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Chemical Traits of Fermented Alfalfa Brown Juice: Its Implications on Physiological, Biochemical, Anatomical, and Growth Parameters of Celosia

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Abstract: Brown juice is a byproduct of fractionated green biomass during leaf protein isolation. It represents approximately 45%–50% of the total pressed fresh biomass. Disposal of brown juice is a serious issue in leaf protein production due to its high biological oxygen demand and carbohydrates content. The current study aimed to find a possible potential use of brown juice. Therefore, chemical and biochemical properties of brown juice—derived from alfalfa green biomass—were determined before and after fermentation by lactic acid bacteria. Additionally, the growth stimulation potential of fermented brown juice on plumed cockscomb (*Celosia argantea* var. plumose 'Arrabona') plants were tested. Celosia seedlings were sprayed at different rates of fermented brown juice (i.e., 0.5%, 1%, 2.5%, 5%, and 10%) and tap water was applied as control. The results revealed that lactic acid bacteria successfully enhanced the stabilization of brown juice via reducing sugars content and increasing organic acids content. After fermentation, contents of glucose monomers were 15 times lower; while concentrations of lactic and acetic acids increased by 7- and 10-fold, respectively. This caused a reduction in the pH of fermented brown juice by 13.9%. Treating Celosia plants at lower rates of fermented brown juice (up to 1.0%) significantly induced their growth dynamics and antioxidant capacity. Higher values of vegetative parameters were measured in treated plants compared to control. The brown juice treatments caused significant changes in histological parameters as well. The activity of catalase and peroxidase increased in plants that received fermented brown juice especially at low rates. Moreover, an increase in water-soluble protein and phenol was measured in different tissues of plants sprayed with fermented brown juice. Malondialdehyde content was lowered in treated plants compared to control. Fermented brown juice at high rates slightly reduced the amount of photosynthetic pigments; however, this reduction was not reported for low rates of fermented brown juice. These results surely illustrate the potential use of fermented alfalfa brown juice as a growth stimulator for crops particularly at rates below 2.5%.

Keywords: deproteinized leaf juice; fermentation; lactic acid bacteria; plant nutrition; antioxidant capacity; ornamental plants

1. Introduction

Due to the continuous growth in the global population (7.2 billion) and malnutrition, the global demand for the protein will increase in the next years [1]. The lack of protein supply has existed as a health problem for many years and is considered as one of the main types of malnutrition in developing and developed countries [2]. Over the next decades, a dramatic increase in the global protein demand is expected and overall protein consumption is predicted to nearly double by 2050. These rapid changes will create serious and accelerated pressure on land and water resources and their scarcity [3]. To meet the increased protein demand there are several approaches to introduce novel protein sources or alternatives [4,5]. The extraction of proteins from forage crops such as alfalfa, clover or grass is a potential process for the production of leaf protein concentrates (LPC), which can be utilized as feed or food but also hydrolyzed into amino acids for the cosmetics or pharma industries [6]. Alfalfa or lucerne plant is well known as the king of forage. It is a perennial flowering plant belonging to the legume family Fabaceae. This plant has several advantages including high-quality leaf protein (50%–60%), strong adaptability, high nutritional value, good taste, wide distribution, and stable productivity [7]. It can also yield crude protein 2-, 3-, and 4-fold higher than peas, soybean, and wheat, respectively [8]. Therefore, alfalfa nowadays is considered as the most promising crop for LPC. Isolation of leaf protein in form of LPC aims to extract solid or insoluble proteins (i.e., the protein of mitochondria, chloroplasts, nucleoprotein, and cell wall) and soluble proteins (i.e., the soluble fraction of mitochondrial proteins, chloroplast matrix, and cytoplasm proteins). Therefore, the thermal treatment of green juice obtained by pressing the fresh biomass is needed to coagulate these types of proteins. During coagulation of leaf protein, a brown liquid byproduct is produced, and it is referred to as "brown juice". One kilogram of fresh alfalfa biomass can produce up to 500 g of brown juice [9]. These large amounts of brown juice are rich in protein and phenols as well as micronutrients. Plant phenolic compounds are known to be able to modulate important physiological routes like signal transduction and transcriptional regulation. Phenolic compounds in brown juice associated with auxin bioregulators [10] prove that the disposal of these amounts of valuable brown juice is a waste. Disposal is high in its costs and will waste the nutritional value of this byproduct, which would be easily adaptable to the circular economy concept; a technology that generates no further waste by utilizing all the produced renewable resources [11–13]. The main product, the leaf protein produced by coagulation, is widely studied [14]; however, the brown liquid, also known as whey or brown juice [15], has limited literature especially in the case of the plant nutrition aspect. Brown juice is mentioned in some articles as DPJ (Deproteinized Plant Juice) [16] or deproteinized leaf extracts or leaf juice, deproteinized whey [17] as a byproduct of plant protein-producing technologies. DPJ can be applied for several purposes; for instance, as a fertilizer for plants, milk for calves, excellent fodder for cattle and rabbits, medium for microbial growth, and also for in vitro rhizogenesis [18–21]. The dry matter and protein content of brown juice range from 13% to 15% and 16%–20%, respectively, whereas the cellulose content is 25%–30% [18]. The alfalfa brown juice has a dry matter content of 4%–8% which is influenced by the species, varieties, weather conditions, phenophase, methods of harvest, and processing.

Several microorganisms like lactic acid bacteria (LAB) are useful, having advantageous features, and can be found in a range of locations from soil and natural water, to the surface of plants up to the human intestinal tract [22]. These microorganisms have been applied for decades in the fermentation processes of raw materials because of their beneficial effects. It has been validated that ferments containing lactic acid bacteria (or other PGPB—plant growth-promoting bacteria) (isolated from different sources) have plant growth-promoting properties [23]. Lactic acid bacteria containing ferments were proven to be effective biofertilizers, biocontrol agents, and biostimulants because they promote plant health, growth, and resilience as they improve nutrient availability [24], however, the

functional roles of these bacteria in the phytomicrobiome have not been discovered yet [25]. *Celosia* genus is native to tropical America and Africa. *Celosia argentea* is a food crop in West Africa as well as a medicinal plant in China and India with considerable pharmacological properties [26]. Among 13 green leafy vegetables, *Celosia argentea* was one of the few that had exceptionally high iron (13.5 mg 100 g^{-1}), calcium (188 mg 100 g^{-1}), sodium (240.6 mg 100 g^{-1}), ascorbic acid (26 mg 100 g^{-1}), and β-carotene (4.42 mg 100 g^{-1}) content. The edible portion of *Celosia argentea* was found to be 55 g 100 g^{-1} fresh weight which was one of the highest, while its moisture and protein content was found to be 87.6 and 3.2 g 100 g^{-1}, respectively [27]. Plumosa Group of *Celosia argentea* is an attractive ornamental plant characterized by a wide range in size and color of flowers. Plumosa cultivars can grow from dwarf to tall. The inflorescence of narrow pyramidal, plume-like, is consistent with tiny, vivaciously colored (e.g., orange, red, purple, yellow) flowers.

This research aimed to enhance the stability of stored brown juice through fermentation by lactic acid bacteria; assess physiochemical traits of alfalfa brown juice before and after fermentation; determine whether the different fermented brown juice concentrations have any impact on the formation of the stem's anatomy; and evaluate the potential of fermented brown juice as a growth stimulator using *Celosia argantea* var. *plumosa* as a model plant.

2. Materials and Methods

2.1. Brown Juice Production and Its Characteristics

2.1.1. Source of Alfalfa Biomass

A field experiment of alfalfa (*Medicago sativa* L. var. Hunor-40) was carried out during 2017 and 2018, under the GINOP (2.2.1-15-2017-00051) project labeled Proteomill [28], at the experimental farm of Tedej Zrt., Hajdúnánás, Hungary. The seeds were sown on chernozem soil at the rate of 25 kg ha^{-1}. All recommended agronomic practices such as irrigation, weed control, and fertilization were done. The alfalfa fresh biomass was used as a source for brown juice. The first cut of alfalfa plants was carried out in the middle of May 2018 directly before the flowering stage since at this time protein in alfalfa biomass is at its highest content. Plants were harvested early morning and directly transferred in special boxes to the laboratory to avoid the degradation of protein by protease enzyme.

2.1.2. Extraction of Brown Juice

Alfalfa fresh biomass was fractionated into the fiber, leaf protein concentrate (LPC), and deproteinized plant juice (DPJ, brown juice) as follows: fresh biomass was pressed and pulped mechanically using Angel Juicer (5500, Angel Ltd., Praha, Czech Republic) into fiber and green juice fractions. Later, the green juice was thermally treated at 80 °C in order to coagulate mainly the chloroplastic and cytoplasmic proteins. After thermal coagulation, the mixture was left at room temperature for approximately 10 min, then the coagulant was separated from brown juice using moistened 100% natural unbleached cotton cloth filter (pore size = 10 microns).

