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Optimizing Nutrient Solution Regime to Enhance Yield, Biochemical Composition, and Antioxidant Capacity in *Andrographis paniculata*

Running Title: The Role of Nutrient Solution Regime on Plant Growth Indices

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ABSTRACT

The quality and quantity of the nutrient solution and its application duration are critical factors influencing essential growth processes such as photosynthesis and enzyme activity in plants. This research sought to investigate the impact of nutrient supplementation on the yield, biochemical composition, and antioxidant systems of the medicinal plant *Andrographis paniculata* (AP). To achieve this, an experiment was conducted using a randomized complete block design (RCBD) by four nutrient solution regimes (every day, every three days, every five days, and every week) and five repetitions. Seedlings at the 8-leaf stage were placed in pots filled with a 50:50 mixture of sand and vermiculite, and maintained under greenhouse conditions for 30 days. The morphological analysis revealed that the highest height of the plant (55.20 cm) and leaf dry weight (6.12 g) were recorded after three days of nutrient solution application. Additionally, the comparison of root lengths indicated that the shortest and longest roots were observed after one and three days of nutrient solution application, respectively. The biochemical analysis demonstrated that the highest levels of chlorophyll, FRAP, total phenols, and flavonoids were achieved after three days of nutrient solution application. Consequently, the findings suggest that administering the nutrient solution every three days in a nutrient-deficient sandy medium is an effective strategy for enhancing the yield and physiological attributes of the *Andrographis paniculata* plant.

Keywords: Andrographis, Antioxidants, Flavonoids, FRAP, Proline

Abbreviation:

DPPH; 2,2-diphenyl-1-picrylhydrazyl, FRAP; ferric reducing antioxidant power, LDW; leaf dry weight, PH; plant height, RL; root length

INTRODUCTION

Andrographis paniculata is a notable medicinal species belonging to the Acanthaceae family. This erect annual herb is characterized by its dark green, four-angled stem, lance-shaped petiolate leaves, small white flowers, a long linear capsule, and tiny yellow-brown seeds [1]. The plant extract, rich in active principles, contains three main components, namely diterpenes, flavonoids, and stigmasterols [2, 3]. The plant has a wide range of properties pharmaceuticals including anti-HIV [4], anti-H1N1 [5], anticancer [6], anti-hepatitis [7], anti-inflammatory [8], blood purifier, and antidiarrheal [9]. These compounds are mainly produced by the aerial organs of the plant, especially the leaves.

In general, the growth and development of plants are influenced by their genotype, their environment, and the interaction of these factors. Therefore, one of the main environmental factors in agricultural planning to achieve high yield and optimal quality, especially in the case of medicinal plants, is the evaluation of different plant nutrition systems [10]. Nutrition during the vegetative phase, which is the basis of the generative phase, is very important. Adequate nutrition during vegetative growth improves the plant's ability to cope with adverse environmental conditions. The transition from the vegetative phase to the generative phase and meeting the plant's needs during this period will increase the number of fertile flowers and increase the number and weight of seeds in the plant, which ultimately leads to increased yield. Therefore, nutrient use will be more efficient when nutrients are carefully selected [11-13].

A critical aspect of plant nutrition is to maintain soil fertility, ensuring that it can supply nutrients in a timely and adequate manner for optimal plant growth [14]. Fertilizer applications should be carefully managed to reduce costs and mitigate environmental impacts [14]. Low soil fertility affects all aspects of plant growth, including photosynthesis [15], enzyme

activity and secondary metabolite production, root formation [16], and plant yield [17]. However, the impact of low soil fertility on nutrient content, particularly that of immunomodulatory compounds, is less well understood. Upon identifying a nutrient deficiency, whether through morphological or physiological indicators, visible symptoms, or tissue analysis, it must be corrected. In such cases, the foliar application of the necessary nutrient can serve as an effective strategy, particularly in soils where the availability of mineral nutrients diminishes swiftly following fertilizer application.

The nutrition of plants plays a critical role in plant growth and development. Both nutrient deficiencies and excesses can lead to significant secondary metabolite changes. Therefore, the concentration of these metabolites is likely to be closely linked to the availability of nutrients. The fundamentals of managing plant nutrition entail understanding the nutritional needs of plants and applying fertilizers to meet those needs [17]. Micronutrients are essential in minimal quantities, whereas macronutrients are required in greater quantities. Each nutrient has a specific function in plant growth and development. Deficiencies of common nutrients in plants can cause a variety of symptoms. To enhance the effective utilization of nutrients and minimize environmental repercussions, accurate application is essential. The internal levels of critical nutrients and their absorption have been thoroughly researched [11, 12, 17]. Adequate nutrition is one of the most important factors for plant growth and productivity. Proper nutrition is a critical element influencing plant growth and productivity. Sufficient nutritional intake leads to alterations in various physiological, biochemical, and phytochemical responses within plants, thereby enhancing all plant functions including improved photosynthesis, respiration, protein synthesis, and nucleic acid metabolism [17, 18].

