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Optimizing the Electrical Conductivity of a Nutrient Solution for Plant Growth and Bioactive Compounds of *Agastache rugosa* in a Plant Factory

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Abstract: The objective of this study was to determine the proper electrical conductivity (EC) of a nutrient solution (NS) for accumulating bioactive compounds of *Agastache rugosa* without decreasing plant growth. Six-week-old seedlings were transplanted in a deep flow technique system with Hoagland NS with a 2.0 dS·m⁻¹ EC for the initial week. From eight days after transplanting, the plants were treated with six EC treatments of 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 dS·m⁻¹ for three weeks. Plant growth parameters, leaf gas exchange parameters, the relative chlorophyll value, and the ratio of variable to maximum fluorescence (F_v/F_m) were measured, and the rosmarinic acid (RA), tilianin, and acacetin concentrations were analyzed at 28 days after transplanting. The results showed that almost all plant growth parameters were maximized at 2.0 and 4.0 dS·m⁻¹ and minimized at 8.0 dS·m⁻¹ compared with the other EC treatments. The relative chlorophyll and F_v/F_m values were maximized at 2.0 and 4.0 dS·m⁻¹. Similarly, leaf gas exchange parameters were increased at 2.0 and 4.0 dS·m⁻¹. The RA content exhibited significantly higher values at 0.5, 1.0, 2.0, and 4.0 dS·m⁻¹ compared with other treatments. The tilianin and acacetin contents exhibited the significantly highest values at 4.0 and 0.5 dS·m⁻¹, respectively. These results suggest optimal EC treatment at 4.0 dS·m⁻¹ for increasing bioactive compounds in *A. rugosa* plants without decreasing plant growth. Excessively high or low EC induced salinity stress or nutrient deficiency, respectively. Furthermore, among the plant organs, the roots of *A. rugosa* contained the highest RA concentration and the flowers contained the highest tilianin and acacetin concentrations, which revealed a higher utilization potential of the roots and flowers for bioactive compounds.

Keywords: acacetin; chlorophyll; Hoagland solution; photosynthesis rate; rosmarinic acid; tilianin

1. Introduction

Agastache rugosa (Fisch. & C.A.Mey.) Kuntze is a popular species of the Lamiaceae family, which includes both herbal medicines and spices [1]. Secondary metabolites in *A. rugosa* plants include essential oils; phenolic compounds, such as rosmarinic acid (RA), ferulic acid, chlorogenic acid, and caffeic acid; and flavone glycosides, such as acacetin, tilianin, sesquiterpenes, triterpene, and diterpenes, which have the capability to prevent disease due to their anti-allergic, antifungal function, anti-inflammatory, antioxidant function, and antidepressant effects [1,2]. Rosmarinic acid is the major active component of *A. rugosa*. The rosmarinic acid activities consist of antioxidant activity, astringent features, an antimutagenic ability, an anti-inflammatory capacity, an antiviral capacity, and antimicrobial properties [3–5]. Previous studies [5–7] have indicated that acacetin and tilianin are important active compounds in *A. rugosa*, and have antiatherogenic, anti-inflammatory, vasorelaxant, and antihypertensive effects. Therefore, the bioactive compounds in *A. rugosa* are very important for

medical applications. *A. rugosa* plants are mostly harvested from outside cultivation or wild regions. Nevertheless, the secondary metabolites of *A. rugosa* plants, as well as other medicinal plants grown outdoors, are very hard to control, so the concentration of secondary metabolites may vary widely under different environments and cultivation conditions, such as location, season, light intensity, photoperiod, temperature, and electrical conductivity (EC) [8,9]. The capacity to manage environmental conditions by plant factory technology has permitted producers to control their production systems to maximize plant growth and development, as well as quality [10]. An optimal nutrient supply is an essential factor for improving plant growth and development, plant physiology, and quality under a hydroponic culture system in a plant factory or greenhouse [8,9,11].

The plant growth of pak choi was demonstrated to be restricted by an imbalanced ion composition, an excessively low or high concentration of a nutrient solution (NS) (0.3, 0.6, and 1.2 dS·m⁻¹ or 9.6 dS·m⁻¹, respectively) because of nutritional inadequacy or ion toxicity, and salinity stress [8]. The salt concentration and electrolyte concentration index of the solution are determined by EC. Moreover, the EC of the NS indicates the ion availability for the plants in the root zone [9,12]. A high EC of the NS has been shown to reduce plant growth, osmotic potential, and absorbing NS because of salt stress. In contrast, a low EC of the NS has been demonstrated to degrade the yield and plant growth because of nutrient deficiency [8,11–13]. The total phenol, chlorogenic acid, and kaempferol contents were maximized at an EC of 2.0 dS·m⁻¹, whereas 3.0 dS·m⁻¹ significantly increased the fresh and dry weights of carrot [14]. An increase in EC from 0.5 to 2.5 dS·m⁻¹ was accompanied by a significant increment of the antioxidant capacity and total phenolic content per shoot of *Crepidiastrum denticulatum*; however, the number of leaves, fresh weight, and leaf area were significantly higher at 2.0 and 2.5 dS·m⁻¹ than those at 0.5, 1.0, and 1.5 dS·m⁻¹ [12]. When the EC of the NS was either excessively high or low, the antioxidant enzyme activities were significantly increased, whereas the fresh weights were significantly decreased at low and high ECs because of nutrient inadequacy and salinity stress, respectively [8]. Therefore, an excessively low EC of the NS can lead to nutrient inadequacy, while an excessively high EC can lead to salinity stress and ion toxicity.

The objective of this study was to clarify the effects of different ECs of the NS on the growth; leaf gas exchange parameters; chlorophyll content; and RA, tilianin, and acacetin concentrations and contents in plant organs and the whole plant of *A. rugosa*. Furthermore, we tried to determine the optimal EC of the NS to achieve the highest accumulation of RA, tilianin, and acacetin concentrations and contents in hydroponically-grown *A. rugosa* without plant growth limitations.

