

## SUSTAINABLE STRATEGIES FOR BOOSTING ALFALFA YIELD AND QUALITY UNDER DROUGHT CONDITIONS

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**Abstract.** Climate change is increasing the frequency and severity of drought stress in forage production systems, posing a serious threat to global food security and livelihoods. This study, conducted at the University of Debrecen, Hungary, to investigate the effects of trichoderma (*Tricho Immun*) and its combination with foliar fertilizer containing calcium, silicon and zinc (*Ino green*) on fresh herbage yield, protein content and protein yield of alfalfa under drought stress. The experiment followed a randomized complete block design with three treatments: control (T0), *Tricho Immun* combined with foliar fertilizer containing calcium, silicon and zinc (T1), and *Tricho Immun* alone (T2), each replicated three times. Data collected were subjected to analyses of variance using Genstat, where significantly different means were separated at a probability of 5% using the least significant difference. The results show that the biostimulant with or without nutrient management strategies significantly improved fresh herbage yield, protein content and protein yield of alfalfa compared to the control under moderate drought stress conditions. The combined application of *Tricho Immun* and foliar fertilizer containing calcium, silicon and zinc (T1) produced the highest fresh herbage of 17415 kg ha<sup>-1</sup> and protein yield of 3883 kg ha<sup>-1</sup>, followed by *Tricho Immun* (T2) alone with fresh herbage yield of 16135 kg ha<sup>-1</sup> and protein yield of 3661 kg ha<sup>-1</sup>. Notably, T1 increased fresh herbage and protein yields by 22.5% and 33.4%, respectively, while T2 boosted these traits by 13.5% and 25.8% over the control. Moreover, T2 elevated protein concentration by 10.6%, followed by T1 with 8.7% over the control. These improvements may be associated with improved plant physiological responses under drought stress conditions. Overall, our findings suggest that integrating *Trichoderma* spp. with foliar fertilizer containing calcium, silicon and zinc can be an effective strategy to improve forage productivity and quality of alfalfa under moderate drought stress.

**Keywords:** *Medicago sativa*, biological fungi, foliar fertilization, protein content, sustainable forage production, water deficit

### INTRODUCTION

Climate change is increasing the frequency and severity of drought stress in forage production systems, posing a serious threat to global food security and human livelihoods. Intensified drought stress significantly limits physiological processes in plants such as alfalfa, including photosynthesis, nutrient uptake, and biomass accumulation, ultimately reducing both yield and forage quality (BIBI and RAHMAN, 2023; APPIAH et al., 2024). The excessive use of chemical fertilizers and pesticides is linked to high economic costs and environmental challenges, intensifying the search for sustainable and resilient agronomic practices to maintain crop productivity under adverse environmental conditions (BIBI and RAHMAN, 2023; CSÓTÓ et al., 2024). Therefore, there is a need for eco-friendly, sustainable strategies to mitigate the impact of climate-induced damage on alfalfa productivity.

Biostimulants, such as beneficial microbes, have emerged as novel tools for improving crop productivity under adverse environmental conditions. Among these, *Trichoderma* spp. are globally recognized for their roles as plant growth promoters, biofertilizers, and biocontrol agents (CALVO et al., 2014; DU JARDIN et al., 2015). These beneficial fungi enhance nutrient uptake and improve the solubilization of nutrients such as phosphorus, iron, potassium, and zinc by producing siderophores and organic acids (CSÓTÓ et al., 2024). Furthermore, they activate plant defence mechanisms and improve drought tolerance by modulating physiological and biochemical pathways, including osmotic adjustment and antioxidant activity. Previous studies in various cropping systems have demonstrated that *Trichoderma*-based biostimulants significantly improve photosynthetic efficiency, chlorophyll content, and yield, even under severe water deficit conditions (RAWAL et al., 2022; ROUPHAEL and COLLA, 2021; APPIAH et al., 2024). ZHANG et al. (2019) demonstrated that inoculating alfalfa with *Trichoderma* spp. positively influences soil nutrient dynamics, rhizosphere microbial communities, and plant growth, suggesting its potential to enhance both productivity and forage quality.

