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Fermented Alfalfa Brown Juice Significantly Stimulates the Growth and Development of Sweet Basil (*Ocimum basilicum* L.) Plants

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Abstract: Fertilization management is a key issue in plant nutrition to produce plants with good quality and quantity. Deproteinized leaf juice or brown juice (BJ) is a by-product during the isolation of leaf protein from biomass crops such as alfalfa. The idea of using BJ as a biostimulant fits well in the aspect of circular economy since BJ is currently a problematic issue of the leaf protein production approach. Fractionation of one-kilogram fresh biomass results in approximately 500 cm^3 BJ. Due to fast spoil of fresh BJ, if left on room temperature, it is found that fermentation of fresh BJ using lactic acid bacteria and reducing its pH increases its stability and storage on room temperature. In the present study, we examined the effect of fermented alfalfa BJ on vegetative, physiological, and anatomical properties of the versatile sweet basil (*Ocimum basilicum* L. 'Bíborfelhő') plants. Sweet basil seedlings were sprayed at different doses of fermented alfalfa BJ (i.e., 0.5%, 1.0%, and 2.5%) and tap water served as a control (0.0% BJ). The results revealed that foliar application of fermented alfalfa BJ significantly improved the biometrical features of sweet basil plants. Plants treated with fermented BJ showed significantly higher values of all the measured parameters compared to the control (0.0%), except for the number of leaves per plants where control plants (0.0%) had more leaves. However, the leaves of control plants (0.0%) were smaller than treated plants as data of leaf area showed. Fermented alfalfa BJ significantly increased the content of photosynthetic pigments (chl a and chl b), relative chlorophyll (SPAD value), lengths of stem and root, fresh masses of stem, root, and leaves, volumes of stem and root, and leaf area. Despite all rates of fermented BJ displayed higher values over control plants (0.0%), the rate of 0.5% was the best one supported by results. Application of fermented alfalfa BJ influenced the anatomical parameters as well. These findings demonstrate the possible use of fermented alfalfa BJ as a promising novel plant biostimulant.

Keywords: sweet basil; alfalfa brown juice; fermentation; biostimulation; chlorophyll pigments; histological changes

1. Introduction

The increase in the world's population leads to several issues that we already have to face, and we must find sustainable solutions for them to save our life on the planet. One of these concerns is the sustainable supply of high-quality protein. To solve the protein issue, several approaches were proposed to identify novel protein sources or alternatives [1–3]. Alfalfa (*Medicago sativa* L.) leaf protein concentrate (LPC) is a promising element either in human or animal diet as the human population of the Earth (7.2 billion) is growing rapidly causing a high demand for animal protein [4,5]. The isolation of leaf protein resulted in four products, i.e., green juice, fiber, leaf protein concentrate, and deproteinized juice or referred to as brown juice (BJ). The amount of produced BJ during the isolation of leaf protein and the utilization of this product is a huge obstacle for the wider recognition of LPC production. Fractionation of one-kilogram fresh biomass results in 450–550 mL BJ [6–8]. Therefore, disposal of BJ is a big challenge that the leaf protein isolate approach faces. Another reason making disposal of BJ risky is the high content of several bioactive components such as sugars, free amino acids, minerals and vitamins that BJ contains [7,9,10]. Therefore, finding an alternative use of BJ is urgent due to environmental concerns, besides maximizing the benefit from this waste that is very rich in several valuable compounds and nutrients. Instead of dumping it we could make a valuable product, thus making a step towards the circular economy concept [11,12]. The BJ contains about 40% carbohydrates (mainly monosaccharides, like glucose and fructose) and 3% nitrogen-based on dry mass [12]; additionally, numerous biologically active components like phenols, amino acids, macro- and microelements and biostimulators, etc. [7,11,13]. However, fresh BJ cannot be stored at room temperature, after a few days it gets spoiled at pH 5–6 due to its high sugar content. In our previous study, inoculation of BJ with lactic acid bacteria was not only found to increase BJ stability at room temperature but also substantially improved the nutritional characteristics of BJ [7] through converting sugars into organic acids decreasing the pH to almost 4.5. Therefore, BJ seems to be an ideal component in animal feeding programs as well as plant nutrition and soil stabilization. Several authors have previously suggested BJ as a plant fertilizer, fodder, and growth medium for microbes [10,14–16]. Fermented BJ can be applied as a very effective foliar biostimulant. We have observed remarkable effects of fermented alfalfa BJ on the growth dynamic of plumed cockscomb [7]. Additionally, lactic acid bacteria as a plant growth-promoting bacteria represents an additional benefit of fermentation of BJ as it promotes plant growth [12,17].

Sweet basil (*Ocimum basilicum* L.) is a well-known annual herb, member of the Lamiaceae family. It is one of the 150 species of the Ocimum genus, a very important medicinal, spice and fresh vegetable, culinary herb and industrial plant, cultivated for aromatic and medicinal use on large areas in many countries [18–23]. It is native to India, Asia and Africa, but grows in many regions of the world, including Italy, Thailand, Vietnam and Laos [24]. Sweet basil is popularly used in traditional Chinese medicine, because of its selected purified components and antiviral activity [25]. The latest scientific developments revealed the strong pharmacological action and nutritional aspects because of antioxidant content [25], additionally, it is a rich source of acylated and glycosylated anthocyanins being a valuable source for the food industry [26].

