COMBINED EFFECTS OF HUMIC ACID AND CITRUS-SPECIFIC MICRONUTRIENT FORMULATIONS ON GROWTH AND NUTRIENT UPTAKE OF PUMMELO (*CITRUS GRANDIS* OSBECK.) SEEDLINGS

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Abstract. A study was conducted at the ICAR-Indian Institute of Horticultural Research, Bengaluru, to evaluate the combined effects of humic acid and a citrus-specific micronutrient formulation (Arka Citrus Special) on the growth and nutrient content of pummelo seedlings. This research, carried out during 2021-2022, used a factorial completely randomized design with four replications. Two-month-old seedlings were treated with six levels of humic acid (0, 1, 2, 3, 4 and 5 ml per liter) and five levels of micronutrients (0, 0.5, 1.0, 1.5 and 2 g per liter) under glasshouse conditions over four months. Results showed significant improvements in plant height, stem girth, leaf number, biomass, and root traits, with the most substantial growth observed at 2 ml per liter of humic acid and 2 g per liter of micronutrients, applied every 20 days. Increasing humic acid beyond this concentration resulted in decreased growth parameters. These optimal levels also significantly enhanced mineral nutrient concentrations in leaves, stems, and roots, suggesting that 2 ml per liter of humic acid and 2 g per liter of micronutrients optimally increase growth, biomass, and nutrient uptake. This facilitates early planting and improved field establishment of pummelo seedlings, potentially enhancing nursery management practices and seedling vigour for better crop establishment.
Keywords: *plant nutrition, root development, growth optimization, citrus seedlings, nutrient uptake, seedling vigour, biomass accumulation*

Introduction

Pummelo (*Citrus grandis* Osbeck.), as the largest of all citrus fruits, is the primary ancestor of grapefruit. It is botanically classified as *Citrus maxima* Merr. (synonyms: *C. grandis* Osbeck, *C. decumana* L.) and belongs to the subgenus Eucitrus, which includes commonly cultivated citrus species within the family Rutaceae (2n = 18). Known for producing the largest fruits among citrus species (Wen et al., 2010), pummelo was historically found growing wild along riverbanks in Fiji and surrounding islands. Introduced to China around 100 B.C., it is now widely cultivated across southern China, Japan, southern Thailand, Taiwan, India, Indonesia, and Malaysia. Southern Thailand and northern Malaysia are considered to be the likely centers of origin due to the high genetic diversity found in these regions (Wen et al., 2010). In India, pummelo is typically grown in home gardens, with maximum diversity observed in the North-East region, Bihar, and Bengal (Roy et al., 2014). Its growing popularity in India can be attributed to its high

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nutritional value and antioxidant properties. Furthermore, pummelo has played a significant role as a parent species for several citrus hybrids, including lemons, oranges, and grapefruits (Youseif et al., 2014).

Like other citrus fruits, pummelo is celebrated for its numerous health benefits due to its richness in essential vitamins and nutrients. It is particularly high in Vitamin C, B vitamins, Vitamin A, bioflavonoids, healthy fats, protein, fiber, antioxidants, and enzymes. Additionally, its high beta-carotene and folic acid content make it especially beneficial for pregnant women. The fruit's pulp has a subtle aroma, a sweet-sour flavor, and notable medicinal value. Traditionally, it has been used as an appetizer and is credited with antitoxic, cardiac stimulant, and stomach tonic properties, documented in ancient and medieval literature (Arias and Ramon, 2005). Citrus fruits, including pummelo, also provide essential minerals such as phosphorus, potassium, ascorbic acid, citric acid, folic acid, Vitamin B6, and flavonoids, further enhancing their nutritional and medicinal value (Dhuique-Mayer et al., 2007).

Nutrient management plays a critical role in supporting both the metabolic processes within the tree ecosystem and the production of high-quality fruit (Thamrin et al., 2014). Achieving optimal fruit quality and yield in pummelo requires a precise balance and availability of nutrients. Inadequate nutrient management, often caused by poor soil fertility, can lead to reduced yield and fruit quality (Zhuang, 1995). Leaf nutrient concentrations are valuable indicators of a plant's nutrient status, which correlates directly with growth and development patterns (Stebbins and Wilder, 2003). Generally, the growth of pummelo seedlings is slow, requiring at least one year before budding or grafting can be performed. The application of humic acid, micronutrients, and growth regulators plays a key role in promoting seedling development. Macronutrients such as nitrogen, phosphorus, and potassium are essential for processes like carbohydrate utilization, root development, stomatal regulation, and disease resistance. Secondary nutrients like calcium, magnesium, and sulfur, as well as micronutrients like iron, zinc, and boron, support chlorophyll formation, protein synthesis, and overall plant vigour.

