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Stinging Nettle (*Urtica dioica* L.) as an Aqueous Plant-Based Extract Fertilizer in Green Bean (*Phaseolus vulgaris* L.) Sustainable Agriculture

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Abstract: Plant-based fertilizers, such as liquid plant extracts, contribute to the cultivation of vegetables, particularly in organic production. The objective of this study was to determine if aqueous nettle extract could be successfully used as a fertilizer, applied on the soil and foliarly, in green bean production under field conditions. The hypothesis was that it could successfully replace mineral fertilizers and be integrated into sustainable and organic agriculture. The experiment was conducted at two climatically and pedologically different locations (Zadar and Poreč) throughout two growing seasons (spring and autumn). Two types of aqueous nettle extracts were used: a short-term extract (SE) was prepared by the extraction of wild stinging nettle (183 g 10 L^{-1} of water) in water for 24 h, while a long-term extract (LE) was prepared at the same ratio with water extraction for 14 days. Both extracts were diluted with water at a ratio of 1:3 (extract:water) before use. The SE was applied foliarly, and LE, by pouring it onto the soil. The abovementioned treatments were compared with mineral fertilization with urea (U) and control (no fertilization (C)). Foliar fertilization with SE proved to be almost as efficient as fertilization with LE, poured onto soil, showing a positive effect on green bean vegetative parameters. Furthermore, aqueous nettle extracts showed a positive effect on the iron accumulation in the leaves.

Keywords: legumes; foliar application; organic fertilizer; soil fertilizer; aqueous extract

1. Introduction

The successes of modern agricultural production have, for a long time, been associated with the frequent use of mineral fertilizers and pesticides [1]. In recent years, consumer attention has focused on food quality and control, leading to increased demand for organically produced foods. Vegetables are an important part of everyday meals, so it is advisable to understand their nutritional and chemical properties [2]. Recent studies suggest that the mineral content in vegetables has been declining [3].

Green beans (*Phaseolus vulgaris* L.) are a polyphenol-rich food and have a positive effect on human health in controlling obesity, diabetes and inflammatory processes in the body [4]. They are also rich in vitamin C, fiber, carbohydrates, minerals and proteins [5]. Green beans have a short growing period (60 to 80 days), and are very easy to grow as they thrive in different soil types. Green beans can be cultivated at both higher and lower altitudes and in dry as well as humid climates, and they also enrich the soil with nitrogen, and are therefore desirable in crop rotation [6].

To ensure high yields in conventional production, plants need to be fertilized before and during cultivation [7]. While the increased use of mineral fertilizers and pesticides affects plant nutrition, it may also reduce soil microbial cultures and threaten sustainable production and ecosystem health [8]. On the other hand, soil management without fertilization leads to significant yield losses. Since vegetables are very sensitive to the accumulation of toxic substances and are mostly used fresh or slightly heat-treated, there is now a growing demand for organically grown vegetables that do not contain pesticide residues, traces of heavy metals and other harmful components. In organic farming, soil fertility is achieved and maintained in a number of ways. Corrective fertilization is attained by adding manure, compost and compost teas [9], vermicompost, peat, ash and stone meal [10], mixtures of plant and fish waste, spent mushrooms, manure [11,12], and seaweed [13–15].

Plant-based fertilizers also have a positive effect on plant growth and development and on the control of plant diseases and pests [16]. They are rich in nutrients that are rapidly released and therefore important for soil biological activity [17]. Most organic plant-based fertilizers used as fertilizers or pesticides [18–22] are largely the result of traditional knowledge, which is passed down from generation to generation. Some of the most commonly recommended preparations are aqueous plant extracts made from stinging nettle [16,23–27].

Stinging nettle (*Urtica dioica* L.) is a perennial plant of the *Urticaceae* family that often grows as a weed in neglected places, such as along roads and river valleys, and near settlements [28,29]. In organic farming, it is traditionally used in the form of aqueous extracts, so-called "vegetable soups", for use as fertilizers or bioinsecticides [7,10].

Aqueous nettle extract is rich in nitrogen, phosphorus, calcium, magnesium and iron and promotes plant growth [16]. When the extract is prepared by soaking nettle plants in water for 14 days, it is used as an organic fertilizer (long-term extract) [24]. A short-term nettle extract can be prepared by soaking the plant in water for 24 h [23] and used for foliar application; it is believed to have a repellent effect on insects [18,19]. There are no field studies on the effects of the short-term extract as a fertilizer; therefore, this research is interesting as a comparison of long-term and short-term plant-based fertilizers with mineral fertilizer.

The objective of this study was to test the effects of long-term and short-term aqueous nettle extracts on green bean vegetative growth, yield and chemical composition. The applied treatments were compared with nitrogen mineral fertilizer (urea 46% N) and a control treatment (no fertilizer). The hypothesis was that aqueous nettle extract could successfully replace mineral fertilizers in green bean production and be integrated into sustainable and organic agriculture.

2. Materials and Methods

2.1. Preparation of Aqueous Nettle Extract

Wild raw material of stinging nettle was collected from a meadow in Valtura (N 44°53′59″; E 13°54′32″). The nettles were cut at the upper third of the shoot and dried at room temperature (22 °C) in the dark until constant mass, and then stored in special double paper bags (Carta Ltd., Osijek, Croatia). The extract was prepared by immersing 183.00 ± 1.00 g of chopped dry herb in 10 L of water, as previously described by Peterson and Jensen [24], and was left in a plastic vessel at ambient temperature with occasional stirring. Two aqueous extracts were prepared, differing in the duration of extraction, aimed at different methods of application. The short-term extract (SE) was macerated for 24 h, while the long-term extract (LE) was macerated for 14 days. Before application, the prepared extracts were filtered through a plastic sieve with 2 mm pores and diluted with distilled water at a 1:3 ratio.

