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Foliar Application of Urea and Amino Acids Regulates Growth, Photosynthesis, Pigments, Antioxidant Activity, and the Essential Oil Content and Composition of Basil (*Ocimum basilicum* L.)

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Abstract: Basil (*Ocimum basilicum* L.) is a prominent medicinal and aromatic plant, widely recognized for its bioactive compounds and substantial economic value across the pharmaceutical, culinary, and industrial sectors. In light of increasing global demand and environmental challenges, this study explores novel approaches to enhance its sustainable production and improve its quality. Urea is the most common form of nitrogen (N) for foliar application due to its quick absorption, affordability, high solubility, as well as relatively low cost per N unit. Amino acids are an organic form of N and play a role in plant protein structure, stress tolerance, and the biosynthesis of secondary metabolites. This research aimed to evaluate the effects of urea (0, 1, and 2 g L^{-1}) and an amino acid-based biostimulant (AAB) (0, 4, and 8 mg L^{-1}), applied foliarly, on the growth, photosynthesis, pigments, antioxidant activity, and essential oil production of basil (*Ocimum basilicum* L.). The best results in terms of leaf number, area, and fresh and dry weight were observed with the combination of 2 g L^{-1} urea and 8 mg L^{-1} AAB. The growth enhancement due to this treatment may be attributed to stimulatory effects on photosynthesis and N content. Chlorophyll, carotenoids, anthocyanins, photosynthesis, stomatal conductance, total phenols, and total flavonoids increased with urea application up to 1 g L^{-1}. Additionally, AAB application up to 8 mg L^{-1} increased total chlorophyll, carotenoid, total phenols, and total flavonoids, while photosynthesis and anthocyanin content increased with 4 mg L^{-1} AAB. Although urea did not significantly affect essential oil content and yield, AAB application increased both. Finally, the combination of 1 g L^{-1} urea and 8 mg L^{-1} AAB had the most effective impact on improving content and yield of essential oil, total phenol, flavonoid, anthocyanin, and antioxidant activity, with a relatively high percentage of estragole.

Keywords: amino acid-based biostimulant; foliar application; basil; nitrogen; urea

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1. Introduction

Herbs and aromatic plants play a significant commercial role due to their secondary metabolite compounds and phenolics with human-health properties [1,2]. Basil (*Ocimum basilicum* L.), belonging to the Lamiaceae family, is an edible medicinal plant known for its great mineral and micronutrient content, as well as various biological activities [3]. It is commonly used in fresh and dried forms to enhance the flavor and aroma of food products such as tomato paste, ketchup, sauce, and soup [4]. Basil is also cultivated in home gardens as a medicinal plant and a vegetable. Basil extract has been found to have potential antidiabetic effects [5] and exhibits antimicrobial [6], antibacterial [7], anti-spasmodic [8], antioxidant, and anti-inflammatory [9] properties. Traditionally, it has been used to treat various ailments such as headaches, common colds, skin abnormalities, and fever [10]. The positive properties of basil have been associated with the chemical profile of its essential oil, which mainly contains linalool, eugenol, and estragole, among other molecules [11,12].

However, the chemical profile of basil essential oil seems to be dependent on several factors, including nutritional and growth conditions, biostimulant treatments, agricultural practices, and processing techniques [13–18]. These factors are critical in shaping the chemical profile and therapeutic potential of the essential oil derived from basil.

Nitrogen (N) is a pivotal nutrient for plant development because of its involvement in various essential processes such as the production of chlorophyll, protein and nucleic acid biosynthesis, and enzyme activity [19]. An N deficiency negatively affects plants' capacity to perceive light and impairs the activity of photosystem II, leading to reduced plant growth and yield [20,21]. Urea is a commonly used form of nitrogen for foliar treatments to sustain plant nutrition due to its rapid absorption, high solubility, and relatively low cost per N unit [22]. The small molecular size of urea allows better penetration through the cuticular membrane of leaves compared to other N fertilizers [23,24]. A study showed that foliar application of urea decreased nitrate levels and improved the quality of edible parts in vegetables such as lettuce leaves [25]. In grapes, foliar application of urea has been shown to enhance the aromatic properties of grape juice [26,27]. However, there have been limited studies on the effect of urea fertilizer on the essential oil from medicinal and aromatic plants. Souri et al. [28] demonstrated that the application of pelleted urea fertilizer improved various physiological and plant growth parameters, such as chlorophyll level, plant height, leaf area, shoot fresh weight, pot yield, and the concentration of potassium (K) and N in basil plants. This suggests that evaluating different forms of N as the source of this essential element is an interesting research area concerning controlling or stimulating secondary metabolism in plants.

Amino acids are molecules involved in crucial physiological processes, such as osmotic regulation and the activity of plant growth regulators like auxin and gibberellin, detoxification of toxins and heavy metals, and improving plant tolerance to many different environmental stressors [29–31]. Likewise, amino acids induce nutrient uptake and translocation, participating in the metabolism and biosynthesis of vitamins [32]. The application of amino acids also stimulates the biosynthesis of secondary metabolites and antioxidant enzymes [33,34]. Therefore, an amino acid-based biostimulant (AAB) has been used for improving plant growth, development, and crop yield. It has been reported that AAB application enhances chlorophyll content, contributes to protein synthesis and storage, and increases plant tolerance to abiotic stresses [35]. Some studies have shown that the foliar application of AABs can enhance iceberg lettuce growth, mineral content (N, K, phosphorus (P), and magnesium (Mg)), bioactive compounds (such as phenols and flavonoids), and antioxidant capacity [36]. Some specific amino acids, such as phenylalanine and tyrosine, are involved in the shikimate/phenylpropanoid pathway, a crucial biosynthetic pathway for secondary compounds in plants [37]. Due to this plethora of beneficial effects, amino acids are incorporated as essential components in biostimulants [38]. Therefore, in this context, the use of AABs can be considered a valuable and environmentally friendly approach for improving herb nutritional and pharmaceutical properties.

Foliar application presents a promising strategy for optimizing crop yield and quality, particularly in crops like basil, where effective nutrient management is essential for robust growth and the production of bioactive compounds [39]. Foliar application of urea and amino acid-based biostimulants has gained considerable attention for its potential to improve plant growth, nutrient uptake, and overall crop productivity [40]. Urea, a readily accessible nitrogen source, is commonly applied through foliar sprays to address nutrient deficiencies, particularly in environments with limited soil fertility [41]. Amino acid-based biostimulants, on the other hand, have been shown to enhance plant tolerance to abiotic stresses, stimulate metabolic activity, and improve nutrient use efficiency [42]. When used together, these substances can have synergistic effects, boosting both physiological and biochemical plant responses [43].

