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STUDY OF THE INFLUENCE OF ORGANOMINERAL FERTILIZER- AMELIORANTS BASED ON NATURAL ALUMINOSILICATES AND BIOLOGICALLY ACTIVE SUBSTANCES ON THE GROWTH OF *PHASEOLUS VULGARIS*. PART 2.

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Abstract. The study presents the results of investigating the influence of novel organomineral fertilizer-ameliorants based on expanded vermiculite and biologically active substances (humic substances, the microalga *Chlorella vulgaris*, and an animal-derived protein hydrolysate) on the growth of common bean (*Phaseolus vulgaris*). Pot experiments were conducted on degraded soils of the Otyrar district of the Turkestan region. It was demonstrated that the application of the fertilizer-ameliorants promoted an increase in total biomass, root mass, leaf number, and plant height compared to the control. The Vermi-KB treatment produced the most pronounced effect, leading to an 80.6% increase in plant dry biomass compared to the control. The use of Vermi-CV also showed a pronounced positive result (an increase in raw biomass by 58.0%). The results confirm the effectiveness of the developed fertilizer-ameliorants and their potential for increasing the fertility of degraded soils.

Keywords: common bean (*Phaseolus vulgaris*), organomineral fertilizers, soil amendment, expanded vermiculite, humic substances, Chlorella vulgaris, protein hydrolysate.

INTRODUCTION

Kazakhstan is one of the largest arid countries in Eurasia, where the combination of an arid climate, historical plowing of the steppes and modern intensive land use creates high soil vulnerability [1-2].

In the arid and semi-arid regions of southern Kazakhstan, the limited leaching regime, combined with excessive application of mineral salts and inefficient irrigation, accelerates secondary salinization and alkalization, with salts rising capillarily and accumulating in the upper soil horizons [3].

In this regard, the development of fertilizer-ameliorants based on natural materials allows farmers to provide nutrients to their crops and minimize the use of synthetic chemical fertilizers and pesticides.

In the work of the authors [4], research was conducted to study the influence of organomineral fertilizer-ameliorants based on expanded vermiculite and humic substances on the growth of corn plants. This work is a continuation of the study of the influence of new organomineral fertilizer-ameliorants on the growth and development of agricultural crops on different types of soil. For the production of organomineral fertilizer-ameliorants, expanded vermiculite was used as a carrier of biologically active substances. Humic substances, *Chlorella vulgaris* microalgae and domestically produced animal protein hydrolysate were used as biologically active compounds and plant growth stimulants.

The use of *Chlorella* spans a wide range of human activities: in agriculture as a plant growth stimulant, in the food industry, in medicine and cosmetology, for wastewater treatment and water body rehabilitation, as well as for oxygen production and biofuel generation. The ability of green algae to enhance plant physiological activity and development offers promising prospects for their practical application [5, 6].

Biopolymer wastes of animal origin can be efficiently processed into biofertilizers rich in amino acids, peptides, and beneficial microorganisms. Such fertilizers not only provide nutrients to plants but also improve soil structure and microbiological balance, thereby reducing the need for chemical fertilizers and supporting sustainable agriculture. The application of protein hydrolysates as fertilizers, feed additives, and biomaterials aligns with the concept of a circular economy and sustainable agriculture.

The aim of this study is to develop an organomineral fertilizer-ameliorants based on expanded vermiculite and biologically active substances, as well as to investigate their effect on the growth of common beans.

MATERIALS AND METHODS

The organomineral fertilizer-ameliorants was produced using industrially expanded vermiculite (Kulantau deposit, Avenue LLP). Expanded vermiculite of grade M-150 was used in the study with the following characteristics: bulk density – up to 140 kg/m^3, particle size distribution – from 0.1 to 7.0 mm, and water-holding capacity – 345.0%. The moisture content of the initial material ranged from 0.15 to 0.8%.

As biologically active substances, commercially produced potassium humate (Black Biotechnology LLP), a strain of the planktonic green alga *Chlorella vulgaris* SKO (Scientific and Technological Water Center LLP), and an animal-derived protein hydrolysate (Bionika 25 LLP) were used, followed by their immobilization on expanded vermiculite.

