

## Article

# Foliar Spraying with Endophytic *Trichoderma* Biostimulant Increases Drought Resilience of Maize and Sunflower

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**Abstract:** Microbial biostimulants that promote plant growth and abiotic stress tolerance are promising alternatives to chemical fertilizers and pesticides. Although *Trichoderma* fungi are known biocontrol agents, their biostimulatory potential has been scarcely studied in field conditions. Here, the mixture of two endophytic *Trichoderma* strains (*Trichoderma afroharzianum* TR04 and *Trichoderma simmonsii* TR05) was tested as biostimulant in the form of foliar spray on young (BBCH 15–16) maize (5.7 ha) and sunflower (5.7 and 11.3 ha) fields in Hungary. The stimulatory effect was characterized by changes in plant height, the number of viable leaves, and the chlorophyll content, combined with yield sensor collected harvest data. In all trials, the foliar treatment with *Trichoderma* spores increased photosynthetic potential: the number of viable leaves increased by up to 6.7% and the SPAD index by up to 19.1% relative to the control. In extreme drought conditions, maize yield was doubled (from 0.587 to 1.62 t/ha,  $p < 0.001$ ). The moisture content of the harvested seeds, as well as sunflower height, consistently increased post-treatment. We concluded that foliar spraying of young plants with well-selected endophytic *Trichoderma* strains can stimulate growth, photosynthesis, and drought tolerance in both monocot maize and dicots sunflower crops in field conditions.

**Keywords:** *Trichoderma afroharzianum*; *Trichoderma simmonsii*; drought tolerance; chlorophyll content; maize yield; fungal biostimulant; foliar spray



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## 1. Introduction

The overuse of synthetic chemical fertilizers and pesticides poses significant environmental challenges, contributing to biodiversity loss and raising concerns about soil health, plant integrity, and food safety [1–4]. Additionally, climate change—characterized by severe droughts, storms, and declining biodiversity—further exacerbates these issues by reducing crop yields, diminishing soil fertility, and increasing the prevalence of pests and disease outbreaks [5–8]. In response to these challenges, sustainable crop production must strike a delicate balance by minimizing reliance on chemical fertilizers and pesticides while ensuring stable yields under increasingly adverse environmental conditions [7].

Beneficial microorganisms offer a promising solution by promoting plant growth and alleviating environmental stresses through diverse mechanisms. These microorganisms enhance nutrient uptake and improve the solubilization of phosphorus, potassium, iron, and

zinc by producing organic acids and siderophores. Additionally, they help mitigate biotic and abiotic stresses by synthesizing phytohormones, inducing systemic resistance, boosting the activity of defense-related enzymes, and increasing the accumulation of osmotically active substances in plants [9–13]. Furthermore, these fungi can mitigate abiotic stress by activating endogenous plant defense responses and altering plant metabolism [14–16].

Among these microorganisms, *Trichoderma* spp. stand out as versatile fungi capable of functioning as biocontrol agents, plant growth stimulators, and biofertilizers [17–19]. These fungi enhance nutrient availability by producing organic acids and siderophores, which foster robust root development [11]. Furthermore, *Trichoderma* spp. directly promote plant growth through phytohormone production [10,20] and mitigate abiotic stresses by activating endogenous plant defenses and modifying plant metabolism [14–16].

The biocontrol and biostimulant properties of *Trichoderma* spp. have been investigated across various crops, including maize and sunflower, through soil or seed treatments [21–25]. While most research has been conducted in controlled environments, further field studies are necessary to validate these findings and assess the long-term impacts of *Trichoderma* applications. This aspect is particularly significant, as *Trichoderma* spp. predominantly colonize root tissues; however, endophytic strains have also been identified, and their biocontrol potential in annual crops remains to be explored [26,27].

In this study, we evaluated the effects of a commercially formulated endophytic *Trichoderma* biostimulant, applied as a foliar spray, on maize and sunflower under field conditions in Hungary. The product, containing two endophytic strains—*Trichoderma afroharzianum* TR04 and *Trichoderma simmonsii* TR05—was derived from woody grapevine tissues [26]. Our results demonstrate the biostimulant efficacy of this formulation in enhancing crop performance in these key monocot and dicot crops in practice under field conditions.

