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# Effects of Different Bio-fertilizer Treatments on the Growth and Quality of Alfalfa

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## ABSTRACT

**Background:** Alfalfa has small seeds and is intolerant to deep-sowing. We investigated seed emergence of alfalfa variety "Longmu 801" from deep-sowing and its quality improvement through different bio-fertilizer treatments.

**Methods:** Seven fertilization treatments were tested: CK: no fertilization (control), A: amino acid fertilizer, B: amino acid fertilizer + selenium-rich yeast fertilizer diluent, C: amino acid fertilizer + selenium-rich yeast fertilizer dilution + microbial agent, D: amino acid fertilizer + algae extract, E: amino acid fertilizer + microbial agent and F: amino acid fertilizer + selenium-rich yeast fertilizer diluent + microbial agent + algae extract.

**Result:** The bio-fertilizer treatments improved the alfalfa seedling emergence, growth and quality. Treatment E increased the emergence rate, plant height and yield by 10.6%, 41.0% and 68.9%, respectively; decreased the stem-leaf ratio, neutral detergent fiber and acid detergent fiber by 41.7%, 3.7% and 3.17%, respectively and increased crude protein, crude fat and relative feeding value by 2.5%, 0.5% and 8.6%, respectively; compared with that of the control. Soaking alfalfa seeds in 0.2% amino acid fertilizer and applying 13.5 g microbial agent was suggested as the most efficient fertilization technique to promote alfalfa emergence, growth and quality and provide profits to farmers due to its low cost.


**Key words:** Alfalfa growth, Amino acid fertilization, Bio-fertilizer, Microbial agent.

## INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a perennial and high-quality legume with high nutritional value and is known as the "king of forage" (Cao *et al*., 2011). Alfalfa has small seeds and has poor water retention capacity in shallow sowing, resulting in weak germination and growth at the seedling stage. Chemical fertilizers can promote alfalfa growth to some extent. However, their excessive application can damage the ecological environment and threaten the health and safety of humans and livestock (Li *et al*., 2019). Therefore, using bio-fertilizers as a substitute has attracted increasing research attention. Specifically, research on the effects of amino acid fertilizers, agricultural microbial agents, seaweed extracts and selenium fertilizers on crops, such as alfalfa, has gained immense attention worldwide (Kalyan *et al*., 2017).

The Bashang area receives less precipitation annually, legume forage is absent. Therefore, while planting alfalfa, appropriate deep-sowing is conducted to preserve water to improve the growth and quality of alfalfa. The "Longmu 801" alfalfa shows cold resistance, drought resistance, strong salt-alkali resistance, high grass yield and wide adaptability to different climate and soil conditions of the Bashang region (Li, 2003).

Previous studies on alfalfa fertilization have mainly focused on traditional fertilizers such as chemical fertilizers (Li, 2019). Research on the application of new bio-fertilizers to alfalfa has not been extensively conducted. Thus, this study aimed to investigate the effects of different fertilizers (amino acid fertilizers, microbial agents, algae extract and rich selenium yeast) on the growth of the "Longmu 801" alfalfa variety. These bio-fertilizers are inexpensive and environmentally friendly. Thus, exploring the effects of these bio-fertilizers and their combination treatments on the emergence, growth and quality of alfalfa cultivated through deep-sowing is necessary, which would promote efficient alfalfa production in arid and semiarid area.

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## MATERIALS AND METHODS

### Experimental materials

The Longmu 801 alfalfa variety was provided by the College of Animal Science and Technology, Hebei North University. The amino acid fertilizer comprising 13.9% organic matter, 2.3% total nitrogen and multiple amino acids was purchased from Taiwan Feihong Fertilizer Co., Ltd. Agricultural microbial agents comprising *Bacillus amyloliquefaciens*, *Bacillus gelophilus* and *Bacillus subtilis*, were purchased from Tianjin Kunhe Biotechnology Group Co. Ltd. Algae extract composed of alginate, enzymes, plant hormones and other substances was provided by the College of Life Sciences, Hebei University. Selenium-rich yeast comprising sodium selenite, yeast powder and protein was provided by the College of Life Sciences, Hebei University.

