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The Evaluation of the Effects of Zn, and Amino Acid-Containing Foliar Fertilizers on the Physiological and Biochemical Responses of a Hungarian Fodder Corn Hybrid

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Abstract: The benefit of applying foliar fertilizers is that crops can uptake them faster than soil fertilizers. The aim of this study was to test and valuate the effects of one zinc (Zn) and two amino acids-containing (AS) foliar fertilizers on a fodder corn hybrid's physiological and biochemical processes. The experiment was conducted in field conditions. The following parameters of a fodder maize hybrid were measured one, two, three, four, five, and six weeks after the treatments (WAT): physiological (relative chlorophyll content and the effectiveness of PSII); biochemical (activities of superoxide dismutase (SOD); ascorbate peroxidase (APX) and guaiacol peroxidase (POD); the concentration of malondialdehyde (MDA); and proline. The yield increased by 10%, 6%, and 10% at Zn, Zn+AS1, and Zn+AS2 treatments. The yield parameters, such as grain/cob and ear weight, were also significantly higher under the applied three treatments relative to the control. The relative chlorophyll content was significantly higher one, two, and four weeks after Zn-treatment, and some changes were also observed when Zn and amino acid-containing fertilizer were applied in combination. The latter sampling did not show any notable changes. In addition, the activity of SOD increased when Zn-containing fertilizer was applied, although the effect of AS-containing fertilizer did not show. There was a correlation between the SOD activity and some of the yield parameters. The increasing SOD activity indicated a higher yield (t/ha) and a higher cob weight.

Keywords: amino acid; antioxidant response; chlorophyll; foliar fertilizer; PSII; yield; zinc

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1. Introduction

Plants are exposed to various kinds of abiotic and biotic stressors [1–5]. Among biotic stressors, the most important are microorganisms, including viruses, bacteria, and fungi. The abiotic stressors include extreme changes in the plants' environment, such as heat, frost, drought, UV radiation, and nutrient deficiency or toxicity [3,6]. Both types of stressors significantly change plants' metabolism and physiological processes [4,7], resulting in a reduced yield and/or a lower quality yield [8].

Stress is a physiological state when plants' growth, development, and reproduction are below their normal function [9,10]. Nitrogen [11] and zinc [12] deficiencies are among the important abiotic stressors. Nitrogen is a key macro element for normal plant growth and development. Its deficiency upsets the metabolic balance [11], reduces the chlorophyll content and photochemical efficiency of photosystem II (PSII) [13], and induces oxidative stress [14,15]. The appropriate form and amount of nitrogen enhances the yield and yield quality [16,17]. Amino acids are the most effective forms of nitrogen fertilizer to use on plants. Plants need to invest less energy in absorbing amino acids relative to the absorption of nitrate or ammonium ions [18,19]. The application of amino acid foliar fertilizers increases the dry matter accumulation, as well as the chlorophyll and carbohydrates content in broad beans [20], and enhances the productivity of tomatoes [21]. Many plant species, such as tomato [22], wheat [23], and maize [24] are able to absorb amino acids as nitrogen sources. In addition, Brankov et al. [24] showed that amino acids containing foliar fertilizer increase the fresh weight, leaf area index, and plant height of maize. They also stated that the maize yield was 30% higher when plants were treated with amino acids, compared to the non-treated control's yield. The effects of amino acids-containing foliar fertilization were mainly examined under abiotic stress. Bahari et al. [25] showed that applying amino acid-containing foliar fertilizer increased the photosynthetic pigment content of wheat under salinity stress and normal growing conditions (control). They also found that the activity of catalase (CAT) and peroxidase (POD) was higher in amino acid-treated plants (also under normal and stressed conditions), which can mitigate oxidative stress and improve the plants' resistance to salinity stress [25].

Zinc (Zn) is an essential microelement that plays a key role in the structure of enzymes and is involved in many biochemical processes, such as photosynthesis, activation of the enzyme system, and protein synthesis [26]. It is possible to increase the quality of the product by consuming 50 kg/ha of zinc sulfate on farms, and by increasing the consumption of this substance to 100 kg/ha of zinc sulfate if it has not had a good effect on crops. Increased zinc sulfate caused zinc poisoning and prevented the transfer of nutrients as a result of plant disorders [27]. The application of Zn as a foliar fertilizer enhances the photochemical reactions in the thylakoid membrane and the electron transport through the photosystem II (PSII) as well as increasing the rate of photosynthesis and the amount of photosynthetic pigment such as chlorophylls. Higher activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were observed after zinc foliar fertilization in tomatoes [28]. Increased corn-grain yield was also observed due to the use of zinc sulfate fertilizer up to 30%. Zinc deficiency is observed in different areas of corn cultivation in the form of pale-yellow bands in the middle leaf veins, and necrosis.

The application of microelements and amino acids as foliar fertilizer is a common practice. The microelements are utilized better when they are sprayed on the leaves than if they are applied to the soil. The use of zinc-containing foliar fertilizer on zinc-deficient soils can double the zinc concentration of grains [29]. The foliar application of micronutrients at different stages of growth can be considered a potential technique to increase the growth and productivity of sugar beet plants [30]. The application of zinc-sulfate solution ($ZnSO_4$) and ZnEDTA increases the growth and yield of plants [31,32]. Zn-containing foliar fertilizer enhances the maize yield and quality parameters [33,34]. The advantage of using foliar fertilizers is that they are absorbed faster than fertilizers which are applied to the soil, and their effects are faster as well. We do not have enough knowledge about the influences of foliar fertilizers on the plants' physiological and biochemical responses after the application. So, it is important to study the responses of plants to the applied foliar fertilizer for a short and long period after the treatments. Our research aims are to evaluate the Zn-containing foliar fertilizer, amino acid foliar fertilizer, and the combination of the effect on the physiological and biochemical responses and corn yield.

2. Results

The relative chlorophyll content (SPAD units) significantly increased at the Zn treatment one, two, and four WAT. There was also a significant increase in the SPAD units when Zn+AS1 and Zn+AS2 were used three WAT. Additionally, the Zn+AS2 treatment significantly increased the SPAD units one WAT. There were no significant differences in the treatments from week five (Figure 1).

