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Enhancing Alfalfa Productivity with Tuber: Associated and Cellulolytic Bacteria in the Aral Sea Region

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ABSTRACT

Enhancement of soil fertility and yield via application of microbial preparations to increase alfalfa productivity presents a potential sustainable approach to mitigate famine through augmentation of protein-rich food supplies. This study investigates the influence of active strains of tuber-associated and cellulolytic bacteria on alfalfa productivity using traditional microbiological and agronomical methodologies. Varied strains of *Sinorhizobium meliloti* bacteria, in combination with cellulolytic bacteria and mineral fertilizers, were tested on alfalfa plants. Multiple replicates for each treatment were conducted, inclusive of a control group without bacterial application. A significant increase in the germination rate of alfalfa seeds, ranging between 80-90%, was observed following the application of microbial preparations based on cellulolytic bacteria. Variant 4 W (CLB+ *Sinorhizobium meliloti* IMVZH5-1) exhibited a higher density of plants per square meter (1205 pieces). When employing this preparation, the amount of green matter was 47 C/ha, the production of alfalfa was 3 C/ha higher, and the seedling absorption rate was 11.1% higher compared to the control group. The biggest contribution to the rise in the number of inorganic nitrogen substances in the soil came from the application of CLB and ANP fertilizers. The results obtained can be used as a basis to increase the alfalfa yield in the conditions of the Kyzylorda region.

1. INTRODUCTION

In response to the rapid global population expansion, an escalation in food production is imperative to cater to human consumption needs. The paucity of arable land has rendered the expansion of farmed land challenging, necessitating effective management of the remaining cropland for agricultural production in the 21st century [1]. Prioritizing the development and implementation of environmentally friendly agricultural systems and food products has become an exigency in modern agro-industrial production.

Rhizobia, a class of free-living soil microorganisms, have been identified as possessing the capability to fix atmospheric nitrogen, accomplished through symbiosis with suitable leguminous plants. Rhizobia are a genus of Proteobacteria phylum that are polyphyletic, with all of the species falling into the alpha- and beta-proteobacteria classes [2, 3]. This process involves the bacterial nitrogenase enzyme converting dinitrogen into ammonia, a process known as biological nitrogen fixation. By eliciting the overexpression of specific genes, Rhizobia have been found to provide plants with resistance against various diseases and enhance soil solubilization of organic phosphate [1]. The practice of leguminous seed priming with rhizobia, prevalent in several agricultural systems, has been recognized as an efficient method for rhizobial introduction to the legume rhizosphere [4, 5].

Rapidly-growing rhizobium *Sinorhizobium meliloti* works in association with legumes from the Medicago, Melilotus, and Trigonella genera to fix nitrogen from the atmosphere. Among the most effective associations involving nitrogen-fixing bacteria and legume plants is the symbiotic relationship of alfalfa with S. meliloti. In this way, alfalfa aids in the assimilation of nitrogen into the soil, which benefits the environment and the economy by reducing the need for synthetic N fertilizers [6]. A number of studies have explored the role of nodule bacteria in crop productivity over the last 15 years. Thus, Tabande et al. [7] explored the effect of Sinorhizobium meliloty inoculation treatment on the yield, quality, and safety of alfalfa cultivated in zinc-polluted soil. Similarly, Azib et al. [8] studied crops' stress tolerance after Sinorhizobium meliloty treatment.

In research by Al-Barakah et al. [9], the nitrogen content and dry biomass indicators of alfalfa generated by the Sinorhizobium meliloty inoculation method were monitored. Remarkably, Sinorhizobium inoculation decreased copper and zinc stress in alfalfa plants, according to a study by Jian et al. [10]. Different types of alfalfa plant treatment including Sinorhizobium and Pseudomonas strains were utilized to analyze plant growth patterns, physiological indices, and biochemical characteristics under conditions of low phosphorus levels in a study by Fu et al. [11]. It was shown that alkali tolerance, chlorophyll levels, root length, and plant height correlated with alfalfa plants Sinorhizobium treatment [12]. Despite the fact that numerous research groups study the effect of Sinorhizobium treatment on legumes, the impact of nodule bacteria on alfalfa yield in the Kyzylorda region is unknown and further research is needed to explore this phenomenon.

