Influence of Biological Preparations on Germination, Growth, and Development of Alfalfa of the Kokshe Variety in the Hill and Plain Zone of the Akmola Region of Kazakhstan

¹Arman Kalin, ¹Ualikhan Sagalbekov, ²Nina Kazydub, ¹Marden Baidalin and ¹Miras Suraganov

¹Sh. Ualikhanov Kokshetau University, Kokshetau, Kazakhstan ²Omsk State Agrarian University named after P.A. Stolypin, Omsk, Russia

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Corresponding Author: Marden Baidalin Sh. Ualikhanov Kokshetau University, Kokshetau, Kazakhstan Email: marden_0887@mail.ru Abstract: An important role in increasing the production of high-protein feed in the steppe zone belongs to leguminous grasses, such as alfalfa, sainfoin, and melilot. The value of perennial grasses, including alfalfa, is not only in providing high-protein feeds and their productivity in the sharply continental climate of Northern Kazakhstan, but it also has great importance in increasing soil fertility. The purpose of this research was to study the influence of biological preparations on the germination, growth, and development of alfalfa of the Kokshe variety in the conditions of the hill and plain zone of the Akmola region of Kazakhstan. The field experiments were conducted in 2020-2022 in the Kokshetau experimental production farm LLP. The following biological preparations for seed treatment were used: BioSleep BW + Foliar (1 1/ha), Orgamica S + Foliar (1 1/ha), Organit P, Organit N, Biodux + Foliar (2 1/ha) and Foliar. The seed treatment with biological preparations was carried out 12 h before sowing, after which the seeds were dried. The Organit P + Organit N + Biodux + Foliar variant showed the highest rates of germination energy (70.5%) and laboratory and field germination (85 and 52%, respectively). The use of Biosleep BW + Foliar had the best effect on the height of plants and the productivity of green and dry mass, where the height of plants was 57.2 cm and the productivity of green and dry mass was 121 and 48 g/plant, respectively. Thus, when cultivating alfalfa of the Kokshe variety for seeds using elements of organic technology, it is recommended to use Organit P + Organit N + Biodux + Foliar and BioSleep BW + Foliar biological preparations.

Keywords: Alfalfa, Biological Products, Laboratory and Field Germination, Plant Height, Green and Dry Mass

Introduction

The use of perennial legumes in modern agriculture is the key to success in obtaining vegetable protein for the livestock industry. One of the most important highprotein feed crops is alfalfa (Medicago) with a high protein content, low fiber content, and high digestibility. This crop is widespread and of great practical importance in feed production (Albrecht and Beauchemin, 2003).

There are more than 20,000 types of alfalfa in the world. In regions such as the USA, Europe, China, Australia, and North and South America, about 30 million hectares are allocated for alfalfa and the figures are increasing every year (Shafique *et al.*, 2014).

A significant area of land is allocated for alfalfa and sainfoin in Canada since due to the biological fixation of nitrogen in the soil, a sufficient amount of nitrogen is accumulated for subsequent crops in the crop rotation (Wang *et al.*, 2021).

Currently, alfalfa as a high-protein fodder crop is commercially in demand in the food market for animal feed (Kulkarni *et al.*, 2018). In this regard, the amount of minerals in the feed necessary for a living organism is obtained by cultivating legumes, since they contain a sufficient amount of macro and micro elements (Grusak, 2002).

The growth and further development of plants occur much better with the introduction of micro-fertilization and the use of biological products, in particular molybdenum, which has a positive effect on nitrogen fixation and



metabolism. Thus, the introduction of molybdenum in the dose of 0.150-300 g/ha on alfalfa crops in Northern China positively affected the content of vegetable protein in alfalfa (Boelt *et al.*, 2015; Mao *et al.*, 2018).

In Kazakhstan, the conditions of the hill and plain zone of the Akmola region are characterized by insufficient moisture supply, where the average longterm precipitation does not exceed 250-330 mm per year (Musynov and Arinov, 2007). These conditions of the sharply continental climate cause special requirements for the technology of pre-sowing treatment of alfalfa seeds, affecting the effectiveness of their germination and subsequently their productivity.

In this study, we propose to use biological products in the cultivation of alfalfa for seeds. The timely use of plant growth regulators and biological products can significantly increase the yield of seeds, which ultimately will have a positive impact on the economic performance of farms.