Foliar application of fertilizers containing calcium, silicon, zinc and magnesium, and other macro nutrients such as NPK, provides an immediate and direct method for nutrient delivery, bypassing soil-related limitations and supplying essential elements during critical growth stages or stress periods. This approach ensures rapid nutrient absorption, making it particularly effective for addressing nutrient deficiencies or supplementing nutritional needs under drought conditions (SIDIQI and SIDIQI, 2025). Recent evidence indicates that integrating foliar-applied biostimulants with nutrient management practices enhances water use efficiency, nutrient assimilation, and overall crop productivity under deficit irrigation regimes (MOUSAVI et al., 2025). Such synergistic approaches are particularly relevant for alfalfa, where biomass yield and forage quality depend heavily on efficient nutrient utilization and stress resilience. Furthermore, ANZABI et al. (2023) demonstrated that foliar fertilizer containing Silicon further enhances photosynthesis by optimising leaf orientation toward light, which ultimately contributes to improved alfalfa growth and yield under stress conditions. However, despite these promising findings, there is limited field-based evidence on the combined effects of *Trichoderma*-based biostimulants and foliar fertilization on alfalfa productivity and forage quality under drought stress in temperate agro-ecological conditions. Therefore, the current study investigates the integrated application of *Trichoderma*-based biostimulants and foliar fertilization as a critical step toward developing sustainable, climate-resilient strategies for improving alfalfa yield and quality under drought stress.

## MATERIAL AND METHODS

### Description of the experimental site

A field experiment was conducted at the University of Debrecen's research garden in Hungary during the 2024 cropping season. The area has a geographical coordinate of 47°33'02"N; 21°35'56"E. The soil of the study area is homogeneous, classified as Calcic Endofluvic Chernozem according to the IUSS Working Group in 2014, in the World Reference Base for soil. The average precipitation during the 2024 cropping season from March to August was 48.1 mm, slightly lower than the 30-year average of 51.1mm, indicating the cropping season experienced a moderate drought spell compared to the previous 30-year average. The average temperature ranges between 17.0 °C and 19.0 °C, indicating an average of 2.1 °C higher than the 30-year average temperature of 15.9 °C (Figure 1). The soil chemical analysis revealed that the pH was slightly acidic to neutral at 0 – 20 cm depth and moderately basic at 20 – 40 cm. Zn, Si, Na, and organic carbon (OC) were low, while N, P, and K were moderate (Table 1).

Table 1

Soil chemical properties of the experimental site		
Parameters/Depth	0 – 20 (cm)	20 – 40 (cm)
pH (KCl)	6.93	7.46
OC (%)	2.89	2.87
P (mg/kg)	1538	1149
N (mg/kg)	82.2	53.4
K (mg/kg)	638	586
Na (mg/kg)	73.7	68.6
Si (mg/kg)	35.8	28.0
Zinc (mg/kg)	2.77	2.24

\*KCl Potassium chloride soluble

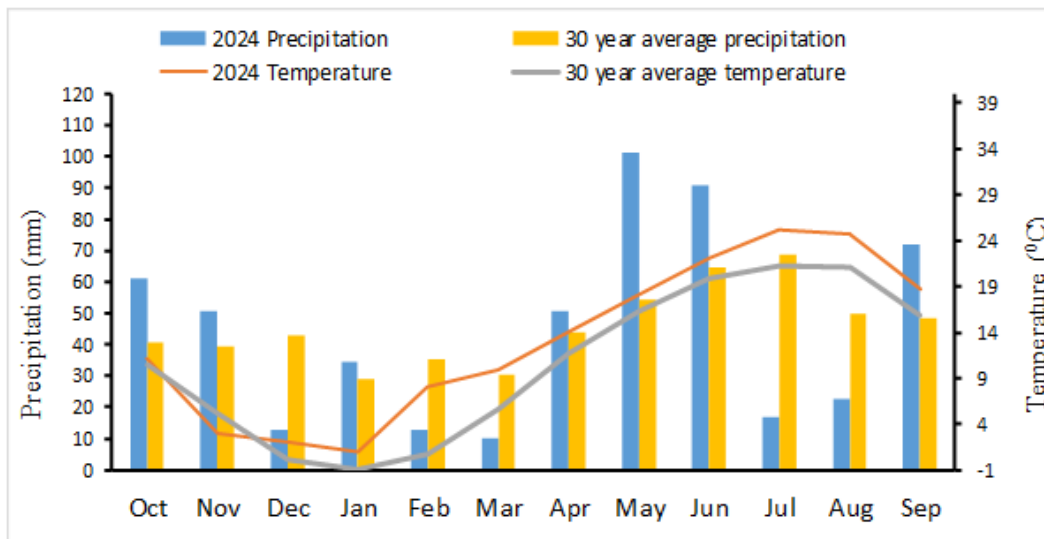


Figure 1: Meteorological data of the University of Debrecen experimental garden during the 2024 cropping season and the average for the past 30 years (Debrecen).