The relevance of legumes, particularly alfalfa, to research is underscored by their agricultural significance and high nutritional value. With the introduction of organic agriculture legislation and a shift towards organic farming, perennial legume crops assume special importance in the Kyzylorda region [13]. Characterized by a continental desert climate with extreme temperature variations and low precipitation, the Kyzylorda region in southern Kazakhstan presents a unique context for alfalfa growth and productivity analysis. The region's sandy desert soils interspersed with pockets of alluvial soils pose salinization challenges to agriculture, necessitating efficient water management for irrigation from the Syr Darya River. The potential of alfalfa to enhance soil fertility through nitrogen fixation aligns with the shift towards organic farming, providing insights into sustainable agricultural practices.

The study aimed to examine the influence of scarification on alfalfa seed germination, ascertain alfalfa seed productivity and germination post bacterial treatment, track the dynamics of nitrogen mineral component concentration in the soil under alfalfa seedlings, determine the total nitrogen content in the alfalfa green mass under varying treatments, and identify the impact of the examined factors on the indicators of alfalfa photosynthetic activity. Scarification, the process of breaking, scratching, or softening the seed coat to enhance germination, can reduce or eliminate the barrier that prevents moisture and gases from reaching the seed's embryo, thereby promoting quicker germination.

This research seeks to add to the existing body of knowledge by investigating the adaptability of alfalfa to harsh conditions and its compatibility with organic farming. This could potentially foster agricultural diversification and resilience, thereby benefiting the region's economic prospects that have historically relied on cotton cultivation.

2. MATERIALS AND METHODS

The work aimed to use biofertilizers to boost alfalfa productivity in the Aral Sea. These "bio" fertilizers contain living bacteria that break down cellulose with the help of cellulases. The object of the research was a year-old alfalfa plant of the variable Agnia variety from the V. R. Williams Research Institute of Feed. Small-scale experiments were conducted at the experimental site of Bolashak University (Kyzylorda city). The studies were conducted in the experimental field of the Department of crop production named after I. A. Stebut of St. Petersburg State Agrarian University in 2012, 2013, and 2014 to identify the most efficient nodule bacteria strains and ascertain their effects on the productivity of alfalfa changeable in the Leningrad region.

Experiments in small land cells were conducted in several configurations in the agro-biotechnological field of Bolashak University in Kyzylorda. Alfalfa nodule bacteria strains and their mixtures with cellulolytic bacteria and mineral fertilizers were employed to create different variants. For seed inoculation, the following strains of nodule *Sinorhizobium meliloti* bacteria were used: the production strain *Sinorhizobium meliloti* 415b, the promising strains *Sinorhizobium meliloti* IMVL5-16 and IM VL 5 (Microbiology), No. 4 W (CLB+ *Sinorhizobium meliloti* IMVL5-1), and ammonium nitrate was used as a mineral fertilizer for alfalfa. In the control variant, no nodule bacteria treatment was applied to the alfalfa seeds. Alfalfa seeds of the "Semirechensk local" type was sown in the cells.

The strains were cultivated on the Luria Bertani (LB) medium at 37°C. Alfalfa seedlings were surface sterilized for 5 minutes with 70% ethanol and then washed with sterile water. The seeds were sown on moist filter paper in Petri dishes and allowed to sprout in a darkened growth environment. The seeds from the previous 20 years must be scarified so that the outer shell is broken before they can be utilized for sowing. To disrupt the integrity of the seeds' outer shell, the seeds were first treated with a biological preparation of CLB before being deposited for five hours. Then the seedlings were injected with the experimental strains, whereas seedlings acting as a control variety were not inoculated. After following this procedure, the sowing was performed.

