



ALFALFA PRODUCTIVITY AND FORAGE QUALITY RELATIONSHIP WITH THE APPLICATION OF BIOLOGICAL PREPARATIONS

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SUMMARY

Increased production of high-quality feed with reduced cost is an important direction in the development of animal husbandry. Perennial leguminous grasses can fulfill the 80% requirements of livestock for roughage. Alfalfa (*Medicago sativa* L.) has the valuable nutrient composition that enriches the protein component of animal diets, as well as high productivity and adaptability to various growing conditions. This research aimed to analyze the green mass yield, as well as evaluate the biochemical composition and nutritional values of alfalfa forage with the application of biological preparations. The variant with Organit P + Organit N + Biodux outperformed other treatments, reaching a green mass yield of 160.0 g/m², which represented a statistically significant increase of 11.8 g/m² (or 8.0%) compared with the control. The use of biopreparations had a noticeable effect on crude protein content, which ranged from 13.73% to 17.75% on the dry matter basis. In the variant with pre-sowing seed treatment using biological preparations (Organit N + Organit P + Biodux), the carotene content was the highest (89.8 mg/kg of feed) and exceeded the control sample by 17.3 mg/kg. The highest crude fiber content (26.4%) recorded was in the variant (Organit N + Organit P + Biodux) versus the control (23.3%).

Keywords: Alfalfa (*M. sativa* L.), seed inoculation, biological preparations, forage quality, correlation, biochemical composition, protein content, crude fibers

Key findings: In alfalfa (*M. sativa* L.), the highest digestible protein content resulted in the pre-sown seeds treated with biological preparations Organit N (12.3%) and Systemica M (12.1%), exceeding the control by 1.69% and 1.43%, respectively. Overall, forage quality and economically valuable traits enhanced by 10%–20%.

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INTRODUCTION

In recent decades, animal husbandry has been facing challenging situations, which may come from a significant reduction in livestock numbers, feed base, grass sowing areas, and, consequently, a shortage of high-protein feeds in terms of composition and nutritional values. Field forage production aimed to increase the proportion of perennial leguminous grasses in forage lands (60%–70%) and implement the advanced harvesting technologies with higher crude protein content (Hennessy *et al.*, 2020). Livestock systems primarily relied on the better pasture use to increase the meat and milk production to meet the growing demands globally. Perennial legumes can interact at different stages of the soil-plant-animal-atmosphere system and proved most effective in mixed herbage up to 50% of legume content (Lüscher *et al.*, 2014; Kebede *et al.*, 2016; Tulu *et al.*, 2023). The advantages of cultivating leguminous crops and moving away from the intensive use of inorganic fertilizers represent a strategic direction in animal husbandry. The silage production from mixtures of perennial leguminous grasses provides substantial economic benefits and contributes to significant cost savings in the livestock sector, highlighting the high economic efficiency of sustainable forage production systems (Rochon *et al.*, 2004; Alehegn *et al.*, 2025).

Perennial legumes' inclusion in crop rotation enhances the yield of subsequent crops, preserves soil fertility, and contributes to weed, disease, and pest control. Integrating perennial leguminous grasses in crop production systems would help in reducing land degradation (Tomić *et al.*, 2024; Gamayunova *et al.*, 2025). Forage lands' productivity can gain enhancement through the use of biological nitrogen by incorporating legumes into grass mixtures. A 1% increase in legume proportion contributes a considerable increase in grassland productivity and boosts nitrogen accumulation by 2–3 kg per hectare, provided that the legume component accounts for at least 30% (Belchenko, 2015; Wuest, 2023).

Among perennial grasses, the leading genus is *Medicago* L., which preserves and

improves the agrophysical properties of soil for subsequent crops in agrophytocenoses. It also plays a key role in the development of sustainable agriculture, enhancing soil fertility through its ability to fix atmospheric nitrogen (Saidi *et al.*, 2021; Hetman *et al.*, 2025). Alfalfa (*Medicago sativa* L.) is a rich source of crude protein with excellent digestibility, and its content crucially depends on the plant development stage, reaching 3500–4000 kg/ha in dry matter. In addition, alfalfa contains the highest levels of macro- and microelements essential for animal health. The green mass yield of alfalfa reaches up to 25 t/ha, and dry matter yield is about 3.5–4.0 t/ha. Low production costs over 3–5 years of cultivation make this crop highly gainful, with profitability reaching up to 200% (Radović *et al.*, 2009).