However, there is limited information on the impact of different N forms in combination with AABs on plant growth and the functional compounds in basil. Therefore, this study aimed to determine the influence of foliar application of urea and AABs on

the growth, morphological characteristics, N accumulation, biochemical responses, and essential oil composition of basil. This research was conducted in a greenhouse, allowing for the precise evaluation of the effect on growth parameters, morphological characteristics, and the accumulation of essential oil constituents in basil (*O. basilicum*). In addition to the above objectives, this research aspires to expand the knowledge on sustainable cultivation practices and high-quality basil production. By optimizing nutrient management through foliar applications of urea and amino acid-based biostimulants, the findings aim to inform agricultural practices that balance productivity with environmental sustainability. Additionally, this study provides valuable insights into enhancing the bioactive compound profile of basil, which could have important implications for the pharmaceutical, culinary, and industrial sectors.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

The study was carried out in the research greenhouse of the Faculty of Agriculture, Lorestan University, Khorramabad, Iran (latitude of 33.43° N, a longitude of 48.26° E, and an altitude of approximately 1170 m above sea level). The experiment was performed from October 2021 to February 2022. Basil (*O. basilicum*) seeds of the Purple Persian basil variety, commonly found in Iran, were obtained from Pakan Bazr Co. in Isfahan, Iran. The plant materials were obtained under the supervision and permission of Lorestan University and according to institutional as well as national guidelines, with all authors complying with all local and national guidelines. Each 5-litre pot was filled with a substrate mixture consisting of loam soil, sand, and cow manure in a ratio of 2:2:1. Six pots were included in each replication, with 162 pots in total. Chemical and physical characteristics of the substrate are detailed in Table 1. Each pot was fertilized with 100 mg of urea per kg of the substrate before sowing the basil seeds to provide the soil with proper N content. Five basil seeds were sown in each pot. After germination and the establishment of seedlings, one plant was maintained in each pot, eliminating the excess seedlings. Pots were maintained in a greenhouse environment with controlled conditions (temperature range of 25–27 °C/15–17 °C during the day/night). The light intensity was set at 500 ± 50 μmol m^{-2} s^{-1} and 65–70% of relative humidity.

Table 1. Some physical and chemical characteristics of the soil.

	pH	EC	Soil Texture	Organic Matter	Carbon Organic	Mg^{+2}	Na^{+}	Fe^{+2}	Cao	Total Porosity	N	P
		(ms/cm)		(%)	(%)	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(%)	(%)	(%)	(mg kg^{-1})
Soil	6.13	1.517	Sandy loam-Clay	1.748	1.04	1437	78.2	5.18	13.75	0.050	0.15	81.6

2.2. Treatments

The basil (*O. basilicum*) plants were subjected to three different concentrations of urea (0, 1, and 2 g L^{-1}) and three different concentrations of AAB (0, 4, and 8 mg L^{-1}). The optimal concentration range for the foliar spray was determined through a thorough literature review and a preliminary experimental study. Urea granular fertilizer with a nitrogen content of 46% was obtained from Razi Company in Iran. The AAB used in the experiment was the commercial product Isabion, manufactured by Syngenta Co. in Basel, Switzerland. Isabion contains 11.7% total nitrogen, 11.5% free amino acids, and 62.5% total amino acids. Foliar spraying of the urea and AAB treatments was performed manually early in the morning. The first application of foliar spraying was carried out when the plants reached four true leaves. Three subsequent foliar applications were performed at 20-day intervals throughout the experiment. Regular watering of the plants was carried out approximately every five days to ensure adequate moisture levels in the pots. Data collection was conducted 10 days after the final foliar spraying, allowing sufficient time for the treatments to exert their effects on the plant.

2.3. Plant Growth Analysis

At the end of the experiment, which was conducted for a total of 110 days after seed sowing, various growth traits of the basil plants were analyzed. These included measurements of the number of leaves per plant, plant height, and leaf area. Four plants were randomly selected for each replication and harvested by cutting them at a height of 5 cm from the soil surface to determine the fresh weight of the aerial parts of the plants, which was measured using a digital scale with a precision of 0.01 g. In addition, the samples were placed in an oven (at 50 °C) and weighed after 72 h to determine the dry weight. Then the leaves were separated from the stems, and the dry weight of the leaves was recorded separately.

2.4. Nitrogen and Nitrate of Leaf

Total nitrogen content was determined using the Kjeldahl method [44]. Nitrate content was determined using the salicylic acid method as described by Cataldo et al. [45]. For this, dried samples were powdered and sieved through a 40-mesh sieve. Then, 0.1 g of the weighed sample was added to distilled water (10 mL) and kept at 45 °C for 1 h. The liquid was filtered using filter paper. Next, the filtered extract (0.2 mL) was mixed with 5% salicylic acid in concentrated sulfuric acid (0.8 mL). After 20 min, 19 mL of NaOH (2 N) was added to the mixture. After cooling, the absorbance of the samples was recorded at 410 nm using a spectrophotometer (METASH model UV-5100, Shanghai Metash Instruments Co., Ltd., Shanghai, China).

2.5. Gas-Exchange and Plant Pigments Determinations

Photosynthesis, stomatal conductance, and transpiration were determined as gas-exchange on fully expanded leaves using an LCA4 (model LCA4, ADC Bioscientific, Ltd. Hoddesdon, UK). Before harvesting the plants at the end of the experiment, five fully expanded leaves from each treatment were randomly selected. Each leaf (attached to the plant) was placed in the chamber for 60 s, and the measurements were conducted on a clear sunny day between 09:00 to 11:00 A.M. The chamber temperature during the measurements ranged from 25–28 °C and the intensity of light was 400 µmol m^{-2} s^{-1}. The leaf chlorophyll and carotenoid content were measured according to the Lichtenthaler method [46]. Samples of 0.1 g of fresh leaves were weighed and ground in a mortar using liquid nitrogen; then the samples were extracted with 10 mL of acetone and centrifuged at 4000× *g* for 15 min. The absorbance of the supernatant solution was read using a spectrophotometer (METASH model UV-5100, Shanghai Metash Instruments Co., Ltd., Shanghai, China) at wavelengths of 470 nm, 662 nm, and 645 nm. The quantity of chlorophyll a (Chla), chlorophyll b (Chlb), carotenoids (Car), and total chlorophyll (Chla + b) were determined and expressed in mg g^{-1} FW.

2.6. Essential Oil Extraction

To extract the essential oil, 20 g of the dried plant was finely ground and heated in a Clevenger flask for 3 h. The essential oil obtained was dehydrated using anhydrous sodium sulfate, then stored in a freezer at −20 °C for subsequent analysis. The essential oil percentage was calculated based on the weight acquired from 100 g of plant material (*w*/*w*). The yield of essential oil was determined by multiplying the essential oil percentage by the dry weight of the aerial parts, and then dividing the result by 100 [47].

2.7. GC/FID and GC/MS Analysis

The essential oil was analyzed using gas chromatography with flame ionization detector (GC/FID) (Agilent Technologies, Inc., Santa Clara, CA, USA). Separation was done using an HP-5 column with specific dimensions (length of 30 m, inner diameter of 0.1 mm, film thickness of 0.40 µm). Helium was used as agas carrier, and both the detector and injector were set to 285 °C. The flow rate of gas carrier was maintained at 1.1 mL/min with 1:60 split ratio. The oven temperature started at 60 °C for 5 min and then enhanced to 280 °C at 7 °C/min rate. The analysis was performed in triplicate.