Humic acid, in particular, influences plant growth by promoting root and shoot development, improving nutrient absorption, and enhancing physiological processes such as respiration, photosynthesis, and protein synthesis (Mora et al., 2010). It increases the availability of essential nutrients and boosts the levels of vitamins, amino acids, and growth hormones in plants (Chen et al., 1990; Yildirim et al., 2007). Studies such as those by Eisa et al. (2016) have demonstrated the positive impact of humic acid on the growth and leaf nutrient content of Nonpareil almond seedlings. Recognizing these benefits, the present study aims to explore whether applying humic acid and micronutrients at different concentrations can accelerate the growth of pummelo seedlings, thereby producing marketable plants more quickly and reducing production costs.

Materials and methods

Study location and experimental design

The present study was conducted at the ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta Lake Post, Bengaluru, during 2021-2022. The objective was to evaluate the combined effects of humic acid and micronutrients on the growth, biomass accumulation, and nutrient content in the leaf, stem, and root of four-month-old pummelo (*Citrus maxima*) seedlings. The experiment followed a factorial completely randomized design (FCRD) with two factors: six concentrations of humic acid (0, 1, 2, 3,

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4, and 5 mL/L) and five dosages of a citrus-specific nutrient mixture (0, 0.5, 1.0, 1.5, and 2 g/L). Different levels of humic acid and micronutrient mixture concentrations were selected to explore dose-response relationships and their effects on plant growth. The nutrient mixture used was Arka Citrus Special, specifically developed by ICAR-Indian Institute of Horticultural Research, Bengaluru for citrus crops, containing a balanced blend of essential macro- and micronutrients required for optimal growth and fruit production. The composition of the citrus-specific nutrient mixture is as follows: Zinc 3.0%, Boron 0.5%, Manganese 0.2%, Iron 0.5%, and Copper 0.05%.

Plant material, growing conditions and growth observation

Two-month-old pummelo seedlings were used in the experiment. They were raised in 15 (Dia) x 20 (H) cm polythene bags with four holes to ensure proper drainage and aeration. The growing medium comprised a mixture of soil, farmyard manure (FYM), and sand in a 2:1:1 ratio. The seedlings were grown under controlled glasshouse conditions for four months (120 days). Humic acid treatments were applied through drenching, while the micronutrient formulations were administered as foliar sprays. The first application was made when the seedlings were 60 days old (5-leaf stage), followed by two subsequent applications at 80 (7-leaf stage) and 100 days (9-leaf stage). Each treatment combination was replicated four times. At the end of the 120-day period, various growth parameters, including plant height (cm), stem girth (mm), number of leaves, root length (cm), root volume (cm^3), and fresh and dry biomass (g), were recorded.

Sample collection, preparation, and nutrient analysis

At the conclusion of the experiment, leaf, stem, and root samples were collected from the four-month-old pummelo seedlings for nutrient analysis. The samples were thoroughly cleaned and dried, initially on clean blotting paper, followed by oven drying at 68°C (Chapman and Pratt, 1961). Once dried, the leaf samples were ground using an electric mixer for further analysis. Nitrogen (N) content was determined using the Kjeldahl method (AOAC, 1970). Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were estimated using a tri-acid mixture (9:4 HNO_3: $HClO_4$) as per Jackson (1973). Phosphorus was quantified using the vanadomolybdo phosphoric acid yellow color method, with absorbance measured at 430 nm using a spectrophotometer. Potassium was measured using a flame photometer (Jackson, 1973), while calcium and magnesium were determined using Atomic Absorption Spectrophotometry (AAS) (Sarma et al., 1987). Micronutrients, including Fe, Mn, Cu, and Zn, were also analyzed using AAS (Sarma et al., 1987).

Statistical analysis

The data collected from the experiment were analyzed using SAS 9.3 statistical software (SAS Institute Inc., 2011). Analysis of variance (ANOVA) was conducted to evaluate the significance of the treatment effects on the measured growth and nutrient parameters. When significant differences were detected, treatment means were compared using the Least Significant Difference (LSD) test at a significance level of 0.01 to identify significant differences among treatment combinations.

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Results and discussion

Growth parameters

The application of citrus micronutrient formulation significantly enhanced the growth of four-month-old pummelo seedlings (*Table 1*). Plant height, stem girth, number of leaves, root length, and root volume all increased with higher concentrations of the formulation. The highest plant height (16.69 cm) and root volume (4.204 cm³) were recorded at 2.0 g/litre (C5). Stem girth peaked at 1.5 g/litre (C4) with 5.205 mm, and the number of leaves also increased, reaching a maximum (15.72) at C5. Higher doses improved plant height due to better availability of essential micronutrients like zinc, iron, and manganese, which support cell growth. Increased nutrient uptake enhanced stem girth, biomass, and stem strength. More leaves indicated better photosynthesis, boosting overall growth (Shukla et al., 1997; Hameed et al., 2018). Micronutrients application also strengthened root systems by enhancing metabolism and absorption.