2.2. Field Experiment and Plant Material

The field experiment with green beans (*Phaseolus vulgaris* L.) cv. "Top Crop" (obtained from MIAGRA Ltd., Križ, Croatia) was conducted in two climatically and pedologically different locations (L) and during two growing seasons (S). The first experimental location was in Poreč, Croatia (N 45°13′18″; E 13°36′11″) and the other in Zadar, Croatia (N 44°9′24″; E 15°26′2″). Seeds were not treated prior to sowing.

The experiment was conducted as a field trial with eight different treatments in a randomized complete block design (RCBD) with four replicates. Green beans were sown in April in the spring growing season (SP) and in August in the autumn growing season (AU) (Table 1). The plot area was 5 m^2 (2.0 m × 2.5 m) and contained four rows. Distance between rows was 0.5 m and 4 cm between plants in a row, resulting in 50 plants per m^2. The experimental plot consisted of two middle rows and was 2.50 m^2 in size. The eight treatments (T) applied were: commercial mineral fertilizer "urea" (46% N) (U); short-term (SE) and long-term (LE) nettle extracts applied one, two or three times at weekly intervals (SE1, SE2, SE3, LE1, LE2 and LE3); and the control (C) (unfertilized plots). Treatment with mineral urea fertilizer (Petrokomija, Ltd., Kutina, Croatia) was applied at N rate of 40 kg ha^{-1} [6] one month after sowing (at the 3–4 leaf stage). The aqueous nettle fertilizers were firstly applied at the stage of the first true leaf on 34 and 28 days after sowing in spring and autumn cultivation, respectively. Thereafter, the applications were repeated once or twice depending on the treatment, but they finished at the flowering stage. The SE aqueous nettle extract was sprayed onto the plants until dripping from leaves; therefore, the amount of extract increased according to the developmental stage of the plant from approximately 0.02 L per plant in the first treatment (1.00 L m^{-2}), up to 0.05 L per plant in the second treatment (2.5 L m^{-2}) and finally 0.09 L per plant in the third treatment (4.5 L m^{-2}). The LE extract was applied to irrigate the soil at a rate of 4.8 L m^{-2} or 0.096 L per plant. Foliar application of LE was avoided due to high NH_4 content (10 times higher than SE), in order to prevent potential leaf toxicity. At each application time, the remaining plots were irrigated and sprayed with the same amount of water. Plant maintenance during the experiment, hoeing and additional drip irrigation were performed as needed. No pesticides nor fertilizers were applied during the experiment, while weeds were removed by mechanical means.

Table 1. Experimental treatments at field locations (Poreč and Zadar).

Location	Growing Season	Sowing Date	First Application	Second Application	Fertilization—Urea	Third Application
Poreč	Spring Autumn	April 26th August 8th	May 30th September 5th	June 6th September 12th	June 6th September 12th	June 13th September 19th
Zadar	Spring Autumn	April 21st August 9th	May 25th September 6th	June 1st September 13th	June 1st September 13th	June 8th September 20th

2.3. Meteorological and Pedological Conditions of Study Sites

The location of Poreč, Croatia (N 45°13′18″; E 13°36′11″) is classified as Cfa according to Köppen, and has a moderately warm and humid climate [30]. The soil is eutric cambisol [31], previously used for barley production (*Hordeum vulgare* L.). During the growing season (April–October), the values of monthly air temperature ranged from 13.3 °C in April to 25.1 °C in July, and the average temperature during that period was 19.1 °C. The lowest mean daily air temperature was 8.9 °C (April 25), while the highest was 28.5 °C (July 21). The lowest precipitation was in July (13.9 mm). The rainiest months were September (156.7 mm) and October (174.1 mm), while the average precipitation was 91.9 mm (Poreč Meteorological Station, measurements 2016) (Figure 1).

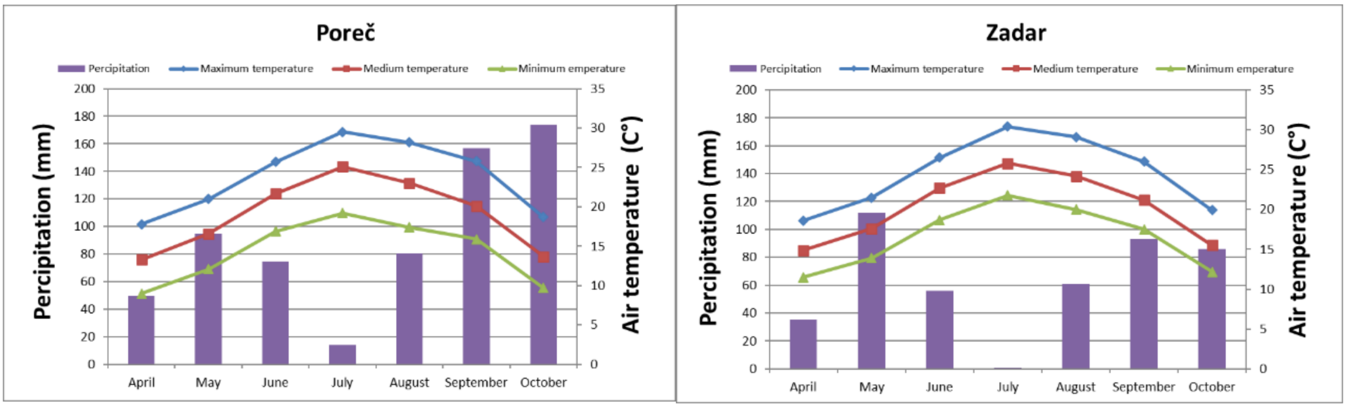

Figure 1. The minimum, maximum and average monthly air temperature and precipitation for Poreč (left) and Zadar (right), 2016.

