

## Effects of nitrogen rates and boron-containing biostimulant application on sunflower (*Helianthus annuus* L.) yield and physiological responses under drought conditions

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### SUMMARY

The objective of the study was to evaluate the effects of nitrogen fertilization and the foliar-applied biostimulant Activstart Szántóföldi B (hereinafter BS) on sunflower (*Helianthus annuus* L.) production under drought conditions. The experiment was established in 2025 in the Újfehértó region, on acidic, low-humus, gleyic brown forest soil. Nitrogen supply was examined at rates of 0, 30, 60, and 90 kg ha<sup>-1</sup>, with biostimulant-treated (applied twice) and untreated control plots. Crop status was monitored during the growing season using SPAD and NDVI measurements, while treatment effects were evaluated based on yield results. Based on yield data, nitrogen fertilization resulted in an almost linear yield increase; however, the rate of increase declined at higher doses. BS application alone produced a significant yield increase of approximately 16%, and in combination with nitrogen resulted in higher yields at all nutrient levels. The most balanced performance was achieved with the combined application of 60 kg ha<sup>-1</sup> nitrogen and BS. SPAD measurements showed no clear relationship between chlorophyll content and nitrogen rates, while biostimulant treatments resulted in more stable values, particularly at lower N levels. NDVI values were only moderately affected by treatments.

**Keywords:** sunflower (*Helianthus annuus* L.); nitrogen management; foliar-applied biostimulant; drought stress; yield response

### INTRODUCTION

Nitrogen (N) and boron (B) are key determinants of sunflower growth and yield stability, as both nutrients are involved in essential physiological processes. Nitrogen plays a central role in protein synthesis and chlorophyll formation, thereby directly supporting vegetative development and maintaining photosynthetic capacity (Shehzad and Maqsood, 2015). Optimal nitrogen management not only contributes to increased yield but may also positively influence the uptake of other nutrients, including boron (Ören & Çelik, 2018). In contrast, excessive nitrogen supply, particularly under water-limited conditions, can inhibit nutrient uptake and reduce nitrogen use efficiency (Wang et al., 2021).

Boron is indispensable for maintaining cell wall structural stability and membrane integrity, which are fundamental for proper organ development and function. Its importance is especially pronounced during the generative growth stage, as it supports pollen germination and pollen tube elongation, thereby directly affecting achene formation (Brown et al., 2002). The combined application of nitrogen and boron may result in synergistic effects, including prolonged phenological phases and improved seed oil quality (Shehzad and Maqsood, 2015). Ensuring sustainable sunflower yields therefore requires adequate nitrogen supply and its integration with other components of crop management, such as precision irrigation practices (Wang et al., 2021).

Drought stress, together with the unfavorable characteristics of sandy soils — particularly low water-holding capacity and enhanced nutrient leaching — can synergistically limit sunflower productivity. Under

such conditions, precision nitrogen management becomes a critical strategy, as appropriately selected nitrogen rates improve physiological performance and may enhance tolerance to abiotic stress (Mahpara, 2019). The application of organic nitrogen sources is particularly advantageous in this context, as they not only supply nutrients but also improve the hydro-physical properties of sandy soils (Helmy and Ramadan, 2009). These improvements promote root system expansion and support osmotic adjustment mechanisms that play a key role in drought tolerance (Egamberdieva et al., 2020).

Non-destructive diagnostic methods are closely integrated into precision crop production, providing rapid, repeatable, and spatially extensive information on crop status. SPAD- and NDVI-based measurements are of fundamental importance in this regard (Cudjoe et al., 2023). The SPAD index is widely used as an indicator of relative chlorophyll content and, indirectly, nitrogen status (Simkó & Veres, 2019). In parallel, vegetation indices — particularly NDVI — are effective tools for monitoring biomass accumulation and photosynthetic activity dynamics (Carvalho et al., 2015). According to the literature, satellite- and UAV-derived NDVI values can be validated with high accuracy against field measurements (Benincasa et al., 2018). Modern approaches increasingly integrate machine learning techniques, which can further enhance the detailed and accurate diagnosis of abiotic stress effects (Barnhart et al., 2020). Overall, the combined interpretation of multiple indicators — such as NDVI, SPAD, and canopy temperature — is considered one of the most reliable approaches for yield optimization (Hnizil et al., 2024).

