

COMPARATIVE ANALYSIS OF PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF *Plectranthus amboinicus* GROWN IN HYDROPONIC AND AEROPONIC SYSTEMS

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Abstract. This study evaluated the effects of hydroponic and aeroponic cultivation systems on the physiological and biochemical characteristics of *Plectranthus amboinicus* under controlled environmental conditions. The 50-day experiment comprised one hydroponic treatment (HYP) and two aeroponic treatments with misting cycles of 30 s on/5 min off (AER1) and 30 s on/15 min off (AER2). Each treatment included three plants as independent biological replicates ($n = 3$; nine plants in total). Plant height, leaf number, branch number, and Soil Plant Analysis Development value were assessed at 0, 10, 20, 30, 40, and 50 days, whereas biomass, water-related traits, and biochemical characteristics were evaluated at harvest. AER1 produced the greatest plant height (48.8 cm), leaf number (42.5 leaves plant⁻¹), fresh weight (82.7 g plant⁻¹), and dry weight (4.26 g plant⁻¹). It also resulted in the highest contents of vitamin C, reducing sugars, titratable acidity, and total flavonoids. In contrast, nitrate accumulation was highest in HYP and decreased by 26.3% under AER1. These findings indicate that, under the tested conditions, the short-interval aeroponic misting regime was associated with greater growth and higher levels of the biochemical parameters measured in *P. amboinicus*.

Keywords: *Plectranthus amboinicus*, aeroponics, hydroponics, physiological traits, biochemical characteristics.

1. Introduction

Plectranthus amboinicus (Lour.) Spreng. is a perennial aromatic herb in the family Lamiaceae with considerable medicinal, nutritional, and phytochemical value [1], [2]. The species contains essential oils, phenolic acids, flavonoids, and other secondary

metabolites that support its antioxidant, antimicrobial, anti-inflammatory, and respiratory health-related properties [2]. Major volatile compounds such as carvacrol and thymol are often associated with its characteristic aroma and biological activities. Because the leaves are the main harvested organ, plant growth, leaf development, chlorophyll status, water balance, and nutrient accumulation are important indicators of both biomass productivity and raw material quality. These physiological traits are strongly affected by cultivation conditions, especially in systems where water, mineral nutrients, oxygen supply, and root-zone environment can be controlled.

Soilless cultivation has become an important strategy for producing high-value crops under controlled conditions [3]. Hydroponics and aeroponics are two advanced approaches that differ mainly in the way the root system receives water, nutrients, and oxygen [4]. In hydroponics, roots are exposed to a nutrient solution, either continuously or periodically. This system can provide stable water and mineral availability, which supports rapid vegetative growth and consistent physiological performance. In aeroponics, roots are suspended in air and supplied with nutrient mist at regular intervals [5]. This approach increases oxygen availability around the roots and may improve root respiration, nutrient uptake, and resource-use efficiency. The difference between a solution-based root environment and a mist-based root environment may lead to distinct patterns of growth, chlorophyll accumulation, water status, and nutrient assimilation in medicinal plants such as *P. amboinicus*.

Earlier studies compared aeroponic and hydroponic cultivation in sunflower [6] and hydroponic system (HYP) and soil cultivation in *P. amboinicus* [7]. However, the effects of aeroponic misting regime on the physiological and biochemical characteristics of *P. amboinicus* remain poorly studied. The present study compared one hydroponic treatment with two aeroponic treatments. In both aeroponic treatments, roots received nutrient mist for 30 s. The interval between misting events was 5 min in aeroponic system 1 (AER1) and 15 min in aeroponic system 2 (AER2). This design assessed the differences between hydroponic and aeroponic cultivation and the effect of misting regime within the aeroponic system. Measurements included plant growth, chlorophyll status, biomass, water-related traits, vitamin C, reducing sugars, titratable acidity, flavonoids, and nitrate content. To our knowledge, this is the first study to compare aeroponic misting regime in *P. amboinicus* through concurrent measurements of physiological and biochemical characteristics. The results provide preliminary information for evaluating suitable misting regimes for the soilless cultivation of this medicinal species.

2. Materials and methods

2.1. Plant materials

Plant materials consisted of healthy and uniform seedlings of *P. amboinicus* provided by the Faculty of Agricultural Technology, University of Engineering and Technology, Vietnam National University Hanoi (Hanoi City, Vietnam). The seedlings were vegetatively propagated and selected at the early vegetative stage with an average height of 10 - 15 cm, 4 - 6 fully expanded leaves, and well-developed root systems. All plants were acclimatized for 3 - 5 days under controlled environmental conditions,

including a temperature of 25 ± 2 °C, relative humidity of 60 - 70%, photosynthetic photon lux density of $250 - 300 \mu\text{mol m}^{-2} \text{s}^{-1}$, and a photoperiod of 16/8 h (light/dark).