These materials were subsequently used in pot experiments to assess their impact on the growth of common bean plants. The following abbreviations are used in the text of the article: Vermi – expanded vermiculite, HS – potassium humate, CV – *Chlorella vulgaris* SKO, KB – protein hydrolysate.

The method of preparing the ameliorant-fertilizer included the following stages. The weighed portions of expanded vermiculite were dried to a constant weight. Then a solution of biologically active substances (BAS) was added to the carrier with constant stirring. The volume of the solution was 80% of the maximum moisture capacity of vermiculite, which ensured its complete and uniform absorption. After processing, the resulting preparation was incubated for 24 hours to achieve equilibrium distribution of the biologically active substances in the carrier matrix. At the final stage, the samples were dried to an air-dry state at room temperature.

Thus, the following biologically active substances were immobilized on expanded vermiculite to obtain ameliorant fertilizers:

1. Humic substances (Vermi-HS).

2. Strain of planktonic green algae *Chlorella vulgaris* (Vermi-CV).

3. Humic substances and a strain of planktonic green algae *Chlorella vulgaris* (Vermi-HS-CV).

4. Hydrolyzed animal protein (Vermi-KB).

Soil. For the vegetation experiment, the soil was collected in the Shauildir rural district of the Otyrar region of the Turkestan region (coordinates 42°42'20"N 68°20'06"E), in the desert soil zone used in irrigated agriculture (figure 1).

The plot is intensively used in agriculture, in recent years, corn and pumpkin crops have been grown here, there is no

crop rotation. It is characterized by a low content of humus (0.78–1.40%), total nitrogen (0.07–0.09%) and phosphorus (0.13–0.15%), and a relatively high content of potassium (2.45–2.52%). In the upper horizon (0–25 cm) there is an increased accumulation of nitrate nitrogen (44.08 mg/kg), mobile phosphorus (37.5 mg/kg) and exchangeable potassium (580 mg/kg). The soil has a slightly alkaline reaction (pH 7.39–7.61). The overall salinity is low (0.16–0.24%), but the sulfate content exceeds the toxic level (1.81–3.26 mg-eq), indicating weak sulfate salinity. According to its particle size distribution, the soil is classified as heavy loam with a high content of physical clay (56.5%). The soil's field capacity (FC), determined in accordance with RD 52.33.219–2002 [7], was 25%.

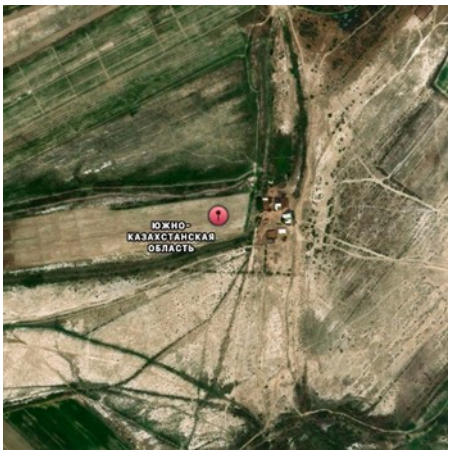

Figure 1 – Soil sampling site, Shaulder village, Otyrar district, Turkestan region (42°42'20"N 68°20'06"E)

Before setting up the experiment, the soil was prepared in the generally accepted way: dried in the air, cleaned of foreign impurities, sifted through a sieve (2 mm) and thoroughly mixed.

Preparation of substrates and setting up a vegetation experiment.

The procedure for preparing substrates for pot experiments is presented in table 1.

Table 1 - Composition of experimental substrate variants

Substrates	Soil, ml	Vermi, ml	HS, ml	CV, ml	KB, ml	Ameliorant-fertilizer, ml
1. Soil (control)	500.0	-	-	-	-	-
2. Soil + Vermi	450.0	50,0	-	-	-	-
3. Soil + HS	500.0	-	0.41	-	-	-
4. Soil + CV	500.0	-	-	0.41	-	-
5. Soil Vermi - HS	450.0	-	-	-	-	50.0
6. Soil + Vermi - CV	450.0	-	-	-	-	50.0
7. Soil + Vermi - HS - CV	450.0	-	-	-	-	50.0
8. Soil + Vermi - KB	450.0	-	-	-	-	50.0
9. Soil + KB	500.0	-	-	-	0.41	-

The amount of expanded vermiculite and each biologically active substance (BAS) introduced into the substrate precisely corresponded to their calculated mass fraction in the final fertilizer–ameliorants.