2.1.3. Fermentation of Brown Juice

Fermentation of brown juice was necessary to increase the stability of brown juice and its storage period because fresh brown juice rapidly spoils due to high sugar and protein content. After cooling, the brown juice was transferred into a 20-L container and inoculated with AdiSil LG-100 Perfect (Fides Agro, Šardice, Czech Republic) containing heterofermentative lactic acid bacterial cultures (10^{11} CFU g^{-1}, *Pediococcus acidilactici, Lactobacillus paracasei, Lactobacillus plantarum*) at the rate of 0.01 g L^{-1}. The inoculated samples were kept at 35 °C for 48 h.

2.1.4. Determination of Lactic Acid Bacteria

The qualitative measurement of lactic acid bacteria in the fermented brown juice was determined at the end of the fermentation process by methylene blue test [29]. Briefly, 1 mL methylene blue

reagent was added to 10 mL fermented brown juice, and then the samples were incubated at 37 °C for 48 h. The time needed for the disappearing of blue color is an indication of lactic acid bacteria density in the solution.

2.1.5. Chemical Properties of Brown Juice

The pH of brown juice was measured by pH-meter (Mettler Toledo S20 Seven Easy, Switzerland). Electrical conductivity (EC) was determined using EC-meter (Thermo Scientific, Orion Model 209A$^+$ type, Germany). Degree Brix was recorded manually by a refractometer (RBR32-ATC, Germany). The content of macro- and micro-elements in brown juice before and after fermentation was measured using HNO_3-H_2O_2 wet digestion method as described by Kovács et al. [30]. Briefly, 1 g lyophilized brown juice was weighed into a Kjeldahl digestion tube, then 10 mL HNO_3 (99%, VWR International, USA) was added. The mixture was placed on the heater at 100 °C for 45 min; after cooling 5 mL H_2O_2 (30%, Sigma-Aldrich, St. Louis, Missouri,USA) was added for complete oxidation of organic materials and samples were kept on the heater for additional 45 min at 120 °C. After cooling the sample volume was brought to 50 mL using distilled water and then filtered using MN 640 W filter paper. The elemental content of brown juice was measured by ICP-OES spectrometer (Perkin Elmer made OPTIMA 3300 DV, Pittsboro, NC, USA).

Total phenol content in brown juice was determined spectrophotometerically using Ultrospec spectrophotometer (2100 pro, Amersham BioSciences, Amersham, United Kingdom) as previously described by Boór and Bélafiné Bakó [31]. Determination of total N content was carried out by Kjeldahl method [32] (Sparks et al., 1996). Concentrations of glucose and organic acids were determined by HPLC using BioRad (Hercules, CA, USA) Aminex HPX-87H (300 × 7.8 mm) column at 65 °C, and a refractive index detector. The eluent was 5 mmol L^{-1} H_2SO_4 at a flow rate of 0.5 mL min^{-1}. The injection volume was 40 µL. Concentrations of fructose, xylose, and arabinose were determined by HPLC using Phenomenex (Torrance, CA, USA) Rezex RPM-Monosaccharide Pb^{+2} (300 × 7.8 mm) column at 80 °C, and a refractive index detector. The eluent was ultrapure (milli-Q) water at a flow rate of 0.5 mL min^{-1}. The injection volume was 40 µL. Total sugars include monomer sugars and sugar oligomers solubilized. Monomer sugar concentrations were determined by HPLC after a sample preparation of 5 min boiling followed by centrifugation (5000 rpm) to eliminate residual proteins. To determine the oligomer sugar content of the samples, weak acid hydrolysis was performed. The samples were mixed with 8 w/w % H_2SO_4 at a volume ratio of 1:1 and treated at 120 °C in the autoclave for 15 min to decompose sugar oligomers into monomers, which were determined by HPLC.

2.2. Celosia Experiment

This experiment was carried out to assess the potential use of brown juice as a plant growth stimulator. In the present study, Celosia (*Celosia argantea* var. *plumosa* 'Arrabona') was used as a model plant for examining physiological, biochemical, and anatomical responses to fermented brown juice in our department and the National Agricultural Research and Innovation Center (NARIC, Budapest, Hungary). Celosia seeds were obtained from NARIC.

2.2.1. Experimental Design

A greenhouse pot experiment was carried out at the NARIC. The experimental layout was the Randomized Complete Block design (RCB) with 15 replicates. A polyethylene pot (7 × 7 × 8 cm) was filled with potting soil for horticultural crops (Klassman-Deilmann TS 3 FINE type, Geeste , Germany). The physical and chemical properties of potting soil are structure fine, pH (H_2O) 6, N 140 mg L^{-1}, P (P_2O_5) 100 mg L^{-1}, K (K_2O) 180 mg L^{-1}, Mg 100 mg L^{-1}, S 150 mg L^{-1}. Seeds of Celosia were sown in nursery substrate on 16th July 2018 and 4 days later germinated seeds were fertilized using different rates of brown juice. After two weeks, identical and healthy seedlings were transferred to the pots. Fermented brown juice was applied as a foliar application at rates of 0.5%, 1.0%, 2.5%, 5%, and 10%. The final application volume was 250 mL and equally shared among all replicates of the

same treatment. The control plants were sprayed with tap water. Brown juice was applied once a week from starting the experiment on 16th July until 14th August, then we applied brown juice twice a week (Tuesdays and Fridays) until the end of the experiment on 11th September. At the end of the experiment, the following vegetative parameters were measured: root and stem length, root and stem volume, root and stem fresh and dry mass, and the number of leaves.

2.2.2. Determination of Water-Soluble Protein and Antioxidant Enzymes

Water-soluble protein fraction of lyophilized root, stem, and leaf tissues was determined using Coomassie Brilliant Blue G-250 according to Bradford [33] in triplicate with bovine serum albumin as standard. Briefly, 20 mg plant tissue was ground into homogenate in the mortar with quartz sand, then transferred into a volumetric flask, and then suspended in 100 mL distilled water to extract water-soluble protein fraction. The solution was centrifuged at 3000 rpm for 5 min. The supernatant was used for the assay of water-soluble protein content using UV-160A spectrophotometer (Shimadzu, Japan) at 595 nm. Peroxidase activity was determined in lyophilized roots, stems, and leaves of Celosia plants according to Roxas et al. [34]. Briefly, 100 mg plant tissue was macerated in 4 mL of phosphate buffer 0.01 M (pH 6.0). The homogenate was centrifuged at 13,000 rpm for 10 min to collect the supernatant. The supernatant was used to measure peroxidase activity using UV-160A spectrophotometer (Shimadzu, Japan) at 460 nm for 1 min. The unit of peroxidase activity was defined with the increase of one unit of absorbance per mL^{-1} min^{-1} g^{-1} of dry matter. Catalase (CAT) activity in lyophilized Celosia leaves was measured by following the decomposition of hydrogen peroxide at 240 nm according to Woodbury et al. [35]. The reaction included 0.2 mL supernatant, 1.5 mL phosphate buffer (pH 7.8, 0.2 M), and 1 mL distilled water. The colorimetric determination of CAT was conducted by the model UV-160A spectrophotometer (Shimadzu, Japan) at 240 nm. The biochemical reaction was initiated by adding 0.3 mL 0.1 M H_2O_2. The activity of CAT was expressed as μmol H_2O_2 consumed/mg protein/min.

2.2.3. Malondialdehyde Measurement

The malondialdehyde (MDA) content was determined from roots, stems, and leaves of Celosia plants by the method of Zhang and Huang [36]. Briefly, 100 mg lyophilized sample was homogenized in 1 mL 0.1% (w/v) TCA solution using cold mortar and pestle. The homogenates were centrifuged at 10,000 × *g* for 10 min. Then, 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA solution was added into 1 mL of supernatant and incubated at 96 °C for 30 min. The tubes were cooled by transferring into an ice bath. The absorbance of the supernatant was recorded at 532 nm. The standard curve was generated from MDA standard. The concentration of MDA of samples was calculated from the absorbance knowing calibration curve.

2.2.4. Photosynthetic Pigment

The photosynthetic pigment content of Celosia leaves was measured spectrophotomertically based on methods described by Porra et al. [37]. For the sample preparation, the leaf disc was cut and the chlorophyll content was extracted by N'N dimethyl-formamide overnight. The absorbance was measured by spectrophotometer (Amersham Biosciences Ultrospec 2100 Pro UV/Visible) on 663 and 645 nm wavelengths and from these data, the chl a, b, a + b, and a/b ratio were calculated.

2.2.5. Histology

We used three specimens per treatment for the stem's histological examination. Each plant was cut into smaller pieces and the third internodes (from beneath) fixed separately in a mixture of glycerin:alcohol:water (1:1:1) for a week. Then, several cross-sections were prepared using blades, after clarification, they were stained with Toluidin-blue. All analyses were performed under a light microscope (Zeiss Axioscope 2+; Zeiss International, Oberkochen, Ostalbkreis, Germany) with a compatible camera, and the Scope Photo software (Scopetek, München, Germany) was used for processing the images. For the measurement, we used at least 15 different cross-sections per

internodes. The measured parameters were thick at the epidermis, primary cortex, pith, primary and secondary vascular tissue.