Annual green manuring crops produced 110–320 kg/ha of nitrogen, while the total nitrogen production by perennial legumes ranged from 300 to 640 kg/ha (Lyngø *et al.*, 2023). Crop residues of alfalfa, clover, and ryegrass emerged to be highly productive and effective fertilizers that can replace the traditional animal-based fertilizers in organic farming systems. Alfalfa has characteristics of high green mass and dry matter productivity, robust regrowth capacity, high crude protein content (20.4%–24.7%), crude fat (1.1%–2.1%), optimal neutral and acid detergent fiber content (40.2%–47.2% and 33.0%–41.7%, respectively), and high energy value of the feed (Horvat *et al.*, 2022). A comparative biochemical analysis of alfalfa under conventional and organic cultivation technologies showed that dry matter percentage was higher under the conventional system; however, crude protein and fat content were 11% and 0.05% higher, respectively, under organic technology than in the conventional system (Marchiş *et al.*, 2018).

The development of organic crop production and the search for effective agronomic solutions from bioresources remain highly relevant to Kazakhstan's agricultural sector. In this context, the selection of these biological preparations has the aim of providing a comprehensive effect—with Organit N and Organit P optimizing mineral nutrition, Biodux stimulating growth, and Systemica M serving

as a biofungicide—based on their documented success on other crops in the region (Savin and Morgounov, 2025).

Previous studies have shown growth regulators, microbial inoculants, and biofertilizers can improve alfalfa growth, morphology, yield, biomass production, and forage quality (Peng *et al.*, 2025; Baha, 2021). However, most studies have focused on individual aspects of alfalfa performance. Therefore, the novelty of the presented study lies in the integrated assessment of agronomic traits, productivity, and forage quality, following seed treatment with biological preparations under arid conditions, providing insights relevant to similar agroecological environments.

Increasing livestock productivity and ensuring its sustainable development are in close association with the organization of balanced feeding, as well as the elimination of deficits in energy, crude protein, and microelements. The concerned study aimed for a comprehensive assessment of the effect of pre-sowing seed inoculation with biological preparations on the formation of biometric parameters, green mass productivity, and the biochemical composition of alfalfa under the arid conditions of Northern Kazakhstan.

MATERIALS AND METHODS

Agricultural practices and research methodology

The field experiments on alfalfa (*M. sativa* L.) commenced under typical soil and climatic conditions of the region. Primary soil preparation included autumn plowing to a depth of 20–27 cm, followed by harrowing to level the field surface. In spring, pre-sowing cultivation proceeded to a depth of 5–7 cm, with subsequent harrowing to create a fine crumb soil structure and also to preserve moisture. Alfalfa sowing was at the optimal agronomic time (May 15) for the region based on the soil's physical maturity. Sowing continued using a row method. The seed depth ranged from 1.5 to 3.0 cm, depending on soil

texture and seed size. The alfalfa sowing seed rate in the row method was 400–600 seeds/m². Seeds received treatment manually in a separate container 12–24 h before sowing. Biological preparations entailed application at the rate of 2 mL of each individual preparation dissolved in 20 mL of water per 1 kg of seed. For the combined variant, 2 mL of Organit N, 2 mL of Organit P, and 2 mL of Biodux incurred mixing in the same 20 mL of water per 1 kg of seed. After sowing, performing rolling sought to improve seed-to-soil contact and ensure uniform emergence. Field observations and measurements took place during the main growth and developmental stages of plants (Figure 1). The field experiments had a randomized design layout with three replicates. The size of each experimental plot was 20 m².

Meteorological conditions

Meteorological data for the 2023–2024 growing seasons corresponded to the characteristics of a sharply continental climate. During the 2023 growing season, 168.8 mm of precipitation fell, which was 37.9 mm less than the long-term average. The lack of atmospheric precipitation in May contributed to intensive evaporation of moisture required by the plant during the initial regrowth period. During the 2024 growing season, the highest amount of precipitation recorded was 275.4 mm, which played an important role in accumulating productive soil moisture reserves and had a favorable effect on plant growth and development during the vegetation period (Table 1).

Study objects and characteristics of biological preparations

Systemica M: Pre-sowing seed treatment with this biofungicide acts on plant roots throughout the vegetation period, suppressing the development of plant diseases such as powdery mildew, brown rust, and septoria.

Biodux: A growth regulator based on the complex of biologically active polyunsaturated fatty acids of the soil fungus *Mortierella alpina*, capable of forming



Figure 1. Row sowing of alfalfa during 2023–2024.