## 2. Materials and Methods

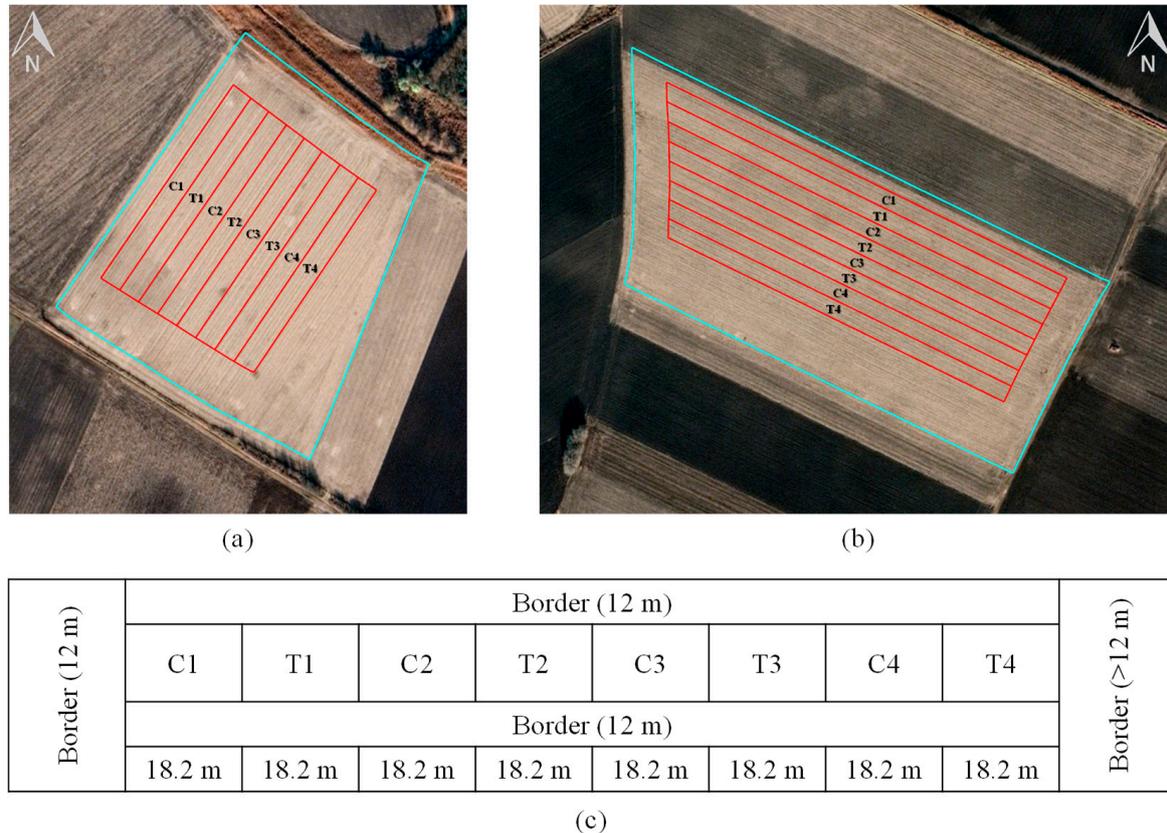
### 2.1. Experimental Sites and Treatments

These experiments were conducted with maize in 2022 at Experimental Site I, with sunflower in 2022 at Experimental Site II, and in 2023, at Experimental Site I. Both experimental sites are located on the outskirts of Hajdúszovát, Hungary, in the South-Hajdúság meso-region of the Great Hungarian Plain.

The soil at both sites is loamy or clay-loam meadow chernozem with a humus content of 3.2–3.5% and a pH of 6.7–7.15 (determined by accredited laboratory). The average altitude of the sites is 92 m above sea level. The GPS coordinates for the center of Experimental Site I (Figure 1a) are 47°23'36" N 21°24'50" E, and for Experimental Site II (Figure 1b), they are 47°22'16" N 21°26'12" E. In all cases, the field was planted with maize the previous year.

The control and the *Trichoderma*-treated plots were set up in four alternating strips (Figure 1). Sunflower NK Neoma (early) hybrid was seeded on the 21st and 22nd April 2022 and 2023. Maize Dekalb DKC4897 (FAO 390–400) hybrid was also seeded on 21 April 2022. Monosem NG Plus 6 (Monosem Inc., Edwardsville, KS, USA) sowing machine was used in all cases. Plant density was 60,500 plants per hectare for sunflower and 74,000 plants per hectare for maize. All experiments were treated one week after applying post-emergent weed control at the BBCH 15–16 phenological stages of the plants [28]. This phase provides sufficient plant surface for effective colonization by the endophytic strains and allows for combination with herbicide treatment. The application was carried out using a John Deere 6120M tractor equipped with AutoTrac and a StarFire 6000 antenna (John Deere Inc., Mannheim, Germany). The navigation system was utilized, with the machine's working width set and tracks recorded for accurate operation. Control plots were sprayed with water in amounts equivalent to the water content of the *Trichoderma* formulation. *Trichoderma*-treated plots were sprayed with a freshly prepared solution of Tricho Immun (Danuba, Szentendre, Hungary), which contained a mixture of *Trichoderma afroharzianum* TR04 and *Trichoderma simmonsii* TR05 conidia ( $2 \times 10^8$  CFU/g) on a substrate consisting of glucose and perlite powder. The viability of the conidia was previously tested in the laboratory. The

