JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2023, 24(3), 134–142
https://doi.org/10.12911/22998993/157568
ISSN 2299–8993, License CC-BY 4.0

Received: 2022.11.26
Accepted: 2023.01.05
Published: 2023.01.15

Effects of Different Biostimulants on Seed Germination of Sorghum Plants

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ABSTRACT
By creating highly productive phytobiomes, selection of new types of biostimulants on the basis of organic substances and microorganism has a decisive role. It could be done by taking into account natural and climatic peculiarities of the region. The article described the importance of sugar sorghum and substantiates the introduction of an adaptive variety to increase the productivity of fodder sorghum and the best option of using growth biostimulants. The results of evaluating the effectiveness of growth biostimulants under laboratory conditions on the main nutritional valuable traits were presented. The treatment of optimal parameters of sugar sorghum seeds with biostimulants in the Research laboratory "Industrial biotechnology" of M. Auezov South Kazakhstan University was determined. It was shown that the "Azotofertil" biostimulator has a high efficiency in pre-sowing seed treatment. For comparative evaluation of potentialities of new biostimulant, MERS biostimulant adapted to climatic conditions was chosen. According to research results, both biostimulants showed high efficiency for seed pre-sowing treatment. The best concentration for treatment of planting material was established. Energy of germination, swelling and the number of germination of seeds of sugar sorghum variety Kazakhstan-16 were determined. In evaluating the activity of biostimulants for efficiency, the dynamics of their friendly germination was traced. At 4% concentration and temperature above 14 °C, the advantage of "Azotofertil" biostimulator based on *Azotobacter chroococcum* strain was proven. Seeds of sugar sorghum variety Kazakhstan-16 showed the best results with 96 ± 3% germination.

Keywords: sorghum, microbiome, nitrogen bacteria, biostimulator, incrustation, germination energy.

INTRODUCTION

The United Nations Food and Agriculture Organization's 2030 "Sustainable Development Goal" program is to ensure the introduction of sustainable technologies for the production of vital products and the implementation of adaptive farming systems such as those that increase productivity and production, which aims to preserve the ecological system, increase the ability to adapt to climate change, extreme weather changes, drought and other disasters, as well as progressively improve soil properties [Methodological note United Nations. 2020].

The non-traditional perennial wild sorghum (Sorghum almum) began research as a forage and for the diversity of forage crops for domestic and farm animals. According to the authors' publications [Marin et al. 2016], such a plant is highly adaptable to drought, pests and various diseases, relatively resistant to soil quality, has a high regenerative capacity and growth after mowing. It is adapted to the traditional technologies of growing crops. Research calculations are presented for several years and can be used on the same plot for a long time (5-6 years). The green mass of the plant, collected in different phases of vegetation, can be used as animal feed both in fresh form and in canned form by making hay. In its unripe form it can be harvested 2–3 times, in years of high humidity it grows constantly and can be harvested every 30–35 days. In this work traditional methods of cultivation of perennial wild sorghum

(*Sorghum almum*) were applied, although there are methods of cultivation with the help of different kinds of natural growth stimulants.

Nutrient content of the sorghum (*Sorgum almum*) plant: total moisture – 73.77%, dry matter – 26.23%, crude protein – 10.57%, gross fat – 2.26%, crude flesh – 38.88%. The nutritive value in nutrient units averaged 0.20 U/kg; in metabolizable energy, 8.53 MJ/kg dry matter. The average carotene content was 53.67 mg/kg; minerals, calcium 0.42%; and phosphorus 0.26%. These indicators are within the average normative values of fodder plants used in animal feed.

The main direction in the study of sorghum remains the creation of a phytobiome of sorghum with effective microorganisms' green mass, having an association of significant biocapacity and high yield of green mass. This type of sorghum must contain digestible nutrients, it is necessary to create technologies of their cultivation, fodder production and haylage from sorghum.

The search for the microorganisms that improve soil fertility and plant nutrition continues to attract attention due to the rising cost of chemical fertilizers and some of their negative effects on the environment. The purpose of this laboratory study was to determine if the effectiveness of microbial plant growth stimulants on seed germination activation energy and nutrient uptake levels. The microbial biostimulants used in the study were Azotobacter chroococcum strains. The selection and application of natural plant allies, in particular, symbiosis or microbiome is extremely relevant. A microbiome is a community of microorganisms that inhabit a particular habitat.