### Experimental design

The experiment was conducted in June 2020. A randomized block design was used with the seven fertilization treatments (Table 1).

Experiments of all treatments were repeated four times in a 9 $m^2$ plot area (3×3 m) with an interval of 0.5 m between groups for later data collection. Seeds in each plot were sown in 10 rows, with a spacing of 30 cm and soil depth of 3 cm.

Alfalfa seeds corresponding to a sowing rate of 22.5 kg/ha were weighed and immersed in 0.2% amino acid fertilizer solution for 12 h at 20°C under dark conditions. Subsequently, they were rinsed thrice with distilled water and placed in a cool place for drying prior to storage.

Further, microbial agents were sprayed evenly in the plot ditch before sowing (13.5 g/plot). Later, when alfalfa seedlings showed branching, 27 mL/plot selenium-rich yeast fertilizer and 0.1% algal extract diluent were sprayed.

### Indicators and methods

### Growth and production performance indicators

### Seedling emergence rate:

Seedling emergence rate (%) =

$$\frac{\text{Actual number of seedlings that emerged}}{\text{Number of seeds sown}} \times 100$$

### Plant height

At the first flowering stage, 10 alfalfa plants were randomly selected from each plot and their height from the ground to the tip of the highest leaf was measured using a ruler.

### Stem-leaf ratio

At the initial flowering stage, 10 alfalfa plants with similar growth trends were randomly selected from each plot, their stems and leaves were separated. Subsequently, the stems and leaves were placed in an oven at 105°C for 15 min and then at 65°C for 24 h. After drying, the mass of stem and leaf was weighed and the stem-leaf ratio (stem dry weight/ leaf dry weight) was calculated.

### Grass yield

Only one alfalfa plant was cut during the early flowering stage and the stubble length was 5 cm. After mowing and measuring the fresh weight in each plot, 1 kg of samples were dried at 105°C for 15 min and later at 65°C to calculate their dry matter content, according to which the hay yield was converted (Yuan *et al*., 2020).

### Nutrition indicators

The treated hay samples were stored in a dry place until further use. Crude protein (CP), crude fat (EE), crude ash (Ash), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods of Gong *et al*. (2020).

### RFV

$$RFV = \frac{DMI\ (BW)\ \% \times DDM\ \%\ (DM)}{1.29}$$

DMI is dry matter intake, DDM is digestible dry matter, BW is the body weight, DM is dry matter. Divided by 1.29 in order to achieve a RFV value of 100 at full flowering (Qaoud *et al*., 2022).

### Statistical analysis

SPSS 22.0 was used for statistical analysis and a single factor random block analysis was used. Multiple comparisons were performed using Duncan's post hoc tests. The results were shown as mean ± standard error for each treatment (Avci *et al*., 2018).

## RESULTS AND DISCUSSION

### Effects of bio-fertilizer treatments on alfalfa growth

### Emergence rate

The seedling emergence rate of each fertilization treatment was significantly higher than that of the control ($P<0.05$), which increased by 13.6%-39.9% compared to that in the control (Fig 1). Treatment E had the highest seedling emergence rate, which was 39.9% higher than that of the control.

### Plant height

Compared with the control, plant height in the bio-fertilizer treatments significantly increased by 11.7%-41.0% ($P< 0.05$) (Fig 2). Further, plant height was the highest in Treatment E and increased by 41.0% compared to that of the control.

### Stem-leaf ratio

The ratio in each fertilization treatment was significantly lower than that of the control ($P<0.05$) (Fig 3). Further, Treatment E (seeds soaked in 0.2% amino acid fertilizer + 13.5 g microbial agent) had the lowest stem-leaf ratio, which was lower by 41.7% than that of the control.