Table 1. Meteorological indicators of the alfalfa growing period for 2023–2024.

Year of observation	Months					Means
	May	June	July	August	September	
Air temperature (°C)						
2023	12.7	18.7	22.8	18.0	12.3	16.9
2024	9.1	20.9	19.5	17.1	10.5	15.4
Long-term average	12.0	17.0	18.7	16.6	11.5	15.1
Precipitation (mm)						
2023	15.8	30.8	20.5	35.5	66.2	168.8
2024	65.1	65.0	67.5	56.0	21.8	275.4
Long-term average	32.7	44.8	63.8	38.8	26.6	206.7
HTC						
2023	0.45	0.57	0.30	0.65	1.80	0.76
2024	3.30	1.07	1.10	1.05	0.94	1.50
Long-term average	0.70	0.84	1.14	0.83	0.62	0.83

prolonged nonspecific systemic resistance in plants and activating growth and biological processes.

Organit P: A safe and effective nutrient mobilizer that improves mineral nutrition of plants by increasing the bioavailability of phosphorus and potassium. The active component was the spores of the strain *Bacillus megaterium* (titer not less than 1×10^9 CFU/ml).

Organit N: Improves the nitrogen nutrition and growth performance of crops due to the ability of *Azospirillum zeae* bacteria to fix atmospheric nitrogen and convert it into forms available for plant uptake. The preparations' inclusion was according to the product line of the company Bionovatic, an

agricultural biotechnology company producing biological products for plant protection and growth stimulation (Kalin et al., 2023a).

Traits measurement

In the dry matter of alfalfa, the total carotene content determination used an organic solvent extraction method, followed by spectrophotometric measurement. Quantitative determination of total nitrogen and crude protein content in alfalfa dry matter succeeded in using the Kjeldahl method, with sulfuric acid digestion and a conversion factor of $N \times 6.25\%$. The dried and ground alfalfa dry matter reached analysis for crude fiber content using the acid-alkali digestion method,

followed by incineration in a muffle furnace at $t = 550\text{ }^{\circ}\text{C}$. In alfalfa feed analysis, nitrogen-free extract (NFE) detection employed the following formula:

$$\text{NFE} = 100 - (\text{CP} + \text{EE} + \text{CF} + \text{CA})$$

Where CP is crude protein, EE is ether extract (crude fat), CF is crude fiber, and CA is crude ash. For the preparation of Organit P: $\text{NFE} = 100 - (13.73 + 2.23 + 19.2 + 8.0) = 56.8$ (1).

The study determined the alfalfa green mass yield by harvesting aboveground biomass from sampling plots at the budding-early flowering stage. Cutting proceeded at a height of 5–7 cm above the soil surface. The harvested green mass incurred weighing and calculation per hectare. The dry matter yield determination continued by collecting a representative sample and drying it to constant weight, with the results expressed in g/m^2 . In experiments with pre-sowing seed treatment 1–2 days before sowing with biological preparations, the experimental scheme and dosages are available in Figure 2.

Statistical analysis

Statistical analysis of the experimental data progressed using analysis of variance (ANOVA) and correlation analysis in STATISTICA version 10 (StatSoft). The significance of differences between treatment means underwent evaluation using the least significant difference test at the 0.05 probability level ($\text{LSD}_{0.05}$). The assessment of data reliability and variability had the coefficient of variation (V%) and standard error of the mean (Sx) calculated.

RESULTS AND DISCUSSION

In this present research on alfalfa (*M. sativa* L.) with seed inoculation in biopreparations, the data entailed recording on various traits, including plant height, plant density, leafiness, green mass yield, and biochemical composition. According to observations during 2023–2024, at the beginning of the flowering stage, average plant height ranged from 58.0

to 64.0 cm. In the first and second years of plant development, a significant increase in plants' density was evident. With seed treatment, the maximum plant density recorded was in the variants Organit P + Organit N + Biodux ($180.6\text{ plants}/\text{m}^2$) and Biodux ($180.3\text{ plants}/\text{m}^2$) compared with minimum values in the variants of the control and Organit N ($170.0\text{ plants}/\text{m}^2$). Leafiness reached 46.2% in the variant Systemica M and 44.9% in Organit P + Organit N + Biodux, which exceeds the control variant by 6.4% and 3.7%, respectively (Table 2). These results revealed an intensive growth and development of alfalfa row crops with the influence of the mentioned biopreparations (Marinova *et al.*, 2023).