In our experiment, these factors were integrated to assess the effects of biostimulant application and different nitrogen rates on the physiological and yield-related parameters of sunflower.

## MATERIALS AND METHODS

The agrotechnical field experiment was established in 2025 in Újfehértó, Hungary, in sunflower (*Helianthus annuus* L.) cultivation. The primary objective of the study was the comprehensive evaluation of the effects of different nutrient supply levels and a boron-containing biostimulant treatment.

The experimental design included four distinct nutrient levels, half of which received a boron-based biostimulant application. This treatment structure allowed for:

- assessment of the effects of nutrient supply levels,
- evaluation of the influence of the boron-based biostimulant on plant physiological traits and yield, and
- identification of potential interactions between nutrient levels and biostimulant application.

The experiment was set up in a randomized complete block design with four replications, using plot sizes of 225 m<sup>2</sup>. Sunflower was sown at a target plant density of 55,000 plants ha<sup>-1</sup>. The effects of nitrogen fertilization were examined at four levels (0, 30, 60 and 90 kg ha<sup>-1</sup> N active ingredient). Nitrogen was applied before sowing and incorporated into the soil in the form of ammonium nitrate (Pétisó, 27% N).

As a biostimulant, Activstart Szántóföldi B (hereinafter BS) was applied at a rate of 2 l ha<sup>-1</sup> using

300 l ha<sup>-1</sup> spray volume, in two applications. The first treatment was applied simultaneously with herbicide application on 10 May, while the second application was carried out at the star bud stage on 10 June using a field sprayer.

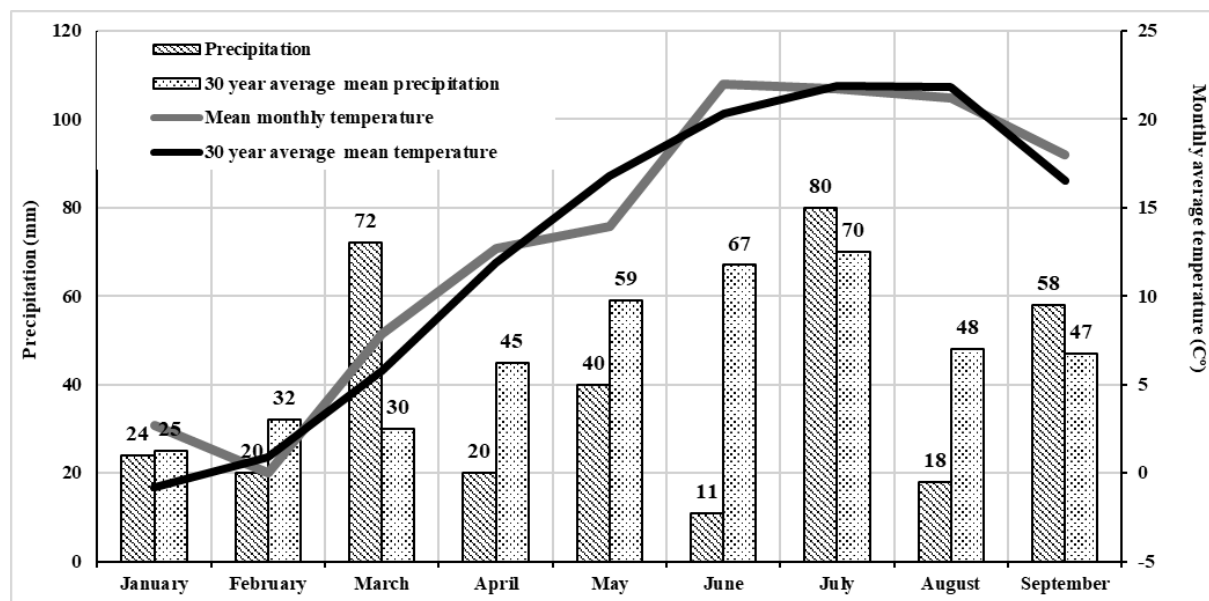
The treatments were as follows:

- 0 N control
- 30 N control
- 60 N control
- 90 N control
- 0 N + BS
- 30 N + BS
- 60 N + BS
- 90 N + BS

Harvesting was performed on 8 September using a grain combine harvester equipped with a sunflower header. Plot-level yield was determined using a slipper scale placed beneath the trailer.