The purpose of this research was to study the influence of biological preparations on the germination, growth, and development of alfalfa of the Kokshe variety in the conditions of the hill and plain zone of the Akmola region of Kazakhstan.

Materials and Methods

Laboratory studies were conducted in the Plant physiology laboratory at Kokshetau University named after Sh. Ualikhanov, Kokshetau. The field experiments were conducted in 2020-2022 in the Kokshetau experimental production farm (OPKh) LLP, Akmola region, Zerendinsky district, Chaglinka village.

The object of the study is the Kokshe variety of alfalfa approved for use in the Akmola region. The winter hardy, seed-yielding variety belongs to the variegated hybrid variety type of alfalfa with a predominance of lilac and light blue color of flowers and is suitable for long-term use.

The meteorological conditions of 2020-2022 corresponded to the definition of continental climate. According to the Chagli weather station, 254.9 mm of precipitation fell in 2020, which is 100.1 mm less than the average long-term norm, 241 mm of precipitation fell in 2021, which is 84.5 mm less than the average long-term norm, and in 2022, precipitation was 100.1 mm lower than the average long-term norm. The precipitation of the cold period (September to March), which played a major role in the accumulation of productive moisture reserves in the soil, amounted to only 71.2 mm, while the deficit of precipitation was 28.8% compared to the long-term norm (Table 1). During the warm period, the precipitation equals 186.5 mm. A relatively small amount of precipitation combined with high temperatures determines low values of air and soil humidity and frequent occurrence of droughts. The maximum moisture reserve is observed in early spring. The aggravating factor contributing to the intensive evaporation of moisture was the lack of precipitation in April and May, where the shortage of precipitation compared to the long-term values was 80.3% against the background of high-temperature conditions.

A distinctive feature of the autumn and winter period is the high temperature of the air, which was lower by 1.6°C compared to the average annual norm.

 Table 1: Meteorological conditions for 2020-2022 (Chagli weather station)

W	eather station	1)					
	Precipitatio	on, mm	Air temperature, °C				
	Average		Average				
Month	long-term	2020-2022	long-term	2020-2022			
2020							
January	11.4	20.7	-17.2	-8.8			
February	11.7	14.6	-15.2	-6.3			
March	17.0	3.3	-6.2	-1.9			
April	20.7	29.7	+4.2	+8.8			
May	37.4	13.5	+11.5	+16.5			
June	52.5	15.0	+17.3	+16.3			
July	75.2	98.4	+18.2	+20.4			
August	41.8	11.9	+16.4	+18.8			
September	27.7	15.3	+10.0	+10.8			
October	29.7	11.6	+3.0	+4.7			
November	16.8	11.1	-5.6	-6.6			
December	13.6	9.8	-12.9	-13.4			
Total	355.5	254.9					
2021							
January	11.7	16.7	-16.4	-18.0			
February	14.0	26.1	-14.1	-14.8			
March	15.7	36.9	-5.7	-7.3			
April	22.7	9.2	+4.4	+4.8			
May	35.0	7.8	+11.9	+17.1			
June	42.4	25.5	+17.0	+17.2			
July	66.7	40.2	+20.1	20.6			
August	36.2	28.0	+16.7	+19.9			
September	26.1	14.2	+10.5	+9.9			
October	25.5	13.6	+3.7	+4.3			
November	16.8	18.0	-5.5	-6.6			
December	12.7	4.8	-13.0	-9.5			
Total	325.5	241.0					
2022	10.2	11.6	-16.0	-12.4			
January February	12.3 13.3	11.0	-16.0	-12.4 -9.2			
March				-9.2			
	16.3	4.7	-6.0				
April	19.0	5.5	+4.5	+8.5			
May	32.8	15.7	+12.2	+13.6			
June	41.0	49.6	+16.7	+17.7			
July	67.0	77.0	+19.0	+19.9			
August	36.5	44.1	+17.1	+16.7			
September	25.2	6.6	+11.0	+13.0			
October	24.3	13.6	+3.7	+4.3			
November	15.8	29.6	-5.1	-8.3			
December	13.1	7.3	12.5	11.5			
Total	316.6	283.8					

The average monthly air temperature was above the norm by 2.7° C, where on some days in May the air temperature reached up to 40.0° C. Due to the circumstances, the sowing and the full germination phase took place in harsh conditions according to the level of moisture availability, which subsequently harmed the formation of the crop. The average monthly air temperature in June and July of the year in question exceeded the average annual norm by $2.0-2.2^{\circ}$ C.