At the second location (Zadar) and based on the climatic data from the nearest meteorological station located in Zadar, Croatia (N 44°9′24″; E 15°26′2″), the climate is considered Mediterranean, with hot dry summers and mild rainy winters [30]. The soil is calcic gleysol [31]. The experimental field was previously used for production of watermelon (*Citrullus lanatus* L.). According to the data from the Zadar meteorological station for 2016 (Figure 1), the values of average monthly air temperature in the study period (April–October) ranged from 14.9 °C in April to 25.8 °C in July, and the average temperature during the entire growing period was 20.3 °C. The lowest mean daily air temperature was 9.0 °C (April 25), while the highest was 28.3 °C (July 11). The average precipitation in the growing period was 63.4 mm, the lowest precipitation was in July (0.7 mm), and the rainiest month was May (118.8 mm).

2.4. Experimental Analysis

2.4.1. Soil Analyses

Soil samples were taken before sowing at both locations randomly from the experimental plots. Surface soil was sampled from the root development zone (0–30 cm), dried, sieved through a 2 mm sieve and prepared for physicochemical characterization according to the standard HRN ISO 11464:2009 (Table 2). Soil pH was determined using a 1:5 soil weight/water volume ratio, organic carbon (OC) was determined by sulfochromic oxidation HRN ISO 14235:2004, and available K_2O and P_2O_5 by the ammonium lactate method [32]. Total nitrogen was determined by the Kjeldahl method [33].

Table 2. Chemical analysis of soil at Poreč and Zadar locations.

Location	pH (H_20)	pH (KCl)	N (%)	P (mg 100 g^{-1})	K (mg 100 g^{-1})	Organic Matter (%)
Poreč	7.82	6.54	0.16	12.64	33.50	2.42
Zadar	8.05	7.15	0.13	8.03	15.00	2.24

2.4.2. Chemical Composition of Aqueous Nettle Extracts

The acidity (pH) of aqueous nettle extracts was determined according to the HRN ISO 10523:2012 method.

Electrical conductivity (EC) was measured using a Mettler Toledo MPC 227 (Mettler-Toledo Ltd., Columbus, OH, USA) conductivity meter with a cell constant control and temperature compensation device (25 °C) according to HRN EN 27888:2008.

Analysis of NO_3-N, NH_4-N and o-PO_4 was performed with a segmented flow system (SFA) and spectrometric detection using a Skalar San+Analyzer following standard procedures HRN EN ISO 13395:1998, HRN EN ISO 11732:2008 and HRN EN ISO 15681-2:2008, respectively. Determination of K was performed using an AAS PerkinElmer 3110 atomic emission spectrometer following the standard procedure HRN ISO 9964-3:1998.

Data quality control assurance for water analysis was implemented using a quality system accredited to the HRN EN ISO/IEC 17025:2007 standard, by participating in the international proficiency testing program, and by using internal reference samples.

The total concentrations of Fe were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) on a Vista MPX AX device (Vista MPX AX, Varian, Palo Alto, Calif.) after microwave-assisted digestion in a mixture of HCl, HNO_3 and H_2O_2 on a MARS Xpress instrument (CEM, Matthews, NC) in closed TFMs with automatic pressure and temperature regulation (HRN ISO 11466:2004).

The chemical composition of stinging nettle aqueous extracts, depending on the extraction duration time, is shown in Table 3.

Table 3. Chemical parameters of aqueous stinging nettle extracts.

Extraction Time	NO_3-N (mg L^{-1})	NH_4-N (mg L^{-1})	P (mg L^{-1})	K (mg L^{-1})	Fe (mg L^{-1})	pH	EC mS cm^{-1}
SE	127.75 ± 3.03	17.96 ± 0.99	17.34 ± 0.89	562.33 ± 18.74	0.06 ± 0.00	7.88 ± 0.02	3.35 ± 0.04
LE	0.63 ± 0.01	111.78 ± 15.45	18.94 ± 2.42	646.00 ± 4.97	0.21 ± 0.01	6.52 ± 0.04	5.42 ± 0.03

Results are expressed as mean values ± standard error; SE: short-term nettle extract; LE: long-term nettle extract.

2.4.3. Vegetative Characteristics and Yield of Green Beans

Vegetative measurements of the legume bean plant were carried out before the start of the bean harvest. A total of 10 plants were analyzed from each experimental plot, i.e., 40 plants per treatment. The stem height was measured with a ruler from the first node to the base of the top leaves. Stem diameter was measured with a digital caliper (CP33659-00, VWR, Monroeville, PA, USA) at the first node of the stem. The number of leaves larger than 1 cm was counted. The leaves were separated from the stem, and the leaf area was measured in the ImageJ program (Rasband, W.S., 1997–2016) on a Cannon LiDE 300 script (Cannon, Tokyo, Japan). Then, plants were dried in a dryer (Inkolab Ltd., Zagreb, Croatia) at 60 °C to a constant weight to determine the dry weight of leaves and stems.

The pod harvest was carried out successively in accordance with technological maturity for green bean consumption. For each calculation plot, the pods were weighed to determine the total yield (kg m^{-2}). In addition, at full harvest, a sample was taken from each plot, and the length and diameter of the pod were measured with a ruler using a digital caliper (CP33659-00, VWR, Monroeville, PA, USA).

2.4.4. Chemical Analysis of Green Bean Leaves

Nitrogen Content and Mineral Composition of Green Bean Leaves

Representative samples of green bean leaves were taken at flowering from each experimental plot, washed in distilled water and dried. Leaf samples were dried at 50 °C until constant mass, grinded and homogenized. The dry matter content was determined gravimetrically by drying at 105 °C until reaching a constant weight (HRN ISO 11465: 2004). The total concentrations of P, K and Fe were determined as described in Section 2.4.2. Total leaf N concentration was measured using the Kjeldahl digestion method (Kjeltec System 1026, Tecator, Höganas, Sweden) [33].

2.5. Statistical Analysis

The differences between the investigated factors for all the measured traits were statistically processed by analysis of variance (ANOVA). The results are expressed as mean ± standard error. For significant effects, the mean values were compared using the Tukey's Honest Significant Difference test (Tukey's HSD) at $p \leq 0.05$ level. Statistical analysis was performed using the GLM procedure of the computer program Statistica v. 13.3.0 (Tibco software, Palo Alto, CA, USA, 2017).