The digestible protein content in the alfalfa dry matter with control was 10.6%, while with the application of Organit N and Systemica M, the said values rose to 12.3% and 12.1%, respectively. In all other variants, the said indicator was below the control. On average over the two-year study, the carotene content across the treated variants ranged from 66.4 to 89.8 mg/kg . The highest carotene content (89.8 mg/kg) appeared with the application of the combined biopreparation variant (Organit N + Organit P + Biodux), which was 23.9% higher than the control variant (72.5 mg/kg). However, in the variants Organit N and Biodux, the carotene values were below the control variant. Past studies also confirmed the highest protein content and nutritional values with the application of biopreparations in alfalfa feed (Soto-Zarazúa *et al.*, 2017). The greater variability of digestible protein and crude fiber may be due to treatment-induced changes in plant morphology, particularly the leaf-to-stem ratio. Since leaves contain more protein and stems contain more fiber, even small changes in plant structure can substantially affect these parameters (Okumus *et al.*, 2021).

Seed inoculation with the biopreparations, such as Organit N and Systemica M, increased the nitrogen concentration in alfalfa samples to 2.84% and 2.79%, respectively. The biopreparations also had a positive effect on ash content and increased to 9.6%, compared with the control



Figure 2. Pre-sowing seed treatment and application rates of biopreparations.

Table 2. Evaluation of agronomic and biological traits depending on the use of biopreparations during 2023–2024.

Variants	Plant height (cm)	Plant density (pcs/m ²)	Leafiness (%)	Green mass yield (g/m ²)
Control	61.0	170.0	43.2	148.2
Organit P	59.5	170.6	43.5	151.1
Organit N	58.0	170.0	42.6	146.2
Biodux	64.0	180.3	43.6	150.7
Systemica M	62.5	170.3	46.2	154.7
Organit P + Organit N + Biodux	59.0	180.6	44.9	160.0
LSD _{0.05}	1.3	0.4	0.1	1.4

value (7.7%). Across all biopreparation variants, variations in fat content and metabolizable energy ranged within 2.23%–2.34% and 9.46–10.45 MJ/kg, respectively. No considerable variations resulted in the content of phosphorus (P), magnesium (Mg), and calcium (Ca). In the variants of Organit N, Biodux, and Organit P, the metabolizable energy content had the amounts of 10.12–10.45 MJ/kg alfalfa dry matter, which was, on average, 3.5% higher than the control (9.90 MJ/kg). The differences in the nutritional value of alfalfa dry matter were nonsignificant, revealing moderate variability (Table 3). Baidalin *et al.* (2024) also reported that with pre-sowing seed treatment of legumes with bacterial strains (Rhizotorphin, Flavobacterin, Mizorin, and Azolene), the significant increase was apparent for crude protein content.

According to the correlation between green mass yield and agronomic traits under alfalfa seed inoculation with biopreparations,

the plant height ($r = +0.59$), plant density ($r = +0.46$), leafiness ($r = +0.54$), and fiber content ($r = +0.36$) demonstrated moderate correlations, while nitrogen content ($r = +0.17$) showed a weak correlation (Table 4). Past studies revealed green mass yield had a positive association with the plant height, plants' density, and plants' leafiness (Serekpavev *et al.*, 2015; Kovács *et al.*, 2023).

During forage harvesting, the primary objective was to preserve the maximum nutritional quality of grasses. Therefore, it was notably essential to determine the optimal harvesting time, since delayed cutting may reduce nutritional values of the forage. Alfalfa cut at the budding stage contains 10% more protein than at the flowering stage (Timoshkin *et al.*, 2020). The protein content in alfalfa dry matter had significant influence from the cutting timings and number of cuttings. In ensuring maximum yield of feed units and digestible protein per hectare, harvesting

Table 3. Biochemical composition and nutritional values of alfalfa dry matter on the use of biopreparations during 2023–2024.