Few studies have examined the concentration of nutrients and simultaneous nutrient solution regimes in the vegetative and generative phases of plant ponds [19]. This study aimed to examine the morphophysiological characteristics of *Andrographis paniculata* as influenced by nutrient solution regimes, to identify the ideal nutrient solution regimes necessary for satisfactory growth and optimal quality within the systems.

MATERIAL AND METHODS

Plant Materials and Growth Conditions

Andrographis paniculata seeds were obtained from the Medicinal Plant Research Center at Shahed University. The seeds underwent a surface sterilization process utilizing a 5% sodium hypochlorite solution for 5 minutes, as described by Talei et al., [20], and were subsequently rinsed thoroughly with distilled water. Following sterilization, the seeds were thoroughly rinsed with distilled water. Subsequently, each seed was placed in individual Petri dishes that were lined with filter paper and moistened with sterile water. These dishes were then incubated in a controlled growth chamber, maintaining a light/dark cycle of 14/10 hours at temperatures ranging from 28 to 30 °C, with a relative humidity of 65-75% [21]. Once the seedlings reached the two-leaf stage, they were transferred to a jiffy medium.

Experimental Technique

To investigate the impact of nutrient solution (NS) regimes on the morpho-physiological traits of *Andrographis paniculata*, a randomized complete block design (RCBD) was employed, consisting of four treatment groups (daily, every third day, every fifth day, and weekly) with five replications. Seedlings, aged 30 days, were transferred into pots containing a 50:50 mixture of sand and vermiculite. These pots were kept in a controlled greenhouse environment, featuring light/dark cycles at temperatures of 28/20°C, relative humidity at 75%, and light intensity ranged 300-400 µmol $m^{-2}s^{-1}$. Following a ten-day cultivation period, the plants were irrigated according to the specified NS regimes (modified Hoagland solution). After 30 days under varying NS conditions (resulting in plants nearly 70 days old and before flowering), measurements were taken for morpho-physiological characteristics, including plant height and root length (PH and RL), leaf dry weight (LDW), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and the total content of phenols and flavonoids.

Leaf Dry Weight

The leaves of the plant were collected and subsequently dried in an oven (Memmert, Germany) at a temperature of 55°C for 72 hours. The dried material was then cut into small fragments and ground into a fine powder.

Determination of Chlorophyll Content

The content of chlorophyll in the leaves was assessed by the Chlorophyll Meter XT SPAD-502 device. Measurements were conducted on three distinct types of leaves: young, mature, and old. The chlorophyll readings from these three leaves were averaged. Measurements were taken in the morning between 9 am and 11 am.

Determination of Proline Content

Proline content was determined utilizing the ninhydrin method as described by Bates et al., [22]. In this procedure, proline was extracted from 0.5 g of fresh leaf tissue using 10 mL of 3% sulfosalicylic acid, followed by filtration through Whatman No. 42 filters. The concentration of proline was then assessed using a Shimadzu model UV-1201 spectrophotometer at a wavelength of 520 nm.

Extraction of Antioxidant Compounds

To extract antioxidant compounds, 0.5 g of fresh leaves from each treatment was finely chopped and transferred into a 150 mL conical flask. Subsequently, 25 mL of distilled water was introduced, and the flask was sealed with aluminum foil. The samples were then subjected to shaking at room temperature for one hour in a dark environment. Following this incubation period, the samples were filtered through Whatman filter paper No. 2, and the obtained extracts were preserved in a refrigerator at -80 °C.

DPPH Free Radical Scavenging Assay

A total of 40 µL of the extract was combined with three mL of a 0.1 mM DPPH methanolic solution, following the procedure outlined by Wong et al., [23]. The resulting mixture was allowed to incubate at room temperature for 30 minutes. Subsequently, the initial absorbance of the DPPH solution in methanol was recorded using a Shimadzu UV-1201 spectrophotometer at a wavelength of 515 nm, continuing until a stable absorbance was reached.

Ferric Reducing Antioxidant Power (FRAP) Assay

An aliquot of 200 µL of the extract was combined with three mL of FRAP reagents, which were formulated using a combination of 300 mM sodium acetate buffer adjusted to pH 3. This solution included 6.10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and 20 mM iron (III) hexahydrate ($FeCl_3 \cdot 6H_2O$) in a ratio of 10:1:1. The resulting mixture was incubated in a water bath for 30 minutes at a temperature of 37°C [23].

Determination of Total Phenolic Compounds Content

The content of total phenolic compounds was assessed utilizing the methodology outlined by Marinova et al., [24]. Initially, one mL of the extract was diluted with 9 mL of distilled water and subsequently mixed with 10 mL of Folin-Ciocalteu phenolic reagent. After allowing the mixture to stand for 5 minutes, 10 mL of 7% Na_2CO_3 was added. The solution was further diluted to a final volume of 25 mL by incorporating 4 mL of distilled water and was permitted to incubate at room temperature for 90 minutes. Following this incubation period, the absorbance was recorded using a spectrophotometer at a wavelength of 750 nm.