As research has shown, manipulating the plant microbiome can lead to reduced plant disease [Bloemberg & Lugtenberg, 2001; Kashyap et al. 2017], increased agricultural production [Bakker et al. 2012; Gouda et al. 2018], and reduced chemical inputs [Aliyu et al. 2019] leading to more sustainable agricultural practices. This goal is seen as vital to sustain the world's growing population. This requires the study of crops in their habitat, i.e., the rhizosphere. The rhizosphere is the area of the soil that is affected by plant roots through rhizoseparation of exudates, mucus, and detached cells. Root exudates contain various compounds, mainly organic acids and sugars, but also amino acids, fatty acids, vitamins, growth factors, hormones, and antimicrobial compounds [Sun et al. 2021]. Root exudates are key determinants of rhizosphere microbiome structure [Ding et al. 2021; Li et al. 2021; Rugova et al. 2017]. The composition of root exudates can vary by plant species and cultivar [Zhang et al. 2015; Mwita et al. 2016], as well as by plant age and developmental stage [Chen et al. 2021; Shi et al. 2021; Chaparro et al. 2013]. In addition, the microbiome affects root exudates, since the plants grown under sterile conditions have a markedly different exudate composition from those affected by microbes.

Of particular interest in the rhizosphere are rhizobacteria that promote plant growth, whose mechanism of action varies [Gislason & de Kievit, 2020]. Nitrogen-fixing bacteria, including free-living (such as Azotobacter spp.) and symbiotic (such as the root nodule Rhizobium rhizobium spp.), provide the plant with a source of fixed nitrogen, and many bacteria of the group will be able to solubilize phosphorus-containing minerals, increasing its bioavailability. Manipulation by microbes of plant hormones, particularly auxins, gibberellins and ethylene, can also lead to growth stimulation or stress tolerance. Many rhizobacteria that promote plant growth act antagonistically to plant pathogens by producing antimicrobial agents or by interfering with virulence factors through effectors supplied by secretion systems [Zboralski et al. 2022]. In particular, actinomycetes are known to produce a wide range of compounds with antibacterial, antifungal, antiviral, nematicidal and insecticidal properties. They are often found as one of the most common classes of bacteria in the soil and rhizosphere and are markedly enriched in endophytic communities.

Despite their complexity and dynamism, especially in vivo, it is important not to lose sight of the plant microbiome when interpreting experimental data, especially in the field [Bhattacharyya et al. 2012]. Genetic modification of plants, for example, may have unintended consequences for the rest of the microbiome, which may or may not be physiologically relevant. The role of the microbiome and its relationship to plant health, productivity, and biogeochemical cycles should be viewed in the same way as the plant itself [Vejan et al. 2016]. An extension of this view is to use molecular breeding or genetic modification of plants to deliberately modulate the microbiome as well as attract disease antagonists and plant growth stimulants to improve agricultural production.

In this regard, it is necessary to find the optimal approach for each crop to obtain the expected yield of the target product, particularly in this study the focus was on the germination rate of sorghum seeds and the yield of green mass for livestock feed. The proposed technology is adapted to the sugar sorghum variety Kazakhstan-16 [Register of the State Commission for Variety Testing].

MATERIALS

Sugar sorghum variety – Kazakhstan-16

The object of research is a variety of sugar sorghum, called "Kazakhstan-16" [Register of the State Commission for Variety Testing], which is intended for silage. It was included in the State Register in 1998. The organizer is the LLP "Kazakh Research Institute of Agriculture and Crop Production". This variety was created as a result of crossing sugar sorghum, general characteristics sorghum is a medium-maturing and high-yielding variety. Yields of green matter correspond to 800–870 cwt/ha, which is resistant to high and low temperature changes. This variety is intended for cultivation in Zhambyl, Pavlodar and South Kazakhstan regions.

Selection of plant growth biostimulants

Biostimulator has a bacterial concentration of 1.0×10^7 CFU/ml^{-1} Azotobacter sp. sub-species Azotobacter sp. presented on the biostimulator "Azotofertil" rDNA with a value of approximately 99.93%. This result was confirmed in the research laboratory "Industrial Biotechnology" and repeated study in the scientific and production association "Ana-Ger" LLP.