The aim of this study was to examine the impact of the fermented BJ on the biometrical, physiological and anatomical features of sweet basil plants.

2. Materials and Methods

2.1. Sources of Brown Juice and Plant Materials

The fresh alfalfa BJ was obtained from the Proteomill Green Protein Biorefinery Factory (Tedej Ltd., Hajdúnánás, Hungary). The fresh BJ was fermented using lactic acid bacteria to avoid its fast spoiling. The physicochemical properties of fresh and fermented BJ as well as the fermentation process were described by Bákonyi et al. [7]. The seeds of sweet basil (*Ocimum basilicum* 'Bíborfelhő') were obtained from the National Agricultural Research and Innovation Center (NARIC, Budapest, Hungary).

2.2. Experimental Study

A pot experiment under greenhouse conditions was carried out at the NARIC to assess the possible growth stimulation effect of fermented BJ using the multipurpose sweet basil as a plant model. The experimental design was the Randomized Complete Block design (RCB) with 15 replicates. A polyethylene pot (7 × 7 × 8 cm) was filled with white peat for young plants (Klassman-Deilmann TS 3 FINE type, Geeste, Germany). The characteristics of growth medium are as follows: fine structure, 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}. The sweet basil seeds were sown in the nursery substrate on 16 July 2018. The germinated seedlings were treated once a week with fermented BJ at different rates (i.e., 0.5, 1, and 2.5%) in the early stage of development (stage 1 BBCH) [27]. On 1 August 2018, the seedlings turned to stage 2 BBCH, all identical and healthy, were transferred to the pots. One pot contained one seedling and each treatment contained 15 pots. Fermented BJ was sprayed on the plants twice a week (on Tuesdays and Fridays) from 15 August till the end of the experiment (11 September) at rates of 0.5, 1.0, and 2.5%. Final application volume of 250 mL BJ was equally shared among all replicates of the same treatment (15 plants). The control plants (0.0%) were sprayed with the equivalent amounts of tap water. The experiment was terminated on 11 September (stage 5 BBCH) and plants were harvested, and samples were collected for the further biometric, physiological and anatomical analyses.

2.2.1. Analysis of Biometric Features of Basil Plants

At the end of the experiment, before flowering (BBCH stage 5), all plants in each treatment were harvested and the following vegetative parameters were measured: root and stem length (cm), root and stem volume (mL), root and stem fresh mass (g $plant^{-1}$), the number of leaves (pcs $plant^{-1}$) and leaf area. Roots were carefully removed from the pots and washed by tap water on a sieve to remove the adhered growth medium particles. Length was measured with a measuring ruler, while volume was determined by a graduated cylinder, fresh mass by OHAUS Pioneer PA214 analytical balance and the number of leaves by counting. The leaf area (cm^2) of sweet basil plants were measured by AreaMeter 350 (Opti-Sciences, Hudson, NY, USA). Six plants were analyzed and represented as a mean ± SD (n = 6). In the case of leaf area, we used the data of nine plants. All leaves of the examined plants were measured.

2.2.2. Determination of Chlorophyll Contents

At the end of the experimental period, the relative chlorophyll values were measured by SPAD 502 chlorophyll meter (Minolta, Japan) using the last fully developed leaves. The chlorophyll-a and -b contents were extracted based on the method of Moran and Porath [28] and determined by the method of Wellburn [29], Vidician and Cachita-Cosma [30]. We used the following formulas: "Chlorophyll a (mg·g^{-1}) = (11.65 a664–2.69 a647)" and "Chlorophyll b (mg·g^{-1}) = (20.81 a647–4.53 a664)". The samples were taken from the last fully expanded leaves and chlorophyll pigments were extracted by 5 mL *N*,*N*-dimethylformamide (DMF) added to 0.05 g leaf disc. The samples were soaked in this solvent for 48 h at room temperature in the dark. After 48 h, the discs were removed and the contents of chlorophyll-a and -b were measured by METEREKSP-830 spectrophotometer. All the measurements were repeated three times for each plant making up to nine measurements for each of the treatments.

2.2.3. Anatomical Analysis

We used three individual plants per treatment for the stem's histological examination. 15 different cross-sections per plant internodes (n = 45) were prepared as described in the following: each plant was cut into smaller pieces and the third internodes (from beneath) fixed separately in Strasburger-Flemming's solution [31], which is 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. Each analysis was performed under a light microscope (Zeiss Axioscope

2+; Zeiss International, Oberkochen, Ostalbkreis, Germany) with a compatible camera, and Scope Photo software (Scopetek, München, Germany) was used for processing the images. The measured parameters were: thickness of the epidermis, primary cortex, pith including primary and secondary vascular tissues.