**Table 1:** Randomized block design table of the experiment.

| Treatment | Fertilization (/plot) |
|---|---|
| CK | 0 |
| A | Seeds soaked in 0.2% amino acid fertilizer |
| B | Seeds soaked in 0.2% amino acid fertilizer+27 mL selenium-rich yeast fertilizer diluent |
| C | Seeds soaked in 0.2% amino acid fertilizer+27 mL selenium-rich yeast fertilizer dilution+13.5 g microbial agent |
| D | Seeds soaked in 0.2% amino acid fertilizer+0.1% algae extract |
| E | Seeds soaked in 0.2% amino acid fertilizer+13.5 g microbial agent |
| F | Seeds soaked in 0.2% amino acid fertilizer+27 mL selenium-rich yeast fertilizer diluent+13.5 g microbial agent+ 0.1% algae extract |

### Yield

The bio-fertilizer treatments significantly affected the alfalfa yield ($P<0.05$) (Fig 4). The alfalfa yield in Treatment D (seeds soaked in 0.2% amino acid fertilizer + 0.1% algae extract) was the highest, which was higher by 68.9% than that of the control.

### Effects of bio-fertilizer treatments on the alfalfa quality

### Crude protein content

The crude protein content in each fertilization treatment was significantly higher than that of the control ($P<0.05$) (Fig 5). Further, it was the highest in Treatment E, which was 2.5% higher than that in the control.

