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Biostimulants as a Tool for Restoration of Disturbed Steppe Ecosystems

Gani Kalymbetov[1], Bakhytzhan Kedelbayev[1*], Abai Sartayev[2], Amir Seitkarimov[2], Bayan Sapargaliyeva[3], Askar Osserbay[1], Zhanara Baymagambetova[1]
Yermek Anarbayev[1], Azhar Abubakirova[4]

[1] M. Auezov State University, Tauke Khan Ave 5, Shymkent, 160012, Kazakhstan
[2] South-West Research Institute of Animal Husbandry and Crop Husbandry, Shymkent, 160012, Kazakhstan
[3] Abai Kazakh National Pedagogical University, 13, Dostyk avenue, Almaty, 050010, Kazakhstan
[4] South Kazakhstan Pedagogical University named after Ozbekali Zhanibekov, Shymkent, 160012, Kazakhstan
* Corresponding author's e-mail: kedelbaev@yandex.ru

ABSTRACT

Modern problems of pasture degradation caused by anthropogenic impact and climate change require the search for new, environmentally safe solutions. The proposed technology of creating cultivated annual pastures with the use of biostimulants is a promising approach to restore and increase the productivity of pasture ecosystems. The study conducted in the south of Kazakhstan demonstrates high efficiency of biostimulants, especially those that fermented cattle manure. In contrast with traditional mineral fertilizers ($N_{20}P_{20}K_{20}$), biostimulants MERS and Bio-Bars contribute not only to the increase in yield of green mass, but also improve soil characteristics by stimulating the growth of beneficial microflora. This makes it possible to create self-regenerating pasture systems resistant to negative environmental impact. The novelty of the study lies in the integrated approach combining the use of biostimulants and fermentation of organic waste. This approach not only increases the efficiency of resource use, but also promotes a closed cycle of production, minimizing the negative impact on the environment.

Keywords: sugar sorghum, biostimulant, manure, green mass, annual cultural pasture.

INTRODUCTION

According to the Sustainable Development Goals until 2030 indicator data portal, there is a need to move towards sustainable food production systems by adopting agricultural practices that increase resilience, productivity and production. These practices will respect ecosystems, helping them to adapt to climate change, natural disasters and soil degradation while improving soil quality [Food and Agriculture Organization of the United Nations 2024].

To provide livestock with feed throughout the production cycle, it is necessary to create a high-yielding forage base. New technologies for pasture restoration in the steppe and forest-steppe zones of Kazakhstan were developed as part of the research. Tests conducted in 2019–2022 showed that multifactorial pastures from alfalfa, awnless brome, turfgrass, sainfoin, sainfoin and other grasses provide abundant yields of green mass (more than 3.33 t/ha) and hay (more than 4.75 t/ha). The use of drought-resistant legumes, cereals and arable crops, as well as their mixtures, not only strengthens the forage base, but also reduces pasture degradation. The inclusion of legumes in pasture vegetation improves its species diversity and possibly increases the protein content of fodder [Shayakhmetova et. al., 2023].

A 20-year crop rotation study showed that sorghum/corn and no-till systems maintain wheat yields. These systems maintain soil chemical and physical properties as well as nitrogen fertilizer use efficiency. Zero tillage achieves maximum wheat yield (7.2 t/ha) with 45% less nitrogen (104 kg/ha) compared to conventional tillage (190 kg/ha) (Ernst et al., 2020, Alvarez and Ernst 2024).

In Pakistan, livestock production and traditional grazing systems are not sustainable due to pasture degradation and declining forage quality. In this study [Khan, Rahman, and Cone, 2020], 10 grass species grown in pastures were evaluated for their nutritional value and methane emission potential. *Jumbo grass (Sorghum bicolour× Sorghum sudanefe)* showed the highest protein content, dry matter digestibility and total gas production with the lowest proportion of methane. The results show that different grass species have different nutritive value and methane emission potential.

Low and irregular rainfall, depleted soil and moisture deficit restrain sorghum cultivation in semi-arid regions. Grain yields of sorghum on sandy and sandy loam soils range from 0.2 to 0.4 t/ha. A study [Kugedera et al., 2024] investigated rainwater harvesting and cattle manure application methods to improve sorghum grain yield under semi-arid conditions. The experiment followed a randomized block design with three rainwater harvesting methods (main factor) and five levels of cattle manure application (sub-factor) and two sorghum varieties (Macia and SV1) (sub-sub-factor). The results showed that the use of linked contours significantly ($p < 0.05$) increased grain yield of sorghum of both varieties. Increase in cattle manure application rates also resulted in significant ($p < 0.05$) increase in grain yield of sorghum compared to control (0 t/ha).

