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Optimal ammonium-nitrate ratio improves polyphenol content, antioxidant activities and nutrient accumulation in methanolic extracts of anthurium

Vanlalruati[1*], Heiplanmi Rymbai[1], SR Assumi[1], Hammylliende Talang[1], MB Devi[1], VK Verma[1], Gunjeet Kumar[2] and M.H. Varun[2]

[1]ICAR Research Complex for NEH Region, Umiam, Ribhoi District, Meghalaya 793 103, India

ABSTRACT

Anthurium is herbaceous genus of the Araceae family possessing significant medicinal values. The leaves were subjected to methanol extraction and further evaluated for their potential phytochemicals and antioxidants. Nutrient solution with high concentrations of NO_3^- resulted in taller plants, bigger leaf area, higher plant biomass and more photosynthetic content. The total phenolic content was determined by using the Folin-Ciocalteu assay and the plants supplied with the 25:75 ratio of NH_4^+:NO_3^- hydroponic solution accumulated maximum total phenol content (20.57±2.50 mg GAE/g FW). Antioxidant activity was calculated based on diphenyl picryl hydrazyl radical scavenging ability with highest antioxidant potential DPPH (0.88±0.40 IC_{50} mg/g) in 100:0 ratios of NH_4^+:NO_3 (IC_{50} 232.90 mg/g). Total flavonoid content was measured by the aluminium chloride colorimetric assay method in which plants treated with 25:75 ratio of NH_4^+:NO_3^- yielded maximum flavonoid content (38.51±1.08 mg QE/g dry weight) whereas 0:100 ratio of NH_4^+:NO_3^- nutrition accumulated maximum flavonol content (33.60±2.63 mg QE/g dry weight). The leaves from plants supplied with 100:0 ratio of NH_4^+:NO_3^- hydroponic solution yielded maximum sugar content (31.235±7.05 mg/g). High concentration of ammonium significantly exhibited greater amounts of antioxidant activity. However, plants with only NO_3^- nutrition showed a lower P and K accumulation than other treatments whilst highest N, Ca and Mg was observed with 25:75 ratios of NH_4^+:NO_3^-. A balanced NH_4^+:NO_3^- ratio (e.g., 25:75 or 50:50) contributes to improved morphological traits, antioxidant capacity and phytochemical content in anthurium.

Key words: Morphological traits, nutrition, macronutrients, flowering, antioxidant activity.

INTRODUCTION

The tropical anthurium (*Anthurium andreanum* Lind.), also known as 'Flamingo Flower' is a member of the family Araceae, which includes more than 100 genera and 1,500 species. It ranked 11th in the global flower trade and is next only to orchids among tropical flowers. It is native to tropical America, Mexico, Costa Rica, Cuba, Brazil and its growth habits vary depending on species (Chen *et al.,* 6). Soilless technology has been adopted for production of best quality flowers throughout the year as it allows a precise control of plant nutrition. Mineral nutrition influences the type and content of secondary metabolites that tends to accumulate in plants. Nitrogen is necessary for the biosynthesis of proteins and nucleic acids and bioavailability of phenolic compounds increased with increased nitrogen supply in wheat grain (Domínguez-Valdivia *et al.,* 9). Higher plants typically absorb two main types of nitrogen: ammonium (NH_4^+) and nitrate (NO_3^-) and an ideal ratio of nitrate to ammonium for plant growth and development is contingent upon various factors such as genotype, growth stage, environmental circumstances and the overall concentration of nitrogen supplied (Guo *et al.,* 14; Hernandez-Perez *et al.,* 17). While higher ratio of ammonium to nitrate in the nutrient solution decreased most growth indices in basil (*Ocimum basilicum*) (Kiferle *et al.,* 20), reducing of ammonium to nitrate ratio at the rate of 25:75 increased the roots fresh and dry weight, stem fresh and dry weight as well as biomass of *Spinacia oleracea* and tomato (Hao and Papadopoulos, 16). Numerous studies have demonstrated that anthurium has anti-inflammatory, antioxidant, and anti-bacterial potential. The flowers and leaves of *Anthurium serocampanense* have been used as an anti-inflammatory agent. Most research on anthurium fertilization primarily examines the impact of nutrient application on growth and yield parameters, with limited focus on phytochemical composition, particularly phenolics and flavonoids (Bartal *et al.,* 3). Therefore, a systematic investigation of variations in bioactive phytochemicals in anthurium is essential. With this background, the current study aims to investigate the effects of different ammonium (NH_4^+) to nitrate (NO_3^-) ratios on the morphological, physiological, and phytochemical properties of anthurium. The study also seeks to identify the optimal NH_4^+:NO_3^- ratio for enhancing growth, phytochemical content, and antioxidant activity in anthurium.