The vegetation experiment was carried out in plastic containers with a volume of 0.8 L. A drainage layer of expanded clay (60 g) and a separating fabric layer were placed at the bottom of the containers. Common bean (*Phaseolus vulgaris*) was used as the test crop. In experimental variants, vermiculite and fertilizer-ameliorants were added in an amount of 10% of the total volume of the substrate. In this case, in variants 3, 4 and 9, the content of biologically active components (HS, CV, KB) corresponded to their formulation in the composition of each specific ameliorant. The control variant was pure soil.

The experiment was carried out in triplicate. The soil moisture in the control variant was maintained at 60% of the FC. To equalize the light conditions, the containers were regularly rotated.

At the end of the vegetation period, a full biometric analysis of the plants was carried out: the length of shoots, the number of leaves were measured, the raw and dry weight of the above-ground and underground parts were determined.

Research methods.

The total humus content was determined using Tyurin's method, based on the oxidation of humus with a sulfate solution of potassium dichromate, the excess of which is titrated with a solution of Mohr's salt [8].

Total nitrogen (N) according to the Kjeldahl method is calculated from the amount of sulfuric acid used for titration of ammonium borate [9].

Determination of mobile forms of phosphorus and potassium was carried out using the Machigin method. The method is based on extracting mobile phosphorus and potassium compounds from the soil with an ammonium carbonate solution at a concentration of 10 g/dm^3 at a soil-to-solution ratio of 1:20, followed by the determination of phosphorus in the form of a blue phosphorus–molybdenum complex using a photoelectric colorimeter, and potassium using a flame photometer [10].

The pH of the soil suspension medium was measured potentiometrically according to [11].

The carbonate content was determined according to [12]. The essence of the method consists of titration with a sulfuric acid solution in an aqueous extract of carbonate ions to pH 8.3, and bicarbonate to pH 4.4.

The exchangeable bases Ca^{2+} and Mg^{2+} were determined complexometrically using Trilon B (EDTA) [13].

Potassium (K^+) and sodium (Na^+) were determined by flame photometry [14]. Sodium was measured at analytical lines of 589.0 and 589.9 nm, and potassium at 766.5 and 769.9 nm.

During the main growth and development phases of common bean plants, biometric measurements and plant sampling were carried out to study growth and development dynamics under the influence of different types of fertilizer–amendments

RESULTS AND DISCUSSION

The development of fertilizer-ameliorants based on natural minerals immobilized by biologically active substances makes it possible to obtain a synergistic effect by enhancing the ameliorative properties of natural minerals and the fertilizing properties of biologically active compounds.

The conducted vegetation experiment allowed us to obtain comprehensive data on the influence of various variants of organomineral fertilizer-ameliorants on the growth and development of common bean plants. The results of biometric measurements, presented in table 2, as

well as in figures 2-7, demonstrate pronounced differences between the experimental variants and the control.

Data analysis shows that the application of pure expanded vermiculite already has a positive effect on bean growth compared to the control soil. An increase was observed in total fresh biomass by 31.13%, stem mass by 28.81%, and leaf mass by 38.82%, which is associated with the improvement of the physicochemical properties of the substrate. Expanded vermiculite increases the soil's water-holding capacity and aeration, thereby creating more favorable conditions for root system development and plant growth as a whole.