2.3. Statistical Analysis

Before the ANOVA test, Levene's Test for Equality of Variances was performed. The Levene's test for different variables at the six treatments of brown juice (i.e., 0%, 0.5%, 1%, 2.5%, 5%, and 10%) was negative, $p < 0.05$, and then the variances showed homogeneity. Results of the experiments were subjected to one-way (for fresh and dry weight, chlorophyll pigments, protein, MDA, POD, and catalase) and two-way (for root and shoot lengths, root and shoot volumes, and number of leaves) ANOVA by 'SigmaPlot 12.0' software and the means were compared by Duncan's Multiple Range Test [38] at $p < 0.05$.

3. Results

3.1. Characteristics of Brown Juice

3.1.1. Chemical Traits of Brown Juice

The fermentation of brown juice significantly changed its chemical properties (Table 1). Inoculation of fresh brown juice by lactic acid bacteria under anaerobic conditions caused a 13.9% reduction in pH. The degree Brix slightly increased after fermentation as it changed from 7.03 to 7.20. Total phenolic content dropped down after fermentation by almost 33.4%. Moreover, EC of fermented brown juice was 25.2% lower than fresh brown juice. Additionally, the density of brown color, that brown juice has, was reduced as its absorbance at 430 nm was diminished by 35.9%.

Table 1. Physiochemical characteristics of alfalfa brown juice before and after fermentation using lactobacillus.

Parameter	Before	After
pH	4.54 ± 0.03	3.91 ± 0.05
Brix † (%)	7.03 ± 0.02	7.20 ± 0.01
Total phenolic content (μg mL^{-1})	36.5 ± 1.19	24.26 ± 0.55
Electrical conductivity (dS m^{-1})	11.13 ± 0.11	8.47 ± 0.06
Color-absorbance (at 430 nm)	0.594 ± 0.006	0.381 ± 0.004
Lactic acid bacteria (CFU × 10^8 per mL)	11.33 ± 4.04	8.00 ± 4.36
Sugars content (g L^{-1})		
Glucose monomer *H*	21.19 ± 0.64	1.33 ± 0.03
Glucose oligomer *H*	2.80 ± 0.58	Nd ‡
Xylose monomer *Pb*	12.0 ± 0.06	nd
Xylose oligomer *Pb*	1.90 ± 0.03	0.60 ± 0.02
Arabinose monomer *Pb*	nd	0.10 ± 0.01
Arabinose oligomer *Pb*	1.50 ± 1.16	0.64 ± 0.01
Fructose monomer *Pb*	3.70 ± 0.02	0.89 ± 0.01
Fructose oligomer *Pb*	nd	nd
Acids content (g L^{-1})		
Acetic acid *H*	1.5 ± 0.02	10.4 ± 0.03
Lactic acid *H*	5.0 ± 0.25	50.1 ± 0.68
Propionic acid *H*	nd	1.2 ± 0.02

Notes: † Degree Brix = water-soluble sugar content (one degree Brix means 1 g of sucrose in 100 mL aqueous solution); ‡ nd = not detected; sample size (n = 6); *H*-samples run on Aminex HPX 87 H column; *Pb*-samples run on Aminex HPX 87 Pb column.

3.1.2. Contents of Sugars and Organic Acids in the Brown Juice

Furthermore, the effect of lactic acid bacteria was not only reflected in the chemical characteristics of brown juice but also was noticed in sugars content. Interestingly, contents of

monomer and oligomer forms of glucose, xylose, arabinose, and fructose reduced after fermentation, except arabinose monomer which was below the detected limit in fresh brown juice and became 0.1 g L^{-1} after fermentation; also, no fructose oligomer was detected either in fresh or fermented brown juice samples (Table 1). The highest decrease was found for glucose monomer as it lowered by 16 times in fermented brown juice compared to fresh brown juice. Fructose monomer, also, was four times lower in fermented brown juice, while arabinose oligomer recorded a decrease of 57.3% (Table 1). In contrast to sugars content, organic acids such as acetic, lactic, and propionic acids were considerably increased after fermentation by lactic acid bacteria. The content of lactic acid was 10-fold higher in fermented brown juice, as the highest recorded increase for any measured organic acid, while acetic acid content changed by seven times higher. Propionic acid content was below the detected limit in fresh brown juice; however, after fermentation it increased, recording 1.2 g L^{-1} (Table 1).

3.1.3. Macro- and Microelements Content of Brown Juice

Content of macro-and microelements of brown juice meaningfully changed due to fermentation by lactic acid bacteria (Table 2). Fermentation of brown juice resulted in a substantial reduction in the concentration of N, P, K, and S by 11%, 32%, 38%, and 21%, respectively. Otherwise, the contents of other elements displayed in Table 2 were found to be considerably higher after treating brown juice with lactic acid bacteria under anaerobic conditions. Interestingly, concentrations of Ca, Mg, Mo, Sr, and Ba were increased by 55%, 63%, 36%, 54%, and 109%, respectively. Furthermore, Na, Mn, Fe, Zn, B, and Al contents were 14.5-, 2.0-, 11.0-, 2.5-, 1.5-, and 5.7-fold higher, respectively, in fermented brown juice than fresh brown juice. No Cu was detected in brown juice either fresh or fermented.

Table 2. Content of macro- and microelements (mg L^{-1}) in alfalfa brown juice before and after fermentation.

Elements	Before	After
N	18.24 ± 0.66 †	16.19 ± 0.01
P	286 ± 30	238 ± 5.98
K	6090 ± 571	5276 ± 153
Ca	1270 ± 70	2326 ± 66.58
Mg	379 ± 16	739 ± 24.83
Na	31.03 ± 8.70	452 ± 15.02
S	352 ± 22	425 ± 6.57
Mn	1.61 ± 0.13	4.66 ± 0.09
Mo	0.29 ± 0.12	0.45 ± 0.01
Fe	2.04 ± 0.45	32.20 ± 0.86
Cu	0.06 ± 0.05	nd ‡
Zn	2.03 ± 0.19	8.59 ± 0.21
Sr	5.46 ± 0.24	8.80 ± 0.23
B	3.69 ± 0.33	11.61 ± 0.33
Al	0.24 ± 0.27	1.67 ± 0.13
Ba	0.39 ± 0.02	0.94 ± 0.02

Notes: † Standard deviation; ‡ not detected; sample size (n = 6).

3.2. Fermented Brown Juice as A Growth Stimulator

The possible utilization of fermented brown juice as a growth stimulator was evaluated. Celosia seedlings were treated with different doses of fermented brown juice through foliar application.

3.2.1. Growth Dynamic of Celosia

Spraying of Celosia seedlings with fermented brown juice significantly induced the development of stems (Figure 1). The application of brown juice at low concentrations had better

effects on plant growth than higher concentrations. Spraying Celosia plants with 0.5% of fermented brown juice resulted in the tallest stem (26.0 cm); however, higher concentrations drastically diminished stem length. For instance, at the rate of 10% fermented brown juice stem length was 15.6 cm (Figure 1A). The root system of Celosia plants responded to fermented brown juice differently to the shoot part. All rates of brown juice resulted in very similar lengths of root systems except the rate of 10% which caused a significant reduction in root systems (16.9 cm). However, the tallest root system was found in control plants sprayed with tap water (Figure 1A).

Although, length of shoot and root systems is considered as a good indicator for plant growth and its response to the newly added fertilizers and/or stimulators, alone it does not precisely describe the real status of plant health. Therefore, to have a comprehensive description of the shoot and root systems, their volumes should be also measured. This is very essential particularly to describe the root system and its architecture as shoot parts respond to growth conditions in a proportional way. Concerning stem volume, similar findings as for its length were reported. At lower rate of fermented brown juice (0.5%) the highest volume of stem (6.0 cm^3) was measured while increasing the rate of fermented brown juice gradually and significantly declined the stem volume and lowest volume (2.3 cm^3) was measured for plants sprayed at 10% fermented brown juice (Figure 1B). Results of root volume presented in Figure 1B displayed that although control plants had the tallest root length, its volume was the lowest among all the treatments. This means that control plants had long roots but unbranched ones with few lateral roots. All treated Celosia plants with fermented brown juice showed higher root volume compared to control plants. The highest root volume was noticed at plants sprayed with 5% of fermented brown juice. Additionally, results show that higher rates of fermented brown juice, i.e., 5% and 10% resulted in higher measured root volumes (Figure 1B).

Fresh mass of different Celosia tissues (roots, stems, and leaves) significantly responded to spraying the plants with different rates of fermented brown juice as shown in Figure 2. Fresh mass of roots, stems, and leaves of all plant parts was higher for plants treated with fermented brown juice compared to control plants sprayed with tap water. The highest fresh mass of roots, stems, and leaves was 4.30, 8.87, and 8.49 g $plant^{-1}$, respectively, that measured at rates of 2.5%, 0.5%, and 5% fermented brown juice, respectively (Figure 2A). Control plants displayed the lowest dry mass of roots, stems, and the number of leaves 0.17, 0.31, and 0.55 g $plant^{-1}$, respectively; while sprayed plants with 2.5% fermented brown juice showed the highest determined dry mass 0.44, 0.64, and 0.86 g $plant^{-1}$, for roots, stems, and leaves, respectively (Figure 2B). All rates of fermented brown juice, except 10%, significantly increased the number of leaves per plant (Figure 2C). Applying fermented brown juice at the rate of 10% significantly decreased the number of leaves not only compared to other fermented brown juice rates but also control plants. The highest number of leaves per plant was 18 and was counted for plants treated with 1% fermented brown juice. However, the differences between treatments of 0.5%, 1%, 2.5%, and 5% of fermented brown juice were not significant.