Based on the meteorological data of 2025 (Figure 1), the growing season was characterized by warm and precipitation-deficient conditions from the perspective of sunflower cultivation. During the spring months, precipitation levels were markedly below the 30-year long-term average. Extreme drought in June (11 mm), combined with a monthly mean temperature of 22 °C, caused severe water stress during the flowering period. The higher amount of rainfall received in July (80 mm) was followed by another dry period in August (18 mm), which also had a significant impact on achene formation. Precipitation of 58 mm in September, together with an average temperature of 18 °C, supported the processes of ripening and senescence; however, the effects of the earlier water deficit on yield could no longer be compensated.

Figure 1. Temperature and precipitation trends in 2025 and the 30-year average for 1991–2020 in Újfehértó region (Újfehértó, 2025)



Sunflower was grown in Újfehértó on acidic, gleyic brown forest soil characterized by moderate nutrient deficiency and low humus content.

The phosphorus and potassium levels of the experimental site were low, making the crop sensitive to nutrient deficiencies and thus well suited for

modelling the effects of different nitrogen supply levels (Table 1). As soil nitrogen content was also very low, the site proved particularly suitable for a provocation-type experiment aimed at evaluating foliar-applied

biostimulant effects under varying nitrogen regimes. Due to poor nutrient background and acidic soil pH, the effectiveness of foliar treatments could be clearly expressed.

Table 1. Soil test results for the sunflower experiment in April 2025 (Újfehértó, 2025)

| pH (KCl) | KA | Total salt % | Humus % | P <sub>2</sub> O <sub>5</sub> mg kg <sup>-1</sup> | Available K mg kg <sup>-1</sup> | Nitrate-N mg kg <sup>-1</sup> | Mg mg kg <sup>-1</sup> | Ca mg kg <sup>-1</sup> |
|----------|----|--------------|---------|---|---------------------------------|-------------------------------|------------------------|------------------------|
| 4.66     | 35 | 0.03         | 2.25    | 65.20   | 197.00                          | <0.100                        | 72.00                  | 202.00                 |
| 4.99     | 32 | 0.03         | 2.09    | 41.40   | 125.00                          | <0.100                        | 28.20                  | 192.00                 |

Plant physiological measurements were conducted using the following instruments: Minolta 502+ for SPAD readings and GreenSeeker for NDVI measurements. Five measurements per plot were recorded for each treatment and replication. SPAD values were measured on 9, 16, and 30 May and on 13 June. NDVI values were recorded on 16 and 30 May and on 13 June, in order to monitor plant nitrogen status and its dynamics during the early stages of the growing season.

Statistical analysis of the measured data was performed using IBM SPSS Statistics 22, and the results were visualized using MS Excel 2016.

**RESULTS AND DISCUSSION**

**SPAD measurement results**

During the 2025 growing season, SPAD measurements conducted in the sunflower stand reflected changes in leaf chlorophyll content across the different nitrogen fertilization levels (0–90 kg ha<sup>-1</sup> N). When evaluated as averages, SPAD values showed only minor differences between the control and the BS treatments, ranging from 35.3 to 41.1. At the beginning of the growing season (9 May), prior to the initiation of treatments, differences were