Thus, most of the period of active vegetation of alfalfa was characterized by high average daily air temperatures exceeding the average annual values, with a shortage of precipitation, which created not quite favorable conditions for the growth, development, and productivity of this crop, from which it can be concluded that the conditions of vegetation were formed in the lack of moisture. Some positive influence on the growth and development of crops in the experiment was provided by precipitation that fell in the third decade of July and the first decade of August in the amount of 73.3 mm, which coincided with the main crop vegetation periods.

The soil can be described as ordinary medium-humus chernozem with a depth of humus horizon of 25-27 cm and an average humus content of 4.01%. The arable soil layer contains 17.9 mg of nitrate nitrogen, 8.6 mg of mobile phosphorus, and 35.0 mg of exchangeable potassium per 100 g of soil. Consequently, the nitrogen content is average, the phosphorus content is low and the potassium content is high. Concerning the mechanical composition, the soil is heavy loamy, the volume weight in the arable horizon is 1.19 g/cm³ and the weight in the m layer on average is 1.30 g/cm³. The humidity of permanent wilting is 12-13%.

The field experiments were performed in four-fold repetition. The agricultural technology in the experiments was zonal. The area of the experimental plot was 20 m^2 and the placement of plots was randomized. The preceding crop was black fallow.

The technique and methodology of performing field observations, records, and analyses were established according to the methodology of Dospekhov (2011). The sowing period was May 17. We used the manual seeder RS-1, with a seeding depth of 2-3 cm. The method of sowing was ordinary and wide-row, the space between the rows was 70 cm. The rate of sowing alfalfa seeds with a wide-row method was 5.0 kg/ha. We used the following biological products for seed treatment: BioSleep BW + Foliar (1 l/ha), Orgamica S + Foliar (1 l/ha), Organit P, Organit N, Biodux + Foliar (2 l/ha) and Foliar. The seed treatment with biological preparations was carried out 12 h before sowing, after which the seeds were dried. The biological preparations were used for the pre-sowing treatment of alfalfa seeds. The experiment design included the following variants:

- 1. Control (seed treatment with water)
- 2. Seed treatment with BioSleep BW + Foliar
- 3. Seed treatment with Orgamica S + Foliar
- 4. Seed treatment with Organit P, Organit N, and Biodux + Foliar
- 5. Seed treatment with Foliar

Regulations for the use of the preparations: The BioSleep BW biological insecticide was used to protect against pests, such as Tychius flavus, using a strain of entomopathogenic mycelial fungus Beauveria bassiana which is effective against a wide range of phytophagous insects. This includes pests of the Lepidoptera order, such as the meadow moth, cabbage moth, black witch moth, fir seed moth, winter moth, etc. It is 90-95% effective against the black witch moth, cabbage moth, and aphids, while harmless to homeothermic animals and bees (Dospekhov, 2011).

Protection against diseases was carried out by the biological preparation Orgamica S, which is a nondangerous microbiological fungicide aimed at suppressing the development of fungal and bacterial plant diseases. The Bacillus amyloliquefaciens strain is a common inhabitant of the soil and manifests its useful properties in the vicinity of the roots and on the surface of the leaves. Upon contact with the surface of the plant, the spores become active and inhibit the growth of harmful objects by exposure to antibiotic enzymes.

For legumes, Biodux is a strong growth stimulant. The seed material was treated and additionally, preparations were sprayed on the leaves during the growing season. The preparation protects plants from a complex of diseases caused by fungal, bacterial, and viral diseases during seed treatment and vegetation. Besides, due to the development of the root system, the digestibility of nutrients improves, which positively affects the effectiveness of organic fertilizers and trace elements. The use of a plant growth regulator has a significant impact on the crop structure (Dospekhov, 2011).

To provide the plant with phosphorus and nitrogen, we used Organit P and Organit N biologics, which include spores of the Bacillus megaterium strain, effective microbiological fertilizers that improve plant nutrition by increasing the biological availability of phosphorus and nitrogen. Bacillus megaterium spores contained in plants are activated when they enter the soil, showing positive properties in the vicinity of the roots. The cultivation of alfalfa with the use of biological preparations led to an increase in yield of up to 15-20%. Organit P and Organit N are safe biological preparations for homeothermic animals and bees (Dospekhov, 2011).

