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INFLUENCE OF *AZOSPIRILLUM FORMOSENSE* STRAINS ON THE GROWTH AND DEVELOPMENT OF *TRITICUM DURUM* DESF. AT THE EARLY STAGES OF ONTOGENESIS

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The evidence of the stimulating effect of the SP-23 *Azospirillum formosense* strain on the germination energy and laboratory germination of *Triticum durum* Desf seeds is presented. Under the influence of SP2 and SP-23 *Azospirillum formosense* strains, linear growth rates and accumulation of fresh weight of *Triticum durum* Desf seedlings significantly increase.
Keywords: *Azospirillum formosense,* seed germination energy, germination, morphometric indicators, ontogenesis, *Triticum durum Desf.*

INTRODUCTION

Currently, obtaining and increasing the yield of environmentally friendly agricultural raw materials in the Republic of Crimea is becoming extremely relevant. The creation and application of biological products based on nitrogen–fixing bacteria is the most effective way to increase plant productivity, crop quality, as well as the preservation of natural soil fertility [1–3]. Microorganisms of the genus *Azospirillum* belong to the group of growth-stimulating, nitrogen-fixing bacteria that play an important role in agrobiotechnology; they were first studied at the beginning of the twentieth century, and came into the field of intensive study in the middle of the same century. They are part of numerous complex microbiological preparations that have a positive effect on the growth and development of many crops. These sticks form highly effective microassociations with plants, and are part of the native microflora of soils that are widespread everywhere. In this regard, the search for strains of mycoorganisms of the genus *Azospirillum* with an increased ability to associate with cultivated plants, intensive nitrogen fixation, as well as growth stimulation is an urgent task of agricultural biotechnology and plant physiology [4–6].

The purpose of this work is to identify the effect of new *Azospirillum formosense* strains on the growth and development of *Triticum durum* Desf. at the early stages of ontogenesis.

MATERIALS AND METHODS

The experimental part of the research was carried out on the basis of the Department of Botany and Plant Physiology and Biotechnology of the Institute of Biochemical Technologies, Ecology and Pharmacy of V. I. Vernadsky Crimean Federal University, in the period of 2021–2022.

Materials for research were seeds and plants of winter wheat, Odari variety (*Triticum dúrum* Desf. CV /Odari/), the seeds of which were selected by average size. After washing in running water, they were kept in a weak $KMnO_4$ solution for 15 minutes, and then washed three times with distilled water.

In accordance with the scheme of the experiment, employees of the NGO Biotechsoyuz inoculated the seeds with strains of Azospirillum formosense microorganisms obtained from the rhizoplane of wild grasses of the flora of the Republic of Crimea.

The scheme of experience:

1. Control – standing tap water.
2. SP-1– *Azospirillum formosense*.
3. SP-2– *Azospirillum formosense*.
4. SP-4– *Azospirillum formosense*.
5. SP-5a– *Azospirillum formosense*.
6. SP-23– *Azospirillum formosense*.

The preparation of *Azospirillum formosense* was diluted in H_2O_{dist} before inoculation in the ratio: 1:9 (10 % preparation) and 1:99 (1 % preparation).

To determine the effect of various *Azospirillum formosense* strains on the germination of wheat seeds of the Odari variety, we laid out seeds after etching in Petri dishes, 50 pieces in each dish, on a double layer of filter paper. 10 ml of H_2O was poured into each Petri dish. Standing tap water served as a control.

The seeds were placed in a thermostat of TS–80–M–2 type for germination (for 3 days in the dark at +20 °C). According to the requirements of the state standard for agricultural crops 12038–84 for durum wheat, the germination energy is determined on 4^{th} day, and the germination of seeds on 8^{th} day.

The energy of seed germination characterizes the amity of the appearance of normal seedlings. Seed germination is understood as the number of normally germinated seeds in a sample taken for analysis, expressed as a percentage [7].

Inoculated wheat seeds were planted in cups with a soil mixture. To prepare the soil mixture, 1 part of the soil and 9 parts of perlite were mixed, the mixture filled the containers for growing plants. The plants were grown in laboratory conditions at a temperature from +22 °C to +24 °C for 2 weeks.

Morphometric parameters were determined on the 4th and 8th days according to the methods generally accepted in plant physiology [8]. As morphometric indicators, the height of plants, the length of the root system, the fresh weight were studied [8].