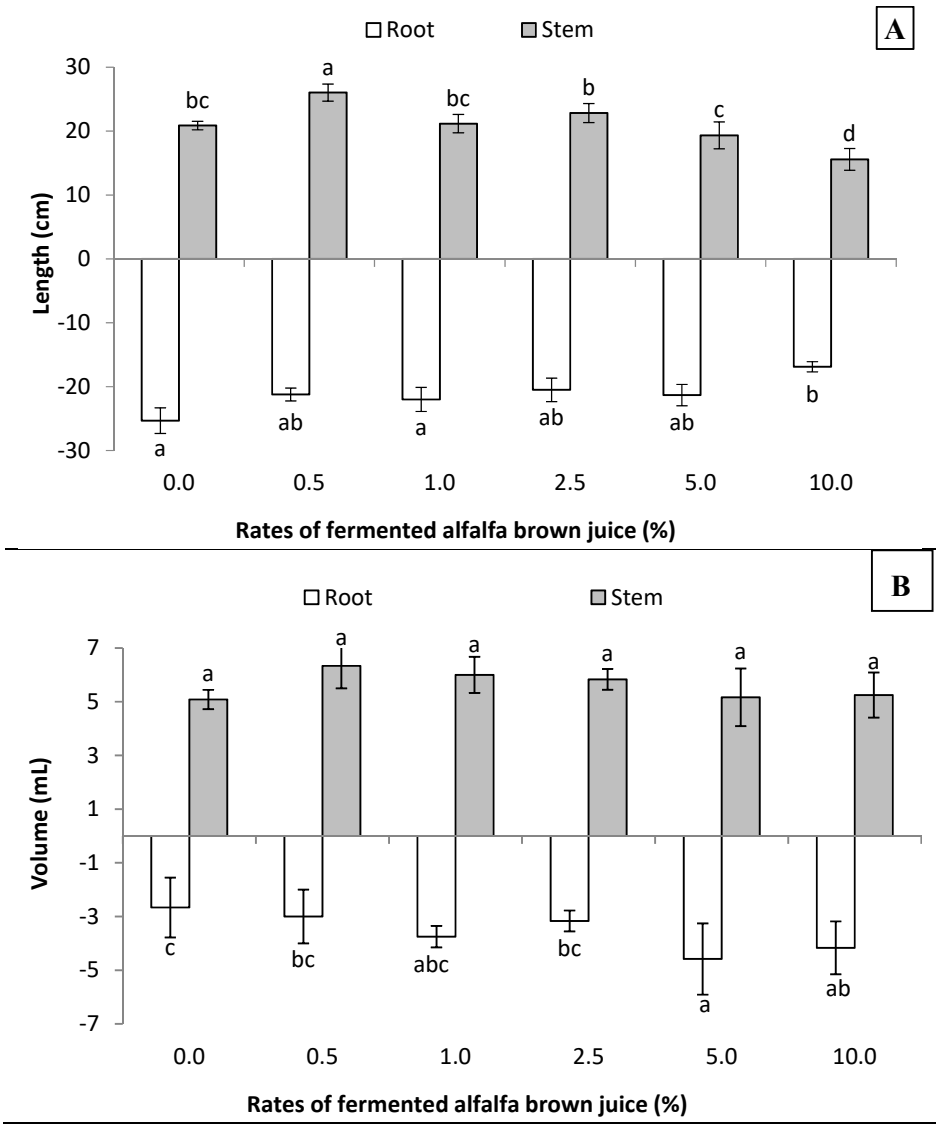
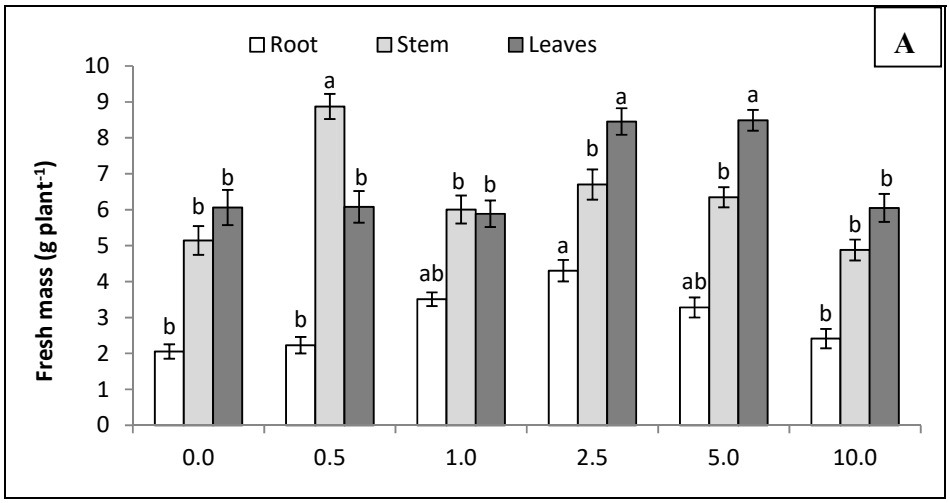


Figure 1. Length (**A**) and volume (**B**) of root and stem systems of Celosia plants fertilized at different rates of fermented alfalfa brown juice applied as a foliar application. Sample size (n = 6). Different letters above the same columns show significant differences at the level of $p < 0.05$.



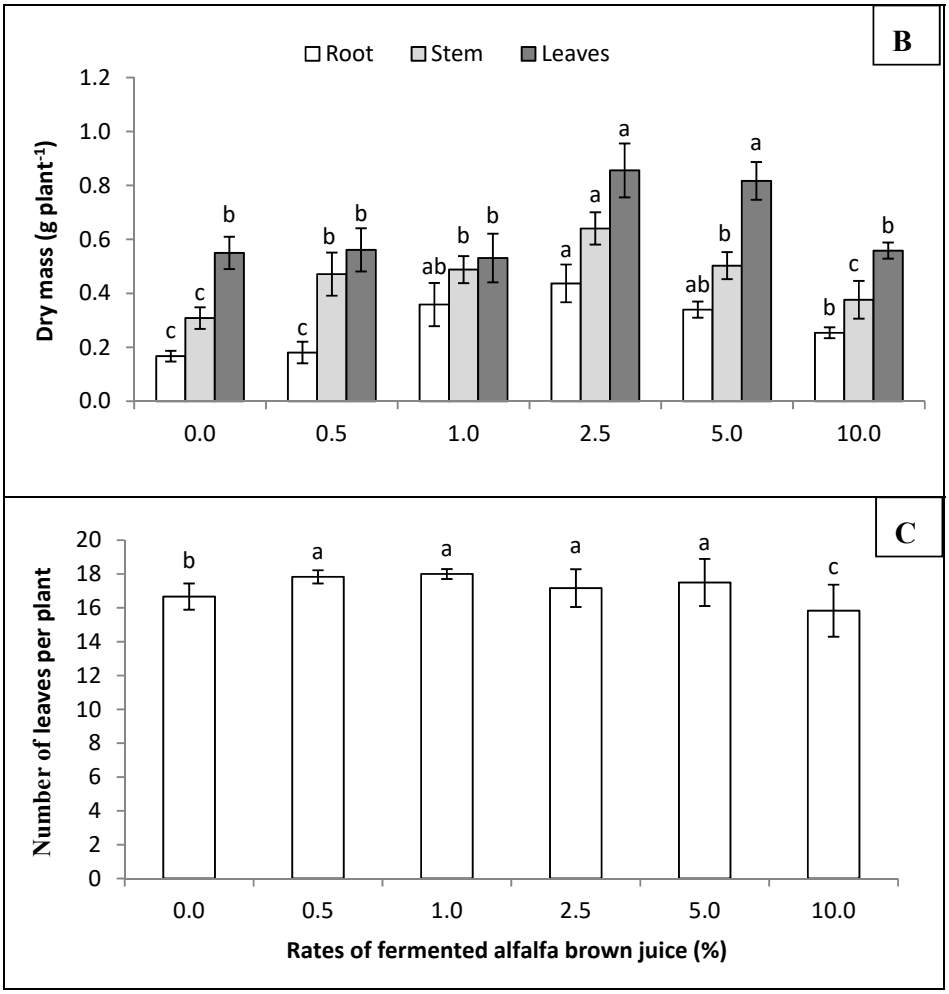


Figure 2. Fresh (**A**) and dry (**B**) masses and the number of leaves (**C**) of different plant tissues (roots, stems, and leaves) of Celosia plants sprayed at different rates of fermented alfalfa brown juice. Sample size (n = 6). Different letters above the same columns show significant differences at the level of $p < 0.05$.