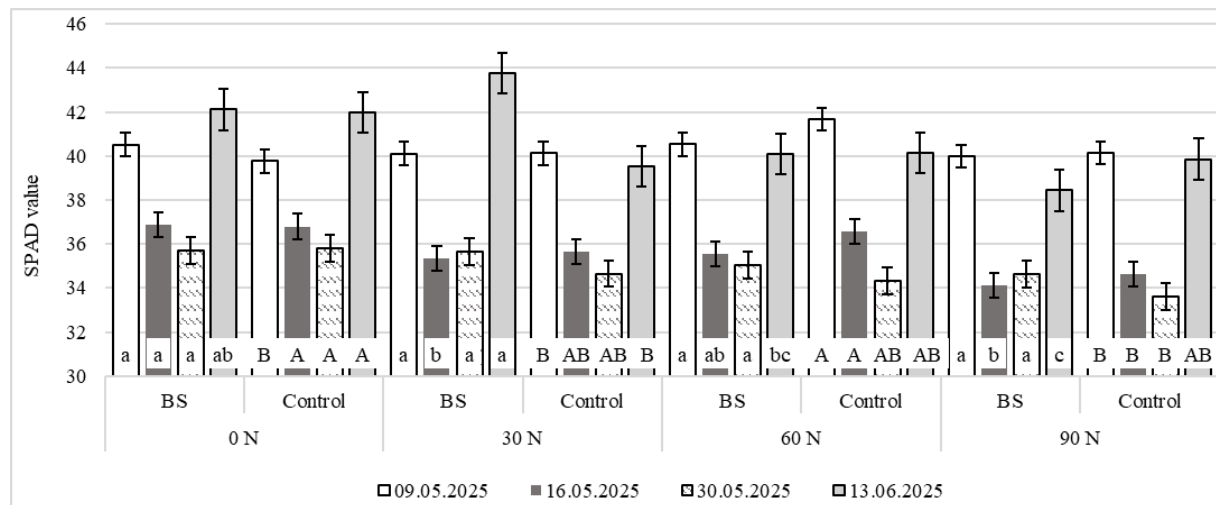
negligible (BS: 40.3; control: 40.4). During the second and third measurement dates (16 and 30 May), SPAD values declined in both the control and BS treatments, which can be attributed to the rapid growth phase and the associated chlorophyll dilution effect.

By the measurement on 13 June, leaf chlorophyll content increased again; however, plots treated with BS exhibited a higher mean SPAD value (41.1), while control plots reached slightly lower values (40.4). The standard error remained low at all measurement dates (0.3–0.5).

Overall, the results indicate that the BS treatment slightly increased leaf chlorophyll content, particularly toward the end of the growing period, which may suggest improved nutrient uptake and enhanced photosynthetic activity.

At the beginning of the growing season (9 May), SPAD values ranged between 39.8 and 41.7 (Figure 2), indicating a uniform initial status in both BS-treated and control plots. During the intensive growth phase (16–30 May), a temporary decline in SPAD values was observed across all treatments, which can be attributed to growth-related chlorophyll dilution and nutrient redistribution within the plants. The lowest values occurred on 30 May, particularly in the 90 N treatments (BS: 34.7; control: 33.6).

Figure 2. Changes in SPAD values as affected by treatments and nutrient levels (Újfehértó, 2025)



Small letters in the figure indicate significant differences among nutrient levels within BS-treated plots measured at the same sampling date. Capital letters indicate significant differences among nutrient levels within the control treatments measured at the same sampling date.



By the measurement on 13 June, leaf chlorophyll content increased again, especially at lower nitrogen levels. The 30 N + BS treatment stood out with the highest SPAD value (43.8), while at the 0 N level the BS treatment also showed a slight advantage (42.1) compared with the control (42.0). At the highest nitrogen level (90 N), no meaningful difference was observed; moreover, SPAD values were slightly lower in the BS-treated plots (38.4–39.9).

The standard error of the measurements ranged from 0.5 to 0.9. Overall, BS treatment had a positive effect on relative chlorophyll content, primarily at the 0 N and 30 N nitrogen levels, where treatment effects were most pronounced. In contrast, excessive nitrogen supply (90 N) was associated with a slight reduction in leaf chlorophyll content, suggesting a potential imbalance in nutrient uptake.

### NDVI measurement results

During the 2025 growing season, sunflower NDVI values were recorded at three sampling dates (16 May, 30 May, and 13 June). NDVI values showed a gradual increase over time as crop development progressed, reflecting increasing leaf area and enhanced photosynthetic activity.

Overall, NDVI values increased steadily over time, indicating intensive early growth, expansion of leaf area, and increasing photosynthetic activity of the crop.