application rate was 1 kg/ha in 300 L of water per hectare, following the manufacturer's protocol [29]. The plot width was 18 m, corresponding to the working width of the Kertitox Revolution (Farmgép, Debrecen, Hungary) trailed field sprayer, which is three times the 6 m working width of the combine harvester (Figure 1c).



**Figure 1.** Experimental design to study the effect of the endophytic *Trichoderma* formulation. Aerial view of Experimental Site 1 (a) and Experimental Site 2 (b). Blue lines indicate the borders of the Experimental Sites, red lines indicate the borders of the plots. (c) Experimental designs C1–4 indicate control plots; T1–4 indicate plots treated with *Trichoderma*.

Meteorological data were collected with a Campbell Scientific (Campbell Scientific Ltd., Logan, UT, USA) meteorological station with Campbell HygroVUE10 temperature and humidity sensor, Kipp&Zonen CMP-11 pyranometer, and PG-200 weighing rain gauge. It is operated by the Centre for Precision Farming R&D Services, FAFSEM, University of Debrecen. The weather station is located in the Agrometeorological Observatory, Debrecen-Kismacs, approximately 20 km from the experimental sites. The annual meteorological diagram was created using monthly resolution data, while the differences during the vegetation period are based on weekly resolution data.

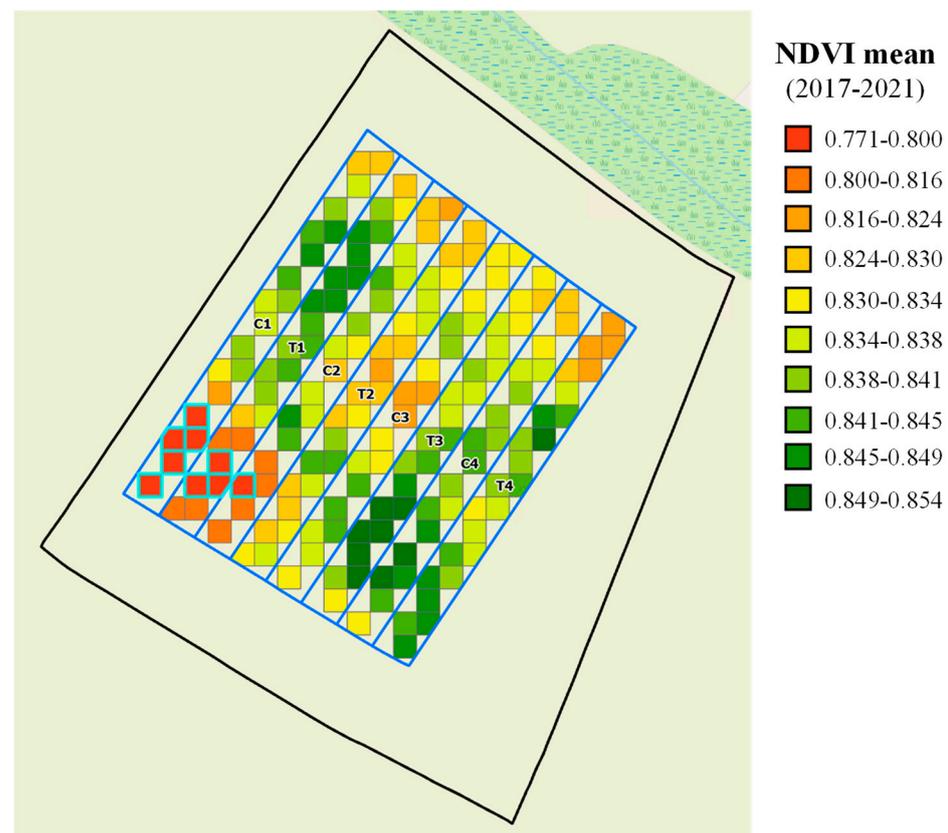
## 2.2. Evaluation of Experimental Site Homogeneity Based on NDVI

Normalized Differential Vegetation Index (NDVI) was calculated using data from Sentinel-2 satellites (launched by the Global Environment and Security Monitoring Program of the European Space Agency). The calculation was based on red and infrared spectral values from 10 m spatial grids, as described by Rouse et al. (1974) [30]:

$$NDVI = \frac{\lambda_{NIR} - \lambda_{RED}}{\lambda_{NIR} + \lambda_{RED}}$$

where  $\lambda_{NIR}$  is the reflectance value of near-infrared (NIR), and  $\lambda_{RED}$  is the reflectance value of red.