In India, it was investigated [Sharma et. al., 2024] how different methods of fertilizer application affect soil quality. 7 methods of fertilizer application were tested: manure, glyricidia (plant), recommended fertilizer dose, recommended fertilizer dose + manure, recommended fertilizer dose + glyricidia, control with rotation, absolute control without rotation. All fertilizer application methods improved soil quality compared to the control. The most effective methods were those using recommended fertilizer dose or glyricidia. In a study [Meyer et al., 2024], applying poultry manure as a fertilizer below the soil surface (subsurface) in southern U.S. pastures increased forage yield and nutritive value by reducing nutrient losses. The 2021–2022 study compared subsurface application of litter to surface spreading. Three forages were studied: sorghum-sudangrass, cocoa, and a mixture of the two. Subsurface application of litter increased protein content by 8–10% in all forages. This method preserves nutrients and increases profitability.

An experiment [Guretzky et al., 2023] conducted from 2017 to 2019 showed that seeding sorghum-sudangrass to smooth bromegrass pasture would help significantly increase forage biomass. Sorghum-sudangrass under-seeding increased forage biomass by 36% compared to pastures without under-seeding. Pastures with alfalfa, red clover, and knotweed produced 7% more forage biomass than pastures fertilized with nitrogen. Fertilizing in early spring or maintaining legumes in pastures with smooth bromegrass may optimize forage biomass. Two types of pastures were studied: nitrogen fertilized smooth bromegrass and mixed legume-grass pastures. Sorghum-sudangrass was sown in two of the six subplots of each main plot. Seeding sorghum-sudangrass to smooth bromegrass pastures can be an effective way to increase forage biomass, especially in late summer. Taking into account the obtained literature and research data, we can definitely say that there is a great need for additional pasture care and effective utilization, using natural and environmentally safe means and measures. In this regard, the author of this study was set the task to use organics to increase soil fertility, activate natural mechanisms of soil regeneration, improve the fodder value of annual pastures, increase the yield of green mass for pasture users.

MATERIALS

Kazakhstan-16 sorghum variety, which is adaptive for the sharply continental climate of Kazakhstan. Biostimulants based on bacterial and plant raw materials under the commercial brand MERS and Bio-Bars. Enriched cattle manure based on vermicompost with the addition of biostimulants (biofertilizer). The soil is light gray loamy soil. Light Calcisol soils are characterized by low natural fertility.

METHODS

Data collection

Data were collected from published peer-reviewed research articles between 2020 and 2024 from Web of Science (http://apps.webofknowledge.com) and Scopus (https://www.scopus.com) databases using keywords and terms such as "sugarcane sorghum", "biohumus", "biostimulant", "green mass" and "annual cultivated pasture". Articles must meet the following criteria:

1. The research must be conducted on irrigated and non-irrigated perennial semi-arid or desert lands. Including studies with pots or "pseudo soils" were also included in our database.
2. Information on the soil concentration of the study field prior to the growing season should be available. Micronutrient levels, salinity, soil microbiota.
3. Those studied only for a short period were excluded from the database.
4. Sustainability management methods should be clearly explained in the study.

Experiment design

Experiments are conducted from 2022 to 2024 in open experimental plots of the research laboratory "Industrial Biotechnology". Mechanism of experimental plot with 4 repetitions per year (5 plots for 4 years) with the use of biostimulants of growth and gradual transition to organics.

Scheme of the experimental plot

Acting factors: chemical fertilizers (control/background), biostimulant 1 - BS1, biostimulant 2 - BS2); biostimulant 1 - BS1 + organics, biostimulant 2 - BS2 + organics. Four replications each. 3 plots for each variant of the factor in each repetition (60 plots in total). Plot size: 1 m^2

Soil management

Soil was loosened to a depth of 8–10 cm. After loosening, the soil was leveled with a disk roller to allow the soil to retain moisture. As a base fertilizer, $N_{20}P_{20}K_{20}$ (background or control) was applied to all experimental fields at a rate of 200 kg/ha, after soil analysis. Experimental plots were additionally treated with biostimulants MERS (biostimulant 1 hereinafter - BS1) and Bio-Bars (biostimulant 2 hereinafter - BS2). Drip irrigation was used for watering.