*Corresponding email : ruth.assumi@gmail.com

[2]ICAR-IARI, New Delhi -110 012

MATERIALS AND METHODS

The experiment was conducted at the ICAR Research Complex for the NEH Region, Umiam, Meghalaya, India (25.6649° N, 91.9029° E) during 2023–2024. To investigate the effect of different ratios of ammonium (NH_4^+) to nitrate (NO_3^-) (100:0, 75:25, 50:50, 25:75, 0:100) on the morpho-physiological and phytochemical properties of anthurium under greenhouse conditions, in a completely randomized design (CRD) with five treatments and four replications (five plants for each replication). Uniform anthurium plants (10-12 cm) of var. Tropical were procured from North Bengal Floritech, Siliguri, West Bengal and were planted in dutch bucket (30 cm × 25 cm × 22 cm) in soilless media incorporated with sterile LECA balls filled with aerated half-strength nutrient solution Hoagland and Arnon (18). The nutrient solutions recipe for each ratio as a treatment is shown in Table 1. Plants with similar physical size were subjected to the nutrient solutions after one week of planting with various NO_3^-/NH_4^+ ratios: T_1 (NH_4^+ : NO_3^- = 100:0),T_2 (NH_4^+ : NO_3^- = 25:75), T_3 (NH_4^+ : NO_3^- = 50:50) (as control), T_4 (NH_4^+ : NO_3^- = 75:25), and T_5 (NH_4^+ : NO_3^- = 0:100).

All anthurium plants were maintained under the concentrations of N (16mM), P (2mM), and K(6mM), Ca (4 mM), Mg (1mM) and S (1mM). Also, microelements were supplied according to Hoagland and Arnon (18). The pH of the nutrient solution was adjusted to 6.0 with dilute NaOH or HCl and was renewed at weekly interval. Morphological traits were measured at vegetative stage and during full flowering stage and averaged. Plant height (cm) was recorded using a measuring scale; spadix diameter was measured with digital vernier calliper (Mitutoyo 500-196-20- 150mm/6 inch Model), leaf area (LA) of the most recent fully expanded leaf was measured using a leaf area meter (LiCOR3100, Li-Cor, Lincoln, NE, USA). For determining dry matter (DM), shoots and roots were dried in a hot air oven at 70°C for 72 hrs and hand weighed. Treatment means were compared by Duncan's Multiple Range Test (DMRT) at 0.05 probability level. All statistical analysis was done By SAS version 9.2. Reducing sugars were estimated by Dinitrosalicylic acid (DNS) method and expressed as percentage. The total phenolic content was determined by using the Folin-Ciocalteu assay (Cakmak, 5) and expressed as mg gallic acid equivalents (GAE). Total flavonols in the leaf sample extracts were determined according to the method of Miliauskas *et al*. (25) and expressed as quercetin equivalent (mg QE/g). For determination of antioxidant potential (DPPH Free Radical Scavenging Ability), the reactive oxygen species scavenger of each extracts solution on DPPH radicals was resoluted. The antioxidant activity of the extract was expressed as IC_{50}. The antioxidant capacity of samples was determined and expressed as µmol TE/g DW (Ahamad *et al*., 2; Benzie and Strain, 4). A fully randomized design using five NH_4^+ NO_3 ratio treatments (100:0, 75:25, 50:50, 25:75, 0:100). All analyses were performed using SAS version 9.2 with a significant threshold of ±=0.05. Tukey's HSD test was used to confirm important comparisons. Components were held based on the description of emperor standards, flexion points in the scree diagram, and variance of at least 5%. Pearson's correlation coefficients were calculated to quantify the relationships between characteristics. The analysis was primarily performed in SAS 9.2 and was complemented with R 4.1.2 for additional analysis and visualization, thereby generating numbers using GraphPad Prism 9. This comprehensive approach provided robust detection of therapeutic efficacy and considered potential disruptive factors and data structure complexity.

