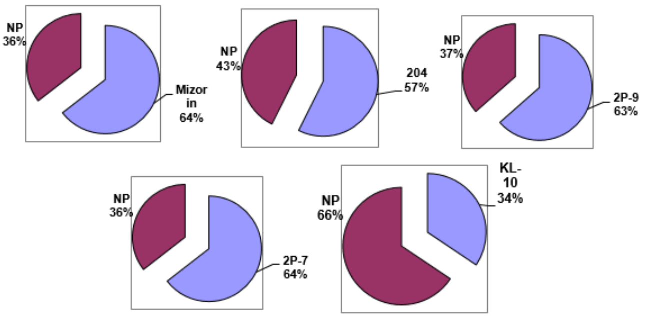

Fig. 2. Share of the studied elements of the corn fertilizer system in increasing grain yield, %

Strain 2P-9, when applied together with mineral fertilizers, proved to be positive not only in terms of agronomic efficiency, but also in economic way – the increase of profitability compared to the control version was 69%, and the cost reduction was 0.84 rubles/kg.

Bacterial preparations Mizorin, 2P-9 and 2P-7 were most beneficial with an energy component – the energy efficiency indicators were 5.25-5.30 in the experiment, and the energy consumption for production was 3.26-3.29 GJ/ton and was the lowest.

Maximum economic efficiency was achieved with the combined use of the Azorizin-8 biological product with mineral fertilizers in a dose of $N_{80}P_{160}K_{160}$ on watermelon crops – increase of profitability amounted to 123.3% (in the case without fertilizers), and the prime cost decreased by 377 rub/t. Agrofil action was slightly lower, but in combination with the $N_{80}P_{160}K_{160}$, the economic results are quite high – profitability is of 163.9%.

IV. CONCLUSION

Summing up, we would like to draw attention to the high efficiency of studied strains of nitrogen-fixing bacteria in the conditions of the Rostov region on chernozem soils. The Azorizin-8 strain performed better in experiments with watermelon; the 2P-7 strain – with corn. The studied bacterial agents provided maximum yield in both cultures when combined with mineral fertilizers. The effectiveness of the bacterial fertilizers application was manifested in cutting the mineral fertilizers' nitrogen down by 30-40 kg/ha and reducing its negative impact on the environment.

Therefore, according to the results, the following elements of the fertilizer system are recommended for a wide industrial use: Azorizin-8 + $N_{60-80}P_{120-160}K_{120-160}$ for watermelon; 2P-9 + $N_{30}P_{40}$ for corn.

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