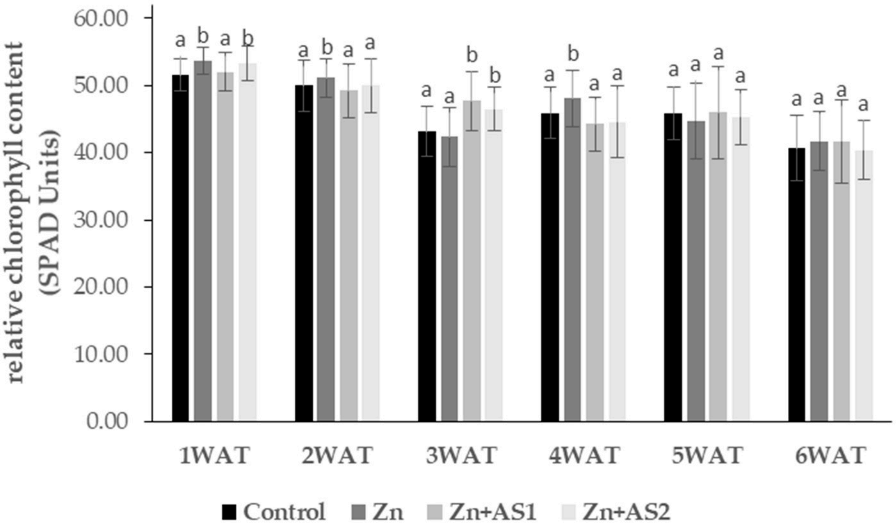

Figure 1. The effects of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the relative chlorophyll content (SPAD units) in the youngest and fully developed leaves of maize 1,2, 3, 4, 5, and 6 WAT. $n = 200 \pm$ S.D. Lower case letters (a, b) show the significant differences among the treatment based on sampling time. WAT: week(s) after treatment.

None of the applied treatments had a significant effect on basic fluorescence (F_o), except Zn-treatment (five WAT), which significantly decreased the F_o by 11.8%, compared to the control (Figure 2). The maximum fluorescence (F_m) significantly decreased at the Zn+AS1 and Zn+AS2 treatments one WAT. In addition, there was a significant increase in F_m when plants were treated with Zn+AS2 three WAT (Figure 3). The variable fluorescence (F_v) was significantly lower at Zn+AS1 one WAT and the Zn and Zn+AS2 treatments five WAT. No changes were observed at other sampling times (Figure 4). The maximum photochemical efficiency of photosystem II (F_v/F_m) (PSII) only changed one WAT when the Zn+AS1 treatment was used (Figure 5).

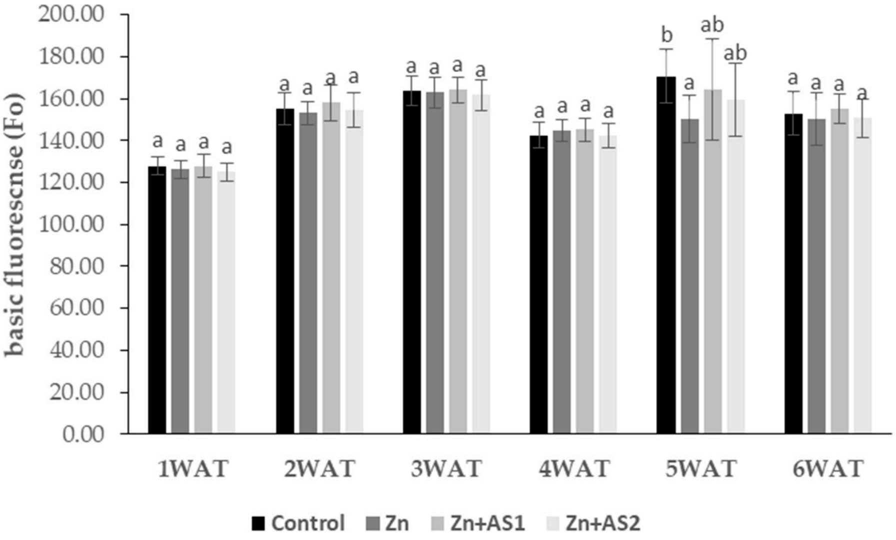

Figure 2. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the basic fluorescence (F_o) in the second youngest and fully developed leaves of maize 1, 2, 3, 4, 5, and 6 WAT. $n = 20 \pm$ S.D. Lower case letters (a, b) show the significant differences among the treatment based on sampling time. WAT: week(s) after treatment.

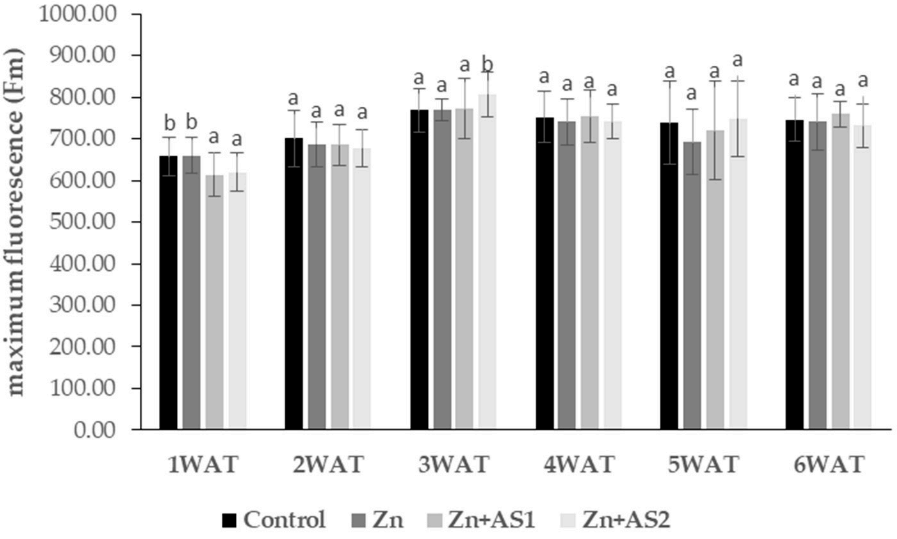

Figure 3. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the maximum fluorescence (F_m) in the second youngest and fully developed leaves of maize 1, 2, 3, 4, 5, and 6 WAT. *n* = 20 ± S.D. Lower case letters (a, b) show the significant differences among the treatment based on sampling time. WAT: week(s) after treatment.

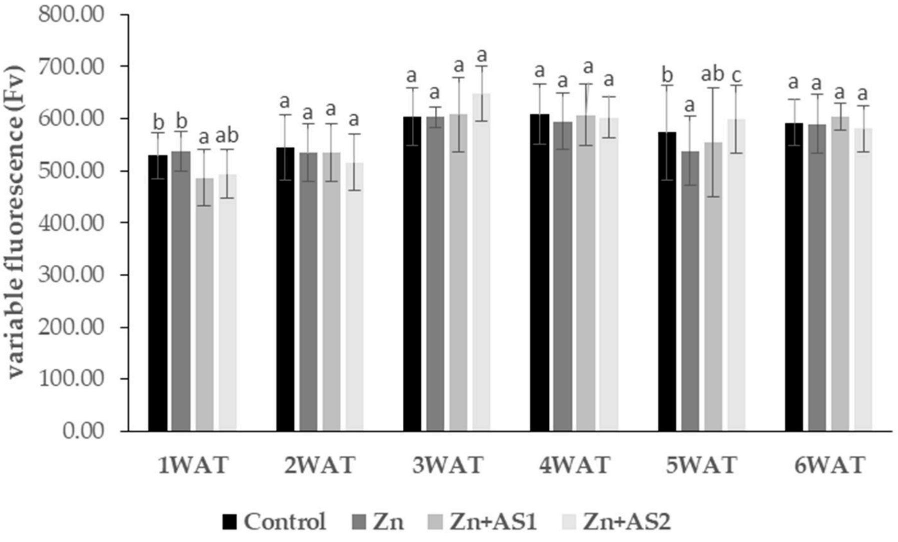

Figure 4. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the variable fluorescence (F_v) in the second youngest and fully developed leaves of maize 1, 2, 3, 4, 5, and 6 WAT. *n* = 20 ± S.D. Lower case letters (a, b, and c) show the significant differences among the treatment based on sampling time. WAT: week(s) after treatment.