### Experimental Design and Treatment

The experiment was arranged in a randomized complete block design (RCBD) with three treatment levels replicated three times. A plot size of (3 × 4m = 12m<sup>2</sup>) was employed with 1 m alleys between plots and 2m between replications. *Tricho Immun* was applied a day after cutting into the soil. In contrast, foliar fertilizer containing calcium, silicon, zinc and magnesium (Ino Green) was also applied a week after regrowth to the leaves. Table 2 represents the treatments used.

Table 2

Treatment labels and levels used for the experiment		
Code	Treatment Levels	Dosage
T0	Control (no biostimulant application)	-
T1	Tricho Immun + Ino Green	3 + 3 L ha <sup>-1</sup>
T2	Tricho Immun	3 L ha <sup>-1</sup>

\*Tricho Immun is the first internal Trichoderma. Composition: Patented strains of *Trichoderma afroharzianum* (TR04), *Trichoderma simmonsii* (TR05), 200 million (2x10<sup>8</sup>) CFU g<sup>-1</sup>. \*Ino Green is a liquid foliar fertilizer that contains calcium in calcite form and silicon. Composition: N (66g L<sup>-1</sup>), Ca (448 g L<sup>-1</sup>), Si (56 g L<sup>-1</sup>), Zn (24 g L<sup>-1</sup>), Mn(8 g L<sup>-1</sup>)

### **Classification of Drought Stress Conditions through the Pálfaí Drought Index (PaDI)**

The drought stress severity during the cropping season was evaluated using the Pálfaí Drought Index (PaDI). Based on the formula proposed by PÁLFAI and HERCEG (2012), the cropping season was classified as moderate drought stress with a Pálfaí drought index value of 6.5 PaDI °C 100 mm<sup>-1</sup>.

### **Data Collection**

The parameters measured included fresh herbage yield, protein content, and protein yield. Fresh herbage yield was determined on a plot basis by harvesting all plants, collecting them, and weighing them using a scale. Prior to protein content analysis, dried samples were ground to pass through a 1 mm mesh sieve and assessed for nitrogen content via the Kjeldahl method. Protein content was calculated as N × 6.25.

Protein yield was derived by multiplying dry matter yield × protein concentration

### **Data analysis**

The collected data were subjected to one-way analysis of variance using Genstat statistical software (edition 18). Means were separated using the least significant difference test at the 5% probability level. Duncan's multiple range test (DMRT) was employed to rank treatment performances from highest to lowest. Pearson correlation analysis was performed to examine relationships among the measured parameters.

## **RESULTS AND DISCUSSIONS**

### **Protein content**

The protein content data for alfalfa are presented in Figure 2. Analysis of variance revealed a significant ( $p < 0.05$ ) effect of treatment application on protein content under drought stress conditions. Treatment T2 recorded the highest protein content (22.69%), followed by T1 (22.3%), while the untreated control (T0) had the lowest (20.5%). These values represent significant increases of 10.6% (T2) and 8.7% (T1) when compared to untreated plots (T0). Under drought stress conditions, nitrogen fixation and assimilation are limited, resulting in low protein contents as observed in our control plots. In contrast, the pronounced increases in T2 likely stem from *Trichoderma*'s capacity to enhance nitrogen metabolism, sustain physiological processes, improve root architecture, boost nitrogen-metabolism-associated enzymatic activities, and foster symbiosis with nitrogen-fixing bacteria (CSÓTÓ et al., 2024). These results align with prior research demonstrating that biostimulants enhance nutrient use efficiency and metabolic stability, yielding protein increases of over 10% under drought (AKDAĞ et al., 2024).