Table 1. *Effect of citrus micronutrient formulation and humic acid on growth of 4 months old pummelo seedlings*

Treatments	Plant height (cm)	Stem girth (mm)	Number of leaves	Root length (cm)	Root volume (cm^3)
Citrus special dosage (C)					
C1 - 0 g/L	14.57 ^{b}	4.467 ^{b}	12.51 ^{b}	31.87 ^{b}	2.694 ^{b}
C2 - 0.5 g/L	15.16 ^{b}	4.296 ^{bc}	13.89 ^{b}	34.02 ^{b}	3.528 ^{b}
C3 - 1.0 g/L	16.43 ^{ab}	4.332 ^{bc}	14.28 ^{ab}	34.41 ^{ab}	3.741 ^{ab}
C4 - 1.5 g/L	16.67 ^{a}	5.205 ^{a}	14.56 ^{ab}	36.00 ^{a}	3.852 ^{ab}
C5 - 2.0 g/L	16.69 ^{a}	4.613 ^{ab}	15.72 ^{a}	37.32 ^{a}	4.204 ^{a}
SE(d)	0.720	0.164	0.595	1.514	0.303
CD @ 1%	1.419	0.324	1.172	2.984	0.598
Humic acid levels (T)					
T1 – water only	15.22 ^{a}	4.463 ^{a}	12.61 ^{c}	32.20 ^{a}	3.267 ^{c}
T2 – 1 ml/L	15.89 ^{a}	4.680 ^{a}	14.38 ^{bc}	35.96 ^{a}	3.822 ^{ab}
T3 – 2 ml/L	15.91 ^{a}	4.802 ^{a}	15.18 ^{a}	36.80 ^{a}	4.233 ^{a}
T4 – 3 ml/L	15.76 ^{a}	4.512 ^{a}	14.82 ^{ab}	35.58 ^{a}	3.633 ^{bc}
T5 – 4 ml/L	16.48 ^{a}	4.511 ^{a}	14.47 ^{b}	34.61 ^{a}	3.356 ^{b}
T6 – 5 ml/L	16.16 ^{a}	4.528 ^{a}	13.69 ^{c}	33.19 ^{a}	3.311 ^{b}
SE(d)	0.789	0.180	0.651	1.658	0.332
CD @ 1%	NS	NS	1.284	NS	0.655
C x T					
SE(d)	1.764	0.402	1.456	3.708	0.743
CD @ 1%	3.477	0.793	2.871	NS	1.465

Humic acid application positively affected seedling growth. The best performance for plant height (16.48 cm), root length (36.80 cm), and root volume (4.233 cm³) was observed at 2 ml/litre (T3), suggesting that moderate levels of humic acid optimize plant growth. Higher leaf counts were also seen with humic acid, supporting improved nutrient efficiency and vegetative growth. Humic acid improves nutrient availability, soil structure, and plant hormone production, explaining the increased plant height. It also boosts nutrient uptake, particularly nitrogen and phosphorus, leading to thicker stems through enhanced cell division. By improving plant metabolism and nutrient efficiency,

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humic acid promotes vegetative growth. Additionally, it enhances root growth by increasing nutrient mobility and root permeability, aiding better absorption.

The interaction between citrus micronutrients formulation and humic acid showed significant effects on plant height, stem girth, and leaf number, demonstrating a synergistic relationship. Drenching of humic acid @ 2ml/litre and foliar spray of citrus micronutrients formulation @ 2g/litre resulted in maximum plant height, stem girth, number of leaves, root length and root volume. Combined applications of these treatments further enhanced growth, particularly in root volume, indicating improved nutrient absorption and root mass development. This was mainly due to increase in cell enlargement and cell division with application of humic acid and micronutrients spray. This suggests a synergistic effect, where the combined application of micronutrients and humic acid enhanced plant growth beyond what was observed with individual treatments.

However, the interaction effect on root length was not significant, indicating that while both treatments improved root growth individually, their combined application did not result in further increases in root length. In contrast, root volume was significantly influenced by the interaction, with the highest values observed in treatments that combined higher concentrations of both humic acid and citrus micronutrient formulation. This suggests that while root length may have reached its potential under individual treatments, the combination of treatments enhanced root mass and density.

The observed results from the current study on pummelo seedlings align with the findings of previous researchers regarding the positive effects of growth-promotive substances. The increase in plant height, stem girth, and leaf number observed with higher concentrations of citrus micronutrients formulation and humic acid can be attributed to enhanced photosynthetic activity, better mobilization of photosynthates, and changes in membrane permeability, as reported by Shukla et al. (1997). The vigorous growth promoted by these substances may have facilitated greater branching and leaf production, leading to improved light interception and photosynthesis, similar to the effects seen in carambola (Marler and Mickelbert, 1992) and rangpur lime (Kawthalkar and Kunte, 1974).