Table 1: Nutrient solution composition for two N-form treatments; the concentrations are for making 1000 L of standard nutrient solution with distilled water

Fertilizer	NH_4^+ : NO_3^- ratios/ element concentration (mM L^{-1})				
	100:0	75:25	50:50	25:75	0:100
KNO_3	-	6	0	4	-
$Ca(NO_3)_2 \cdot 4H_2O$	-	4	4	4	4
$NH_4H_2PO_4$	-	2	-	-	-
KH_2PO_4	2	-	2	2	2
NH_4Cl	16	-	8	4	-
$MgSO_4 \cdot H_2O$	1	1	1	1	1
KCl	4	-	-	-	6
$CaCl_2$	4	-	-	-	-

RESULTS AND DISCUSSION

The different NH_4^+:NO_3^- ratios significantly (p < 0.05) affected the morphological and quality attributes of anthurium (Table 2). Plants treated with NH_4^+:NO_3^- ratios of 25:75 exhibited maximum plant height (31.75±2.39 cm), leaf area (183.38±6.79 sq.cm), fresh shoot biomass (66.133±1.42g) and fresh root biomass (36.158±1.42g) whereas lowest values were observed in the 75:25 ratio of NH_4^+:NO_3^- for all these morphological attributes. Numerous studies have demonstrated improved growth characteristics at increased nitrate ratios. In *Citrullus lanatus*, an increased in nitrate to ammonium ratios was observed to correspond with a rise in plant biomass while the presence of ammonium (NH_4^+) has been reported to have detrimental effect on the growth of *Ocimum basilicum* (Kiferle *et al.,* 20). Altered acid-base equilibrium, decreased protein glycosylation and energy expenditure in exporting excess ammonium are associated with ammonium toxicity (Liu *et al.,* 23). The lower dry matter content of plants in response to higher NH_4^+ as N source may be associated with decrease in plant height, leaf area, fresh weight of shoots and roots.

The different NH_4^+:NO_3^- ratios significantly (p < 0.05) affected the total phenolic content of anthurium (Table 3). Nutrient solution with high concentrations of NO_3^- resulted in greater amounts of phenolic content and the plants supplied with the 75:25 ratio of NH_4^+:NO_3^- accumulated maximum total phenols (20.57±2.50 mg GAE/g FW. The result of present study is in agreement with some previous works in pea (Chun *et al.*, 7). Ammonium might be altering intracellular acidity affecting the biosynthesis of metabolites including phenyl propanoid pathway (8). On a wide scale, high phenolic content is directly associated to increased plant resistance to pests and diseases, and thus precise regulation of N supplementation could provide agroecosystem benefits by reducing pesticide use in agriculture (Liu *et al.,* 23).

The total flavonoid and flavonol content of anthurium was significantly influenced (P < 0.05) by N forms on the macro and micronutrient concentrations (Table 3). The hydroponic solution with 25:75 ratio of NH_4^+ : NO_3^- resulted in maximum flavonoid content (38.51±1.08 mg QE/g dry weight whereas plants treated with 0:100 ratio of NH_4^+ : NO_3^- accumulated maximum flavonol content (33.60±2.63 mg QE/g dry weight). The concentration of flavonoid and flavonol decreased with increased proportion of NH_4^+ and lowest flavonoid and flavonol content in anthurium was recorded in plants treated with NH_4^+ : NO_3^- ratio of 100:0 (24.93±4.21 mg QE/g DW and 27.90±1.24 mg QE/g DW respectively). Nutrient solution with high NO_3^- levels resulted in greater accumulation of flavonoid and flavonol compounds, significantly. These results correspond to a phytochemical analysis in *Prunella vulgaris* (Fattahi *et al.,* 11; Marschner, 24).