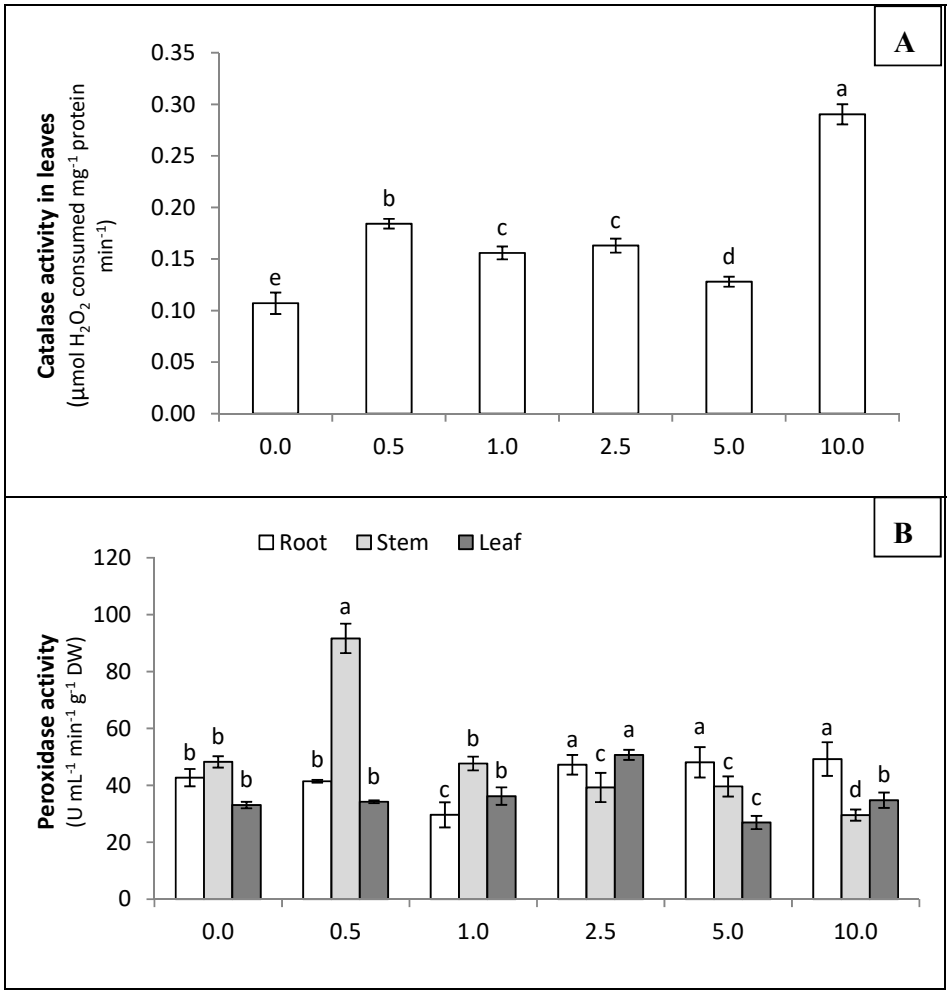
3.2.2. Antioxidant Capacity of Celosia Plants Treated with Fermented Brown Juice

Spraying Celosia plants with fermented brown juice significantly induced the activity of catalase (CAT) enzyme in the leaves (Figure 3A). All treated plants had higher activities of CAT enzyme compared to control plants (sprayed with tap water). However, increasing the rate of applied brown juice gradually reduced the CAT activity up to 5%, but this reduction was still higher than the control. Treated Celosia plants at the rate of 10% achieved the highest CAT activity among all treatments (0.290 μmoL H_2O_2 consumed mg^{-1} protein min^{-1}).

Different Celosia plant tissues (i.e., root, stem, and leaf) showed a significant response of peroxidase enzyme activity (POD) to added fermented brown juice (Figure 3B). Higher rates of fermented brown juice above 1.0% resulted in higher POD activity in the root system than both lower rates and control plants. The root POD activity in treatments of 2.5%, 5%, and 10% of fermented brown juice was higher than lower rates and the control; however, no statistically significant differences were calculated among these treatments. Interestingly, applying fermented brown juice at the rate of 1% resulted in the lowest determined activity of POD in the root system among all treatments including the control plants. The activity of POD in the stem tissue of Celosia plants was totally in contrast to POD activity in the root system (Figure 3B). The high rates of fermented brown juice above 1% showed lower POD activity in the stem than low rates (i.e., 0.5% and 1%) and control plants. The lowest POD activity in stems was noticed when plants were sprayed at 10% fermented

brown juice, while the highest measured POD activity in the stem was found for plants that received 0.5% fermented brown juice (Figure 3B). Except for treatments of 2.5% and 5% fermented brown juice, all other treatments including control plants showed similar POD activity in leaf tissue without significant differences. The highest leaf POD activity was measured in the leaves of treated plants with 2.5% fermented brown juice, while at the rate of 5% fermented brown juice the lowest leaf POD activity was determined (Figure 3B).

Malondialdehyde (MDA) content in different tissues of Celosia plants was measured as a marker for the degree of lipid peroxidation of unsaturated fatty acids due to oxidative stress. In the root system of Celosia plants, the highest measured value of MDA content was denoted in control plants. All treated plants with fermented brown juice had lower root MDA content than control plants. However, the response of treated plants with fermented brown juice hesitated as no clear trend was seen. The lowest applied rate 1% fermented brown juice showed the lowest root MDA content, while the highest root MDA content was measured in the root system of plants sprayed with 2.5% fermented brown juice (Figure 3C). In contrast to the root system, stem MDA content was found to increase as the rate of fermented brown juice increased up to 5% then reduced at the rate 10% recording the lowest MDA content in stem tissue among all treated plants with fermented brown juice. However, the lowest MDA content in the stem was displayed in control plants. Leaves of control plants showed higher MDA content than plants that received different rates of fermented brown juice. No significant differences were found in the MDA content of leaves of plants sprayed at the rates of 1%, 2.5%, and 5% fermented brown juice (Figure 3C). However, the lowest leaf MDA content was determined in the leaves of plants treated with 0.5% fermented brown juice.



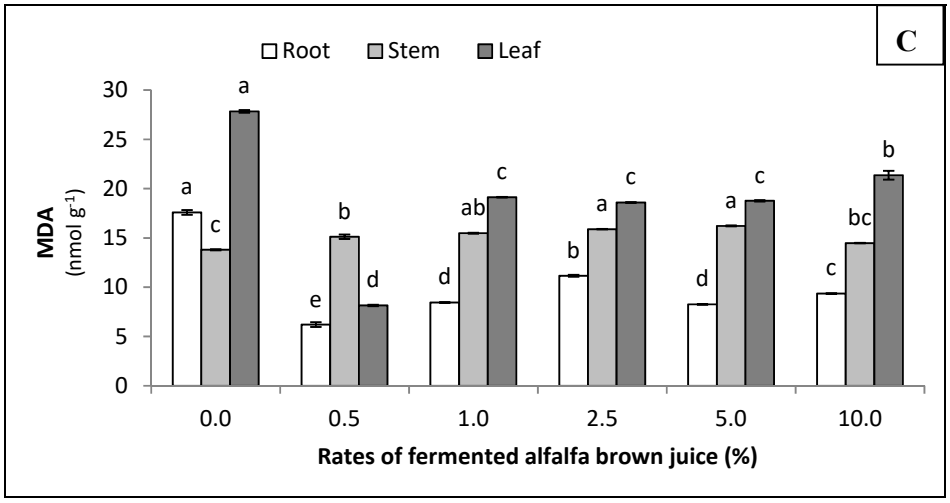


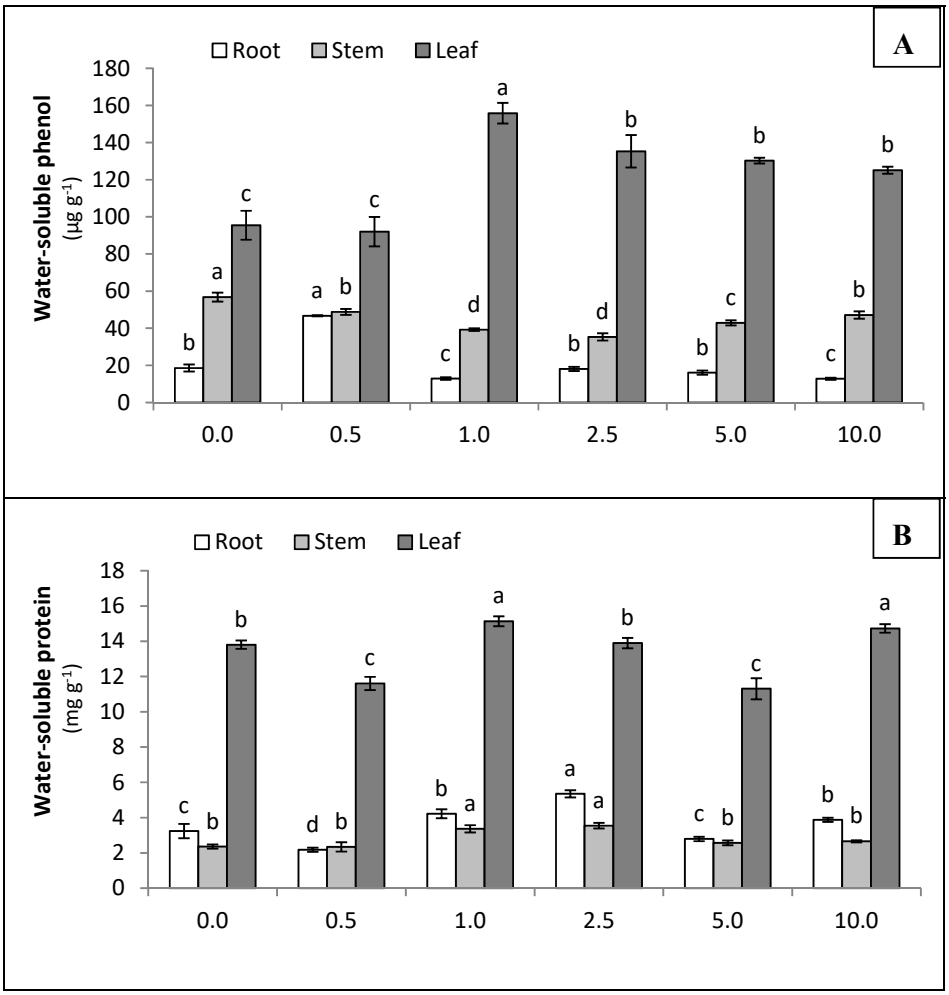
Figure 3. The activity of catalase (**A**) and peroxidase (**B**) and malondialdehyde content (**C**) in different plant tissues (roots, stems, and leaves) of Celosia plants sprayed at different rates of fermented alfalfa brown juice. Sample size ($n = 6$). Different letters above the same columns show significant differences at the level of $p < 0.05$.