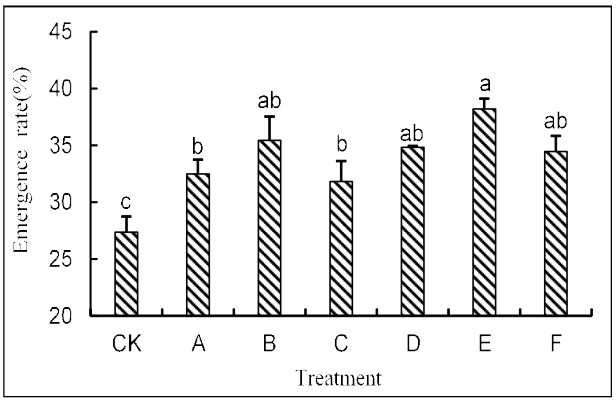


**Fig 1:** Effects of the bio-fertilizer treatments on seedling emergence rate of alfalfa.

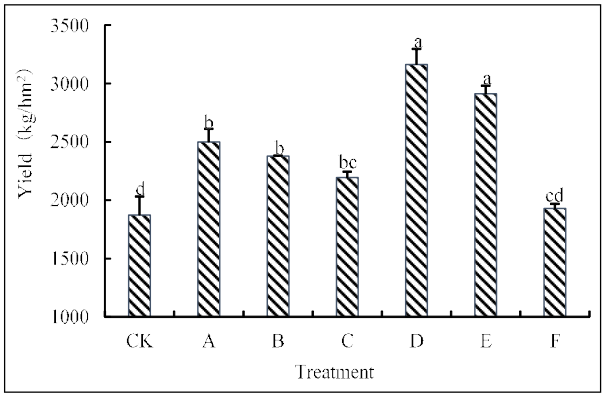


**Fig 4:** Effects of the bio-fertilizer treatments on the grass yield of alfalfa.

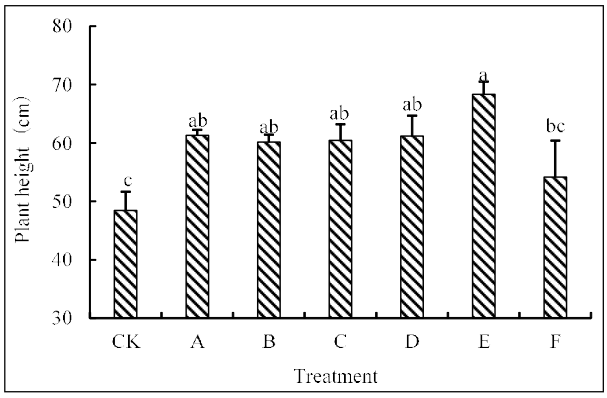


**Fig 2:** Effects of the bio-fertilizer treatments on the plant height of alfalfa.

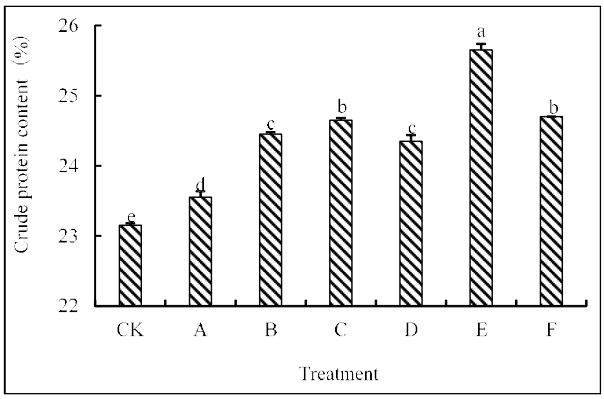


**Fig 5:** Effects of the bio-fertilizer treatments on the crude protein content of alfalfa.

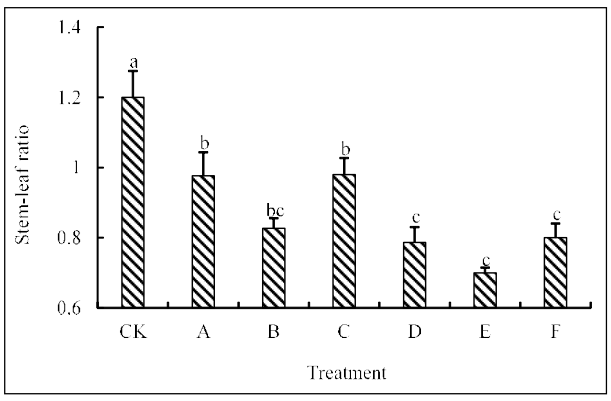


**Fig 3:** Effects of the bio-fertilizer treatments on the ratio of stem to leaf of alfalfa.

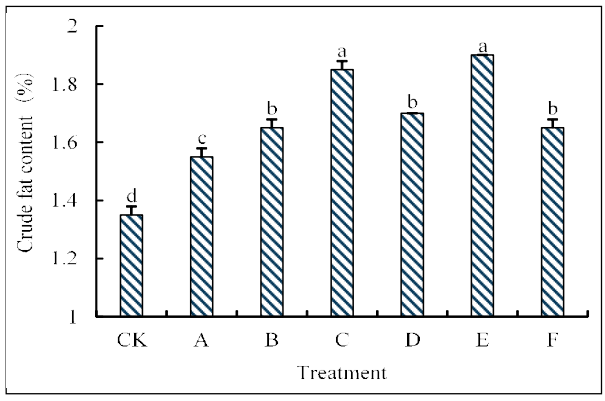


**Fig 6:** Effects of the bio-fertilizer treatments on the crude fat content of alfalfa.

### Crude fat content

The crude fat content was significantly higher in each fertilization treatment than that in the control (P<0.05) (Fig 6). In Treatment E, the EE content was the highest, which was 0.6% higher than that in the control.

### Crude ash content

The crude ash content in each fertilization treatment was significantly lower than that in the control (P<0.05) (Fig 7). It was the lowest in Treatment E, which was lower by 0.5% than that of the control.

### Neutral detergent fiber content

The neutral detergent fiber content in each fertilization treatment was significantly lower than that in the control (P<0.05) (Fig 8). It was the lowest in Treatment E and significantly lower than that in the other treatments; further, it was lower by 3.7% compared with the control.

### Acid detergent fiber content

The acid detergent fiber content in each fertilization treatment was significantly lower than that of the control (P<0.05) (Fig 9). It was the lowest in Treatment E, which was 3.1% lower than that of the control.

### Relative feeding value

The relative feeding value of each fertilization treatment was significantly higher than that of the control (P<0.05) (Fig 10). Further, it was the highest in Treatment E, which was 8.6% higher than that in the control.

### Effects of the bio-fertilizer treatments on alfalfa growth

The seed emergence rate in Treatment A was 13.6% higher than that in the control, indicating that appropriate amounts of amino acid fertilizers could significantly improve seedling emergence from deep-sowing. These results were consistent with those of Li *et al*. (2017).

Further, the plant height and yield of alfalfa in Treatment A increased by 26.4% and 33.4%, respectively and the stem-leaf ratio decreased by 18.6% compared with that of the control. These results were consistent with those of Liu *et al*. (2021).

In our study, compared with Treatment A, Treatment B improved the alfalfa height and yield in the leaf-expanding stage and reduced the stem-leaf ratio, thus, indicating that the combined application of amino acid and selenium-rich yeast fertilizers significantly promoted alfalfa growth.