Biofertilizer preparation and biostimulant dosage

The optimal dosage for both biostimulants is 0.05% [Kalymbetov et al., 2023].

The manure is washed in advance with running water from urea. Then, $N_{20}P_{20}K_{20}$ is scattered in layers (5 layers) every 15 cm. Biostimulants are diluted with distilled water 1 part of biostimulant to 30 parts of water. The resulting aqueous solution is sprayed on cattle manure. It is left to ferment for 10 days. This method improves the characteristics of biofertilizer. Microbiota activity increases and manure fermentation is enhanced [Baymagambetova et al., 2023].

Meteorological data collection

Meteorological data obtained from the Republican Center for Hydrometeorology [Agrometeorological forecasts – Kazhydromet, 2024]. The experiment was implemented in a region with arid climate. The analysis of weather indicators for 2022–2024 years (periods of research) demonstrated that the experimental site had appropriate conditions for perennial pastures: temperature, humidity and precipitation corresponded to the norms (Table 1).

According to the SPI data, on average for 2022-2024, the climate was moderate for its region. But at times there were abnormal abrupt changes in climate. Abnormal changes are shown in Table 2.

Data processing and analysis

Differences between background (control) and each treatment (BS1 and BS2), as well as between individual treatments, and green mass yield were evaluated using one-factor analysis of variance (ANOVA, Tukey's criterion, $p \leq 0.05$). Variation within treatments was determined by calculating standard deviation (±SD) values. Correlation coefficient values were calculated using Spearman's nonparametric criterion for chemical and biological properties. All statistical analyses were performed using Statistica PL 13 and MiniTab 21.1.1 software.

Table 1. Classification of moisture conditions by values standardized precipitation index (SPI)

2.00 and above	extremely humid
1.50…1.99	very humid
1.00…1.49	optimally humid
0.99…-0.99	near normal
-1.00…-1.49	precipitation deficit
-1.50…-1.99	drought
-2.00 and less	extreme drought

Table 2. Abnormal changes in air temperature of Turkestan region [Agrometeorological forecasts – Kazhydromet, 2024]

Period, years	October			November			December			January			February			March			April	
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II
2021–2022	+5.1	-0.1	-1.3	-5.0	-3.1	1.8	5.9	-4.6	2.9	3.7	6.3	2.1	1.8	2.8	6.2	4.0	0.7	-3.1	6.6	5.3
2022–2023	-0.9	2.0	0.7	-0.6	-1.1	3.3	-7.2	-1.3	-0.9	3.4	-11.0	-4.7	2.2	2.0	4.3	6.7	4.5	4.0	2.3	-0.5
2023–2024	2.1	0.5	3.7	2.0	4.8	7.0	3.2	-3.7	5.3	7.0	1.6	1.0	3.2	2.3	-3.8	-0.3	-3.7	0.3	-1.8	0.3

Note: – above normal air temperature anomaly, – air temperature anomaly near the norm, air temperature anomaly below normal

RESULTS AND DISCUSSION

Seed germination in cultivated pasture

The effect of interaction of experimental year and treatment methods on the studied indicators was not statistically significant. However, according to the results of analysis of variance, in both years of the study there were significant differences between the control and variants of application of biostimulants of soil treatment in the creation of cultivated annual pastures. The novelty of these experiments is that in the south of Kazakhstan and in general on pasture lands of the country were not used biostimulants of plant growth to increase yield and green mass yield.

The direct effect of biostimulant growth MERS was evaluated by the density of crops, the results are shown in Table 3, that both with treatment BS1 and in BS2, all experimental methods had a higher density compared to the control $N_{20}P_{20}K_{20}$ (background). The number of plants per 1m^2 area ranged from 93 to 104±2.3 ($p < 0.05$) seedlings per square meter.