3.2.3. Phenolic, Protein, and Photosynthetic Pigments Contents

The results of water-soluble phenol content are depicted in Figure 4A. Different plant tissues of Celosia plants possessed different water-soluble phenol contents as the root system showed the lowest content, while the highest water-soluble phenol content was measured in leaves. The addition of fermented brown juice as a foliar application to Celosia plants significantly affected the water-soluble phenol content in the root system. The highest water-soluble phenol content (46.7 μg g^{-1}) was measured in the root system of plants that received 0.5% fermented brown juice, while, when plants were allowed to grow in the presence of 10% fermented brown juice, the water-soluble phenol content was 12.8 μg g^{-1} (Figure 4A). The root system of the control plant displayed 18.6 μg g^{-1} water-soluble phenol content. In stem tissues, water-soluble phenol content in plants treated with fermented brown juice showed lower water-soluble phenol content than control plants. Increasing the rate of fermented brown juice up to 2.5% gradually increased the content of water-soluble phenol in stem tissues, then a linear increase was recorded when rates of fermented brown juice were increased up to 10%. The highest stem water-soluble phenol content (56.8 μg g^{-1}) was measured for control plants (Figure 4A). Except for treatment of 0.5% fermented brown juice, all fermented brown juice rates showed higher water-soluble phenol content in leaf tissues. The highest water-soluble phenol content (μg g^{-1}) was measured in leaves of plants that received 1% fermented brown juice; then a gradual decrease was noticed with increasing the rate of fermented brown juice up to 10% (Figure 4A).

The content of water-soluble protein was higher in leaf tissue followed by the root system, while the lowest content was denoted in stem tissue. Significantly, the application of fermented brown juice improved water-soluble protein content in the root system. Treatments of 1%, 2.5%, and 10% fermented brown juice displayed higher water-soluble protein content than control and treatments of 0.5% and 5% (Figure 4B). The lowest water-soluble protein content (2.18 mg g^{-1}) was measured in the root system of plants treated with 0.5% of fermented brown juice. Similar results were found in stem tissue of Celosia plants, where all treated plants with fermented brown juice had higher water-soluble protein content than control except treatment of 0.5% fermented brown juice. Although the content of water-soluble protein in leaves was higher than measured in the root system, the trend in which roots and leaves responded to spraying with fermented brown juice was almost the same. Leaf water-soluble protein contents in plants of treatments of 0.5% and 5% were the lowest among all treatments including the control. Other fermented brown juice rates enhanced the water-soluble protein content in leaf tissue over the control plants (Figure 4B).

Significant differences were noticed in a few cases among treatments for chlorophyll pigments content (Figure 4C). Content of *chl a* was reduced gradually with increasing the rate of applied fermented brown juice. Application of fermented brown juice at low rates (i.e., 0.5%) significantly improves the *chl a* content recording the highest value among all other treatments but was similar to control plants. On the other hand, the *chl b* content was found to respond negatively to increasing the rate of applied fermented brown juice as a gradual significant reduction was noticed. Content of total *chl a + b* displayed a similar tendency as it slightly decreased with increasing the rate of fermented brown juice. The low rate of fermented brown juice showed a slightly higher content than the control, but this increase was not significant (Figure 4C). Except treatment of 0.5% fermented brown juice, carotenoids content in all treatments including the control showed similar values as no significant differences were statistically measured. The lowest carotenoids content was determined in leaves of plants that received 0.5% fermented brown juice (Figure 4C).



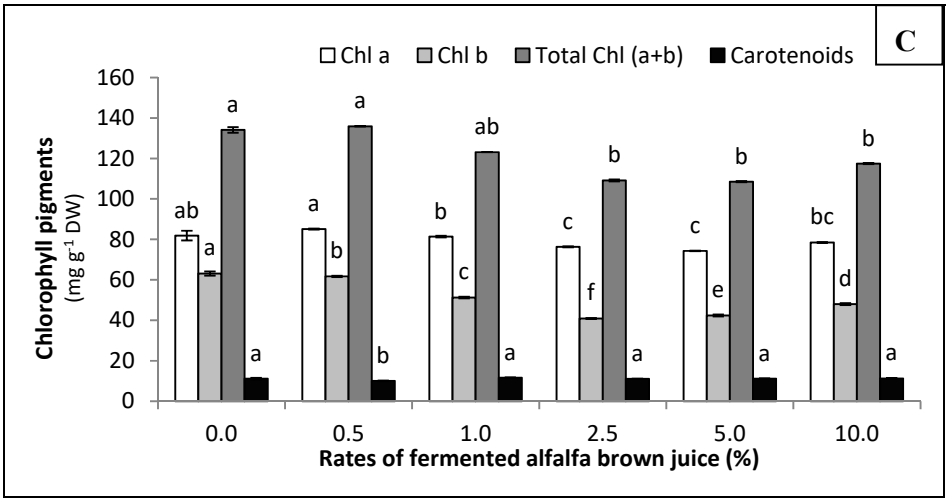


Figure 4. Water-soluble phenol (**A**), water-soluble protein (**B**), and chlorophyll pigments contents (**C**) in different plant tissues of Celosia plants sprayed at different rates of fermented alfalfa brown juice. Sample size (n = 6). Different letters above the same columns show significant differences at the level of $p < 0.05$.

3.2.4. Anatomical Features of Celosia Stem after Brown Juice Application

Regarding the cross-sections, 10–15 cm from the apex were analyzed, and the tissue structure was representative of an older Celosia's stem anatomy, with successive cambia [39,40]. Stems were covered by the epidermis (single row); beneath its primer cortex containing angular collenchyma (four to six cells thick) was visible. In the pith primary vascular bundles were located surrounded by a cylinder of anomalous cambium. Secondary and primary vascular tissues were separated by the conjunctive tissue [41]. Both the conjunctive tissue and the inner part of the pith were composed of parenchymatous cells (Figure 5).

There is no fundamental difference in the tissue structure in connection with the treatment, but there are significant differences in the thickness of the tissues, which support the differences that are visible to the naked eye too (e.g., thicker, stiffer stem). All levels of concentration caused a reduction in the thickness of the epidermis, while it was the 1% treatment that caused a reduction to a greater extent. The thickness of the primary cortex reinforced with angular collenchyma was decreased by most treatments, except the 0.5% and 10% treatments, where statistically verified thickening was observed. The proportion of pith involved vascular tissues increased for all treatments. The more concentrated brown juice treatments resulted in significantly thicker primary vascular tissues, except the 1% and the 10% treatments. Growing of the secondary vascular tissue was the highest at the 0.5% treatment, where the new successive cambium formed almost entirely closed xylem and phloem, significantly contributing to the strength of the stem (Table 3).

To sum up, it can be stated that the brown juice treatments (applied as foliar) influence the proportions of the Celosia stem's tissue. As a result of the treatments, the thinning of the epidermis and the intense growth of the vascular tissues (especially the secondary vascular tissue) can be projected. The growth rate of secondary tissues within the pith is the highest at 0.5% treatment.

Table 3. Impact of different concentrations of fermented brown juice on stem tissue of *Celosia argentea* var. *plumosa* (μm) (mean ± SD, n = 45).