For further characterization, a Varian 3400 gas chromatography/mass spectrometry (GC/MS) system with HP-5 silica column was employed (Agilent Technologies, Inc., Santa Clara, CA, USA). The oven temperature was set the same as during the GC-FID analysis. Helium was used as the gas carrier with 1.1 mL/min flow rate, and the split ratio was set to 1:60. The transfer line temperature was 260 °C. Flame ionization detection was performed with specific settings, including a linear velocity of 31.5 cm/s and a voltage of 70 eV, covering a mass range of 40–450 amu. The identification of the constituents of the essential oil was accomplished by comparing their mass spectra with those available in Wiley and NIST05 libraries, as well as comparing their retention indices (RI) with those reported in the Adams book [48,49].

2.8. Antioxidant-Related Parameters

The antioxidant capacity was measured using the FRAP method, as described by Benzie and Strain [50]. A solution of iron chloride ($FeCl_3.6H_2O$) (20 mM, 2.5 mL) and acetate buffer (300 mM, 25 mL, pH of 3.6) was prepared. The solution was shaken for 30 s. Then, 150 µL of the plant extract was mixed with 2850 µL of fresh FRAP reagent and vortexed. After incubating for 15 min at room temperature, the absorbance was read at 593 nm. The antioxidant capacity of samples was expressed in mM using a $FeSO_4.7H_2O$ calibration curve.

For total phenol content, 1 g of the dried plant sample was ground in 2 mL of 80% methanol using a mortar. The mixture was then shaken at room temperature for 72 h. Afterward, it was filtered through filter paper, and 15% sodium carbonate solution and Folin–Ciocalteu reagent were added. The absorbance of the solutions was recorded at 755 nm using a UV-vis spectrophotometer (METASH model UV-5100, Shanghai Metash Instruments Co., Ltd., Shanghai, China). Total phenol was expressed as mg of gallic acid equivalents g^{-1} of dry weight (DW) [51].

Total flavonoids were determined using the calorimetric method. The plant extract, obtained as described above, was added to sodium carbonate (0.5 mL) and incubated for 5 min. Subsequently, 0.5 mL of aluminium chloride (10%) was added to the homogenate, followed by another 6-min incubation. After adding 2 mL of sodium hydroxide (1 M), the absorbance of the resulting solution was determined at 510 nm using a spectrophotometer (METASH model UV-5100, Shanghai Metash Instruments Co., Ltd., Shanghai, China). Total flavonoids was expressed as mg of quercetin equivalents g^{-1} of dry weight (DW) [52]. Total anthocyanin was determined using the Wagner [53] method. For this, leaf tissues were ground in a mortar containing methanol. The resulting extract was then kept at room temperature in the dark for 24 h. After this period, the samples were centrifuged at a rate of 4000× *g*, and the absorbance of the supernatant solution was recorded using a spectrophotometer (METASH model UV-5100, Shanghai Metash Instruments Co., Ltd., Shanghai, China) at 550 nm. The concentration of total anthocyanin was calculated in µmol g^{-1} FW.

2.9. Experimental Design and Data Analysis

The trial was designed as a two-way factorial arrangement using a completely randomized design (CRD), with three replications for each treatment, which consisted of five pots. The first factor involved foliar spraying of urea and the second factor included foliar spraying of the AAB. The variance analysis (ANOVA) was performed based on a two-way factorial design CRD. Comparison of means was carried out using Duncan's Multiple Range Test (DMRT) with SAS 9.4 software.

3. Results

3.1. Plant Growth Analysis

Significant effects were observed on the number of leaves, plant height, and leaf area in response to foliar application of urea, AAB, and their interaction ($p \leq 0.01$) (Table S1). The highest plant height was observed with the application of 1 g L^{-1} urea without AAB,

followed by the treatment of 2 g L^{-1} urea and 8 mg L^{-1} AAB. Conversely, the lowest plant height was observed when no urea or AAB was applied (Figure 1A). Similarly, the highest number of leaves and leaf area were associated with the foliar application of 2 g L^{-1} urea and 8 mg L^{-1} AAB, which did not show significant differences with the treatment of 2 g L^{-1} urea alone. In contrast, the lowest values in the number of leaves and leaf area were observed without urea and AAB application (Figure 1B,C).