Different ammonium and nitrate ratios of NH_4^+:NO_3^- (p < 0.01) had significantly affected the antioxidant capacity of anthurium, as determined by the FRAP and DPPH assay (Table 3). The highest antioxidant activity in both DPPH (0.88±0.40 IC_{50} mg/g) and FRAP (2.828±6.48 µmol TE/g dry weight) methods was exhibited by extracts of anthurium harvested from 100:0 ratios of NH_4^+:NO_3^- while the lowest antioxidant activity by was exhibited by 0:100 ratio of NH_4^+:NO_3^- by DPPH assay (0.32±0.03 IC_{50} mg/g) and 25:75 ratio of NH_4^+:NO_3^- by FRAP assay (2.196±6.39 µmol TE/g dry weight). High concentration of ammonium in the nutrient solution significantly exhibited a greater amounts of antioxidant activity in anthurium treated plants in the hydroponic solution. Our experiment is also in consistent with previous findings of various researchers (Fanasca *et al.,* 10; Jakovljevic *et al.,* 19). The study found that higher NO_3^- ratios resulted in increased accumulation of total phenolics, flavonoids, and flavonols, likely due to the efficient assimilation of NO_3^- into amino acids that serve as precursors for

Table 2: Morphological attributes of anthurium in response to different NH_4^+ : NO_3^- ratios under hydroponic system.

Treatment ($NH4^+$:$NO3^-$)	Plant height (cm)	Leaf area (Sq. m.)	Stalk diameter (cm)	Shoot biomass (g)	Root biomass (g)
T1(0:100)	27.13±2.09[ab]	142.20±19.16[b]	0.608±0.08[a]	60.440±3.11[a]	31.053±3.37[a]
T2 (25:75)	31.75±2.39[a]	183.38±6.79[a]	0.568±0.09[a]	66.133±1.42[a]	36.158±1.42[a]
T3 (50:50)	24.88±2.95[bc]	151.30±14.42[b]	0.613±0.13[a]	59.343±6.02[a]	29.268±5.93[a]
T4 (75:25)	20.75±2.66[c]	102.68±5.93[c]	0.495±0.06[a]	47.463±4.17[b]	17.013±3.82[b]
T5 (100:0)	27.63±3.98[ab]	165.30±6.12[ab]	0.588±0.04[a]	63.033±4.13[a]	33.108±4.21[a]

Table 3: Phytochemical and antioxidant capacity of anthurium in response to different NH_4^+ : NO_3^- ratios under hydroponic system.

Treatment (NH_4^+ : NO_3^-)	Total phenol content (mg GAE/g FW)	Flavonoid (mg QE/g DW)	Flavonol (mg QE/g DW)	DPPH (IC_{50} mg/g)	FRAP (µmol TE/g DW)	SPAD
T1 (0:100)	13.37±2.63[b]	31.61±1.10[b]	33.60±2.63[a]	0.32±0.03[c]	2.424±10.87[c]	60.09±1.36[a]
T4 (25:75)	20.57±2.50[a]	38.51±1.08[a]	31.08±0.81[a]	0.53±0.50[b]	2.196±6.39[bc]	57.09±1.07[a]
T3 (50:50)	9.98±3.00[b]	30.94±2.48[b]	29.24±0.60[a]	0.45±0.01[bc]	2.336±5.27[c]	54.52±2.29[a]
T2 (75:25)	12.15±1.88[b]	31.18±1.74[b]	30.11±1.07[a]	0.56±0.02[b]	2.637±8.05[b]	56.81±3.78[a]
T5 (100:0)	11.48±2.07[b]	24.93±4.2 [c]	27.90±1.24[a]	0.88±0.40[a]	2.828±6.48[a]	43.44±3.76[b]

the phenylpropanoid pathway (Fritz *et al.,* 12; Hachiya and Noguchi 15; Krapp *et al.,* 21).