Seed germination study showed that the highest increase of 18% was achieved with the combined application of biostimulant and organic fertilizer (cattle manure) compared to the control (Table 2). In addition, the use of biostimulants had no effect on biochemical and physiological properties of sorghum. Our results are in agreement with the findings of other studies that also demonstrated the positive effects of biostimulants. For example, Kayisoglu et al. [2024] and Saithalavi et al. [2021] found that sorghum seed nutritive value increased during germination studies clearly describe the physicochemical changes occurring during germination. The effect of germination on macronutrient content was analyzed. The effect of germination on the content of anti-nutritional factors. Evaluated the effect of germination on the content of biologically active compounds. And other similar studies application of biostimulants improved stress tolerance of sorghum [Ennoury et al., 2023; Wazeer et al., 2024]. The use of biostimulants in combination with manure improved soil characteristics, increasing soil fertility and productivity without negatively affecting the soil environment and soil microbiota. On the contrary, joint application of biostimulant and manure increases ammonifixing, phosphatase and catalase activity of microorganisms in soil [Baimagambetova et al., 2024]. Effective use of the proposed technology, increases the quality of soil and thus improves the characteristic of pastures on

Table 3. Effects of growth biostimulant application on sorghum seed germination, density and tillering before harvest

Objects of research	Density of plants us stage of sorghum seed germination		Sorghum seed germination		Sorghum density before mowing	
	Number per 1 m^2	+/- comparison	%	+/- comparison	Number per 1 m^2	+/- comparison
Control	71±4.2b	–	71±2.2	–	70±1.3 c	–
BS1	95±3.5c	21	92±2.4a	7	96±5.5 b	31
BS2	93±4.7ab	23	94±3.1ab	11	93±4.7 c	27
BS1 + organics	101±6.1a	19	104±4.8a	18	103±4.1 a	41
BS2 + organics	104±2.3b	25	105±3.6a	16	104±7.8 c	43
p-value	0.0304	–	0.0092	–	0.014	–
Coefficient of variation (%)	8.14	–	5.43	–	9.74	–

Note: Organics – cattle manure. Mean values in columns followed by different letters are significantly different in terms of comparison of Tukey's adjusted mean at $p \leq 0.05$. Data are presented as mean values ± standard deviation (SD).

grass, which can be included in the program for sustainable development of agro-industrial complex.

Determination of soil microbiological activity

The research conducted by the authors revealed a close relationship between the size of soil aggregates of migratory-micellar post-agrogenic Calcisol and the intensity of soil-biological processes. It was found that in small aggregates (<1 mm) there is an increased activity of microorganisms, especially in the processes of mineralization of complex organic compounds. Analysis of the species composition of microbiota showed an increase in the number of microorganisms involved in the carbon cycle in the transition from large to small aggregates. At the same time, the activity of nitrogen cycle microorganisms reaches a maximum in medium-sized aggregates (1.0–5.0 mm), which can be defined as "biologically valuable".

A sample of humus horizon of humus horizon of Calcisol migratory-micellar quasi-gleyey clayey of undisturbed composition with the size of 25×25×35 cm was taken in the territory [Baimagambetova et al., 2024]. Drying of soil during microbiological sowing on elective media gives distorted, as a rule, underestimated results of microorganisms number. The number of bacteria decreases by 5–10 times and the qualitative composition of microorganisms changes [Baymagambetova et al., 2023]. Therefore, sieving into aggregates was performed from fresh samples of natural moisture content. Soil was sieved on sieves: 0.25 mm; 0.5 mm; 1 mm; 2 mm; 3 mm; 5 mm; 7 mm, pre-sterilized with 70% ethyl alcohol.

The following agrochemical parameters were determined in each size fraction in the research laboratory "Industrial Biotechnology": pH of aqueous and saline soil suspensions [Sokolov, 1975]; exchangeable calcium and magnesium content according to Shollenberg [Sokolov, 1975], Ca^{2+} and Mg^{2+} concentration on AAnalyst 200 atomic absorption spectrometer; exchangeable potassium content according to Maslova [Sokolov, 1975], potassium concentration on Flafo-4 flame photometer; mobile and total phosphorus compounds were determined by spectrophotometric method [Sun et al., 2024; Sokolov, 1975].

The number of microorganisms of different ecological and trophic groups was determined according to [Nidhin and Chattopadhyay, 2022], by the method of limiting dilutions of soil suspension and surface sowing on selective nutrient media - meat-peptone agar (MPA) for ammonifiers, starch-ammonia agar - SAA (for amylolytic bacteria and actinomycetes), oligotrophic microorganisms on Lockheed soil agar, oligonitrophilic microorganisms on Ashby medium [Wolny-Kołładka et al., 2022].

It was important to study the dynamics of phosphorus mobilizing (PM) microorganisms cultured on glucose-aspartate medium. The small amount of FM found in the initial soil (6.1 million CFU/g soil) increased both under the influence of biostimulants and cattle manure (Figure 1).