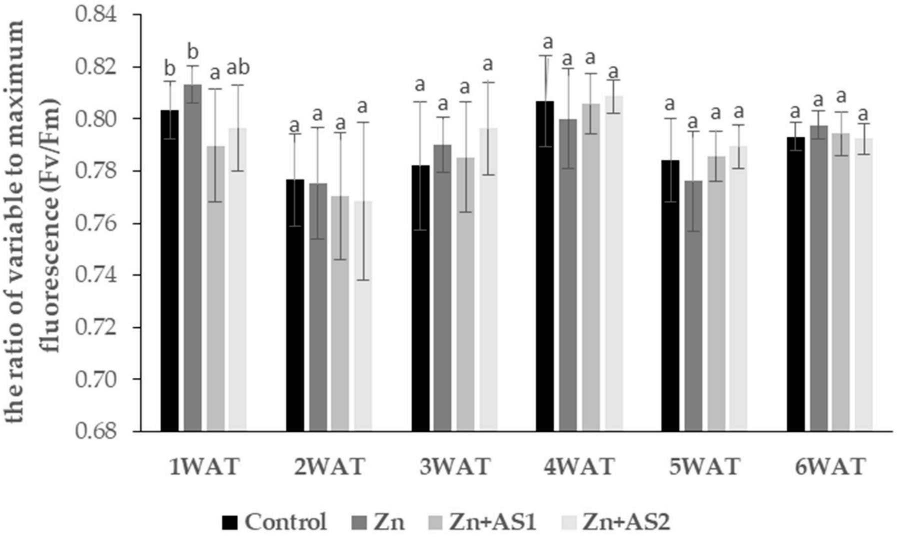

Figure 5. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the ratio of variable to maximum photochemical efficiency of PSII in the second youngest and fully developed leaves of maize 1, 2, 3, 4, 5, and 6 WAT. $n = 20 \pm$ S.D. Lower case letters (a, b) show the significant differences among the treatment based on sampling time. WAT: week(s) after treatment; PS: photosystem.

The proline content in leaves was significantly increased when zinc-containing foliar fertilizer (Zn) was used in combination with amino acid-containing (AS1 and AS2) foliar fertilizers one and two WAT relative to the control. Additionally, the Zn+AS2 treatment also affected proline three WAT. None of the treatments showed efficacy from 4 WAT (Figure 6).

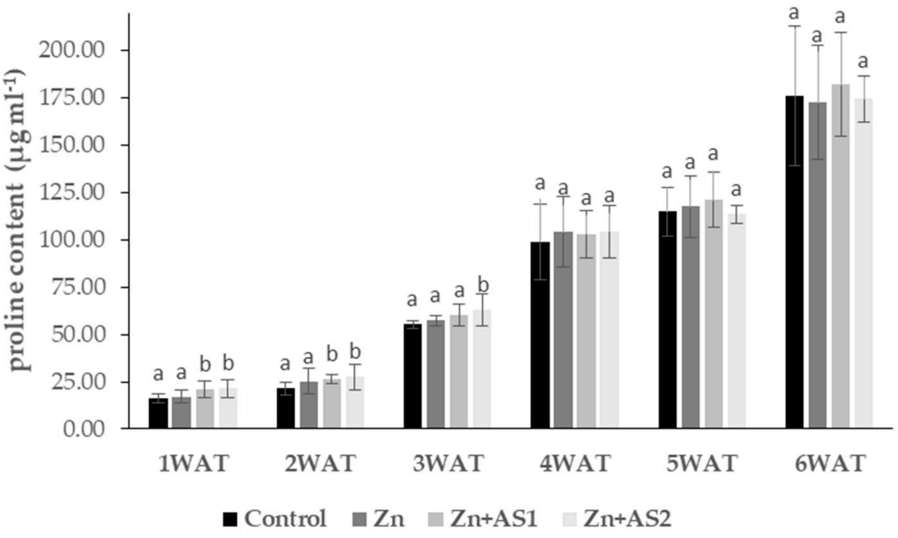

Figure 6. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the proline content in corn leaf, $n = 12 \pm$ S.D. Lower case letters (a, b) show the significant differences among treatments based on the independent *t*-test ($p < 0.05$). AS: amino acid; FW: fresh weight; Zn: zinc; WAT: week(s) after treatment.

The MDA concentration significantly increased one WAT at all three applied treatments, compared to the control. There were no significant changes in the MDA concentration two WAT. However, the MDA concentration significantly decreased three WAT. Zn+AS2 treatment significantly increased the MDA concentration, while Zn treatment alone substantially reduced MDA four WAT. There were no significant differences among the treatments at five and six WAT (Figure 7).

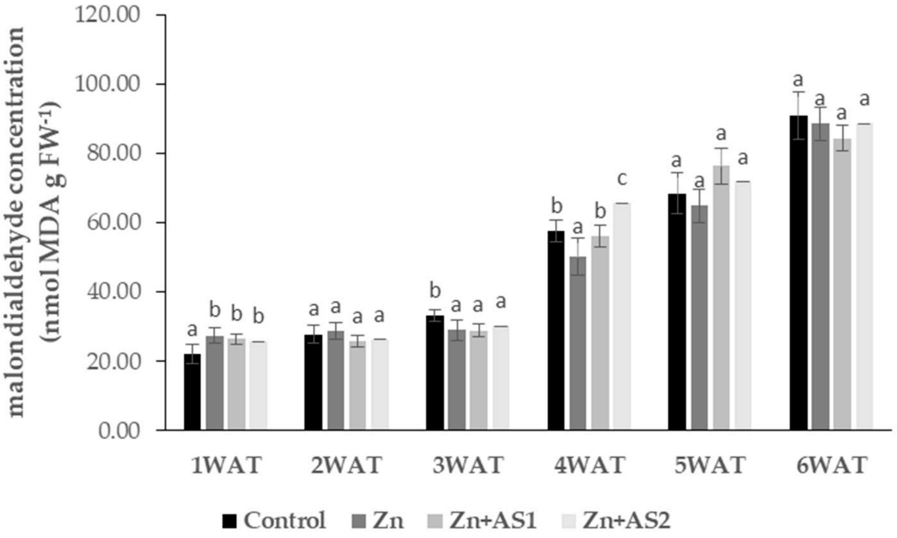

Figure 7. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the malondialdehyde concentration of corn leaf, $n = 12 \pm$ S.D. Lower case letters (a, b, and c) show the significant differences among treatments based on the independent *t*-test ($p < 0.05$). AS: amino acid; FW: fresh weight; Zn: zinc; WAT: week(s) after treatment.