Different nitrogen types have varying effects on plant photosynthetic rates and that higher levels of NO_3^- have increased effect on chlorophyll levels (Table 3). Anthurium plants fertilized with the 0:100 ratios of NH_4^+:NO_3^- exhibit highest photosynthetic pigments (60.09±1.36 SPAD) while lowest photosynthetic pigments (43.44±3.76 SPAD) was observed in 100:0 ratios of NH_4^+ : NO_3^-. These results are in conflict with some findings mentioned in other scientific papers and this may be due to unrevealed matters in uptake of both forms of N. Several previous studies reported that using NO_3^- as main N source, increases the availability of nitrogen and magnesium which enhanced photosynthetic content. Similar results were found by Liu *et al.* (22). Reduced rates of net photosynthesis were also reported in species *Catharanthus roseus* that receive ammonium as their primary nitrogen source as overconcentration of NH_4^+ has been linked to its detrimental impact on net photosynthetic rate (Guo *et al.,* 13). The reducing sugar was significantly influenced ($P < 0.05$) by N forms on the macro and micronutrient concentrations and their content in anthurium ranges from 15.465±2.25- 31.235±7.05 mg/g (Table 3). The leaves from plants supplied with 100:0 ratio of NH_4^+:NO_3^- hydroponic solution yielded maximum sugar content (31.235±7.05 mg/g) followed by 75:25 ratio of NH_4^+:NO_3^- (26.878±2.96). Sugar accumulation could be enhanced by fertilization and absorption of potassic fertilizers. Potassium (K) fertilization increased soluble sugar content by regulating trehalose metabolism.

Different nitrogen types have varying effects on macro and micronutrient content (Table 4). The anthurium plants treated with an NH_4^+:NO_3^- ratio of 25:75 exhibited the highest leaf N (3.403±0.21 %, leaf Ca (2.908±0.28 %) and leaf Mg content (2.885±0.15 %) while leaf P (1.24±0.14 %) and leaf K (3.95±0.32 %) contents was highest in NH_4^+:NO_3^- ratio of 100:0. According to earlier studies, plant species that received ammonium as their primary nitrogen source have higher concentrations of anions like P and lower concentrations of cations like Ca and Mg than species that are fed with nitrate as their primary nitrogen source. Applications of ammonium, however, can also improved accumulation of phosphate and sulfate while nitrate inhibits the uptake of these vital anions (Marschner, 24). Plants use K accumulation as a coping mechanism to mitigate NH_4^+ toxicity, which is most likely the cause of the high content of K in plants cultivated in high ammonium environments. The proportion of NH_4^+ : NO_3^- produced balanced N, P and K in plant organs as compared to the sole NO_3^- as N source (0:100 ratio of NH_4^+ : NO_3^-. Eustoma plants fed with NH_4^+ as a source of N showed a decrease in Mg and K contents (Hachiya and Noguchi 15). High NH_4^+ ratios were associated with increased reducing sugar content, likely due to enhanced potassium (K) uptake, as K plays a critical role in sugar metabolism and transport, indirectly influencing phenolic and flavonoid biosynthesis (Cakmak 5, Marschner, 24). These findings highlight the importance of optimizing NH_4^+ : NO_3^- ratios to enhance the medicinal and ornamental value of anthurium through improved phytochemical content and antioxidant activity.

Antioxidants and nutritional properties were grouped according to PC loadings. PCA was found effective in grouping the nutrient solution composition based on antioxidant content and nutrient properties of anthurium. Based on the PCA figures, nutrient solution compositions can be selected for specific antioxidant compounds and nutrient properties. A scree diagram showing the contribution of each component can be found in Figure 1. In PC1, almost all the nineteen assays have positive loadings except DPPH, FRAP, phosphorus and potassium whereas in PC2, DPPH, FRAP, flavonoids, total phenolic content, shelf life, SPAD, flavonols, nitrogen, magnesium and calcium showed positive loadings (Fig. 2). Out of the 19 principal component axis, four had Eigen values greater than one and all together accounted for 80.92% of the total variability. The first major component was 38.74% of the percentage dispersion, which was strongly reduced with plant height, leaf area, total carbohydrates, reduced sugar, firing biomass, root

Table 4: Biochemical and nutrient accumulation of anthurium in response to different NH_4^+ : NO_3^- ratios under hydroponic system.