To evaluate the homogeneity of the experimental sites, firstly, NDVI images were created, and then, cloud-covered and cloud-shadowed images were ignored by the Fmask algorithm [31]. Research and practical experience have shown that a stronger correlation existed between NDVI values and yield at certain phenological phases [32]. Consequently, only NDVI time points where the field-level NDVI values exceeded 0.7 were used for homogeneity analysis. Annual average NDVI values were calculated using  $10 \times 10$  m Sentinel-2 raster data for the years from 2017 to 2021. Each year, the analysis included more than five images. The raster data were polygonized using ArcGIS Pro before analysis. To accurately assess NDVI within experimental plots, Sentinel-2 imagery (10 m resolution) was spatially intersected with plot polygons. Grids with less than 80% overlap were excluded to minimize mixed-pixel effects. Subsequently, average NDVI values were calculated for each plot and categorized into five classes (0.2 NDVI intervals) to highlight NDVI differences in the plot. While Experimental Site I exhibited a range of NDVI values, with 5.14% of pixels in the 0.6–0.8 category, all pixels at Experimental Site II were classified as high NDVI (0.8–1.0) (Figure 2).



**Figure 2.** Average NDVI mean values with natural breaks coloring method, using 80% overlapping (Experimental Site I). Light blue polygon outline color shows the lower average NDVI values (below 0.8). C1–4 indicate control plots; T1–4 indicate Trichoderma-treated plots.

### 2.3. Measurements of Photosynthetic Potential and Plant Height

To assess photosynthetic potential, the number of viable leaves was counted, and chlorophyll content was measured weekly from treatment until harvest using a SPAD 502Plus chlorophyll meter (Konica Minolta Sensing Inc., Osaka, Japan). At harvest, plant height for sunflowers was manually measured using a Bosch GR 500 (Robert Bosch Tools GmbH, Leinfelden-Echterdingen, Germany) leveling staff. Due to severe drought damage in 2022, maize plant height could not be assessed. For each plot, SPAD measurements

were taken on 20 randomly selected plants. Four points on four upper leaves of each plant were taken per plant and averaged. Viable leaf numbers were also counted on these same 20 plants. Plant height was measured similarly on 20 plants per plot right before harvest. Data from 80 control and 80 Trichoderma-treated plants were used to calculate the average and standard error for each data point.