2. Materials and Methods

2.1. Plant Materials and Seedlings

Agastache rugosa (Danong Seed Co., Ltd., Seoul, Korea) seeds were sown in a rockwool media tray (60 × 40 cm in size, 240 holes, UR rockwool, Suwon, Korea) and covered with vermiculites. Afterward, the seed tray was translocated to a germination room, in which the air temperature and relative humidity were maintained at 22 ± 2 °C and 65 ± 10% during the day and 18 ± 1 °C and 75 ± 10% during the night, respectively. The fluorescent lamps (TL5 14W/865, Philips, Seoul, Korea) generated a photosynthetic photon flux density (PPFD) of 180 ± 10 μmol·m⁻²·s⁻¹ on the tray. The seedlings of *A. rugosa* were immersed daily in water for 1 minute. At 2 weeks after sowing, the seedlings were immersed for 1 minute in Hoagland NS with 1.2 dS·m⁻¹ EC daily. The mineral salts used for the A Hoagland nutrient solution were FeEDTA: 4.898 g·L⁻¹; Ca(NO₃)₂·4H₂O: 191.880 g·L⁻¹; and KNO₃: 61.310 g·L⁻¹ and those employed for the B Hoagland nutrient solution were H₃BO₃: 0.603 g·L⁻¹; MnSO₄·5H₂O: 0.435 g·L⁻¹; ZnSO₄·7H₂O: 0.044 g·L⁻¹; CuSO₄·5H₂O: 0.010 g·L⁻¹; Na₂MoO₄·2H₂O: 0.004g·L⁻¹; KNO₃: 61.310 g·L⁻¹; MgSO₄·7H₂O: 100.610 g·L⁻¹; and NH₄H₂PO₄: 23.470 g·L⁻¹.

2.2. Electrical Conductivity Experiment and Growth Conditions

Six-week-old seedlings with the same uniformity were selected and transplanted into six hydroponic culture systems (0.58 m (W) × 12.5 m (L) × 0.11 m (H)) in a plant factory which was maintained at the average temperature of 22 ± 3 °C and average relative humidity of $70 \pm 10\%$. The plants were grown under white light-emitting diode (TL5 14W/865, Philips, Seoul, Korea) irradiation of 200 ± 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD with a 14/10 h (day/night) photoperiod for four weeks. Hoagland NS with 2.0 $\text{dS}\cdot\text{m}^{-1}$ EC was supplied for the initial week to avoid low or high EC transplant shock. From 8 days after transplanting (DAT) onwards, the plants were grown in six EC treatments of 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 $\text{dS}\cdot\text{m}^{-1}$ for three weeks. The EC was adjusted by the Hoagland NS. A portable conductivity meter and pH tester combo (HI98129, Hanna instrument, Woonsocket, RI, USA) was used to measure the EC and pH values of the NS. The tank had a volume of 50 liters. The six EC treatments were as follows:

EC0.5 $\text{dS}\cdot\text{m}^{-1}$: diluted Hoagland nutrient solutions A (62.5 mL) and B (62.5 mL) in 50 liters deionized water;

EC1.0 $\text{dS}\cdot\text{m}^{-1}$: diluted Hoagland nutrient solutions A (125 mL) and B (125 mL) in 50 liters deionized water;

EC2.0 $\text{dS}\cdot\text{m}^{-1}$: diluted Hoagland nutrient solutions A (250 mL) and B (250 mL) in 50 liters deionized water;

EC4.0 $\text{dS}\cdot\text{m}^{-1}$: diluted Hoagland nutrient solutions A (500 mL) and B (500 mL) in 50 liters deionized water;

EC6.0 $\text{dS}\cdot\text{m}^{-1}$: diluted Hoagland nutrient solutions A (750 mL) and B (750 mL) in 50 liters deionized water;

EC8.0 $\text{dS}\cdot\text{m}^{-1}$: diluted Hoagland nutrient solutions A (1000 mL) and B (1000 mL) in 50 liters deionized water.

2.3. Measurement of Growth Parameters

All plant growth characteristics of *A. rugosa* were determined at 28-DAT with six measurements per replication ($n = 6$). The number of leaves both longer than 1 cm and wider than 1 cm was counted, and the leaf area was measured by a leaf area meter (Li-3100, LiCor, Lincoln, NE, USA). The leaf width, leaf length, root length, and stem length were manually determined by a measuring tape. The root fresh weight and shoot fresh weight were measured by a digital scale (ARG224 OHAUS, Sigma-Aldrich. Co. LLC, Seoul, Korea). After that, samples were immediately placed in a drying oven (HB-502M, Hanback Sci, Suwon, Korea) at 70 °C for seven days. Afterward, a digital scale (ARG224 OHAUS, Sigma-Aldrich. Co. LLC, Seoul, Korea) was used to measure the root dry weight and shoot dry weight.

2.4. Relative Chlorophyll Value and Chlorophyll Fluorescence (F_v/F_m) Measurement

The relative chlorophyll value was measured at 28 DAT on the third intact leaves from the plant apex by a portable chlorophyll meter (502, Minolta Camera Co., Ltd., Tokyo, Japan). The ratio of variable to maximum fluorescence (F_v/F_m) was measured at 28 DAT on the third intact leaves from the plant apex by using a portable fluorometer (Fluorpen Pen FP 100, Photon System Instruments Ltd, Drasov, Czech Republic). Chlorophyll fluorescence and relative chlorophyll values were measured by three and six measurements ($n = 3$ and $n = 6$) in each replication.

2.5. Leaf Gas Exchange Measurement

The leaf gas exchange parameters, which consisted of the net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration rate (T_r) of *A. rugosa*, were measured at 28 days after transplanting by using a portable photosynthesis measurement system (LICOR 6400, Licor. Inc. Nebraska, NE, USA) that had previously been warmed for 30 minutes and

calibrated in the ZERO IRGA mode. The optimal conditions of the leaf chamber were established as follows: 25 °C leaf temperature, 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD, 60% relative humidity, 400 $\mu\text{mol}\cdot\text{mol}^{-1}$ ambient CO_2 concentration, and 500 $\text{cm}^3\cdot\text{s}^{-1}$ air flow rate. Leaf gas exchange parameters were measured on the third intact leaves from the plant apex. The measurement process was conducted automatically. Four measurements were used in each EC treatment per replication ($n = 4$).