Further, Chen *et al*. (2020) reported that the yield of sweet corn increased by 7.11% and 8.24% under seaweed

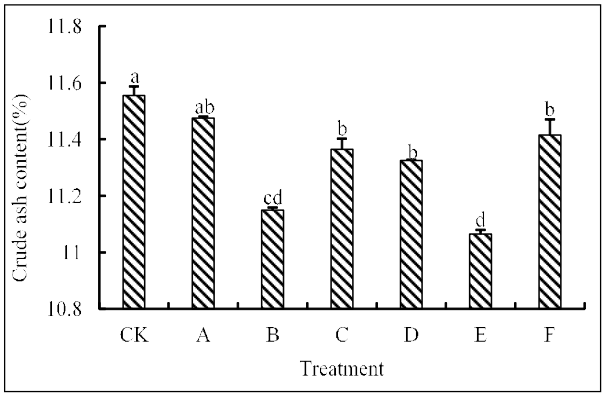


**Fig 7:** Effects of the bio-fertilizer treatments on the crude ash content of alfalfa.

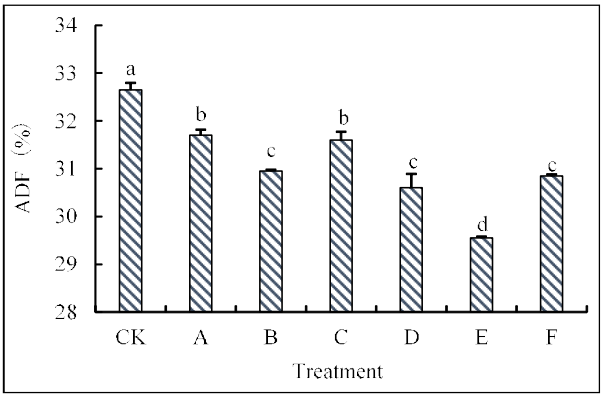


**Fig 9:** Effects of the bio-fertilizer treatments on the ADF of alfalfa.

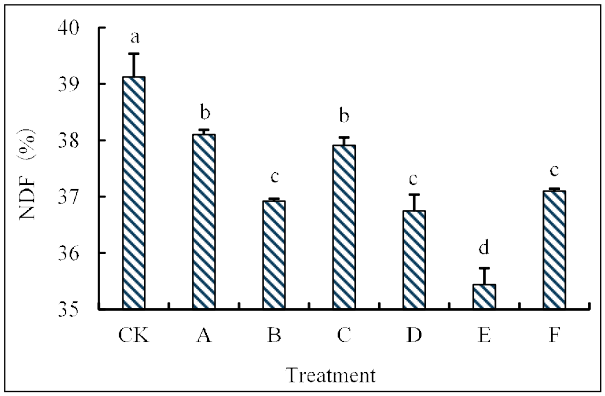


**Fig 8:** Effects of the bio-fertilizer treatments on the NDF of alfalfa.

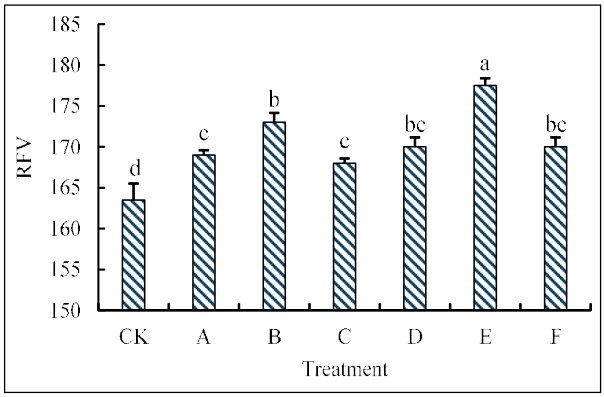


**Fig 10:** Effects of the bio-fertilizer treatments on the RFV of alfalfa.

extract treatment. In this study, compared with Treatment A, application of Treatment D at the later stage improved the alfalfa grass yield, thus, indicating that the combined application of amino acid fertilizer and algae extract had a significant promoting effect on alfalfa growth. However, the grass yield of alfalfa after applying algae extract was significantly higher than that of selenium-rich yeast fertilizer, possibly because the seaweed extract was easier to be absorbed and utilized by plants than the selenium-rich yeast fertilizer.