2.4. Obtaining Harvest Data

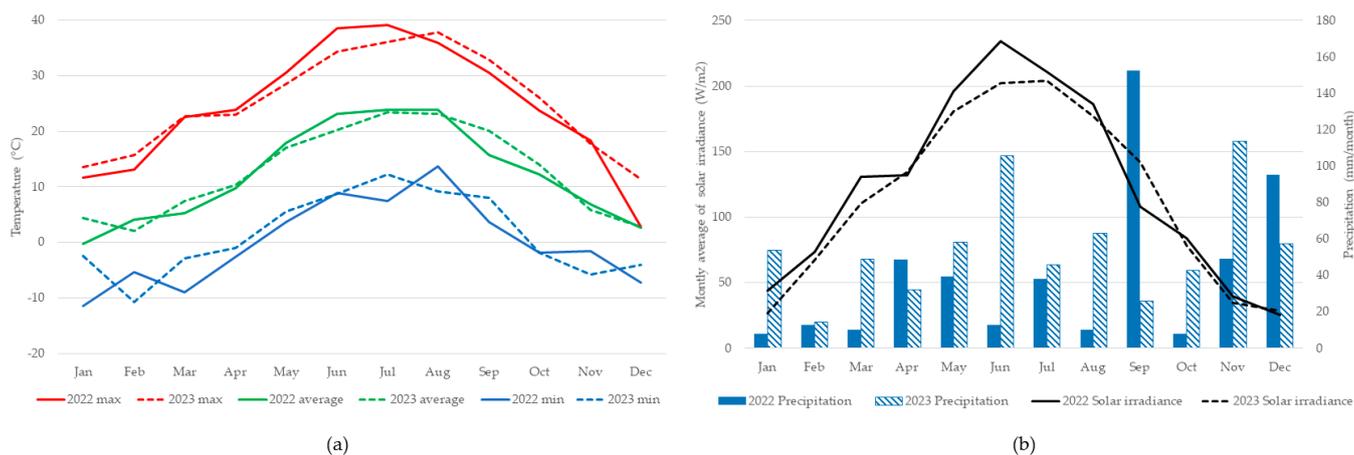
The 2022 maize experiment harvest was gathered on 10 October 2022, while the 2022 and 2023 sunflower experiment harvests were gathered on 5 September 2022 and 13 September 2023, respectively. A John Deere S770i combine harvester equipped with an intelligent yield sensor, AutoTrac, and a StarFire 6000 RTK antenna (John Deere Inc., Mannheim, Germany) was used for all harvests. Yield and moisture content data were collected at one-second intervals and filtered using box plot analysis to eliminate outliers. Grid cells were excluded from analysis if they had less than 80% overlap with their assigned treatment area (Figure 2). Additionally, grid cells from Experimental Site I with predominantly low NDVI values (below a threshold of 0.8) were removed, as these areas were considered to have low productivity (Figure 2). Harvester data were spatially aligned with Sentinel-2 NDVI grids, and average yield and moisture values were calculated for each grid cell using the “Summarize Within” function in ArcGIS Pro.

2.5. Statistical Analysis

Data processing and visualization were performed using MS Excel 2016, while descriptive statistics and hypothesis testing were conducted in IBM SPSS 29. The normality assumption for parametric tests was assessed using Q–Q plots and the Shapiro–Wilk test. Homogeneity of variances (homoscedasticity) was evaluated using Levene’s test. When both assumptions were satisfied, the parametric *t*-test was employed. The non-parametric Mann–Whitney test was used for comparisons when the data did not meet the normality or homogeneity assumptions.

3. Results

The 2022 growing season was characterized by severe drought and elevated temperatures, particularly during the critical period from May to August (Figure 3).



**Figure 3.** Monthly weather data of the experimental years 2022 and 2023. (a) Average, minimal and maximal temperatures. (b) Monthly average of solar radiation and precipitation.

The average annual precipitation is 600 mm in this region [33], but it was only 484.6 mm in 2022. A more abundant 662.2 mm precipitation was measured in 2023. Furthermore, the pre-sowing period (October 2021 to March 2022) was drier in 2022, with a precipitation deficit of 156 mm compared to the following year. The growing season of 2022

also had higher average temperatures (+1.5 °C) and increased solar irradiance compared to 2023 (Figure 3).

### 3.1. Homogeneity of the Experimental Sites

A five-year pre-experimental analysis revealed that all plots at Experimental Site II had highly stable and consistent NDVI values, ranging from 0.813 to 0.845 within the 0.8–0.9 category (Figure 2). However, at Experimental Site I, a small but significant proportion (5.5%) of plots showed lower NDVI values, falling within the 0.7–0.8 range. These plots were excluded from further analyses to ensure homogeneity within the dataset (Figure 2).

### 3.2. Plant Growth

Given the significant drought stress in 2022, which led to the premature death of corn plants, measuring plant height was not feasible. As the plants did not reach maturity, measuring height would not have provided meaningful data on growth vigor but rather on the timing of desiccation. The sunflower plants were shorter by 50 cm in 2022 compared to 2023. The application of *Trichoderma* species containing commercial products mitigated these drought effects, resulting in a 10 cm increase in sunflower height in 2022 and a 4 cm increase in 2023 (Table 1).