Determination of Total Flavonoids

The total flavonoid content was evaluated using the methodology outlined by Marinova et al., [24]. A one mL sample of the extract was introduced into a flask containing four mL of distilled water, to which 0.3 mL of 5% $NaNO_2$ was subsequently added. After 5 minutes, 0.3 mL of 10% $AlCl_3$ was incorporated into the mixture, which was then brought to a final volume of 10 mL by incorporating an additional 2.4 mL of distilled water. The absorbance of the resulting solution was recorded at a wavelength of 510 nm.

Statistical Analysis

The initial raw data were subjected to normality testing using SPSS software version 26. Subsequently, the data were analyzed through variance analysis, and mean comparisons were conducted following Duncan's multiple range test at a significance level of $P \leq 0.05$.

RESULTS

Impact of Nutrient Solution Regimes on the Morphological Traits of *Andrographis paniculata*

The variance analysis indicated that the supply of nutrient solutions significantly influenced plant height (PH), root length (RL), and leaf dry weight (LDW) at a significance level of $P \leq 0.01$ (Table 1). The findings demonstrated a negative correlation between PH, RL, and LDW with the nutrient solution regimes one-month post-application ($P \leq 0.01$). Leaf dry weight served as an indicator for assessing the morphological response to nutrient solution application. After one month, a notable increase in leaf dry weight was observed across all plants subjected to nutrient solutions. The application of nutrient solutions every third day resulted in a significant enhancement of leaf dry weight by 17.11% in comparison to the control group.

Table 1 Analysis of variance on some morpho-physiological characteristics of *Andrographis paniculata* under different NS regimes.

S. O. V	df	Mean Square								
		PH	RL	LDW	T. Chl	Proline	DPPH	FRAP	T. Phenol	Flavonoid
NS regimes	3	387.87 **	15.78 **	4.26 **	99.82 *	128.88 *	2.56 ^{ns}	8.53 *	0.001 **	285.33 *
Test error	16	13.08	3.98	0.50	23.16	57.620	4.21	1.65	0.000	65.08
CV (%)	---	8.29	15.40	11.82	10.27	12.80	22.16	13.67	24.62	14.28

PH; plant height, RL; root length, LDW; leaf dry weight, T. Chl; total chlorophyll, DPPH; 2, 2-diphenyl-1-picrylhydrazyl, FRAP; ferric reducing antioxidant power assay, T. Phenol; total phenol.

The plants reached their peak height of 55.20 ± 0.58 cm on the third day following the application of the nutrient solution, while the minimum height recorded was 33.80 ± 1.36 cm on the seventh day. On the third day, there was a notable increase in plant height of 61.23% compared to the height measured after seven days of nutrient solution application (Fig. 1a). Furthermore, the results demonstrated a significant variation in root length across the different nutrient solution treatments, with the longest root length of 14.00 ± 1.02 cm observed after one day of nutrient solution application, and the shortest root length of 10.80 ± 1.02 cm recorded after three days (Fig. 1b). The differences in leaf dry weight due to the various nutrient solution treatments were found to be highly significant. Additionally, the plants exhibited the highest leaf fresh weight of 308.00 ± 12.41 g and the lowest of 174.00 ± 21.00 g at three and seven days of nutrient solution application, respectively. Ultimately, the maximum leaf dry weight recorded was 6.98 ± 0.3 g at three days, while the minimum was 4.74 ± 0.38 g at seven days of nutrient solution application. After three days of nutrient solution application, the leaf dry weight showed an increase of 67.90% compared to the weight measured after seven days (Fig. 1c).