2.6. Analysis of Acacetin, Tilianin, and Rosmarinic Acid Concentrations and Contents

The whole plant, as well as the roots, stems, leaves, and flowers, of *A. rugosa* samples from each replication were separated and immediately put in liquid nitrogen at 28 days after transplanting, placed in a deep freezer at $-70\text{ }^\circ\text{C}$, and dried at $-50\text{ }^\circ\text{C}$ in the dry freezer (TFD5503, IL Shinbiobase Co. Ltd, Gyeonggi-do, Korea) for 4 days. Acacetin, tilianin, and RA concentrations were analyzed by three measurements ($n = 3$) in each replication per EC treatment. Plant organs were subsequently milled to a fine powder with a blender. Afterward, the powder was sieved through mesh sieves. The freeze-dried powder (0.1 g) was blended with 80% (v/v) MeOH (1.5 mL) by vortexing the sample and it was then sonicated for 60 min. The solution was centrifuged at $12,000\times g$ for 10 min at $4\text{ }^\circ\text{C}$ using a microcentrifuge (R17 Plus, Hanil Scientific Co., Ltd., Gimpo, Korea). Before high-performance liquid chromatography (HPLC) analysis, the supernatant was filtered by being passed through a $0.45\text{ }\mu\text{m}$ filter for HPLC analysis. The HPLC analysis was conducted at $30\text{ }^\circ\text{C}$ by using a C18 column ($250\text{ mm}\times 4.6\text{ mm}$, $5\text{ }\mu\text{m}$; RS tech, Daejeon, Korea). Additionally, 0.20% (v/v) acetic acid (solvent A) and 100% methanol (solvent B) were used as the solvent systems. The gradient profile of solvent B was as follows: 0–15 min: 45%; 15–20 min: 45%–55%; 20–45 min: 55%–80%; 45–50 min: 80%; 50–52 min: 80%–45%; 52–60 min: 45% (total of 60 min). The injection volume was 20 μL , the flow rate was upheld at one mL/min, and the detection wavelength was 275 nm. The acacetin, tilianin, and RA from Sigma-Aldrich Corporation (Sigma-Aldrich, Co., Ltd., Seoul, Korea) were used as standard compounds. The tilianin, acacetin, and RA concentrations were determined by reversed-phase HPLC-ultraviolet analysis (1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) [3,5]. The average values were determined from three independent replicates of each plant organ (root, leaf, stem, and flower). The acacetin, tilianin, and RA concentrations ($\text{mg}\cdot\text{g}^{-1}$ plant organ and whole plant dry weight (DW)) were determined. The RA, tilianin, and acacetin concentrations in each plant organ (root, leaf, stem, and flower) ($\text{mg}\cdot\text{g}^{-1}$ DW) were the mean mg RA, tilianin, and acacetin concentrations per gram plant organ dry weight (DW). The RA, tilianin, and acacetin concentrations in the whole plant ($\text{mg}\cdot\text{g}^{-1}$ DW) were the mean mg RA, tilianin, and acacetin concentrations per gram plant DW. The RA, acacetin, and tilianin contents of the whole plant ($\text{mg}/\text{plant DW}$) were the mean RA, acacetin, and tilianin concentrations ($\text{mg}\cdot\text{g}^{-1}$ plant DW) multiplied by the whole plant DW (g).

2.7. Statistical Analysis

The EC experiment was performed with two replications using completely randomized designs. The SPSS 20.0 soft program (SPSS 20, SPSS Inc., Chicago, IL, USA) was used to determine differences among the means of all EC treatments. One-way ANOVA was conducted. Significant differences among the means of treatment groups at the 5% level were compared using Tukey's multiple range test.

3. Results

3.1. Plant Growth Parameters

The results showed that the variation in the ECs of the NS significantly influenced the plant growth parameters of *Agastache rugosa* (Table 1). The leaf length at $8.0\text{ dS}\cdot\text{m}^{-1}$ was 7.73 cm, which was the lowest value among all EC treatments. There was a decreasing trend in the leaf width and number of leaves at 6.0 and $8.0\text{ dS}\cdot\text{m}^{-1}$ compared with the other treatments and these results were not significantly different from 0.5 to $4.0\text{ dS}\cdot\text{m}^{-1}$ treatments. The leaf area was not significantly different from 0.5 to $4.0\text{ dS}\cdot\text{m}^{-1}$ treatments and no significant differences were found among 0.5, 6.0, and 8.0

$\text{dS}\cdot\text{m}^{-1}$, but the $4.0\text{ dS}\cdot\text{m}^{-1}$ treatment was the highest and $8\text{ dS}\cdot\text{m}^{-1}$ treatment was the lowest. The stem length of $4.0\text{ dS}\cdot\text{m}^{-1}$ treatment was the longest compared to the other treatments. Stem lengths at $1.0, 2.0,$ and $4.0\text{ dS}\cdot\text{m}^{-1}$ were longer than the other treatments. The shoot fresh weight (SFW) at 0.5 and $8.0\text{ dS}\cdot\text{m}^{-1}$ was lower than the other treatments. The SFW was the lowest for $8.0\text{ dS}\cdot\text{m}^{-1}$ treatment among all the EC treatments. A similar pattern was observed for the root fresh weight (RFW). The RFW was minimized with the $8.0\text{ dS}\cdot\text{m}^{-1}$ treatment. The RFW at $2.0, 4.0,$ and $6.0\text{ dS}\cdot\text{m}^{-1}$ was higher than the other treatments. The root length value under 2.0 and $4.0\text{ dS}\cdot\text{m}^{-1}$ was larger than other EC treatments. In particular, the root length at $2.0\text{ dS}\cdot\text{m}^{-1}$ was 17.6 cm longer than that at $8.0\text{ dS}\cdot\text{m}^{-1}$. The shoot dry weight (SDW) and root dry weight (RDW) at $8\text{ dS}\cdot\text{m}^{-1}$ were the lowest among all the EC treatments. The overall growth parameters were minimized at $8\text{ dS}\cdot\text{m}^{-1}$ treatment (Table 1).

3.2. Relative Chlorophyll Value and Chlorophyll Fluorescence (F_v/F_m)

The variation in the ECs of the NS had a significant influence on the F_v/F_m and relative chlorophyll values. The relative chlorophyll value was significantly increased at 2.0 and $4.0\text{ dS}\cdot\text{m}^{-1}$ treatments, while the F_v/F_m value was higher from 2.0 to $6.0\text{ dS}\cdot\text{m}^{-1}$ compared with the other treatments. The F_v/F_m and relative chlorophyll values were significantly minimized at $0.5\text{ dS}\cdot\text{m}^{-1}$ (Figure 1A,B).

