INFLUENCE OF BACILLUS MEGATERIUM TO PROMOTE GROWING OF COTTON (GOSSYPIUM HIRSUTUM L.)

Yulya Bataeva[1], Damelya Magzanova[1], Adelia Baimukhambetova[1,2,*], Lilit Grigoryan[1], Daria Vilkova[1,3]

[1] *Astrakhan State University, Astrakhan, Russia*
[2] *Moscow State University of Food Production, Moscow, Russia*
[3] *Cherepovets State University, Cherepovets, Russia*

**Corresponding author. Email: baimukhambetova.mail@gmail.com*

ABSTRACT

The article presents the results of studying the influence of Bacillus megaterium on the growth and development of Gossypium hirsutum cotton plant in the field conditions of the Astrakhan Region. In the wild plants have to cope with several adverse environmental conditions, such as water scarcity, high salt concentrations in the soil, extreme temperatures, nutrient deficiencies and pathogen attacks. However, plants can interact with several soil microorganisms, including plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi, which make the plant more resistant to such stresses. Bacillus-based products represent the most important class of microbial products for phytosanitary use available on the market.

Field studies and microbiological analysis of the soil were carried out on the basis of the All-Russian Research Institute of Irrigated Horticulture and Melon Growing (VNIIOB) and Astrakhan State University. To compare the results, in the experiment the chemical mineral fertilizer Amofoska was used in the concentration of the working solution. Distilled water was used as a control substance. The weight of the crop was calculated at the end of the vegetation season. The research revealed that Bacillus megaterium has growth-promoting effect on cotton culture. Germination of seeds treated with bacillus was 96%. Plant biometrics indicate that plants treated with bacillus show the highest values in terms of leaf weight, leaf area and root length relative to the control. Thus, the number of buds and flowers increased by 5.1 and 3.1 pieces in comparison to the control sample and the one treated with mineral fertilizer, respectively. The length of the root also increased by 9.8 and 2.4 cm. The weight of cotton treated with bacillus exceeds the control variant by 46 g. and mineral fertilizer by 48.4 g, respectively. When abundantly poured for the second time, this indicator exceeded the weight of the control sample by 8 g and after treatment with mineral fertilizer - by 32 g.

Keywords: *Bacillus megaterium, cotton plant, raw cotton weight, growth-promoting effect, Astrakhan Region.*

INTRODUCTION

In Russia, the cotton industry is one of the leading sub-sectors of the textile industry. Its share, among all fabrics and yarns produced, is 60%. Cotton is used not only in textile, but also in many other industries: consumer goods manufacturing, medical, food, aviation, automotive, space [1-3].

In order to wean Russia of dependence on cotton fiber imports, it is necessary to revive the cultivation of cotton in the South of Russia, in particular in the Astrakhan Region, as the most favorable one for its cultivation.

The need to increase agricultural cotton production and environmental protection necessitated studying of new sustainable technologies.

Beneficial bacteria associated with soil and plants can promote plant growth and resistance to stress, therefore, are a sustainable alternative to overuse of chemicals in agriculture. Representatives of the genus Bacillus are among such beneficial bacteria used as microbial pesticides, fungicides or fertilizers. Bacillus-based products are the most important class of microbial products for phytosanitary use available on the market [6-9].

Gram-positive bacteria Bacillus megaterium are found in soils and are members of the microbiome of several host plants around the world, acting mainly as biological control agents [10-14]. These strains effectively colonize soils and plant tissues and produce a wide range of biologically active compounds that are involved in promoting plant growth [15-21] and antiphytopathogenic activity [22]. Bacillus species are also a source of a wide range of metabolites and enzymes of biotechnological and industrial interest [23]. Nevertheless, one of the most important characteristics of bacillus strains is their ability to form spores, thereby increasing their ability to withstand a wide range of stressful conditions and allowing their usage as inoculants of "plant growth promoters" (plant growth-promoting bacteria - PGPR) for agriculture and bioremediation processes. Thus, obtaining spore-forming and stress-resistant strains Bacillus megaterium with a high level of ability to promote plant growth is important for the development of new products that will be used in agricultural/bioremediation plants, especially those that are subjected to stressful conditions, which is very important in the arid climate of the Astrakhan Region

The purpose of this work is to study the influence of Bacillus megaterium on the development of agricultural crops of the Malvaceae family on the example of cotton (Gossypium hirsutum).