5 seeds were sown in each vessel, the repetition was threefold.

Statistical processing of the obtained data was carried out by calculating the arithmetic mean and the standard error of the arithmetic mean, the standard deviation, the Student's reliability criterion for comparing two samples. All measurements and studies were carried out on equipment that had passed metrological verification and expertise [9].

RESULTS AND DISCUSSION

The result of the study proved that preparations with SP-1 and SP-2 strains inhibited the germination of wheat seeds both when using a concentration of 10 % and a concentration of 1 %. The germination energy of wheat seeds in the variant with SP-1 at a concentration of 10 % reached 42.4 %, which is 18.1 % less than in the control variant (Fig. 1). When using a microbiological preparation of the SP-1 strain at a concentration of 1 %, the significant difference between the control and experimental variants was 4.9 %.

We found that a microbiological preparation with the SP-2 strain had an inhibitory effect on the germination energy of wheat seeds. For example, inoculation of seeds with a 10 % preparation significantly reduced germination energy by 2.3 % in experimental variants compared with control variants with 1 % – by 2.4 % (fig. 1).

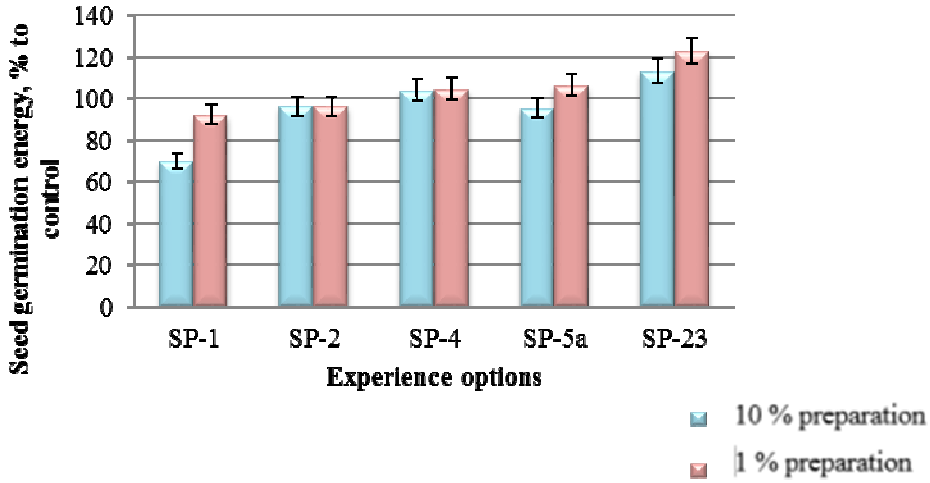

Fig. 1. The effect of *Azospirillum formosense* strains on the energy of seed germination of *Triticum durum.*

When analyzing experimental data of studying the effect of a microbiological preparation, containing the *Azospirillum formosense* SP-4 strain, we found that both concentrations did not have a significant effect on the germination energy of durum wheat seeds. The use of the preparation, which included the *A. Formosense* SP-5a strain, showed that the high concentration of the preparation (10 %) significantly inhibited, and the concentration of 1 % preparation significantly stimulated the energy of seed germination.

It was found that in experimental variants with inoculation of wheat seeds with a microbiological preparation, containing the *Azospirillum formosense* SP-23 strain, the germination energy was significantly higher than in control variants in which no treatment with the preparation was carried out. It was shown that in variants with a preparation concentration of 1 %, the experimental variants exceed the control ones by an average of 23 %. If in the control variant the seed germination energy was 62.5 %, then in the

experimental variants in which inoculation with microorganisms was carried out, this indicator was 76.7 % (see fig. 1).

When studying the effect of preparations, containing various *Azospirillum formosense* strains, on the laboratory germination of *Triticum durum* seeds, data similar to the germination energy were obtained (fig. 2). The pattern established in the study of the effect of microbiological preparations on germination energy can also be traced in determining germination. Thus, preparations with SP-1 and SP-2 strains significantly inhibited the germination of wheat seeds both when using a concentration of 10 % and a concentration of 1 %. Laboratory germination of wheat seeds in the variant with SP-1 in the concentration of the preparation 10 % reached 67.2 %, which is 25.2 % less than in the control variant. When using a microbiological preparation of the SP-1 strain at a concentration of 1 %, the significant difference between the control and experimental variants was 9.9 %. The preparation containing the SP-2 strain also had an inhibitory effect on the laboratory germination of wheat seeds (fig. 2).