The activity of guaiacol peroxidase (POD) significantly decreased one WAT, while significantly increasing two WAT in the case of Zn and Zn+AS1 treatments. A significant increase was also observed at the Zn+AS2 treated plants three and four WAT. Furthermore, the POD activity significantly increased five WAT and significantly decreased six WAT at Zn+AS2 treatments, compared to the control (Figure 8).

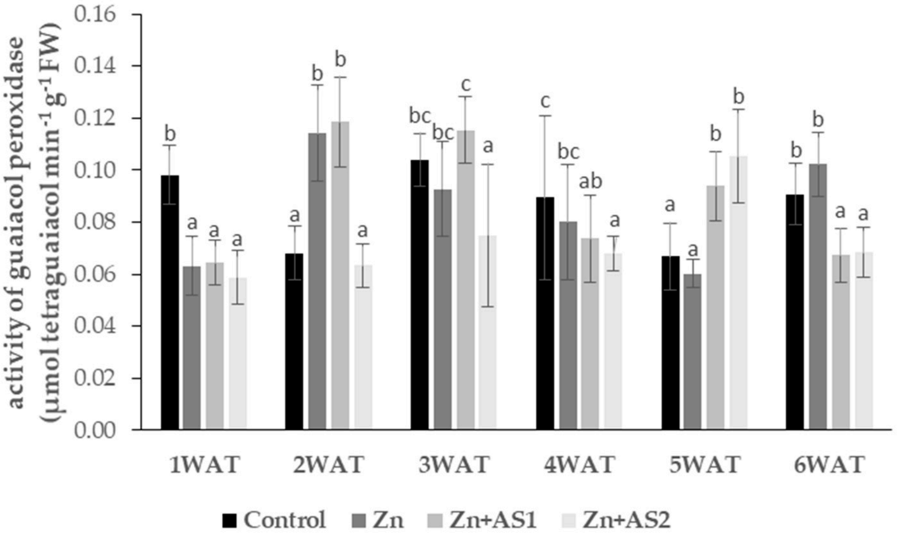

Figure 8. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on guaiacol peroxidase activity, $n = 12 \pm$ S.D. Lower case letters (a, b, and c) show the significant differences among treatments based on the independent *t*-test ($p < 0.05$). AS: amino acid; FW: fresh weight; Zn: zinc; WAT: week(s) after treatment.

There was no significant difference in the APX activity one and two WAT, compared to the control. The APX activity significantly increased at the Zn+AS1 and Zn+AS2 treatments three WAT. Additionally, the Zn+AS1 treated plants had significantly higher APX activity four WAT. There was a significant difference between five WAT (Zn+AS1 and Zn+AS2) and six WAT (Zn+AS2), compared to the control (Figure 9).

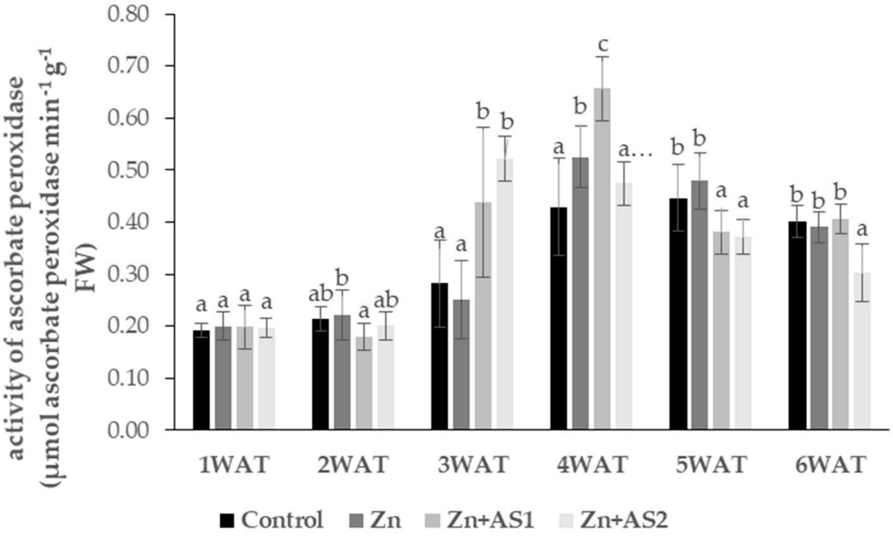

Figure 9. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the activity of ascorbate peroxidase, $n = 12 \pm$ S.D. Lower case letters (a, b, and c) show the significant differences among treatments based on the independent *t*-test ($p < 0.05$). AS: amino acid; FW: fresh weight; Zn: zinc; WAT: week(s) after treatment.

The superoxide dismutase activity was significantly higher at all three applied treatments than the control one WAT. Compared to the control, the Zn+AS1 treatments also increased the SOD activity two, three, and four WAT. On the other hand, the Zn+AS2 treatment significantly decreased the SOD activity three WAT. Moreover, a high increase was observed in the SOD activity five (Zn+AS1 and Zn+AS2) and six WAT (all treatments) (Figure 10).

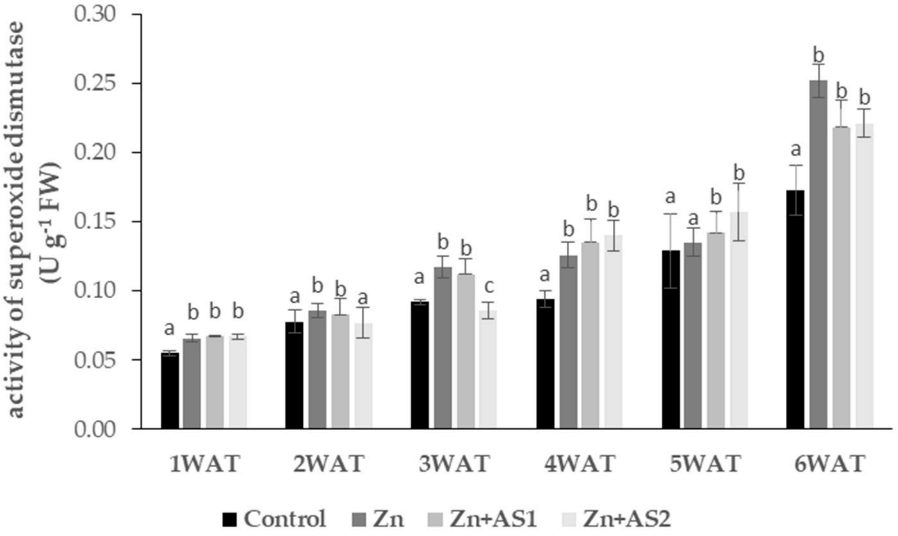

Figure 10. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the activity of superoxide dismutase, $n = 12 \pm$ S.D. Lower case letters (a, b) show the significant differences among treatments based on the independent *t*-test ($p < 0.05$). AS: amino acid; FW: fresh weight; Zn: zinc; WAT: week(s) after treatment.