**Table 1.** Effects of *Trichoderma* TR04 and TR05 strains on the plant height of sunflower at harvest.

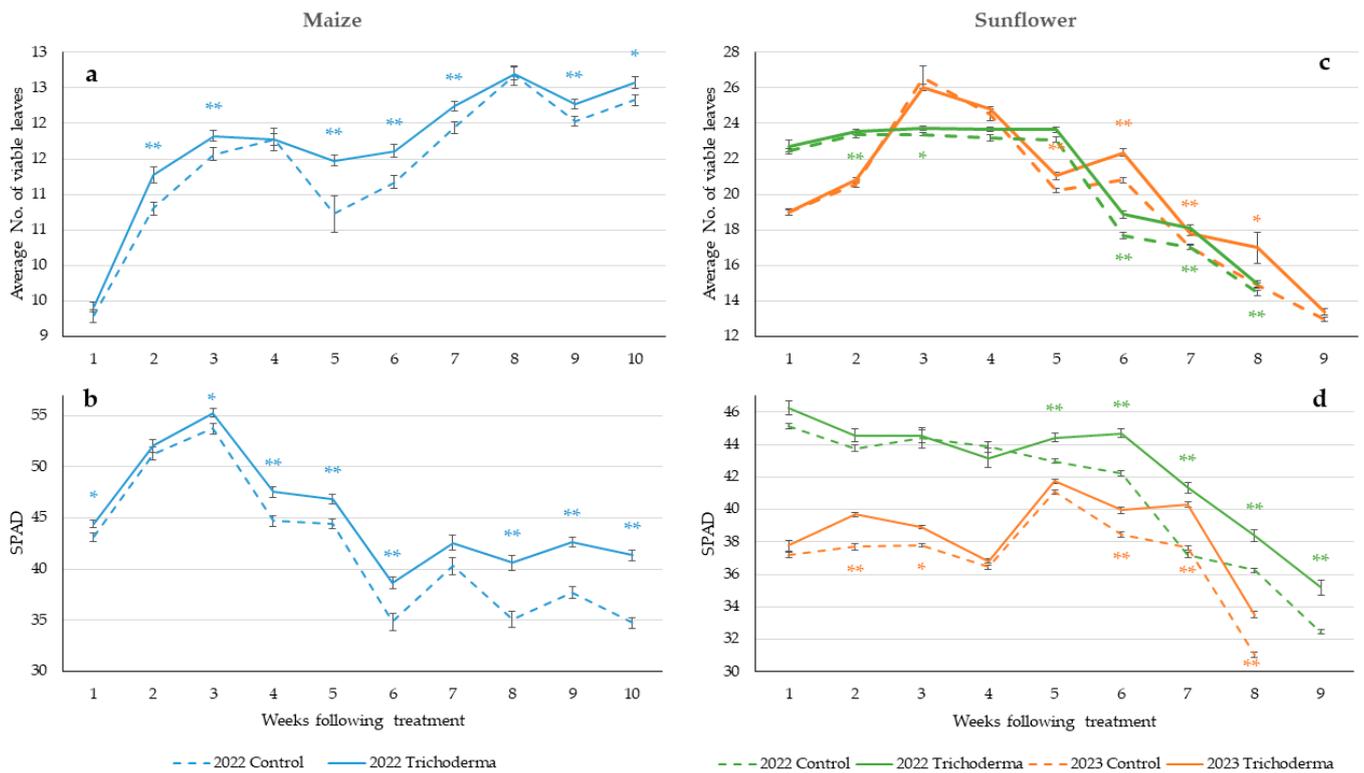
	Treatment	Sunflower (2022) (Mean ± SE)	Sunflower (2023) (Mean ± SE)
Plant height (cm)	<i>Trichoderma</i>	120.4 ± 2.14	174.9 ± 0.89
	Control	110.5 ± 3.37	171.1 ± 0.93
<i>t</i> -test <i>p</i>		0.014	0.003

### 3.3. Photosynthetic Potential

To evaluate the impact of treatment on photosynthetic potential, the number of viable (green) leaves was determined, and SPAD values were measured. Maize plants treated with *Trichoderma* spp. exhibited significantly increased leaf viability, with the effect becoming apparent as early as two weeks after the foliar application and persisting throughout the growing season (Figure 4a). The application of *Trichoderma* spp. to sunflower plants significantly increased viable leaf numbers, thereby stimulating plant development and prolonging leaf longevity. A statistically significant increase in leaf number was detected three weeks after treatment in 2023 and five weeks after treatment in 2022. This positive effect of *Trichoderma* spp. on leaf development and viability was maintained until the end of the vegetation period (Figure 4b).

*Trichoderma* treatment significantly increased SPAD values, indicating enhanced chlorophyll content in both maize and sunflower plants. Maize plants showed a rapid response to *Trichoderma* treatment, with increased SPAD values observed shortly after application. This positive effect persisted throughout the entire growing season (Figure 4c). Sunflower plants exhibited a delayed response, with significant increases in SPAD values observed five weeks post-treatment in 2022 and two weeks post-treatment in 2023 (Figure 4d).