2.2. Methods

Growth conditions: Plants were cultivated under controlled conditions using one hydroponic system and two aeroponic systems. Each experimental condition comprised nine plants distributed among three independent cultivation containers, with three plants per container. Each container constituted an independent experimental unit ($n = 3$), and the plants within each container were treated as subsamples. The HYP consisted of rectangular plastic containers measuring $60 \times 20 \times 18$ cm, each filled with 10 L of half-strength Hoagland nutrient solution. Plant roots were continuously immersed in the nutrient solution. The two aeroponic systems used closed root chambers of the same size ($60 \times 20 \times 18$ cm), in which roots were suspended in air and supplied with half-strength Hoagland solution via mist nozzles. Each aeroponic chamber was equipped with two full-cone mist nozzles positioned 20 cm apart and approximately 10 cm below the root zone. The nozzles operated at 0.25 MPa with a flow rate of 0.12 L min^{-1} per nozzle and produced droplets with a mean diameter of approximately 80 μm . Each 30-s spray event delivered 0.12 L of nutrient solution per chamber. AER1 used a 30-s spray period followed by a 5-min interval and completed approximately 262 spray events per day. Its gross daily spray volume was $31.4 \text{ L chamber}^{-1}$. AER2 used a 30-s spray period followed by a 15-min interval and completed approximately 93 spray events per day. Its gross daily spray volume was $11.1 \text{ L chamber}^{-1}$. Drainage from each chamber returned to its corresponding 10-L reservoir. In all treatments, pH was maintained at 5.8 - 6.2, and electrical conductivity was kept at $1.2 - 1.4 \text{ mS cm}^{-1}$, as previously described, with minor adjustments [8]. The growth room was maintained at 25 ± 2 °C, 60 - 70% relative humidity, $250 - 300 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, and a 16/8 h light/dark photoperiod.

Sample collection: Plant height, leaf number, branch number, and Soil Plant Analysis Development (SPAD) values were measured non-destructively on the same tagged plants at 0, 10, 20, 30, 40, and 50 days after the beginning of cultivation. The same group of nine plants per treatment was monitored throughout the experiment. At day 50, all plants were harvested for leaf area, fresh weight, dry weight, water content, dry matter content, nitrate content, and biochemical analyses. No plants were harvested at the intermediate measurement dates. Fully expanded leaves from the middle canopy region were selected for chlorophyll and biochemical analyses. For growth measurements, whole plants were carefully removed from the cultivation systems and rinsed with distilled water to eliminate residual nutrient solution before recording morphological parameters. Fresh leaf samples used for biochemical analyses were immediately frozen in liquid nitrogen and stored at -20°C until analysis to minimize metabolic degradation.

Physiological parameters measurement: Physiological parameters were measured throughout the experimental period to assess plant growth, water status, and photosynthetic performance. Plant height (cm) was measured from the stem base to the apical meristem using a ruler, while leaf number (leaves plant⁻¹) and branch number (branches plant⁻¹) were recorded by counting fully expanded leaves and primary lateral

branches longer than 1.5 cm. Leaf area was determined at the final harvest through digital image analysis with ImageJ software (National Institutes of Health, Bethesda, MD, USA) [9]. Fully expanded leaves were placed flat on a white background and photographed from a perpendicular position. A metric ruler was included in each image for spatial calibration. Leaf boundaries were traced manually, and the enclosed area was calculated with ImageJ [9]. Mean leaf area was expressed as $\text{cm}^2 \text{ leaf}^{-1}$. Fresh weight (FW, g) was measured immediately after sampling, and dry weight (DW, g) was obtained after oven-drying samples at 60 °C until constant weight. Leaf water content (%) and dry matter content (%) were calculated from FW and DW. SPAD value was measured with a SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan). At each sampling date, three fully expanded leaves at comparable positions in the middle canopy were selected from each plant. Three readings were recorded from the central region of each leaf. The midrib, major veins, leaf margins, and damaged areas were excluded from the measurement points. The nine readings obtained from each plant were averaged and expressed as one plant-level SPAD value. Nitrate content was expressed as mg kg^{-1} FW and determined using a spectrophotometric method after extraction from fresh leaf samples.