The application of biologically active substances without a carrier showed mixed results. Potassium humate (HS) in its pure form had a significant positive effect, increasing the total raw weight by 41.56% and the leaf weight by 51.05%. At the same time, the application of pure *Chlorella vulgaris* suspension (CV) led to negative dynamics of root system development to -8.33% of the control and the lowest total dry weight among all variants by 18.91%. This may be due to the rapid degradation and insufficient preservation of algae cells in the soil without a protective carrier, which leads to competitive or allelopathic interactions in the early stages of plant development.

a

b

Figure 2 – Pot experiments of the common bean plants (a). Measurement of the biometric data of the common bean plants (b)

The most indicative results are those of the application of ameliorant fertilizers, where expanded vermiculite acted as a carrier for biologically active substances. The combination of vermiculite with *Chlorella vulgaris* showed high efficiency. This variant demonstrated the maximum increase in the raw mass of stems and leaves by 59.66% and 70.04%, respectively (figure 3). There is also a significant increase in total raw and dry biomass by 57.98% and 73.27%, respectively (figure 6).

The greatest effect in terms of accumulation of dry biomass is observed in the variant with an ameliorant-fertilizer based on expanded vermiculite and Vermi-KB protein hydrolysate. The increase in total dry mass relative to the control was 80.55%, which is the maximum value among all the studied variants. Also, in this variant the highest absolute values of dry mass of stems 0.15 g, and leaves 0.13 g, as well as the maximum stem width of 2.53 mm were recorded (figure 6). Such a significant effect is probably due to the direct entry into the soil of readily available forms of nitrogen (amino acids and peptides) from the hydrolysate, which, being immobilized on expanded vermiculite, prevents their rapid leaching and provides prolonged nutrition to the plant.

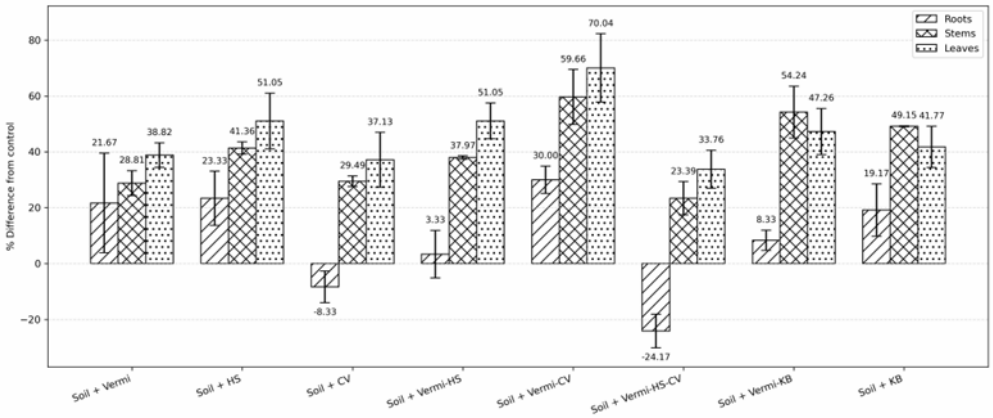

Figure 3 – Effect of substrate composition on the raw mass of vegetative organs of common beans

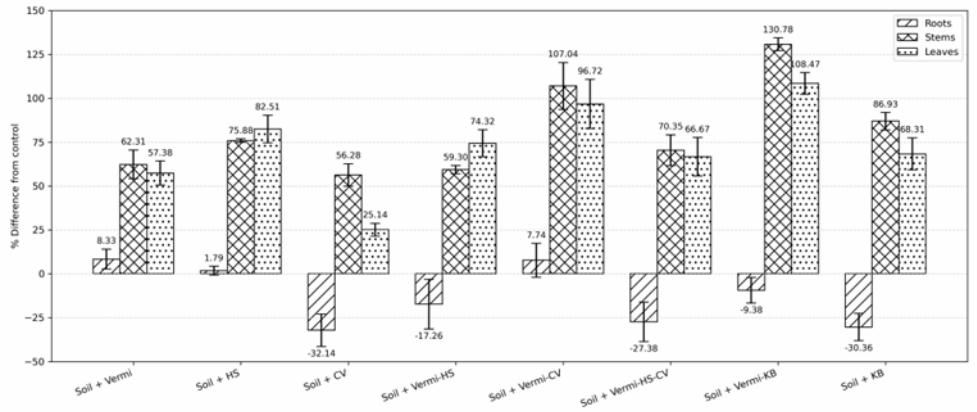

Figure 4 – Effect of substrate composition on dry mass of vegetative organs of common beans