None of the applied treatments had significant effects on the measured quality parameters of maize (moisture% protein%, starch%, and oil%). In contrast, a significant difference was observed in yield. The number of grains per cob, as well as the weight of grains per cob, significantly increased in all the applied three treatments, compared to the control. The largest yield increase was at the Zn+AS2 treatment. Additionally, there was no significant difference in thousand-seed weight, compared to the control. The yield (t/ha) showed a significant increase in all three treatments, compared to the control. However, these three treatments (Zn, Zn+AS1, and Zn+AS2) were not significantly different (Table 1).

Table 1. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the quality and yield parameters of maize, $n = 40 \pm$ S.D.

Parameters	**Treatments**			
	Control	**Zn**	**Zn+AS1**	**Zn+AS2**
Moisture %	12.6 ± 1.30^{a}	13.3 ± 1.34^{a}	13.2 ± 1.14^{a}	13.2 ± 0.59^{a}
Protein %	5.47 ± 0.49^{a}	5.36 ± 0.36^{a}	5.42 ± 0.50^{a}	5.30 ± 0.33^{a}
Starch %	64.7 ± 0.51^{a}	64.7 ± 0.64^{a}	64.6 ± 0.48^{a}	64.4 ± 0.57^{a}
Oil %	3.22 ± 0.42^{a}	3.11 ± 0.33^{a}	3.25 ± 0.34^{a}	3.40 ± 0.36^{a}
Grain/cob (db)	557 ± 43.2^{a}	$596 \pm 30.8^{b,c}$	583 ± 32.2^{b}	608 ± 43.6^{c}
Grain/cob (g)	245 ± 30.9^{a}	269 ± 21.7^{b}	261 ± 19.4^{b}	269 ± 19.8^{b}
Thousand-seed weight (g)	37.0 ± 5.92^{a}	30.0 ± 4.79^{a}	43.0 ± 6.81^{a}	28.5 ± 4.56^{a}
Grain weight/cob (g) 14%	24 ± 28.3^{a}	257 ± 15.9^{b}	248 ± 17.6^{b}	256 ± 16.09^{b}
Thousand-seed weight at 14% moisture (g)	416 ± 34.8^{a}	430 ± 23.0^{a}	425 ± 40.2^{a}	422 ± 28.3^{a}
Ear weight (g)	275 ± 33.6^{a}	309 ± 22.9^{b}	296 ± 23.9^{b}	306 ± 22.5^{b}
Cob weight (g)	30.6 ± 5.48^{a}	36.2 ± 6.15^{b}	34.6 ± 4.98^{b}	35.3 ± 2.62^{b}
Grain:cob	12.6 ± 1.30^{a}	13.3 ± 1.34^{a}	13.2 ± 1.14^{a}	13.2 ± 0.59^{a}
Yield t/ha at 14% moisture	17.3 ± 2.09^{a}	18.9 ± 1.25^{b}	18.4 ± 1.30^{b}	19.0 ± 1.19^{b}

Lower case letters (^{a}, ^{b}, ^{c}) show the significant differences among treatments based on the independent *t*-test ($p < 0.05$). AS: amino acid; FW: fresh weight; Zn: zinc.

3. Discussion

Most research focuses on the effects of foliar fertilization, amino acid and microelements, such as Zn, and fertilizers under stress conditions on plants' physiological and biochemical responses. There is less knowledge about what happens in plants after the application of foliar fertilizer. Awad et al. [35] stated that zinc-containing foliar fertilizer significantly increases the relative chlorophyll content in carrot leaves under zinc-deficient conditions. In addition, other research studies showed the positive effect of zinc foliar fertilization on the chlorophyll content of citrus [36] and canola [37] under zinc deficiency. Furthermore, reports indicate that the application of zinc as a foliar fertilizer could increase leaf chlorophyll content under normal, not microelements deficient, growing conditions. The Zn-foliar fertilizer significantly increased the chlorophyll content of mung beans [38], wheat [39], peppermint [40], and maize [41]. Based on the obtained data, it can be concluded that maize did not show any nitrogen or other nutrient deficiency (e.g., iron or zinc) in any of the applied treatments, as none of the treatments reduced the relative amount of chlorophyll, compared to the control treatment. Furthermore, the inappropriate time of fertilization or unsuitable weather conditions can cause harmful or negative effects of foliar fertilizer on plants [42]. The application of Zn-containing foliar fertilizer significantly increased the relative chlorophyll content of one, two, and three WAT in this experiment (Figure 1). Applying amino acids as foliar fertilizers increases the fresh matter, leaf area index, and plant height of maize in a field experiment [24]. Tardos et al. [43] showed that chlorophyll-a and chlorophyll-b concentrations were significantly higher in maize leaf when amino acid fertilizer was sprayed at 7-leaf, tasseling, and milk stages, compared to the non-treated control. In this experiment, the relative chlorophyll content significantly increased one, three, and four WAT (Figure 1).

The abiotic and biotic stresses influence the photosynthetic pigments content and the process of photosynthesis as well. To examine the impacts of treatments on photosynthesis efficiency, chlorophyll fluorescence measurements are one of the most common practices [44]. Three characteristics such as basic (F_o), maximum (F_m), and variable fluorescence (F_v) were measured, and two were calculated (F_v/F_m and F_v/F_o) in this study. Among the chlorophyll fluorescence characteristics, F_v/F_m is one of the most often used in scientific papers [45]. Romanowska-Duda et al. [46] stated that the examination of the maximal efficiency of photosystem II (PSII) could be a rapid and reliable method to measure the plants' reaction to fertilization. The Zn-containing foliar fertilizer had a significantly negative effect on F_O only five WAT. Additionally, the F_m and F_v were significantly lower one week after the Zn+AS1 and Zn+AS2 treatments. A significant increase was observed at the Zn+AS2 treatments three WAT in the case of F_m (Figures 2 and 3). Furthermore, the Fv was significantly lower at Zn treatment, and significantly higher at Zn+AS2 treatment five WAT (Figure 4). The calculated parameters such as F_v/F_m and F_v/F_o were significantly changed only one week after Zn+AS1 treatments (Figures 5 and 11). This suggests that the plants were not able to utilize the received nutrients immediately in the metabolic processes; instead, such a high amount of nutrients acted as a stress factor.