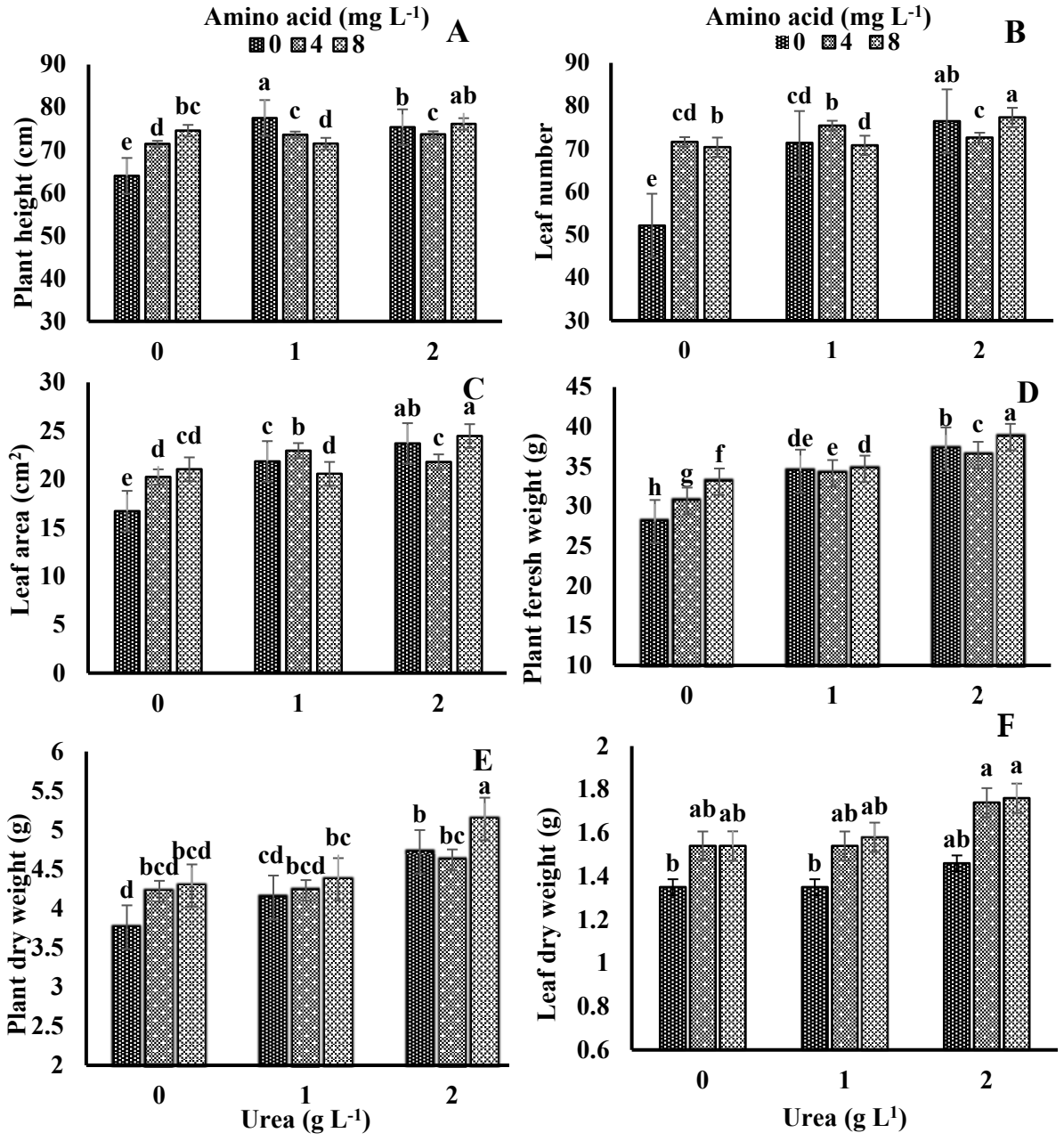


Figure 1. The response of plant height (**A**), leaf number (**B**), leaf area (**C**), plant fresh weight (**D**), plant dry weight (**E**), and leaf dry weight (**F**) of basil to different levels of urea and amino acid stimulant; the values are means of replications. Different letters show significant differences among different treatments ($p \leq 0.05$); vertical bars represent the standard deviation.

The biomass of basil plants also exhibited significant differences due to urea, AAB, and their interaction ($p \leq 0.01$) (Table S1). The combination of 2 g L^{-1} urea and 8 mg L^{-1} AAB resulted in the highest fresh and dry weight, which were higher than the control treatment without foliar spraying (Figure 1D,E). Concerning leaf dry weight, the highest value was recorded under the application of 2 g L^{-1} urea and 8 mg L^{-1} AAB, although this was not significantly different from the combination of 2 g L^{-1} urea and 4 mg L^{-1} AAB. Both treatments significantly increased dry matter when compared to the control (Figure 1F).

3.2. Plant Pigments Determinations and Gas-Exchange

The study examined the impact of different experimental factors on plant pigments, specifically chlorophyll, carotenoids, and anthocyanins. Chlorophyll a content was found to be significantly influenced by urea ($p \leq 0.01$), AAB ($p \leq 0.05$), and their interaction ($p \leq 0.01$) (Table S2). The most effective condition for increasing chlorophyll a content was noticed in the interaction of 2 g L^{-1} urea and 4 mg L^{-1} AAB, followed by the application of 2 g L^{-1} urea and 8 mg L^{-1} AAB (Figure 2A). However, the content of chlorophyll b did not show any significant response to the experimental factors or their interactions (Table S2). Meanwhile, the accumulation of total chlorophyll (a + b) was significantly influenced by urea, AAB, and their interaction ($p \leq 0.01$) (Table S2). The highest total chlorophyll content was observed under the application of 0 g L^{-1} urea and 8 mg L^{-1} AAB, as well as 2 g L^{-1} urea and 4 mg L^{-1} AAB (Figure 2B). Carotenoid concentration was significantly influenced by the AAB and the interaction of urea and the AAB ($p \leq 0.01$) (Table S2). The application of 0 g L^{-1} urea and 8 mg L^{-1} AAB resulted in an increased in carotenoid concentration compared to other treatments, while the lowest carotenoid content was obtained when no urea or AAB was applied (Figure 2C).

Stomatal conductance was significantly influenced by urea application ($p \leq 0.01$) (Table S2). As depicted in Figure 2D, stomatal conductance increased with the application of 1 mg L^{-1} urea and then decreased with higher foliar spray concentration. However, the transpiration rate was not significantly affected by any experimental factors or their interaction (Table S2). Conversely, the photosynthesis was significantly affected by urea spraying, AAB, and their interaction ($p \leq 0.01$) (Table S2). The combination of 2 g L^{-1} urea and 8 mg L^{-1} AAB resulted in the highest photosynthesis rate, which had just a significant difference with the treatment of 2 g L^{-1} urea and 4 mg L^{-1} AAB as well as control. The lowest photosynthesis rate was observed in the absence of urea and AAB application (Figure 2E).

3.3. Nitrogen and Nitrate Content

Taking into account that the experimental factors are commonly used to fulfil the plant's nitrogen requirement, this study measured the nitrogen and nitrate content in the edible parts of basil plants. The nitrogen and nitrate content of basil plants was strongly influenced by foliar spraying of urea and the interaction between urea and AAB ($p \leq 0.01$), while the response to the AAB alone was not significant (Table S3). The highest nitrogen content was observed when 2 g L^{-1} urea was applied without AAB, followed by the foliar application of 2 g L^{-1} urea and 8 mg L^{-1} AAB. In contrast, the lowest nitrogen content was recorded in the control treatment without foliar spraying of urea and AAB (Figure 3A). Nitrate content increased in plants sprayed with 1 or 2 g L^{-1} urea without foliar spraying of AAB compared to other treatments. Conversely, the lowest nitrate content was detected in the control treatment without foliar spraying of urea and AAB (Figure 3B).