### **Fresh herbage yield**

The application of treatment significantly affected the fresh herbage yield of alfalfa. Amongst the treatments, T1 produced the highest fresh herbage yield of 17415 kg ha<sup>-1</sup>, followed by T2 with a yield of 16135 kg ha<sup>-1</sup>, whereas T0 had the lowest yield of 14210 kg ha<sup>-1</sup> Figure 3. The results revealed that there were no differences between the treatments (T1 and T2); their performance was statistically similar. Comparatively, the analysis confirmed that T1 significantly outperformed the control by increasing fresh herbage yield by 22.5% under drought stress, underscoring a pronounced synergistic effect. This substantial improvement is attributable to the pivotal role of *Trichoderma* spp. in enhancing root architecture, water acquisition, and nutrient uptake under drought, synergized with foliar fertilizers that preserve photosynthetic efficiency, metabolic homeostasis, and hormonal balance, collectively enhancing plant height, chlorophyll content, and canopy expansion for superior herbage yield. These results align with prior research, reporting a 20.5% fresh yield increase in alfalfa via

biostimulants, with maximal gains in later harvests amid intensified drought (Appiah et al., 2024). Stamatov (2020) demonstrated yield boosts in wheat and sunflower using leaf fertilizers and biostimulants, while De Beer et al. (2023) showed that biostimulants like fulvic acid and bioflavonoids elevate N uptake efficiency, herbage quantity, and quality.

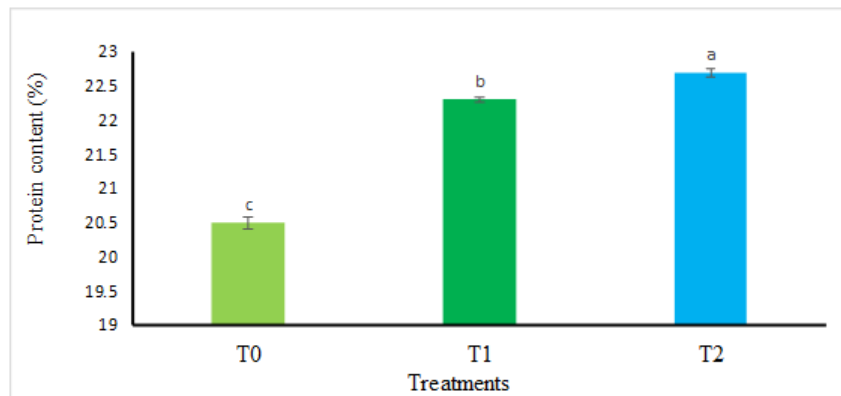


Figure 2: Effect of biostimulant treatment with or without foliar fertilizer on protein content of alfalfa crop cultivated during the 2024 cropping season. Columns represent annual average mean values of genotype (2) and repetitions (3)  $\pm$  standard error (n = 12). Different letters mean significant differences between treatments at p = 5%

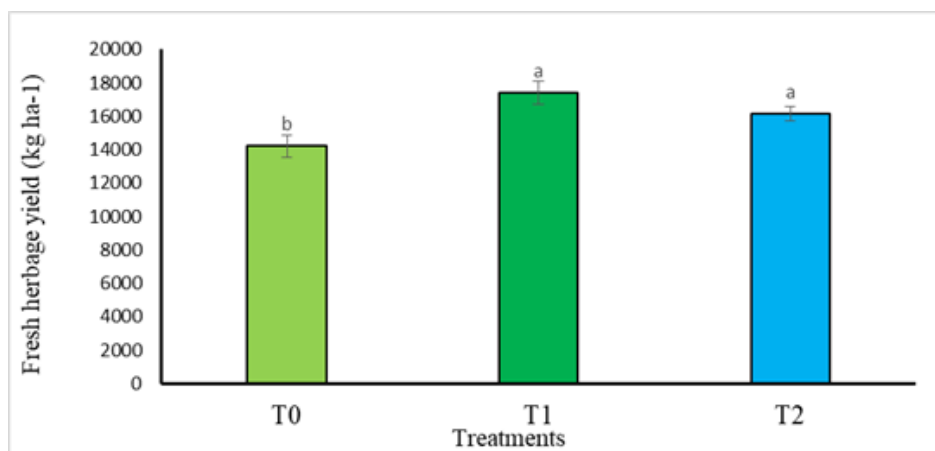


Figure 3: Effect of biostimulant treatment with or without foliar fertilizer on fresh herbage yield (kg ha<sup>-1</sup>) of alfalfa crop cultivated during the 2024 cropping season. Columns represent annual average mean values of genotype (2) and repetitions (3)  $\pm$  standard error (n = 12). Different letters mean significant differences between treatments at p = 5%

