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ORIGINAL ARTICLE

Efficacy of Application of Biostimulants Based on Non-pathogenic Microorganisms when Growing Okra in Arkansas

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ABSTRACT

The results of the experiment on the cultivation of okra vegetable crops in a farm in Osceola, Arkansas, USA using innovative biostimulants based on non-pathogenic microorganisms are presented.

It is shown that the use of biostimulants contributed to a significant improvement in the morphological characteristics of plants, such as plant height, the number of side branches, leaves, fruits. In the green fruits of okra, the content of vital elements-nitrogen, sulfur, potassium, and especially iron, zinc, copper, magnesium and phosphorus-increased compared with the control variant, while the content of nitrates was significantly reduced, which suggests that biostimulants studied based on non-pathogenic microorganisms are promising.

INTRODUCTION

Due to the insufficient provision of the population with vital nutrients, such as macro and microelements, vitamins, amino acids, etc., nowadays, the creation of high-quality food is of particular relevance, since human health is largely determined by nutritional status for him, nutrients are available in whole, original nature, product.

Unlike the self-regulating natural ecosystem, where the circulation of chemical elements is almost closed, in an agroecosystem, management of the circulation is conducted by a person from outside. Unbalanced and excessive use in agriculture of mineral fertilizers, pesticides, herbicides, etc. leads to a significant accumulation of harmful substances in plants, which reduces the nutritional value of agricultural products and most often affects the organisms, damaging the immune system.

A large role in the biogeochemical circulation of chemical elements belongs to living organisms, especially soil microorganisms. They create soil fertility and have been successfully engaged in this for millions of years. Fertile soil is a community of hundreds of species of living organisms. In the soil, the roots of plants, like a clutch,

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are dressed with a live layer of microbial cells - bacteria and fungi, both beneficial and harmful.

Obtaining with the help of biostimulants containing non-pathogenic microorganisms, environmentally safe plant products with the optimum content of vital chemical elements, vitamins and amino acids is one of the most important tasks nowadays.

Biostimulants are materials, other than fertilisers, that promote plant growth when applied in low quantities. The word biostimulant was first defined by Kauffman, et al. [1]. This word was increasingly used over the following years, expanding the range of substances [2-4]. Biostimulants available in the marketplace include bacteria, fungi, seaweed, higher plants, animals and humate-containing raw materials. Bacteria interact with plants in all possible ways [5]. Plant growth-promoting rhizobacteria are multifunctional and influence all aspects of plant life: nutrition and growth, morphogenesis and development, response to biotic and abiotic stress, interactions with other organisms in the agroecosystems [6-12].

Mycorrhizal fungi establish symbioses with over 90% of all plant species. The Arbuscule-Forming Mycorrhiza (AFM) are a widespread type of endomycorrhiza [13,14]. There is growing interest in using mycorrhiza for plant nutrition (for both macronutrients, especially P and micronutrients), water balance, biotic and abiotic stress protection of plants [15-20]. Hyphal networks can interconnect not only fungal and plant partners but also individual plants within a plant community [21,22].

Many scientists [23,24] note that the use of non-pathogenic soil bacteria living on the roots of plants is a very promising direction and opens up significant opportunities for organic farming. Bacteria belonging to the genus Bacillus, and especially strains of *Bac. subtilis*, are effective for the biological control of many plant diseases caused by soil pathogens [25-28].

The biostimulants based on non-pathogenic microorganisms studied by us are also biopreparations characterized by potentially high growth-stimulating activity.

The purpose of this study was to study the effect of innovative biostimulants based on non-pathogenic microorganisms on the morphological indicators of okra plants and on the content of essential macro-microelements in green fruits when grown in the State of Arkansas (USA).

MATERIALS AND METHODS

Place of experience

Microfield experience with okra was conducted in Arkansas on the territory of the farmer's land plot from Osceolla. This farm is located in the southeastern part of the state, characterizing the almost flat plain side with alluvial soils. The climate is humid subtropical with hot, humid summers and cold, less humid, winters; in July, the average maximum is 34°C, a minimum of 23°C; in January, the average maximum is 11°C, the average minimum is 0°C. The average annual rainfall varies between 1000 and 1500 mm.