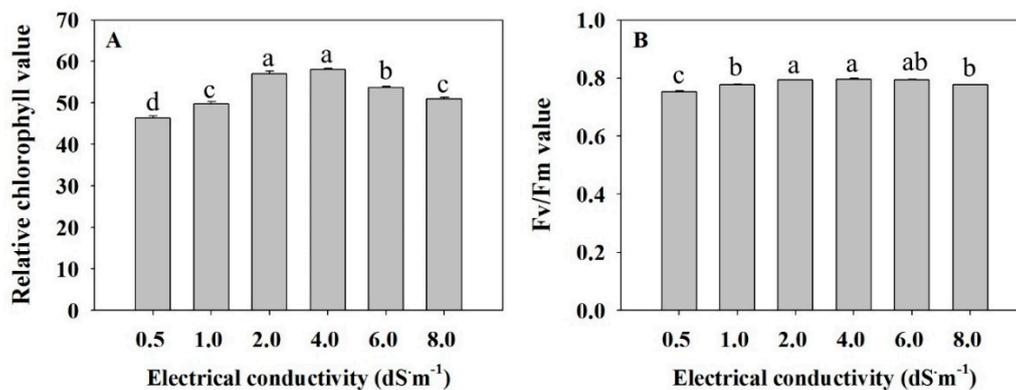


Figure 1. Relative chlorophyll value (A) and chlorophyll fluorescence (F_v/F_m) (B) of *A. rugosa* at different electrical conductivity (EC) treatments ($0.5, 1.0, 2.0, 4.0, 6.0,$ and $8.0\text{ dS}\cdot\text{m}^{-1}$). The data show the means and the vertical bars show the standard error ($n = 6$ and $n = 3$). Different letters show significant differences among EC treatments (Tukey's test, $p \leq 0.05$).

Table 1. Plant growth parameters of *Agastache rugosa* grown at different electrical conductivity (EC) treatments (0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 dS·m⁻¹) at 28 days after transplanting.

EC ^w (dS·m ⁻¹)	Leaf Length (cm)	Leaf Width (cm)	Number of Leaves	Leaf Area (cm ²)	Stem Length (cm)	SFW (g/plant)	RFW (g/plant)	Root Length (cm)	SDW (g/plant)	RDW (g/plant)
0.5	9.23 ± 0.29 ab	8.28 ± 0.25 ab	71.33 ± 4.09 ab	739.07 ± 43.95 abc	37.27 ± 0.42 cd	20.77 ± 1.38 bc	8.94 ± 0.78 bc	40.32 ± 1.28 cd	2.40 ± 0.11 bc	0.55 ± 0.05 cd
1.0	9.08 ± 0.30 ab	8.77 ± 0.29 a	75.00 ± 1.71 ab	841.33 ± 53.71 ab	40.28 ± 0.69 bc	24.04 ± 1.61 ab	10.89 ± 0.66b c	45.80 ± 1.27 bc	2.58 ± 0.15a bc	0.61 ± 0.02 bc
2.0	9.72 ± 0.31 a	8.87 ± 0.16 a	74.67 ± 3.79 ab	838.07 ± 56.59 ab	41.87 ± 0.52 ab	24.66 ± 1.22 ab	14.61 ± 1.29 a	54.47 ± 2.26 a	2.79 ± 0.18 ab	0.73 ± 0.03 ab
4.0	9.32 ± 0.25 a	8.45 ± 0.25 a	84.50 ± 4.55 a	921.88 ± 52.22 a	43.05 ± 0.31 a	26.89 ± 1.57 a	15.29 ± 1.03 a	48.21 ± 1.01 ab	3.07 ± 0.14 a	0.74 ± 0.03 a
6.0	8.13 ± 0.18 bc	7.52 ± 0.17 b	67.17 ± 3.20 b	685.25 ± 26.78 bc	38.28 ± 0.60 cd	23.44 ± 0.39 ab	12.34 ± 0.38 ab	44.45 ± 1.35 bc	2.57 ± 0.04 abc	0.61 ± 0.01 bc
8.0	7.73 ± 0.18 c	7.42 ± 0.09 b	68.00 ± 2.72 b	611.75 ± 24.95 c	34.83 ± 0.50 e	17.94 ± 0.92 c	8.39 ± 0.73 c	36.87 ± 1.30 d	2.24 ± 0.07 c	0.48 ± 0.02 d
Significance ^z	***	***	*	***	***	***	***	***	**	***
L ^y	***	***	NS	**	**	NS	NS	*	NS	NS
Q ^x	***	***	*	***	***	***	***	***	***	***

^w electrical conductivity; NS: not significant ($p > 0.05$); ^z significant at * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$; ^y L: linear; ^x Q: quadratic in regression analysis. Data are the means ± SE ($n = 6$). Different letters indicate significant differences among treatments at the level of 5%, according to Tukey's test. SFW: shoot fresh weight; RFW: root fresh weight; SDW: shoot dry weight; and RDW: root dry weight.

3.3. Leaf Gas Exchange Parameters

The EC of the NS significantly affected the net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration rate (T_r) (Figure 2). When the EC of the NS was increased to $8.0 \text{ dS}\cdot\text{m}^{-1}$, P_n , C_i , g_s , and T_r were significantly decreased. A higher photosynthesis rate was observed at 2.0 and $4.0 \text{ dS}\cdot\text{m}^{-1}$, and there was no significant difference between 1.0 and $6.0 \text{ dS}\cdot\text{m}^{-1}$; however, the photosynthesis rate was minimized at $8.0 \text{ dS}\cdot\text{m}^{-1}$ (Figure 2A). Furthermore, the g_s at 2.0 and $4.0 \text{ dS}\cdot\text{m}^{-1}$ was significantly higher than that of the other treatments and there were no significant differences among 0.5 , 1.0 , 6.0 , and $8.0 \text{ dS}\cdot\text{m}^{-1}$ (Figure 2B). However, the C_i value from 1.0 to $4.0 \text{ dS}\cdot\text{m}^{-1}$ was higher than that of the other treatments (Figure 2C). A similar pattern was observed for T_r , showing that T_r from 1.0 to $4.0 \text{ dS}\cdot\text{m}^{-1}$ was significantly higher than the other treatments (Figure 2D). The leaf gas exchange parameters exhibited the significantly lowest values of $4.41 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for P_n , $0.026 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for g_s , $107.07 \mu\text{mol CO}_2 \text{ mol}^{-1}$ for C_i , and $0.49 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for T_r at $8 \text{ dS}\cdot\text{m}^{-1}$ (Figure 2A,D).