### Protein yield

Protein yield was significantly ( $p < 0.01$ ) affected by the treatment application. Numerically, T1 was observed with the highest protein of 3883 kg ha<sup>-1</sup>, followed by T2 with a protein yield of 3661 kg ha<sup>-1</sup>, whereas T0 recorded the least protein yield of 2910 kg ha<sup>-1</sup>. The data also

reveal that the performance of treatments T1 and T2 were statistically similar. Furthermore, T1 significantly enhanced protein yield under drought stress by 33.4%, whereas 25.8% enhancement in protein yield was observed under T2 when compared to untreated plots (Figure 4). These substantial gains demonstrate that T1 and T2 markedly enhanced alfalfa productivity under drought, likely via improved water uptake, hormonal stimulation supporting growth and protein synthesis, and better nutrient metabolism. These findings align with studies demonstrating that biostimulants improve nutrient uptake efficiency, stimulate root development, and enhance plant stress tolerance through the activation of metabolic pathways and antioxidant defence systems (CALVO et al., 2014; AKDAĞ et al., 2024).

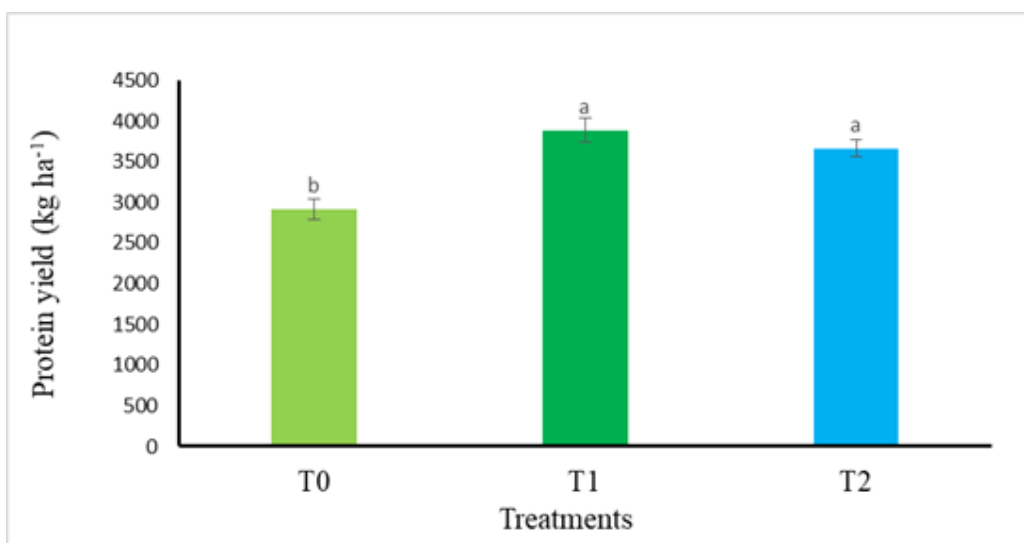


Figure 4: Effect of biostimulant treatment with or without foliar fertilizer on protein yield (kg ha<sup>-1</sup>) of alfalfa crop cultivated during the 2024 cropping season. Columns represent annual average mean values of genotype (2) and repetitions (3) ± standard error (n = 12). Different letters mean significant differences between treatments at p = 5%

## CONCLUSIONS

The results of our study demonstrate that the application of biostimulants with or without foliar fertilizers as a sustainable strategy can significantly improve alfalfa yield and forage quality under drought conditions. The combined application of Tricho Immun and foliar fertilizer containing calcium, silicon and zinc (T1) showed the greatest effectiveness by increasing fresh herbage yield and protein yield by 22.5% and 33.4%, respectively, indicating a synergistic effect. Overall, integrating Trichoderma-based biostimulants with targeted foliar fertilizer containing calcium, silicon, and zinc offers a robust and sustainable approach to improve alfalfa resilience and productivity in water-limited environments.

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