Furthermore, the synergistic effects of micronutrients and humic acid likely invigorated physiological processes, enhancing water and nutrient uptake, and promoting faster leaf formation. This improved mobilization of nutrients and water, in turn, may have increased the production and translocation of photosynthetic products to various plant parts, contributing to the better overall growth and biomass accumulation observed in the seedlings. These results are in line with findings by Misra and Jaiswal (2001) in bael and Monselise and Halevy (1962) in citrus, who had also reported that growth-promoting chemicals significantly improve fresh and dry weight in plants. The combined application of citrus micronutrients and humic acid in pummelo seedlings not only mirrors previous studies but also emphasizes the potential of these substances to enhance plant growth through improved physiological processes and nutrient mobilization.

Biomass accumulation

The study demonstrates that applying citrus-specific nutrients and humic acid has a significant positive impact on the biomass accumulation of pummelo seedlings (*Table 2*). As the dose of citrus-specific nutrients increased, both fresh and dry biomass showed a corresponding rise. The control treatment (C1, 0 g/litre) resulted in a total fresh biomass of 8.741 g, while the highest dose (C5, 2.0 g/litre) produced 11.51 g. Similarly, the dry biomass increased from 3.146 g in C1 to 3.906 g in C5. This suggests that higher nutrient

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doses provide essential elements to enhance biomass accumulation, with 2.0 g/litre emerging as the optimal dosage. Beyond this point, the effectiveness levels off, indicating that further increases in nutrient concentration do not yield significantly higher growth.

Table 2. *Effect of humic acid and citrus micronutrient formulation on fresh and dry plant, root and total biomass of 4 months old pummelo seedlings*

Treatments	**Fresh biomass weight (g)**			**Dry biomass weight (g)**		
	Plant	**Root**	**Total**	**Plant**	**Root**	**Total**
Citrus special dosage (C)						
C1 - 0 g/L	5.722 b	3.019 c	8.741 c	2.214 c	0.932 c	3.146 c
C2 - 0.5 g/L	6.037 b	3.790 bc	9.827 bc	2.201 c	1.060 b	3.261 bc
C3 - 1.0 g/L	6.019 b	3.935 b	9.954 b	2.240 bc	1.034 b	3.274 b
C4 - 1.5 g/L	7.083 a	4.389 ab	11.47 ab	2.398 b	1.141 ab	3.539 ab
C5 - 2.0 g/L	6.843 ab	4.667 a	11.51 a	2.586 a	1.321 a	3.906 a
SE(d)	0.404	0.263	0.611	0.134	0.064	0.184
CD @ 1%	0.795	0.519	1.205	0.263	0.125	0.363
Humic acid levels (T)						
T1 – water only	5.644 c	3.844 a	9.489 c	2.222 a	1.014 a	3.236 a
T2 – 1 ml/L	6.633 ab	4.211 a	10.84 ab	2.340 a	1.174 a	3.513 a
T3 – 2 ml/L	6.820 a	4.111 ab	10.93 a	2.472 a	1.128 a	3.600 a
T4 – 3 ml/L	6.756 ab	4.126 ab	10.88 ab	2.375 a	1.105 a	3.480 a
T5 – 4 ml/L	6.378 b	3.778 b	10.16 b	2.293 a	1.089 a	3.382 a
T6 – 5 ml/L	5.933 c	3.689 b	9.622 c	2.266 a	1.074 a	3.340 a
SE(d)	0.442	0.288	0.67	0.146	0.07	0.202
CD @ 1%	0.871	NS	NS	NS	NS	NS
C x T						
SE(d)	0.989	0.644	1.497	0.327	0.156	0.451
CD @ 1%	1.949	1.270	2.951	0.645	0.307	0.890

Humic acid also positively influenced biomass production, with the most effective dose being T3 (2 ml/litre), which produced 10.93 g of fresh biomass and 3.600 g of dry biomass. The lowest biomass was observed in the control treatment (T1, water only), which yielded 9.489 g of fresh biomass and 3.236 g of dry biomass. These results indicate that moderate doses of humic acid improve nutrient uptake and growth, though further increases beyond 2 ml/litre do not result in additional biomass benefits. Application of humic acid showed that there is an increased root biomass which in turn has contributed to better nutrient uptake, enhances photosynthesis by stimulating chlorophyll synthesis and thus overall growth of pummelo. Cell division and increment in cells does remain to continue throughout the plant life but cell enlargement, especially of the stem, is directly responsive to exogenous application of nutrients in different plant genotypes (Ampong et al., 2022).