	Epidermis	Cortex	Pith	Primary Vascular Bundle	Secondary Vascular Tissue
Cont.	31.44 ± 5.28 [a]	356.14 ± 57.69 ab	1873.30 ± 295.29 b	236.47 ± 79.43 b	221.47 ± 51.79 b
0.5%	30.81 ± 6.72 a	374.10 ± 99.50 ab	1903.79 ± 187.39 b	271.65 ± 73.59 ab	295.59 ±76.55 a

1%	25.83 ± 3.59 b	322.42 ± 61.76 bc	2033.30 ± 205.45 b	242.23 ± 41.22 b	212.56 ± 51.56 b
2.5%	28.50 ± 4.42 ab	261.69 ± 19.64 d	2011.11 ± 198.88 b	303.98 ± 88.94 a	230.18 ± 73.69 b
5%	31.43 ± 3.81 a	339.46 ± 68.14 b	2227.77 ± 310.33 a	348.30 ±122.47 a	256.93 ± 66.78 a
10%	28.69 ± 3.49 ab	392.07 ± 91.05 a	1915.50 ± 209.11 b	310.12 ± 102.47 a	274.80 ± 89.62 a

Notes: Different letters in each column indicate statistically significant differences ($p < 0.05$).

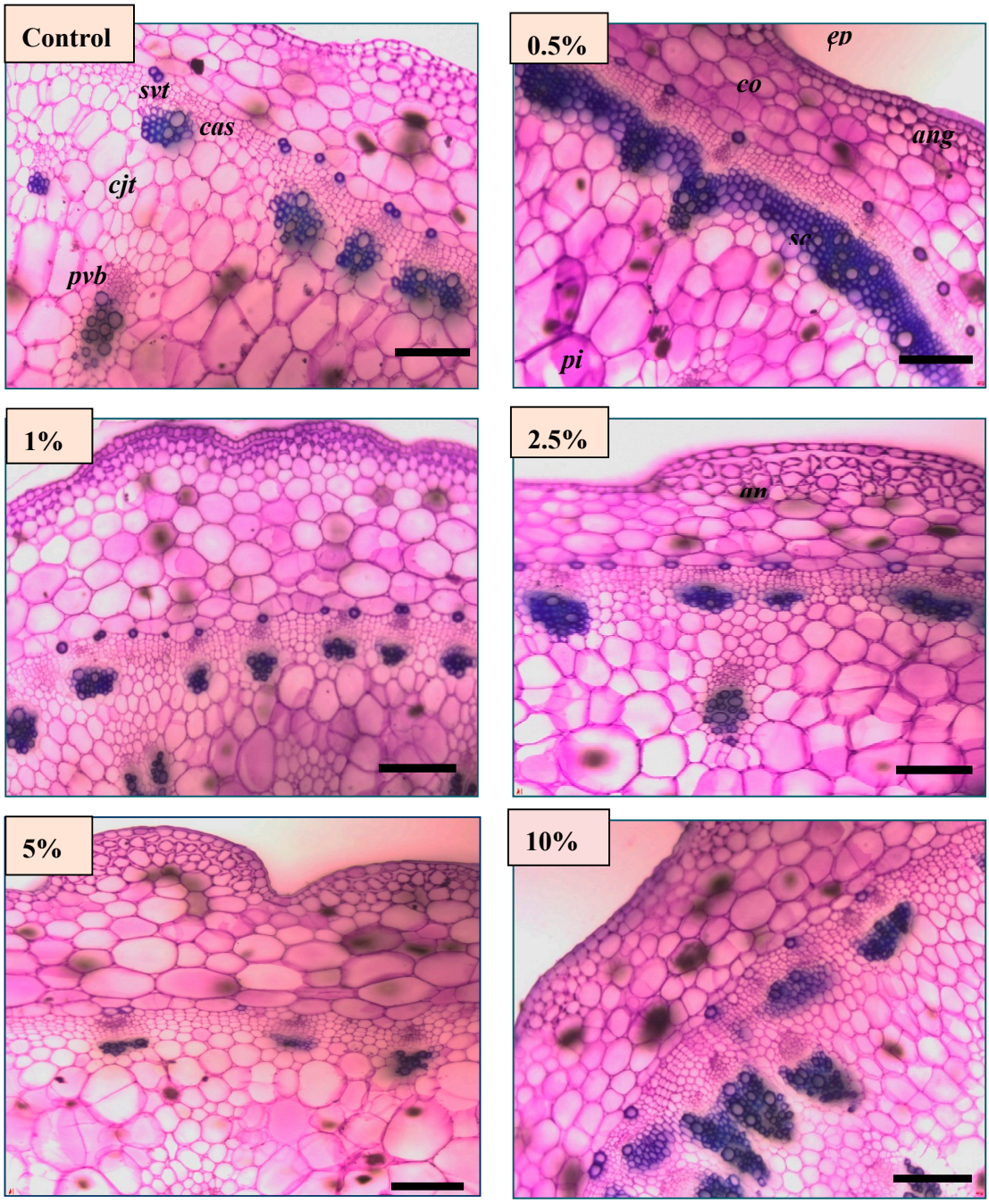


Figure 5. Anatomical sections of *Celosia argentea* var. *plumosa* stem. ***ep*** epidermis, ***co*** cortex, ***ang*** angular collenchyma, ***pi*** pith, ***sc*** successive cylinder (***svt*** secondary vascular tissue), ***ca*** cambium, ***cjt*** conjunctive tissue, ***pvb*** primary vascular bundle after spraying Celosia plants with different rates of fermented brown juice (i.e., control, 0.5%, 1%, 2.5%, 5%, and 10%). *Scale bar is* 200 μm.

4. Discussion

Recently, isolation of protein from plant green leaves has gained increasing attention as an attempt to bridge the gap between protein production and demand due to the dramatic increase in population and the increase in living standards. Brown juice (referred to as deproteinized plant juice or DPJ as well) is a byproduct generated during the coagulation of soluble protein in green juice through thermal treatment. Brown juice has gained less attention than LPC and press cake. It represents nearly 50% of pressed and pulped fresh biomass [9]. Therefore, these huge amounts could be an obstacle facing the acceleration of this approach and its acceptance by both politicians and the public. Disposal of alfalfa brown juice is a serious issue in LPC production due to its high biological oxygen demand (BOD) and carbohydrates content [42]. Due to its richness in free amino acids, peptides, soluble sugars, vitamins, and many macro- and microelements, it can be directed towards animal feeding and production of many chemicals [43]. Additionally, it can be used as a fertilizer, growth stimulator and/or growth medium for microorganisms [42]. Although some pieces of literature have been reporting the possible utilization of brown juice as a ruminant feed [44], few studies have been focusing on brown juice as a fertilizer [42,45].

During our recent experiments on LPC production from alfalfa biomass, it became clear that the storage of the brown juice at room conditions leads to fast spoiling. Therefore, we had to store it below 4 °C. This may be due to its high carbohydrate content, which represents a suitable environment for bacteria to grow [42]. Therefore, converting sugars into organic acids and subsequent decrease in the pH of brown juice through fermentation using lactic acid bacteria seemed to be an ideal solution since fermented brown juice is stable and this facilitates its handling.

Lactic acid bacteria have long been known for their role in the fermentation of carbohydrates. Consequently, it has wide applications in medicine and food processing. Nowadays, lactic acid bacteria have been found to play an important role in agriculture, bioenergy production, and bioremediation of the environment [46]. In the present study we, firstly, aimed to stabilize alfalfa brown juice through reducing its water-soluble sugars content and pH using lactic acid bacteria. Accordingly, sugars content in brown juice was reduced after fermentation, because lactic acid bacteria use sugars as energy and carbon sources [47]. As shown in Table 1, most of the sugars in brown juice were found to be below the quantification limits after fermentation indicating that lactic acid bacteria consumed them. Comparable results have been previously presented by many researchers [48,49]. On the other hand, organic acids (e.g., lactic, acetic, and propionic acids) were increased in fermented brown juice causing a subsequent reduction in pH (Table 1). Novik, et al. [46] reported lactic acid as the main acid produced after fermentation of water-soluble sugars such as glucose and fructose either monomer or oligomer by lactic acid bacteria. Similar findings were cited by Bautista-Trujillo et al. [48], who observed a decrease in pH of maize silage after inoculation by lactic acid bacteria due to the increase in the production of organic acids, mainly lactic and acetic acids. Moreover, they reported an increase of 46.3% in lactic acid content. However, lactic acid content was found to increase by 8-fold after fermentation compared to unfermented brown juice (Table 1). This high increase in lactic acid content may be attributed to the initial low pH of fresh brown juice (4.54), which helps to hydrolyze the oligo- and polysaccharides, therefore they become available for lactic acid bacteria [48]. Additionally, another possible reason for high lactic acid content could be attributed to the high Mn content in brown juice. Cheng et al. (2014) stated that applying Mn to Jerusalem artichoke juice enhanced the lactic acid production by lactic acid bacteria up to 12 g L^{-1}. Moreover, Dimitrovski et al. [49] stated that the fermentation of Jerusalem artichoke tuber juice by lactic acid bacteria reduced its pH from 6.5 to 4.7 after 30 h. In the current study, at the end of the fermentation process, the pH of brown juice was 3.91. Lactic acid bacteria significantly reduced the absorbance of brown juice by 35.9%. This result was supported by that previously cited by Kwaw et al. [50]. They studied the effect of different strains of lactic acid bacteria on colorimetric properties of mulberry juice, reporting a 6.9% reduction in the color. They referred this reduction to the increase in content of the monomeric anthocyanin. Although an increase in total phenolic content has been previously reported for fermented mulberry juice [50] and pomegranate juice [51], our results displayed a decrease of 33.4% after inoculation of brown juice by lactic acid bacteria. Except N, P, K,

and S other macro- and microelements were higher in fermented alfalfa brown juice (Table 2). The reduction in concentrations of N, P, K, and S could be attributed to the fact that they are essential elements for the growth of lactic acid bacteria. However, similar findings were described by Kim [52], who stated that fermented kale juice had higher elemental composition than unfermented juice. Moreover, he cited significant differences between kale juice fermented by different lactic acid bacterial strains. The increase in the concentration of microelements, in particular, may be due to the increase of brown juice acidity. Although, the concentration of macronutrients (e.g., N, P, and K) was reduced after fermentation, the content of macro- and microelements is still high, and this makes the fermented brown juice a potential growth stimulator. On the whole, these results are supported by earlier findings of Ream et al. [45]. They reported that alfalfa brown juice contains a relatively high content of N and K, in addition to small amounts of P, Ca, Mg, and other microelements.