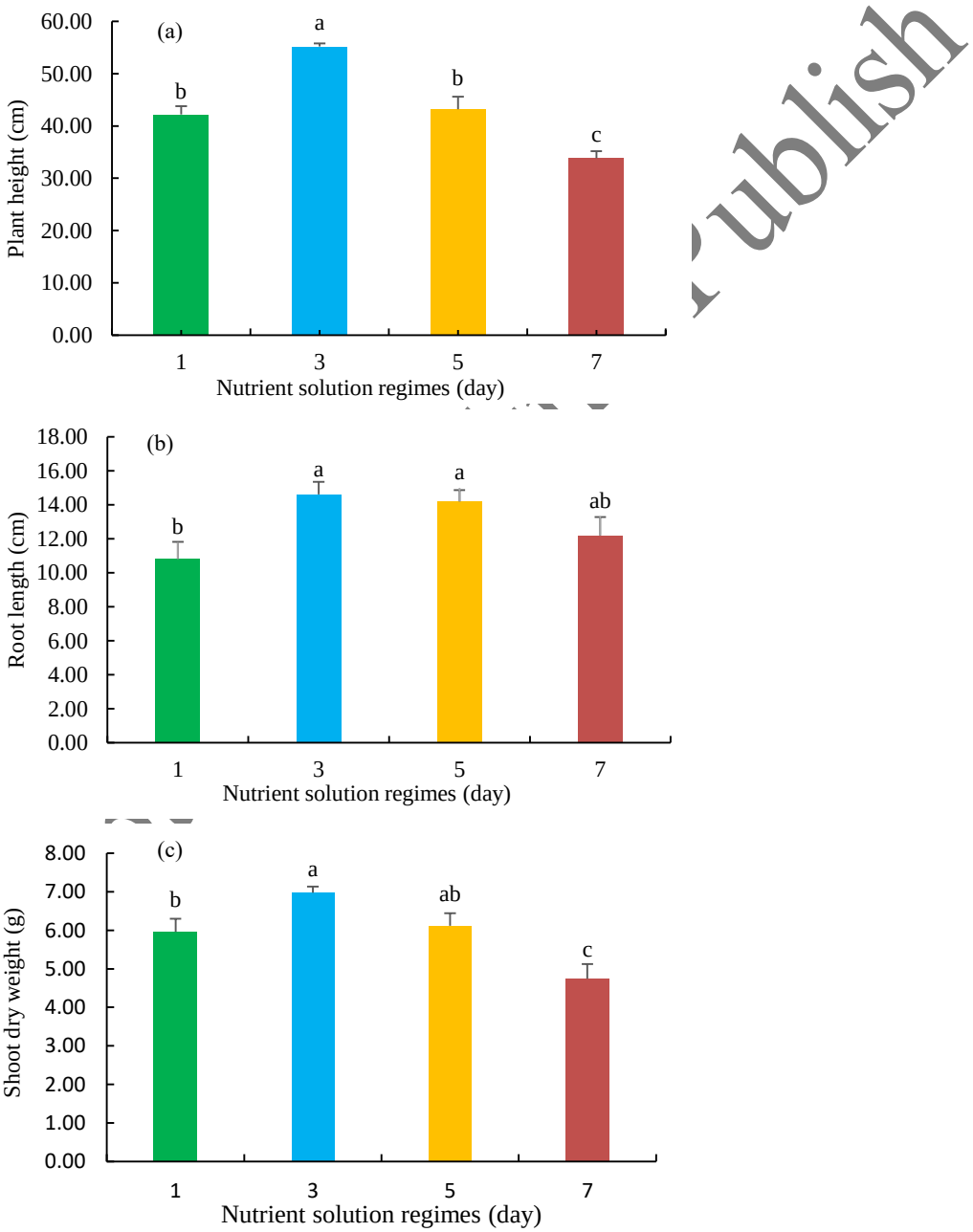

Fig. 1 Mean comparison of morphological traits in *Andrographis paniculata* under various nutrient solution (NS) regimes. a) plant height; b) root length and c) leaf dry weight. The error bars represent the standard error of the mean (SEM) based on three replicates, while distinct letters indicate significant differences in nutrient irrigation durations, as assessed by Duncan's multiple comparison test at P≤ 0.01.

Impact of Various Nutrient Solution Regimes on Chlorophyll and Proline Content in *Andrographis paniculata*

The variance analysis revealed that the supply of nutrient solutions significantly influenced chlorophyll and proline levels ($P \leq 0.05$) (Table 1). Chlorophyll and proline content served as indicators of the physiological response to nutrient solution application. After one month, a notable increase in both chlorophyll and proline content was observed across all plants subjected to nutrient solution feeding. The chlorophyll content significantly improved to 52.80 ± 2.71 µg. g^{-1} FW with a three-day NS regime compared to the control, while the proline content reached 218.57 ± 7.36 µg. g^{-1} FW with a five-day NS regime in comparison to the control. The highest chlorophyll content recorded was 52.80 ± 2.71 µg. g^{-1} FW at the three-day NS feeding, while the lowest was 40.67 ± 1.84 µg. g^{-1} FW at the one-day feeding (Fig. 2a). Proline content varied significantly among the different NS regimes, ranging from 66.67 ± 2.84 µg. g^{-1} FW at the five-day feeding to 218.57 ± 7.36 µg. g^{-1} FW at the one-day feeding (Fig. 2b).