METHODOLOGY

Clearing of seed infestation with fusarium, helminthosporiosis, alternaria infection

To remove pathogenic substances from the surface of seeds, a method of ozonation on an OZON-10000 device (made in Russia) was used. When using it, these oxidized sub+stances pass into the gaseous phase or precipitate and pose no danger in the future. Presowing treatment with ozone is the safest and environmentally friendly method, because no harmful substances and impurities remain after treatment.

Technology of seed preparation with the use of growth biostimulants

The main purpose of seed pretreatment is to create maximum coverage of grain surface with growth stimulants. In the research laboratory (RL) "Industrial Biotechnology", the degree of retention of the drug on the surface of the seeds was determined. To apply a thin film of biostimulant substance on the surface of seeds dissolved in water, the method of incrustation was used.

Effect of the studied biostimulants on soaking, swelling and germination of sorghum seeds

To intensify the process of seed awakening to vitality the main condition is penetration of vegetative moisture, which transforms nutrients into dissolved chemical state and directs them to the germ. Soaking is a given action of seed preparation for further germination in order to activate all viability processes and related physiological, biological, water-coordination processes. To regulate the process of absorption by the plant of incoming water, which precedes germination, the method of swelling was used.

Methods for determining the germination and germination energy of seeds.

According to GOST 12038 – "Methods for determining germination" for the result of the analysis take the arithmetic mean of the results of determining the germination of all the analyzed samples. For reliability, four repetitions of the test were carried out. In determining the germination of the seeds on four samples, the deviation of the test results of individual samples did not exceed the arithmetic mean.

Statistical analyses

Experiments were performed in 4 replications, under laboratory conditions, according to the requirements of the respective methods. The results were processed statistically and presented as mean values and their standard deviations ($x\pm\delta$). Significance of differences was assessed using ANOVA analysis of variance, with a significance level of $p \leq 0.05$.

RESULTS

Sampling was carried out according to GOST 12036 (GOST – State Standard, Russian National Standard). When determining the seeding characteristics of seeds treated with biostimulants filter paper was used as a bed. From sorghum seeds of purity according to GOST 12037, four samples of 100 seeds in each sample were taken. To determine germination, one sample was separated from them and separated into selected seeds and waste. From the selected seeds, samples were prepared for germination. There were 84 samples in total.

Determination of seed germination

Germination testing under laboratory conditions was carried out as follows. From the test sample, 100 pieces of seeds were selected and evenly distributed on filter paper so that they were in equal contact with the liquid. Then the paper was moistened and left for a certain time for moisture absorption and germination of seeds. During the germination process, constant ventilation was provided in the thermostats. The lids of Petri dishes were opened every day for a few seconds. After 4 days, the germinated seeds were evaluated and counted to determine the germination energy and germinability. The setting the seeds for germination and the germination energy or germination rate were counted in one day. When counting germination energy, only normally germinated and obviously rotten seeds were counted and removed, and when counting germination energy, normally germinated, swollen, hard, rotten and abnormally germinated seeds were counted separately. The arithmetic mean value of sorghum seed germination was determined in accordance with (Table 1) GOST 12038 – Methods for determining germination.

Table 1. Arithmetic mean value of sorghum seed germination

Arithmetic mean value of germination, %	Permissible deviation of the analysis results of individual samples from the average for the analysis of 4-100 seeds, %
99 or 1	-2
from 97 to 98 from 2 to 3	±3
from 95 to 96 from 4 to 5	±4
from 92 to 94 from 6 to 8	±5
from 88 to 91 from 9 to 12	±6
from 83 to 87 from 13 to 17	±7
from 75 to 82 from 18 to 25	±8
from 62 to 74 from 26 to 38	±9
from 39 to 61	±10

Technology of seed preparation using growth biostimulants

Seed pre-treatment with solutions of biostimulants are treated to improve the immune system of plants, protection from pests and stimulation of friendly sprouting of seeds in the soil. Encrusted film creates a protective shell against pathogens, which increases the germination of seeds, plant resistance to extreme weather and environmental factors, in addition to optimizing the water as well as nutrient balance of the crop. For this purpose, it is necessary to find the optimal concentration of biostimulant when soaking the seeds. Firstly, 100 pieces of sorghum grains were soaked in such a way that moisture was supplied in sufficient quantities to vital organs such as the endosperm and the germ. Before soaking, the seeds were preliminarily cleaned with distilled water from dirt and dust, and pathogens were removed using an ozonator. Distilled water with pH = 5.6–6 was used for the control variant. Soft water up to pH 7 can also be used.