In our study, Treatment E (seeds soaked in 0.2% amino acid fertilizer and 13.5 g microbial agent) significantly improved the emergence rate, plant height and grass yield, reduced the stem-leaf ratio and the promoting effect was better than other bio-fertilizer treatments, possibly because the microbial agent could reduce soil alkalinity, which promotes microbial activities and plant growth.

Treatments C and F improved the seedling emergence rate and grass yield of alfalfa. Additionally, significant reductions in the stem-leaf ratio were observed, but the effects were not as significant as those observed in Treatments B, D and E. This may be due to the inhibitory effect of simultaneously applying selenium-rich yeast fertilizer diluent and algae extract on the alfalfa growth.

In conclusion, applying 0.2% amino acid fertilizer and 13.5 g microbial agent as the base fertilizer had the most significant effect on the growth indexes of alfalfa, such as seedling emergence rate, plant height, stem-leaf ratio and grass yield.

**Effects of bio-fertilizer treatments on alfalfa quality**

Treatment A significantly increased the CP content, EE content, RFV and reduced crude ash, NDF and ADF contents compared to the control. Thus, Treatment A could not only significantly improve the alfalfa grass yield, but also alfalfa quality and palatability effectively, which was similar to the results of Liu *et al*. (2021).

Treatment B promoted CP content, EE content and RFV and decreased the crude ash, NDF and ADF contents significantly, indicating that combined application of amino acid fertilizer and selenium-rich yeast fertilizer had better effect than only amino acid fertilizer. Guo *et al*. (2011). Found that spraying selenium fertilizer on alfalfa leaf could significantly increase the CP and EE content of alfalfa and reduce the crude fiber content.

In this study, combined application of amino acid fertilizer and algae extracts increased the quality of alfalfa significantly; The application effect of the algae extract on alfalfa quality was higher than that of the rich selenium yeast, possibly because algae extracts could be easily absorbed by plants than the selenium-enriched yeast fat, which was similar to the results of Jiang *et al*. (2002).

Huang *et al*. (2021) found that appropriate dosage of microbial agents significantly increased soluble protein and soluble sugar content. In this study, the Treatment E was significantly better than other bio-fertilizer treatments. This might due to the microorganisms that transform nutrients, especially some rare soil elements, into metabolites that are beneficial to crops and promote plant growth.

Alfalfa quality was significantly higher in Treatment F (seeds soaked in 0.2% amino acid fertilizer + 27 mL selenium-rich yeast fertilizer diluent + 13.5 g microbial agent + 0.1% algae extract) than that in the control and Treatment A (seeds soaked in 0.2% amino acid fertilizer). However, it was lower than that in Treatment E (seeds soaked in 0.2% amino acid fertilizer + 13.5 g microbial agent), indicating that the excessive variety and amount of bacterial fertilizer will inhibit the growth of alfalfa in the field.

In conclusion, Treatment E had the most significant effect on improving alfalfa quality, our results could provide a more scientific planting method for improving the deep-sowing method and alfalfa quality.

## CONCLUSION

Bio-fertilizer treatments significantly improved the seed emergence rate of alfalfa from deep-sowing. Treatment E showed the best promoting effect on alfalfa seedling emergence rate, which was 39.9% higher than that in the control. Further, treatment E had the best effect on increasing alfalfa yield and grass yield increased by 68.9%.

The bio-fertilizer treatments significantly improved the quality of alfalfa. Treatment E showed the highest effect with the CP content, EE content and RFV being 2.5%, 0.5% and 8.6% higher and NDF and ADF being 3.7% and 3.1%, lower than those observed in the control, respectively.

Therefore, soaking alfalfa seeds in 0.2% amino acid fertilizer could increase the germination rate of alfalfa; Soaking alfalfa seeds in 0.2% amino acid fertilizer and applying 15 kg/ha microbial agent was suggested as the most efficient fertilization method to promote the emergence and growth of alfalfa and improve its quality. These bio-fertilizers are environmentally friendly and inexpensive, thus, assuring increasing economic benefits to farmers.

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**Conflict of interest:** None.

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