3. Results

3.1. The Effect of Nettle Extracts on Green Bean Vegetative Growth Parameters and Yield

The application of different fertilization treatments, and cultivation in different locations and growing seasons significantly influenced the morphological parameters of green beans, with the exception of leaf area (Table 4).

Table 4. Influence of fertilization treatment, location and sowing season on green bean vegetative growth parameters.

	Stem Height (cm)	Stem Diameter (mm)	Leaf Area (dm^2)	Pods Total Yield ($kg\ m^{-2}$)
Treatment (T)				
C	28.1 ± 10.04 [ab]	4.9 ± 0.87 [b]	84.8 ± 46.12	1.13 ± 0.78 [b]
U	31.0 ± 11.30 [a]	6.1 ± 0.96 [a]	152.7 ± 77.91	1.41 ± 0.43 [a]
SE1	28.2 ± 11.94 [ab]	5.3 ± 0.80 [b]	112.1 ± 57.52	1.14 ± 0.69 [b]
SE2	29.7 ± 10.05 [ab]	5.2 ± 1.09 [b]	99.5 ± 47.17	1.11 ± 0.69 [b]
SE3	25.2 ± 10.83 [b]	5.4 ± 0.90 [ab]	105.1 ± 76.21	1.12 ± 0.58 [b]
LE1	29.1 ± 9.34 [ab]	5.3 ± 0.92 [b]	103.4 ± 51.45	1.17 ± 0.68 [ab]
LE2	32.6 ± 10.60 [a]	5.5 ± 0.76 [ab]	120.0 ± 62.97	1.08 ± 0.58 [b]
LE3	30.6 ± 13.38 [ab]	5.2 ± 0.74 [b]	100.4 ± 49.55	1.16 ± 0.59 [ab]
Location (L)				
Poreč	33.2 ± 11.09 [a]	5.7 ± 0.88 [a]	132.1 ± 68.81 [a]	1.57 ± 0.60 [a]
Zadar	25.2 ± 9.37 [b]	5.0 ± 0.85 [b]	85.1 ± 38.03 [b]	0.77 ± 0.25 [b]
Season (S)				
Spring (SP)	20.7 ± 6.81 [b]	5.2 ± 0.82 [b]	84.9 ± 60.98 [b]	1.39 ± 0.75 [a]
Autumn (AU)	37.2 ± 7.80 [a]	5.6 ± 0.98 [a]	134.8 ± 50.42 [a]	0.94 ± 0.27 [b]
ANOVA				
T	**	***	NS	**
L	***	**	***	***
S	***	**	***	***
T × L	NS	NS	NS	NS
L × S	NS	**	***	***
T × S	NS	NS	NS	NS
T × L × S	NS	NS	NS	**

Results are expressed as mean ± standard error. Treatments (T): Urea (U); short-term nettle extract (SE); long-term nettle extract (LE); number of nettle extract applications on vegetation, one, two or three (SE1, SE2, SE3, LE1, LE2, LE3) and control (C). Location (L). Season (S): spring (SP), autumn (AU). Data were subject to ANOVA. Analyses of variance between factors: non-significant (NS) or significant at reported p-value (*** for $p \leq 0.001$ and ** for $p \leq 0.01$). Means with the same letter within the column are not significantly different at $p \leq 0.05$ by Tukey's Honest Significant Difference (HSD) test.

The effect of different fertilization treatments as well as control cultivation on the stem height did not differ from each other, except for the SE3 treatment. Plants treated with LE2 resulted in statistically equal stem height values to plants treated with urea. (Table 4). Furthermore, bean stems were taller in Poreč compared with Zadar, and in autumn compared with the spring growing season. The interaction of the investigated factors had no significant effect on plant height (Table 4).

Stem diameter was larger in plants treated with urea compared with the unfertilized control, SE1, SE2, LE1 and LE3 (Table 4). Significantly wider stems were observed in Poreč and in the autumn season. A significant interaction of location and growing season on stem diameter was observed, and the widest stems were found for plants grown in Poreč in the autumn period, compared with other location–season combinations (Figure 2A).