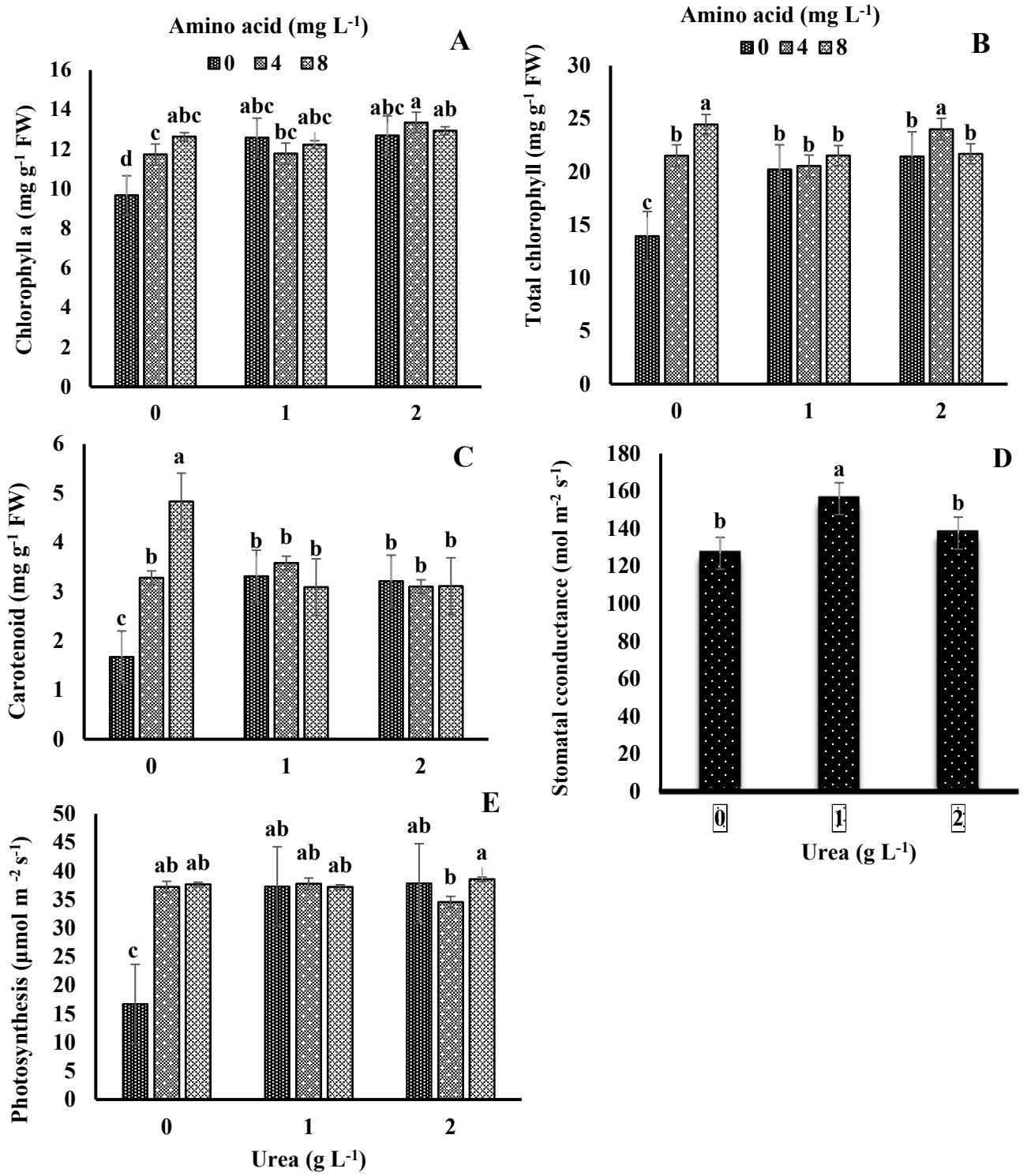


Figure 2. The response of chlorophyll a (**A**), total chlorophyll (**B**), carotenoid (**C**), stomatal conductance (**D**), and photosynthesis rate (**E**) of basil to different levels of urea and amino acid stimulant; the values are means. Different letters show significant differences among different treatments ($p \leq 0.05$); vertical bars represent the standard deviation.

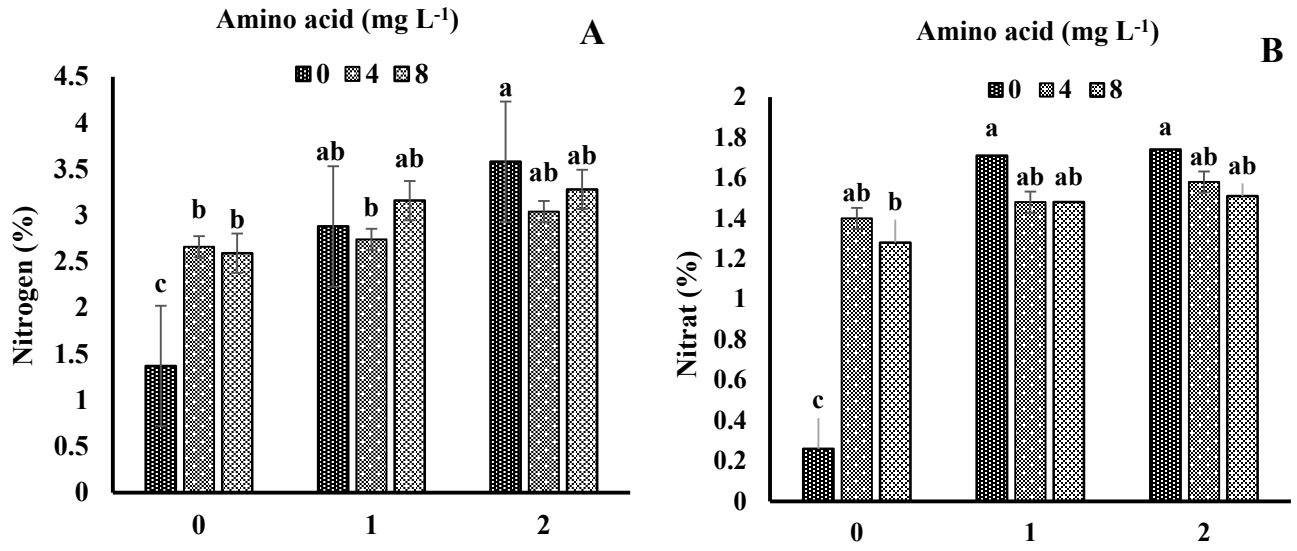


Figure 3. The response nitrogen (**A**) and nitrate (**B**) of basil to different levels of urea and amino acid stimulant; the values are means. Different letters show significant differences among different treatments ($p \leq 0.05$); vertical bars represent the standard deviation.

3.4. Antioxidant-Related Parameters

Total phenolic content in the leaves was effectively modulated by the foliar application of urea, AAB, and their interaction ($p \leq 0.01$) (Table S3). The highest concentration of total phenols was found in the interaction of foliar spraying with 1 g L^{-1} urea and 4 mg L^{-1} AAB, with no significant difference compared to the treatment of 1 g L^{-1} urea and 8 mg L^{-1} AAB. This treatment significantly boosted the total phenolic content compared to the control treatment without foliar spraying (Figure 4A). Similarly, the content of total flavonoid was significantly affected by urea, AAB, and their interaction ($p \leq 0.01$) (Table S3). The most effective treatment, as shown in Figure 4B, was the combination of 1 g L^{-1} urea and 8 mg L^{-1} AAB, which strongly increased the total flavonoid concentration by 75.94% compared to the control treatment.

Antioxidant activity, measured using the FRAP test, was also significantly influenced by urea, AAB, and their interaction ($p \leq 0.01$) (Table S3). The treatment of 1 g L^{-1} urea and 8 mg L^{-1} AAB exhibited the highest antioxidant activity in the basil leaves. The lowest antioxidant activity was observed in the basil leaves under the control treatment (Figure 4C). Total anthocyanin content also exhibited significant differences in response to urea, AAB, and their interaction ($p \leq 0.01$) (Table S3). The interaction of 2 g L^{-1} urea and 8 mg L^{-1} AAB led to an increase in total anthocyanin content in the plant tissue compared to other treatments, while the lowest amount of anthocyanin was found when no urea or AAB was applied (Figure 4D).

