





## Article

# Fermented Deproteinized Alfalfa Juice Modified with Fly Ash Filtrate as an Alternative Nutrient Source for Winter Wheat (*Triticum aestivum* L.)

Péter Makleit <sup>1</sup>, Andrea Kovács Balláné <sup>2</sup>, Nóra Bákonyi <sup>1</sup>, Éva Domokos-Szabolcsy <sup>1</sup>, Gábor Miklós Fári <sup>1</sup> and Szilvia Veres <sup>1,\*</sup>

- <sup>1</sup> Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Crop Science, Department of Applied Plant Biology, University of Debrecen, 4032 Debrecen, Hungary; pmakleit@agr.unideb.hu (P.M.); bakonyi@agr.unideb.hu (N.B.); szabolcsy@agr.unideb.hu (É.D.-S.); fari@agr.unideb.hu (G.M.F.)
- <sup>2</sup> Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Agricultural Chemistry and Soil Science, University of Debrecen, 4032 Debrecen, Hungary; kovacs@agr.unideb.hu
- \* Correspondence: szveres@agr.unideb.hu

**Abstract:** An alternative method of plant nutrition involves the utilization of different by-products. In this study, a combination of two by-products was applied to investigate this method: fermented deproteinized alfalfa juice (FDAJ), a by-product of alfalfa leaf protein production, and fly ash filtrate from a wood-fired power plant. A pot experiment was conducted with winter wheat in an open-sided greenhouse in sandy soil. The aim was to evaluate the efficacy and usability of the combination of these by-products (SFDAJ) for enhanced plant nutrition via spraying or irrigation. Prior to overwintering, photosynthetic pigments, relative chlorophyll content, specific leaf area, and shoot dry weight were measured. At full maturity, we determined morphological parameters, yield, and the element content of the grain. Significant differences were observed between treatments at full maturity. The application of SFDAJ resulted in 73.2% of the yield having the optimal nutrient supply. Compared to the treatment with no nutrients added, the application of SFDAJ increased yields by 260%. Our results show that SFDAJ alone is suitable for providing enhanced nutrient supply in soils with good nutrient supply or in extensive cultivation technology. When supplemented with fertilizer, it can be used on soils with low nutrient supply or in intensive cultivation technology. Based on our results of field applications of SFDAJ, the addition of 10 v v<sup>-1</sup>% FDAJ via irrigation is recommended.

**Keywords:** deproteinized plant juice; brown juice; fly ash; by-product utilization; plant nutrition



Academic Editor: Jianbin Zhou

Received: 29 December 2024

Revised: 23 January 2025

Accepted: 26 January 2025

Published: 28 January 2025

**Citation:** Makleit, P.; Balláné, A.K.; Bákonyi, N.; Domokos-Szabolcsy, É.; Fári, G.M.; Veres, S. Fermented Deproteinized Alfalfa Juice Modified with Fly Ash Filtrate as an Alternative Nutrient Source for Winter Wheat (*Triticum aestivum* L.). *Agronomy* **2025**, *15*, 339. <https://doi.org/10.3390/agronomy15020339>

**Copyright:** © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Plant nutrition is an essential agrotechnical factor in crop production. The cost of plant nutrition accounts for a significant portion of overall production expenses. Plant nutrition quality determines the quantity and composition of the crop product [1]. Chemical fertilizers are becoming increasingly costly to produce and purchase. Moreover, they pose significant environmental risks, including soil acidification, soil degradation, and nitrogen leaching [2], and resource exploitation is threatening some natural resources such as rock phosphates [3]. In addition to conventional organic fertilizers, biofertilizers and various by-products for nutrient supply can be used as alternatives to chemical fertilizers. The use of by-products for plant nutrition is a rational solution for several reasons. They are produced in increasing quantities, and most of them are not reused or composted

but simply deposited in landfills. They can be used directly without modification or after processing through various methods such as composting, burning, pyrolysis, and anaerobic fermentation, which reduce their negative effects and improve their nutrient availability [4]. In using these alternatives, their risks, mainly their potential toxicity, and cost-effectiveness must be taken into account [5]. Moreover, the use of by-products is in line with the principles of circular farming, as they do not increase the CO<sub>2</sub> footprint [6].

Protein extraction from plants, especially from leaves, holds promise as a sustainable solution for protein-rich feeding of monogastric animals, particularly if the leaf protein concentrate (LPC) has amino acid profiles similar to soybean [7]. The LPC is usually produced by squeezing the plant material, mainly leaves; pressing the green juice; heating to coagulate the proteins; and filtering [8].

One of the by-products of LPC production is brown juice [9], or deproteinized plant juice (DPJ). In the application of alfalfa as a potential biorefinery crop, the DPJ used is called deproteinized alfalfa juice (DAJ) [10]. DPJ yield from plant material is around 450–550 mL kg<sup>-1</sup> [10,11]. The composition of DPJ and DAJ depends on several environmental factors, such as the plant material and the extraction method [12,13]. Although DAJ contains high amounts of minerals, and its application is clearly beneficial for plant nutrition, few publications address its use.

Due to its short shelf life and high production, its preservation is of interest, as well as the testing of its effects on plant species grown over large areas, such as wheat. The use of DAJ as fertilizer immediately after LPC production is not always possible. Due to its high carbohydrate content and the presence of proteins, putrefaction is induced [14]. Lactic acid fermentation may be a suitable preservation method. In this approach, the use of lactic acid-producing bacteria reduces the free sugar content and pH of DAJ, and the product, fermented deproteinized alfalfa juice (FDAJ), can be preserved for a longer period [12]. Although this is a viable technique for preserving DAJ, its low pH is unfavorable for plant nutrient supply [15]. Therefore, before agricultural use, the pH of FDAJ solutions should be adjusted to a level that is optimal for plant nutrition (pH: 6.0–6.5) [16].

Solutions comprising wood ash or fly ash hold promise in FDAJ application. These by-products are available in large quantities in biomass power plants and have high mineral content [17]. An FDAJ solution supplemented with fly ash solution (SFDAJ) is more valuable for plant nutrition.

The aim of our experiment was the evaluation of the effects of two by-products, namely FDAJ solutions supplemented with fly ash solutions applied at different concentrations via foliar spraying and irrigation, on different growth, physiological, and morphological parameters of wheat (*Triticum aestivum* L.). The effect of the application of by-products was compared to the use of conventional nutrient solutions or deionized water in a breeder pot system. The findings of this study contribute to the development of best practices for the field application of FDAJ.

## 2. Materials and Methods

### 2.1. Plant Growth and Treatments

A commercial wheat hybrid, Hystar (Asur Plant Breeding, Estrées-Saint-Denis, France), was used in experiments, which were performed from autumn 2021 to summer 2022. The total duration of the experiment was 293 days. The experiment was carried out in a completely randomized design with four replicates of each treatment. The plants were grown in pots with a diameter of 20 cm in an open-sided greenhouse, except in winter when the pots were moved outside for vernalization and to protect the plant roots from freezing. The pots contained 3 kg of sandy soil with low nutrient content (Table 1). The soil had a low pH, salt, and especially lime content, as well as a moderate humus content.

**Table 1.** Basic characteristics of the experimental soil.

Characteristics	Quantity
pH (KCl)	5.35
K <sub>A</sub>	<25
Density (kg m <sup>-3</sup> )	1400
Water-soluble total salt (m m <sup>-1</sup> )%	<0.02
Lime (m m <sup>-1</sup> )%	<0.100
Humus content (m m <sup>-1</sup> )%	0.99
Phosphorus pentoxide (mol m <sup>-3</sup> ) (AL-soluble)	2.21 × 10 <sup>-7</sup>
Potassium oxide (mol m <sup>-3</sup> ) (AL-soluble)	1.07 × 10 <sup>-6</sup>
Nitrate (mol m <sup>-3</sup> ) (KCl-soluble)	2.78 × 10 <sup>-8</sup>
Sodium (mol m <sup>-3</sup> ) (AL-soluble)	8.43 × 10 <sup>-7</sup>
Magnesium (mol m <sup>-3</sup> ) (KCl-soluble)	7.50 × 10 <sup>-7</sup>
Sulphur (mol m <sup>-3</sup> ) (KCl-soluble)	4.64 × 10 <sup>-8</sup>
Manganese (mol m <sup>-3</sup> ) (EDTA-soluble)	3.08 × 10 <sup>-7</sup>
Zinc (mol m <sup>-3</sup> ) (EDTA-soluble)	1.94 × 10 <sup>-8</sup>
Copper (mol m <sup>-3</sup> ) (EDTA-soluble)	3.41 × 10 <sup>-8</sup>

Twenty seeds were sown in each pot to approximately 2 cm depth. Initially, 20 plants were grown, and after the first (autumn) sampling, 16 plants remained. After overwintering, the number of plants per pot was reduced to 12 by selecting 4 additional plants. No fertilizer was used in the experiment. The soil was irrigated every other day with deionized water up to the field water capacity. In the autumn, the plants received a deionized water age of less than 19 days (BBCH 12). After irrigation with deionized water until the first autumn sampling, the plants were treated four times, once a week (Table 2), with four replicates in each treatment. During the second sampling period, from spring to summer 2022, the plants were treated once a week for one month and twice a week for the remaining period with the treatments described in Table 2, with four replicates in each treatment.