Productivity analysis was used to gauge how well nodule bacteria were performing. For each cell, the number of plants in the 1 m^2 of soil was calculated. The degree of acetylene-reducing ability of root nodules was used to calculate nitrogen-fixing (nitrogenase) activity, which was represented in micromoles of ethylene produced by one plant's nodules every hour. Roots with nodules were put in glass vials, and 10 percent acetylene was introduced via a rubber membrane after the air was drawn out. One sample required one hour of incubation. An Agilent Technologies 6855 Network GC System (USA) gas chromatograph was used to examine the gas mixture.

During the budding stage, the number of photosynthetic pigments was measured. Five randomly chosen plants of one variant's middle layer of leaves were chosen. The level of photosynthesis was assessed using the following formula. The plant-filled jar was put in a hermetically sealed space that was being blasted with atmospheric air at a rate of 15 L/min. One L/min of gas was taken into the gas analyzer at the chamber's output. Through a water filter, a KG-2000 incandescent bulb was used to illuminate the chamber. After recording the rate of CO_2 uptake by entire plants, the chamber was opened, the plants were cut, and then it was closed once more to measure the rate of soil respiration with roots. Using the Lowry technique in the budding phase, the protein content of alfalfa leaves was evaluated; the quantitative and qualitative contents of amino acids were assessed using ion-exchange liquid-column chromatography and an automated analyzer.

The choice of specific measurements in the study was made with the intention of comprehensively assessing the effects of the treatments on alfalfa productivity. Each chosen measurement reflects a different aspect of the plant's performance, providing insights into its physiological, biochemical, and metabolic responses to the applied treatments. These chosen measurements collectively provide a comprehensive understanding of the treatment effects on alfalfa productivity. Nitrogenase activity directly addresses the plant's nitrogen supply, photosynthetic pigments give insights into energy capture and utilization, and protein content relates to the nutritional value of the plant. By examining these aspects, the study can holistically evaluate the efficiency of the applied biofertilizers, bacteria strains, and mineral fertilizers in enhancing various facets of alfalfa growth, making informed conclusions about their potential to boost overall productivity.

3. RESULTS

Cellulolytic (cellulose-degrading) bacteria (CLB) comprehensively contribute to plant growth: they increase the seed germination ability and have a great influence on the further development of alfalfa. This contributes to a large accumulation of plant green mass throughout the growing season.

The research showed that CLB had a favorable impact on seed germination (Table 1).

The largest amount of alfalfa per 1 m^2 of the area was recorded when using variant No. 4 W (CLB+ *Sinorhizobium meliloti* IMVL5-1) – 1205 pieces. This variant also had a high germination rate (absorbability) of alfalfa – 74.3%.

The smallest indicators for the number of plants (1025 pieces) and germination rate (63.2%) were found to be in the control variant. In this variant, the strains of cellulolytic nodule bacteria were not used. In the heyday phase of the second mowing, the yield of alfalfa was determined. Table 2 shows the height of the alfalfa culture after bacterial treatment and the amount of green mass.

Table 1. General provisions of the scarification influence on the germination of alfalfa seeds

Experiment Variants	Number of Plants, Pieces/m^2	Germination, %
No. 1 Zh (control version)	1025	63.2
No. 2 Zh (CLB+ ANP fertilizer)	1123	69.3
No. 3 Zh (CLB+ Sinorhizobium meliloti IM VL 5)	1143	70.5
No. 4 Zh (CLB+ Sinorhizobium meliloti IM VL 5-1)	1205	74.3
No. 5 Toad (CLB + Sinorhizobium meliloti 24)	1134	70.0

Table 2. General provisions of alfalfa seed productivity and germination after bacterial treatment

Experiment Variables	Height, cm	Yield of Green Mass, C/ha	Height, cm	Yield of Green Mass C/ha
	I-place		II-place	
No. 1 Zh (control version)	60.1	300/17.25	57.1	295/16.75
No. 2 Zh (CLB+ ANP fertilizer)	64.3	328/19.25	60.2	320/18.75
No. 3 Zh (CLB+ *Sinorhizobium meliloti* IM IN W)	63.5	350/19.25	61.3	334/18.55
No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1)	68.5	349/20.00	64.5	342/19.75
No. 5 Toad (CLB + Sinorhizobium meliloti 24)	65.6	348/20.25	63.5	341/19.5

The maximum yield of blue juveniles in the first quarter was obtained in variants No. 3 Zh and No. 4 Zh (350 and 349 C/ha, respectively). The height of plants in these variants was 68.5 and 68.3 cm, respectively (Figure 1).