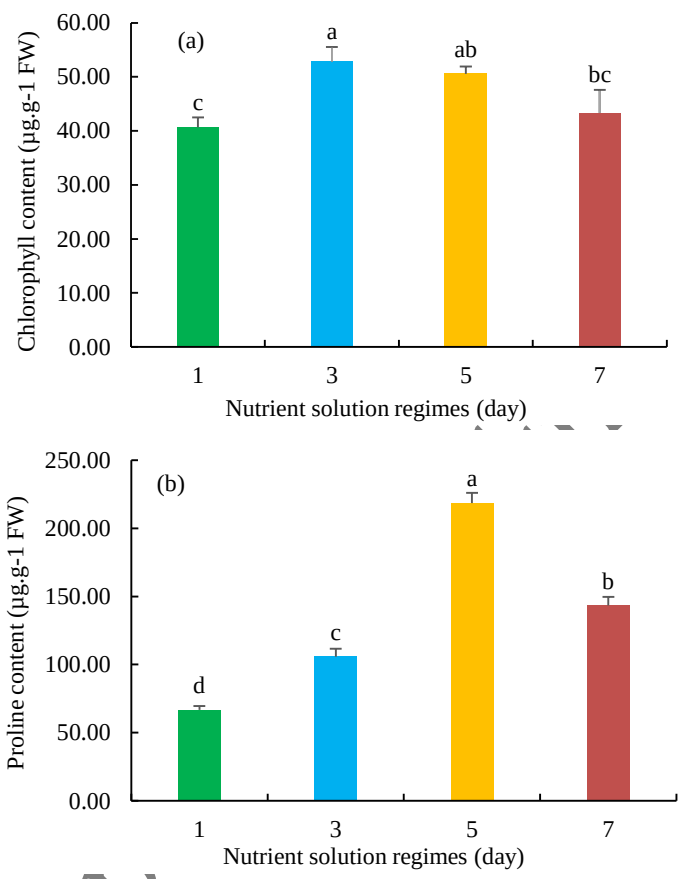

Fig. 2 Mean comparison of chlorophyll and proline content in *Andrographis paniculata* under various nutrient solution (NS) regimes. a) chlorophyll content and b) proline content. The error bars represent the standard error of the mean (SEM) based on three replicates, while distinct letters indicate significant differences in nutrient irrigation durations, as assessed by Duncan's multiple comparison test at $P \leq 0.01$.

Impact of Various Nutrient Solution Regimes on Antioxidant Systems in *Andrographis paniculata*

Analysis of variance indicated a notable difference among the various nutrient solutions regarding FRAP, total phenolic content, and flavonoid levels in the plants, whereas no significant differences were observed in DPPH content (Table 1). The highest values for total phenols, DPPH, and FRAP were recorded in plants subjected to three days of nutrient solution application. Although no significant differences were noted among the nutrient solutions concerning DPPH, the highest DPPH value (5.07%) was achieved during the three-day nutrient solution regime. The findings demonstrated a significant difference in FRAP levels across the different nutrient solution regimes ($P \leq 0.05$). Specifically, the highest FRAP value (96.03±0.63%) was observed at three days of nutrient solution feeding, while the lowest value (90.10±0.70%) was noted at one day (Fig. 3a). The total phenolic content varied significantly among the nutrient solution regimes, ranging from (0.071±0.01 mg/g) on the third day to (0.029±0.008 mg/g) on the seventh day of nutrient solution feeding (Fig. 3b).

Additionally, a significant difference was found in total flavonoid content across the different nutrient solution regimes, with the highest value (1982.67±13.71 mg/g) recorded at three days and the lowest (1823.00±12.93 mg/g) at one day of nutrient

solution feeding (P≤0.05) (Fig. 3d). Notably, the most frequent nutrient solution regime (everyday feeding) led to a significant reduction in both phenolic and flavonoid levels.

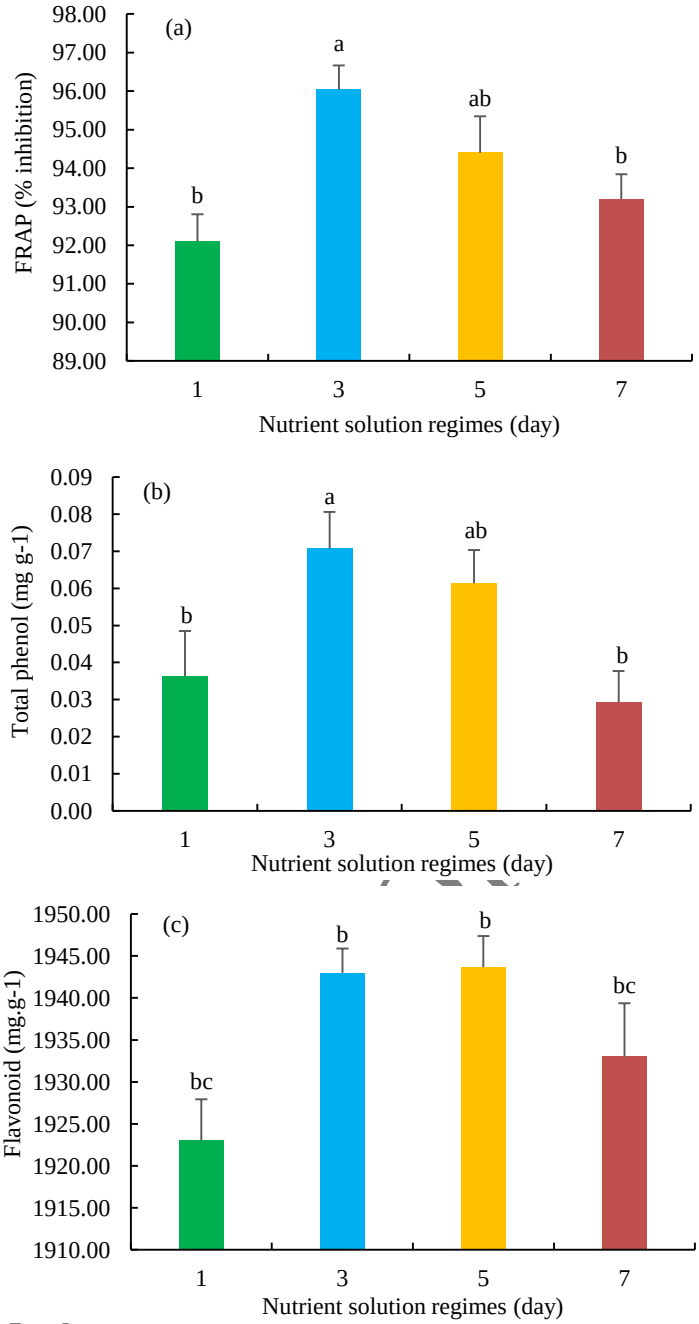

Fig. 3 Mean comparison of antioxidative systems in *Andrographis paniculata* under various nutrient solution (NS) regimes. a) FRAP; b) total phenol; and c) flavonoid compounds. The error bars represent the standard error of the mean (SEM) based on three replicates, while distinct letters indicate significant differences in nutrient irrigation durations, as assessed by Duncan's multiple comparison test at P≤ 0.01.