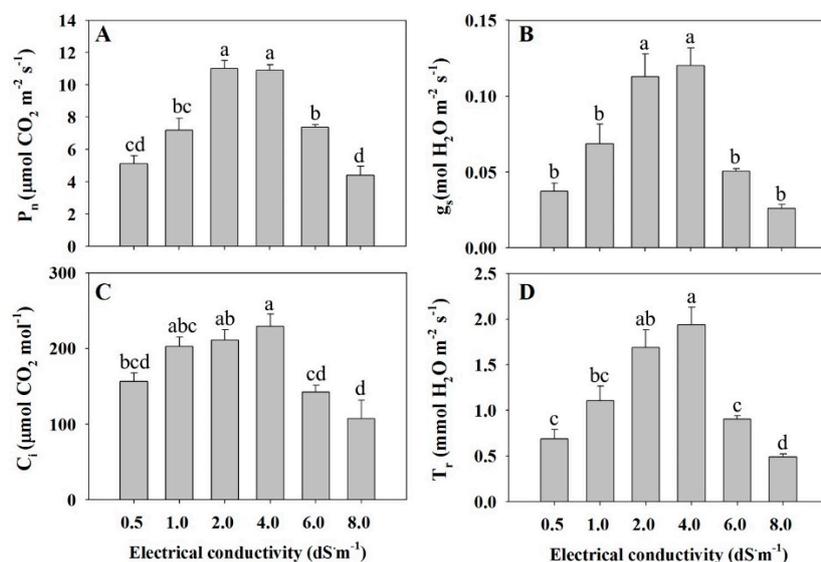


Figure 2. Net photosynthetic rate (P_n) (A), stomatal conductance (g_s) (B), intercellular CO_2 concentration (C_i) (C), and transpiration rate (T_r) (D) at different electrical conductivity (EC) treatments (0.5 , 1.0 , 2.0 , 4.0 , 6.0 , and $8.0 \text{ dS}\cdot\text{m}^{-1}$). The data represent the means and the vertical bars show the standard error ($n = 4$). Different letters show significant differences among EC treatments (Tukey's test, $p \leq 0.05$).

3.4. Acacetin, Tiliandin, and Rosmarinic Acid Concentrations and Contents

The RA, tiliandin, and acacetin concentrations of *A. rugosa* were separately analyzed in the leaves, flowers, stems, and roots (Table 2). In addition, the RA, tiliandin, and acacetin concentrations of *A. rugosa* were analyzed in the whole plant (Figure 3). The RA concentration in leaves was significantly higher at 0.5 and $1.0 \text{ dS}\cdot\text{m}^{-1}$ compared with the other treatments. The RA concentration in flowers showed no significant difference among all EC treatments; however, the RA concentration in flowers was higher at lower EC (0.5 , 1.0 , and $2.0 \text{ dS}\cdot\text{m}^{-1}$) compared with higher EC treatments. The RA concentration in stems was significantly highest at $1.0 \text{ dS}\cdot\text{m}^{-1}$ compared with the other treatments. The RA concentration in roots was significantly highest at $0.5 \text{ dS}\cdot\text{m}^{-1}$ compared with the other treatments. These results revealed an increasing trend in the RA concentration of plant organs at low EC treatments. The RA concentration was maximized in roots compared with other organs of *A. rugosa* (Table 2).

Table 2. Rosmarinic acid (RA), tilianin, and acacetin concentrations (mg g^{-1} DW) in leaves, flowers, stems, and roots in *A. rugosa* grown at different electrical conductivity (EC) treatments (0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 $\text{dS}\cdot\text{m}^{-1}$) at 28 days after transplanting.

EC ^w ($\text{dS}\cdot\text{m}^{-1}$)	RA Concentration in Plant Organs (mg g^{-1} DW)				Tilianin Concentration in Plant Organs (mg g^{-1} DW)				Acacetin Concentration in Plant Organs (mg g^{-1} DW)			
	Leaves	Flowers	Stems	Roots	Leaves	Flowers	Stems	Roots	Leaves	Flowers	Stems	Roots
0.5	3.566 a	4.735	3.992 de	21.141 a	0.667 c	3.294 c	0.962 ab	0.056 b	0.059 b	0.199 d	0.019 b	ND
1.0	3.231 a	4.687	6.722 a	15.752 b	0.639 c	4.971 b	1.062 a	0.134 a	0.011 d	0.327 c	0.012 cd	ND
2.0	2.232 b	4.401	6.064 b	17.013 b	0.969 b	5.108 b	0.938 ab	0.046 d	0.169 a	0.232 d	0.013 c	ND
4.0	1.510 c	3.425	3.507 e	10.163 c	1.273 a	5.795 a	0.762 c	0.032 f	0.008 d	0.344 c	0.010 d	ND
6.0	1.436 c	3.658	5.180 c	12.122 c	1.289 a	5.913 a	0.857 bc	0.052 c	0.028 c	0.555 a	0.019 b	ND
8.0	1.909 bc	3.944	4.510 d	10.389 c	1.470 a	5.837 a	1.050 a	0.037 e	0.013 d	0.492 b	0.026 a	ND
Significance ^z	***	NS	***	***	***	***	***	***	***	***	***	ND
L ^y	***	*	NS	***	***	***	NS	*	NS	***	**	ND
Q ^x	***	*	NS	***	***	***	***	NS	NS	***	***	ND

^w electrical conductivity; NS: not significant ($p > 0.05$); ^z significant at * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$; ^y L linear; ^x Q: quadratic in regression analysis. Data are the mean of three measurements ($n = 3$). Different letters indicate significant differences among treatments at the level of 5%, according to Tukey's test. ND: not detected; RA: rosmarinic acid; DW: dry weight.