3.5. Essential Oil Analysis

The percentage and the yield of essential oil were significantly influenced by urea, AAB, and their combination ($p \leq 0.01$), as indicated in Table S3. The highest essential oil percentage and yield were observed in the basil leaves treated with foliar spraying of 1 g L^{-1} urea and 8 mg L^{-1} AAB. This treatment led to a notable 1.96 times increase in the percentage of essential oil and a significant 2.3 times increase in the essential oil yield compared to the control treatment (Figure 4E,F). The analysis of essential oil through GC-MS revealed the presence of 30 different compounds, with linalool and estragole as the main components. Other notable constituents included α-bergamotene, germacrene D, and T-cadinol (Table 2). The interaction between urea and AAB foliar application significantly impacted the percentage of different components of essential oil. The highest level of linalool (15.55%) was obtained with the application of 1 g L^{-1} urea and 4 mg L^{-1} AAB, which did not significantly differ from the treatment of 2 g L^{-1} urea and 0 mg L^{-1} AAB.

The highest amount of estragole (59.74%) was found under the application of 2 g L^{-1} urea and 8 mg L^{-1} AAB, while the content of α- bergamotene was highest under the interaction of 0 g L^{-1} urea and 8 mg L^{-1} AAB. Increasing the urea concentration to 2 g L^{-1} seemed to affect the accumulation of α-bergamotene in the essential oil negatively. Germacrene D showed the highest accumulation with 2 g L^{-1} urea and 4 mg L^{-1} AAB, as well as 0 g L^{-1} urea and 8 mg L^{-1} AAB. The content of T-cadinol was also highest with the combination of 0 g L^{-1} urea and 8 mg L^{-1} AAB (Table 3).

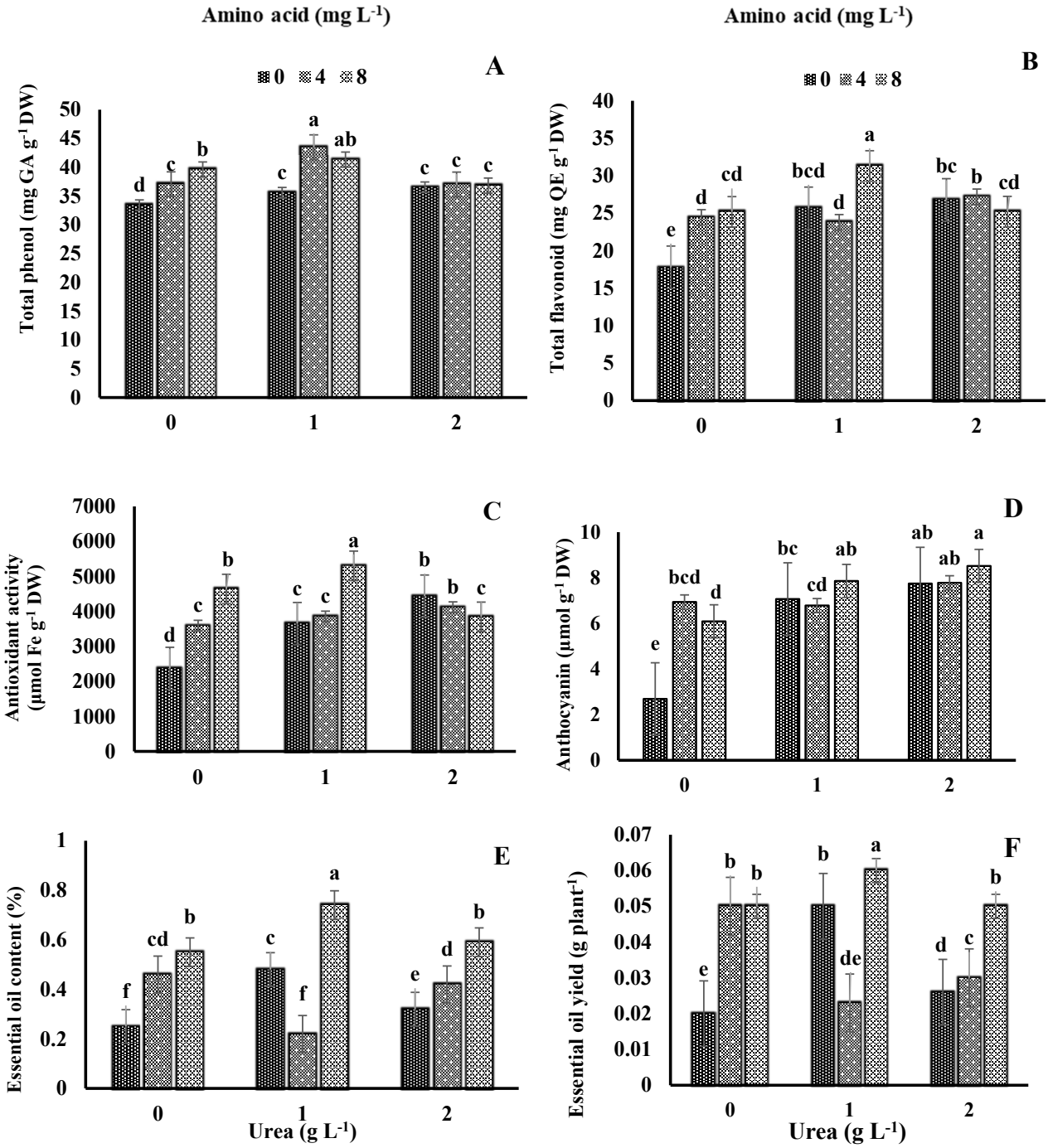


Figure 4. The response of total phenolics (**A**), total flavonoids (**B**), antioxidant activity (**C**), and anthocyanin (**D**), essential oil content (**E**), and essential oil yield (**F**) of basil to different levels of urea and amino acid stimulant; the values are means. Different letters show significant differences among different treatments ($p \leq 0.05$); vertical bars represent the standard deviation.

Table 2. Constituents of the essential oil in *O. basilicum*.

No	Oil Constituents	RI [a]	LIT RI [b]	ID [c]	No	Oil Constituents	RI	KI	ID [c]
1	*β*-Myrcene	988	986.53	RI, MS	16	*γ*-Muurolene	1449	1454.83	RI, MS
2	Cineole	1031	1030.18	Std	17	*Z*-*β*-Farnesene	1460	1458.66	RI, MS
3	*β*-Ocimene	1040	1042.22	RI, MS	18	Germacrene D	1474	1489.74	Std
4	*L*-Fenchone	1088	1092.18	RI, MS	19	α-Selinene	1498	1502	RI, MS
5	Linalool	1101	1104.44	Std	20	*α*-Bulnesene	1509	1498.22	RI, MS
6	Camphor	1146	1151.33	RI, MS	21	*γ*-Cadinene	1514	1509.55	RI, MS
7	Isoborneol	1164	1173.24	RI, MS	22	*δ*-Cadinene	1525	1517.47	RI, MS
8	Terpinen-4-ol	1177	1182.54	RI, MS	23	Nerolidol	1554	1567.66	RI, MS
9	Estragole	1200	1218.63	Std	24	Spathulenol	1572	1568.52	RI, MS
10	Copaene	1378	1376.77	RI, MS	25	Caryophyllene oxide	1573	1571.84	Std
11	Bornyl acetate	1283	1286.33	RI, MS	26	Isoaromadendrene epoxide	1579	1578.33	RI, MS
12	Methyl eugenol	1400	1405.88	Std	27	Cubenol	1601	1609.44	RI, MS
13	Caryophyllene	1415	1419.84	RI, MS	28	T-Cadinol	1644	1645.22	RI, MS
14	*α*-Bergamotene	1430	1437.44	RI, MS	29	*β*-Eudesmol	1649	1654.64	RI, MS
15	*β*-Gurjunene	1449	1453.99	RI, MS	30	*α*-Bisabolol	1691	1699.2	RI, MS

[a] RI, linear retention indices on HP-5 column, experimentally determined using homologue series of n-alkanes. [b] Relative retention indices taken from Adams. [c] Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, Adams and NIST 05; RI: by comparison of retention index with those reported in literature; Std: by comparison of the retention time and mass spectrum of available authentic standard.