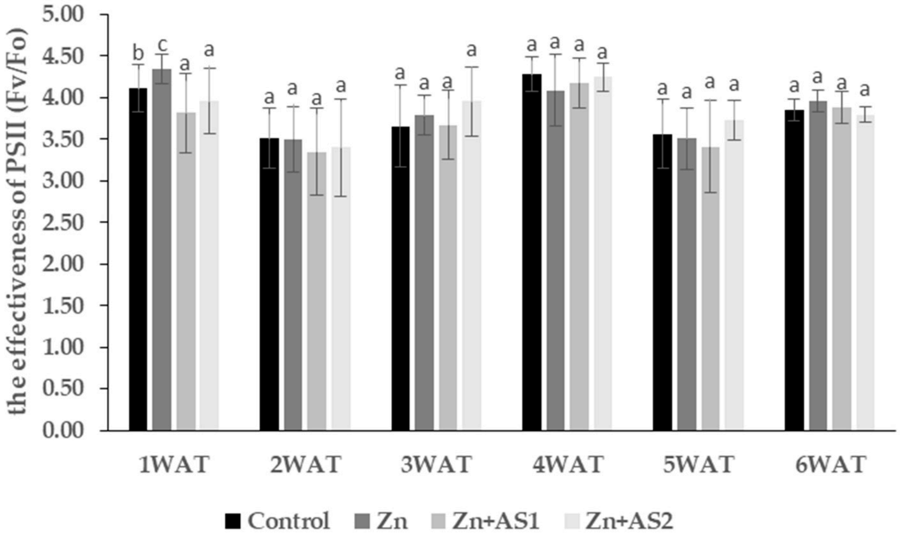

Figure 11. The effect of different foliar treatments (Zn, Zn+AS1, and Zn+AS2) on the effectiveness of PSII in the second youngest and fully developed leaves of maize 1, 2, 3, 4, 5, and 6WAT. *N* = 20 ± S.D. Lower case letters (a, b, and c) show the significant differences among the treatment based on sampling time. WAT: week(s) after treatment.

Proline is called a stress amino acid, so-called because it can indicate stress conditions [47]. The proline concentration was significantly higher at the Zn+AS1 and Zn+AS2 treatments one WAT and two WAT, and it was also higher at Zn+AS2 three WAT in this study (Figure 6). The proline concentration in the leaf was mainly examined under stress conditions. There is no literature on how proline concentration changed after the foliar fertilizer application. This study proves that the application of Zn and amino acid-containing fertilizer simultaneously increases the proline concentration in maize's leaf one and two weeks after the treatments. This result suggests that the foliar fertilizer is a stress factor because the plants could not utilize all the applied nutrients and use them in their metabolism processes.

The concentration of malondialdehyde (MDA) is usually used to present the rate of lipid peroxidation, which is a stress indicator of membrane damage [48]. The use of Zn-containing foliar fertilizers decreased the concentration of MDA and hydrogen peroxide relative to the controls under oxidative stress conditions that were induced by drought in tomatoes [49]. The MDA concentration was significantly higher 1WAT and significantly lower three WAT in all three treatments relative to the control. In addition, significant changes were also observed four WAT, where the MDA concentration was lower at Zn and significantly higher at Zn+AS2 treatments.

Foliar application of a high amount of micro fertilizers increases the number of reactive oxygen species. The application of micronutrients enhances the enzymatic defense systems of plants in response to abiotic stresses [50]. Zinc-containing fertilizer increased the activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) in tomatoes [28]. The activity of SOD and POD were notably higher when celery was treated with copper, zinc, and boron foliar fertilizer, compared to the control [51]. The micronutrient plays an important role in plant physiology, and their doses are a key factor in their functionality [52]. Amino acids have various roles in crops, such as reducing the stress responses in plants, nitrogen sources, hormone precursors [53], and antioxidant metabolism [54]. Santi et al. [55] showed that maize plants grown in a diluted nutrient solution containing a mixture of free amino acids presented changes, and included several transcripts-encoding transcription factors, among them, those related to cellular organization, stress, transport, metabolism, and hormonal signaling. The antioxidant enzyme activities varied in this study. The POD activity was significantly lower (Figure 8), while the APX activity did not change (Figure 9), and the SOD activity was significantly higher one WAT (Figure 10). Peroxidase activity is one of the key factors in stress responses that regulate ROS levels [56]. This enzyme transforms highly reactive ROS such as hydrogen peroxide (H_2O_2) into harmless molecular oxygen and water [57]. This means that when the concentration of H_2O_2 is high, the activity of POD increases. The higher concentration of H_2O_2 can be explained by the higher activity of SOD [58] because SOD converts superoxide into H_2O_2 and molecular oxygen [59]. However, in this study, higher SOD activity resulted in lower POD activity. On the other hand, the activity of APX did not change significantly one WAT. The APX activity was significantly higher at the Zn+AS1 and Zn+AS2 treatments three WAT, and significantly lower five WAT. The proper functioning of SOD reduced the number of ROS and increased the yield [60]. Singh et al. [61] reported that Zn-containing foliar fertilizer increased the SOD activity. Several studies have shown a strong positive correlation between Cu/Zn SOD activity and the amount of Zn that is available to plants [62–64].

The application of zinc [65] and amino acid foliar fertilizers has impacts on yield and yield quality [24]. However, Steward et al. [66] found a limited yield response of maize to Zn-containing foliar fertilizer. They assumed that their data provide evidence for target growth stages to increase micronutrient uptake, and for the mobilization of the applied micronutrient to tissues with physiological demand. In a three-year field experiment, an 18% yield was reported when maize was sprayed with Zn-containing fertilizer [67]. Furthermore, Teixeira et al. [67] stated that the application of amino acid foliar fertilizer increased soybean production by 21% in a greenhouse and 22% in a field experiment. In this study, the yield increased by 10%, 6%, and 10% at the Zn, Zn+AS1, and Zn+AS2 treatments. The yield parameters such as grain/cob and ear weight were also significantly higher under the applied three treatments than the control (Table 1). On the contrary, the applied treatments did not affect the quality parameters such as protein%, starch%, and oil% in this experiment. Such unresponsiveness could be reversed if foliar fertilizers are applied under abiotic stress conditions. The positive impacts of Zn-, and amino acid-containing foliar fertilizer on quality parameters were induced under stress conditions such as drought stress, and not under normal growing conditions [68].