When evaluated as averages across nitrogen fertilization levels (0–90 kg ha<sup>-1</sup> N), NDVI values at the first measurement date (16 May) were identical in both

treatments ( $0.28 \pm 0.004$ ), suggesting that during the early developmental stage there were no substantial differences in canopy cover or stand density.

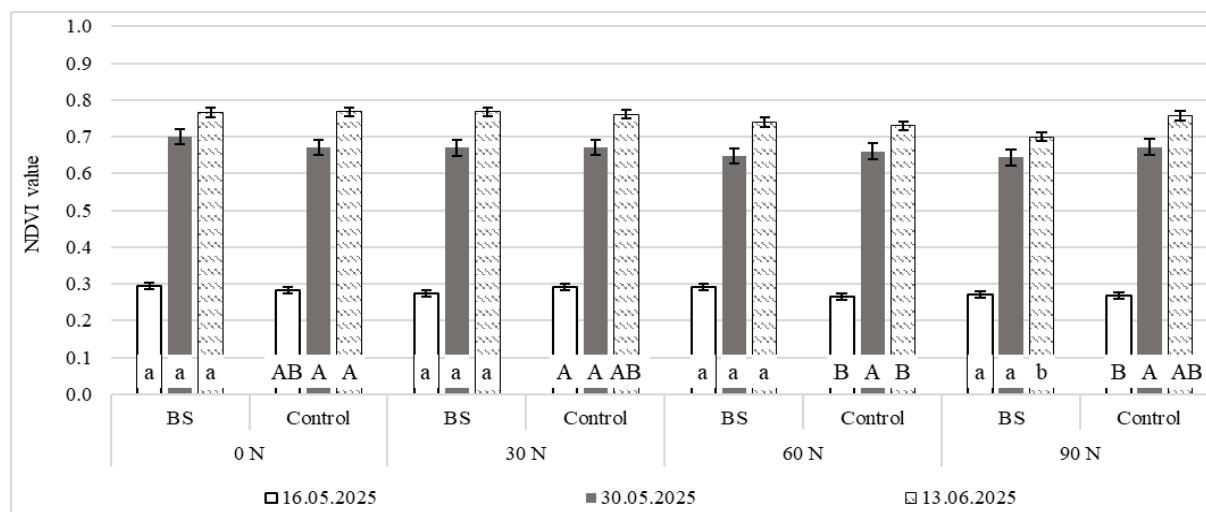
On 30 May, during the rapid vegetative growth phase, NDVI values increased to  $0.67 \pm 0.01$  in both treatments. During this period, moderate temperatures (mean 22 °C) and several low-intensity precipitation events (5–11 mm) favored biomass accumulation; consequently, no meaningful difference was observed between the control and BS treatments.

By the measurement on 13 June, NDVI values reached their maximum:  $0.74 \pm 0.006$  in the BS-treated plots and  $0.75 \pm 0.006$  in the control plots. Despite high temperatures and decreasing precipitation, the crop still exhibited full canopy development at this stage; however, NDVI values in BS-treated plots remained slightly lower.

Overall, the temporal pattern of NDVI values indicated that BS application did not result in a significant difference in crop development dynamics during the investigated period.

At the first measurement date on 16 May (Figure 3), NDVI values ranged between 0.27 and 0.30, reflecting the early vegetative stage of sunflower development. The highest value was recorded in the 0 N + BS treatment ( $0.30 \pm 0.009$ ), while the lowest value was associated with the 90 N nutrient level ( $0.27 \pm 0.009$ ). At this stage, cool and moderately rainy weather conditions still limited crop growth, and no significant differences were observed between the control and BS treatments.

Figure 3. Changes in GreenSeeker NDVI values as affected by treatments and nutrient levels (Újfehértó, 2025)



Small letters in the figure indicate significant differences among nutrient levels within BS-treated plots measured at the same sampling date. Capital letters indicate significant differences among nutrient levels within the control treatments measured at the same sampling date.