2.3. Statistical Analysis

Results of the experiments were subjected to one-way ANOVA by SigmaPlot 12.0 and IBM SPSS Statistics 24 software and the means were compared by Tukey Test [32] at $p \leq 0.05$. Before the ANOVA test, in SPSS the Levene's Test for Equality of Variances was performed in the case of anatomical data. SigmaPlot 12.0 automatically ran a check test for Equality of Variance. The Equality of Variance test for different variables at the four treatments of BJ (i.e., 0.0, 0.5, 1.0, and 2.5%) were negative, $p \leq 0.05$, and the variances showed homogeneity.

3. Results

3.1. Plant Biometric Features

Results of root and stem length of sweet basil are presented in Figure 1. The treated plants with fermented BJ had taller stems and roots compared to the control plants (0.0%). Most importantly, fermented BJ enhanced plant development resulting in higher values of both root and stem. Despite all treated plants showed a taller stem than control plants (0.0%), increasing the rate of applied fermented BJ slightly reduced the stem length. However, the differences in stem length between treatments of fermented BJ were not significant. Treated plants with 0.5% fermented BJ showed an increase of 50.4% in stem length compared to control (0.0%) as the highest measured increase. Similarly, root length showed a relationship as root length gradually increased with increasing the rate of the applied fermented BJ. The application of fermented BJ significantly increased root length compared to the control (0.0%); however, no significant differences in root length were reported between different rates of fermented BJ (Figure 1).

Stem volume of sweet basil plants follows a dose-relationship response to fermented BJ; increasing rates of fermented BJ significantly and gradually caused an increase in stem volume. The highest increase in stem volume (38.6%) compared to untreated control plants (0.0%) was recorded by 2.5% BJ (Figure 2). Similar effect was noticed for root volume, as it increased upon spraying plants with increased rates of fermented BJ. Nevertheless, the highest rate of fermented BJ (2.5%) showed the same value as for treatment of 0.5% fermented BJ, which was two-fold higher than the control plants (0.0%). The highest root volume was 1.16 mL and was found when plants received 1.0% fermented BJ.

Results of stem and root fresh mass are presented in Figure 3. The fresh mass of stem gradually increased as a result of increased rates of fermented BJ. Significantly, all fermented BJ increased the stem fresh mass. While control plants (0.0%) possessed a stem fresh mass of 0.73 g plant^{-1}, treated plants with 2.5% fermented BJ showed a stem fresh mass of 1.52 g plant^{-1} as the highest recorded value. The differences between fermented BJ treatments were not significant. In contrast to stem fresh mass, root fresh mass increased as rate of fermented BJ increased up to 1.0%, then decreased at 2.5% fermented BJ. However, all treatments showed significant increases in the fresh mass of root system in comparison to control plants (0.0%) (Figure 3). Plants received 1.0% of fermented BJ, which was almost three-fold higher in their root fresh mass than control plants (0.0%).

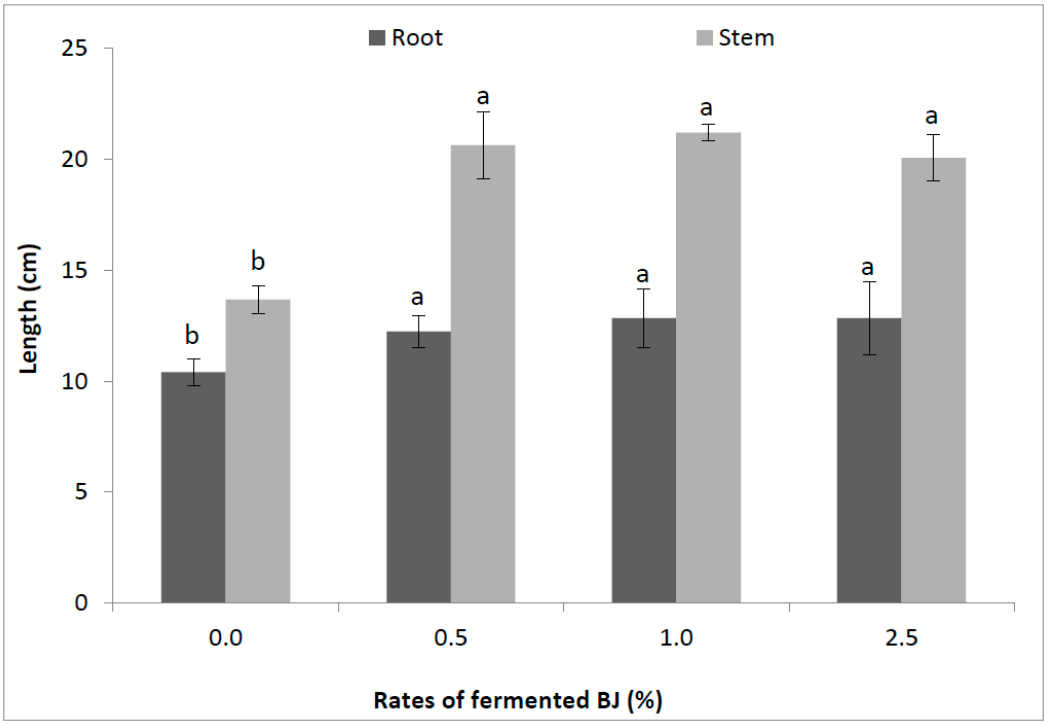

Figure 1. Root and stem length of sweet basil plants treated with different rates of fermented BJ. Sample size $n = 6$ (mean ± SD). Different letters above the same columns show significant differences at the level of $p \leq 0.05$.