Table 3. Mean comparison of the interaction of urea and amino acid on *O. basilicum* essential oil constituents.

Urea (g/L)	Amino Acid (mg/L)	Cineole	Linalool	Estragole	Copaene	Methyleugenol	Caryophyllene	α-Bergamotene	cis-β-Farnesene	Germacrene D	α-Bulnesene	γ-Cadinene	Caryophyllene Oxide	T-Cadinol
0	0	1.35 ^{b}	13.57 ^{b}	49.38 ^{bc}	3.10 ^{b}	0.88 ^{d}	1.89 ^{b}	4.05 ^{cd}	1.20 ^{c}	3.84 ^{bc}	1.24 ^{d}	1.95 ^{bc}	0.58 ^{e}	5.74 ^{abc}
	4	1.08 ^{c}	13.62 ^{b}	50.57 ^{abc}	0.28 ^{f}	2.74 ^{b}	1.50 ^{c}	4.14 ^{cd}	1.37 ^{bc}	3.26 ^{cd}	1.45 ^{c}	2.29 ^{abc}	0.70 ^{de}	6.36 ^{abc}
	8	0.5 ^{e}	3.83 ^{e}	47.08 ^{bc}	3.59 ^{a}	0.27 ^{f}	2.47 ^{a}	6.45 ^{a}	2.05 ^{a}	5.3 ^{a}	1.85 ^{a}	3.06 ^{a}	1.49 ^{a}	8.44 ^{a}
1	0	0.67 ^{d}	6.57 ^{d}	54.09 ^{ab}	2.43 ^{c}	0.27 ^{f}	2.20 ^{ab}	3.91 ^{d}	1.90 ^{ab}	4.06 ^{b}	1.60 ^{b}	2.25 ^{abc}	1.18 ^{b}	6.37 ^{abc}
	4	1.83 ^{a}	15.55 ^{a}	48.33 ^{bc}	2.22 ^{cd}	0.26 ^{f}	1.89 ^{b}	4.56 ^{bc}	1.54 ^{abc}	3.24 ^{cd}	0.94 ^{e}	1.95 ^{bc}	0.78 ^{cd}	5.36 ^{bc}
	8	0.42 ^{e}	10.95 ^{c}	54.27 ^{ab}	1.18 ^{e}	0.57 ^{e}	1.86 ^{b}	4.34 ^{bcd}	1.71 ^{abc}	4.03 ^{b}	0.99 ^{e}	2.31 ^{abc}	0.78 ^{cd}	7.05 ^{abc}
2	0	1.10 ^{c}	14.92 ^{ab}	55.15 ^{ab}	1.98 ^{d}	0 ^{g}	1.48 ^{c}	3.13 ^{e}	1.17 ^{c}	2.65 ^{de}	1.12 ^{d}	1.42 ^{c}	0.90 ^{c}	4.52 ^{c}
	4	0.67 ^{d}	9.97 ^{c}	43.35 ^{c}	0.40 ^{f}	3.22 ^{a}	2.44 ^{a}	4.78 ^{b}	1.46 ^{bc}	5.88 ^{a}	1.54 ^{bc}	2.58 ^{ab}	1.30 ^{b}	7.93 ^{ab}
	8	1.15 ^{bc}	11.3 ^{c}	59.74 ^{a}	0 ^{g}	1.51 ^{c}	1.09 ^{d}	2.71 ^{e}	0.65 ^{d}	2.2 ^{e}	0.87 ^{e}	2.02 ^{bc}	0.81 ^{cd}	6.77 ^{abc}

Means accompanied by the same letter are not significantly different at $p \leq 0.05$ (LSD test).

4. Discussion

4.1. Plant Growth

In the present study, we found that the spraying of urea and AAB on basil leaves increased the biomass of the edible part of the plant. Specifically, the combination of 2 g L^{-1} urea and 8 mg L^{-1} AAB resulted in the highest increases in fresh and dry weight. Meanwhile, the application of 2 g L^{-1} urea and 8 mg L^{-1} AAB resulted in the highest value of leaf dry weight, which had no significant difference with the other treatments, except when compared to the application of 2 g L^{-1} urea and 0 mg L^{-1} AAB and the control treatment. Additionally, we observed the highest number of leaves and leaf area under the interaction of 2 g L^{-1} urea and 8 mg L^{-1} AAB, as well as 2 g L^{-1} urea and 0 mg L^{-1} AAB. Our findings align with previous studies that showed foliar application of urea improved growth and yield in sunflowers [54], and had positive effects on N content and the growth and yield of *Diplotaxis tenuifolia* (L.) [55]. Other studies also demonstrated that the use of amino acids like glutamine and glycine in basil plants leads to an enhancement in fresh and dry weight as well as the accumulation of K, Mg, calcium (Ca), iron (Fe), and zinc (Zn) [56].

4.2. Plant Pigments and Photosynthesis

Given that plant nitrogen nutrition is crucial for chlorophyll synthesis, which is closely linked to photosynthesis, we also investigated the levels of plant pigments, photosynthesis, and stomatal conductance. We found that regardless of the AAB treatment, chlorophyll a, total chlorophyll, carotenoid, and anthocyanin content, as well as photosynthesis rate and stomatal conductance, increased with the application of urea up to a concentration of 1 g L^{-1}. However, applying 2 g L^{-1} of urea in most cases did not significantly increase these traits. Moreover, applying AAB up to 8 mg L^{-1} increased total chlorophyll and carotenoid when no urea was applied. The photosynthesis rate and anthocyanin content increased by applying 4 mg L^{-1} AAB, but the highest concentration (8 mg L^{-1}) did not significantly impact these traits. These findings indicate that the use of 8 mg L^{-1} AAB benefits the accumulation of plant pigments. The enhanced plant biomass may be primarily linked to increased chlorophyll biosynthesis, higher photosynthesis, and enhanced protein biosynthesis [19,28]. Additionally, photosynthesis increase was observed with higher leaf nitrogen content in *Saccharum officinarum* [57]. The N also improves leaf growth and expansion, thus increasing the photosynthetic area and stimulating the biosynthesis of proteins involved in cell growth, division, cell wall, and cytoskeleton biosynthesis [58]. In *Zea mays*, a 29% increase in leaf area was noted under conditions of high nitrogen supply compared to low nitrogen levels [59]. In the present study, we found that foliar application of urea and AAB significantly increased leaf nitrogen content and boosted photosynthetic activity. This aligns with the previous findings of Allison et al. [60], who showed that the nitrogen content of leaves positively influences photosynthesis, which is correlated with the distribution of nitrogen in pigment, photosynthetic enzymes, and the size, number, and composition of chloroplasts. Furthermore, AABs have been shown to enhance plant productivity by improving photosynthesis and increasing the uptake of essential nutrients [61].