By the second measurement on 30 May, NDVI values increased substantially (0.64–0.70), corresponding to the rapid vegetative growth phase. The highest NDVI value was observed at the 0 N nutrient level ( $0.70 \pm 0.021$ ), whereas at the 90 N level NDVI reached  $0.64 \pm 0.021$ . This trend suggests that higher nitrogen rates may have temporarily reduced

canopy cover, likely due to accelerated early growth and increased water demand. During this period, air temperatures (on average 22–23 °C) and intermittent low precipitation events favored leaf area development.

At the third measurement date on 13 June, the crop reached full canopy development, with NDVI values

ranging between 0.70 and 0.77. The highest value was again recorded at the 0 N nutrient level ( $0.77 \pm 0.012$ ), while lower values at the 90 N level ( $0.70-0.76 \pm 0.012$ ) can be interpreted as a consequence of drier and warmer conditions combined with increased water use.

Increasing nitrogen fertilization did not result in a linear increase in NDVI; in fact, higher nitrogen rates occasionally exerted a slightly unfavorable effect on canopy cover. The biostimulant treatment resulted in higher NDVI values at most nutrient levels; however, these differences were not statistically significant. The cool and precipitation-deficient conditions in spring, followed by a warm and dry period in June, clearly influenced sunflower development and the dynamics of reflectance values.

**Yield and grain moisture results**

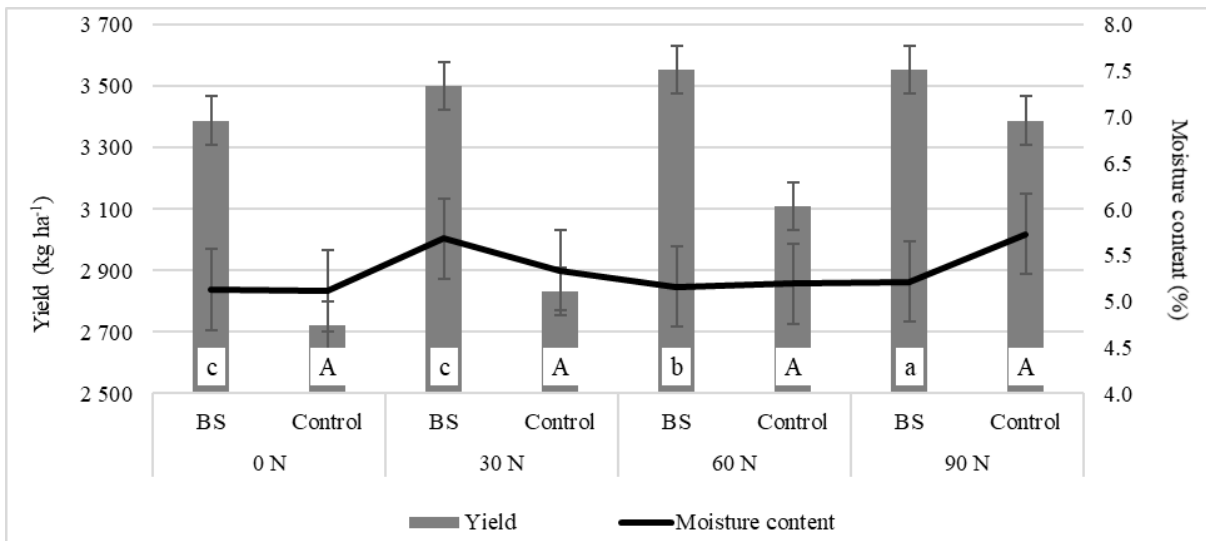
In 2025, sunflower yield performance was evaluated exclusively in relation to different nitrogen

application rates, as the experimental site had received 40 t ha<sup>-1</sup> of farm manure in 2022, and therefore no additional phosphorus or potassium fertilization was required.

When evaluated as averages across nitrogen fertilization levels (0–90 kg ha<sup>-1</sup> N), the mean yield of the control plots was 3013.9 kg ha<sup>-1</sup>, while the BS-treated stands achieved an average yield of 3500 kg ha<sup>-1</sup>. The resulting yield increase of 486.1 kg ha<sup>-1</sup> corresponded to a 16.1% improvement compared with the untreated control. Yield measurements were associated with a standard error of  $\pm 38.9$  kg ha<sup>-1</sup>.