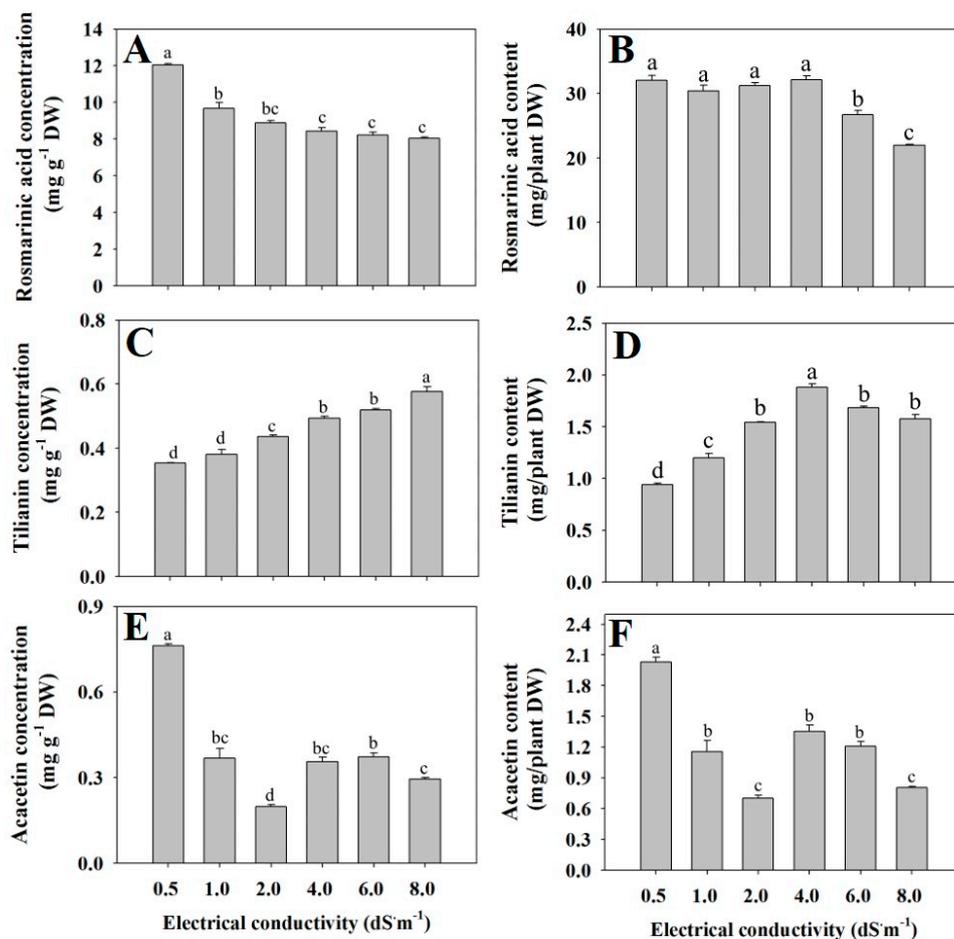


Figure 3. Rosmarinic acid (A,B), tiliainin (C,D), and acacetin (E,F) concentrations and contents of *A. rugosa* at different electrical conductivity (EC) treatments (0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 dS·m⁻¹). The data show the means and the vertical bars show the standard error ($n = 3$). Different letters show significant differences among EC treatments (Tukey's test, $p \leq 0.05$).

The tiliainin concentration in leaves was significantly higher at 4.0, 6.0, and 8.0 dS·m⁻¹ compared with the other treatments. However, the tiliainin concentration in flowers had an increasing tendency as EC increased. The tiliainin concentration in stems was lower at 4.0 and 6.0 dS·m⁻¹ than the other treatments. The tiliainin concentration in roots was maximized at 1.0 dS·m⁻¹ and minimized at 4.0 dS·m⁻¹ compared with the other treatments. The tiliainin concentration was maximized in flowers compared with other organs of *A. rugosa* (Table 2). The significantly highest acacetin concentration was observed at 2.0 dS·m⁻¹ in leaves, 6.0 dS·m⁻¹ in flowers, and 8.0 dS·m⁻¹ in stems. The acacetin concentration was undetectable in roots. The acacetin concentration was maximized in flowers compared with other organs of *A. rugosa* (Table 2).

The significantly highest value of RA concentration in the whole plant was observed at 0.5 dS·m⁻¹ (Figure 3A); however, the RA content in the whole plant was significantly higher at 0.5, 1.0, 2.0, and 4.0 dS·m⁻¹, and significantly lowest at 8.0 dS·m⁻¹. This is because the RA content is dependent on SDW and RDW (Figure 3B). The tiliainin concentration in the whole plant tended to increase with increasing EC (Figure 3C); however, the significantly highest tiliainin content was found at 4.0 dS·m⁻¹ since the tiliainin content is highly dependent on SDW and RDW (Figure 3D). The acacetin concentration in the whole plant was significantly highest at 0.5 dS·m⁻¹, and lowest at 2.0 dS·m⁻¹ (Figure 3E). A similar pattern was observed for the acacetin content (Figure 3F). This study suggests that controlling the EC of the NS in the root zone at 4.0 dS·m⁻¹ promoted both RA and tiliainin contents per plant, and at 0.5 dS·m⁻¹ promoted the acacetin content per plant of *A. rugosa* (Figure 3B,F).

4. Discussion

4.1. Plant Growth Parameters

Plant growth and quality have been shown to be affected by the NS composition ratio and nutrient concentration in a hydroponic culture system [8,9]. In the present study, we found that almost all plant growth parameters were significantly decreased at $8.0 \text{ dS}\cdot\text{m}^{-1}$ compared to the other treatments, possibly because of salinity stress at $8.0 \text{ dS}\cdot\text{m}^{-1}$ treatment. A similar result was reported by Pecanha et al., [15] who reported that the higher EC of $10.0 \text{ dS}\cdot\text{m}^{-1}$ significantly decreased the biomass of lettuce because the high salinity concentration reduced the osmotic potential in the NS.