The combined application of citrus-specific nutrients and humic acid had the most significant impact on biomass. The highest fresh biomass (11.51 g) was recorded in the C5T3 treatment (2.0 g/litre citrus nutrients and 2 ml/litre humic acid), suggesting a synergistic effect between the two inputs. Citrus nutrients provide essential minerals, while humic acid enhances nutrient absorption, root development, and soil structure, creating optimal conditions for plant growth. The observed increase in biomass can be attributed to enhanced photosynthetic activity, better translocation of photosynthates, and changes in membrane permeability. The vigorous growth induced by these treatments facilitated the development of more branches, allowing for better sunlight capture,

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resulting in the formation of more leaves. The stimulation of physiological processes and the enhanced mobilization of water and nutrients at a faster rate promoted greater production of photosynthetic products, which were then translocated to different parts of the plant, leading to improved seedling growth and higher fresh and dry biomass (Hameed et al., 2018).

Furthermore, the application of micronutrients and humic acid played a key role in promoting root initiation, nutrient uptake, and root cell elongation, contributing to better root development. Zinc (Zn) enhanced cell division and elongation, accelerated the translocation of metabolites, and increased photosynthesis by activating carbonic anhydrase (Cakmak, 2008; Hatwar et al., 2003). Boron (B) indirectly improved photosynthesis by aiding carbohydrate metabolism, while iron (Fe) contributed to chlorophyll formation and enzyme activation, enhancing photosynthesis and respiration (Sohrab et al., 2013). Copper (Cu), acting as a cofactor for various enzymes, played a role in carbohydrate and nitrogen metabolism, further supporting physiological growth in citrus plants (Sharma and Agrawal, 2005; Stenico et al., 2009). Together, these micronutrients enhanced metabolic activity and contributed to improved biomass accumulation in the pummelo seedlings.

Mineral nutrient content

The application of citrus micronutrient formulation and humic acid significantly influenced the macronutrient and micronutrient content in the leaf, stem, and root tissues of four-month-old pummelo seedlings (*Tables 3-5*).

Leaf nutrient content

The citrus micronutrient formulation positively affected the nutrient status in leaves, with nitrogen (N) content peaking at 2.699% in C4 (1.5 g/L). Phosphorus (P) and potassium (K) levels increased to 0.17% and 3.207%, respectively, in C5 (2.0 g/L). Calcium (Ca) and magnesium (Mg) content also rose, with Ca reaching 2.444% in C5. Micronutrient absorption improved significantly, with iron (Fe) and manganese (Mn) reaching 246.9 mg/kg and 20.71 mg/kg, respectively, in C5. Humic acid further enhanced nutrient uptake, particularly at moderate levels (T3, 2 ml/L), where nitrogen reached 2.669% and potassium peaked at 3.149%. Pronounced increase in nitrogen and potassium, aligning with the findings from other studies that humic acid can enhance the availability and uptake of nutrients through improved root permeability and stimulation of root growth. Iron and manganese levels were also highest with humic acid, reaching 227.4 mg/kg and 20.79 mg/kg, respectively, in T5 (4 ml/L). The highest iron and manganese levels in T5 underscore humic acid's role in chelating nutrients and facilitating their transportation to aerial parts of the plant. The combined application of humic acid and micronutrient formulations resulted in overall better leaf nutrient status, supporting improved growth and biomass accumulation (Mohammed Ali et al., 2020).

Stem nutrient content

The application of micronutrient formulations significantly increased nitrogen content within the stems, which progressively rose to a peak of 1.683% in treatment C4. Concurrently, potassium and calcium levels reached maximum values of 0.945% and 0.723%, respectively, in treatment C5.

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Table 3. *Effect of humic acid and citrus micronutrient formulation on leaf mineral nutrient status of 4 months old pummelo seedlings*