Characteristics of plants okra

Okra (*Latin Abelmoschus esculentus*) is a very valuable vegetable crop that has many names, including gombo, okra and ladies' fingers. This is an annual herb from the Malvova family, widely distributed in Africa, North America and India, and is found in Europe, Russia and Ukraine. It is a thermophilic, moisture-loving and sun-loving plant.

By chemical composition, okra fruits are similar to legumes. Okra is an ideal vegetable for weight loss, it is called a vegetarian's dream because of the huge amount of useful and nutrients contained in its fruits: raw protein (1.5-2%), sugar (2.2-6.1%), dietary fiber, vitamins C (14-35 mg/100 g), B6, K, A, folic acid, iron, calcium and potassium. Mature seeds contain up to 20% oil, resembling olive oil.

Dietary fiber and plant oxy mucus is absorbed in the small intestine, which helps regulate blood sugar levels. This vegetable also strengthens the capillary walls, washes away excess cholesterol and toxins from the body. Okra is recommended for use in such diseases as atherosclerosis, sore throat, depression, chronic fatigue, diabetes, cataracts, asthma, peptic ulcer and others.

Characteristics of bio stimulants used

Offered biostimulants are safe for humans, plants, animals and the environment. They enhance the immunity of plants, respectively, increase the resistance of plants to adverse environmental factors, including a significant increase in temperature in hot summer conditions. They significantly reduce the development of root rot and phytopathogens of any type and source.

The composition of biostimulants, in addition to non-pathogenic microorganisms that make up the active ingredient of the preparation, includes nutrient medium and organic-mineral carrier. In the experiment used two biostimulant (Bioelements Ltd, York, UK), slightly different in composition of microorganisms. Both biostimulants contained spore-forming bacteria *Bacillus subtilis*, which live in fertile soil - black soil, and fungus (*Trichoderma harzianum*) - antagonist of pathogens. In addition to the above-mentioned microorganisms, a mycorrhizal fungus (*Glomus intraradices*) was added to the first preparation, and a symbiotic nitrogen-fixing bacterium (*Azotobacter chroococcum*) in the second.

From a technological point of view, the bacteria endospores of *Bac. subtilis* is more effective than living cells, because they are more resistant than vegetative cells, and retain their viability for many years under the right conditions of drug storage. In addition, endospores are resistant to extreme pH values, much more resistant to drying to form powders and relatively easy to produce using industrial fermentation technology.

Preparations of biostimulants is a powder that can be stored in a dry cool place at a temperature from + 5°C to + 25°C for 3 years. Dry the drug is pre-diluted in the appropriate amount of non-chlorinated water and poured into the sprayer. The solution should be used within 24 hours, preferably in cloudy weather or in the morning.

The methodology of the experiment

The scheme of our experience provided for two options: 1) control 2) biostimulants. On plots measuring 20 m2, okra seeds were sown in early May. When sowing, a solution with biostimulator No. 1 was applied at a dose of 1 kg/ha 1.5 months after sowing, okra plants of the experimental variant were watered with biostimulator No. 2 at the rate of 0.5 kg/ha. During the growing season, grown plants were visually observed, and in early October, an assessment of the main morphological parameters of plants was carried out and green okra fruits were selected for chemical analysis.

The chemical elemental composition of green okra fruits was determined in the United States at the Waypoint Laboratory (Tennessee, Memphis). The S, P, K, Mg, Ca, Na, B, Zn, Mn, Fe and Cu contents of green okra fruits were assessed using an ICP-MS on a mass spectrometer Optima 7300 (Perkin Elmer, USA), N was assessed using an LECO 528 and nitrates were assessed using Colorimetric Cadmium Reduction Lachat 8500.

RESULTS AND DISCUSSION

Plants of okra of the experimental and control variants five months after sowing differed significantly in their morphological characteristics (Figure 1). As can be seen from the figure, on each plant of an okra there are at the same time green and overripe fruits, fruits in the stage of seed ripening, new flowers and buds. For food purposes, collect green fruit no larger than 10 cm in the period from August to November.

The introduction of biostimulants contributed to the better development of okra plants. Thus, the height of plants increased by an average of 38% (from 73 cm in the control variant to 101 cm in the experimental one), the number of side branches per plant increased by an average of 75% (from 4 in the control variant to 7 in experienced), the number of leaves per plant is on average 45% (from 22 in the control variant to 32 in the experimental one), the number of seeds in one ripe fruit is on average 65% (from 32 in the control variant to 53 pieces in an experienced one), the number of green fruits of different sizes on one plant is on average 39% (from 23 pieces in the control variant up to 32 pieces in an experienced one) (Table 1).