Notably, a high EC reduced the osmotic potential of the NS, which may reduce the water uptake and turgor pressure in the plant. Furthermore, a high EC may limit cell expansion due to the diminished water uptake and turgor pressure. A high EC may lead to the retention of toxic ions in the root zone, so ion imbalance may affect plant growth. Therefore, plant growth was decreased by a high EC [15,16]. Moreover, the increase in Cl^- and Na^+ may limit the Ca^{2+} and K^+ uptake of roots because the hydrated radii of potassium and calcium are higher than that of sodium. A low K^+/Na^+ selectivity ratio may result from a high-affinity potassium transport system. Therefore, ion imbalance occurs under salinity stress [17,18]. According to Degl'Innocenti et al. [18], plant growth was significantly decreased in barley species because the osmotic potential was reduced and ion imbalance arose around the roots. We found that the effect of high and low EC stress of the NS on *A. rugosa* in this study was negligible because of the lower EC ($0.5 \text{ dS}\cdot\text{m}^{-1}$) and higher EC ($8.0 \text{ dS}\cdot\text{m}^{-1}$) shock in the root zone of the plant treated from 8 days after transplanting. In our study, the highest EC treatment at $8.0 \text{ dS}\cdot\text{m}^{-1}$ had the lowest biomass production, leaf size, leaf area, and root and stem lengths due to salinity stress, ion imbalance, toxicity, lower water uptake, and turgor pressure in the plant, and the variation in osmotic potential around the roots. In addition, the ion ratio and concentration of the NS may affect the uptake and translocation between each part of the plant. Therefore, the growth of *Agastache rugosa* was improved under the optimal EC treatments (2.0 to $4.0 \text{ dS}\cdot\text{m}^{-1}$).

4.2. Relative Chlorophyll Value and Chlorophyll Fluorescence (F_v/F_m)

In the present study, the relative chlorophyll and F_v/F_m values of *Agastache rugosa* decreased in response to a high electrical conductivity ($8.0 \text{ dS}\cdot\text{m}^{-1}$) due to the effects of salinity stress and toxicity under the very high concentration of nutrient solution and low electrical conductivity (0.5 and $1.0 \text{ dS}\cdot\text{m}^{-1}$) caused by the nutrient inadequacy of nitrogen, magnesium, and iron, which are important factors for the synthesis of chlorophyll and maintenance of the chloroplast structure and function [19–21]. In addition, the leaf chlorophyll concentration and dark color in tomato leaves were increased at high EC ($4.5 \text{ dS}\cdot\text{m}^{-1}$) of the NS [13]. Chlorophyll a and b, and the chlorophyll content were significantly decreased in nitrogen deficiency treatment [21]. A similar result was reported by Shah et al. [22], who revealed that the total chlorophyll was decreased at a low nutrient supply. Furthermore, Garriga et al. [23] found that the chlorophyll content was gradually reduced with an increasing salinity concentration. The relative chlorophyll value of cucumber was increased at a higher EC of the NS [24]. The maximum chlorophyll fluorescence (F_v/F_m) was increased by adding more nitrogen in winter wheat [25]. This agreed with our results, for which the relative chlorophyll value in *A. rugosa* was higher at 2.0 and $4.0 \text{ dS}\cdot\text{m}^{-1}$ treatments and F_v/F_m ratio was higher at 2.0 , 4.0 , and $6.0 \text{ dS}\cdot\text{m}^{-1}$ treatments, compared with the other treatments, probably because *A. rugosa* avoided EC shock right after being transplanted by conducting EC stress from 8 days after transplanting. These study results suggest that an excessively low EC ($0.5 \text{ dS}\cdot\text{m}^{-1}$) may lead to nutrient deficiency and an excessively high EC ($8.0 \text{ dS}\cdot\text{m}^{-1}$) may lead to salinity stress in *A. rugosa*. Therefore, nutrient deficiency and salinity stress could reduce the relative chlorophyll and F_v/F_m values in *A. rugosa*.

4.3. Leaf Gas Exchange Parameters

A reduction of the photosynthesis rate often results in low assimilation production [26]. In our study, the net photosynthetic rate (P_n), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration rate (T_r) of *A. rugosa* were enhanced under medium EC treatments (2.0 and 4.0 $\text{dS}\cdot\text{m}^{-1}$) compared to the other EC treatments. In this respect, Ding et al. [8] indicated that an excessively high EC leads to salinity stress and an excessively low EC leads to nutrient deficiency. The salinity concentration was shown to decrease the water uptake and increase abscisic acid (ABA) in roots, which resulted in stomatal closure [27,28]. CO_2 uptake is negatively affected by stomatal closure [29]. The photosynthesis rate of some plant species is decreased by stomatal closure due to the influence of salinity [8,28,30].

The leaf photosynthesis rate, stomatal conductance, leaf transpiration, and intercellular CO_2 concentration of some plants have also been seen to be significantly decreased by a higher EC of the NS in a hydroponic culture system, such as pak choi [8], lettuce [30], and papaya [16]. Coinciding with these former studies, our results also showed that leaf gas exchange parameters (P_n , C_i , g_s , and T_r) were significantly decreased at a high EC of 8.0 $\text{dS}\cdot\text{m}^{-1}$ because the salinity stress was increased with an increasing EC of the NS. The influence of salinity on *A. rugosa* resulted in a decreased water uptake and increased ABA, which reduced stomatal conductance and the photosynthesis rate. Moreover, the leaf photosynthesis response is mainly based on the leaf nitrogen content. Leaf nitrogen deficiency has been shown to reduce the chlorophyll and net photosynthetic rate [26,31]. In our study, leaf gas exchange parameters were reduced under an excessively low EC (0.5 $\text{dS}\cdot\text{m}^{-1}$) compared with the other treatments. These study results coincide with previous reports on pak choi [8], olive [31], and sorghum plants [26].

4.4. Acacetin, Tiliandin, and Rosmarinic Acid Concentrations and Contents

The RA concentration was higher than the tiliandin and acacetin concentrations in the whole plant of *A. rugosa* (Figure 3). A similar result was determined by Tuan et al. [5] who reported that the RA concentration was higher than the tiliandin and acacetin concentrations of *A. rugosa*. Furthermore, RA is an important phenolic compound and was concentrated in the hairy roots of *A. rugosa* [3]. This also coincides with our results, for which the RA concentration was found to be the highest in the roots of *A. rugosa* compared with other plant organs (Table 2). Similarly, RA was maximized in the roots compared with other organs of *A. rugosa* [32]. In contrast, the RA concentration was maximized in flowers of *A. rugosa* [5], probably due to the different harvest times or types of varieties.