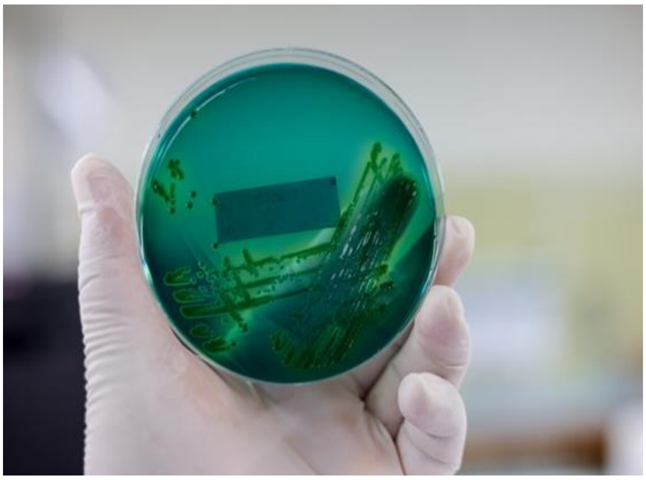

Figure 1. No. 4 Cellulolytic Bacteria

In the second trench in variant No. 4 W (CLB+ *Sinorhizobium meliloti* IMVZH5-1), the height of alfalfa (64.5 cm), the productivity of green mass and hay (342 and 19.75 C/ha, respectively) are higher than other practical and control options.

Alfalfa benefits from the availability of nutrients throughout the whole vegetative period, but notably during the crucial stages of its growth and development. The mineral forms of nitrogen are particularly significant since they have a direct impact on the vegetative part's growth, as well as several biochemical activities that raise the dry matter content and yield.

Table 3 shows the dynamics of nitrogen mineral component concentration (mg/kg) for different variants.

Table 3. Nitrogen mineral component concentration dynamics (mg/kg) for different variants in the experimental soil

Experiment Variants	Mineral Nitrogen Content, mg/kg
No. 1 Zh (control version)	13.2
No. 2 Zh (CLB+ ANP fertilizer)	21.7
No. 3 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5)	15.3
No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1)	18.5
No. 5 Toad (CLB + *Sinorhizobium meliloti* 24)	17.8

The application of CLB and the ANP fertilizer had the greatest impact on the rise in the number of mineral nitrogen compounds in the soil, as it was demonstrated by the findings of experimental data analysis. The usage of bio-preparations also raised nitrogen levels, but they were unable to override the effects of applying CLB + ANP fertilizer. With the addition of No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1), the greatest rates among bio-preparations were attained.

The most important criterion for the level of metabolic activities is the amount and quality of crop productivity. Its structure and development are determined by biological traits, growth techniques, and particularly nutritional circumstances. Only through a thorough investigation of the impact of fertilizers on the development and growth of plants is it feasible to regulate plant critical activity processes in the desired range. The amounts of nutrients in various plant organs reveal the ideal levels of macro- and micronutrients in those tissues during certain growth and development phases. They support optimum cell metabolism, making them potential markers of the nutrient status of plants mineral nutrition [14].

According to the findings of the study, it can be concluded that the application of No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1) resulted in much greater nitrogen content in the leaves of alfalfa than the fertilizer-free form (Table 4).

Table 4. Content of total nitrogen in the green mass of alfalfa according to different variants

Experiment Variants	Content of Total N (% of dry matter)
No. 1 Zh (control version)	1.36
No. 2 Zh (CLB+ ANP fertilizer)	2.08
No. 3 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5)	2.19
No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1)	2.44
No. 5 Toad (CLB + *Sinorhizobium meliloti* 24)	2.31

Comparing the results obtained, it can be stated that the implementation of nodule bacteria significantly improves the content of total nitrogen in alfalfa green mass. The highest nitrogen rates were observed when utilizing variant No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1). Variants No. 3 and No. 5 showed similar results. However, variant No. 2 was not as successful.