Treatment (NH_4^+ : NO_3^-)	Reducing sugar (mg/g)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
T1 (0:100)	15.465±2.25[c]	3.048±0.33[ab]	0.398±0.01[c]	2.918±0.44[c]	1.065±0.21[a]	1.100±0.13[a]
T4 (25:75)	16.110±4.22[c]	3.403±0.21[a]	0.758±0.21[b]	3.650±0.13[ab]	2.908±0.28[a]	2.885±0.15[a]
T3 (50:50)	18.508±3.92[bc]	2.680±1.01[ab]	0.543±0.19[bc]	3.368±0.23[ac]	1.878±0.60[a]	1.920±0.40[a]
T2 (75:25)	26.878±2.96[ab]	2.380±0.45[ab]	0.433±0.11[bc]	3.260±0.10[ac]	0.948±0.26[b]	1.313±0.43[ab]
T5 (100:0)	31.235±7.05[a]	1.885±0.40[b]	1.240±0.14[a]	3.950±0.32[a]	0.640±0.17[b]	0.855±0.57[b]

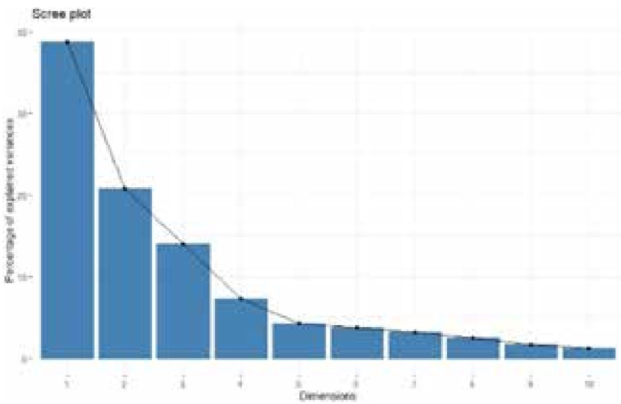

Fig. 1. Scree plot showing the contribution of each component of anthurium (*Anthurium andreanum* Lindl.).

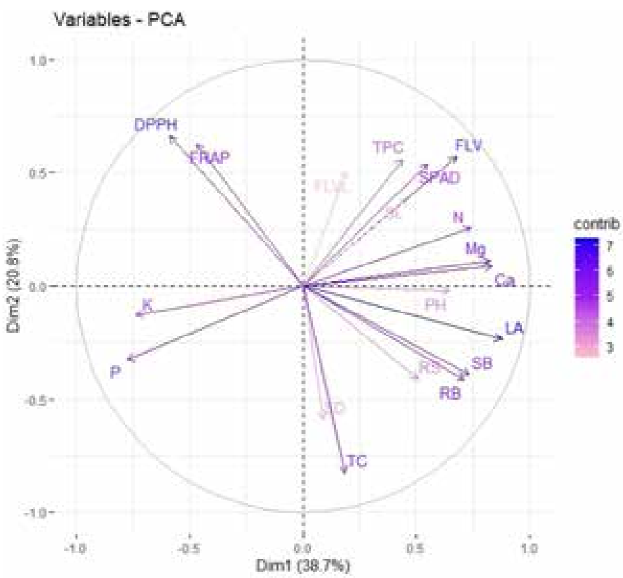

Fig. 2. Principal Component Analysis (PCA)-biplot of different Ammonium-Nitrate ratio treatments based on the variance in the growth and quality traits of anthurium. The first two components explained 38.7 % and 20.8 % of the variances, respectively. Arrows indicate the strength of the trait influence on the first two PCs. The varying color intensities and lengths of the arrows represent the contribution of the traits to the first two components in the PCA.

biomass, total phenol content (TPC), SPAD, sheet nitrogen content, leaf phosphorus content, and leaf catasium content. The second factor with a significant positive load accounted for 20.77% of the variation caused mainly by plant height, leaf surface, sugar reduction, bud biomass, root biomass, total phenol content and flavonoids and flavonoids. Variable correlation diagrams within circles at both factor levels represent articles of various quantitative and qualitative properties (Ahamad *et al.,* 1; Dixon and Paiva 8).