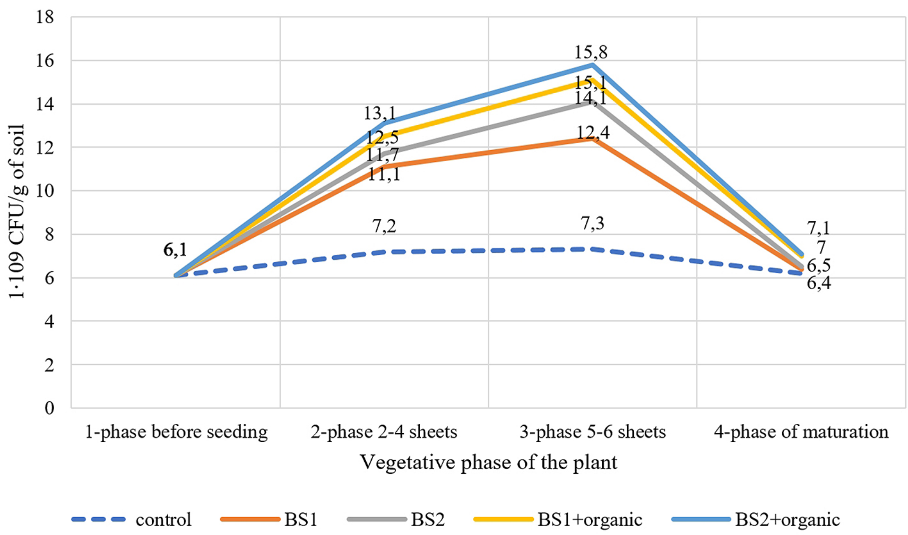

Figure 1. Dynamics of the number of soil phosphorus-mobilizing microorganisms under the influence of biostimulants and their solution with manure during sorghum vegetation. Numbers of microorganisms (million CFU in 1 g of soil), confidence interval $p < 0.05$. 1 - before sowing; 2 - phase of 2–4 leaves; 3 - phase of 5–6 leaves (panicle formation); 4 - maturation phase

Dynamics of phosphate-mobilizing microorganisms in variants BS1, BS2, BS1 + organic and BS2 + organic differed positively (15.8 million CFU/1 gram of soil). But it should be noted that during the vegetative phase a close reliable relationship of the number of phosphorus-mobilizing microorganisms from the presence in the soil of fermented manure in the composition of BS1 + organic and BS2 + organic was found, but after ripening the regularity was not established. One fact must be considered, fermented manure (Paez et al., 2022) with biostimulants shows prolonged action compared to single applications of biostimulants. In its phosphorus compound is uniformly released in mineral form and such process does not activate phosphorus-mobilizing microorganisms. When studying the effect of biofertilizers on the dynamics of oligotrophic microorganisms, it was noticed that the highest number of oligotrophs in all variants was within the low value (1.4–3.3 million CFU/1 gram of soil). This is the main indicator of soil improvement as oligotrophs inhabit only in micronutrient poor soils. Similar studies have been conducted worldwide [Ghosh et al., 2024; Di et al., 2018], in all the studies one thing is noticeable that sorghum plant itself gradually regenerates the soil, retains moisture, creates a favorable environment for *Rhizosphere bacteria*.

The study of mineral nitrogen and phosphorus compounds on the background with the control variant showed a moderate increase in the number of ammonificators (Figure 2) in the phase of 1–4 leaves and at the beginning of panicle formation (budding). The dynamics of ammonificator content in soil at BS1 application was similar to