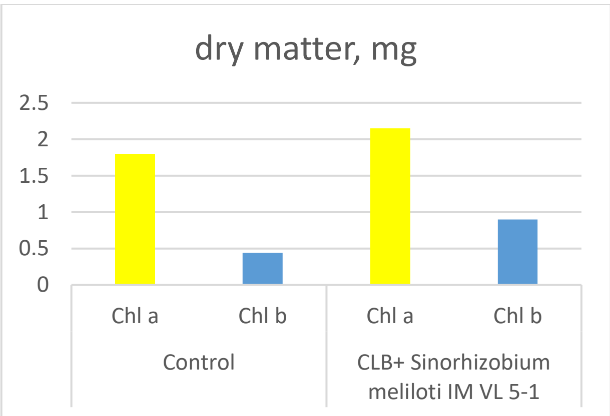

Figure 2. Alfalfa chlorophyll content after inoculation with CLB+ *Sinorhizobium meliloti* IM VL 5-1

The growth in yield is influenced by the buildup of dry matter by plants, which is directly correlated with the photosynthetic activity of the leaves. In contrast to the control variant, suspensions of microorganisms used for seed inoculation had a favorable influence on the accumulation of photosynthetic pigments in alfalfa leaves in the studies (Figure 2). Plants whose seeds were inoculated with combination No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1) showed the highest benefit (the content of chlorophylls a and b almost rose significantly compared to the control). There is no question that the presence of more chlorophyll in the leaves was a result of the introduced strains' beneficial influence on rhizobia, whose functional activity is directly correlated with the rate of nitrogen fixation.

Since nitrogen is mostly found in amino acids and proteins and the primary CO_2 absorption enzyme makes up more than half of the soluble protein content of the photosynthetic cell, the intensity of photosynthesis of plant leaves often corresponds strongly with the nitrogen level in them. Undoubtedly, the key factor contributing to the increase in plant photosynthesis intensity was the intensification of nitrogen fixation following pretreatment application. A more active symbiotic apparatus that develops on plant roots during the preparation inoculation may also raise the “necessity” for absorption from the root system, hence enhancing the photosynthetic activity of leaves. The extensive unloading of phloem element terminals in nodules, which results in a concentration gradient of carbon transport forms, predominantly sucrose, in the conducting system and speeds up their outflow from leaves, constitutes the physiological foundation of such a “necessity”. This, thus, speeds up the uptake of carbon during photosynthetic processes by removing the constraints on photosynthesis imposed by its byproducts following the feedback principle.

Thus, in plants that have been inoculated, the efficient operation of the symbiotic system promotes the accumulation of photosynthetic pigments and heightens photosynthesis. Since photosynthetic carbon absorption is the foundation of a plant organism's biological productivity, including that of those capable of symbiotic nitrogen fixation, the buildup of organic materials aids in the active creation of plant biomass. One of the key factors determining a plant product's biological value is its amino acid makeup. Comparing the variant of inoculation with CLB+ *Sinorhizobium meliloti* IM VL 5-1 to the control variant, the total content of amino acids in the leaves for which the analysis was conducted increased drastically. At the same time, a rise in the quantities of the amino acid’s methionine, histidine, arginine, and tyrosine was seen. These amino acids are found in modest amounts in plant leaves and are thought to be one of the constraints on the pace of protein synthesis, particularly in generative organs. Lysine, the most lacking necessary amino acid for both humans and animals, was also found in higher concentrations (Table 5).