MATERIAL AND METHODS OF RESEARCH

Based on the sequencing of the 16 S rRNA gene at the Kurchatov Institute - GosNIIgenetics Research Institute, the bacteria used in this study were identified as Bacillus megaterium Q57-31. This strain of bacteria was isolated from the nodules of a legume plant - Vigna (Vigna cylindrica), grown on the soils of the Astrakhan Region, using the plate method on solid media.

The experiment was conducted in open ground conditions on the territory of the All-Russian Research Institute of Irrigated Horticulture and Melon Growing (VNIIOB) with cotton seeds (*Gossypium hirsutum*) of AC 1 variety.

The AC-1 cotton variety was obtained from a hybrid population with the original forms: chimbai 4031 × AC-6. The variety is early-ripening (the vegetation season is 110-114 days), the bush is semi-spreading; the fiber type is 5, the fiber yield is 35-37%, the productivity is up to 3.2 t/ha of cotton. The new AC-1 cotton variety makes it possible to obtain in the conditions of the south of Russia a yield of raw cotton exceeding the standard (AC-5) by 15-20% with a high yield of type 4-5 fiber. With defoliation, the variety is fully suitable for combine mechanized harvesting [24].

During the experiment, seed germination, plant height, root length were evaluated [25]. The height of the plants was measured from the soil surface to the crown using a ruler. The number of leaves per plant was determined by counting them on all plants, followed by calculating the arithmetic mean. The area of the leaf surface was calculated by mathematical method [26]. The weight of the leaves was measured on laboratory electronic scales. During the budding period, the number of buds (red and green) was calculated, during the blooming period - the number of flowers. The weight of the crop was calculated at the end of the vegetation season. From ten plants of each variant, 5 bolls were collected.

Before bedding-out, the seeds were soaked for 2 hours in bacterial suspensions and solutions of phytostimulating compounds under laboratory conditions. Before bedding-out, the territory was divided into seed-plots of 20 cm wide and 5 meters long. The experiment was carried out in 2 variants, which differed in the number of treatments. So, in the 1st variant, the treatment was carried out for1 time at the stage of 4-6 leaves, in the second variant - 2 times - at the stage of 4-6 leaves, budding and flowering. Each variant was performed in three repetitions. In the experiment, the seeds and sprouts were treated with five different phytostimulation objects: culture of cyanobacteria, in the concentration of 0.5 g / 10 ml; bacteria of *Bacillus* genus, at the concentration 107c/ml; EPIN (epibrassinolid), at the concentration of 10-7; chemical fertilizer Amofoska, at the concentration of working solution; the control, soaking and treatment with distilled water were applied.

The treatment was carried out by pouring liquids directly next to the root of the plant.

For the selection of soil samples, the envelope method was used, at depths of 0-20 cm and 20-40 cm [27]. Microbiological analysis of the soil was carried out in ASU laboratory of biotechnology, by quantifying ecological and trophic groups of microorganisms by the method of limiting dilutions in solified media [28, 29].

The obtained results were processed by generally accepted methods of mathematical statistics using Excel and Biostat programs [25, 26].

THE OBTAINED RESULTS AND THEIR DISCUSSION

The soils of the Astrakhan Region lack moisture, contain high concentrations of salts, which creates extreme conditions for the existence of living organisms. This leads to the formation of specific microbial communities [30, 31].

Within this study, adding organic and bioorganic fertilizers had the main goal of increasing the amount of nutrients and improving germination and growth of cotton in alluvial meadow, heavy loam, dark-coloured, slightly saline soil. The authors studied seed germinating ability, biometrics of cotton during budding and plant yield at the end of the vegetation season, as well as the microbiological composition of the incubated soil and its humidity.