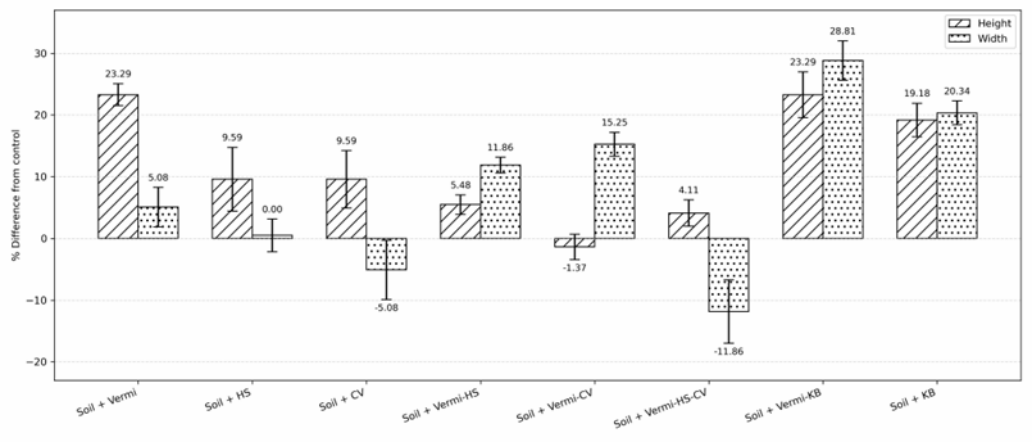

Figure 5 – Effect of substrate composition on the growth parameters of common bean (*Phaseolus vulgaris*) compared to the control

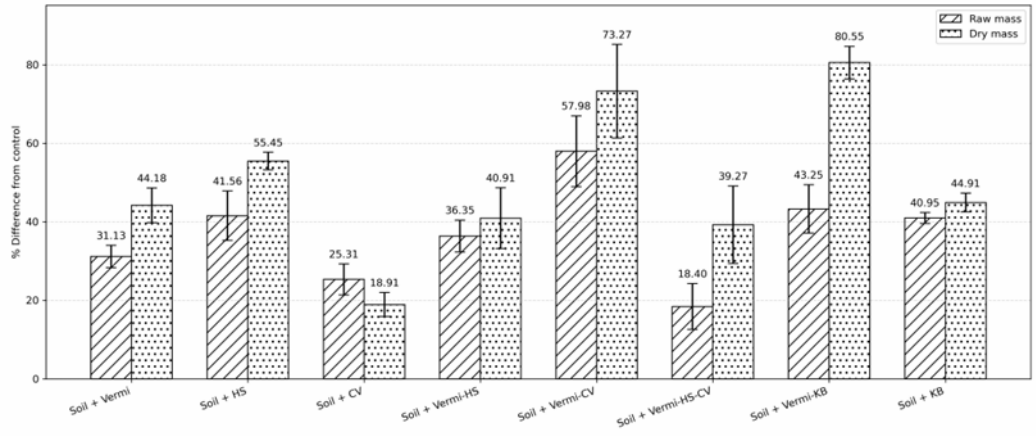

Figure 6 – Effect of substrate composition on the accumulation of total raw and dry biomass of common bean (*Phaseolus vulgaris*) compared to the control

