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Physiological and morphological performance of acid lime (*Citrus aurantifolia* Swingle) seedlings as influenced by leaf litter amendments and humic acid concentrations under nursery conditions

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Abstract

Production of healthy and vigorous seedlings is fundamental for the successful establishment of citrus orchards. The present investigation was undertaken to assess the combined effect of different leaf litter substrates and humic acid concentrations on the morphological growth and physiological efficiency of acid lime (*Citrus aurantifolia* Swingle) seedlings. The experiment was conducted under nursery conditions using a Randomized Block Design comprising thirteen treatments, including soil alone (control) and soil amended with mango, sapota, or karonda leaf scrap at different levels in combination with humic acid seed priming (1500 and 2000 ppm). Observations on growth and physiological parameters were recorded at 150 days after sowing (DAS). Among the treatments, soil amended with mango leaf scrap (100 g) and humic acid at 2000 ppm recorded significantly higher plant height (11.01 cm), number of leaves (13.22), and stem girth (1.86 mm) compared to the control. The same treatment also exhibited maximum Leaf Area Index (2.09) and Leaf Area Duration (32.43 cm² days), indicating enhanced photosynthetic capacity and sustained assimilatory activity. Improved root and shoot biomass accumulation resulted in higher seedling vigour indices and survival percentage. The study demonstrates that integration of mango leaf litter with an optimum concentration of humic acid can substantially improve seedling quality in acid lime nurseries.

Keywords: Acid lime, leaf area index, leaf area duration, seedling vigour, root–shoot ratio, organic amendments

1. Introduction

Acid lime (*Citrus aurantifolia* Swingle) is an economically important citrus crop cultivated extensively in tropical and subtropical regions of India. The success of commercial orcharding largely depends on the availability of uniform, vigorous, and physiologically efficient planting material. However, conventional nursery practices often result in seedlings with weak root systems, poor biomass accumulation, and low field establishment potential (Naik, 1949) [5]. Improving early seedling growth through sustainable and cost-effective nutrient management strategies is therefore essential.

In recent years, the use of organic residues and biostimulants has gained considerable attention in nursery production systems. Leaf litter amendments contribute to soil organic carbon, improve physical properties, and release essential nutrients during decomposition, thereby enhancing the rhizosphere environment. The nutrient composition and decomposition rate of leaf litter vary with botanical origin, which may influence seedling growth responses differently. Mango, sapota, and karonda leaf litters are commonly available organic residues in citrus-growing regions, yet their comparative effectiveness in citrus nurseries has not been adequately evaluated.

Humic acid, a major component of soil organic matter, is known to stimulate plant growth by enhancing nutrient availability, improving root membrane permeability, and promoting root proliferation (Nardi *et al.*, 2002) [6]. Several studies have reported that humic substances positively influence root architecture and biomass accumulation, leading to improved shoot

growth and physiological efficiency in fruit crops (Canellas *et al.*, 2002; Zandonadi *et al.*, 2007)^[3, 8]. Despite these documented benefits, information on the interactive effects of leaf litter substrates and humic acid concentrations on citrus seedling physiology remains limited.

The present study was therefore undertaken to evaluate the morphological and physiological responses of acid lime seedlings to different leaf litter amendments in combination with humic acid seed priming, to identify an optimum nursery management practice for producing high-quality planting material.

2. Materials and Methods

2.1 Experimental site and plant material

The experiment was conducted at the nursery of Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, Madhya Pradesh. Seeds of acid lime cv. *Kagzi* were used for the study.

2.2 Treatments and experimental design

The experiment consisted of thirteen treatments laid out in a Randomized Block Design with three replications. The treatments included soil alone as control and soil amended with mango, sapota, or karonda leaf scrap at 100 g or 200 g per kg of soil, combined with humic acid seed priming at two concentrations (1500 ppm and 2000 ppm). Leaf scrap materials were shade-dried, powdered, and thoroughly mixed with the potting soil before sowing.

2.3 Seed priming and nursery management

Seeds were primed in respective humic acid solutions for the prescribed duration, shade-dried, and sown in nursery containers. Uniform cultural practices were followed throughout the experimental period.

2.4 Observations recorded

Morphological parameters such as plant height, number of

leaves, stem girth, and fresh and dry weights of shoots and roots were recorded at 150 DAS. Physiological parameters, including Leaf Area Index (LAI) and Leaf Area Duration (LAD), were calculated using standard growth analysis procedures (Watson, 1952)^[7]. Derived indices such as root-shoot ratio, Seedling Vigour Index I and II, and survival percentage were computed using established formulae.