Biochemical parameters measurement: Biochemical parameters were measured at the final harvest with three independent biological replicates per treatment. Ascorbic acid content was determined by iodine titration with starch as the endpoint indicator according to a previous report [10]. Titratable acidity was determined according to TCVN 5483:2007 with standardized NaOH and phenolphthalein. Reducing sugar content was measured by the 3,5-dinitrosalicylic acid method at 575 nm [11]. Total flavonoid content was determined by the aluminum chloride colorimetric method at 415 nm [12]. Nitrate content was measured by the salicylic acid method at 410 nm [13]. Each extract was measured three times as technical replicates, and the mean value was used for statistical analysis.

Relative change analysis: Relative changes in physiological and biochemical parameters under aeroponic systems were calculated using the hydroponic treatment as the reference control. The percentage change for each parameter was determined according to the following equation: $\text{Relative change (\%)} = [(\text{Treatment value} - \text{Hydroponic value}) / \text{Hydroponic value}] \times 100$. Positive values indicated an increase relative to the hydroponic system, whereas negative values indicated a reduction. The analysis was conducted using mean values obtained at the final harvest stage for each cultivation system.

Data analysis: Growth parameters were analyzed with a linear mixed-effects model. Cultivation system, measurement time, and their interaction were included as fixed effects. Cultivation unit and plant nested within cultivation unit were included as random effects to account for repeated measurements from the same plants. Tukey-adjusted pairwise comparisons were applied after significant fixed effects. Biochemical parameters measured only at final harvest were analyzed by one-way analysis of variance followed by Tukey's honestly significant difference test. Differences were considered significant at $P < 0.05$ [14].

3. Results and discussion

3.1. Growth and physiological characteristics of *Plectranthus amboinicus* under hydroponic and aeroponic systems

The growth performance of *P. amboinicus* differed among cultivation systems during the experimental period. At the beginning of cultivation, no significant differences were observed among treatments for plant height, leaf number, branch number, or SPAD values. However, differences became evident after 10 days of cultivation and gradually increased over time. Plants grown under AER1 showed the highest values for most growth parameters throughout the experiment. At 50 days, plant height reached 48.8 cm in AER1, compared with 45.3 cm in AER2 and 41.5 cm in the HYP (Figure 1A). Similar trends were observed for leaf number and branch number. AER1 produced 42.5 leaves plant⁻¹ and 7.5 branches plant⁻¹ at harvest, whereas HYP-grown plants produced 33.1 leaves plant⁻¹ and 5.1 branches plant⁻¹ (Figures 1B, 1C). SPAD values also increased under aeroponic conditions, particularly in AER1, which maintained the highest chlorophyll index during most sampling periods (Figure 1D). The progressive differences among treatments indicate that the cultivation system influenced plant performance throughout the experimental period. At harvest, plants grown under AER1 had 17.6%, 28.4%, 47.1%, 20.9%, and 39.7% higher plant height, leaf number, branch number, fresh weight, and dry weight, respectively, than those grown under HYP. However, Szabó-Nagy et al. (1994) reported a higher growth rate in hydroponically grown sunflower seedlings than in aeroponically grown seedlings, although aeroponics induced thicker roots, greater lateral-root development, and changes in root redox activity [6]. The contrasting responses indicate that the effects of aeroponics depend on plant species, developmental stage, and system configuration. In the present study, the superior performance of AER1 relative to AER2 suggests that the shorter misting regimes provided a more favorable balance between root aeration and water and nutrient availability. Nevertheless, root oxygen status and nutrient uptake rates were not measured; therefore, this explanation remains to be experimentally verified.

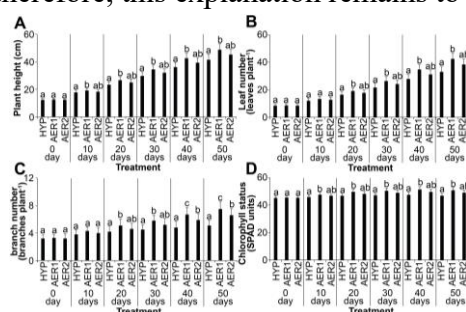


Figure 1. Growth and physiological characteristics of *Plectranthus amboinicus* cultivated under hydroponic and aeroponic systems during 50 days of cultivation. (A) Plant height, (B) leaf number, (C) branch number, and (D) SPAD values

HYP represents the hydroponic system, AER1 represents aeroponic system 1 with a misting cycle of 30 s on and 5 min off, and AER2 represents aeroponic system 2 with a misting cycle of 30 s on and 15 min off. Data are presented as mean $\pm$ standard deviation. Different lowercase letters indicate significant differences among treatments within the same sampling time according to Tukey's honest significant difference test at p -value < 0.05 .