The correlation analysis revealed significant relationships among most of the measured characteristics at P≤0.01 (Table 2). A strong positive correlation was identified among the three morphological characteristics. Furthermore, a positive correlation was observed between FRAP content and both phenols and flavonoids. The relationship between chlorophyll content and antioxidant systems was also positively significant, whereas the correlation between proline content and antioxidant systems was negatively significant. The strongest positive correlation (r=0.880) was noted between chlorophyll content and FRAP, while the weakest negative correlation was found between proline content and leaf dry weight (r = -0.451).

Table 2 Correlation coefficient (r) among measured traits of *Andrographis paniculata* under different NS regimes.

	PH	LDW	RL	T. Chl	Proline	DPPH	FRAP	Phenol	Flavo
PH	1								
LDW	0.821 **	1							
RL	0.483 *	0.562 **	1						
T. Chl	0.444	0.604 *	0.519	1					
Proline	0.134	-0.451 **	0.168	0.395	1				
DPPH	0.139	-0.185	-0.314	0.719 **	-0.541 **	1			
FRAP	0.470	0.284	0.393	0.880 **	0.214	0.432	1		
Phenol	0.523	0.388	0.603 *	0.785 **	-0.742 **	0.213	0.601 *	1	
Flavo	0.477	0.125	0.421	0.357	0.644 **	0.545	0.582 *	0.576	1

The significant correlation is indicated by ** (P≤0.01) and * (P≤0.05). PH; plant height, LDW; leaf dry weight, RL; root length, *DPPH;* 2, 2-diphenyl-1-picrylhydrazyl*, FRAP; Ferric reducing antioxidant power assay, Flavo; Flavonoid*

Discussion

Andrographis paniculata is a significant medicinal plant that has been utilized in traditional medicine across South Asian countries. The plant's therapeutic properties are attributed to the presence of diterpenes, flavonoids, and stigmasterols. The nutrient solution supplies essential micro and macro elements, as well as various bioactive compounds, in a manner that promotes plant growth by enhancing the physical, chemical, and biological characteristics of the soil [25]. This study demonstrated that varying nutrient solution (NS) regimes had a considerable impact on the morphological traits and antioxidant systems of *Andrographis paniculata*.

The development of shoots and roots serves as essential indicators in agriculture, as demonstrated by various studies. The growth and progression of *Andrographis paniculata* are affected by varying nutrient solution conditions. Parameters such as plant height, root length, leaf dry weight, and antioxidant systems were modified under various NS conditions. This study aligns with the findings of Ma et al., [26] and Traenkner et al., [27], confirming that mineral nutrition plays a critical role in the growth and development of plants under different nutrient solution regimes. These findings are particularly significant, as plants that received the highest number of feeding days (seven days) exhibited reduced yields. The low dry matter content observed during the seven days of NS feeding suggests that the plant height may be linked to the reduced moisture content in plant tissues, potentially due to compromised root function. Our findings align with those of El-Nakhel et al., [28], who investigated the impact of macronutrient deficiency on lettuce plants and found that reduced nutrient availability led to a decrease in fresh weight. Similar observations were made by Murphy and Pill [29], Wieth et al., [30], and Pannico et al., [31] in their studies on arugula, red microgreens, and lettuce, respectively. Consequently, the outcomes of our research can be linked to the nutrient deficiency experienced by the plants subjected to more than three days of nutrient solution (NS) feeding.

The observed increase in antioxidant activity after three days of NS feeding corresponds with the rise in both fresh and dry weights noted during the same treatment period, as these factors, along with chlorophylls, are crucial pigments for the photosynthetic process [32]. The elevated antioxidant activity recorded during the three-day NS feeding can be attributed to the high levels of antioxidant compounds present in this treatment, including chlorophyll and phenolic compounds. Conversely, flavonoid compounds were found to accumulate more significantly in plants subjected to five days of NS feeding. It can be inferred that while flavonoids are recognized as key antioxidant components, other compounds may also play a role in the overall antioxidant mechanisms observed in plant studies [33]. Thus, although nutrient stress is anticipated to enhance the levels of phytochemical compounds as part of the plants' defense strategies against such stress [28, 32], the literature presents conflicting results, suggesting that plant responses to nutritional stress can vary based on species and the intensity of the stress.