In the current study, positive and negative correlations were found between 19 different properties (Fig. 3). This figure shows that correlation coefficients with absolute measurements above 0.22 and 0.28 are statistically significant and are 5 or 1% probability. Significant correlations with "r" values above ±0.50 between quality indicate the relative effect of each character. The association analyses showed that plant height had a significant positive correlation with leaf area (r = 0.69), shoot biomass (r = 0.63), root biomass (r = 0.62), total phenol content (r = 0.58), r = 0.58). The leaf surface recorded a very significant positive correlation with shoot biomass (0.82), root biomass (r = 0.82), and a significant positive correlation with flavonoids (0.51), nitrogen (r = 0.55), calcium (r = 0.57), and magnesium (r = 0.59). A significant positive correlation was observed for stem diameters with total carbohydrates (r = 0.55). Very significant and positive correlations were recorded for shoot biomass with root biomass (r = 1.00) and nitrogen (r = 0.63). Significant and positive correlations were found for the overall phenol content with flavonoids (r = 0.67) and flavonols (r = 0.62). Flavonoids with SPAD (r = 0.65), nitrogen (r = 0.60) and Mg (r = 0.63); DPPH with FRAP (r = 0.56) and potassium (r = 0.53). SPAD showed a significant positive correlation with N (r = 0.54), Ca (r = 0.63), and Mg (r = 0.63), but a very significant but negative correlation with P (r = −0.83). Flavonols and FRAP showed no significant correlations with phytochemical

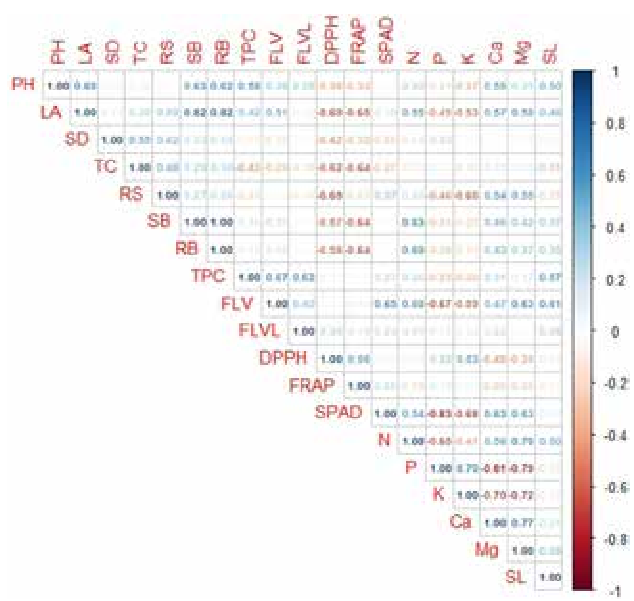

Fig. 3. Pearson correlation coefficients among 19 quantitative traits of anthurium evaluated during 2023-2024. The correlation coefficients with the absolute values more than 0.22 and 0.28 were significant at the statistical probability level of 5 and 1%, respectively.

and antioxidant content. Nitrogen showed a significant positive correlation with Ca (r = 0.56) and Mg (r = 0.70). P was significantly positively correlated with K (r = 0.70), while it was significantly but negatively correlated with Ca (r = −0.81) and Mg (r = −0.79). K was significantly negatively correlated with Ca (-0.70) and Mg (r = -0.72). Ca observed highly significant positive correlation with Mg (r=0.77).

The results revealed that higher NO^{3-} ratios improved morphological traits, phytochemical content, and chlorophyll levels, while higher NH^{4+} concentrations enhanced antioxidant activity and reducing sugar content. Additionally, higher NH^{4+} increased P and K levels while higher NO^{3-} boosting N, Ca, and Mg. A balanced NH^{4+} : NO^{3-} ratio (e.g., 25:75 or 50:50) yielded the best overall results. Management of nitrogen source could contribute to improved morphological traits, antioxidant capacity and phytochemical content in anthurium. In this study, nitrate and ammonium mixture gave better results than sole application of nitrate or ammonium., production and yield can be increased using a predominantly NO_3^- source fertilizer in the early vegetative growth stage.

AUTHORS' CONTRIBUTION

Conceptualization of research (V); Designing of the experiments (V,HR); Contribution of experimental materials and execution of field/ lab experiments, data collection (V, SA,HT); Analysis of data and interpretation (GK,VH); interpretation of manuscripts (V, MD,VV).

DECLARATION

The authors declares that they have no conflict of interest.

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