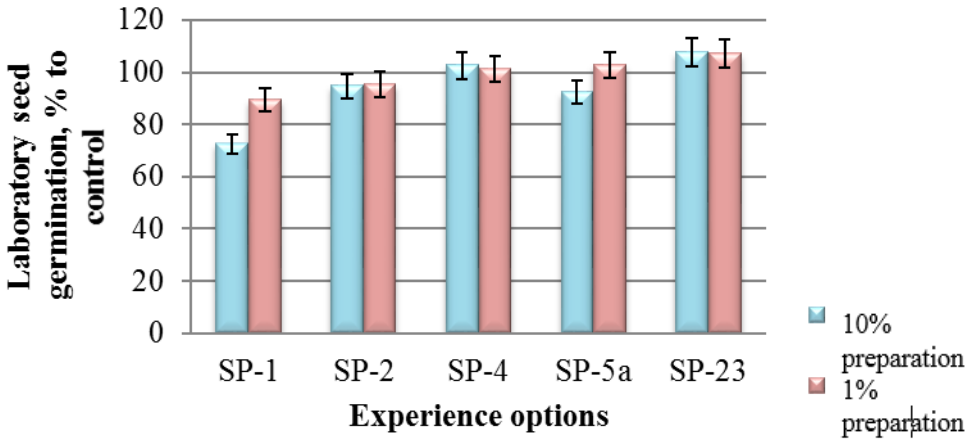

Fig. 2. The effect of *Azospirillum formosense* strains on laboratory germination of *Triticum durum* seeds.

Analyzing the experimental data obtained when studying the effect of a microbiological preparation, containing the *Azospirillum formosense* SP-4 strain, it was found that both concentrations did not have a significant effect on the laboratory germination of durum wheat seeds. The use of the preparation, which included the *A. Formosense* SP-5a strain, showed that a high concentration of the preparation (10 %) significantly inhibited, and a preparation concentration of 1 % significantly stimulated laboratory seed germination (see fig. 2).

In experimental variants with the inoculation of wheat seeds with a microbiological preparation, containing the *Azospirillum formosense* SP-23 strain, the laboratory germination of seeds was significantly higher than in the control variants in which no treatment was carried out using the preparation. It was shown that in variants with a preparation concentration of 1 %, the experimental variants exceed the control ones by an average of 7.3 %, the germination rate reaches 100 %. If in the control variant the

laboratory germination of seeds was 93.4 %, then in the experimental variants in which 1 % inoculation with a preparation of microorganisms was carried out, this figure was 100 % (see fig. 2).

Thus, analyzing the obtained data on the effect of *Azospirillum formosense* strains on the germination of *Triticum durum* Desf. seeds, it can be concluded that a microbiological preparation containing strain SP-23 had a stimulating effect on both germination energy and laboratory germination. At the same time, in variants with a preparation concentration of 10 %, the experimental variants exceed the control ones by 7.3–8.0 %, with a concentration of 1 % – 6.6– 4.2 %, respectively.

The effect of *Azospirillum formosense* strains on the morphometric parameters of *Triticum durum* Desf.

When studying the effect of microbiological preparations, containing new strains of *Azospirillum formosense*, on the height of 8-day-old plants, the data obtained were presented in Fig. 3.

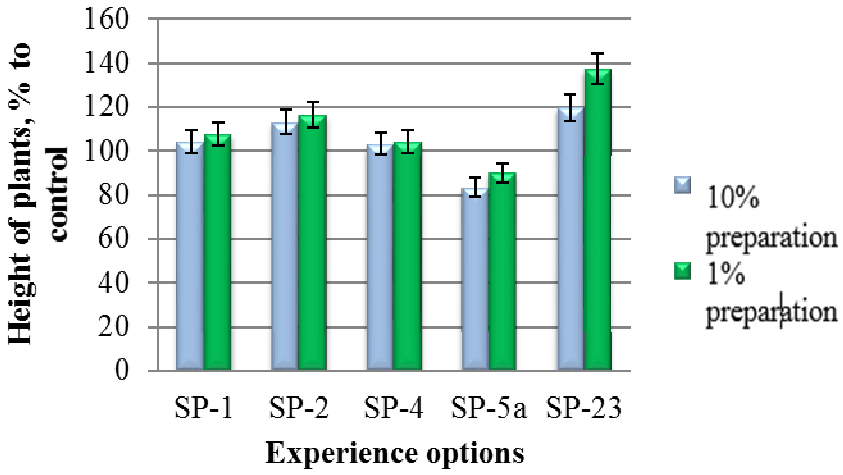

Fig. 3. The effect of *Azospirillum formosense* strains on the height of 8-day-old *Triticum durum* plants.