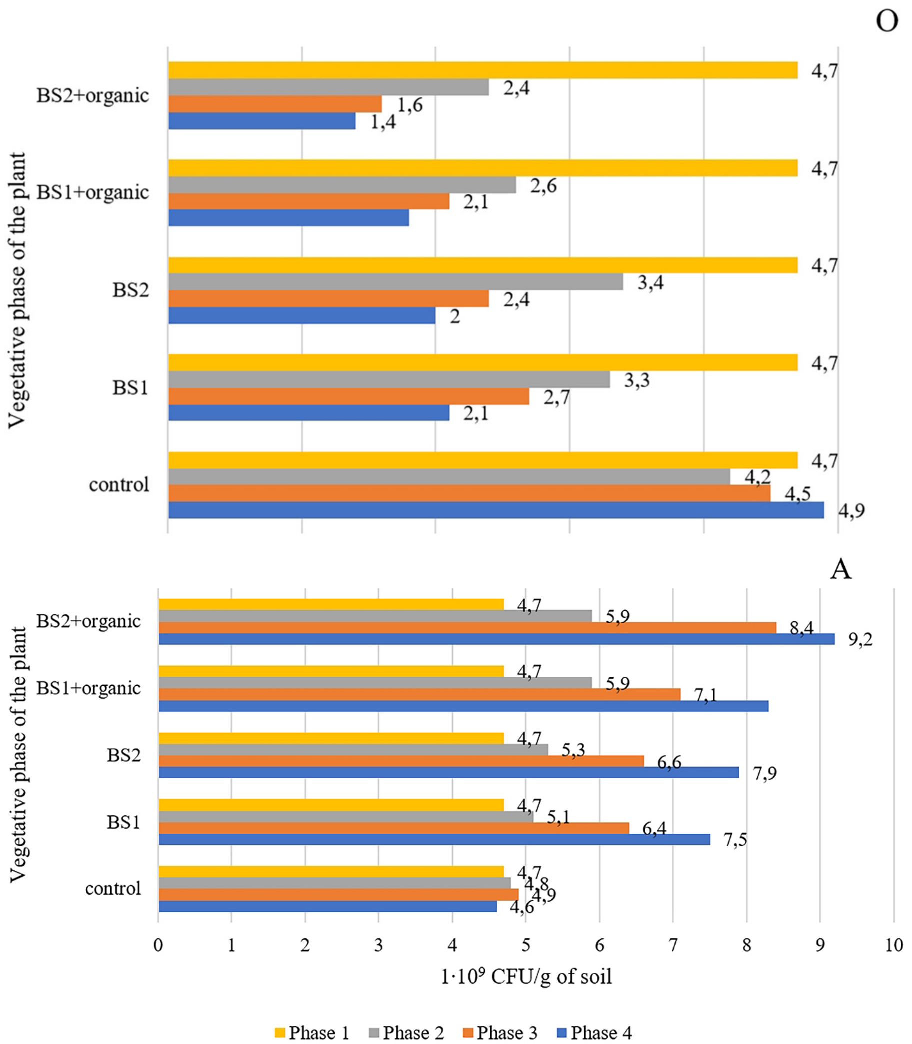

Figure 2. Dynamics of ammonifying (A) and oligotrophic (O) microorganisms number under the influence of biostimulants and their solution with manure during sorghum vegetation. Numbers of microorganisms (million CFU in 1 g of soil), confidence interval $p < 0.05$

BS1+organic, while the trend of abundance in the variant with BS2+organic showed a direct dynamics of ammonificator growth effect (7.9–9.2 million CFU). This phenomenon can be explained by the special properties of BS2 during manure fermentation, which determines the amount and "quality" of nitrogen, as well as the degree of its assimilation. In fermented manure, nitrogen is in nitrate and ammonium forms. Microorganisms process this form of nitrogen at different rates. Such a phenomenon has a prolonged action, thereby providing the soil and plant than during the growing season (Figure 3).

The number of oligonitrophilic microorganisms in soil before biofertilizer application (BS1, BS2, BS1 + organic, BS2 + organic) was 1.5 million CFU in 1 gram. After biofertilizer treatment, their number increased sharply to 25.3 million CFU in 1 gram. This is explained by the fact that before biofertilizer application there was a sharp deficit of mineral form of nitrogen in the soil. Another factor to consider is that during rapid plant growth and in the phase of butanization the soil is deficient in nitrogen due to maximum consumption by plants, in addition, there are denitrifying microorganisms, which in turn process mineral nitrogen into molecular form [Qiu et al., 2024]. A decrease in the number of oligonitrophils or an increase in denitrifiers is an indicator of decreasing soil fertility.

Phenological development of sorghum under the influence of growth biostimulants

The first sprouts in sorghum treated with growth biostimulant appeared in 4–6 days [Kalymbetov et al., 2023]. For the Kazakhstan-16 variety, the most effective option for harvesting is after the 6th leaf, before flowering. At this age,