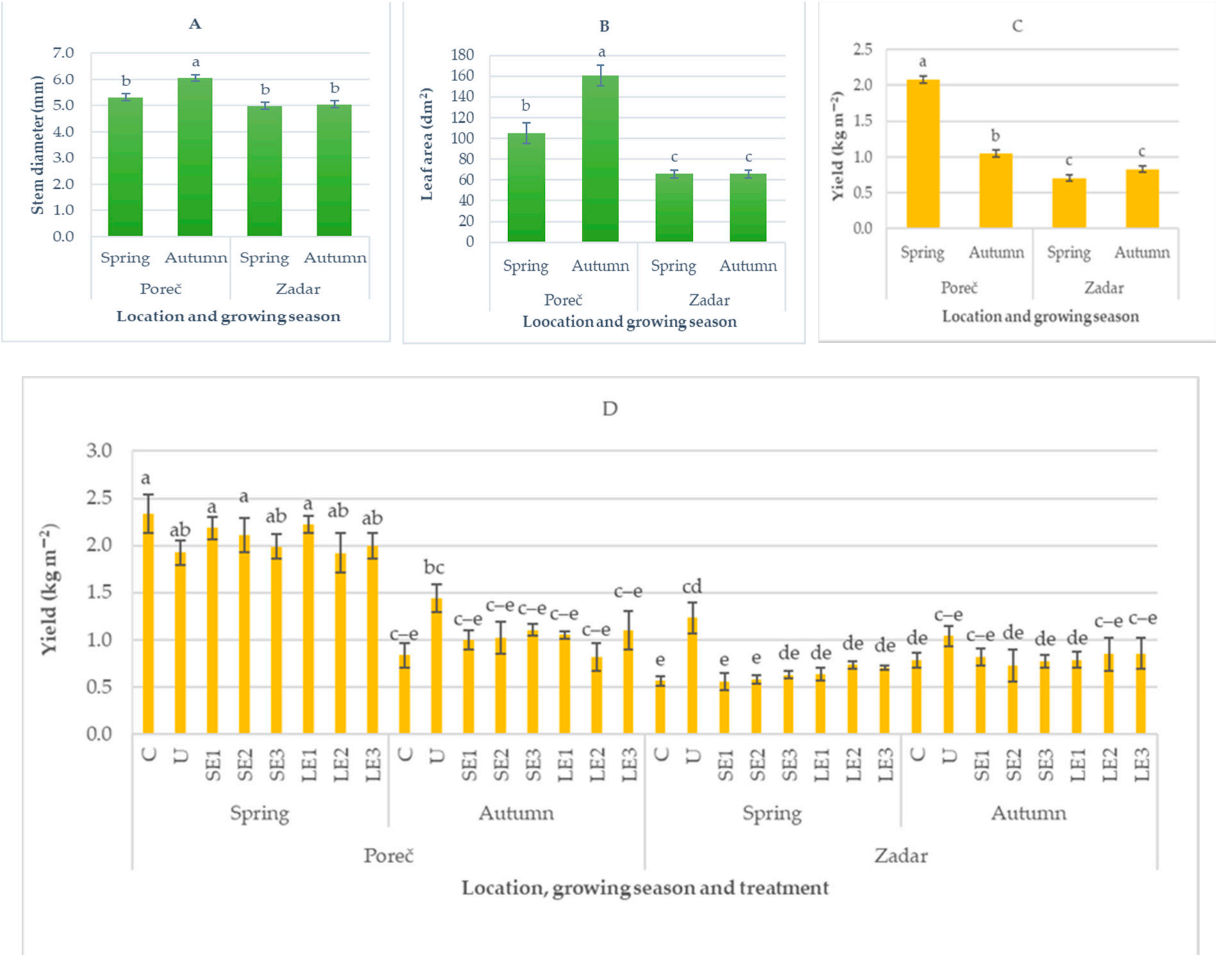

Figure 2. Influence of the interaction of three factors, treatment (T), location (L) and sowing season (S), on green bean vegetative growth parameters: (**A**) steam diameter, L × S; (**B**) leaf area, L × S; (**C**) yield, L × S; (**D**) yield, T × L × S. Results are expressed as mean ± standard error. Means with the same letter within the column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

The leaf area of green beans was not affected by the fertilization treatments (Table 4). However, the leaf area of green beans was larger in Poreč compared with Zadar, and in plants grown in autumn compared with the spring season (Table 4). The interaction of L × S had a significant effect on leaf area (Table 4). The largest leaf area was found in bean plants grown in Poreč in the autumn season, while the smallest leaf area was measured in the spring season in Zadar (Figure 2B).

A higher pod yield was found in green beans fertilized with urea compared with unfertilized plants and SE1, SE2, SE3, and LE2 treatments (Table 4). The yield was higher at the Poreč location compared with Zadar and in the spring growing season compared with autumn, respectively (Table 4). Significant interactions were found for L × S and T × L × S combinations (Table 4).

The highest yield was found at the Poreč location in spring. A significant interaction among treatments, location and time season (T × L × S) has shown that the highest green bean yields were obtained at the Poreč location in the spring season compared with all other combinations (Figure 2D). Urea treatment at Poreč in the spring season was similar to SE3, LE2, and LE3 treatments in the spring season at the same location (Figure 2D). The green bean yields obtained at Poreč and Zadar in the autumn season were not affected by the applied fertilization treatments; however, in the spring season at Zadar, urea increased pod yields compared with C, SE1 and SE2 (Figure 2D).

3.2. The Effect of Nettle Extracts on the Total Nitrogen and Mineral Composition of the Green Bean Leaf

Table 5 shows the values of total nitrogen and mineral composition (P, K, and Fe) of green bean leaves. The application of urea increased total N in green bean leaves compared with other fertilization treatments; however, the interactive effect of the tested factors was significant for the L × S and T × L × S combinations.

Table 5. Influence of treatment, location and sowing season on total nitrogen and mineral composition of green bean leaf.

	Total Nitrogen (% N DM)	P ($g\ kg^{-1}$ DM)	K ($g\ kg^{-1}$ DM)	Fe ($g\ kg^{-1}$ DM)
Treatment (T)				
C	3.03 ± 0.54 [b]	2.98 ± 0.54	11.58 ± 2.33	212.3 ± 134.72 [ab]
U	3.52 ± 0.53 [a]	2.72 ± 0.87	11.68 ± 3.13	161.15 ± 45.98 [b]
SE1	3.02 ± 0.54 [b]	2.83 ± 0.76	10.78 ± 2.60	207.37 ± 121.01 [ab]
SE2	3.02 ± 0.59 [b]	2.87 ± 0.72	11.01 ± 2.68	241.71 ± 97.13 [ab]
SE3	2.96 ± 0.51 [b]	2.77 ± 0.67	11.56 ± 3.10	256.58 ± 239.46 [ab]
LE1	3.13 ± 0.39 [b]	2.85 ± 0.81	12.28 ± 3.11	195.39 ± 91.88 [ab]
LE2	2.84 ± 0.54 [b]	2.79 ± 0.80	11.32 ± 2.04	209.73 ± 144.15 [ab]
LE3	2.91 ± 0.54 [b]	2.75 ± 0.64	11.20 ± 2.74	319.16 ± 167.01 [a]
Location (L)				
Poreč	3.31 ± 0.38	3.22 ± 0.35 [a]	13.40 ± 1.73 [a]	160.82 ± 81.46 [b]
Zadar	2.83 ± 0.57	2.48 ± 0.82 [b]	9.73 ± 2.17 [b]	280.69 ± 162.67 [a]
Season (S)				
Spring (SP)	2.75 ± 0.49	2.55 ± 0.29	11.87 ± 2.34	184.42 ± 113.11 [b]
Autumn (AU)	3.41 ± 0.35	3.13 ± 0.91	10.92 ± 2.99	271.27 ± 161.45 [a]
ANOVA				
T	***	NS	NS	**
L	NS	***	***	***
S	NS	***	NS	***
T × L	NS	**	NS	NS
L × S	***	***	***	NS
T × S	NS	NS	NS	NS
T × L × S	**	NS	NS	NS