**Table 2.** Treatments and their abbreviations.

Treatments	Marking
Irrigation with deionized water	DW
Irrigation with nutrient solution	NS
Irrigation with a nutrient solution in half concentration	half NS
Irrigation with 5% fermented DAJ completed with fly ash solution	5W
Irrigation with 10% fermented DAJ completed with fly ash solution	10W
Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration	half NS+5S
Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration	half NS+10S
Spraying with 2.5% fermented DAJ solution and irrigation with deionized water	DW+2.5S

The number and timing of treatments and samplings are summarized in Table 3.

**Table 3.** Summary of treatments and samplings in the experiment.

Autumn treatments
Irrigation with deionized water every two days in September
Treatments according to Table 2; once a week, in October
First sampling at the end of October
Spring treatments
Treatments according to Table 2; once a week, in March
Treatments according to Table 2; twice a week, from April to June
Second sampling at the end of June

## 2.2. Preparation and Amount of Solutions for Treatments

The nutrient solution was prepared according to Treeby et al. (1989) [18] by adding  $10^{-3}$  mol L<sup>-1</sup> Fe-EDTA (Merck KGaA, Darmstadt, Germany) to the diluted solution. The FDAJ was obtained from field-grown alfalfa plants originating 20 km away from Hajdúnánás-Tedej, Hungary (geohash: u2x0pwz62y90). The DAJ was fermented by adding 0.01 g L<sup>-1</sup> AdiSil LG-100 Perfect inoculum (Bioferm CZ, Ltd., Brno, Czech Republic) and glucose (Merck KGaA, Darmstadt, Germany); (12 g L<sup>-1</sup>) at room temperature until the solution reached pH = 4. The fly ash used in the experiment was obtained from a biomass power plant in Hungary (Szakoly, Hungary; geohash: u2rxdxjp2tt). The solution was prepared by adding 50 g of fly ash to 1000 mL of deionized water. This solution was stirred for 4 h using an automatic stirring machine and then filtered through filter paper. The pH of this solution was 12.5, making it particularly suitable for FDAJ pH adjustment. To obtain the optimum pH of 6.0, 229 mL and 377 mL of the fly ash solution were added per 1000 mL of 5% and 10% FDAJ solution, respectively. Thus, the final concentration of the FDAJ solution was 3.86% and 6.23% after the addition of the fly ash solution. For each treatment, the volume of solution used for irrigation and foliar spraying was similar (150 mL and 15 mL for each pot, respectively). Pots treated via irrigation received 15 mL of deionized water through foliar spraying. The element contents of the different FDAJ concentrations supplemented with fly ash filtrate, as well as the element content of fly ash and fly ash filtrate, are presented in Supplementary Material.

Table 4 shows the total amount of plant nutrients applied to the plants in one pot of the treatments. In the experiment, a total of 3.6 L of solution was applied by watering and a total of 0.36 L by spraying per pot (0.15 L/watering; 0.015 L/spraying). No plant nutrients were added in DW treatment.

**Table 4.** Total amount of plant nutrients applied to the plants in one pot of the treatments (mg).

Element	NS	Half NS	5W	10W	Half NS+5S	Half NS+10S	DW+2.5S
N	1008.000	504.000	2581.992	4172.731	762.199	921.274	164.445
P	222.984	111.492	29.772	48.110	114.473	116.303	1.993
K	1055.653	527.827	1779.001	2907.335	705.727	818.560	45.975
Ca	1442.808	721.404	806.562	1322.946	802.060	853.699	10.017
Mg	218.628	109.314	42.743	69.077	113.854	116.222	2.772
S	693.410	346.705	445.320	731.596	391.237	419.865	3.287
Cu	0.457	0.229	0.047	0.043	0.234	0.233	0.002
Fe	2.009	1.004	0.277	0.450	1.030	1.049	0.018
Mn	1.105	0.553	0.167	0.270	0.570	0.580	0.001
Zn	1.178	0.589	0.281	0.454	0.617	0.634	0.002

Table 4. Cont.

Element	NS	Half NS	5W	10W	Half NS+5S	Half NS+10S	DW+2.5S
Mo	0.032	0.016	0.097	0.155	0.025	0.031	0.002
B	0.039	0.019	1.094	1.789	0.128	0.198	0.003
Na	-	-	36.180	59.472	3.618	5.947	0.186
Cl	63.817	31.909	-	-	31.909	31.909	-
Al	-	-	0.068	0.112	0.007	0.011	0.005
Ba	-	-	0.454	0.745	0.045	0.075	0.004

Footnote: NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

The element content of the soil in a pot (3 kg soil) should always be added to the amount of nutrients available to the plants: N: 1.31 mg; P: 57.52 mg; K: 121.66 mg; Mg: 76.71 mg; S: 18.69 mg; Mn: 71.01 mg; Zn: 5.31 mg; Cu: 9.12 mg; Na: 81.09 mg.

Considering the proportion of nutrients in the solutions applied to plants in pots, the 5W and 10W treatments show a lower proportion of phosphorus and calcium and a higher proportion of boron compared to the NS and half NS treatments. The nutrient ratios of the applied half NS+5S and half NS+10S solutions are most similar to the nutrient ratios of the NS and half NS solutions.

### 2.3. Samplings, Measurements, and Data Evaluation

The experiment involved two samplings times, the first in October 2021 at BBCH stage 14–15 and the second in June 2022 at BBCH stage 89.

#### 2.3.1. Measurements at the First Sampling

In the first sampling, four shoots were removed from each pot. The parameters were measured in the youngest fully developed leaves. The relative chlorophyll content (SPAD values) was measured using a SPAD-502 chlorophyll meter (Konica Minolta Inc., Tokyo, Japan). The absolute chlorophyll and carotenoid content was measured, following the pigment extraction method of Moran and Porath (1980) [19] and the spectrophotometric method of Wellburne [20], using a spectrophotometer (Metertec SP-830, Metertech Inc., Taipei, Taiwan). Briefly, 50 mg fresh leaves were isolated and placed in 5 mL dimethyl-formamide (Merck KGaA, Darmstadt, Germany) solution for 72 h in the dark. After that, the solution was filtered, and absorbances were measured at 480, 647, and 664 nm. The amount of pigments was calculated from the absorbance values. The specific leaf area (SLA) was determined using the thermogravimetric method. Leaf discs of known amount and diameter were dried to a constant weight, and then their area per unit weight was calculated [21]. The dry weight of the shoots was measured after drying at 65 °C using a drying oven (Mettler UM-100; Mettler GmbH, Büchenbach, Germany) until a constant weight was reached. The leaf discs' dry matter content was determined to measure the SLA and the absolute chlorophyll content, which were used to measure shoot weights. Measurements were performed in four replications.

#### 2.3.2. Measurements at the Second Sampling

During the second sampling, the shoot and spike length, the number of leaves, the number of spikelets in spikes, the number of grains in spikes, and the diameter of the shoot

at the base and under spikes were measured using a caliper and ruler. For the second sampling, 12 plants were used in each replicate.

The total carbon, nitrogen, and sulfur contents of the grains were determined via dry combustion using an Elementar CNS instrument (vario Max cube, Elementar Analysensysteme GmbH., Langenselbold, Germany). The phosphorus, potassium, calcium, magnesium, manganese, and zinc contents of the grain were determined after digestion with  $\text{HNO}_3 + \text{H}_2\text{O}_2$  (Merck KGaA, Darmstadt, Germany) using a CEM MARS 6 (CEM Corporation, Matthews, NC, USA) microwave digester. Briefly, 0.1 g grain and 5 cm<sup>3</sup>  $\text{HNO}_3$  were measured and digested for 50 min. Phosphorus was measured using the spectrometric method via molybdenum blue colorimetry [22] on a Thermo Scientific Gallery 861 spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The K, Ca, Mg, Mn, and Zn contents of the grains were determined using a Thermo Scientific ICE 3500 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) atomic absorption spectrometer, and K was measured using the emission method. Before measurement, the grains were ground in a laboratory mill (Model: IKA A11B, IKA-Werke GmbH., Staufen, Germany). The element content was determined in the four replicates of each treatment.