4. Materials and Methods

4.1. Details of the Field Experiment

The experiment was conducted at the Demonstration Garden of the Institute of Land Use, Engineering and Precision Farming Technology (Hungary; 47°55′N, 21°60′E, 111 m asl). All data were obtained in 2020. The experimental plant was a Hungarian fodder corn hybrid (*Zea mays* L. cv Armagnac—FAO490) under irrigated conditions. The irrigation was carried out with a dripping irrigation system that was controlled by remote sensing. The total irrigated amount was 104.04 mm during the important period (8 July 2020–12 September 2020) of the growing season. The study examined four different foliar fertilizer treatments (control: distilled water; Zn: Zn-containing foliar fertilizer, Zn+AS1: the combination of Zn-containing foliar fertilizer and AS1-containing foliar fertilizers, and Zn+AS2: the combination of Zn-containing foliar fertilizer and AS2-containing foliar fertilizers) which were repeated four times (randomized block design). The water-soluble Zn concentration of Zn-containing foliar fertilizer was 10.2%. The composition of amino acids-containing foliar fertilizer is shown in Table 2.

Table 2. The composition of examined amino acid-containing foliar fertilizers (AS1 and AS2).

Composition	AS1	AS2
Total amino acids (m/m%)	47.00	21.67
N (m/m%)	3.20	8.0
P (m/m%)	3.90	0.5
K (m/m%)	3.20	0.2
Alanine %	5.26	2.64
Arginine %	1.52	<0.10
Aspartic acid %	3.80	1.18
Cysteine %	<0.04	<0.10
Glycine %	2.98	0.28
Glutamine %	12.95	16.15
Histidine %	0.96	0.13
Leucine + isoleucine %	2.27	0.23
Lysine %	2.14	<0.10
Methionine %	0.90	<0.10
Phenylalanine %	1.42	<0.10
Proline %	4.23	1.04
Serine %	2.19	-
Threonine %	2.24	-
Tryptophan %	0.34	-
Tyrosine %	1.71	-
Valine %	2.29	-

The seeds were sowed on 23 April 2020, and the treatments were applied on 19 June 2020, as follows, for the Zn-containing foliar fertilizer: 4.8 L ha^{-1}, AS1: 5L ha^{-1}, AS2: 3L ha^{-1}. The relative chlorophyll content (SPAD units), the effectiveness of photosystem II (PSII), and the leaf samples (top fully expanded leaf) for enzyme assays were taken every week for six weeks after the treatments (WAT). The leaf samples were immediately frozen in liquid nitrogen and stored at −80 °C until processing.

The soil samples were collected from the experimental field on 1 April, before the growing season, and analyzed by the accredited HL-LAB Environmental and Soil Testing Laboratory (Debrecen, Hungary). The plasticity index, according to Arany (K_A), which describes the soil type, was 45 (clay loam) [69]. Additionally, the following soil parameters were measured: average pH_{KCl} of 7.64 [70]; humus content of 2.12%; carbonated lime content of 16.3%; Al-lactate soluble P_2O_5 content of 321 mg kg^{-1}; Al-lactate soluble K_2O of 256 mg kg^{-1} (MSZ, 1999); and Al-lactate soluble Na content of 55.67 mg kg^{-1}. The KCl-soluble $N\text{-}NO_3 + NO_2$ (all nitrate + nitrite) [71] content was 5.3 mg kg^{-1}, while the KCl-soluble Mg content was 451.6 mg kg^{-1}, and the S content was 8.84 mg kg^{-1}. In addition, the potassium-chloride EDTA-soluble Cu, Mn, and Zn contents were 1.99, 60.07, and 2.70 mg kg^{-1}, respectively.

Data on average precipitation and average air temperatures during the growing season are shown in Figure 12.

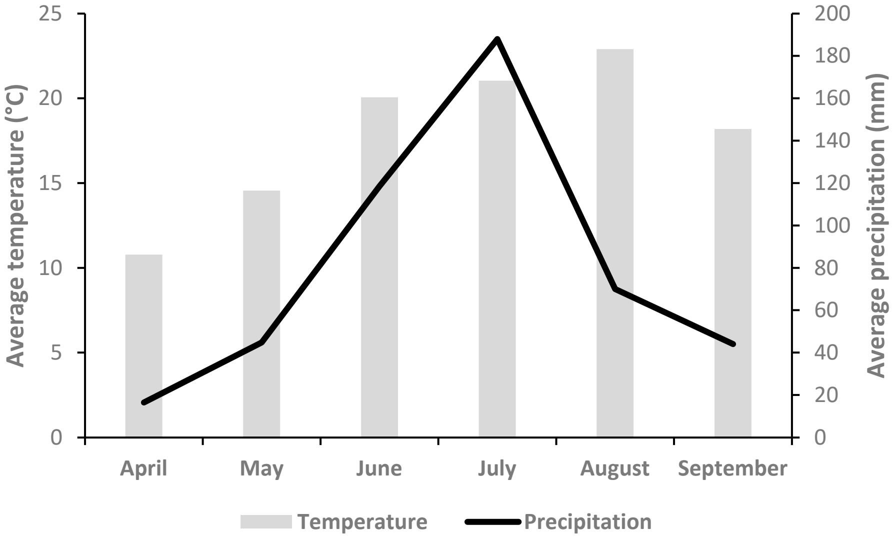

Figure 12. Precipitation and temperature data of the growing season (April 2020 and September 2020) in the experimental site.

4.2. Relative Chlorophyll Content (SPAD Units)

SPAD-502+ Chlorophyll Meter (Konica Minolta, Japan) readings were used to measure the relative chlorophyll content presented in SPAD Units. The measurements were taken one, two, three, four, five, and six WAT on the second top fully expanded leaf in five replications per plant. Ten plants per plot were used. The number of repetitions was 200 in the case of relative chlorophyll content measurements.

4.3. The Photochemical Efficiency of Photosystem II (PSII)

To determine the efficiency of photosynthesis, chlorophyll fluorescence was used as an indirect method [72]. The parameters of the rapid phase of chlorophyll fluorescence induction were determined using an OS5p+ fluorometer (Opti-Sciences, Hudson, NH, USA). Before the measurements, the leaves were dark-adapted using clips for 20 min. During the measurement, the dark-adapted leaf was illuminated with low light and the level of the basic fluorescence (F_0) was measured, and then the instrument detected the maximum fluorescence (F_m) after applying a saturation light pulse (6000 µmol m^{-2} s^{-1}). F_m falls back to F_0 after about 20 s in the dark. The difference between F_m and F_0 is the variable fluorescence (F_v). The F_v/F_m ratio was used to characterize the maximum photochemical efficiency of PSII.