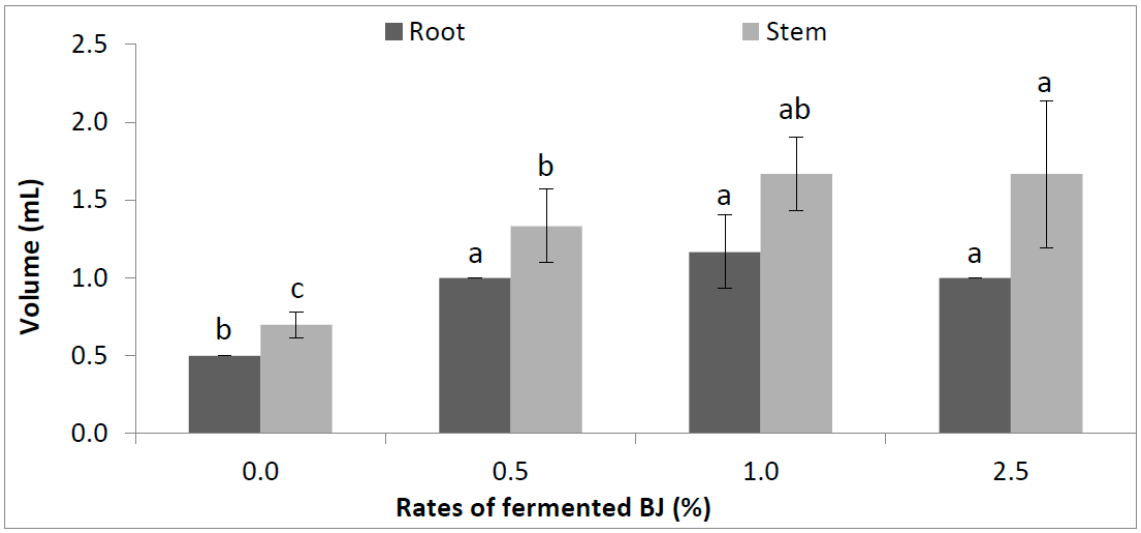

Figure 2. Stem and root volume of sweet basil plants treated with different rates of fermented BJ. Sample size $n = 6$ (mean ± SD). Different letters above the same columns show significant differences at the level of $p \leq 0.05$.