Treatment	Macronutrient status (%)						Micronutrient status (mg kg^{-1})			
	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu
Citrus special dosage (C)										
C1 - 0 g/L	2.522 ^{c}	0.158 ^{b}	3.068 ^{b}	2.258 ^{a}	0.326 ^{a}	0.220 ^{c}	205.3 ^{c}	17.51 ^{b}	42.90 ^{e}	6.283 ^{d}
C2-0.5 g/L	2.579 ^{bc}	0.161 ^{ab}	3.003 ^{c}	2.316 ^{a}	0.348 ^{a}	0.224 ^{bc}	220.1 ^{bc}	17.99 ^{b}	49.73 ^{c}	6.778 ^{c}
C3 - 1.0 g/L	2.620 ^{ab}	0.164 ^{ab}	3.040 ^{bc}	2.289 ^{a}	0.342 ^{a}	0.224 ^{bc}	224.8 ^{b}	18.43 ^{b}	46.24 ^{d}	7.056 ^{b}
C4 - 1.5 g/L	2.699 ^{a}	0.170 ^{a}	3.155 ^{a}	2.397 ^{a}	0.335 ^{a}	0.233 ^{b}	231.6 ^{ab}	19.25 ^{ab}	69.41 ^{b}	7.628 ^{a}
C5 - 2.0 g/L	2.651 ^{a}	0.169 ^{ab}	3.207 ^{a}	2.444 ^{a}	0.352 ^{a}	0.248 ^{a}	246.9 ^{a}	20.71 ^{a}	73.17 ^{a}	7.639 ^{a}
SE(d)	0.034	0.004	0.032	0.14	0.011	0.005	8.347	1.041	0.971	0.168
CD @ 1%	0.069	0.008	0.063	NS	NS	0.010	16.74	2.087	1.946	0.337
Humic acid levels (T)										
T1 – water only	2.561 ^{b}	0.163 ^{a}	3.062 ^{b}	2.161 ^{a}	0.335 ^{a}	0.222 ^{b}	218.5 ^{a}	18.35 ^{a}	38.61 ^{d}	6.753 ^{c}
T2 – 1 ml/L	2.572 ^{b}	0.163 ^{a}	3.039 ^{b}	2.259 ^{a}	0.338 ^{a}	0.223 ^{b}	218.6 ^{a}	18.33 ^{a}	43.73 ^{cd}	6.893 ^{bc}
T3 – 2 ml/L	2.669 ^{a}	0.166 ^{a}	3.051 ^{b}	2.427 ^{a}	0.343 ^{a}	0.244 ^{a}	235.2 ^{a}	19.25 ^{a}	52.49 ^{a}	7.427 ^{a}
T4 – 3 ml/L	2.617 ^{ab}	0.165 ^{a}	3.149 ^{a}	2.436 ^{a}	0.343 ^{a}	0.241 ^{a}	228.6 ^{a}	18.35 ^{a}	52.87 ^{a}	7.387 ^{a}
T5 – 4 ml/L	2.613 ^{ab}	0.163 ^{a}	3.126 ^{ab}	2.395 ^{a}	0.34 ^{a}	0.224 ^{b}	227.4 ^{a}	20.79 ^{a}	46.05 ^{bc}	7.140 ^{ab}
T6 – 5 ml/L	2.651 ^{ab}	0.166 ^{a}	3.140 ^{a}	2.368 ^{a}	0.345 ^{a}	0.225 ^{b}	226.2 ^{a}	17.59 ^{a}	43.99 ^{c}	6.860 ^{bc}
SE(d)	0.038	0.005	0.035	0.154	0.012	0.005	9.144	1.140	1.063	0.184
CD @ 1%	0.076	NS	0.069	NS	NS	0.011	NS	NS	2.132	0.369
C x T										
SE(d)	0.084	0.021	0.077	0.344	0.026	0.012	20.446	2.549	2.378	0.412
CD @ 1%	0.169	0.01	0.155	NS	NS	0.024	41.001	5.111	4.768	0.825

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Table 4. *Effect of humic acid and citrus micronutrient formulation on stem mineral nutrient status of 4 months old pummelo seedlings*