### 2.3.3. Data Evaluation

Data were analyzed using the statistical software SPSS 23.0. Descriptive statistics were used, followed by analysis of variance and Duncan's test, or Kolmogorov–Smirnov and Mann–Whitney tests, depending on the distribution of the data. Principal component analysis (PCA) was used to examine the relationships between the variables.

## 3. Results

### 3.1. Results of First Sampling

As shown in the results listed in Table 5, a significant difference was only found in the shoot dry weight between the half NS and DW+2.5S treatments, with the difference in the former considered more significant. The SFDAJ treatments did not increase the shoot dry weight. In the DW treatment, the shoot dry weight was the same as in the other treatments.

**Table 5.** Values of the shoot dry weight, specific leaf area (SLA), and relative chlorophyll content (SPAD values) of experimental plants with different treatments at first sampling (10/21); (n = 4; ±SE; significant differences at  $p \leq 0.05$  are indicated with different letters).

Treatments	Shoot's Dry Weight mg Shoot <sup>-1</sup>	Specific Leaf Area (SLA) mm <sup>2</sup> mg <sup>-1</sup>	Relative Chlorophyll Content (SPAD Values)
DW	260.09 ± 14.92 <sup>ab</sup>	5.24 ± 0.20 <sup>ab</sup>	30.04 ± 0.84 <sup>a</sup>
NS	273.46 ± 14.56 <sup>ab</sup>	4.80 ± 0.26 <sup>a</sup>	35.75 ± 1.49 <sup>d</sup>
half NS	294.93 ± 27.40 <sup>b</sup>	5.17 ± 0.14 <sup>ab</sup>	33.84 ± 0.62 <sup>bcd</sup>
DW+2.5S	233.93 ± 11.70 <sup>a</sup>	4.84 ± 0.26 <sup>a</sup>	29.55 ± 1.79 <sup>a</sup>
half NS+5S	272.60 ± 14.22 <sup>ab</sup>	5.24 ± 0.20 <sup>ab</sup>	32.55 ± 0.97 <sup>abcd</sup>
half NS+10S	267.54 ± 21.76 <sup>ab</sup>	4.64 ± 0.23 <sup>a</sup>	35.14 ± 0.59 <sup>cd</sup>
5W	243.45 ± 13.67 <sup>ab</sup>	5.63 ± 0.29 <sup>b</sup>	31.83 ± 0.83 <sup>abc</sup>
10W	237.67 ± 22.28 <sup>ab</sup>	4.76 ± 0.26 <sup>a</sup>	30.61 ± 0.88 <sup>ab</sup>

Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

The SLA of the 5W treatment was significantly larger than that of the NS, DW+2.5S, half NS+10S, and 10W treatments. The relative chlorophyll content differed considerably between treatments, with significantly higher values in the half NS, NS, and half NS+10S treatments and significantly lower values in the DW and DW+2.5S treatments.

Significant differences in chlorophyll-a content were observed between treatments, with lower values in the 5W and 10W treatments than in NS or half NS treatments, alone or in combination (Table 6). The NS and half NS treatments resulted in higher chlorophyll-b contents than the other treatments.

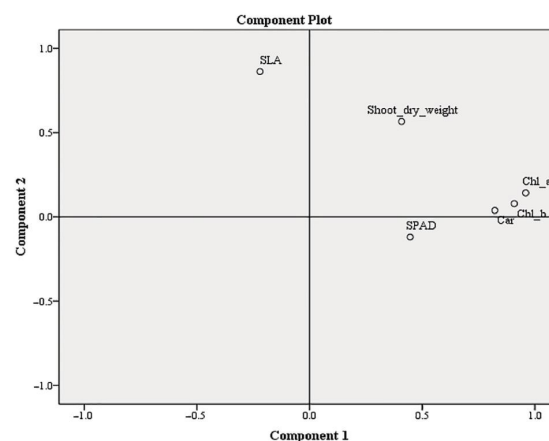
**Table 6.** Chlorophyll-a, chlorophyll-b, and carotenoid content ( $\text{mg g}^{-1}$  fresh weight) of experimental plants with different treatments at first sampling (10/21) ( $n = 4$ ;  $\pm$ SE; significant differences at  $p \leq 0.05$  are indicated with different letters).

Treatments	Chlorophyll-a $\text{mg g}^{-1}$	Chlorophyll-b $\text{mg g}^{-1}$	Carotenoids $\text{mg g}^{-1}$
DW	$20.72 \pm 0.37$ abc	$7.19 \pm 0.17$ a	$1.71 \pm 0.03$ bc
NS	$22.77 \pm 0.49$ d	$8.09 \pm 0.25$ bc	$1.67 \pm 0.05$ b
half NS	$23.04 \pm 0.47$ d	$8.41 \pm 0.28$ c	$1.82 \pm 0.02$ c
DW+2.5S	$20.72 \pm 0.39$ abc	$6.97 \pm 0.18$ a	$1.78 \pm 0.02$ bc
half NS+5S	$21.48 \pm 0.31$ bcd	$7.39 \pm 0.20$ ab	$1.76 \pm 0.04$ bc
half NS+10S	$21.60 \pm 0.67$ cd	$7.49 \pm 0.37$ ab	$1.73 \pm 0.06$ bc
5W	$19.44 \pm 0.38$ a	$6.67 \pm 0.27$ a	$1.46 \pm 0.02$ a
10W	$19.78 \pm 0.81$ a	$6.79 \pm 0.36$ a	$1.51 \pm 0.04$ a

Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

The 5W and 10W treatments resulted in lower carotenoid content than the other treatments. Among the remaining treatments, a significant difference in carotenoid content was only observed in NS and half NS treatments, with the latter exhibiting higher values.

According to the PCA results in Figure 1, the first of the two latent components explains 47.79% of the variances and the second 17.95%. The first latent component includes the three photosynthetic pigments, which therefore vary together. The second latent component refers to the SLA value and shoot dry weight.



**Figure 1.** Component plot of parameters (SLA: specific leaf area; SPAD: relative chlorophyll content; Chl\_a: chlorophyll-a; Chl\_b: chlorophyll-b; Car: carotenoids and shoot dry weight) measured in the first sampling (10/21).

### 3.2. Results of Second Sampling

The effects of the different treatments on the morphological, growth, and yield parameters of the experimental plants at the second sampling are presented in Tables 7–10. The SFDAJ treatments alone and in combination with half NS treatment significantly reduced the shoot length compared to NS and half NS treatments but led to significantly higher shoot length than DW and DW+2.5S treatments.

**Table 7.** Effect of different treatments on the shoot and spike length of experimental plants at second sampling (06/22) (n = 4;  $\pm$ SE; significant differences at  $p \leq 0.05$  are indicated with different letters).

Treatments	Shoot Length (mm)	Spike Length (mm)
DW	442.50 $\pm$ 18.09 <sup>a</sup>	38.13 $\pm$ 9.81 <sup>ab</sup>
NS	592.81 $\pm$ 7.16 <sup>d</sup>	58.44 $\pm$ 5.70 <sup>d</sup>
half NS	595.31 $\pm$ 12.00 <sup>d</sup>	59.06 $\pm$ 10.20 <sup>d</sup>
DW+2.5S	428.44 $\pm$ 15.57 <sup>a</sup>	35.94 $\pm$ 8.00 <sup>a</sup>
half NS+5S	543.75 $\pm$ 8.90 <sup>c</sup>	49.06 $\pm$ 7.58 <sup>c</sup>
half NS+10S	529.38 $\pm$ 9.00 <sup>bc</sup>	42.50 $\pm$ 5.77 <sup>b</sup>
5W	500.31 $\pm$ 14.29 <sup>b</sup>	41.25 $\pm$ 8.66 <sup>ab</sup>
10W	543.75 $\pm$ 5.45 <sup>c</sup>	49.06 $\pm$ 5.54 <sup>c</sup>

Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

**Table 8.** Effect of different treatments on the leaf number and shoot diameters of experimental plants at second sampling (06/22) (n = 4;  $\pm$ SE; significant differences at  $p \leq 0.05$  are indicated with different letters).