Results are expressed as mean ± standard error. Treatments (T): Urea (U); short-term nettle extract (SE); long-term nettle extract (LE); number of nettle extract applications on vegetation, one, two or three (SE1, SE2, SE3, LE1, LE2, LE3), and control (C). Location (L). Season (S): spring (SP), autumn (AU). DM, dry matter. Data were subject to ANOVA. Analyses of variance between factors: non-significant (NS) or significant at reported *p*-value (*** for $p \leq 0.001$ and ** for $p \leq 0.01$). Means with the same letter within the column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

At the Poreč location, total N in leaves was higher in the autumn season compared with the spring season, and the same trend was observed in Zadar (Figure 3A). Three-way ANOVA generally showed that application of urea positively affected leaf N content in Poreč in spring and autumn, as well as in Zadar in autumn, although the effect was not always significant compared with other fertilization treatments (Figure 3B).

No effect of fertilization treatments was found for P and K content in green bean leaves (Table 5). The three applications of LE nettle-based extract (LE3) had a positive effect on Fe content in leaves of green beans, and it was higher compared with urea application.

While the P and K were higher at the Poreč location, the influence of season was observed with a higher content of Fe in autumn compared with the spring season.

The interaction of location and season (L × S) was significant for the content of P at the Poreč location in the autumn season (Figure 3D).

The interaction of location with all the treatments (L × T) affected the P content in Poreč, while no effect of the fertilization treatments on the leaf P content in Zadar was found, except for the unfertilized control (Figure 3C).

Similarly to N and P content in bean leaves, the interaction between factors L × S was significant for K content as well (Table 5). The highest K content in bean leaves was found at the Poreč location in both growing seasons, while the lowest amount of K was in plants grown in autumn in Zadar (Figure 3E).

The content of Fe was affected by all tested factors, but no interaction among them was observed (Table 5).