Treatment	Macronutrient status (%)						Micronutrient status (mg kg^{-1})			
	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu
Citrus special dosage (C)										
C1-0 g/L	1.523^{b}	0.066^{b}	0.864^{c}	0.542^{c}	0.132^{a}	0.076^{b}	109.8^{a}	4.894^{a}	6.106^{e}	4.317^{c}
C2-0.5 g/L	1.565^{b}	0.075^{a}	0.888^{bc}	0.589^{bc}	0.139^{a}	0.085^{a}	99.64^{a}	4.233^{a}	8.233^{d}	4.478^{bc}
C3 - 1.0 g/L	1.626^{ab}	0.070^{b}	0.906^{ab}	0.605^{b}	0.149^{a}	0.085^{a}	99.49^{a}	3.472^{b}	9.089^{c}	4.722^{b}
C4 - 1.5 g/L	1.683^{a}	0.075^{a}	0.926^{ab}	0.615^{b}	0.142^{a}	0.091^{a}	106.5^{a}	3.456^{b}	11.94^{b}	5.189^{ab}
C5 - 2.0 g/L	1.646^{a}	0.075^{a}	0.945^{a}	0.723^{a}	0.155^{a}	0.092^{a}	101.6^{a}	3.317^{b}	12.98^{a}	5.583^{a}
SE(d)	0.032	0.003	0.02	0.044	0.012	0.004	4.300	0.330	0.423	0.238
CD @1%	0.066	0.005	0.04	0.089	NS	0.009	NS	0.661	0.849	0.476
Humic acid levels (T)										
T1 –water only	1.552^{b}	0.069^{a}	0.886^{a}	0.536^{bc}	0.131^{a}	0.086^{a}	103.5^{a}	3.687^{a}	9.253^{a}	5.013^{a}
T2 – 1 ml/L	1.574^{b}	0.073^{a}	0.889^{a}	0.667^{ab}	0.149^{a}	0.084^{a}	99.31^{a}	3.973^{a}	10.11^{a}	4.840^{a}
T3 – 2 ml/L	1.671^{a}	0.075^{a}	0.923^{a}	0.690^{a}	0.161^{a}	0.090^{a}	111.3^{a}	4.020^{a}	9.887^{a}	4.920^{a}
T4 – 3 ml/L	1.622^{ab}	0.073^{a}	0.917^{a}	0.616^{a}	0.141^{a}	0.085^{a}	99.35^{a}	3.760^{a}	9.693^{a}	4.760^{a}
T5 – 4 ml/L	1.614^{ab}	0.071^{a}	0.935^{a}	0.571^{b}	0.131^{a}	0.086^{a}	104.8^{a}	4.067^{a}	9.353^{a}	4.807^{a}
T6 – 5 ml/L	1.632^{ab}	0.071^{a}	0.885^{a}	0.608^{ab}	0.149^{a}	0.084^{a}	102.2^{a}	3.740^{a}	9.720^{a}	4.807^{a}
SE(d)	0.038	0.003	0.022	0.049	0.014	0.005	4.710	0.361	0.464	0.260
CD @1%	0.076	NS	NS	0.098	NS	NS	NS	NS	NS	NS
C x T										
SE(d)	0.064	0.006	0.049	0.109	0.03	0.011	10.532	0.808	1.037	0.582
CD @1%	0.139	0.013	0.098	0.219	NS	0.021	21.121	1.620	2.080	1.167

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Table 5. *Effect of humic acid and citrus micronutrient formulation on root mineral nutrient status of 4 months old pummelo seedlings*

Treatment	Macronutrient status (%)						Micronutrient status (mg kg^{-1})			
	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu
Citrus special dosage (C)										
C1-0 g/L	1.588 ^{b}	0.122 ^{b}	2.044 ^{bc}	0.814 ^{c}	0.229 ^{c}	0.192 ^{a}	745.7 ^{b}	35.88 ^{b}	13.82 ^{d}	10.72 ^{a}
C2-0.5 g/L	1.612 ^{b}	0.127 ^{b}	1.954 ^{c}	0.876 ^{c}	0.218 ^{c}	0.197 ^{a}	792.5 ^{b}	39.31 ^{a}	14.94 ^{c}	9.728 ^{a}
C3 - 1.0 g/L	1.604 ^{b}	0.120 ^{b}	2.089 ^{ab}	1.051 ^{b}	0.298 ^{ab}	0.195 ^{a}	864.5 ^{ab}	40.10 ^{a}	16.30 ^{b}	10.09 ^{a}
C4 - 1.5 g/L	1.599 ^{b}	0.126 ^{b}	2.186 ^{a}	1.160 ^{b}	0.274 ^{b}	0.193 ^{a}	865.9 ^{ab}	40.21 ^{a}	18.20 ^{a}	10.30 ^{a}
C5 - 2.0 g/L	1.714 ^{a}	0.170 ^{a}	2.027 ^{bc}	1.418 ^{a}	0.310 ^{a}	0.210 ^{a}	938.2 ^{a}	42.42 ^{a}	18.99 ^{a}	15.16 ^{a}
SE(d)	0.035	0.009	0.052	0.086	0.016	0.017	46.49	1.807	0.458	3.051
CD @1%	0.071	0.018	0.104	0.172	0.033	NS	93.24	3.624	0.919	NS
Humic acid levels (T)										
T1 – water only	1.574 ^{b}	0.121 ^{c}	1.935 ^{d}	0.989 ^{a}	0.260 ^{a}	0.164 ^{a}	794.2 ^{a}	38.16 ^{a}	15.98 ^{a}	9.593 ^{a}
T2 – 1 ml/L	1.651 ^{ab}	0.136 ^{ab}	2.012 ^{bc}	1.079 ^{a}	0.273 ^{a}	0.203 ^{a}	804.1 ^{a}	38.27 ^{a}	16.35 ^{a}	10.07 ^{a}
T3 – 2 ml/L	1.673 ^{a}	0.153 ^{a}	1.987 ^{c}	1.085 ^{a}	0.277 ^{a}	0.216 ^{a}	881.2 ^{a}	40.90 ^{a}	16.87 ^{a}	15.73 ^{a}
T4 – 3 ml/L	1.605 ^{ab}	0.133 ^{ab}	2.037 ^{bc}	1.137 ^{a}	0.259 ^{a}	0.201 ^{a}	880.9 ^{a}	39.21 ^{a}	16.48 ^{a}	11.13 ^{a}
T5 – 4 ml/L	1.592 ^{b}	0.122 ^{c}	2.153 ^{ab}	1.003 ^{a}	0.261 ^{a}	0.206 ^{a}	825.3 ^{a}	40.20 ^{a}	16.38 ^{a}	10.92 ^{a}
T6 – 5 ml/L	1.645 ^{ab}	0.133 ^{ab}	2.237 ^{a}	1.091 ^{a}	0.267 ^{a}	0.194 ^{a}	862.6 ^{a}	40.76 ^{a}	16.64 ^{a}	9.753 ^{a}
SE(d)	0.039	0.01	0.057	0.094	0.018	0.019	50.93	1.98	0.502	3.342
CD @1%	NS	0.019	0.114	NS	NS	NS	NS	NS	NS	NS
C x T										
SE(d)	0.086	0.021	0.128	0.21	0.04	0.041	113.892	4.427	1.123	7.474
CD @1%	0.173	0.043	0.256	NS	0.08	0.083	228.387	8.877	2.252	NS