Parameters	Protein (%)	Carotene (mg/kg)	Nitrogen (%)	Crude fiber (%)	Ash (%)	Fat (%)	NFE (%)	Digestible protein (%)	Metabolizable energy (MJ/kg)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)
Control	15.74	72.5	2.52	23.3	7.7	2.30	50.9	10.6	9.90	0.31	3.27	2.78	0.45
Organit P	13.73	74.6	2.20	19.3	8.0	2.23	56.8	8.9	10.45	0.29	3.13	2.54	0.44
Organit N	17.75	66.8	2.84	21.7	9.6	2.31	48.63	12.3	10.12	0.31	3.21	2.82	0.46
Biodux	15.75	66.4	2.52	21.2	8.1	2.31	52.61	10.7	10.18	0.30	3.62	2.54	0.43
Systemica M	17.44	82.5	2.79	25.1	9.0	2.34	46.08	12.1	9.65	0.32	3.17	2.76	0.45
Organit N + Organit P + Biodux	14.35	89.8	2.30	26.4	8.0	2.34	48.88	9.51	9.46	0.31	3.37	2.77	0.46
V (%)	9.3	10.5	9.2	11.2	7.4	2.04	6.83	12.8	7.2	7.6	6.76	6.21	9.4
Sx	1.4	6.4	0.22	2.5	0.6	0.04	3.49	1.5	0.73	0.02	0.21	0.16	0.05

Table 4. Correlation matrix of agronomic traits and feed quality with the use of biopreparations during 2023–2024.

Traits	Green mass yield (g/m ²)	Plant height (cm)	Plant density (plants/m ²)	Leafiness (%)	Crude protein (%)	Fiber (%)	Fat (%)	Ash (%)
Plant height (cm)	+0.59	1						
Plant density (pcs/m ²)	+0.46	+0.34	1					
Leafiness (%)	+0.54	-0.37	-0.37	1				
Crude protein (%)	+0.17	-0.30	-0.17	+0.29	1			
Fiber (%)	+0.36	+0.41	+0.43	+0.17	-0.05	1		
Fat (%)	+0.03	-0.12	-0.13	-0.15	+0.26	-0.2	1	
Ash (%)	-0.01	-0.30	-0.07	+0.19	+0.29	-0.17	+0.29	1

alfalfa should be at the end of the budding stage and at the beginning of flowering during the first cutting. At this stage, it maintained the highest protein level, low-fiber content, good digestibility, and sufficient energy values. The biopreparations application at the early flowering stage provided the highest crude protein content versus with the control in alfalfa (Kalin *et al.*, 2023b).

The crude protein content in the variants treated before sowing with Organit N and Systemica M showed the highest values, reaching 17.75% and 17.44%, respectively, and exceeding the control sample by 2.01%–1.70%. The variants Biodux, Organit N + Organit P + Biodux, and Organit P showed lower values of protein (15.75%, 14.35%, and 13.73%, respectively) than the control. The

agronomic practices influenced the crude fiber content as follows: the control variant was 23.3%, while lower values were evident in the variants Organit P, Biodux, and Organit N, ranging from 19.3% to 21.7%. However, the higher values (25.1% to 26.4%) resulted in the variants Systemica M and Organit N + Organit P + Biodux.

Analysis of the biochemical composition of alfalfa samples over two years showed protein content increased in the variants Organit N (by 12.8%) and Systemica M (by 10.8%) compared with the control group. However, the variant Organit P revealed a decrease in protein content (by 12.8%) relative to the control group. This phenomenon may be because the protein content was largely genetically influential and also

dependent on the cutting time and the plant development stage at the time of harvesting. Zotikov and Zubareva (2024) reported pre-sowing seed treatment with Organit P, Organit N, Pseudobacterin 3, and Biodux provided the greatest yield increase in soybeans.

The highest yield (160.0 g/m²) and yield increase (11.8 g/m²) emerged with the tank mixture (Organit P + Organit N + Biodux). With an increase in green biomass productivity, forage quality may decline; however, in this study, the use of biopreparations resulted in a simultaneous increase in both indicators by 3%–15%. The integration of biological preparations into the cultivation technology is an effective approach to improve the agroecosystem productivity and product quality. According to Fomin *et al.* (2022), the application of growth stimulants, microbiological fertilizers, and a biofungicide improved the photosynthetic activity and grain yield and quality in winter wheat crops.

CONCLUSIONS

With the rational use of forage resources, the crop producers should focus on generating high-protein feed products. The use of biopreparations in alfalfa (*M. sativa* L.) cultivation technology positively affects plant growth and development and improves the biochemical composition of hay. The pre-sowing seed treatment with biopreparations Organit N and Systemica M increased crude protein content by an absolute 1.70% to 2.01% and reduced fiber content by up to 17%, thereby improving the forage quality and nutritional values of alfalfa hay. For agricultural production, pre-sowing treatment with Organit N or Systemica M is a high recommendation for increasing crude protein content, while the combination of Organit P + Organit N + Biodux is the best option for increasing total forage biomass.

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