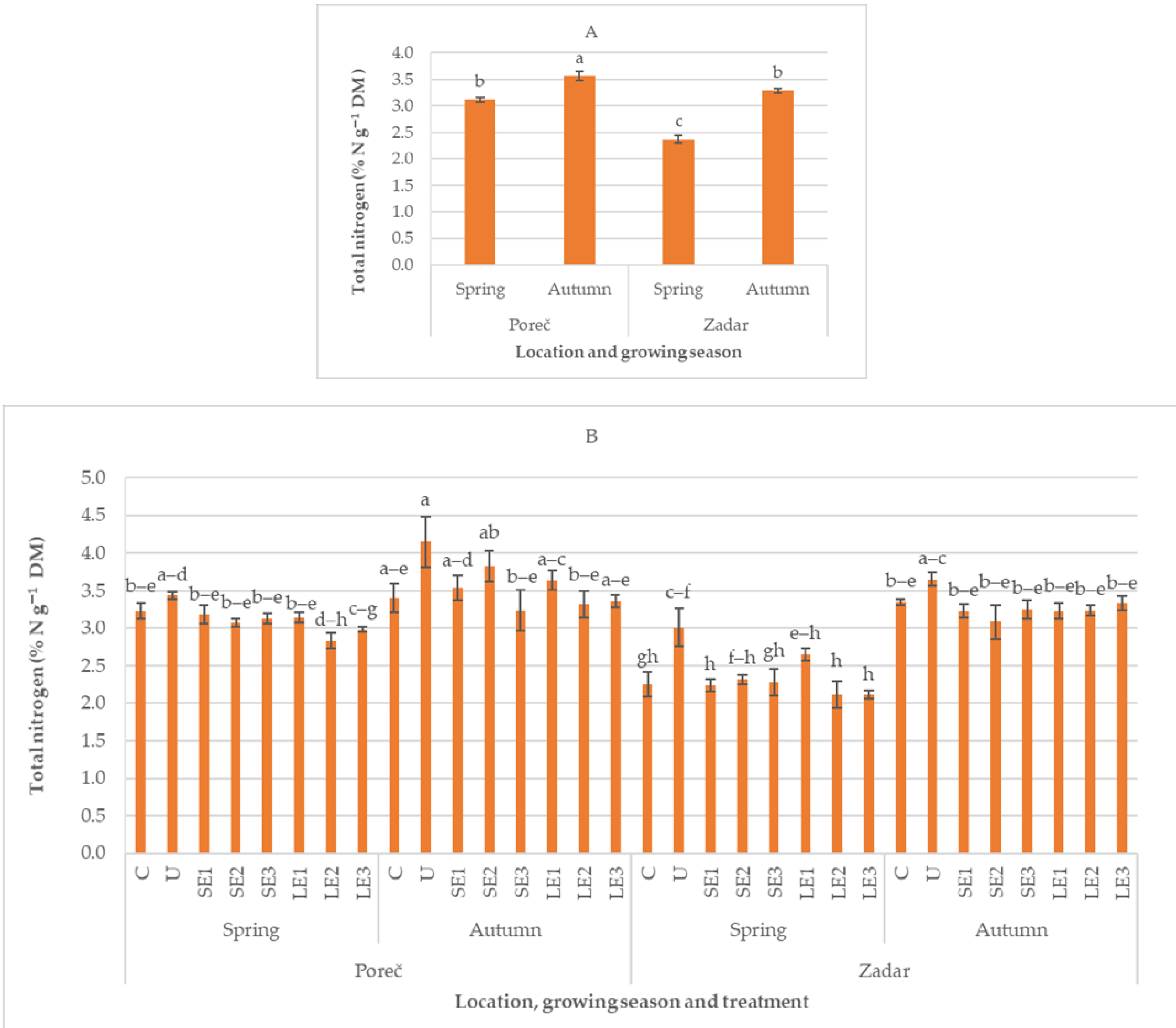

Figure 3. *Cont.*

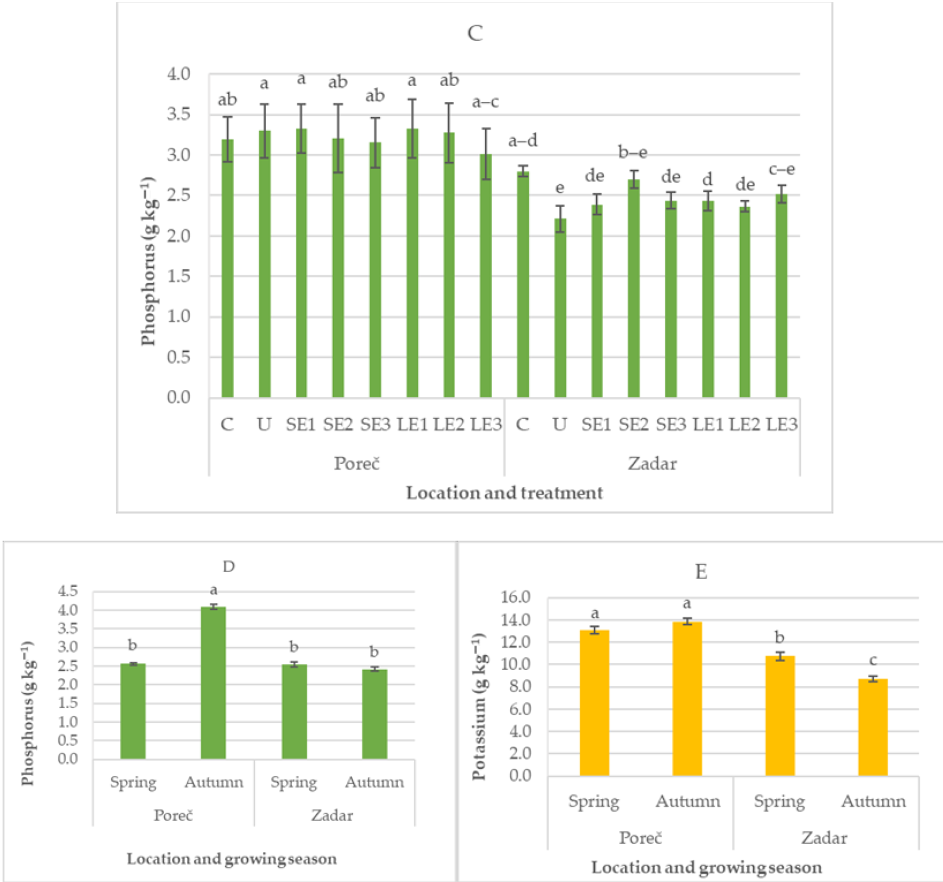

Figure 3. Influence of the interaction of three factors, treatment (T), location (L) and sowing season (S), on total nitrogen and mineral composition of green bean leaf: (**A**) total nitrogen, L × S; (**B**) total nitrogen, T × L × S; (**C**) phosphorus, T × L; (**D**) phosphorus, L × S; (**E**) potassium, L × S. Results are expressed as mean ± standard error. Results are expressed as mean ± standard error. Means with the same letter within the column are not significantly different at $p \leq 0.05$ by Tukey's HSD test.

4. Discussion

Studies have already proved that stinging nettle is a source of many vitamins, minerals, amino acids and carbohydrates, which are also present in its extracts, and that this seems to lead to increased growth in nettle-treated plants [34,35]. For this reason, chemical analysis of two nettle extracts (SE and LE) was performed before their application onto green bean plants. Of particular interest is N, which stimulates plant growth and increases yield, but its effect is variable, depending on the doses and forms applied [35]. In this study, it was shown that the SE nettle extract was rich in nitrogen in the NO_3 form, while the LE extract had more nitrogen in the NH_4 form, similar to studies by Peterson and Jensen [25,26]. The presence of phosphorus and potassium was higher in LE compared with SE, in contrast to the results of Peterson and Jensen [25,26]. Previous research has proved that aqueous nettle extracts are rich in iron [24], and the presence of this microelement, in this study, was 2.5 times higher in LE than in SE and generally much lower than the results of Peterson and Jensen [24]. The aqueous nettle extract (nettle broth and tea) analyzed by Rivera et al. [16] showed higher levels of Ca, Mg, P and N compared with the extracts described here. In this research, the pH and EC values of LE were similar to the results of Peterson and Jensen [25], as well as chemical analyses of nettle tea and nettle soup by Rivera et al. [16].

Species of the *Fabaceae* family are generally not considered to require intensive fertilization due to the possibility of nitrogen fixation from the air via symbiotic bacteria [6]. Some authors recommend fertilization with urea at the 3–4 leaf stage [36]. In this experiment, the application of different fertilizations, at different locations and in different growing seasons, significantly influenced the stem height, stem diameter, yield, total nitrogen and iron content of the bean plants. Applied fertilization treatments U and LE2 had similar effects on stem height and diameter. Urea treatment showed significantly higher yield, vegetative growth parameters (stem height and diameter) and total nitrogen compared with other fertilization treatments. This was expected, considering that urea as a nitrogenous fertilizer contains 46% nitrogen in the amide form [37]. Accordingly, studies of nitrogen fertilization with mineral fertilizers or their combination with a biofertilizer resulted in higher yield, biomass and nutritional value of snap bean crops [38].

In the present research, aqueous nettle extracts showed a positive effect on some morphological properties. LE2 had the same effect on stem height as U, both resulting in the highest plants. Stem diameter was the largest with the U treatment, but did not differ significantly from SE3 and LE2. Total yield was the highest after U application, but once again, did not differ significantly from LE1 and LE3. The presented results suggest that aqueous nettle extracts, especially long-term extracts, could be effective as organic nitrogen fertilizers.