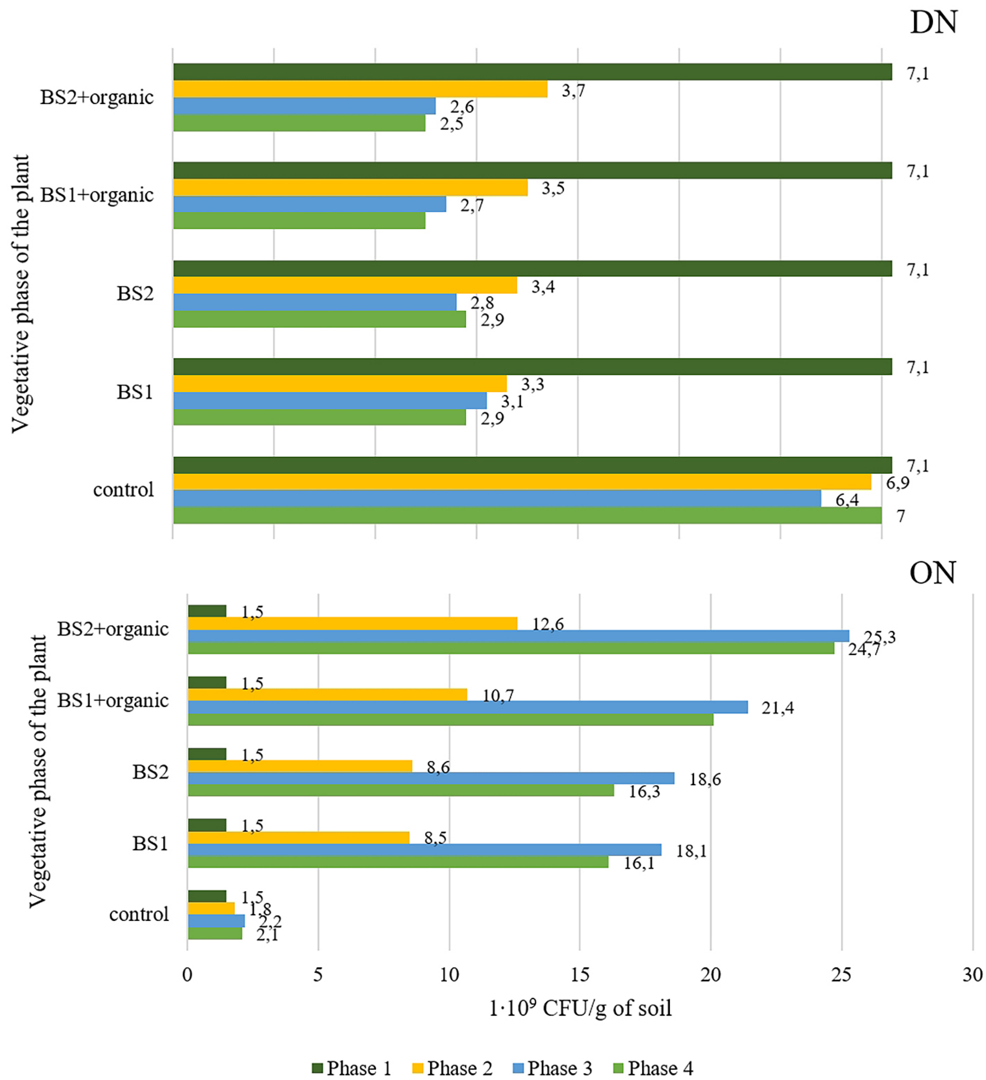

Figure 3. Dynamics of oligonitrophil (ON) and denitrifier (DN) abundance before and after biofertilizer application. Numbers of microorganisms (million CFU in 1 g of soil), confidence interval $p < 0.05$

the stalks are still juicy and tender. Livestock consumes it without residue. The timing and methods of harvesting have a great influence on the quality and nutritional value of hay. Early harvesting makes it possible to get more nutritious forage. The best hay will be when it will be harvested on the 45–50th day after sprouting or on the 10–12th day before flush. In the south of Kazakhstan, the growing season is 180 days. In the experimental plots, mowing was done manually. At the same time, plants were conditioned and left in the swaths. Due to the fact that sorghum has a large percentage of sugar in the stems, the cut plant is difficult to dry out. Therefore, on the second or third day after mowing, the hay should be turned, and after drying, the hay is skirled. This method of drying makes it possible to extract hydrocyanic acid from the sorghum stalk. The plants should not be over-dried as the leaves are lost. The first cut was received on June 5 with 1 m^2 about 2–3 kg of green mass, the second cut 35 days after the first cut the amount of green mass 3–3.5 kg. This is due to good tillering and nutrition of plants with BS + organic. The third mowing was made 30 days after the second mowing. The last calves were left on the pasture for cattle in mowed form. Mowing is necessary for hydrocyanic acid to volatilize. And the mown sorghum remained fresh for a week and did not spoil [Kalymbetov et al., 2023]. The difference between background and BS1 + organic, BS2+organic is significantly high and reliable. The results of phenological development are shown in Figure 4.