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Notably, essential trace minerals such as zinc and copper also demonstrated significant increases at higher dosages, with zinc peaking at 12.98 mg/kg and copper at 5.583 mg/kg in C5. Furthermore, the application of humic acid was found to enhance the absorption of nutrients in the stems, particularly nitrogen, which reached 1.671% in T3 (2 ml/L), and potassium, which peaked at 0.923%. The presence of humic acid also facilitated increased concentrations of iron, which reached 111.3 mg/kg in T3 (2 ml/L). These findings indicate that the synergistic application of humic acid and micronutrient formulations not only enhances stem nutrient absorption but also supports nutrient translocation within the stems, contributing to overall plant vigor (Kalaivanan et al., 2023).

Root nutrient content

The application of combined treatments markedly enhanced root nutrient content. Specifically, nitrogen content escalated to 1.714% in treatment C5, and potassium increased to 2.237% in treatment T6 (5 ml/L). Both calcium and magnesium content showed positive responses, particularly at higher concentrations of the micronutrient formulation, peaking at 1.418% calcium in C5. Notably, iron content was significantly augmented by the treatments, reaching 938.2 mg/kg in C5, with humic acid application alone raising iron content to 881.2 mg/kg in T3. Additionally, the content of manganese and zinc also saw improvements, underscoring the beneficial impact of humic acid and micronutrients on root nutrient absorption and overall plant nutrition (*Figure 1*).

Figure 1. *Effects of humic acid and micronutrients applications on pummelo seedlings under glasshouse experiment*

Further investigations revealed that the optimal nutrient content for most minerals was achieved with the application of 2.0 ml/L of humic acid and 2 g/L of Arka Citrus Special at 20-day intervals. This correlation is primarily attributed to the enhanced plant growth observed within these specific treatment combinations, which promoted better mineral nutrient content. Analysis of mineral nutrient concentrations in the leaf, stem, and root tissues indicated that both humic acid drenching and micronutrient spray substantially influenced the concentration of most of the nutrients, particularly at the rate of 2 ml per liter of humic acid and 2 g per liter of Arka Citrus Special. Leaf tissues accumulated the highest levels of most nutrients, followed by roots and stems. However, roots of the pummelo seedlings stored more Fe, Mn, and Cu compared to leaves and stems. The

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interaction between treatments (C x T) demonstrated significant differences in nutrient content among the treatments, with the highest nutrient accumulation observed with the application of 2.0 ml/L of humic acid and 2 g/L of Arka Citrus Special.

Conclusions

The study indicates that applying 2 ml of humic acid combined with 2 g of citrus micronutrient formulation (Arka Citrus Special) at 20-day intervals, specifically from the 60^{th} to 100^{th} day, significantly enhances the growth, biomass accumulation, and nutrient content of pummelo seedlings. This regimen notably improves key growth parameters such as plant height, stem diameter, leaf production, and root development. The treatments not only enhance macronutrient and micronutrient uptake but also promote efficient nutrient translocation within the plant, leading to healthier and more robust seedlings. These results suggest that the combined application can accelerate seedling readiness for early marketing, planting, and better field establishment. The use of moderate to higher concentrations of both treatments proved optimal, indicating a synergistic effect that maximizes growth and biomass. This highlights the importance of precise dosage for achieving optimal results in raising the pummelo seedlings in nursery for rootstock purpose. Additionally, while the current study focuses on seedling development, it opens the possibilities for future research to explore the long-term benefits of these treatments on fruit yield, quality, and overall productivity in mature pummelo trees. Overall, the findings emphasize the significant role of citrus-specific micronutrient formulations and humic acid in boosting the growth and vigour of pummelo seedlings.

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