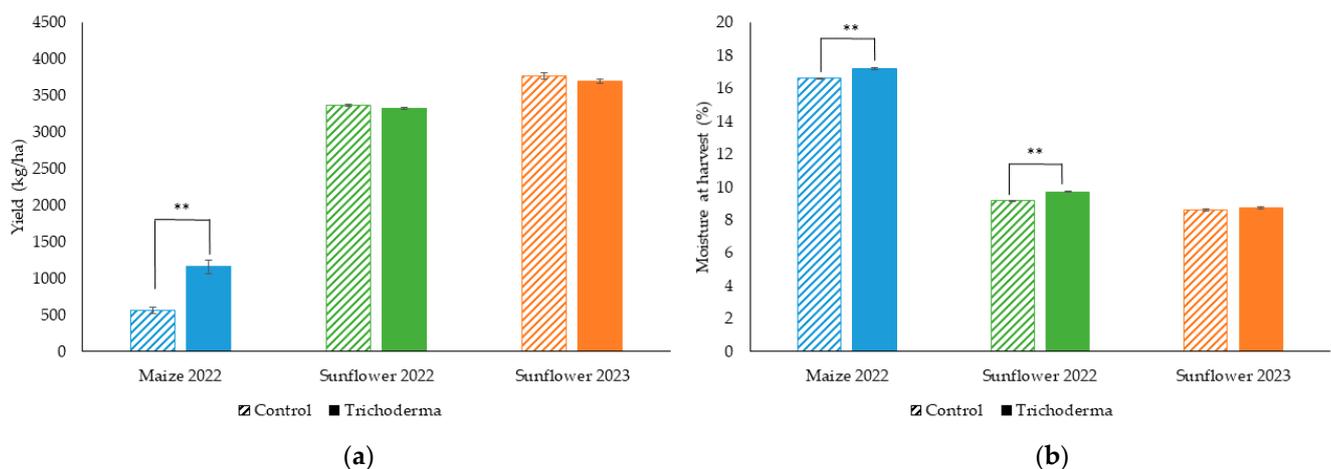
While the foliar application of *Trichoderma* spp. had a positive impact on plant health and photosynthetic parameters, it did not significantly affect NDVI values. The difference in the NDVI between the treated and the control plots was less than 1%, following the treatment on either crop.



**Figure 4.** Changes in average number of viable leaves (a,c) and chlorophyll content (b,d, measured as SPAD values) of maize (a,b) and sunflower (c,d) plants following treatment with *Trichoderma* spp. compared to untreated control plants. Data points represent mean values, and error bars indicate standard error. \*: *t*-test  $p < 0.01$ , \*\*: *t*-test  $p < 0.001$ .

### 3.4. Yields and Moisture Content

The severe drought in 2022 significantly reduced maize yield to an extremely low 0.587 t/ha. However, *Trichoderma* treatment mitigated the negative effects of drought, significantly increasing maize yield to 1.62 t/ha (Figure 5a).



**Figure 5.** Average yield (a) and average moisture content of harvested seeds (b) of maize and sunflower plants treated with *Trichoderma* spp. compared to untreated controls. Error bars represent standard error. \*\*: *t*-test  $p < 0.001$ .

The moisture content of the maize increased significantly from 16.6% to 17.19%, representing only a 0.59% difference between the treated and untreated maize. However,

this increase did not affect the quality of the harvested seeds. Similarly, in the drought year (2022), the moisture content of the sunflower increased slightly to 9.7% compared to the 9.16% measured in the seeds from the control plots, a difference of only 0.54%. In 2023, when normal precipitation was detected, neither the yield nor the moisture content of the sunflower showed any significant changes (Figure 5b).

#### 4. Discussion

Climate change, with its increasing frequency and intensity of extreme weather events such as heatwaves and droughts, poses a critical threat to global food security by reducing crop yields and exacerbating pest and disease outbreaks [34,35]. To address these challenges, sustainable agricultural solutions, including the use of biostimulants, are emerging as viable alternatives to traditional chemicals [36,37]. While traditional agricultural practices often depend on chemical inputs, there is a growing demand for more sustainable and environmentally friendly approaches to secure food production in a changing climate [36,37].