Treatments	Leaf Number (Piece)	Shoot Diameter at the Base (mm)	Shoot Diameter at the Top (mm)
DW	5.00 $\pm$ 0.24 <sup>ab</sup>	0.18 $\pm$ 0.01 <sup>a</sup>	0.09 $\pm$ 0.01 <sup>a</sup>
NS	5.13 $\pm$ 0.24 <sup>ab</sup>	0.22 $\pm$ 0.01 <sup>c</sup>	0.15 $\pm$ 0.00 <sup>c</sup>
half NS	5.44 $\pm$ 0.22 <sup>ab</sup>	0.22 $\pm$ 0.01 <sup>c</sup>	0.14 $\pm$ 0.01 <sup>c</sup>
DW+2.5S	5.88 $\pm$ 0.18 <sup>b</sup>	0.19 $\pm$ 0.01 <sup>ab</sup>	0.10 $\pm$ 0.00 <sup>a</sup>
half NS+5S	5.06 $\pm$ 0.14 <sup>ab</sup>	0.22 $\pm$ 0.00 <sup>c</sup>	0.12 $\pm$ 0.00 <sup>ab</sup>
half NS+10S	4.75 $\pm$ 0.17 <sup>a</sup>	0.22 $\pm$ 0.00 <sup>c</sup>	0.12 $\pm$ 0.00 <sup>ab</sup>
5W	4.88 $\pm$ 0.13 <sup>ab</sup>	0.20 $\pm$ 0.00 <sup>bc</sup>	0.12 $\pm$ 0.00 <sup>ab</sup>
10W	4.81 $\pm$ 0.16 <sup>a</sup>	0.22 $\pm$ 0.01 <sup>c</sup>	0.13 $\pm$ 0.00 <sup>ab</sup>

Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

**Table 9.** Effect of different treatments on shoot and root dry weight of experimental plants at second sampling (06/22) (n = 4;  $\pm$ SE; significant differences at  $p \leq 0.05$  are indicated with different letters).

Treatments	Shoot Dry Weight (g Plant <sup>-1</sup> )	Root Dry Weight (g Plant <sup>-1</sup> )
DW	0.36 $\pm$ 0.03 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>a</sup>
NS	0.68 $\pm$ 0.02 <sup>d</sup>	0.27 $\pm$ 0.02 <sup>d</sup>
half NS	0.73 $\pm$ 0.05 <sup>d</sup>	0.18 $\pm$ 0.01 <sup>c</sup>

**Table 9.** *Cont.*

Treatments	Shoot Dry Weight (g Plant <sup>-1</sup> )	Root Dry Weight (g Plant <sup>-1</sup> )
DW+2.5S	0.33 ± 0.02 <sup>a</sup>	0.13 ± 0.01 <sup>ab</sup>
half NS+5S	0.54 ± 0.02 <sup>c</sup>	0.14 ± 0.01 <sup>b</sup>
half NS+10S	0.49 ± 0.03 <sup>bc</sup>	0.13 ± 0.00 <sup>ab</sup>
5W	0.45 ± 0.03 <sup>b</sup>	0.12 ± 0.01 <sup>ab</sup>
10W	0.53 ± 0.02 <sup>bc</sup>	0.12 ± 0.00 <sup>ab</sup>

Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

**Table 10.** Effect of different treatments on the spikelet number per spike, grain number per spike, weight of grains per spike, and yield per pot of experimental plants at second sampling (06/22) (n = 4; ±SE; significant differences at  $p \leq 0.05$  are indicated with different letters).

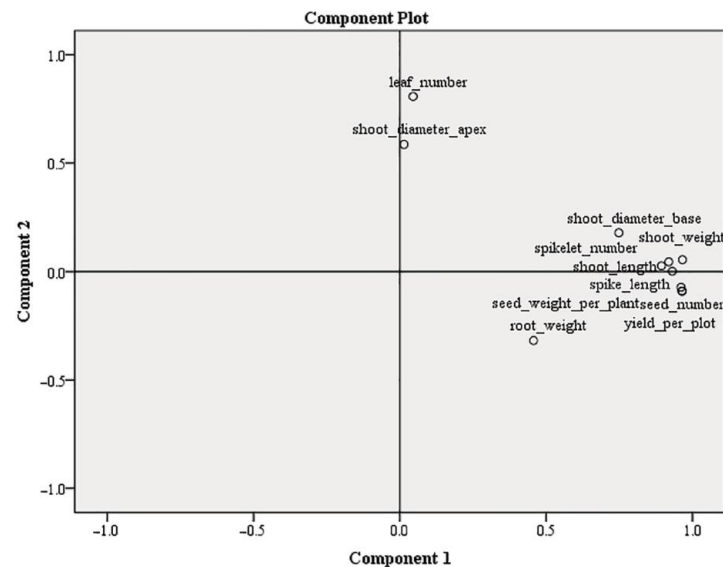
Treatments	Spikelet Number per spike	Grain Number per Spike	Weight of Grains per Spike (g Spike <sup>-1</sup> )	Yield per Pot (g)
DW	3.06 ± 0.29 <sup>a</sup>	9.50 ± 1.44 <sup>a</sup>	0.25 ± 0.05 <sup>a</sup>	2.97 ± 0.54 <sup>a</sup>
NS	5.56 ± 0.13 <sup>de</sup>	22.00 ± 0.71 <sup>de</sup>	0.88 ± 0.04 <sup>d</sup>	10.58 ± 0.43 <sup>d</sup>
half NS	5.81 ± 0.23 <sup>e</sup>	23.88 ± 1.47 <sup>e</sup>	0.90 ± 0.07 <sup>d</sup>	10.82 ± 0.88 <sup>d</sup>
DW+2.5S	3.31 ± 0.24 <sup>a</sup>	7.81 ± 1.01 <sup>a</sup>	0.23 ± 0.03 <sup>a</sup>	2.76 ± 0.34 <sup>a</sup>
half NS+5S	5.00 ± 0.24 <sup>cd</sup>	18.63 ± 0.90 <sup>bc</sup>	0.72 ± 0.05 <sup>c</sup>	8.59 ± 0.54 <sup>c</sup>
half NS+10S	4.56 ± 0.20 <sup>bc</sup>	15.88 ± 0.74 <sup>b</sup>	0.58 ± 0.03 <sup>b</sup>	6.99 ± 0.40 <sup>b</sup>
5W	4.13 ± 0.24 <sup>b</sup>	15.63 ± 0.93 <sup>b</sup>	0.56 ± 0.04 <sup>b</sup>	6.76 ± 0.45 <sup>b</sup>
10W	5.13 ± 0.18 <sup>cd</sup>	19.50 ± 0.63 <sup>cd</sup>	0.64 ± 0.03 <sup>bc</sup>	7.74 ± 0.35 <sup>bc</sup>

Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

NS and half NS treatments also increased the spike length compared to the other treatments. Among the SFDAJ treatments, only the half NS+5S and 10W treatments resulted in a higher spike length compared to the DW and DW+2.5S treatments, and the differences were significant. A higher leaf number was measured in the DW+2.5S treatment, but there was no significant difference between the other treatments. The diameter at the base of the shoot was significantly lower in DW and DW+2.5S treatments than in the other treatments, which were statistically similar. The NS and half NS treatments resulted in a larger diameter at the shoot apex, but there was no significant difference between the other treatments.

Similar to the shoot length, SFDAJ treatments alone or in combination with half NS treatments significantly reduced the shoot dry weight, number of grains, grain weight per spike, and yield per pot, compared to NS and half NS treatments, but resulted in significantly higher values than DW and DW+2.5S treatments. The conversion of the yields per pot, taking into account the area of the pots, into tonnes per hectare, revealed values between 0.88 and 3.45 tonnes per hectare. For the yield results calculated in tonnes per hectare, the same findings as mentioned above are valid for the differences/uniformities between treatments. SFDAJ treatments also reduced the root dry weight and the number of spikelets per spike compared to NS and half NS treatments but exhibited higher values than DW treatment, and the differences were significant.

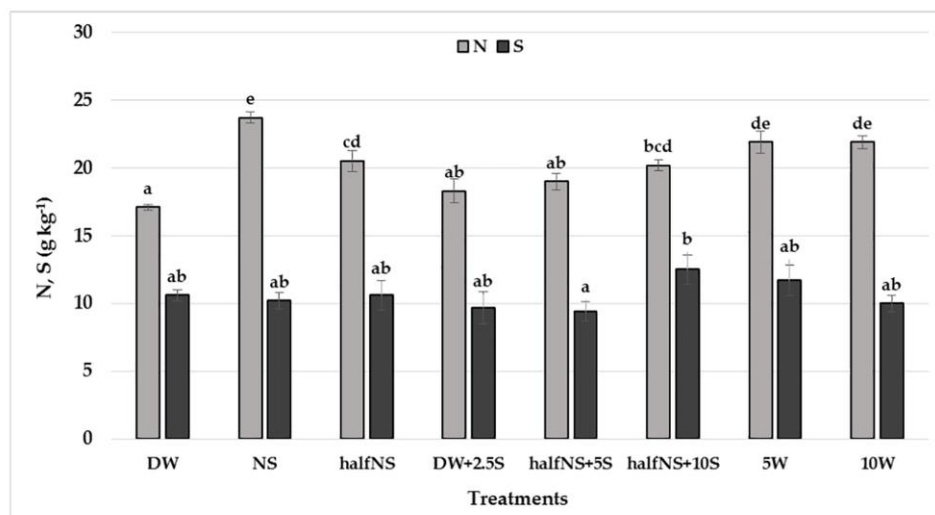
Based on the PCA results in Figure 2, the first of the two latent components explains 63.65% of the variances for the morphological variables and the second 10.40%. The former encompasses morphological variables except leaf number and shoot diameter at the apex. Therefore, the shoot and root weight, spikelet number, shoot length, seed number and weight per plant, yield per pot, and shoot diameter at the base vary together.