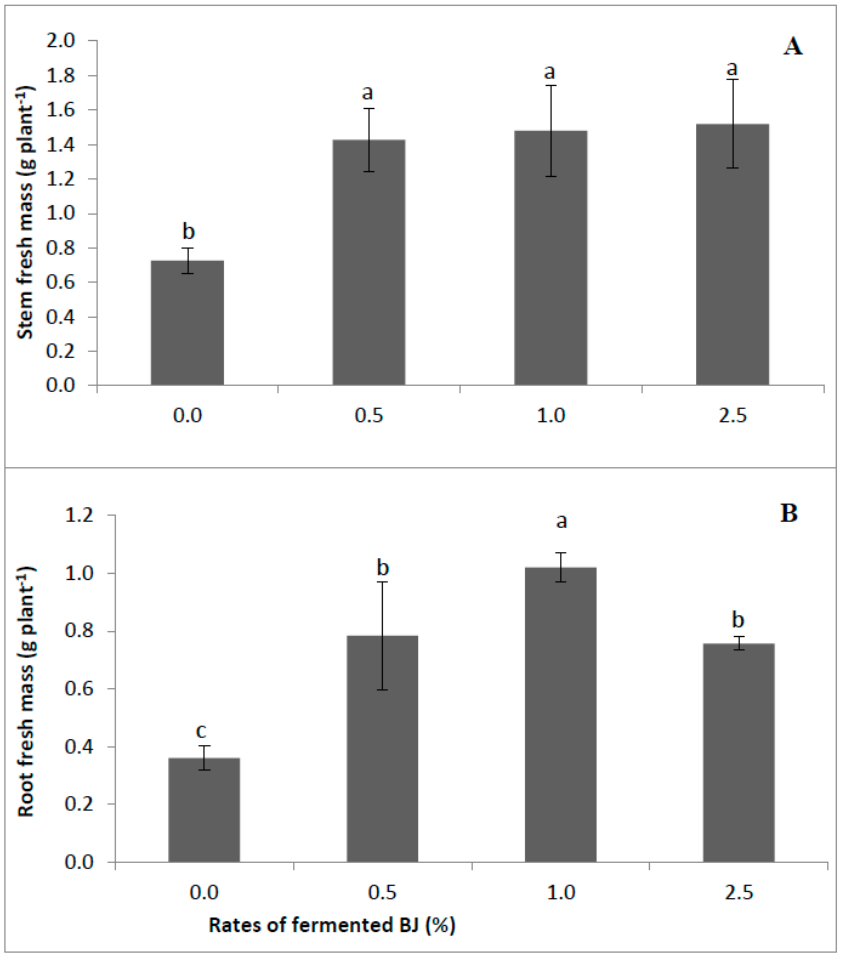

Figure 3. Fresh mass of stem (**A**) and root (**B**) of sweet basil plants treated with different rates of fermented BJ. Sample size $n = 6$ (mean ± SD). Different letters above the same columns show significant differences at the level of $p \leq 0.05$.

Figure 4 shows the results of the leaves fresh mass, number of leaves and leaf area of sweet basil plants after treating them with different rates of fermented BJ extracted from alfalfa biomass. Results showed that fermented BJ significantly improved plant growth as the leaves fresh mass of treated plants was higher than those untreated (control, 0.0%). All fermented BJ rates resulted in significantly higher values of leaves fresh mass than the control (0.0%). However, the highest applied rate of fermented BJ (2.5%) showed a lower fresh mass value than that measured at 0.5 and 1.0% of fermented BJ. The highest leaves fresh mass was recorded at treatment with 0.5 and 1.0% fermented BJ (Figure 4A). Contrarily, the number of leaves per plant gradually reduced with increasing rate of applied fermented BJ (Figure 4B). Control plants (0.0%) possessed the highest number of leaves (22 leaves per plant), while the plants sprayed with 2.5% fermented BJ had 11.7 leaves per plant. The differences among all treatments including the control (0.0%) were statistically significant. Despite control plants (0.0%) possessing a higher number of leaves than the treated plants with fermented BJ, the leaf area of plants sprayed with different rates of fermented BJ was larger than the control plants (0.0%). While the control plants (0.0%) had a leaf area of 71.4 cm^2, the plants of the treatment of 0.5% fermented BJ displayed a leaf area of 131.5 cm^2 (Figure 4C). Moreover, other treatments exhibited significantly higher leaf areas than control plants (0.0%) as well.