Table 1. Leaf development, biomass accumulation, and water status of *Plectranthus amboinicus* at harvest

<i>Treatment</i>	Leaf area (cm²)	FW (g plant⁻¹)	DW (g plant⁻¹)	Water content (%)	Dry matter (%)
HYP	74.9 ± 6.8 ^a	68.4 ± 5.7 ^a	3.05 ± 0.34 ^a	95.5 ± 0.4 ^a	4.5 ± 0.4 ^a
AER1	88.6 ± 7.4 ^c	82.7 ± 6.3 ^c	4.26 ± 0.41 ^c	94.8 ± 0.5 ^a	5.2 ± 0.5 ^b
AER2	81.3 ± 7.1 ^b	76.2 ± 6.0 ^b	3.72 ± 0.38 ^b	95.1 ± 0.4 ^a	4.9 ± 0.4 ^{ab}

HYP, hydroponic system; AER1, aeroponic system with 30 s misting and 5 min interval; AER2, aeroponic system with 30 s misting and 15 min interval. Values are presented as mean ± standard deviation. Different lowercase letters indicate significant differences among treatments within the same sampling time, according to Tukey's honest significant difference (HSD) test ($P < 0.05$).

3.2. Biochemical characteristics of *Plectranthus amboinicus* under hydroponic and aeroponic systems

The biochemical characteristics of *P. amboinicus* differed significantly among the hydroponic and aeroponic systems at harvest (Table 2). AER1 produced the highest contents of vitamin C, reducing sugars, organic acids, and total flavonoids, with values of 52.4 mg 100 g⁻¹ FW, 33.7 mg g⁻¹ FW, 1.15 g 100 g⁻¹ FW, and 27.9 mg QE g⁻¹ DW, respectively. Aeroponic system 2 showed intermediate values for all these biochemical indicators, while the hydroponic system showed the lowest levels. At harvest, AER1 increased vitamin C, reducing sugars, organic acids, and total flavonoids by 35.8%, 35.9%, 40.2%, and 50.8%, respectively, relative to HYP, whereas AER2 produced smaller increases. Previously, Dass et al. (2022) found that soil-grown *P. amboinicus* accumulated higher levels of phenolics, flavonoids, hydroxycinnamic acids, chlorophylls, and carotenoids than hydroponically grown plants [7]. Meanwhile, their hydroponic plants contained higher concentrations of several minerals and greater superoxide dismutase and catalase activities. These results demonstrate that cultivation systems can differentially regulate pigment status, primary metabolism, and secondary-metabolite accumulation. The higher SPAD values and flavonoid content observed under AER1 may be associated with differences in root-zone oxygen availability and nutrient delivery. However, these mechanisms were not directly assessed in the present study.

Table 2. Biochemical characteristics of *Plectranthus amboinicus* under hydroponic and aeroponic systems at harvest

<i>Treatment</i>	Vitamin C (mg 100 g⁻¹ FW)	Reducing sugars (mg g⁻¹ FW)	Titrateable acidity (g 100 g⁻¹ FW)	Total flavonoids (mg QE g⁻¹ DW)	Nitrate content (mg kg⁻¹ FW)
HYP	38.6 ± 3.1 ^a	24.8 ± 2.4 ^a	0.82 ± 0.07 ^a	18.5 ± 1.6 ^a	712.4 ± 58.3 ^c
AER1	52.4 ± 4.2 ^c	33.7 ± 2.9 ^c	1.15 ± 0.09 ^c	27.9 ± 2.1 ^c	524.8 ± 46.5 ^a
AER2	46.1 ± 3.8 ^b	29.6 ± 2.7 ^b	0.97 ± 0.08 ^b	23.4 ± 1.9 ^b	603.7 ± 51.2 ^b

Values are presented as mean ± standard deviation. Different lowercase letters indicate significant differences among treatments within the same sampling time, according to Tukey's HSD test ($P < 0.05$).

Nitrate content showed an opposite trend compared with the other biochemical parameters. The highest nitrate accumulation was recorded in hydroponic plants at 712.4 mg kg⁻¹ FW, followed by AER2 at 603.7 mg kg⁻¹ FW and AER1 at 524.8 mg kg⁻¹ FW. The lower nitrate content in aeroponic treatments may be associated with more efficient nitrate assimilation under tested root-zone aeration. The elevated nitrate concentration in the HYP may be associated with continuous root exposure to nutrient solution, which can enhance nitrate uptake and accumulation in plant tissues [15], [16].