Table 5. Productivity and protein content in alfalfa leaves inoculated with mono- and binary mixtures of microorganisms

Variant	The mass of Green Mass, g/vessel			Protein Content in Leaves, % Dry Matter	Significance of Differences
	1st Mowing	2nd Mowing	Total Mass		
No. 1 Zh (control version)	14.5±0.3	16.8±0.2	31.2	13.4	P=0.000
No. 2 Zh (CLB+ ANP fertilizer)	16.4±0.4	18.5±0.4	36.2	13.6	P=0.000
No. 3 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5)	20.1±0.2	23.5±0.3	42.3	16.9	P=0.000
No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1)	25.3±0.6	29.8±0.3	53.8	21.7	P=0.000
No. 5 Toad (CLB + *Sinorhizobium meliloti* 24)	22.3±0.5	24.3±0.1	46.3	19.4	P=0.000

The variant with a combination of CLB and *Sinorhizobium meliloti* IMVL5-1 (Variant No. 4 W) resulted in the highest germination rate (74.3%) and the largest number of plants per square meter (1205 plants). The control variant, where no cellulolytic nodule bacteria were used, had the lowest germination rate (63.2%) and the smallest number of plants (1025 plants per square meter). Variants No. 3 Zh and No. 4 Zh showed the highest yield of blue juveniles in the first quarter (350 and 349 C/ha, respectively). In the second trench, Variant No. 4 W had the highest height of alfalfa (64.5 cm), green mass productivity, and hay yield (342 and 19.75 C/ha, respectively). The application of CLB and ANP fertilizer had the greatest impact on the increase in the number of mineral nitrogen compounds in the soil. Inoculation with CLB+ *Sinorhizobium meliloti* IM VL 5-1 resulted in a higher nitrogen content in the leaves of alfalfa compared to the fertilizer-free form. The inoculation of alfalfa seeds with nitrogen-fixing microorganisms increased the nitrogenase activity of the root nodules, the intensity of photosynthesis, the growth of the vegetative mass, and raised the protein content and quality of the plants.

As a result, the inoculation of alfalfa seeds with the preparation of nitrogen-fixing microorganisms optimizes the plant's production process by increasing the nitrogenase activity of the root nodules and the intensity of photosynthesis, which in turn promotes the growth of the vegetative mass and raises its protein content and quality. In summary, the application of cellulolytic bacteria, in combination with nitrogen-fixing bacteria, was found to have beneficial effects on the growth, development, and nutrient content of alfalfa plants. The use of specific strains of bacteria and fertilizer had a significant impact on various factors, including seed germination, plant height, yield, and nutrient levels in the soil and plants. The findings of this study suggest that inoculation with CLB+ *Sinorhizobium meliloti* IM VL 5-1 could be a valuable strategy for optimizing the production process of alfalfa plants, increasing yield, and improving the nutritional quality of the crop.

4. DISCUSSION

The implementation of nodule bacteria-based preparations is an eco-friendly method to boost crop yields and enhance soil fertility. Numerous studies show a positive correlation between biologics utilization and enhanced plant growth and development [15-17]. Given its superior value (digestibility and protein content), widespread cultivation, inexpensive cost of cultivation, and seasonal distribution, alfalfa is among the most significant feed legume crop [8]. However, its growth and development are heavily influenced by cultivation conditions, such as water supply, temperature, pathogen infection, and soil contamination [9-11, 18-20]. The present study demonstrated that seed treatment with a preparation containing CLB and *Sinorhizobium meliloti* strains significantly improved the green mass yield and height of alfalfa plants. These data confirm the findings presented in previous research. Thus, a recent study evaluated the development and nodulation of alfalfa plants in conjunction with six different strains of *Sinorhizobium meliloti* under water-scarce conditions [8]. The results showed that compared to uninoculated plants, *Sinorhizobium meliloty* treatment improved the plants' ability to cope with stress. Therefore, in comparison to the non-inoculated control in all water stress levels, the plants that received *Sinorhizobium meliloti* inoculation increased shoot growth, root length, and shoot and root dry weights.