In this dynamics can be clearly seen the application of biostimulants gives impetus to the plant. Such dynamics was shown by BS1 + organic and BS2 + organic. In this regard, it can be reliably stated that the use of pure biostimulant increases the physical strength of the plant and improves phenological characteristics. But at the same time it is observed the effectiveness of use in combination with organic matter (cattle manure). The condition in the control plot (background) was stable. Germination as per the norm 7±1 days, the 1st mowing after sprouting 50±2.2 days. Then the subsequent 2 cropping with a gap of 15 days from each other. On the 4th harvest in the control variant did not have time, as the first frosts began, sorghum could not grow up to 5–6 leaves for cutting. But the dead stalks of sorghum after frosting are eaten by livestock before and under snow.

Creation of cultivated annual pastures with the use of biostimulants helps to restore perennial pastures in summer periods, providing fresh green mass for livestock. It is trampling, silent grazing, tearing of young branches of perennial grasses that prevents them from blooming and reproduction. At such times, if there is a pre-prepared pasture in such a form, as suggested by the authors, the load on perennial pastures will be reduced. The task set by the authors has been fulfilled. The proposed technology of growth biostimulator application will help to stop pasture degradation.

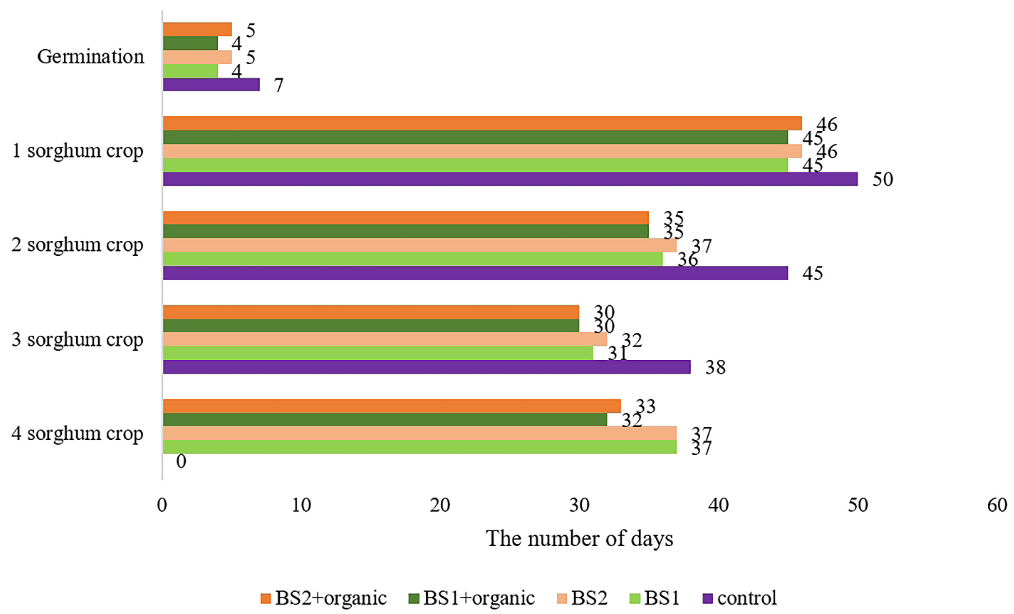

Figure 4. Dynamics of vegetation period and readiness for sorghum cutting

CONCLUSIONS

The use of chemical fertilizers ($N_{20}P_{20}K_{20}$) as in this study gives standard, and sometimes lower results. This is due to the fact that recently there are often climatic anomalies. In this regard, the authors recommend the use of biological stimulants. In this study, MERS and BioBars, based on organic matter, were experimented with. Other growth biostimulants can be used. Which do not harm the environment. In this experimental study, the authors simultaneously used a method to improve the beneficial characteristics of biostimulants using fermentation of cattle manure. Since manure contains natural microorganisms that can fertilize and prepare humus, thereby improving the quality characteristics of biostimulants and preventing soil degradation by enriching them with mineral elements. Microorganisms are becoming key players in the formation of sustainable agroecosystems of the future. Their active use in closed-loop systems and controlled agriculture allows you to optimize the cycle of nutrients and increase productivity. Research into microbiomes in such systems provides the basis for developing new technologies aimed at creating sustainable and resource-efficient methods of food production.

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