Fertigrain Foliar was used as a micronutrient to increase the vegetative formation of plants, eliminate the deficiency of trace elements, anticipate an increase in the productivity of legumes and increase the ability to restore. The composition of the Fertigrain Foliar preparation includes 0.75% of zinc, 0.50% of manganese, 0.10% of boron, 0.10% of iron, 0.10% of copper, 0.02% of molybdenum and 0.01% of cobalt. It is used during the growing season by spraying the plant. The treatment is carried out in the phase of maximum vegetative development of the plant.

The graphs were drawn and mathematical data processing was carried out in the statistica software (data analysis software system), version 10.

Results

According to the results of the seedling count, the highest indicator of laboratory germination during germination in petri dishes as close as possible to field conditions was observed in the sample treated with Organit P, Organit N, and Biodux + Foliar. The germinated seeds averaged 76-85%, while in the control variant, this value equaled 75%, which is 10% lower compared to the variants treated with biological preparations, except for the variant treated with Orgamica S + Foliar (Table 2, Figs. 1-3).

Based on the data presented in Fig. 1, it was noted that the lowest germination energy of alfalfa seeds equaled 63.5% in the Orgamica S + Foliar variant and 64.5% in the control variant. The maximum seed germination energy in the experiment was noted in the variant with BioSleep BW + Foliar seed treatment (77%).

According to the data presented in Fig. 2, the lowest laboratory germination of alfalfa seeds averaged 70.5% in the Orgamica S + Foliar variant and 74.5% in the control variant. The maximum laboratory seed germination in the experiment was noted in the variant with the Organit P, Organit N, and Biodux + Foliar seed treatment (84.5%). The laboratory germination in the control variant was 75%, in the BioSleep BW + Foliar variant 77%, in the Organit S + Foliar variant 70%, in the Organit P, Organit N, Biodux + Foliar variant 76%, respectively. The highest rate of laboratory germination in petri dishes was noted in the sample treated with Organit P, Organit N, and Biodux + Foliar (85%).

According to the data presented in Fig. 3, the treatment of seeds with biological preparations had a positive effect on the seedling length. Thus, in the control variant, the average seedling length was 46 mm and in all experimental variants, it increased to 52-59.5 mm. The exception was the variant where the seeds were treated with Orgamica S + Foliar. There, the seedling length was minimal in the experiment, equaling 43.5 mm (Fig. 4).

After the end of the germination period, the seedling length was measured by putting them over millim paper. The greatest seedling length was observed in the variant treated with Organit P, Organit N, and Biodux + Foliar (59.5 cm), where the length of the sprouts that came to the surface in the control variant was 46 cm (Fig. 4).

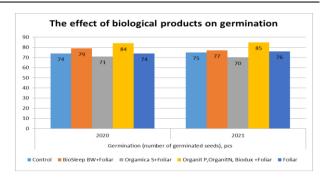


Fig. 1: The effect of biological products on the seed germination energy in 2020-2021

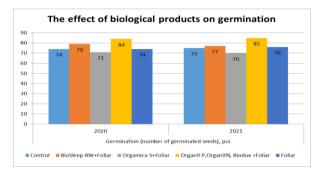


Fig. 2: The effect of biological preparations on laboratory seed germination in 2020-2021

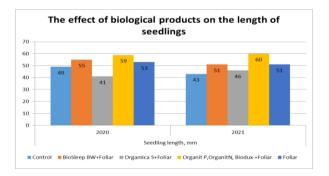


Fig. 3: The effect of biological preparations on the seedling length, 2020-2021

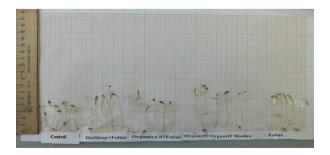


Fig. 4: The length of alfalfa seedlings grown in petri dishes

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		Germination energy, %		Germinatio	on, %	Seedling length, mm		
No.	Variants	2020	2021	2020	2021	2020	2021	
1	Control	65	64	74	75	49	43	
2	BioSleep BW + Foliar	78	76	79	77	55	51	
3	Orgamica S + Foliar	63	64	71	70	41	46	
4	Organit P, Organit N, Biodux + Foliar	71	70	84	85	59	60	
5	Foliar	67	66	74	76	53	51	