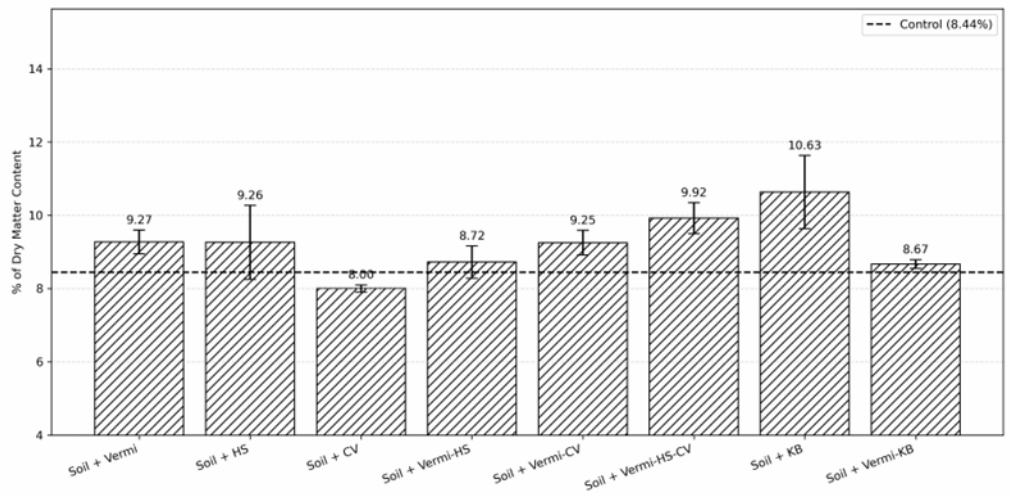

Figure 7 – Dry matter content in common bean plants depending on the composition of the substrate

Table 2 – Effect of different substrates on morphometric growth parameters of common bean plants

Substra-tes	Total raw weight, g	Raw weight of roots, g	Raw weight of stems, g	Raw weight of leaves, g	Number of leaves, pcs.	Stem width, mm	Plant height from root collar to stem, cm	Total dry weight, g	Dry weight of roots, g	Dry weight of stems, g	Dry weight of leaves, g	Total dry matter content, %
1. Soil (control)	2.17	0.40	0.98	0.79	5	1.97	24.33	0.18	0.06	0.07	0.06	8.44
2. Soil + Vermi	2.85	0.49	1.27	1.10	5	2.07	30.00	0.26	0.06	0.11	0.10	9.27
3. Soil + HS	3.08	0.49	1.39	1.19	6	1.97	26.67	0.29	0.06	0.12	0.11	9.26
4. Soil + CV	2.72	0.37	1.27	1.08	4	1.87	26.67	0.22	0.04	0.10	0.08	8.00
5. Soil + Vermi-HS	2.96	0.41	1.36	1.19	5	2.20	25.67	0.26	0.05	0.11	0.11	8.72
6. Soil + Vermi-CV	3.43	0.52	1.57	1.34	6	2.27	24.00	0.32	0.06	0.14	0.12	9.25
7. Soil + Vermi-HS-CV	2.57	0.30	1.21	1.06	5	1.73	25.33	0.26	0.04	0.11	0.10	9.92
8. Soil + Vermi-КВ	3.11	0.43	1.52	1.16	6	2.53	30.00	0.33	0.05	0.15	0.13	10.63
9. Soil+ КВ	3.06	0.48	1.47	1.12	4	2.37	29.00	0.27	0.04	0.12	0.10	8.67

An interesting finding is the reduced effectiveness observed with the combined immobilization of humic substances and *Chlorella* on vermiculite (Vermi-HS-CV). In most parameters, this treatment performed worse not only than Vermi-CV and Vermi-KB (figure 3–5) but also than pure expanded vermiculite. This may be explained by possible sorption or chemical interactions between the components (HS and CV cells), which reduce their biological availability and activity.

Thus, the obtained results clearly indicate that not a simple addition, but namely immobilization of biologically active substances on the matrix of expanded vermiculite is the key factor of efficiency. The best results were shown by the fertilizer-ameliorants Vermi-CV and Vermi-KB (figure 5-7), which makes them the most promising for further research and practical application in the conditions of irrigated agriculture on saline and degraded soils of Southern Kazakhstan.

CONCLUSION

Based on the research conducted, the following conclusions can be drawn:

New compositions of organomineral fertilizer-ameliorants have been developed based on expanded vermiculite immobilized with humic substances, a suspension of Chlorella vulgaris and a hydrolyzed protein of animal origin.

It has been established that all developed fertilizer-ameliorants have an effect on the growth indicators of common beans, however, the degree of influence varies significantly depending on the composition.

The greatest stimulating effect on growth processes and accumulation of biomass of common beans is provided by ameliorants based on vermiculite in combination with Chlorella vulgaris (Vermi-CV) and protein hydrolysate (Vermi-KB).