The number of microorganisms was determined on the 4th day of cultivation. The total number of colonies was calculated (Table 1). In Petri dishes, colonies in 100 visual fields were first calculated, then the arithmetic mean of two sequences was determined. To estimate microorganisms in 1g of absolutely dry soil, the number of cells in 1g of wet soil is divided by the amount of absolutely dry soil contained in 1g of wet soil.

Table 1. Microbiological composition of soil

Nutrient medium I + II repetition	**Total microbial number (TMN)** (CFU/g)	
	0-20 cm	**20-40 cm**
Med. Czapek	0.3·107	0.9·107
Med. Gauze	2.9·107	1.05·107
Med. RPA (fishpeptone agar)	9.6·107	9.2·107
Med. PA:10 (nutrient agar)	6.8·107	0.9·107

Analysis of the total number of physiological groups of microorganisms in the RPA medium showed at both depths predominance of saprophytic microorganisms (9.6·107 CFU/g and 9.2·107 CFU/g), participating in the destruction of organic substances in the soil. The smallest total number of microorganisms was observed in Czapek medium, i.e. saccharolytic microorganisms.

Microscopic examination of the obtained colonies showed the presence of various cell morphotypes: cocci, rods, filamentous forms. Fungi are present in some media. Thus, under a microscope endogenous sporulation was observed in Czapek medium. Sporangium - a sac with conidia (spores). Presumably this is a fungus of the Mucor genus (Mucor).

Microbiological examination of soil samples revealed a wide variety of physiological groups of microorganisms involved in the nitrogen, phosphorus and carbon cycle.

In the conducted experiments, special attention was also paid to the qualitative acceleration of the rate of cotton development. The results of the studies indicate a positive effect of bacilli, cyanobacteria and epin on the growth and development of cotton in comparison with the control variant. The results of germination of cotton seeds (on the 14th day) showed that these biofertilizers produce a result of more than 90%. The lowest germination rates were marked during treatment with mineral fertilizer.

A characteristic reaction of the organism of cotton plants developing under normal conditions is the increased growth of the main stem, formation of sympodial, i.e., fruiting branches, and consequently, the number of fruits. In the vegetation experiment, phenological observations were carried out describing the plant growth, as well as its development by phases.

At the budding stage, the number of leaves, buds and flowers was measured, the weight of leaves and leaf area were calculated, and the length of the plant root was also taken into account (Table 2).

The results of single treatment of plants with bacillus (at the stage of 4-6 leaves) showed improved indicators in all parameters in comparison to the control. Thus, the number of buds and flowers increased by 5.1 and 3.1 items in comparison to the control sample and the one treated with mineral fertilizer, respectively. The length of the root also increased by 9.8 and 2.4 cm. High figures of the number of leaves and their biomass give us reason to note the activity of the photosynthesis process, as well as the intensive accumulation of substances necessary for plants, which directly affect the yield and productivity of agricultural crops. At the same time, double processing, on the contrary, reduces the indicators against all the other studied samples.

The results of cotton productivity were evaluated by the number of green and red bolls (Figure 1).