Microbial biostimulants, particularly those based on *Trichoderma* species, have shown promise in mitigating the adverse effects of climate change on crop production [38]. These fungi form beneficial associations with plant tissues by producing plant hormones such as indole-3-acetic acid, gibberellic acid, abscisic acid, ethylene, jasmonic acid, and salicylic acid, as well as biostimulant metabolites like lactones and hydrophobins, which regulate growth and stress responses [18,39,40]. These interactions enhance root growth, improve water and nutrient absorption [41], and boost photosynthesis and carbohydrate metabolism, optimizing energy utilization for development [42–44]. *Trichoderma* spp. also produces siderophores, increasing iron availability crucial for metabolic functions, including photosynthesis [45]. Additionally, endophytic *Trichoderma* species have also been shown to induce systemic resistance, thereby enhancing their defense against pathogens and environmental stresses [27,40,46]. While the mechanisms of *Trichoderma* spp. in laboratory settings are well understood, its field application in crops like maize and sunflower remains underutilized. This is partly attributed to the difficulty in achieving field homogeneity, a critical factor for accurate evaluation of biostimulant efficacy in large-scale experiments evaluations [47]. Remote sensing technology employing vegetation indices, such as NDVI, can effectively assess field variability and inform experimental design, ultimately improving the reliability of field trials [48,49].

Maize and sunflower field crops were evaluated under homogeneous field conditions (5.7 and 11.3 ha, respectively). Field homogeneity was assessed using NDVI data from the previous five years. A preceding dry pre-sowing period (January–March 2022; 30.8 mm precipitation) compared to 2023 (117 mm), coupled with increased temperatures (+1.5 °C) and solar radiation during the 2022 growing season, contributed significantly to the severity of the drought. *Trichoderma* treatment significantly improved the plant's growth and photosynthetic capacity in sunflower plants. The increased number of viable leaves and higher SPAD index, indicative of enhanced chlorophyll content, were observed two weeks post-application and persisted throughout the growing season. These findings suggest that foliar *Trichoderma* treatment with endophytic strains can promote plant vigor and optimize photosynthetic efficiency. These differences highlight the sunflower's inherent drought tolerance due to its efficient water use and resilience mechanisms [50,51].

For field crops such as maize and sunflower, microbial biostimulants, including *Trichoderma* products, are typically applied through seed treatment or direct soil application of the root colonizing strains [25,52–55]. While these fungi are typically considered to colonize only the roots of plants [56], their presence has been detected in other plant tissues above the soil [57,58], suggesting that foliar spraying could also be an effective treatment method. While seed and soil treatments are common, foliar applications offer greater flexibility for targeted interventions during specific growth stages or in response to stress or control diseases. Disease symptom expression was not detected in the field of the studied crops. In vitro confrontation tests indicated high biocontrol indexes of both applied *Trichoderma*

strains toward several plant pathogen fungi [26]. Field tests necessary for the registration of the product with plant protection effect are underway. Preliminary results indicate the disease *Trichoderma* formulation tested in this study can repress the *Fusarium* head blight and sunflower foliar disease. The biocontrol activity of the product may further increase the yield and quality parameters of the harvested grains. Foliar spraying provides the potential for reducing environmental impact and operational costs by enabling combined applications with herbicides. However, ensuring compatibility between *Trichoderma* strains and herbicides is crucial for maintaining efficacy stressors [59].

In summary, microbial biostimulants, particularly *Trichoderma*-based products, represent a sustainable and flexible solution for enhancing crop resilience and productivity under drought stress, with further research needed to optimize their field applications and integration into agricultural practices.

## 5. Conclusions

Foliar spraying with the endophytic *Trichoderma* TR04 and TR06 strains stimulates abiotic stress tolerance of the monocot maize and the dicot sunflower under field conditions and would likely have the same effect with other annual crops as well. Pest control that includes spraying with low-cost microbial agents in combination with compatible pesticides (e.g., with post-emergent weed control) may lead to reduced costs and a smaller carbon footprint.

**Author Contributions:** Conceptualization, A.C., P.R. and E.S.; data curation, A.C., P.R., L.K. and E.S.; formal analysis, E.F., L.K. and E.S.; investigation, G.T., P.R. and A.Z.; methodology, A.C., G.T., P.R. and E.S.; resources, A.C., P.R., L.K. and E.S.; software, A.C., P.R. and V.T.; supervision, A.C. and E.S.; validation, A.C., L.K. and E.S.; visualization, A.C. and P.R.; writing—original draft, A.C., G.T., P.R., A.Z., V.T., E.F., L.K. and E.S.; writing—review and editing, A.C., P.R., L.K. and E.S. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data presented in this study are available upon request from the corresponding author due to the ongoing nature of our research. Additional field data are currently restricted to our research team.

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**Conflicts of Interest:** Author Péter Riczu was employed by the company KITE Zrt. E.S. receives a royalty for the *Trichoderma* product “TrichoImmun” tested in this study. The rest of the authors declare no conflicts of interest.

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