Figure 1 Okra with (left) and without (right) biostimulants.

Table 1: Morphological parameters of okra plants 5 months after sowing.

Parameters	Average in Control and in Experiment (count)		Changes to control, %
	Control	Experiment	
the height of plants, cm	73	101	38%
the number of side branches per plant	4	7	75%
the number of leaves per plant	22	32	45%
the number of green fruits of different sizes on one plant	23	32	39%
the number of seeds in one ripe fruit	32	53	65%

Since the yield of okra fruits is determined by the number of leaves on the stem, since flowers are located precisely in the axils of the leaves and how many leaves grow on a plant, so many fruits will be on it, therefore the number of leaves is one of the important characteristics of the plant. Fruits at the pyramidal okra with 5-7 facets, 20-25 cm long, they are called a multi-seed box, inside of which there are rounded seeds with a diameter of 5-6 mm. Okra bloom begins two months after the emergence of shoots. Gather its fruits many times (every two or three days) before the onset of frost.

The chemical elemental composition of green fruits okra. The green fruits of okra determined the content of 12 vital chemical elements. The results were evaluated from the standpoint of the relationship of the studied elements to living organisms. According to A. Leninger's classification

[29], the necessary inorganic macroelements include nitrogen, sulfur, potassium, calcium, phosphorus, sodium, magnesium, etc.

The results of determining the content of macro-microelements in green fruits grown in the experience of okra are presented in table 2.

It was established that in the fruits of an okra grown with the use of biostimulants, as compared with the control variant, the nitrogen content increased from 2.06% to 2.23% (1.1 times), sulfur-from 0.23% to 0.25 % (1.1 times), phosphorus-from 0.50% to 0.65% (1.3 times), magnesium-from 0.34% to 0.40% (1.2 times). The potassium content (3.38% in the control variant and 3.42% in the experimental one), calcium (0.99% in both variants) and sodium (0.02% in both variants) in the fruits did not practically change when using biostimulants.

According to A.P. Avtsyn, et al. [30], zinc, manganese, iron, copper are among the most important essential (vital) microelements, and boron is conventionally essential. As can be seen from table 2, under the influence of biostimulants applied in green fruits of okra, the zinc content increased from 53 mg/kg to 64 mg/kg (1.2 times), iron - from 48 mg/kg to 68 mg/kg (in 1.4 times), copper-from 6 mg/kg to 9 mg/kg (1.5 times). The boron content increased slightly-from 25 mg/kg to 26 mg/kg, and the manganese content, on the contrary, decreased from 14 mg/kg (in the control variant) to 13 mg/kg (in the experimental variant).

The determination of nitrates in green fruits of okra showed that their level when using biostimulants decreased significantly from 374 mg/kg to 222 mg/kg (1.7 times).

CONCLUSION

Thus, innovative biostimulants based on non-pathogenic microorganisms contributed to the improvement of morphological parameters of okra plants - the number of lateral branches, leaves, green fruits on one plant and seeds in ripened fruits, as well as plant height, increased. The accumulation of many essential macro- microelements in the green fruits of okra, especially phosphorus, magnesium, zinc, iron, copper, also increased, while the level of accumulation of nitrates in them significantly decreased.

Table 2: Contents of essential elements in green okra fruits.

Indicators	Average in Control and in Experiment	
	Control	Experiment
N, %	2.06 ± 0.21	2.23 ± 0.20
S, %	0.23 ± 0.03	0.25 ± 0.03
P, %	0.50 ± 0.05	0.65 ± 0.06
K, %	3.38 ± 0.37	3.42 ± 0.31
Mg, %	0.34 ± 0.04	0.40 ± 0.05
Ca, %	0.99 ± 0.10	0.99 ± 0.10
Na, %	0.02 ± 0.00	0.02 ± 0.00
B, mg/kg	25 ± 2.4	26 ± 2.9
Zn, mg/kg	53 ± 4.8	64 ± 5.8
Mn, mg/kg	14 ± 1.3	13 ± 1.2
Fe, mg/kg	48 ± 4.3	68 ± 6.2
Cu, mg/kg	6 ± 0.5	9 ± 0.8

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