 Table 2: The effect of biological preparations on the germination energy, laboratory germination of seeds, and seedling length, 2020-2021

Table 3:	The effect of biological	preparations on field	germination of seeds,	plant heigh	nt, and leaf and stem ma	ss of alfalfa (on average for 2020-2022)	
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			Plant he	Plant height, cm			Green mass, g/plant				Dry mass, g/plant			
	Field germination, %		1 st year of life		2 nd year of life		1 st year of life		2 nd year of life		1 st year of life		2 nd year of life	
Variants	2020	2021	2020	2021	2021	2022	2020	2021	2021	2022	2020	2021	2021	2022
Control	41	40	33	33	44.5	47	46	42	81	86	11	10	21.5	22
Std. Dev	2.507		9.597				21.358				6.365			
F-ratio	4.750		1.059				24.055				4.444			
Variances														
P-Variances	±0.03		±0.7				±0.09				±0.35			
BioSleep	46	45	39	36	48	57.5	59	52	96	103	13	12	22	26
BW + Foliar	1 7 5 0		10 50 4				24.055				< 111			
Std.Dev	4.750		10.594				24.055				6.444			
F-ratio	3.590		1.218				1.268				1.025			
Variances P-Variances	0.11		.07				0.65				±0.96			
Orgamica	±0.11 38	36	±0.7 40	31.5	39	42	±0.65 47	39	106	105	±0.96 11	8	27	29
S + Foliar	30	50	40	51.5	39	42	4/	39	100	105	11	0	21	29
S + Fonal Std. Dev	4.659		9.207				33.283				10.535			
F-ratio	3.454		1.086				2.428				2.739			
Variances	5.454		1.000				2.420				2.15)			
P-Variances	±0.12		±0.87				±0.09				±0.05			
Organit P,	52	51	43.5	37	50.5	57	69	66	117	120	15	13	35	38
Organit N,	02	01	1010	21	0010	0,	07	00		120	10	10	00	20
Biodux + Foli	iar													
Std. Dev	2.927		9.825				28.441				12.777			
F-ratio	1.363		1.047				1.773				4.029			
Variances														
P-Variances	± 0.49		± 0.03				±0.27				±0.01			
Foliar	44	42	38	36	44.5	52.5	58	48	98	102	12	12	22.5	/26,5
Std. Dev	3.817		10.279				25.303				7.371			
F-ratio	2.318		1.147				1.403				1.341			
Variances														
P-Variances	±0.28		±0.34				±0.51				±0.57			

The mathematical data processing was carried out in the statistica software (data analysis software system), version 10, www.statsoft.com. The diagram of the span of the length of alfalfa seedlings is shown in (Fig. 5).

The results of the regression analysis are presented in (Fig. 6).

To determine the field germination, we used alfalfa seeds of the Kokshe variety treated with biological preparations before sowing. 140 germinating seeds were sown per 1 linear m (Table 3).

Field germination in the control variant was 41%, in the BioSleep BW + Foliar variant 46%, in the Orgamica S + Foliar variant 38%, in the Organit P, Organit N, and Biodux + Foliar variant 52%, and in the Foliar variant 44%.

The plant height in the control variant was 46.8 cm, in the BioSleep BW + Foliar variant 57.2 cm, in the Orgamica S + Foliar variant 39 cm, in the Organit P, Organit N and Biodux + Foliar variant 50 cm and in the Foliar variant 44.6 cm.

The productivity of the green mass in the control variant was 86 g/plant, in the BioSleep BW + Foliar variant 121 g/plant, in the Orgamica S+Foliar variant 62 g/plant, in the Organit P, Organit N and Biodux + Foliar variant 72 g/plant and in the Foliar variant 107 g/plant.

The productivity of dry mass in the control variant was 28 g/plant, in the BioSleep BW + Foliar variant 48 g/plant, in the Orgamica S + Foliar variant 18 g/plant, in the Organit P, Organit N, and Biodux + Foliar variant 24 g/plant and the Foliar variant 32 g/plant.