The results of the study to optimize the concentration of biostimulants are shown in Tables 2–3. Biostimulator "Azotofertil" variant-4 with 4% concentration showed the best result among 10 samples of different concentrations. All variants below 4% concentration of "Azotofertil" biostimulator showed weak effect on seed viability. All variants above 4% showed inhibitory character, the growth process gradually stopped. Inhibitory character is explained by the fact that the amount of water that awakens the seeds decreases. A large amount of stimulant in the sample suspends the fermentation process inside the grain. The second biostimulator "MERS" variant-5 with concentration of 5% showed similar results. All analyses are shown in Figure 1, as well as Tables 2 and 3.

When the seeds were germinated on a bed of filter paper, the dishes were disinfected with alcohol before use. Petri dishes were sterilized in a desiccator at 130 °C for 1 h. Seeds were spread on two or three layers of moistened paper in Petri dishes. Seeds were germinated on filter paper at 25 °C, in a dark place, with germination energy of 4 days and germinating capacity of 8 days. Precooling to 0 °C was used as an additional condition for seeds in the dormant state. The experiments to determine germination and germination energy of sorghum seeds treated with biostimulants were carried out in Research Laboratory "Industrial Biotechnology" of M. Auezov SKU.

Table 2. The results of determining the optimal concentration of biostimulator Azotofertil

№	Variants	Quantity, pcs	Determination of optimal concentration on seed germination, pcs Variants Quantity, pcs				
			1 h	2 h	3 h	4 h	5 h
1.	Control (H_2O)	100	-	17	26	39	51
2.	Azotofertil						
	(1) 1%	100	-	21±3	33±3	47±2	56±3
	(2) 2%	100	29±2	41±2	55±1	68±2	79±2
	(3) 3%	100	42±3	54±2	70±2	87±3	93±2
	(4) 4%	100	49±2	63±1	79±1	94±1	99-2
	(5) 5%	100	40±3	54±2	68±3	86±2	91±2
	(6) 6%	100	27±3	43±3	57±1	65±2	80±2
	(7) 7%	100	23±1	35±2	50±2	62±3	71±3
	(8) 8%	100	21±2	37±2	49±3	53±2	62±2
	(9) 9%	100	16±3	25±3	36±2	41±2	56±3
	(10) 10%	100	10±2	21±1	33±2	37±2	50±2

Table 3. Results of determining the optimal concentration of biostimulant MERS

№	Variants	Quantity, pcs	Determination of optimal concentration on seed germination, pcs				
			1 h	2 h	3 h	4 h	5 h
1.	Control (H_2O)	100	-	17	26	39	51
2.	MERS						
	(1) 1%	100	-	18±2	28±2	40±2	51±2
	(2) 2%	100	-	20±3	30±1	45±2	53±2
	(3) 3%	100	27±3	39±1	51±2	66±2	74±3
	(4) 4%	100	41±2	52±2	67±2	79±1	91±2
	(5) 5%	100	51±2	66±1	79±1	90±1	97-1
	(6) 6%	100	39±2	51±3	64±2	81±2	90±1
	(7) 7%	100	25±1	41±2	55±2	62±2	76±2
	(8) 8%	100	21±2	33±2	47±3	55±3	68±2
	(9) 9%	100	19±2	27±3	35±2	40±2	55±2
	(10) 10%	100	16±1	24±3	31±2	38±2	49±2

The main factor, and the first in the sequence controlling the process of seed germination, is the entry of water inside and swelling of the seed endosperm, and structural, metabolic changes. The process of water ingress into the grain is represented by three stages. The first two stages of swelling are reversible. Moistening and then drying out can still keep the seed alive. The last third stage is the beginning of deep biological, physiological and enzymatic processes. When water enters, the volume of the grain increases by 35% of the initial volume. Physical properties change, the hard grain changes into a soft and elastic state. Tannins and various salts are extracted from the shell.