When evaluating the combined effects of nitrogen fertilization and biostimulant application on yield (Figure 4), experimental results demonstrated that both nutrient supplementation and biostimulant treatment individually increased yield; however, their combined application proved to be the most favorable.

Figure 4. Effects of treatments and nutrient levels on yield and grain moisture content (Újfehértó, 2025)



Small letters in the figure indicate significant differences among nutrient levels within BS-treated plots measured at the same sampling date. Capital letters indicate significant differences among nutrient levels within the control treatments measured at the same sampling date.

The mean yield of the 0 N control plots was 2722.2 kg ha<sup>-1</sup>, whereas at the same nutrient level the BS-treated stands achieved 3388.9 kg ha<sup>-1</sup>, representing an increase of +666.7 kg ha<sup>-1</sup>. At the 30 kg ha<sup>-1</sup> N rate, yield increased from 2833.3 kg ha<sup>-1</sup> in the control to 3500 kg ha<sup>-1</sup> in the BS-treated plots, corresponding to a difference of +666.7 kg ha<sup>-1</sup>. At the 60 kg ha<sup>-1</sup> N level, yield rose from 3111.1 kg ha<sup>-1</sup> to 3555.6 kg ha<sup>-1</sup> (+444.5 kg ha<sup>-1</sup>), while at the highest nitrogen rate (90 kg ha<sup>-1</sup> N) the difference between the control and BS treatment was +166.7 kg ha<sup>-1</sup> (3388.9 kg ha<sup>-1</sup> and 3555.6 kg ha<sup>-1</sup>, respectively).

With increasing nitrogen rates, yield increased in both treatment groups; however, BS-treated plots consistently achieved higher yields at all nutrient levels. The highest yield (3555.6 kg ha<sup>-1</sup>) was recorded at both 60 and 90 kg ha<sup>-1</sup> N in combination with BS

application. With a standard error of 77.5 kg ha<sup>-1</sup>, the differences among treatments were statistically reliable.

Under the drought- and heat-stress conditions of the 2025 growing season, nitrogen fertilization combined with biostimulant application improved nutrient use efficiency and stress tolerance in sunflower. Based on the results, the 60 N + BS combination proved to be the most favorable and balanced treatment level, representing an agronomically efficient management strategy.

**CONCLUSIONS**

The 2025 growing season, characterized by pronounced drought and heat stress, clearly demonstrated that sunflower yield performance and



yield stability are fundamentally determined by year-specific climatic effects and by the nutrient management strategy adjusted to these conditions. Based on the results, nitrogen fertilization alone had a positive effect on yield; however, with increasing application rates the magnitude of yield increase declined. This response indicates the limited nitrogen use efficiency of sunflower and the strongly constrained nutrient uptake under water-deficit conditions.

Foliar-applied biostimulant (BS) treatment increased yield at all examined nutrient levels. Its effect was particularly pronounced at low (30 N) and medium (60 N) nitrogen rates. The outcomes of the combined treatments suggest that BS application primarily improved nutrient uptake efficiency and stress tolerance rather than excessively stimulating vegetative growth. This interpretation was supported by plant physiological measurements, as SPAD values indicated more stable chlorophyll content, while NDVI dynamics appeared to be predominantly governed by weather conditions.

Based on the investigations, the 60 N + BS treatment proved to be the most balanced and economically justifiable solution. The application of higher nitrogen levels under drought conditions did not result in proportionate yield increases and may have increased plant sensitivity to stress.

On the basis of these findings, it is recommended that nitrogen fertilization in sunflower production be adjusted to the water availability of the given growing season and to site-specific soil conditions, while avoiding excessive nitrogen rates. In drought-prone areas, the use of moderate nitrogen levels supplemented with biostimulants is justified, as this approach may improve nutrient use efficiency and enhance yield stability. For future research, it would be advisable to extend investigations across multiple growing seasons and different soil types, as well as to further elucidate the mechanisms of action of biostimulants, with particular emphasis on the interactions between water stress and nutrient uptake.

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