According to the findings of another investigation, the control variant's nitrogen content and alfalfa dry biomass were considerably lower than those produced by the *Sinorhizobium meliloty* inoculation treatment [9]. The present study showed that variant No. 4 Zh (CLB+ *Sinorhizobium meliloti* IM VL 5-1) treatment produced roughly the same outcome as the studies discussed, increasing alfalfa dry mass and plant growth. Alfalfa grown on soil polluted with zinc benefited from *Sinorhizobium meliloty* inoculation treatment because it produced less toxic hydrogen peroxide and malondialdehyde, which improved the crop's quantity, quality, and safety for consumption [7]. Similarly, *Sinorhizobium* inoculation reduced copper and zinc stress in alfalfa plants, which resulted in greater root and above-ground plant length and dry weight compared to the control [10, 21]. The putative ability of biologicals to remediate the soils of the Kyzylorda region was out of the scope of the present study.

However, the studied strains show the promise to increase soil fertility in the Aral Sea region, and future research could focus on the possibility to implement these findings into agricultural practices. It was shown that under limited phosphorus availability, compared to uninoculated plants, the treatment of alfalfa plants with *Sinorhizobium* alone or in combination with *Pseudomonas* dramatically increased plant growth, as well as physiological parameters, and biochemical features [11, 22]. These results were confirmed in the present study as *Sinorhizobium* treatment of alfalfa generally enhanced the crop's morphological indicators in all variants except the control. Finally, *Sinorhizobium*-inoculated alfalfa plants also showed higher alkali resistance, higher levels of chlorophyll, comparatively longer roots, greater plant height, and more branches, according to Xing et al. [12]. Similarly, the present results indicated increased photosynthesis levels in *Sinorhizobium*-treated crops in all variants but the control.

According to the data obtained in the present study, cellulose-degrading bacteria also contribute to the increased alfalfa yield. These results find endorsement in literature as well, as cellulases produced by these microorganisms have vast agro-technological implementations [23, 24]. It was demonstrated that adding cellulases to crops could improve plant development, including improved seed germination and protective benefits. They are thought to primarily act as biocontrol agents that facilitate disease inhibition and/or activate plant defense systems [25, 26]. The precise relationship between these enzymes and plants has not yet been fully understood. Similarly, in the present study, the exact mechanism of cellulose-degrading bacteria's effect on alfalfa growth and development is not determined. A hypothesis is made that celluloses produced by the studied stains act as a scarification agent facilitating seed germination.

However, cell expansion, seed germination, and seed protection are only a few biological processes that cellulases appear to be engaged in that might be connected to plant development. According to the literature, plants must use hydrolytic enzymes like cellulases to weaken their cell walls in order to develop properly [23]. These findings can be illustrated with the example of coffee seed germination. Coffee tree seeds take a long time to germinate because they are sown immediately after the harvest of the crop which falls during a cold season, besides the fact that parchment acts as a barrier to prevent the seed from absorbing water [27, 28].

Coffee seeds from the cultivar "Acaiá do Cerrado" were soaked in cellulase solution at various doses in an experiment to determine the impact of exogenous administration of the enzyme on coffee seed germination. When the seeds were exposed to the solution for 144 hours, it was discovered that the cellulase enzyme offered the highest index of speed germination and the highest germination percentage [27]. Another study showed that during the germination of lettuce seeds, cellulase contributes to the weakening of the endosperm cap and the extension of the radicle [29, 30]. The present study showed that the implementation of cellulose-degrading bacteria improved plant growth, however, additional research is needed to link the obtained results with the cellulase impact on seeds' integrity.

Additionally, seeds that had been pretreated with cellulases showed improved resistance. For example, cellulases may have direct impacts on *P. splendens*, possibly by releasing glucans that might serve as plant endogenous elicitors or by releasing poisonous compounds from the seeds that prevented *P. splendens* from germinating [23]. Given that cellulose is a key component of pathogenic oomycetes cell walls, cellulases seem to be crucial in their antagonistic interactions [23]. The present research implemented bacterial strains only. However, numerous cellulolytic fungi, in addition to bacteria, are known to play a crucial role in agriculture by promoting improved seed germination, quick plant growth and flowering, superior root systems, and higher crop yields [31-33]. These fungi include *Trichoderma sp., Geocladium sp., Chaetomium sp*., and *Penicillium sp*. Despite the fact that these fungi affect plants directly (perhaps through growth-promoting diffusible substances) and indirectly (by reducing plant infections and diseases), it is not yet known how these fungi support greater plant performance [31, 33]. Future studies regarding the effect of cellulose-degrading microorganisms on alfalfa growth in the Aral Sea region should focus not only on the crop parameters but on the mechanisms of cellulases interplay with seeds' outer layers and putative antagonistic interactions with soil microbiota. It would be also beneficial to study cellulose-degrading fungi for possible implementation in agricultural practices.