One of the key mechanisms that plants employ in response to stress is the accumulation of compatible soluble substances, such as proline. Proline serves as an amino acid, osmoprotectant, and a crucial signaling molecule that accumulates within the cytosol of plants. It plays a vital role in stabilizing and safeguarding membranes, protein enzymes, and various proteins. In this investigation, it was observed that plants exhibited a significant increase in proline levels over five days of NS feeding. According to El Boukhari et al., [34], this accumulation of proline may serve a protective function against nutritional stress, safeguarding proteins from ionic damage and preventing the degradation of proteins, membranes, and subcellular structures. Numerous studies have indicated that proline accumulation is typically linked to stress adaptation in plants, with the enhanced production of secondary metabolites under stress conditions believed to offer cellular protection against oxidative damage [35-37].

The observed increase in leaf dry weight every three days of NS feeding can be attributed to the elevated levels of antioxidant compounds. During both short and long-term NS feeding, there was a decline in antioxidant compounds such as FRAP,

phenols, and flavonoids; however, during medium-term NS feeding (specifically on days three and five), there was an increase in the production of these antioxidant compounds. Our findings are consistent with those of Petropoulos et al., [12], who reported that ten NS treatments resulted in high yields and bioactive compounds, including polyphenols. Notably, the peak increase in antioxidant compounds was recorded at three days of NS feeding, which may be regarded as the critical threshold for NS feeding in this particular plant.

CONCLUSION

The utilization of a nutrient solution represents a valuable agricultural technique aimed at enhancing both yield and the concentration of antioxidant compounds. The levels of these antioxidant compounds in medicinal plants are shaped by genetic factors as well as environmental conditions, which are contingent upon the cultivation environment. Consequently, optimizing these growing conditions to maximize the production of antioxidant compounds is possible. Findings from this research indicate that a three-day application of nutrient solution feeding is an effective method for boosting yield, biochemical properties, and antioxidant levels in the *Andrographis paniculata* plant. This increase in the production of secondary metabolites, such as soluble flavonoids, plays a crucial role in safeguarding cellular structures against environmental stressors. Evidently, for medicinal plants intended for direct pharmaceutical use, the quality and concentration of active compounds hold greater significance than the overall yield.

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REFERENCES

1. Chen X., Ren J., Yang J., Zhu Z., Chen R., Zhang L. A critical review of Andrographis paniculata. Medicinal Plant Biology. 2023;2(1):1-12.
2. Valdiani A., Mihdzar A.K., Tan S.G., Talei D., Puad M.A., Nikzad S. Nain-e Havandi (Andrographis paniculata) present yesterday, absent today: A plenary review on underutilized herb of Iran's pharmaceutical plants. Molecular Biology Reports. 2012;39:5409-5424.
3. Rajani M., Shrivastava N., Ravishankara M.N. A rapid method for isolation of andrographolide from Andrographis paniculata Nees (Kalmegh). Pharmaceutical Biology. 2000;38(3):204-209.
4. Basak A., Banik U.K., Basak S., Seiah N.G., Li S. Evaluation of anti-proprotein convertase activity of diterpene andrographolid derived products. Regulation of carcinogenesis, angiogenesis and metastasis by the proprotein convertases (PCs): a new potential strategy in cancer therapy, Springer. 2006:137-154.
5. Ko H.C., Wei B.L, Chiou W.F. The effect of medicinal plants used in Chinese folk medicine on RANTES secretion by virus-infected human epithelial cells. Journal of Ethnopharmacology. 2006;107(2):205-210.
6. Chun J.Y., Tummala R., Nadiminty N., Lou W., Liu C. Andrographolide, an herbal medicine, inhibits interleukin-6 expression and suppresses prostate cancer cell growth Genes & Cancer. 2010;1(8):868-876.
7. Dumrongsak P., Nadege B., Catherine A., Georges M., Alail B., Helene M. Effect of Andrographis paninculata extract and andrographolide on hepatic cyt P450 mRNA expression and monoxygenase activity after in vivo administration to rats and in vitro in rat and human hepatic cultures. Chemico Biological Interactions. 2009;179(2-3):247-255.
8. Chao W.W., Kuo Y.H., Lin B.F. Anti-inflammatory activity of new compounds from Andrographis paniculata by NF-KB transactivation inhibition. J Agric Food Chem 2010;58(4):2505-12.
9. Kumar B., Verma S.K., Singh H. Effect of temperature on seed germination parameters in Kalmegh (Andrographis paniculata Wall. ex Nees.) Ind Crops Prod. 2011;34:1241-1244.
10. Bayer A., Whitaker K., Chappell M., Ruter J., van Iersel M., editors. Effect of irrigation duration and fertilizer rate on plant growth, substrate solution EC and leaching volume. International Symposium on Growing Media and Soilless Cultivation 1034; 2013.
11. Schjoerring J.K., Cakmak I., White P.J. Plant nutrition and soil fertility: synergies for acquiring global green growth and sustainable development. Springer; 2019; 1-6.
12. Petropoulos S.A., El-Nakhel C., Graziani G., Kyriacou M.C., Rouphael Y. The effects of nutrient solution feeding regime on yield, mineral profile, and phytochemical composition of spinach microgreens. Horticulturae. 2021;7(7):162.
13. Tang L. Soil Fertility, Plant Nutrition and Nutrient Management. MDPI; 2024;1- 34.
14. Withers P.J., Doody D.G., Sylvester-Bradley R. Achieving sustainable phosphorus use in food systems through circularisation. Sustainability. 2018;10(6):1804.
15. Rychter A.M., Rao I. Role of phosphorus in photosynthetic carbon metabolism. Handbook of photosynthesis. 2005;2:123-48.
16. Rao I.M., Miles J.W., Beebe SE, Horst WJ. Root adaptations to soils with low fertility and aluminium toxicity. Annals of Botany. 2016;118(4):593-605.
17. Smith M.R., Reis Hodecker B.E., Fuentes D., Merchant A. Investigating nutrient supply effects on plant growth and seed nutrient content in common bean. Plants. 2022;11(6):737.
18. Tozlu I., Moore G.A., Guy C.L. Effects of increasing NaCl concentration on stem elongation, dry mass production, and macro- and micro-nutrient accumulation in Poncirus trifoliata. Functional plant biology. 2000;27(1):35-42.
19. Garcia C.B., Grusak M.A. Mineral accumulation in vegetative and reproductive tissues during seed development in Medicago truncatula. Frontiers in Plant Science. 2015;6:155788.