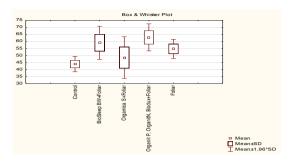
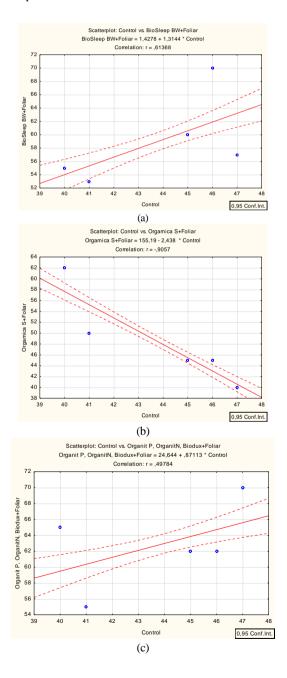


Fig. 5: Diagram of the span length of alfalfa seedlings grown in petri dishes



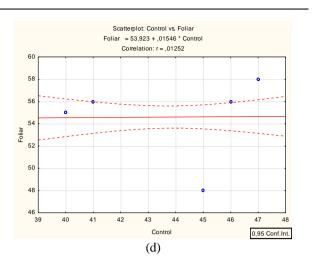


Fig. 6: The regression equation

According to Table 3, the maximum number of plants per 1 linear m was observed in the seeds treated with Organit P, Organit N, and Biodux + Foliar. In this case, the average field germination of seeds was 51.5%. The minimum number of seedlings was observed in the variant treated with Orgamica S + Foliar (37%), while the average field germination was obtained in the control variant (41%).

Discussion

The study of the effect of biological preparations on the seed productivity of alfalfa showed that they differed in comparison with the control variant in laboratory and field germination. As a result of the study, it was found that the average seed germination energy of the studied variables in the control variant was 64.5%. The maximum seed germination energy in the experiment was noted in the variant with seed treatment with Organit P, Organit N, and Biodux + Foliar (70.%).

Our experimentally obtained results on biological efficacy and mechanism of action are consistent with previous studies by other authors on different field crops.

Studies by Glazunova *et al.* (2019) have shown that the biological insecticide BioSleep BW based on a strain of the fungus Beauveria bassiana with a consumption rate of 2-3 l/ha is biologically effective on cereal crops by 78.3%, against grain aphids and cereal leaf beetle, which is similar to the use of a chemical preparation.

Organit N, Organit P, and Orgamica S biological preparations can fix atmospheric nitrogen and convert it into forms suitable for plant consumption, improve plant nutrition by increasing the biological availability of phosphorus, and inhibit the growth of harmful objects through the action of special enzymes due to

the colonies of bacteria Bacillus megaterium and amyloliguefaciens. The complex Bacillus of biologically active polyunsaturated fatty acids of the soil fungus Mortierella alpina contained in the biological preparation Biodux managed to form and activate growth and biological processes in plants. The complex application of Organit N, Organit P, Biodux, and Orgamica S biological preparations in the cultivation of safflower showed that the crude fat content in the seeds was 28.8% in the control variant and 30.0% when using the biological preparation. At the same time, the yield in the control variant was 0.600 t/ha, and with biological preparations, it rose to 0.764 t/ha (Nasiev et al., 2021).

The use of Fertigrain Foliar micronutrients with zinc (0.75%), manganese (0.50%), boron (0.10%), and molybdenum (0.02%) positively affected the structure of wheat yield elements. In the variants treated with Fertigrain Foliar, the excess grain yield over the control variant varied in the range of 6.8-15.7% (Sevov and Delibaltova, 2013). In our studies, the combined use of Fertigrain Foliar increased field germination by 44-52%, plant height by 44.6-57.2 cm, and productivity of green mass by 107-121 g/plant compared to the control variant.

The conducted studies found that the growth processes and productivity of the plant were the best in the variant with Organit N + Organit P + Biodux + Foliar.

Conclusion

The use of biological preparations before sowing and during the growing season can increase laboratory and field germination of seeds and improve plant growth and productivity. Thus, the Organit P + Organit N + Biodux + Foliar variant showed the highest rates of germination energy (70.5%) and laboratory and field germination (85 and 52%, respectively). The use of BioSleep BW + Foliar had the best effect on the height of plants and the productivity of green and dry mass, where the height of plants was 57.2 cm and the productivity of green and dry mass was 121 and 48 g/plant, respectively.

Thus, when cultivating alfalfa of the Kokshe variety for seeds using elements of organic technology, it is recommended to use Organit P + Organit N + Biodux + Foliar and BioSleep BW + Foliar biological preparations comprehensively.

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Author's Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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