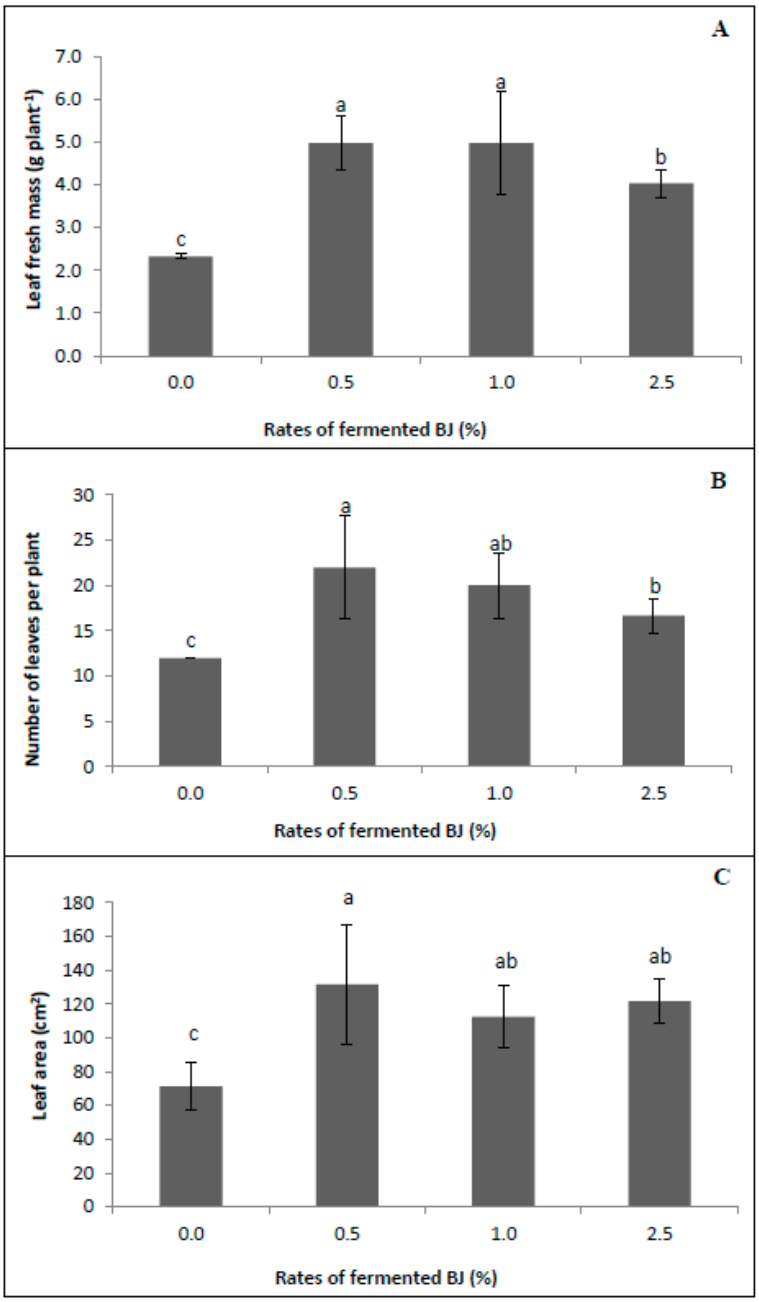

Figure 4. (**A**) Leaves fresh mass; (**B**) number of leaves; (**C**) leaf area of sweet basil plants treated with different rates of fermented BJ. Sample size $n = 6$ (mean ± SD). Different letters above the same columns show significant differences at the level of $p \leq 0.05$.

3.2. Contents of Photosynthetic Pigment

The results showed that the content of chlorophylls increased upon spraying sweet basil plants with fermented BJ (Table 1). The contents of chlorophyll-a (chl a) and chlorophyll-b (chl b) in the control (0.0%) leaves were 6.90 and 1.95 mg g^{-1}, respectively, and significantly increased to 8.04 (chl a) and 2.66 (chl b) mg g^{-1} after treating plants with 0.5% (chl a) and 2.5% (chl b) fermented BJ, respectively. The total content of chlorophylls ranged from 8.86 to 10.60 mg g^{-1}, 2.5% BJ application increased the chlorophyll content by 19% compared to the control (0.0%). Chlorophyll a/b ratio in treated plants with fermented BJ ranged from 2.97 to 3.50; while the control plants (0.0%) possessed a chlorophyll a/b

ratio of 3.52 (Table 1). The SPAD value showed a significant, 28% increase when leaves were treated with 0.5% concentration of fermented alfalfa BJ in comparison to the control (0.0%).

Table 1. Content of chlorophyll in sweet basil leaves (mg g^{-1}) and their relative changes (%) compared to the control plants (0.0%) (n = 9).

	Chlorophyll-a	Chlorophyll-b	Chlorophyll-a/b Ratio	Total Chlorophyll	SPAD Value
0.0%	6.91 b	1.96 b	3.52 a	8.86 a	27.00 b
0.5%	8.05 a (16.52%)	2.46 ab (25.78%)	3.26 ab	10.51 ab (18.57%)	34.60 a (28.15%)
1.0%	6.94 ab (0.56%)	2.00 ab (2.26%)	3.47 ab	8.95 ab (0.93%)	32.40 ab (20.00%)
2.5%	7.93 ab (14.86%)	2.67 a (36.12%)	2.97 b	10.60 b (19.56%)	31.60 ab (17.04%)

Different letters (a, b, ab) in the same columns show significant differences at the level of $p \leq 0.05$.

3.3. Anatomical Traits of Sweet Basil

The cortex is made up of angular collenchyma (two to four cells thick) and typical parenchymatous cells. The pith consists of mainly parenchymatous cells, but the ratio of primary and secondary vascular tissues is dependent on the treatments. Treatments 0.5% and 1.0% resulted in well-developed secondary vascular tissues (Figure 5).