Water absorption is divided into two phases: water absorption phase and swelling phase. During the water ingestion phase, dry seeds pass from a dormant state and awaken, absorbing water drops from the air or from the environment where they are located to an excitable moisture content, which is clearly calculated for each plant. For sorghum seeds to swell, 35% moisture of the seed weight is sufficient. For ordinary corn, swelling is 40%; for wheat, swelling is 60%. The ingress of water activates the respiration of the grain (at the end of swelling, it increases by 2–3 times). At this stage, dry seeds absorb a certain amount of water with maximum force; this force reaches several tens of atmospheres. This value in weeds is much greater, reaching several hundred atmospheres. This explains why weeds grow quickly.

The rate at which seeds absorb water depends on the variety and climate of the area where sorghum is grown and the composition of the grain.

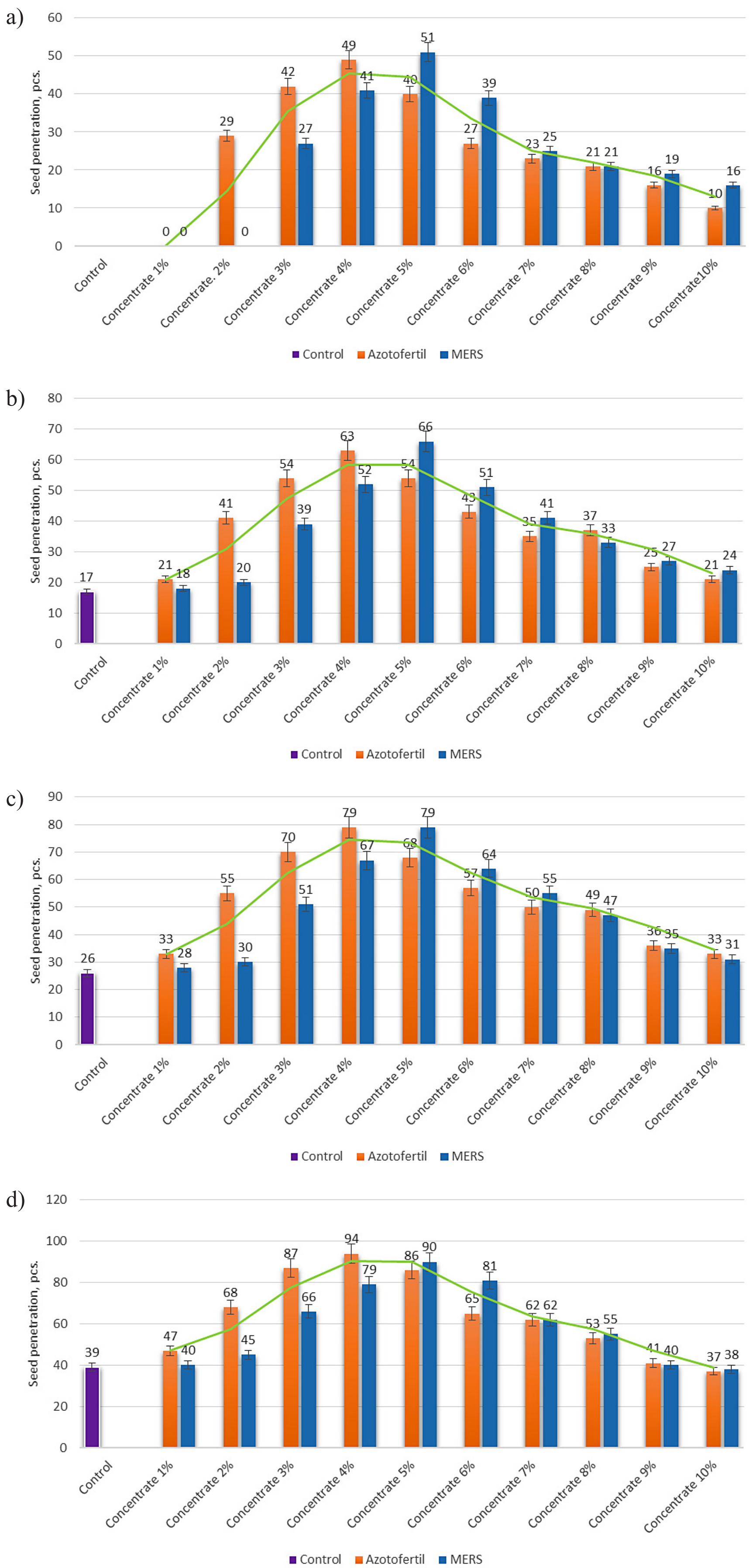

Figure 1. Determination of optimal concentration and soaking time on seed germination (a) for 1 hour; (b) for 2 hours; (c) for 3 hours; (d) for 4 hours of soaking

e)

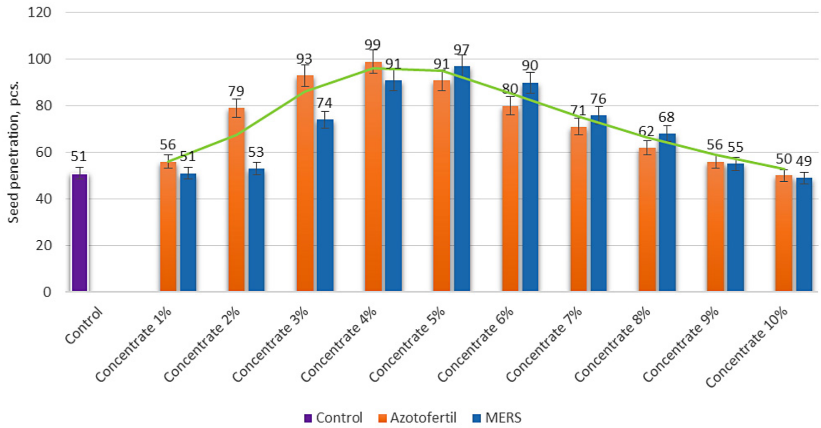

Figure 1. Cont. Determination of optimal concentration and soaking time on seed germination (e) for 5 hours of soaking

The sorghum seeds grown in dry and hot regions absorb water faster than those grown in temperate climates. Water temperature has no less of an effect. Below 10 °C development stops, the optimal temperature is 12–14 °C. If the temperature is above 15 °C, the microorganisms in the sorghum seed develop vigorously. They absorb soluble oxygen in the water, thereby inhibiting the embryo's vital activity.