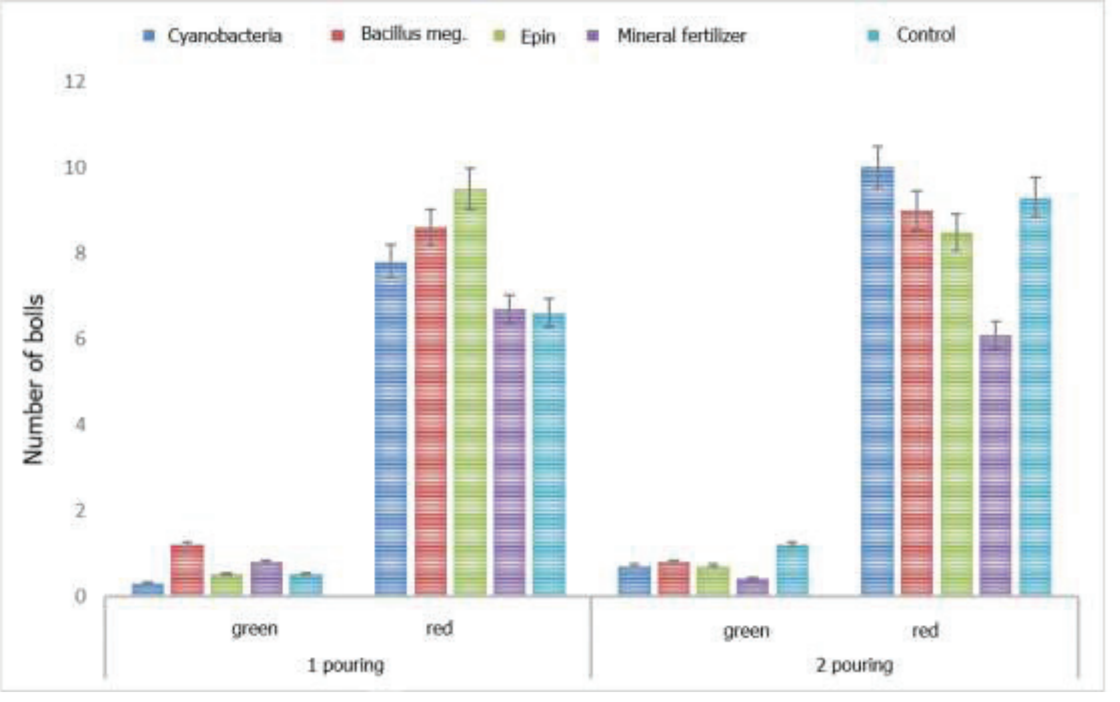

Figure 1. Cotton productivity (number of bolls).

Among the green bolls with a single pouring, the productivity of cotton treated with bacillus showed the highest indicator, among the red bolls - productivity increased on average by 2.0 and 1.9 pcs. in comparison to the control and mineral fertilizer, respectively.

Table 2 Biometrics of cotton

	Variant	Buds and flowers, pcs	Number of leaves, pcs	Weight of leaves, g	Leaf area, cm2	Root length, cm
Cyanobacteria	1	12.00±1.85	23.10±1.64	35.70±1.40	53.50±1.87	20.80±1.50
	2	20.00±1.19	33.70±0.53	48.80±1.61	39.00±1.89	26.70±0.87
Bacteria of Bacillus megaterium genus	1	19.10±1.64	32.60±1.24	37.95±1.57	45.25±1.52	24.6±1.54
	2	15.00±1.15	23.40±0.87	30.40±1.28	38.11±1.11	25.3±0.95
Epin	1	19.60±1.43	35.80±1.13	37.71±1.34	51.74±0.85	26.5±1.26
	2	18.10±1.33	25.45±1.46	29.42±1.87	45.45±1.03	25.70±1.56
Mineral fertilizer	1	16.00±1.52	25.20±1.32	54.50±1.92	34.00±1.15	22.20±1.15
	2	15.20±2.08	22.30±1.54	24.10±1.94	36.30±0.40	24.90±1.41
Control	1	14.00±1.41	22.20±1.88	25.40±1.74	20.90±1.35	14.80±1.17
	2	16.30±0.57	29.00±1.62	28.10±1.82	35.20±1.15	25.60±1.01