4.4. Determination of Proline Content of Leaves

Proline in corn leaves was determined using 0.1 g fresh tissue ground in liquid nitrogen (LN) and 2 mL of ethanol (70% (*v*/*v*)) [73,74]. One mL (1mL) of reaction mixture (1% ninhydrin in 60% (*v*/*v*) acetic acid) was reacted with 500 µL of sample solution in a 1.5 mL Eppendorf tube and heated (95 °C) on a hot plate with agitation for 20 min, after which the reaction was stopped in ice immediately and centrifuged (12,000× *g* for 1 min). The absorbance of the supernatant was read at 520 nm. The amount of proline was calculated using ethanol as a blank and the proline standard curve was plotted from the absorbance values [75].

4.5. Determination of Malondialdehyde (MDA) Concentration

To determine the corn leaves' malondialdehyde concentration, the methods of Heath and Packer [76] were used. Fresh corn tissue (100 mg) was powdered using LN, and a mortar and pestle. The homogenized tissue powder was ground in a 1 mL thiobarbituric acid (TBA, 0.25% (*w*/*v*)) and trichloroacetic acid (TCA, 10% (*w*/*v*)) mixture. The solution was centrifuged at 10,800× *g* for 25 min at 4 °C. Afterwards, 0.2 mL of supernatant was reacted with 0.8 mL of TCA (20% (*w*/*v*)) and TBA (0.5% (*w*/*v*)) mixture in a clean Eppendorf tube. The reaction mixture was mixed well using a vortex and placed in a water bath (95 °C), and the reaction was stopped using ice after 30 min. This was followed by centrifugation at 10,800× *g* for 10 min at 4 °C. The absorbance was read at 532 nm and 600 nm. The concentration of MDA was calculated using its extinction coefficient (155 $\text{mM}^{-1}\text{cm}^{-1}$).

4.6. Antioxidant Enzymes Assays

The enzyme extract from corn leaf samples for POD and APX activity was prepared based on Pukacka and Ratajczak [77]. One g (1 g) of fresh leaf sample was powdered in LN and ground with a mortar and a pestle in a 5 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM of ethylenediaminetetraacetic acid (EDTA); 2% (*w*/*v*) polyvinylpyrrolidone (PVPP); 0.1% (*w*/*v*) Triton X-100; and 1 mM of ascorbate. The enzyme extracts were kept at a constant 4 °C and centrifuged (15,000× *g* for 20 min). Afterwards, the supernatant was pipetted into a clean Eppendorf tube.

The APX activity was determined according to the method of Mishra et al. [78] with some modifications. One ml (1 mL) of reaction mixture had 470 µL of 50 mM phosphate buffer (pH 7.0); 200 µL of 0.5 mM sodium ascorbate; 50 µL of 0.1 mM EDTA; and 30 µL of enzyme extract, as previously described. In addition, the reaction was initiated with 250 µL of 0.1 M H_2O_2. The APX activity was determined by measuring the decrease in absorbance at 290 nm (2.8 $\text{mM}^{-1}\text{cm}^{-1}$) for 5 min at 20 °C against a blank (50 mM phosphate buffer).

A method of Zieslin and Ben-Zaken [79] was performed to measure the POD activity. The reaction mixture contained 50 µL of 0.2 M H_2O_2; 100 µL of 50 mM guaiacol; 340 µL of distilled H_2O; 500 µL of 80 mM phosphate buffer (pH 5.5); and 10 µL of enzyme extract. An increase in absorbance as a result of tetraguaiacol formation was measured at 470 nm for 3 min at 30 °C against the blank (50 mM phosphate buffer). The absorbance of the reaction mixture was read at 470 nm for 3 min at 30 °C. An extinction coefficient of 26.6 $\text{mM}^{-1}\text{cm}^{-1}$ was used to determine the peroxidase activity.

The SOD activity was measured by a photochemical reduction in nitro blue tetrazolium chloride (NBT) [80], according to the method of Beyer and Fridovich [81]. A fresh leaf sample (400 mg) was crushed with liquid nitrogen and 4 mL of 50 mM phosphate buffer (0.1 mM EDTA, 1% PVPP (*w*/*v*); 1 mM phenylmethylsulfonyl fluoride (PMSF) at pH 7.8) was added to each sample. The samples were centrifuged at 10,000× *g* for 15 min. One SOD unit was defined as the amount of enzyme that was required to inhibit the light-induced reduction in nitro blue tetrazolium (NBT) by 50%, compared to tubes without plant extract. After centrifugation, 25 µL of the supernatant as plant extract; 25 µL of NBT (9 mM); 25 µL of riboflavin (0.25 mM); 250 µL of methionine (0.16 M); and 2.675 mL of phosphate buffer (pH 7.8, 50 mM) was stirred, kept at room temperature, and after 15 min the absorbance of the mixture was measured at 560 nm. A total of 2.7 mL of phosphate buffer without plant extract was added to the blank tubes. All other components were the same as described above.

4.7. Measurements of Quality Parameters and Yield

The quality parameters of grains (oil, protein, starch contents) were measured with a DA 7250 At-line NIR Instrument (Perkin Elmer, Waltham, MA, USA). Two hundred grams of whole corn grains were used in 40 repetitions per treatment.

4.8. Statistical Analysis

The research data were analyzed using IBM SPSS Statistics 25 (Armonk, NY, USA) software. For normality tests, Kolmogorov–Smirnov and Shapiro–Wilk tests [82] were applied. The data were evaluated by a one-way ANOVA [83], and the differences among means were tested using the Tukey HSD test [84].

5. Conclusions

The impacts of Zn and amino acid-containing foliar fertilizers were examined on a Hungarian fodder corn hybrid. The relative chlorophyll content was significantly higher one, two, and four weeks after Zn-treatment, and some changes were also observed when Zn and amino acid-containing fertilizer were applied in combination. Although the treatments had positive effects on yield and yield parameters such as cob weight, the quality parameters did not change significantly relative to the control. The treatments, rather than the relative chlorophyll content, had less influence on the fluorescence characteristics. The measured and calculated fluorescence parameters significantly changed mostly one WAT, compared to the control. The activity of antioxidant enzymes and the concentration of proline and malondialdehyde also changed after Zn and amino acid-containing foliar fertilizers application, compared to the control. The data of this research suggest that the plants were not able to utilize the received nutrients immediately in the metabolic processes; instead, such a high amount of nutrients acted as a stress factor after a short time of foliar fertilizer application. This means that the applied AS and Zn treatments effects were measured on yield and not on measured physiological parameters, e.g., antioxidant enzymes activities and characteristics of PSII. In practice, this means that farmers do not see the effect of Zn and AS-containing foliar fertilizers directly or within a short time after their application, because their effects are reflected in yield parameters.

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Abbreviations

amino acid-containing foliar fertilizers type 1 (AS1); amino acid-containing foliar fertilizers type 2 (AS2); ascorbate peroxidase (APX); catalase (CAT); liquid nitrogen (LN); photosystem II (PSII); superoxide dismutase (SOD); week(s) after treatment (WAT); Zn (zinc).

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