The chemical composition of sorghum seeds also greatly affects the amount of water absorption. Protein absorbs the most water, up to 180%; starch, about 70%; and fiber, about 30%. The amount of protein in sorghum grain is 10.6 grams out of 100 grams. The amount of water is 9 grams. From this value, the amount of water absorbed can be calculated. (Protein+water)×180% = total amount of water absorbed: (10,6+9)×180 = 35%. The results of the study of the swelling of seeds of sorghum variety Kazakhstan-16 in the presence of biostimulants are shown in Table 4.

After determining the optimal concentration of both biostimulants, the process of seed water absorption was studied at room temperature according to encrustation technology (Table 2). Azotofertil" biostimulator with 4% concentration showed the best result; absorption of working solution corresponded to authors' expectations – 35±1%. Biostimulator "MERS" with concentration of 5% corresponded to 31±2% of seed swelling (Table 3). Here, it should be noted that in healthy seeds water absorption is not more than 35%. If seeds continue to absorb more liquid, it means that there are spoiled or rotting seeds among the tested ones.

Another important thing to consider is the enzymatic activity inside the grain. Primary water absorption of grain is a physical process, gradually passing to chemical, that is, the mechanism of enzymes is manifested here. Stable chemicals that are insoluble in water-such as proteins, fats, and other compounds-are transformed into soluble forms during enzyme activity.