The results obtained in the present study are consistent with research by Peterson and Jensen [25,26] but opposite to the results of Rivera et al. [16]. Studies by Rivera et al. [16] showed that the use of aqueous nettle extracts as fertilizers three times a week during the whole vegetation period had no effect on lettuce leaf area, number of leaves, fresh weight or dry matter in relation to control plants treated with water. In contrast, Peterson and Jensen [25,26] showed a positive effect of aqueous nettle extract on barley and tomato growth. Their study showed a 20% increase in plant growth and a doubling increase in the root length and fresh weight of barley [25]. A study was conducted two years later with aqueous nettle extract on tomato, and showed a 60% increase in dry shoot mass and a 20% increase in dry root mass [26]. Godlewska et al. [27] proved the effectiveness of aqueous nettle leaf and root extracts in stimulating the growth of white head cabbage seedlings. Kim et al. [9] obtained similar results, with increased roots and shoots of lettuce, sweet corn and soybean plants fertilized with aerated compost tea (rice straw, vermicompost and Hinoki cypress bark), and Dozet et al. [39] demonstrated higher yields of soybean with the foliar application of nettle extract compared with the control variant. Aqueous nettle extract also significantly affected lettuce fresh weight and root length, increasing growth [40]. In contrast, studies of lettuce treated with aqueous nettle tea and soup [16] did not show significant vegetative values compared with control water treatment.

In this study, the influence of the treatments on total nitrogen was not so different in the aqueous nettle extract treatments and the control treatment. Although nettle extract is rich in nitrogen, the analysis of total N in the green bean leaves after nettle treatment was significantly lower compared with the urea -fertilized variants in our research, which is also confirmed by Hartz, Smith and Gaskell [41], inferring that organic fertilizers had lower N availability compared with mineral fertilizers. In this research, the LE nettle extract had a significant amount of nitrogen in the ammonium form (NH_4), and the SE nettle extract with the nitrate form of nitrogen (NO_3); however, this did not affect the bean plants treated with LE or SE-1, -2, or -3, regardless of the number of applications. Studies by Peterson and Jensen [26] showed that lettuce plants treated with aqueous nettle extract had 10% more total N than those treated with a nutrient solution with the same mineral composition, and also the proportion of inorganic and organic nitrogen was higher by 50% compared with the control; however, the intake of P and K was lower. In this study, only bean leaf iron content was significantly higher in the plants treated with LE3 nettle extract compared with the plants treated with urea. Bean plants treated with other SE and LE nettle extracts showed higher iron content compared with urea treatment but they did not differ significantly. This iron content increase when nettle extract is applied may be related to the presence of this microelement in the extract and/or due to the dilution effect (where the same amount of Fe is diluted in more leaf biomass) [42]. At the Zadar location, the iron content in green bean plants was higher than in Poreč, and the soil had an alkaline soil reaction. In contrast, some similar studies have shown that plant uptake of iron is less in alkaline soils [43,44]. According to Peterson and Jensen [24,25], nettle extract raises the pH in the soil, which causes the plant to absorb less iron. Nettle extract contains a significant amount of organic matter and organically chelated Fe [24].

When comparing the two nettle extracts (SE and LE), no significant difference was found between long and short treatment in stem height, stem diameter, yield, total nitrogen and iron content. Since nitrogen is flushed out, the plant receives it better at intervals than with a single fertilization. For this reason, the number of treatments was researched in this study (1, 2, 3). Although there is a relative but not statistical difference between the LE2 treatment and LE3, LE2 enhanced the plant height by 6.5% and caused wider stem diameter by 5.7%, which confirms that the addition of nitrogen twice during intensive plant growth gave better results compared with fields treated three times before legume flowering.

Regardless of the treatment, the yield and all the vegetative parameters were significantly higher at the Poreč location, probably due to the initially higher parameters of soil analysis. The green bean plants grown in Poreč had a higher value of phosphorus, which is correlated with the initial higher values in the soil at the beginning of the experiment. The available P in the soil depends on the acidity of the soil and the soil temperature, and consequently where the values of soil temperature and acidity are lower, phosphorus is less available [45]. Furthermore, the climatic factor at the location of Poreč being classified as Cfa according to Köppen, meaning a moderately warm and humid climate [30], had a significant impact on all morphological parameters as well as mineral composition (P, K) in relation to the other experimental location in Zadar.

When considering the two growing seasons (spring and autumn), vegetative parameters were significantly higher in autumn (plant height 79%, stem diameter 8%, leaf area 58%) compared with the spring growing season probably due to higher rainfall in autumn compared with spring (from May to October). However, the yield was 34% lower in autumn compared with the spring growing season, which was expected because in October higher amounts of rainfall were recorded as well as a drop in temperature, which shortened the vegetation.

5. Conclusions

In the presented paper, two different aqueous extracts of stinging nettle and their influence as fertilizers on green bean cultivation were studied with respect to the yield, vegetative parameters (stem height and diameter, and leaf area) and chemical parameters in leaves (total N, P, and Fe). Foliar fertilization with the SE extract was found to be almost equivalent to fertilization with the LE extract poured onto the soil regarding the yield and vegetative parameters such as plant height and diameter. The influence of aqueous nettle fertilization proved to be significant with respect to two pedologically and climatically different growing sites. The results of this field study contribute significantly to new knowledge on the effect of aqueous nettle extract as a plant-based fertilizer. Of particular importance also is the indication of the effect of SE as a fertilizer, whose application and preparation is much faster under field conditions. In view of the results obtained, there is a need for further field trials with a larger amount of nettle extract on legumes and other vegetable species. Although nettle fertilization with aqueous nettle extract cannot compare with mineral fertilization in terms of the yield realized, the application of plant-based fertilizers in combination with mineral fertilizers could maintain high yields and reduce water pollution as a result of their use in sustainable agriculture in the future.

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