The use of the preparation, which included the *A. Formosense* SP-5a strain, found that a high concentration of the preparation (10 %) significantly inhibited the growth of wheat plants in the same way as the preparation concentration of 1 %.

It was found that in the experimental variants with the inoculation of wheat seeds with a microbiological preparation, containing the *Azospirillum formosense* SP-23 strain, the plants of wheat were significantly higher than in the control variants, in which the preparation was not treated. It was shown that in variants with a preparation concentration of 1 %, the experimental variants exceed the control ones by an average of 37.4 %. If in the control variant the height of the seedlings was 9.1 cm, then in the experimental

variants, in which inoculation with microorganisms was carried out, this figure was 12.5 cm.

Preparations of strains of microorganisms *Azospirillum formosense* SP-2 had a stimulating effect on the height of 8-day-old wheat plants, both at a concentration of 10 % and at a concentration of 1 %. The height of the above-ground part of wheat plants in the variant with a concentration of the preparation of 10 % reached 10.3 cm, which is 1.2 cm higher than in the control variant (see fig. 3). When using a microbiological preparation of strain SP-2 at a concentration of 1%, the significant difference between the control and experimental variants was 16.5 %.

Thus, analyzing the obtained data on the effect of *Azospirillum formosense* strains on the height of the above-ground part of of *Triticum durum* Desf., we can conclude that the microbiological preparation with the SP-23 strain had the greatest stimulating effect. At the same time, in variants with a preparation concentration of 10 %, the experimental variants exceed the control ones by 19.8 %, with a concentration of 1 % – 37.4 %, respectively.

When studying the effect of microbiological preparations, containing new strains of *Azospirillum formosense,* on the length of the root system of 8-day-old plants, the data obtained are shown in Fig. 4.

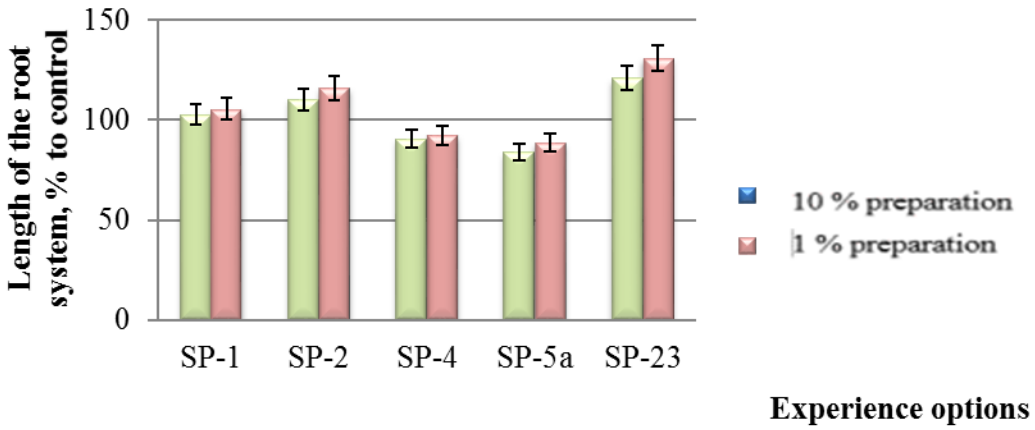

Fig. 4. The effect of *Azospirillum formosense* strains on the length of 8-day-old plants of *Triticum durum.*

It was shown that the use of a microbiological preparation, containing the *Azospirillum formosense* SP-1 strain, at different concentrations did not have a significant effect on the length of the root system of durum wheat plants. The use of the preparation, which included *A. formosense* SP-5a and SP-4 strains, showed that both high concentration of the preparation (10 %) and a preparation concentration of 1 % significantly inhibited the growth of the root system (see fig. 4).