Enzymatic vital activity drops sharply under the influence of antiseptic substances (dressing agents, stimulants and various hormones) which inhibit embryo vital activity and enzyme formation process, which subsequently leads to stopping swelling and germination processes. In connection with this process, when using antiseptic substances before sowing, it is necessary to remember about the reduction of enzymatic activity, and this leads to a slowing down of the germination process. This is well demonstrated by the data in Tables 2 and 3: as the dose of growth stimulant in the solution increases, the minimum

Table 4. Swelling of seeds of sorghum variety Kazakhstan-16

№	Variants	Dry weight, g	Average weight of 100 seeds after soaking, g					Water absorbed, ml*
			1 h	2 h	3 h	4 h	5 h	
1	Qontrol (H_2O)	2,0	2,03	2,06	2,13	2,2	2,27	0,27
2	Azotofertil (4%)	2,0	2,15	2,2	2,27	2,31	2,35	0,35
3	MERS (5%)	2,0	2,09	2,14	2,2	2,26	2,31	0,31

Note: *1 ml distilled water = 1 g weight.

Table 5. Results of in vitro germination of encrusted seeds under different temperature regimes in thermostat

№	Variant name	Temperature					
		t + 5 °C		t + 14 °C		t + 25 °C	
		Germination rate %					
		for 1 day	for 5 day	for 1 day	for 5 day	for 1 day	for 5 day
1	Control (H_2O)	-	-	31±4	62±3	59±3	90±2
2	Azotofertil (4%)	-	-	56±2	85±2	74±3	97±2
3	MERS (5%)	-	-	45±3	73±2	67±3	92±2

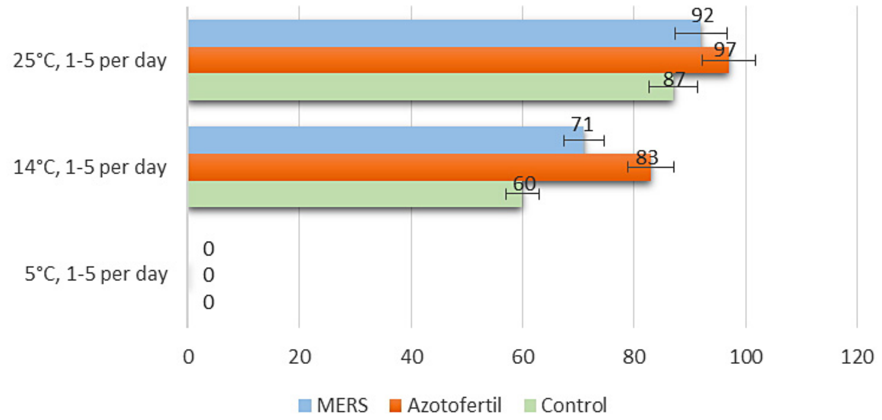

Figure 2. Dependence of seed germination on temperature

amount of water that awakens the enzymatic process inside the seeds decreases.

Germination of sorghum seeds was determined in different gradations of temperature changes from +5 °C, +14 °C and +25 °C for 1-5 days, the data obtained are shown in Table 5 and Figure 2.

The results of the study of seed germination at different temperature regimes are shown in Table 5. The effect of "Azotofertil" biostimulator at a temperature of 14 °C on seed germination reached 85±2%, and at temperature 25 °C seed germination reaches up to 97±2%. On this basis, it can be argued that the germination period of sorghum seeds is proportional to the change in temperature. The conducted research proves that the most optimal germination parameter is temperature 23–25 °C. The obtained research data corresponds to all expected results.

CONCLUSIONS

On the basis of the research, it was discovered that the "Azotofertil" biostimulator with a concentration of 4% showed a very good result. The authors recommend usage in the cultivation of sorghum in fodder production. The treatment of biostimulator increases seed germination, accelerates friendly germination, increases soil fertility, improves plant nutrition, thereby having a favorable effect on the surrounding ecosystem.

In this work, the optimal parameters of the effect of growth biostimulant on the activation energy of seed germination of sugar sorghum variety of the Kazakhstan-16 were determined. When using the "Azotofertil" biostimulator based on strain *Azotobacter chroococcum* at a concentration of 4% and at temperatures above 14 °C, inoculated sorghum seeds showed the best results. A change in these parameters strongly affects the growth and yield of green mass of sorghum. The results indicate that the biostimulants based on *Azotobacter chroococcum* can be effectively used. In the future, they can be further evaluated as components of integrated plant growth management strategies.

Acknowledgements

Our special gratitude to the scientific organizations Research Laboratory "Industrial Biotechnology" and Scientific-Production Association LLP "Ana-Zher" for their assistance and support of this research work.

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