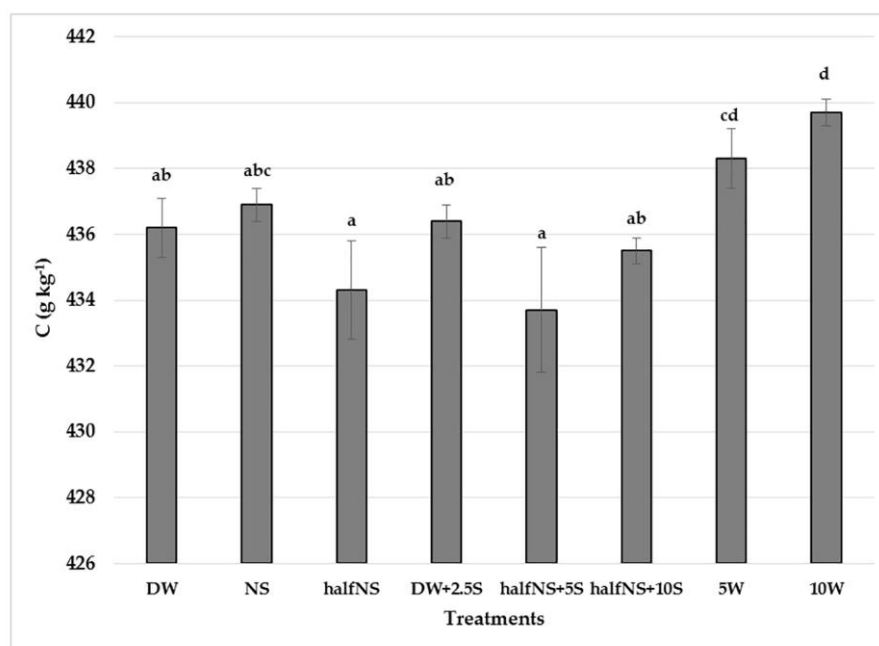
**Figure 2.** Component plot of morphological parameters (shoot and root weight, spikelet number, shoot length, seed number and weight per plant, yield per pot, and shoot diameter at the base and at the apex and leaf number) in the second sampling (06/22).

The nitrogen, carbon, and sulfur contents of the grains in the different treatments are presented in Figures 3 and 4. The nitrogen content varied between the treatments. In those with SFDAJ applied via irrigation, the same nitrogen content was obtained as in the NS and half NS treatments. Treatments involving the application of SFDAJ via foliar spraying resulted in significantly higher nitrogen levels than the DW treatment but only when combined with half NS treatment and applied at 10% concentration. Treatments using SFDAJ application via foliar spraying in combination with half NS treatment exhibited no positive effect compared to the half NS treatment. The carbon content of the treatments also differed significantly. The application of SFDAJ via irrigation increased the carbon content compared to all other treatments except NS. The other treatments did not differ in carbon content. Regarding the sulfur content, a difference was only observed between half NS+5S and half NS+10S treatments.

The data in Table 11 indicate that SFDAJ treatments via irrigation had a negative effect on the phosphorus concentration in wheat grain. These treatments resulted in significantly lower phosphorus concentration values than NS, half NS, half NS+5S, and even DW treatments. Potassium concentrations significantly varied between the treatments. The 10W treatment increased the potassium concentration significantly compared to most treatments, except for the half NS and half NS+10S treatments, which revealed similar results. The treatments had no effect on the calcium concentration in the grains. SFDAJ application significantly increased the magnesium concentration when applied via foliar spraying and combined with half NS treatment, compared to its application using irrigation. SFDAJ treatments resulted in the same manganese and zinc concentrations as NS and half NS treatments. Manganese and zinc concentrations were highest in the DW and DW+2.5S treatments, with the differences considered significant.



**Figure 3.** Effect of different treatments on nitrogen and sulfur content (N, S g kg<sup>-1</sup>) of wheat grains at second sampling (06/22) (n = 4; ±SE; significant differences at  $p \leq 0.05$  are indicated with different letters). Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; halfNS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; halfNS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; halfNS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.



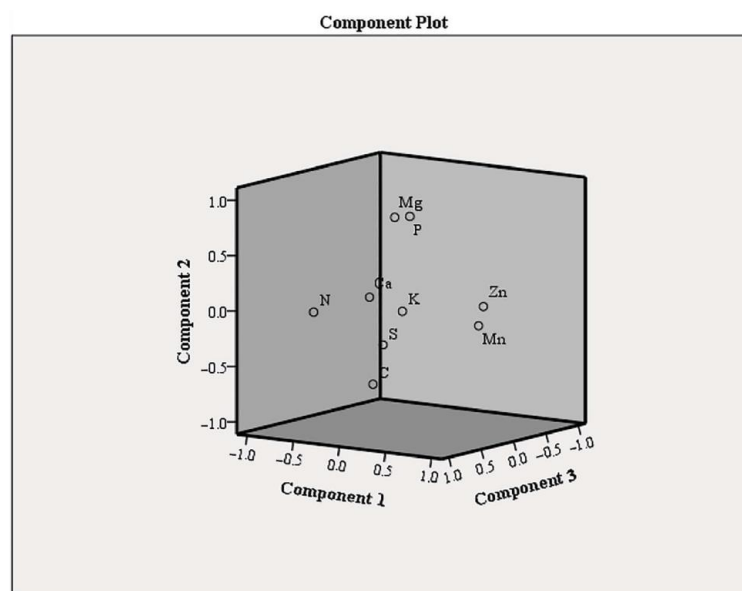
**Figure 4.** Effect of different treatments on the carbon (C g kg<sup>-1</sup>) content percentage of wheat grains at second sampling (06/22) (n = 4; ±SE; significant differences at  $p \leq 0.05$  are indicated with different letters). Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; halfNS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; halfNS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; halfNS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

**Table 11.** Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), and zinc (Zn) content of wheat grain treated with SFDAJ (g kg<sup>-1</sup>; mg kg<sup>-1</sup>; n = 4; ±SE; significant differences at  $p \leq 0.05$  are indicated with different letters).

Treatments/ Elements	P (g kg <sup>-1</sup> )	K (g kg <sup>-1</sup> )	Ca (g kg <sup>-1</sup> )	Mg (g kg <sup>-1</sup> )	Mn mg kg <sup>-1</sup>	Zn mg kg <sup>-1</sup>
DW	1.69 ± 0.12 <sup>bc</sup>	6.10 ± 0.13 <sup>abc</sup>	1.83 ± 0.01 <sup>a</sup>	1.23 ± 0.05 <sup>abc</sup>	69.25 ± 3.12 <sup>c</sup>	35.49 ± 0.45 <sup>cd</sup>
NS	1.97 ± 0.09 <sup>d</sup>	5.93 ± 0.23 <sup>ab</sup>	1.78 ± 0.19 <sup>a</sup>	1.35 ± 0.11 <sup>bc</sup>	51.90 ± 4.21 <sup>b</sup>	28.30 ± 1.90 <sup>ab</sup>
half NS	1.64 ± 0.09 <sup>bc</sup>	6.46 ± 0.17 <sup>bcd</sup>	2.10 ± 0.38 <sup>a</sup>	1.39 ± 0.05 <sup>bc</sup>	30.90 ± 4.89 <sup>a</sup>	28.35 ± 1.50 <sup>ab</sup>
DW+2.5S	1.53 ± 0.09 <sup>ab</sup>	5.98 ± 0.13 <sup>abc</sup>	1.83 ± 0.04 <sup>a</sup>	1.11 ± 0.16 <sup>ab</sup>	72.48 ± 5.57 <sup>c</sup>	39.82 ± 2.21 <sup>d</sup>
half NS+5S	1.85 ± 0.09 <sup>cd</sup>	5.79 ± 0.07 <sup>a</sup>	1.59 ± 0.08 <sup>a</sup>	1.29 ± 0.01 <sup>abc</sup>	41.58 ± 3.04 <sup>ab</sup>	28.55 ± 1.75 <sup>ab</sup>
half NS+10S	1.55 ± 0.07 <sup>ab</sup>	6.51 ± 0.17 <sup>cd</sup>	1.81 ± 0.13 <sup>a</sup>	1.40 ± 0.10 <sup>c</sup>	51.15 ± 5.98 <sup>b</sup>	33.47 ± 0.770 <sup>bc</sup>
5W	1.36 ± 0.05 <sup>a</sup>	5.78 ± 0.15 <sup>a</sup>	2.01 ± 0.23 <sup>a</sup>	1.07 ± 0.05 <sup>a</sup>	53.00 ± 2.65 <sup>b</sup>	26.53 ± 2.33 <sup>a</sup>
10W	1.33 ± 0.02 <sup>a</sup>	7.00 ± 0.29 <sup>d</sup>	1.78 ± 0.38 <sup>a</sup>	1.11 ± 0.06 <sup>ab</sup>	37.48 ± 1.75 <sup>a</sup>	29.72 ± 1.30 <sup>ab</sup>

Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

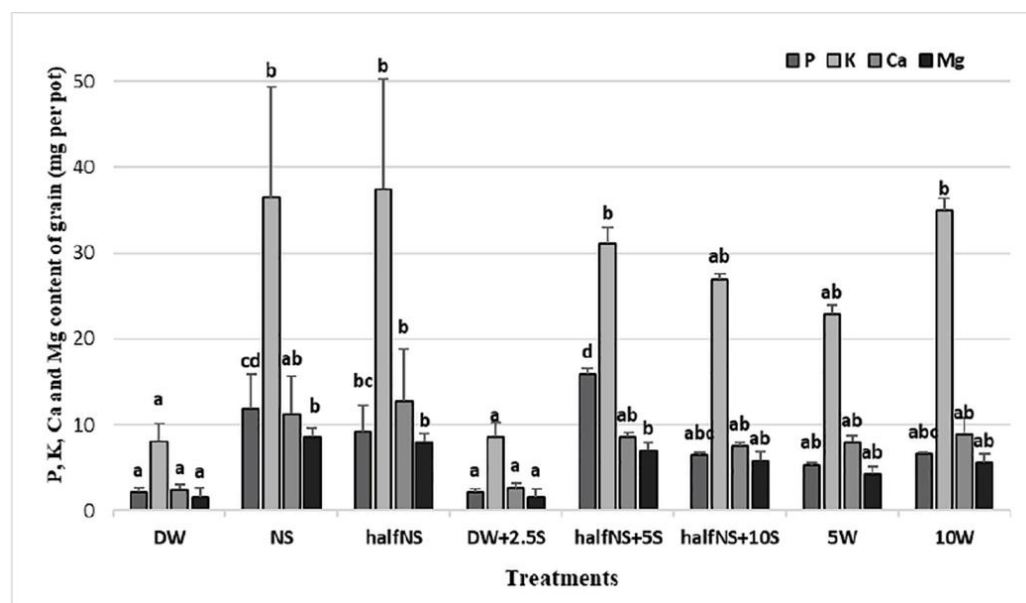
Figure 5 shows the PCA analysis results of the measured elements. The first explains 23.33% of the variances, the second 20.97%, the third 17.73%, and the fourth 12.47%. The first of the four latent components encompasses Mn, Zn, and N content. An inverse association was observed between the N content and the Mn and Zn contents, i.e., the higher the N content, the lower the Mn and Zn content. The second latent variable includes the P, Mg, and C contents. The P and Mg contents exhibited similar change trends: when one increased or decreased, the other underwent similar changes, but an increase in C content exhibited an inverse association with an increase in P and Mg content. The third latent variable includes the K and Ca content, indicating that the variation in the amounts of these two elements is correlated. The fourth latent variable is only mathematically meaningful, not scientifically.



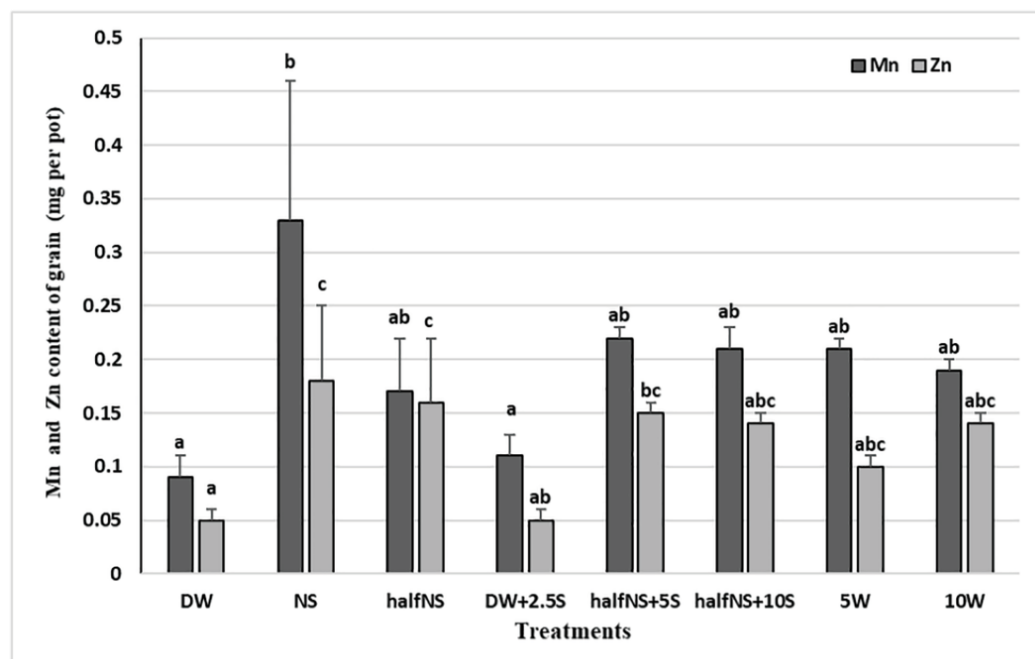
**Figure 5.** Component plot of elements (C, N, S, K, P, Ca, Mg, Zn, Mn) measured in the second sampling (06/22).

The results in Figures 6 and 7 illustrate the total concentration of elements incorporated into the wheat grains in different treatments. The NS treatment can be considered optimal in terms of both the amount of plant nutrients and their proportions. In the half NS treatment, the proportion of plant nutrients is also optimal. Therefore, SFDAJ treatments should be

compared to these treatments. If we take into account the amount of nutrients supplied to each pot by watering and/or spraying, it can be concluded that the half NS+5S and half NS+10S treatments are closest to the nutrient ratio of the NS and half NS treatments. In the 5W and 10W treatments, compared to the above, the ratio of calcium and phosphorus is lower, and the ratio of boron is higher. The amount of phosphorus incorporated into the grains was lower in the DW and DW+2.5S treatments than in the NS, half NS, and half NS+5S treatments. The low phosphorus content of the 5W and 10W treatments account for the lower incorporated phosphorus in the grains in these treatments. In the SFDAJ treatments, the amount of potassium incorporated into the grains—with the exception of the DW+2.5S treatment, which has a low potassium input—was the same as in the NS and half NS treatments. The amount of calcium and magnesium incorporated into the grains was lower in DW and DW+2.5S treatments than in NS and half NS treatments. There was no significant difference between the treatments containing SFDAJ (except the DW+2.5S treatment) and the NS and half NS treatments in the incorporated amount of calcium and magnesium. The amounts of manganese and zinc incorporated into the grains were lower in the DW and DW+2.5S treatments than in the other treatments, which, however, did not differ in these parameters. It follows that the SFDAJ treatments (with the exception of the DW+2.5S treatment) are as effective as the NS and half NS treatments for the supply of the tested elements, with the exception of the phosphorus supply. The difference between the amount of all applied nutrients (see Table 4) and the amount incorporated into the grain yield was incorporated into the other parts of the plants (shoot and root system).



**Figure 6.** The total potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) content incorporated into wheat grains in different treatments (grain yield per pot multiplied by the concentration of element: mg yield of pot<sup>-1</sup>; n = 4; ±SE; significant differences among data at  $p \leq 0.05$  are indicated with different letters). Footnote: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.



**Figure 7.** The total manganese (Mn) and zinc (Zn) content incorporated into wheat grains in different treatments (grain yield per pot multiplied by the concentration of element: mg yield of pot<sup>-1</sup>; n = 4; ±SE; significant differences among data at  $p \leq 0.05$  are indicated with different letters). Foot-note: DW: Irrigation with deionized water; NS: Irrigation with nutrient solution; half NS: Irrigation with a nutrient solution in half concentration; 5W: Irrigation with 5% fermented DAJ completed with fly ash solution; 10W: Irrigation with 10% fermented DAJ completed with fly ash solution; half NS+5S: Spraying with 5% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; half NS+10S: Spraying with 10% fermented DAJ completed with fly ash solution and irrigation with a nutrient solution in half concentration; DW+2.5S: Spraying with 2.5% fermented DAJ solution and irrigation with deionized water.