4.3. Antioxidant-Related Parameters

The antioxidant activity of *O. basilicum* leaves increased with the application of urea up to a level of 2 g L^{-1}, when no AAB was applied. Specifically, the application of urea at a rate of 1 g L^{-1} led to an increase in the total phenols and flavonoids content while a higher concentration of urea (2 g L^{-1}) did not increase these traits. Moreover, foliar spraying of AAB increased the total phenols, flavonoids, and antioxidant activity when the urea was not applied. However, under the conditions of the 2 g L^{-1} urea application, the amino acid treatment did not positively impact these traits. Ultimately, the highest accumulation of total flavonoid and antioxidant activity was obtained under the application of 1 g L^{-1} urea and 8 mg L^{-1} AAB. This suggests that the increase in antioxidant activity under the combination of 1 g L^{-1} urea and 8 mg L^{-1} AAB may be related to the accumulation of flavonoids. Previous studies have reported a positive correlation between phenolic compounds, flavonoids, and antioxidant activity [62–64]. It has also been noted that the lower content of nutrients in solution positively regulates the accumulation of phenolic and flavonoid compounds [65–67]. Additionally, the foliar application of AAB has been shown to significantly increase the yield, dry matter, accumulation of N, P, K, and Mg, phenolic compounds, antioxidant activity, and total flavonoids in iceberg and romaine cultivars of lettuce [36]. Other reports also indicate an enhancement in the level of phenolic compounds, total flavonoids, and antioxidant activity in *Achillea millefolium* L. [68] and lettuce [69] leaves due to the use of biostimulants.

4.4. Essential Oil

Our study indicated that while the application of urea did not positively affect the content and yield of essential oil, the application of AAB led to an increase in both. Specifically, the application of 1 g L^{-1} urea and 8 mg L^{-1} AAB was the most effective combination in improving the content and yield of essential oil and positively regulating leaf dry weight. Since essential oil yield is determined by the product of essential oil percentage in the leaf,

an increase in dry weight of leaf can lead to an increase in the yield of essential oil [70]. Previous research also supports the positive effect of amino acids on essential oil content and composition in plants [71]. Additionally, it has been reported that supplying nitrogen affects leaf growth, improves the interception of solar energy, and influences carbon fixation by increasing photosynthetic pigments, enhancing the activity of Krebs cycle enzymes, increasing simple sugars, balancing hormones, and regulating the relationship between source and sink, ultimately leading to an increase in the secretory glands of essential oil, biosynthesis of terpenoid compounds, and storage capacity of essential oil [72].

The application of urea fertilizer and AAB at different levels led to significant changes in the essential oil composition of the basil plant. This is in line with the understanding that nitrogen plays a crucial role in accumulating essential oil compounds, particularly terpenoid compounds. Previous research has also reported that linalool, α-bergamotene, and T-cadinol were the most important constituents of basil essential oil under the application of 100 mg L^{-1} phenylalanine [73]. The α-bergamotene was also one of the major constituents of the essential oil of basil plants treated with urea and Fe nano-complex [74]. Furthermore, nitrogen application contributes to the production of carbon skeletons through essential coenzymes ATP, NADPH, and CoA, playing a pivotal role in terpenoid biosynthesis [75]. Additionally, amino acids serve as substrates for various secondary metabolites, with specific amino acids being substrates for different compounds. For example, tryptophan is a substrate for auxins, alkaloids, phytoalexins, and indole glucosinolates, while tyrosine is a substrate for quinones, betalins, and isoquinoline alkaloids [76]. Numerous studies have demonstrated the positive and significant effects of foliar application of AABs on the amount of total phenols, essential oil, and their composition in various plant species such as *Melissa officinalis* L. [77] *Coleus blumei* L. [78] and *O. basilicum* L. [73]. Moreover, nitrogen impact on essential oil production through carbon metabolism and the formation of acetyl-CoA in the mevalonate pathway is well documented [79]. The positive effects of different nitrogen concentrations on essential oil content and chemical composition have also been reported in *Petroselinum crispum* [80], *Nigella sativa* [81] and *Satureja hortensis* [82]. Given these findings, foliar spraying can be recommended as an alternative solution to reduce soil application, thus potentially reducing nitrogen leaching.

5. Conclusions

In conclusion, this study highlights the significant role of foliar application of urea and AABs in enhancing plant physiological traits and overall growth. The findings demonstrate that the combination of 2 g L^{-1} urea and 8 mg L^{-1} AAB was most effective in promoting plant growth and improving yield characteristics, likely due to the synergistic effects on photosynthesis, pigment production, and nutrient assimilation. Specifically, chlorophyll content, carotenoid levels, and stomatal conductance showed positive responses to urea application up to a concentration of 1 g L^{-1}, with diminishing returns observed at higher concentrations. Additionally, AAB application enhanced chlorophyll, carotenoids, and anthocyanins, with the highest photosynthesis rate observed at 4 mg L^{-1} AAB, reflecting its role in optimizing plant metabolism. Furthermore, the positive impact of 1 g L^{-1} urea on phenolic compounds, including total phenols and flavonoids, was complemented by increased antioxidant activity and flavonoid content following AAB treatment. Although urea did not significantly affect the essential oil yield, AAB enhanced both its yield and composition, with estragole concentration notably increased. The combination of 1 g L^{-1} urea and 8 mg L AAB emerged as the most effective treatment for improving both the quality and yield of essential oils, as well as enhancing other key phytochemicals like phenolics and antioxidants. These results suggest that foliar application of urea and AABs not only contribute to improving plant growth and productivity but also hold potential for optimizing the production of high-value secondary metabolites. Future research should explore the comparative effectiveness of foliar versus root application, assess potential reductions in nitrogen fertilizer usage, and evaluate the economic viability of foliar nitrogen application in agricultural systems, especially considering both crop yield and quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14122950/s1, Table S1. Analysis of variance of the effect of urea and amino acid on growth and biomass of basil. Table S2. Analysis of variance of the effect of urea and amino acid on plant pigments and gas exchange of basil. Table S3. Analysis of variance of the effect of urea and amino acid on nitrogen, nitrate, gas exchange, and essential oil content and yield of basil. Table S4. Analysis of variance of the effect of urea and amino acid stimulant on basil essential oil constituents.

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