The Vermi-KB variant demonstrated maximum efficiency, providing an increase in the total dry biomass of plants by 80.55% compared to the control, as well as the highest absolute values of the dry mass of vegetative organs.

The results confirm the prospects of using vermiculite as an effective carrier for biologically active substances and emphasize the relevance of further research on the developed organomineral fertilizer-ameliorants under field conditions for the rehabilitation of degraded and saline agricultural lands.

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ТҮЙІН

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ТАБИҒИ АЛЮМОСИЛИКАТТАР МЕН БИОЛОГИЯЛЫҚ БЕЛСЕНДІ ЗАТТАРДЫҢ НЕГІЗІНДЕ ЖАСАЛҒАН ОРГАНОМИНЕРАЛДЫҚ ТЫҢАЙТҚЫШ-МЕЛИОРАНТТАРДЫҢ PHASEOLUS VULGARIS ӨСІМДІГІНІҢ ӨСУІНЕ ӘСЕРІ. 2 БӨЛІМ.

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Жұмыста кеңейтілген вермикулит және биологиялық белсенді заттар (гуминдік заттар, микробалдырлар *Chlorella vulgaris*, гидролизденген жануар ақуызы) негізіндегі жаңа органоминералды тыңайтқыш-мелиоранттарының кәдімгі бұршақтың (*Phaseolus vulgaris*) өсуіне әсерін зерттеу нәтижелері берілген. Түркістан облысы Отырар ауданының бұзылған топырақтарында құмыра тәжірибелері жүргізілді. Тыңайтқыш-мелиоранттарын енгізу бақылаумен салыстырғанда өсімдіктердің жалпы биомассасының, тамыр массасының, жапырақ санының және биіктігінің өсуіне ықпал ететіні көрсетілді.

Өсімдіктердің құрғақ биомассасы 80,6%-ға өскен Vermi-КВ нұсқасын қолданғанда ең жоғары нәтиже алынды. Vermi-CV қолдану да айқын оң нәтиже көрсетті (шикі биомассаның 58,0%-ға артуы). Нәтижелер әзірленген тыңайтқыш-мелиоранттардың тиімділігін және олардың бұзылған топырақтардың құнарлылығын арттыруға мүмкіндіктерін растайды.

Түйінді сөздер: бұршақ, органоминералды тыңайтқыштар, мелиорант, кеңейтілген вермикулит, гуминді заттар, *Chlorella vulgaris*, ақуыз гидролизаты.

РЕЗЮМЕ

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ИЗУЧЕНИЕ ВЛИЯНИЯ ОРГАНОМИНЕРАЛЬНЫХ УДОБРЕНИЙ-МЕЛИОРАНТОВ НА ОСНОВЕ ПРИРОДНЫХ АЛЮМОСИЛИКАТОВ И БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ НА РОСТ РАСТЕНИЙ PHASEOLUS VULGARIS. ЧАСТЬ 2.

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В работе представлены результаты изучения влияния новых органоминеральных удобрений-мелиорантов на основе вспученного вермикулита и биологически активных веществ (гуминовые вещества, микроводоросль Chlorella vulgaris, гидролизат белка животного происхождения) на рост фасоли обыкновенной (Phaseolus vulgaris). Лабораторные опыты проведены на деградированных почвах Отырарского района Туркестанской области. Показано, что внесение органоминеральных удобрений-мелиорантов способствует увеличению общей биомассы, массы корней, количества листьев и высоты растений по сравнению с контролем. Наиболее высокий эффект получен при использовании варианта Vermi-КБ, где сухая биомасса растений увеличилась на 80,6 %. Использование Vermi-CV также показало выраженный положительный результат (увеличение сырой биомассы на 58,0 %). Результаты подтверждают эффективность разработанных органоминеральных удобрений-мелиорантов и их потенциал для повышения плодородия деградированных почв.

Ключевые слова: фасоль обыкновенная, органоминеральные удобрения, мелиорант, вспученный вермикулит, гуминовые вещества, Chlorella vulgaris, гидролизат белка.

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