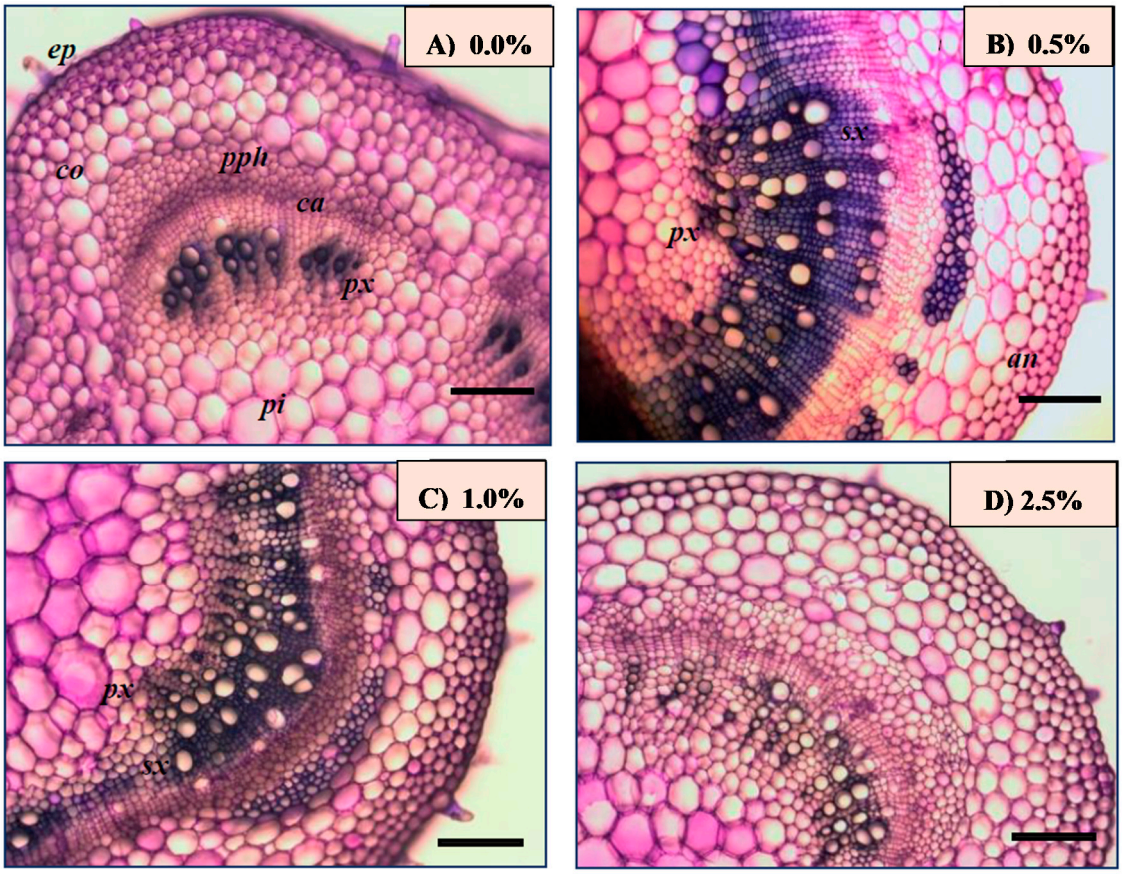

Figure 5. Anatomical sections of sweet basil stem. Subfigures show samples treated with different rates of fermented BJ such as (**A**) 0.0%, (**B**) 0.5%, (**C**) 1.0%, (**D**) 2.5%. ***ep*** epidermis, ***co*** cortex, ***ang*** angular collenchyma, ***pi*** pith, ***pph*** primary phloem ***ca*** cambium ***px*** primary xylem ***sx*** secondary xylem, after treating with different rates of fermented BJ (i.e., control (0.0%), 0.5%, 1.0%, 2.5%). Scale bar is 200 µm.

Differences which are visible in the tissues caused by the treatments were proven true by our measurements. All levels of concentration increased the thickness of the epidermis, however only two tests were significant. The impact of all treatments was a decrease in the thickness of the primary cortex.

Treatments 0.5% and 1.0% increased both the extension of the pith and the proportion of vascular tissue in it, which were proven statistically significant. Growth of the secondary vascular tissue was the highest at the 0.5% treatment. It can be concluded that a greater brown juice concentration, which was 2.5% in this analysis, may block the development of secondary vascular tissues (Table 2).

Table 2. Anatomical traits of basil stem tissue (epidermis, cortex, pith involved primary and secondary vascular tissues) (μm) after treatment with fermented BJ. Sample size n = 45 (Mean ± SD).

BJ Rate	Epidermis	Cortex	Pith	Vascular Tissue
0.0%	23.75 ± 5.55‡ b	273.55 ± 48.18 a	2202.89 ± 239.13 c	234.41 ± 53.75 c
0.5%	27.92 ± 7.81 a	259.39 ± 73.72 a	2529.03 ± 280.56 b	548.68 ± 107.39 a
1.0%	27.37 ± 8.22 ab	260.97 ± 51.36 a	2684.10 ± 259.04 a	539.41 ± 96.54 a
2.5%	28.88 ± 7.54 a	242.16 ± 56.36 a	2101.83 ± 162.30 c	290.38 ± 59.46 b

Different letters (a, b, ab) in the same columns show significant differences at the level of $p \leq 0.05$.

Consequently, the use of an incremental concentration of brown juice can increase the thickness of the stem and the vascular tissue in it but only to a certain extent.

4. Discussion

The BJ is largely produced during the isolation of leaf protein from several green leafy crops. The storage of fresh BJ in room temperature is the main concern due to the high content of carbohydrates particularly in the form of monosaccharaides. Lactic acid bacteria showed a considerable effect on reducing BJ pH as a result of organic acids production under an anaerobic condition and consequently increased the stability and handling of BJ at a pH of 4.5–4.8 [7].

Fermented BJ can be exploited as an organic fertilizer or growth stimulator due to its richness in free amino acids, soluble sugars, vitamins, organic acids and other nutrients. In addition, lactic acid bacteria—as plant growth promoting bacteria—represent an additional benefit for using fermented BJ as a fertilizer [7,33]. In the present study, fermented BJ proved its efficiency as a promising plant growth stimulator as it caused a significant increase in photosynthetic pigments including chl a, chl b, total chlorophyll and relative chlorophyll (SPAD value). Similar findings have been previously reported by Bergstrand et al. [34] who proved in their study on nitrogen speciation in pot experiment of sweet basil fertilized by different organic manures, i.e., blood meal + Baralith®Enslow and poultry manure, that the plant-based organic fertilizer treatment induced the chlorophyll content. Moreover, applying biofertilizers, i.e., Nitrajin (including Azotobacter, Azospirillum and Pseudomonase), increased the amount of photosynthetic pigments and the leaf area of sweet basil [35]. Additionally, Ertani et al. [36] mentioned that the extract prepared from alfalfa biomass using enzymes contained growth stimulant compounds like triacontanol and indole-3-acetic acid, which significantly improved the relative chlorophyll and growth of maize plants under salt-stress conditions.