Thus, the positive effect of the preparation containing cellulose-degrading bacteria and *Sinorhizobium meliloty* on alfalfa crops can be attributed to the symbiotic interactions between these microorganisms and the plant. Cellulase-producing bacteria putatively enhance alfalfa seed germination by weakening the outer layers of the seeds and promoting their growth. Additionally, cellulases play an important role in bacterial antagonistic interactions and protect the alfalfa seeds and plants from pathogenic microorganisms. Last but not least, cellulases degrade the organic matter and enrich the soil with elements crucial for alfalfa development.

The research underscores the need for further investigations to uncover the intricate interplay between microorganisms and plants, particularly regarding cellulase interactions with seeds and potential antagonistic effects on soil microbiota, ultimately enhancing our understanding of these processes for agricultural applications. The positive correlation between *Sinorhizobium meliloty* treatment and alfalfa green mass yield is most likely due to the symbiosis between the strain and the host plant. It is highly likely that improved nitrogen fixation, owing to *Sinorhizobium meliloty* penetration into the alfalfa legumes, boosts the plant's metabolism and enables increased resistance to harsh growth conditions. Future studies could elaborate on the exact mechanisms of plant-bacterial interactions between alfalfa and microorganisms in the preparation studied.

5. CONCLUSIONS

The research has elucidated that inoculation of alfalfa seeds, specifically of the Agnia variety, with nodule bacteria biologics substantially elevates its productivity. A distinct microbial preparation, Variant No. 4 W (CLB+ *Sinorhizobium meliloti* IMVZH5-1), stands out by presenting a superior plant density, an enhanced seed germination rate, and a notable yield increase compared to other methods. Furthermore, the application of CLB and the ANP fertilizer was found to intensify the concentration of mineral nitrogen compounds in the soil. Significantly, seed inoculation has also been shown to augment the accumulation of photosynthetic pigments in the alfalfa leaves. Most remarkably, the use of the specific combination CLB+ *Sinorhizobium meliloti* IM VL 5-1 led to a marked rise in the levels of both chlorophyll and amino acids in the alfalfa leaves.

This investigation holds a pioneering stature by elucidating the robust efficacy of a specific microbial preparation, particularly in the context of the Kyzylorda region. By revealing the positive synergistic effects derived from the combined use of nodule and cellulolytic bacteria in seed inoculation, it offers a groundbreaking perspective on agronomic enhancements. Such findings underscore the potential for elevating agricultural output through innovative biological interventions.

For farmers, especially those based in regions akin to Kyzylorda, these findings offer a tangible blueprint to significantly amplify alfalfa yields. The enriched nitrogen content and bolstered photosynthetic pigments emerging from this research indicate a potential for producing a more nutritious livestock feed. Given that alfalfa is a primary dietary staple for many livestock species, the cascading benefits of its enhanced yield and nutritional profile could manifest in the meat and dairy sectors, leading to healthier livestock and potentially increased meat and dairy outputs.

Future investigations should delve deeper into the long-term ramifications of such inoculation techniques on the overarching soil health, considering the potential for soil microbial equilibrium disturbances. It is also crucial to explore any resistance that alfalfa might develop against common pests or diseases due to this inoculation. Direct impacts of heightened amino acid and chlorophyll content on livestock health and productivity warrant rigorous study. Additionally, expanding this research to encompass a variety of alfalfa strains and geographic contexts can provide insights into its broader applicability. Lastly, a comprehensive understanding of the ecological implications, especially in terms of interactions of the introduced bacteria with native soil microbial communities, is essential for ensuring sustainable agricultural practices.

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