20. Talei D., Mihdzar A.K., Khanif M.Y., Saad M.S., Valdiani A.R. Effects of different surface sterilizers on seed germination and contamination of king of bitters (Andrographis paniculata Nees.). American-Eurasian journal of agricultural and environmental sciences. 2011;10:639-643.
21. Talei D., Valdiani A., Maziah M., Sagineedu S.R., Abiri R. Salt stress-induced protein pattern associated with photosynthetic parameters and andrographolide content in Andrographis paniculata Nees. Bioscience, biotechnology, and biochemistry. 2014;(ahead-of-print):1-8.
22. Bates L.S., Waldren R.P., Teare I.D. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39(1):205-207.
23. Wong S.P., Leong L.P., Koh J.H.W. Antioxidant activities of aqueous extracts of selected plants. Food chemistry. 2006;99(4):775-783.
24. Marinova D., Ribarova F., Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy. 2005;40(3):255-260.
25. Martin C.C.S. Enhancing soil suppressiveness using compost and compost tea. Organic amendments and soil suppressiveness in plant disease management: Springer; 2015;25-49.
26. Ma Q., Bell R., Biddulph B. Potassium application alleviates grain sterility and increases yield of wheat (Triticum aestivum) in frost-prone Mediterranean-type climate. Plant and Soil. 2019;434:203-16.
27. Tränkner M., Tavakol E., Jákli B. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. Physiologia plantarum. 2018;163(3):414-431.
28. El-Nakhel C., Pannico A., Kyriacou M.C., Giordano M., De Pascale S., Rouphael Y. Macronutrient deprivation eustress elicits differential secondary metabolites in red and green-pigmented butterhead lettuce grown in a closed soilless system. Journal of the Science of Food and Agriculture. 2019;99(15):6962-6972.
29. Murphy C., Pill W. Cultural practices to speed the growth of microgreen arugula (roquette; Eruca vesicaria subsp. sativa). The Journal of Horticultural Science and Biotechnology. 2010;85(3):171-176.
30. Wieth A.R., Pinheiro W.D., Duarte TdS. Purple cabbage microgreens grown in different substrates and nutritive solution concentrations. Revista Caatinga. 2020;32:976-85.
31. Pannico A., Graziani G., El-Nakhel C., Giordano M., Ritieni A., Kyriacou M.C., et al. Nutritional stress suppresses nitrate content and positively impacts ascorbic acid concentration and phenolic acids profile of lettuce microgreens. Italus Hortus. 2020;27:41-52.
32. Becker C., Urlić B., Jukić Špika M., Kläring H.P., Krumbein A., Baldermann S., et al. Nitrogen limited red and green leaf lettuce accumulate flavonoid glycosides, caffeic acid derivatives, and sucrose while losing chlorophylls, β-carotene and xanthophylls. PloS one. 2015;10(11):e0142867.
33. Pereira C., Dias M.I., Petropoulos S.A., Plexida S., Chrysargyris A., Tzortzakis N., et al. The effects of biostimulants, biofertilizers and water-stress on nutritional value and chemical composition of two spinach genotypes (Spinacia oleracea L.). Molecules. 2019;24(24):4494.
34. El Boukhari M.E.M., Barakate M., Drissi B., Bouhia Y., Lyamlouli K. Seaweed extract biostimulants differentially act in mitigating drought stress on faba bean (Vicia faba L.). Journal of Plant Growth Regulation. 2023;42(9):5642-5652.
35. Karray-Bouraoui N., Harbaoui F., Rabhi M., Jallali I., Ksouri R., Attia H., et al. Different antioxidant responses to salt stress in two different provenances of Carthamus tinctorius L. Acta Physiologiae Plantarum. 2011;33:1435-1444.
36. Arif Y., Singh P., Siddiqui H., Bajguz A., Hayat S. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiology and Biochemistry. 2020;156:64-77.
37. Zhang Q., Dai W. Plant response to salinity stress. Stress physiology of woody plants: CRC Press; 2019;155-173.