In our study, the RA concentration was significantly highest at 0.5 $\text{dS}\cdot\text{m}^{-1}$ in roots. Likewise, the RA concentration in the whole plant was the highest at 0.5 $\text{dS}\cdot\text{m}^{-1}$ among all treatments. Moreover, the results of our study indicated that *A. rugosa* under low EC treatments exhibited a trend of an increasing RA concentration because RA is a phenylpropanoid with a very strong antioxidant capacity [33]. A group of genes that are involved in the biosynthetic pathway of RA consisting of phenylalanine ammonia-lyase: PAL, 4-coumarate: CoA ligase: 4CL, and cinnamate 4-hydroxylase: C4H, was strongly produced under nitrogen deficiency [34]. These results are consistent with previous reports, such as those for the RA concentration in *Prunella vulgaris*, which was increased with a low nutrient supply [35]. The phenolic acid content in the plant was increased under nutrient deficiency (nitrogen, phosphorus, and potassium) [36]. The total polyphenols content in lettuce was maximized under nitrogen deficiency [37]. Furthermore, the RA of sweet basil was significantly highest at the low NO_3^- concentration of 0.5 $\text{mol}\cdot\text{m}^{-3}$ compared with 5.0 and 10.0 $\text{mol}\cdot\text{m}^{-3}$ in the NS in a hydroponic system [38]. The RA concentration in green and red perilla was significantly highest at 1.0 $\text{dS}\cdot\text{m}^{-1}$ compared to at 2.0 and 3.0 $\text{dS}\cdot\text{m}^{-1}$ [9].

The highest tiliandin and acacetin concentrations were detected in the flowers of *A. rugosa* compared with other plant organs (Table 2). Similarly, tiliandin and acacetin concentrations were maximized in the flowers of *A. rugosa* compared with other plant organs [5]. Moreover, total flavonoids were maximized in the flowers compared to other organs of *A. rugosa* [32]. The results in our experiment indicated that

the tilianin concentration in the whole plant increased when increasing the EC levels from 0.5 to 8.0 $\text{dS}\cdot\text{m}^{-1}$ (Figure 3C). The tilianin concentration in the whole plant of *A. rugosa* was maximized at 8.0 $\text{dS}\cdot\text{m}^{-1}$. The same result was reported by Ding et al. [8], who found that the treatment of 9.6 $\text{dS}\cdot\text{m}^{-1}$ increased the antioxidant enzyme activity and ascorbic acid content in pak choi compared to the other treatments because of toxicity and salt stress. The values of electrolyte leakage, lipid peroxidation, and some antioxidants (superoxide dismutase and peroxidase) were maximized at 8.0 $\text{dS}\cdot\text{m}^{-1}$ compared with the other treatments [39]. The antioxidant activity in artichoke was maximized at 6.9 and 6.45 $\text{dS}\cdot\text{m}^{-1}$ [40]. The phenol concentration, antioxidative capacity, total soluble solids, organic acids, and carotenoids in tomatoes were increased at a high EC (6.5 and 10 $\text{dS}\cdot\text{m}^{-1}$) [41] because the high EC of the NS induced salt stress [8]. In addition, the ascorbic acid, α -carotene, lutein, and polyphenol contents of pepper grown at high EC conditions were significantly increased in a hydroponic culture system [17]. Moreover, the lycopene concentration of red ripe tomato fruits was higher at a higher EC (4.5 $\text{dS}\cdot\text{m}^{-1}$) compared with a lower EC (2.3 $\text{dS}\cdot\text{m}^{-1}$) [13]. Secondary metabolites in *Hypericum pruinatum* were increased at a high nitrogen dose [42]. A high EC of the NS could lead to salt stress [8]. Salt stress enhanced the increase of hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-) in cells, which may have induced the accumulation of some oxidative stress parameters, such as protein oxidation and lipid peroxidation [43], thereby increasing reactive oxygen species accumulation and the production of secondary metabolites in *A. rugosa*. This agreed with our results, for which tilianin was increased at high EC treatments.

The results in this study showed that 4.0 $\text{dS}\cdot\text{m}^{-1}$ increased both RA and tilianin contents, and 0.5 $\text{dS}\cdot\text{m}^{-1}$ maximized the acacetin content in *A. rugosa* (Figure 3B,D,F). However, excessively low EC treatment (0.5 or 1.0 $\text{dS}\cdot\text{m}^{-1}$) restricted leaf gas exchange parameters, chlorophyll, and the tilianin content, probably due to nutrient inadequacy, while excessively high EC treatment (6.0 and 8.0 $\text{dS}\cdot\text{m}^{-1}$) limited chlorophyll, the photosynthesis rate, RA and acacetin contents, and plant growth, probably due to salinity stress and toxicity. The EC treatment at 2.0 $\text{dS}\cdot\text{m}^{-1}$ reduced tilianin and acacetin contents. Therefore, the optimal EC treatment was 4.0 $\text{dS}\cdot\text{m}^{-1}$ for plant growth and the accumulation of RA and tilianin contents in *A. rugosa*.

5. Conclusions

The optimal EC treatment is 4.0 $\text{dS}\cdot\text{m}^{-1}$ for growth and major bioactive compound accumulation because RA and tilianin contents were increased under 4.0 $\text{dS}\cdot\text{m}^{-1}$ and the amount of RA and tilianin contents in *A. rugosa* were higher than the acacetin content. *A. rugosa* grown under a hydroponic culture system in a plant factory could achieve high bioactive compounds by controlling the nutrient NS. Moreover, these results also provide quality information on each part of the *A. rugosa* plant (roots, flowers, leaves, and stems), according to the user's demands for RA, tilianin, and acacetin contents. Moreover, this study provides information about the effects of variation in the ECs of the NS on plant growth, photosynthesis, the chlorophyll content, and bioactive compounds in *A. rugosa*. Therefore, the study data are useful information for optimizing the NS to improve plant biomass and bioactive compounds.

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