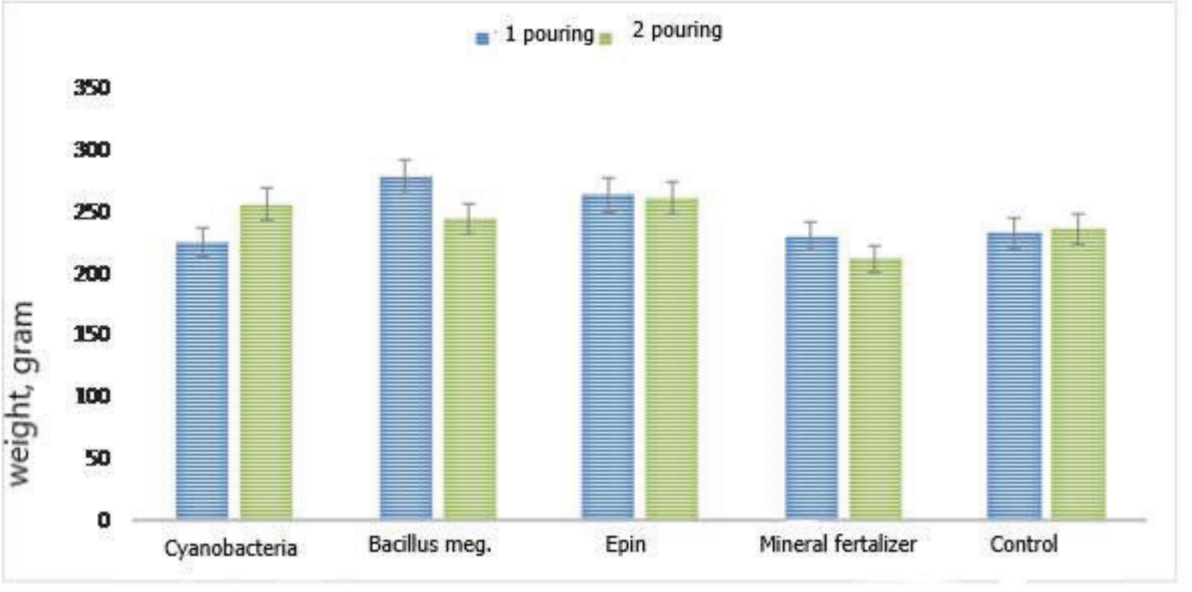

Figure 2. Cotton yield.

When poured twice the highest index of red buds is observed in cotton treated with cyanobacteria, its productivity increased by 1.2 against the control and by 1.1 against the mineral fertilizer.

At the end of the vegetation season, the yield was assessed by the weight of the cotton obtained in all variants. The weight of cotton treated with bacillus exceeds all variants when poured once but loses to epin and cyanobacteria with a double pouring (Figure 2).

In the experiment with one pouring, the weight of cotton treated with bacillus exceeds the control variant by 46 g. and with mineral fertilizer by 48.4 g, respectively. With repeated pouring, these indicators exceed the weight of the control sample by 8 g. and the yield after treatment with mineral fertilizer by 32 g.

Thus, to obtain a large mass of cotton yield when treated with bacillus, single pouring is most suitable. The volumes of cotton grown after such treatment are competitive with plants that were poured twice with cyanobacteria and with the same scheme of epin.

CONCLUSION

Thus, as a result of the conducted research, it was found that the studied soil is slightly acidic, nonsaline, with a predominance of saprophytic microorganisms, with the presence of microscopic fungi. The positive effect of bacillus on the growth and development of cotton culture in the Astrakhan Region has been proved. High rates were obtained with a single pouring.

AUTHORS' CONTRIBUTIONS

The article "The influence of bacillus megaterium on the stimulation of cotton growth" was written by a team of authors, all authors took an equal part in the theoretical analysis of the problem and in conducting the study.

Bataeva Yu.V. is responsible for all aspects of the project, the integrity of all parts of the article and its final version. Magzanova D.K. analyzed and summarized the literature data on the research problem, was responsible for the experimental project. Baymukhambetova A.S. was responsible for conducting a comparative analysis of the effect of various fertilizers on biometric indicators and cotton yields and drafting a manuscript. Grigoryan L.N. performed a microbiological analysis of the soil composition and made valuable comments when revising the article. Vilkova D.D. conducted the analysis and systematization of experimental data

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