In experimental variants with the inoculation of wheat seeds with a microbiological preparation with the *Azospirillum formosense* SP-23 strain, it was shown that the length of the wheat root system was significantly higher than in the control variants. Therefore, in

variants with a preparation concentration of 1 %, the experimental variants exceed the control ones by an average of 31.1 %. If in the control variant the length of the roots was 10.6 cm, then in the experimental variants in which inoculation with microorganisms took place, this figure was 13.9 cm.

Thus, analyzing the obtained data on the effect of *Azospirillum formosense* strains on the length of the root system of *Triticum durum* Desf., it can be concluded that the microbiological preparation with strain SP-23 had the greatest stimulating effect. At the same time, in variants with a preparation concentration of 10 %, the experimental variants exceed the control ones by 20.8 %, with a concentration of 1 % – 31.1 %, respectively.

When analyzing the experimental data on the effect of a microbiological preparation, containing strains of *Azospirillum formosense* SP-5a and SP-23, it was found that both concentrations did not have a significant effect on the fresh weight of the above-ground parts of durum wheat plants. When using preparation, which included strains of *A. formosense* SP-5a and SP-23, it was shown that both a high concentration of the preparation (10 %) significantly inhibited, and a concentration of the preparation 1 % – the fresh weight of the above-ground part increased (fig. 5).

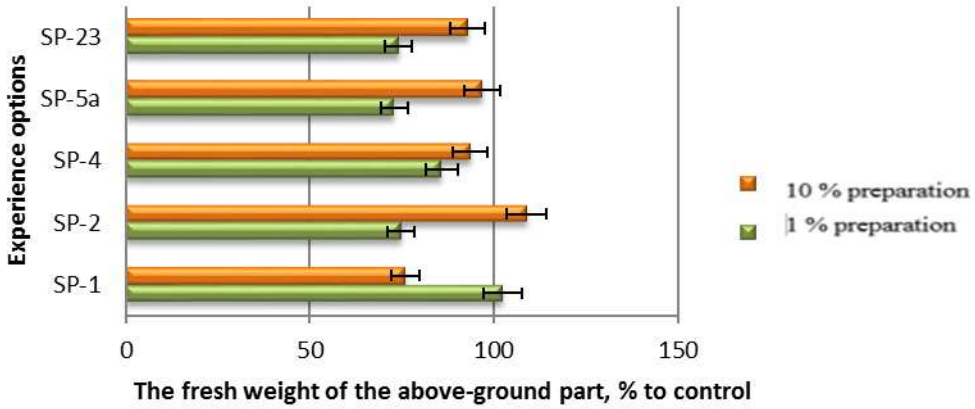

Fig. 5. The effect of *Azospirillum formosense* strains on the fresh weight of 8-day-old plants of *Triticum durum.*

Preparations of strains of microorganisms *Azospirillum formosense* SP-2 had a stimulating effect on the fresh weight of the above-ground part of 8-day-old wheat plants, as when using a concentration of 10 % SP-1 showed the best result, and at a concentration of 1 % – strain SP-2 did. The fresh weight of the above-ground part of wheat in the variant with a concentration of the preparation of 1 % reached 0.307 g, which is 8.9 g higher than in the control variant. When using a microbiological preparation, containing the SP-1 strain, at a concentration of 10 %, a significant difference between the control and experimental variants was 2.5 % (see fig. 5).

Thus, by analyzing our data on the effect of *Azospirillum formosense* strains on the fresh weight of the above-ground part of *Triticum durum* Desf., we can conclude that the

microbiological preparation with the SP-2 strain had the greatest stimulating effect. At the same time, in variants with a preparation concentration of 10 %, the experimental variants did not exceed the control, and with a concentration of 1 %, the experimental variants significantly exceed the control ones by an average of 8.9 %, respectively.

When analyzing the experimental data on the effect of a microbiological preparation, containing the *Azospirillum formosense* SP-1 strain, it was found that the concentration did not have a significant effect on the fresh weight of the root system of durum wheat plants. When studying the preparation, which included the strain *A. formosense* SP-1, it was shown that both a high concentration of the preparation (10 %) significantly inhibited, and a concentration of the preparation of 1 % – the mass of the raw substance of the root system (fig. 6).