Biochemical traits differed between HYP and AER, indicating that the cultivation system affected the biochemical composition of *P. amboinicus* under the tested conditions. Previous studies have reported associations between soilless cultivation systems and nutrient partitioning, pigment content, antioxidant metabolism, and secondary metabolite accumulation [8], [17], [18]. These processes could contribute to the differences observed in the present study. However, nutrient uptake rates, root characteristics, and dissolved oxygen concentrations were not measured. The present results therefore do not establish whether nutrient transport or root-zone oxygen availability caused the recorded biochemical responses.



Figure 2. Representative growth performance of *Plectranthus amboinicus* cultivated under different soilless cultivation systems at 50 days after treatment

The upper left panel (A) shows plants grown under aeroponic system 1 with a misting cycle of 30 s on and 5 min off. The upper right panel (B) shows plants grown under aeroponic system 2 with a misting cycle of 30 s on and 15 min off.

The lower panel (C) shows plants cultivated under the hydroponic system.

3.3. Effects of hydroponic and aeroponic systems on plant performance of *Plectranthus amboinicus*

Relative change analysis demonstrated that aeroponic cultivation improved most physiological and biochemical characteristics of *P. amboinicus* compared with the hydroponic system (Table 3). AER1 showed the greatest enhancement in plant performance, particularly for branch number, DW, and total flavonoid content, which increased by 47.1%, 39.7%, and 50.8%, respectively, relative to hydroponics. Leaf number, FW, vitamin C, reducing sugars, and organic acids also increased significantly under AER1. AER2 showed similar trends, although the magnitude of improvement was generally lower than that observed in AER1. SPAD values increased moderately under both aeroponic treatments, while water content showed only minor reductions

compared with hydroponics. These results indicate that aeroponic cultivation promoted stronger vegetative growth and metabolite accumulation without major changes in plant water status (Figure 2).

Table 3. Relative changes in physiological and biochemical parameters in aeroponic systems compared with the hydroponic system.

No.	Parameter	AER1	AER2	No.	Parameter	AER1	AER2
1	Plant height	17.6	9.2	8	Water content	-0.7	-0.4
2	Leaf number	28.4	16.0	9	Dry matter	15.6	8.9
3	Branch number	47.1	29.4	10	Vitamin C	35.8	19.4
4	SPAD value	8.3	4.7	11	Reducing sugars	35.9	19.4
5	Leaf area	18.3	8.5	12	Organic acids	40.2	18.3
6	Fresh weight	20.9	11.4	13	Total flavonoids	50.8	26.5
7	Dry weight	39.7	22.0	14	Nitrate content	-26.3	-15.3

Relative changes were calculated based on the hydroponic treatment as the reference control using the formula: Relative change (%) = [(aeroponic value - hydroponic value) / hydroponic value] × 100. Positive values indicate increases relative to hydroponics, whereas negative values indicate reductions

Nitrate accumulation showed a negative relative change under both aeroponic systems, with reductions of 26.3% and 15.3% in AER1 and AER2, respectively, compared with HYP. Nitrate concentration decreased by 26.3% in AER1 and 15.3% in AER2 relative to HYP. Leaf nitrate concentration is determined by the balance among nitrate uptake, assimilation, and dilution through biomass accumulation [15], [16]. Thus, the lower nitrate levels under aeroponics may have resulted from enhanced nitrate assimilation, greater biomass production, or both, whereas continuous exposure to the nutrient solution may have maintained greater nitrate availability under HYP. Because nitrate reductase activity, total nitrogen uptake, and root-zone oxygen concentration were not measured, the specific mechanism underlying this response cannot be distinguished. In general, the short-interval misting regime used in AER1 produced greater positive changes than the longer-interval misting regime in AER2, which indicates that misting frequency influenced plant physiological and biochemical responses under aeroponic conditions.

3. Conclusions

The cultivation systems affected the growth, chlorophyll status, biomass, and measured biochemical traits of *P. amboinicus* during the 50-day experiment. AER1 produced the highest plant height, leaf number, branch number, leaf area, fresh weight, dry weight, and SPAD value. This treatment also produced the highest ascorbic acid, reducing sugar, titratable acidity, and total flavonoid contents and the lowest nitrate content. Water content did not differ significantly among treatments. Under the tested conditions, the AER1 misting regime was associated with the highest values for most of

the measured growth and biochemical parameters. Essential oil yield and composition, such as thymol and carvacrol contents, were not analyzed. Conclusions concerning the aromatic or medicinal quality of the plants therefore require further chemical analysis.

Note for contributor:

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- Author's contributions: T.T.T. Huyen: conceptualization, supervision; N. T. Thuy: methodology; C. D. Ha: conceptualization, data analysis, writing; D. H. Gioi: software, visualization; N.X. Thanh: conceptualization, review & editing.

Conflict of interest. The authors declare no conflict of interest.

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