Noticeably, all rates of BJ resulted in higher values of stem and root length (Figure 1), stem and root volume, stem and root fresh mass, number of leaves and leaf area. This was in agreement with those documented earlier by El-Ziat et al. [37]. They cited that organic fertilization and humic acid application improved growth parameters of 'Red Rubin' basil plant, i.e., fresh weight, plant height and leaf area, in a greenhouse experiment. Organic fertilization of basil using organic NPK fertilizer (4–3–4) (Organic Fertilizer, Mighty Grow®Fruitdale, AL) resulted in changes in both fresh and dry weight, and in nutrient uptake as well [24]. Onofrei et al. [18] stated that different organic foliar applications, i.e., Fylo®, Geolino Plants&Flowers®, Cropmax®, Fitokondi®, stimulated the content of total phenolic compounds contributing to healthier vegetable production of *Ocimum basilicum* L.

Unique positive changes in secondary vascular tissues observed as a result of 0.5% and 1.0% BJ treatments (Figure 5 and Table 2), which according to our best knowledge have not been published before. However, similar effects were reported on plumed cockscomb (*Celosia argantea* var. plumosa 'Arrabona') plants by Bákonyi et al. [7] who cited significant changes in histological parameters after treating plants with fermented BJ. The tissue structure of the stem of sweet basil analyzed is a typical

structure of a plant at the age of 10–12 weeks. Stems were covered by the epidermis (single row) with a thin layer of cuticle. Contrary to Venkateshappa and Sreenath [38], more types of the trichomes are identified on the surface of the stem, e.g., non-glandular uniseriate hair (composed of three cells) and glandular capitate hairs, which supports the findings of Werker et al. [39] and Nasssar et al. [40].

In a few cases, the application of fermented BJ led to a slight reduction in some measured parameters. However, all rates of fermented BJ were better than the control plants (0.0%, untreated). This beneficial role of fermented BJ is owed to its high content of phytoavailable nutrients such as N, P, K, Ca, Mg, S, Mn, Fe, Cu, Zn, and Mo [7]. Similar findings were reported earlier by Ream et al. [41]. They revealed that BJ at the rate of 1.25 cm enhanced the yield and growth of corn, alfalfa and bromegrass. However, they also stated that higher rates of BJ (2.5 cm) caused a reduction in development and yields of all crops. Another study cited similar results where the application of BJ obtained from alfalfa biomass at low rates positively improved the germination of many crops, i.e., cowpea, mung bean and groundnut. Negative impacts were reported when BJ was used at higher rates, above 10% [33]. The detrimental effect of high rates of BJ may be attributed to the existence of some phytotoxic organic compounds in BJ [6]. Moreover, the reduction in plant growth at a high applied rate of fermented BJ could be due to the high electrical conductivity of BJ [7]. Additionally, spraying plants with acidic solutions (low pH as at the high rate of fermented BJ) is known to lessen the stomatal conductance [42]; therefore, a reduction in plant growth is expected. This hypothesis was supported by the earlier findings of Long et al. [43].

The results obtained from the present study alongside with our previous published work [7] strongly support the hypothesis about prospective studies on BJ either fermented or fresh to improve soil properties through the soil application technique. In alkaline, salt-affected soils, and sandy soils the expected benefits of using fermented BJ would be more due to the high content of macro and microelements, free amino acids [7,42,44]. Additionally, the soluble sugars will facilitate and improve the growth of rhizosphere bacteria supporting them against the unflavored growth conditions [45]. Another substantial result for using fermented BJ via soil application is increasing the soil aggregates due to a high sugar content in BJ. Moreover, the low pH of fermented BJ is an advantage of using fermented BJ as a soil conditioner since it will partially modify the local soil pH around the root system facilitating the uptake of especially microelements by plant roots [43]. However, in our next studies, we are going to work on these hypotheses.

5. Conclusions

This study demonstrates the potentiality of fermented alfalfa BJ to enhance both quantity and quality of one of the most important multipurpose crops, i.e., sweet basil, which is cultivated on large areas in many countries. Our results showed a significant increase in photosynthetic pigments leading to better vegetative growth; and consequently, enhancement in important medical phytochemicals in sweet basil could occur. For a plant such as sweet basil (used as spice and fresh vegetable, and culinary herb), larger leaf area, number of leaves, and thickness of stem (including vascular tissues) as well as fresh weight are very important decisive traits. The results also indicated that concentrations of 0.5 and 1.0% fermented BJ were the most valuable and have a considerable biostimulator effect on leaves as these treatments were more effective improving leaf parameters in comparison to control plants (0.0%).

Additionally, we came to the conclusion based on the results that fermented alfalfa BJ has great potential as a biofertilizer and plant growth promoter. Future experiments are needed to justify more of these findings with other horticultural and agricultural crops.

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