#### 4. Discussion

Experiments and publications on the use of deproteinized, fresh plant juices (DPJs) from different plants in plant nutrition have shown that efficacy is determined by the concentrations used, the plant of origin, and the test plant. Jadhav investigated the association between DPJ concentration and their positive and negative effects on crop production: higher doses of DPJ caused chromosomal aberrations in *Allium cepa* root tips, whereas alfalfa DPJ at lower concentrations had a positive effect on cowpea yield. It improved root nodulation, leaf number, dry matter, and protein content [23,24]. In 2019, Jadhav and co-workers investigated the effect of DPJs derived from different fabaceous plants (*Phaseolus lunatus* and *Vigna unguiculata*) found in India on the growth and photosynthetic pigment content of grassland plants (*Pennisetum typhoides*, *Eleusine coracana*, and *Sorghum vulgare*). Their results show that the effect of DPJs depends on the plant from which they are derived. The DPJs from plants of the *Fabaceae* family used reduced the chlorophyll content of *Pennisetum* and *Elusine* species. On the other hand, the DPJ prepared from *Phaseolus lunatus* increased the chlorophyll content of *Sorghum vulgare* plants, and DPJ prepared from *Vigna unguiculata* plants increased carotenoid content in *Pennisetum typhoides* plants [25]. The toxic effect of large quantities of DAJ applied under field conditions has been demonstrated, and the concentration threshold with no toxicity has been determined [26,27]. Other authors have reported that DPJ of undetermined origin at a concentration of 0.1% induced root formation in tissue cultures of various leguminous plants [28]. In addition, DAJ has demonstrated germination-stimulating effects at low concentrations [29,30].

Lactic fermentation of DPJs allows for longer storage/usage and significantly improves their quality. The only disadvantage of the process is that the use of fermented DPJs as plant nutrients is limited due to the low pH produced. Therefore, fermented DPJs can only be used in low concentrations for a limited period of time. This finding is confirmed by several publications. The application of FDAJ at concentrations of 0.05–0.1 v v<sup>-1</sup>% in hydroponics increased the growth and relative chlorophyll content of maize plants, whereas higher concentrations (i.e., 1.0–5.0 v v<sup>-1</sup>%) had a reverse effect, which is attributed to the low pH in the nutrient solution due to FDAJ application. In other experiments performed on horticultural perlite, the application of FDAJ at 2.5 v v<sup>-1</sup>% via foliar spraying in maize provided a good alternative to nutrient solution treatment, but similar results were not found in sunflower [15]. For herbs and ornamental plants (*Occimum* and *Celosia*), FDAJ has been shown to have a beneficial effect when sprayed with a 1.0–2.5 v v<sup>-1</sup>% solution. Spraying *Celosia argentea* plants with a lower concentration (up to 1.0 v v<sup>-1</sup>%) of FDAJ improved their growth and antioxidant capacity [12]. The application of FDAJ in 2.5 v v<sup>-1</sup>% via foliar spraying increased the fresh weight of stem and root, length of stem and root, and leaf area in *Ocimum basilicum* plants [31].

Considering the low pH of fermented DPJs, successful experiments have previously been carried out to supplement them with wood ash filtrate to change their pH to a pH favorable for plant nutrient uptake and to use the improved DPJ for plant nutrition. Although the higher concentration of alfalfa-derived DPJ could be used effectively in these experiments, the duration of its application was limited (a few weeks). Specifically, FDAJ was supplemented with fly ash solution in sunflowers and corn grown on horticultural perlite, thus eliminating the toxicity of FDAJ and increasing its concentration up to 10% [15].

In the present study, the experiment was performed using SFDAJ, and unlike previous studies, the treatments were applied repeatedly and at higher concentrations during the entire growth period.

The first sampling was carried out because we aimed to investigate whether there was a difference between treatments before overwintering. At the first sampling, no significant differences in plant development were observed between the SFDAJ treatments and the control treatments, which may be attributed to the nutrient content of the grain crops and the low, but existing, nutrient supply capacity of the soil. At the first sampling, the addition of SFDAJ via irrigation or foliar spraying did not reduce the specific leaf area. The relative chlorophyll content of the SFDAJ treatments was significantly lower than the NS treatment, which is ideal for plant nutrition. SFDAJ treatments via irrigation reduced the absolute chlorophyll content of the experimental plants at the first sampling, compared to the nutrient solution treatments. Given the essential role of iron in chlorophyll biosynthesis [32] and the relatively low iron content of DAJ and SFDAJ solutions, which has been established in previous research [15], this is not a surprising result. A significant amount of iron remains in the protein fraction during coagulation in the LPC, which reduces the iron content of DAJ. The SFDAJ treatments via irrigation resulted in significantly lower carotenoid content than the other treatments. Since carotenoids are protective pigments against high light intensity, this raises the possibility that SFDAJ application may make plants more sensitive to this type of stress.

In the second sampling, the SFDAJ treatments were inferior to the nutrient solution treatments in terms of the measured morphological and yield parameters but exhibited significantly better performance than the deionized water irrigation treatments.

The SFDAJ treatments were particularly effective in improving the carbon and nitrogen content of grains. The nitrogen content of the grains reached the values measured in the nutrient solution treatments. Our results reveal that the use of SFDAJ is more beneficial

for increasing the nitrogen and carbon content of grains when applied via irrigation than when using foliar spraying.

SFDAJ treatments also had an impact on the other macro- and micronutrient content of grains. The SFDAJ treatments applied at higher concentrations primarily increased the potassium but decreased the phosphorus concentration compared to distilled water control treatments. The high manganese and zinc concentrations in the treatments with deionized water were unexpected. This may be due to the very low yield of plants treated with deionized water. Another reason may be the acidic soil pH. Irrigation with deionized water alone kept the soil pH acidic, which increased micronutrient solubility and uptake. In the other treatments, the soil pH shifted upwards, thus reducing solubility and uptake of these elements.

To ensure that the difference in crop volume does not affect the results of the amount of nutrients incorporated, we calculated the amount of nutrients incorporated into the yield of a pot in a treatment, determined as the grain yield per pot multiplied by the concentration of an element and expressed in milligrams. According to this parameter, SFDAJ treatments (with the exception of the DW+2.5S treatment) are as effective as the NS and half NS treatments for the supply of the tested elements, with the exception of the phosphorus supply.

## 5. Conclusions

It can be concluded that FDAJ supplemented with fly ash filtrate can be used for enhanced plant nutrition. Although its use does not reach the effectiveness of nutrient solutions with an optimal composition, especially in terms of the amount of grain yield, it does not exhibit any toxic effects. Depending on the intensity of crop production, it can replace the use of fertilizers to a certain extent and even completely at the low-input level of plant production. It can also be used at the 10% concentration without any challenges in field conditions, as evidenced by our experiment. It is recommended to be applied mainly via irrigation to increase its effectiveness. If nutrient supply needs to be achieved using SFDAJ alone, the potential risks of suboptimal nutrient ratios should be considered. Primarily due to the high potassium and low phosphorus and iron content of SFDAJ, it may be necessary to supplement fertilization to balance the nutrients, although the cost of this approach is significantly lower than ensuring the plant's nutrient requirements solely with synthetic fertilizers.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15020339/s1>, Table S1: Composition of fermented, deproteinized alfalfa juice (FDAJ) and its solutions, composition of FDAJ solutions with fly ash filtrate used in the experiment ( $\text{mg L}^{-1}$ ); Table S2: Composition of fly ash and fly ash filtrate ( $\text{mg kg}^{-1}$ ;  $\text{mg L}^{-1}$ ).

**Author Contributions:** Conceptualization, G.M.F. and S.V.; methodology, S.V. and P.M.; software, P.M.; validation, S.V., N.B. and É.D.-S.; formal analysis, P.M.; investigation, A.K.B. and P.M.; resources, N.B. and S.V.; data curation, P.M.; writing—original draft preparation, P.M. and N.B.; writing—review and editing, S.V.; visualization, É.D.-S.; supervision, G.M.F.; project administration, S.V.; funding acquisition, S.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the National Research, Development and Innovation Fund of Hungary, project no. TKP2021-NKTA-32.

**Data Availability Statement:** The raw data supporting the conclusions of this article will be made available by the authors on request.

**Acknowledgments:** The authors acknowledge the determination of soil characteristics by the Regional Agricultural Centre of Measuring Instruments, University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management. The authors would like to acknowledge László

Huzsvai for his help with the statistical analysis. We would also like to acknowledge Tibor Losoncz, manager of the Veolia Bioenergy Plant, Szakoly, for providing the fly ash used.