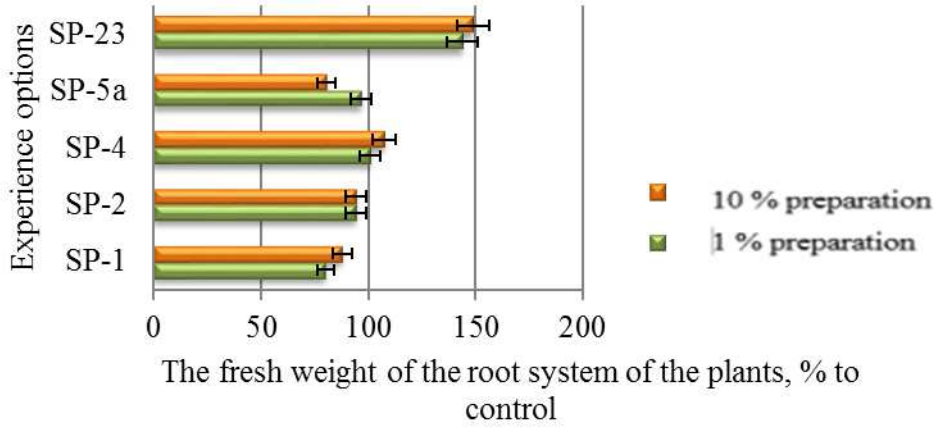

Fig. 6. The effect of *Azospirillum formosense* strains on the fresh weight of the root system of 8-day-old plants of *Triticum durum.*

In experimental variants with the inoculation of wheat seeds with a microbiological preparation of *Azospirillum formosense* SP-23 strain, the fresh weight of the wheat root system was significantly higher than in the control variants. It was shown that in variants with a preparation concentration of 1 %, the experimental variants exceed the control ones by an average of 48.9 %. If in the control variant the fresh weight of the root system was 0.266 g, then in the experimental variants in which inoculation with microorganisms was carried out, this figure was 0.396 g (see fig. 6).

Analyzing the data obtained on the effect of strains of *Azospirillum formosense* on fresh weight of the root system of *Triticum durum* Desf., we can conclude that the microbiological preparation with strain SP-23 had the greatest stimulating effect. At the same time, in variants with a preparation concentration of 10 %, the experimental variants significantly exceed the control ones by 43.6 %, with a concentration of 1 % – by 48.9 %, respectively.

Thus, analyzing the obtained data on the effect of *Azospirillum formosense* strains on fresh weight of the above-ground part of Triticum durum Desf., we can conclude that the microbiological preparation with strain SP-23 had the greatest stimulating effect. At the

same time, in variants with a preparation concentration of 10 %, the experimental variants exceed the control by 15.6 % and with a concentration of 1 % by – 24.5 %, respectively.

CONCLUSIONS

1. The positive effect of new *Azospirillum formosensis* strains on the growth and development of *Triticum durum* Desf has been established at the early stages of ontogenesis.
2. Inoculation of *Triticum durum* Desf seeds by *Azospirillum formosensis* strain SP-23 has a positive effect on their germination. Germination energy and laboratory germination significantly increased by an average of 8.0 – 14.2 % in experimental variants compared to the control.
3. Under the influence of SP-2 and SP-23 *Azospirillum formosense* strains, the linear growth rates of *Triticum durum* Desf. of the experimental variants exceed the control ones by 10.4 – 37.4 %.

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Белоусов В. В. Влияние штаммов *Azospirillum formosense* на рост и развитие *Triticum durum* Desf. на ранних этапах онтогенеза / Белоусов В. В., Чмелёва С. И., Сидякин А. И., Джелдубаева Э. Р., Туманянц К. Н. // Ученые записки Крымского федерального университета им. В. И. Вернадского. Биология, химия. – 2023. – Т. 9 (75), №2. – С. 44–52.

Описаны различные штамм-специфические эффекты действия предпосевной обработки семян *Triticum durum* штаммами *Azospirillum formosense*. Из исследованных штаммов только один (SP-23) достоверно повышает показатели лабораторной всхожести, и положительно влияет на прирост массы сухого и сырого вещества, а так же на увеличение линейных размеров осевых органов растений пшеницы твердой на ранних этапах онтогенеза.

Ключевые слова: *Azospirillum formosense,* энергия прорастания семян, всхожесть, морфометрические показатели, онтогенез, *Triticum durum* Desf.