In the present study, fermented alfalfa brown juice as a growth stimulator was evaluated using the Celosia plant as a model. Brown juice was applied at different rates by foliar application. The foliar application of fermented brown juice showed a significant potential on the development of Celosia seedlings in comparison with control. Noticeably, increasing the application rate of brown juice sprayed on Celosia seedlings led to a considerable reduction in shoot parts, particularly the stem length. From a horticultural point of view, this seems to be a good result since the target is the flower not the vegetative growth of Celosia. Application of brown juice at low rates such as 0.5% and 1.0% enhanced the growth and resulted in high values of stem length, the volume of stem and root, fresh masses of stem and root, and number of leaves. Shorter but more branched root systems were observed when Celosia seedlings were sprayed with brown juice (Figure 2). This phenomenon is supported by data of length and the volume of roots (Figure 1). The beneficial effect of alfalfa brown juice could be attributed to its high content of macro- (i.e., N, P, K, Ca, and Mg) and microelements (i.e., S, Mn, Fe, Cu, Zn, and Mo); all in phyto-available forms (Table 2). Similarly, Ream et al. [45] observed that using brown juice as a fertilizer added at an annual rate of 1.25 cm induced the growth and yield of alfalfa, corn, and bromegrass; while, at the higher rate (2.5 cm) a reduction in yield and plant damage were noticed in all crops. However, they referred to the damage in plant growth caused by high rates of brown juice to unknown reasons; moreover, they considered it a not serious problem since the added amount of brown juice can be controlled. These results are supported by findings of Reddy et al. [42], who earlier stated that the application of alfalfa brown juice at low rates enhanced germination and growth of cowpea, mung bean, and groundnut; while high rates inhibited the germination and reduced the plant growth. They reported that alfalfa brown juice can be used as a fertilizer if it would be added at a lower level than 10%. Additionally, they could not give a reason for such damaging effects of high rates of brown juice, except what previously was mentioned by Pirie [53], who stated that alfalfa brown juice contains some phytotoxic organic compounds.

In our experiment, the reduction in plant growth of treated Celosia plants at high rates of fermented brown juice can be explained by high EC value and low pH of brown juice solutions. Increasing the rates of brown juice caused a gradual increase in EC and a decrease in pH as shown in Table 4. At treatment of 0.5% brown juice, EC (dS m^{-1}) and pH were 0.12 and 4.21, respectively; while, at the highest applied rate 10% they were 1.99 and 4.38, respectively. Low pH is not favorable for the development of plants; it reduces photosynthesis due to a reduction in stomatal conductance [54]. This might explain why we found diminished growth of Celosia plants treated at high rates of brown juice. However, in the current study, the lowest pH was 4.38 when Celosia plants received 10% fermented brown juice. Long et al. [55] had earlier reported a reduction in citrus growth below pH 4, while higher pH did not inhibit the growth and seedlings reached their maximum growth at pH 5. The reduction in growth may be attributed to H^+-toxicity which damages leaves. Absorption of nutrients applied as foliar application depends on the pH of the solution. Extreme pH (below 2 and above 12) was reported to burn the leaves. Moreover, some elements prefer different pH values for their optimum absorption by plant leaves.

Table 4. pH and electrical conductivity (EC, dS m^{-1}) values of alfalfa brown juice solutions before and after fermentation at the beginning of the experiment.

Rates of Brown Juice (%)	pH		EC	
	Before	*After*	*Before*	*After*
0.5	4.65 ± 0.02 †	4.21 ± 0.07	0.15 ± 0.01	0.12 ± 0.03
1.0	4.67 ± 0.02	4.16 ± 0.00	0.28 ± 0.01	0.54 ± 0.58
2.5	4.68 ± 0.01	4.16 ± 0.01	0.67 ± 0.07	0.46 ± 0.02
5.0	4.72 ± 0.01	4.18 ± 0.00	1.20 ± 0.04	0.81 ± 0.05
10.0	4.72 ± 0.01	4.19 ± 0.00	2.26 ± 0.04	1.44 ± 0.12

Notes: † Standard deviation.

Antioxidant enzymes such as CAT and POD are among the most important antioxidant enzymes which play a vital role in scavenging reactive oxygen species generated in cells due to different biotic and abiotic stresses [56]. Thus, enhancing the activity of these enzymes is considered an important step in improving the plants' tolerance to different kinds of stress [57,58]. The results abstracted from this research showed that the application of alfalfa brown juice after fermentation by lactic acid bacteria significantly increased the activity of CAT and POD in different Celosia tissues. However, the low rates of brown juice seemed to be more effective than higher ones, as a reduction in the activities was noticed. These results are confirmed by results of MDA, as treated plants with fermented brown juice had lower MDA content than control plants (untreated plants) regardless of the type of plant tissues. These results demonstrate that fermented brown juice can potentially be exploited as a growth stimulator, particularly at low rates. Besides, fermented brown juice had a significant effect on water-soluble phenol and protein contents, as they were higher in treated plants in comparison to control ones. The high rates of brown juice were found to reduce the photosynthetic pigment content. This could be attributed to low pH at high rates of fermented brown juice. This result was in accordance with that cited by Solati et al. [59], who reported a decrease in chlorophyll content due to low pH.

Brown juice could be very useful as a soil fertilizer/conditioner particularly in alkaline soils and/or sandy soil due to its rich composition in macro- and microelements and sugars. These could induce the microbial growth in soil increasing soil fertility. Additionally, sugars play an important role in soil stabilization through maintaining soil aggregation, which subsequently leads to better water holding capacity [42,60]. While delivering deeper insights into the possible use of alfalfa brown juice as a growth stimulator and trying to precisely determine the most effective rate and application method, there are many issues that should be addressed in the future [61]. Results, undoubtedly, suggest that brown juice tolerance can be plant species dependent; therefore, more studies on different plant species at different rates of brown juice are crucially needed. Additionally, phytotoxicity of brown juice should be the focus of future studies.

5. Conclusions

The present study highlights the possible use of alfalfa brown juice as a growth stimulator. Brown juice is a serious problem in LPC production, where its disposal represents a threat to the environment due to its high content of water-soluble sugars as well as macro- and microelements. Fermentation of brown juice using lactic acid bacteria significantly improved its nutritional value and stability, because these bacteria—as our data showed—produce a significant amount of organic acids i.e., lactic, acetic, and propionic acids through their metabolism making the nutrients more available and the pH of row material (brown juice) lower, thereby stabilizing it. Most water-soluble sugars were under the detectable level after fermentation as the bacteria used them as carbon source. Moreover, the concentration of nutrients increased—showing the effect of bacteria for nutrient availability—after fermentation except N, P, K, and S showed a slight decrease. In this study, treating *Celosia argentea*, a valuable ornamental species with significant food and medical uses, with low rates of fermented brown juice through foliar application significantly improved the growth, as all of the vegetative parameters such as stem and root length, shoot and root volume, fresh mass of stem and

root, and the number of leaves increased. The brown juice treatments in low (0.5%) concentration caused positive changes in histological parameters, in the growth rate of secondary tissues. Additionally, fermented brown juice showed a considerable impact on the antioxidant capacity of Celosia plants, as CAT and POD activities increased while MDA content decreased. Moreover, both water-soluble phenol and protein were found to increase in treated plants with fermented brown juice compared to the control showing the beneficial effect of lactic acid fermentation and chemical properties of brown juice. These results conclude and state the potential use of fermented alfalfa brown juice as a sustainable growth stimulator for crops with a particular interest in horticultural crops. Our data regarding the chemical and microbiological properties of brown juice and the effects (listed plant responses) it triggered confirm the scientific investigations where plant growth-promoting properties of lactic acid bacteria contribute greatly to the maintenance of the health of plants (also strengthening disease resistance) and by consuming these plants, they are also beneficial in human digestive processes. It should be noted that the sample size number was modest in our study that is why it was difficult to draw strong far-going conclusions, however preliminary conclusions support the fact that phytomicrobiome engineering can be a promising strategy for sustainable agriculture, but the data available is limited to understand properly these complex symbiotic relationships. Therefore, examination of fermented alfalfa brown juice's effect on physiological, biochemical, and anatomical parameters of other horticultural and agricultural crops is in progress.

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Conflicts of Interest: The authors declare no conflict of interest.

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