**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## References

- Ishfaq, M.; Wang, Y.; Xu, J.; Hassan, M.U.; Yuan, H.; Liu, L.; He, B.; Ejaz, I.; White, P.J.; Cakmak, I.; et al. Improvement of Nutritional Quality of Food Crops with Fertilizer: A Global Meta-Analysis. *Agron. Sustain. Dev.* **2023**, *43*, 74. [\[CrossRef\]](#)
- Savci, S. Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia* **2012**, *1*, 287–292. [\[CrossRef\]](#)
- Walan, P.; Davidsson, S.; Johansson, S.; Höök, M. Phosphate Rock Production and Depletion: Regional Disaggregated Modeling and Global Implications. *Resour. Conserv. Recycl.* **2014**, *93*, 178–187. [\[CrossRef\]](#)
- Kulhánek, M.; Asrade, D.A.; Suran, P.; Sedlář, O.; Černý, J.; Balík, J. Plant Nutrition—New Methods Based on the Lessons of History: A Review. *Plants* **2023**, *12*, 4150. [\[CrossRef\]](#)
- Withers, P.; Neal, C.; Jarvie, H.; Doody, D. Agriculture and Eutrophication: Where Do We Go from Here? *Sustainability* **2014**, *6*, 5853–5875. [\[CrossRef\]](#)
- Van Raamsdonk, L.W.D.; Meijer, N.; Gerrits, E.W.J.; Appel, M.J. New Approaches for Safe Use of Food By-Products and Biowaste in the Feed Production Chain. *J. Clean. Prod.* **2023**, *388*, 135954. [\[CrossRef\]](#)
- Santamaría-Fernández, M.; Molinuevo-Salces, B.; Kiel, P.; Steinfeldt, S.; Uellendahl, H.; Lübeck, M. Lactic Acid Fermentation for Refining Proteins from Green Crops and Obtaining a High Quality Feed Product for Monogastric Animals. *J. Clean. Prod.* **2017**, *162*, 875–881. [\[CrossRef\]](#)
- Pirie, N.W. Leaf Protein: A Beneficiary of Tribulation. *Nature* **1975**, *253*, 239–241. [\[CrossRef\]](#)
- Pirie, N.W. Leaf Protein and Other Aspects of Fodder Fractionation. In *Leaf Protein and Other Aspects of Fodder Fractionation*; Cambridge University Press (CUP): Cambridge, UK, 1978.
- El-Ramady, H.; Abdalla, N.; Kovacs, S.; Domokos-Szabolcsy, E.; Bákonyi, N.; Fari, M.; Geilfus, C.-M. Sustainable Biorefinery and Production of Alfalfa (*Medicago sativa* L.). *Egypt. J. Bot.* **2020**, *60*, 621–639. [\[CrossRef\]](#)
- Rathor, D.B.M. Studies and analysis of growth of aspergillus niger fungi on deproteinized leaf juice (DPJ). *Life Sci. Inform. Publ.* **2016**, *2*, 192–197.
- Bákonyi, N.; Kisvarga, S.; Barna, D.; O. Tóth, I.; El-Ramady, H.; Abdalla, N.; Kovács, S.; Rozbach, M.; Fehér, C.; Elhawati, N.; et al. Chemical Traits of Fermented Alfalfa Brown Juice: Its Implications on Physiological, Biochemical, Anatomical, and Growth Parameters of Celosia. *Agronomy* **2020**, *10*, 247. [\[CrossRef\]](#)
- Thomsen, M.H.; Bech, D.; Kiel, P. Manufacturing of Stabilised Brown Juice for L-Lysine Production—From University Lab Scale over Pilot Scale to Industrial Production. *Chem. Biochem. Eng. Q.* **2004**, *18*, 37–46.
- Lamont, J.R.; Wilkins, O.; Bywater-Ekegård, M.; Smith, D.L. From Yogurt to Yield: Potential Applications of Lactic Acid Bacteria in Plant Production. *Soil Biol. Biochem.* **2017**, *111*, 1–9. [\[CrossRef\]](#)
- Bákonyi, N.; Barna, D.; Fári, M.G.; Veres, S.; Alshaal, T.; Domokos-Szabolcsy, E.; Makleit, P. Role of Alfalfa Brown Juice in Plant Growth and Development. In *Biostimulants in Plant Protection and Performance*; Elsevier: Amsterdam, The Netherlands; pp. 149–163.
- Ferrarezi, R.S.; Lin, X.; Gonzalez Neira, A.C.; Tabay Zambon, F.; Hu, H.; Wang, X.; Huang, J.-H.; Fan, G. Substrate pH Influences the Nutrient Absorption and Rhizosphere Microbiome of Huanglongbing-Affected Grapefruit Plants. *Front. Plant Sci.* **2022**, *13*, 856937. [\[CrossRef\]](#)
- Johansen, J.L.; Nielsen, M.L.; Vestergård, M.; Mortensen, L.H.; Cruz-Paredes, C.; Rønn, R.; Kjølter, R.; Hovmand, M.; Christensen, S.; Ekelund, F. The Complexity of Wood Ash Fertilization Disentangled: Effects on Soil pH, Nutrient Status, Plant Growth and Cadmium Accumulation. *Environ. Exp. Bot.* **2021**, *185*, 104424. [\[CrossRef\]](#)
- Treeby, M.; Marschner, H.; Römheld, V. Mobilization of Iron and Other Micronutrient Cations from a Calcareous Soil by Plant-Borne, Microbial, and Synthetic Metal Chelators. *Plant Soil* **1989**, *114*, 217–226. [\[CrossRef\]](#)
- Moran, R.; Porath, D. Chlorophyll Determination in Intact Tissues Using *N,N*-Dimethylformamide. *Plant Physiol.* **1980**, *65*, 478–479. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wellburn, A.R. The Spectral Determination of Chlorophylls a and b, as Well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. *J. Plant Physiol.* **1994**, *144*, 307–313. [\[CrossRef\]](#)
- Garnier, E.; Shipley, B.; Roumet, C.; Laurent, G. A Standardized Protocol for the Determination of Specific Leaf Area and Leaf Dry Matter Content. *Funct. Ecol.* **2001**, *15*, 688–695. [\[CrossRef\]](#)
- Thamm, F.; Krámer, M.; Sarkadi, J. Növények És Trágyanyagok Foszfor-Tartalmaének Meghatározása Ammónium-Molibdo-Vanadátos Módszerrel. *Agrokémia Talajt.* **1968**, *17*, 145–156.

23. Jadhav, R.K. Effect Of Deproteinised Juice From *Eichhornia crassipes* L. On Mitotic Cell Division *Allium cepa* L. and *Allium sativum* L. *Bioinfolet* **2009**, *6*, 245–248.
24. Jadhav, R.K. Effect of Deproteinised Juice (DPJ) on Root Growth And Nodulation of Leguminous. *Plants. J. Bionano Front.* **2017**, *10*, 84–87.
25. Jadhav, R.K.; Kadam, R.; Joshi, D. Physiology of Gramineae Crops by Deproteinised Foliage Extract and Its Influence on Photosynthetic Chemistry. *J. Phytochem.* **2019**, *110*, 149–163.
26. Ream, H.W.; Smith, D.; Walgenbach, R.P. Effects of Deproteinized Alfalfa Juice Applied to Alfalfa—Brome grass, Brome grass, and Corn1. *Agron. J.* **1977**, *69*, 685–689. [[CrossRef](#)]
27. Welch, D.A.; Smith, D.; Soberalske, R.M.; Ream, H.W. Growth and Composition of Alfalfa Fertilized in Greenhouse Trials with Deproteinized Juice from Low and High Saponin Alfalfa and from Oat Herbage. *J. Plant Nutr.* **1979**, *1*, 151–170. [[CrossRef](#)]
28. Sreenivasan, E.; Pathak, S.S.; Joshi, R.N. Deproteinized Leaf Juica- a Novel Medium for Rhizogenesis in Vitro. *Curr. Sci.* **1995**, *68*, 586.
29. Patil, R. Effect of Potassium Humate and Deproteinised Juice (DPJ) on Seed Germination and Seedling Growth of Wheat and Jowar. *Ann. Biol. Res.* **2011**, *2*, 26–29.
30. Manwatkar, V.G.; Gogle, D.P. The Effect of Deproteinised Juice (DPJ) on Seed Germination and Seedling Growth of Different Plants (by Paper Towel Method). *Int. J. Life Sci.* **2014**, *2*, 65–68.
31. Kisvarga, S.; Barna, D.; Kovács, S.; Csatóri, G.; O. Tóth, I.; Fári, M.G.; Makleit, P.; Veres, S.; Alshaal, T.; Bákonyi, N. Fermented Alfalfa Brown Juice Significantly Stimulates the Growth and Development of Sweet Basil (*Ocimum basilicum* L.) Plants. *Agronomy* **2020**, *10*, 657. [[CrossRef](#)]
32. Pushnik, J.C.; Miller, G.W.; Manwaring, J.H. The Role of Iron in Higher Plant Chlorophyll Biosynthesis, Maintenance and Chloroplast Biogenesis. *J. Plant Nutr.* **1984**, *7*, 733–758. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.