2.5 Statistical analysis

The data were subjected to analysis of variance (ANOVA) and treatment means were compared at a 5 per cent level of significance.

3. Results and Discussion

3.1 Plant Height

Plant height of acid lime seedlings was significantly influenced by the combined application of leaf litter substrates and humic acid concentrations at 150 DAS. The treatment comprising soil amended with mango leaf scrap (100 g) along with humic acid seed priming at 2000 ppm recorded the maximum plant height (11.01 cm), which was significantly superior to the control treatment (6.07 cm). Other treatments receiving humic acid and leaf litter also showed improved growth, though to a lesser extent.

The enhanced plant height observed under mango leaf scrap combined with humic acid may be attributed to improved nutrient availability and stimulation of cell elongation processes. Humic acid is known to enhance nitrogen uptake and activate metabolic pathways associated with vegetative growth, while decomposing mango leaf litter likely supplied essential nutrients in a sustained manner. Similar improvements in seedling height following humic acid application have been reported in citrus and other perennial fruit crops (Nardi *et al.*, 2002; Mosa *et al.*, 2020)^[6, 4].

Table 1: Effect of leaf scrap and humic acid on plant height (cm) (30, 60, 90, 120, 150 DAS)

Treatment	30DAS	60DAS	90DAS	120DAS	150DAS
T ₁ - Control	0.80	1.62	3.14	3.77	6.07
T ₂ - Soil + Mango leaf scrap 100 g+ humic acid 1500 ppm	1.22	2.43	4.30	6.92	9.85
T ₃ - Soil + Mango leaf scrap 100 g+ humic acid 2000 ppm	2.17	3.24	4.82	9.82	11.01
T ₄ - Soil + Mango leaf scrap 200 g+ humic acid 1500 ppm	1.70	2.56	3.30	4.69	6.85
T ₅ - Soil + Mango leaf scrap 200 g+ humic acid 2000 ppm	1.40	2.29	3.56	7.76	8.10
T ₆ - Soil + Sapota leaf scrap 100 g+ humic acid 1500 ppm	1.10	3.02	4.22	8.99	9.76
T ₇ - Soil + Sapota leaf scrap 100 g+ humic acid 2000 ppm	1.30	2.49	4.23	8.45	9.00
T ₈ - Soil + Sapota leaf scrap 200 g+ humic acid 1500 ppm	0.90	2.46	3.82	7.74	8.62
T ₉ - Soil + Sapota leaf scrap 200 g+ humic acid 2000 ppm	1.67	2.51	4.07	6.07	8.62
T ₁₀ - Soil + Karonda leaf scrap 100 g+ humic acid 1500 ppm	1.59	2.48	4.10	5.86	8.18
T ₁₁ - Soil + Karonda leaf scrap 100 g+ humic acid 2000 ppm	0.62	2.43	3.50	4.80	7.60
T ₁₂ - Soil + Karonda leaf scrap 200 g+ humic acid 1500 ppm	1.50	2.60	3.75	4.59	7.47
T ₁₃ - Soil + Karonda leaf scrap 200 g+ humic acid 2000 ppm	1.02	2.24	3.83	5.78	7.98
SEM ±	0.06	0.13	0.17	0.19	0.26
CD @ 5%	0.16	0.39	0.49	0.55	0.76
CV	7.40	9.34	7.47	5.02	5.40

3.2 Number of Leaves

The number of leaves per seedling varied significantly among treatments. Seedlings grown in soil amended with mango leaf scrap (100 g) and humic acid at 2000 ppm produced the highest number of leaves (13.22), nearly double that recorded in the control (6.78). Increased leaf production was also observed in other humic acid-treated combinations, though mango leaf scrap proved most effective.

An increase in leaf number reflects improved meristematic

activity and nutrient status of the seedlings. Humic substances have been reported to enhance chlorophyll synthesis and nitrogen assimilation, which support leaf initiation and expansion. In addition, mango leaf litter may have improved soil microbial activity, thereby facilitating better nutrient mineralization and uptake. Comparable findings were reported by Adelani (2023)^[1], who observed increased leaf production in citrus seedlings grown with organic leaf litter amendments.

Table 2: Effect of leaf scrap and humic acid on number of leaves (30, 60, 90, 120, 150 DAS)

Treatment	30DAS	60DAS	90DAS	120DAS	150DAS
T ₁ - Control	1.02	2.11	4.77	5.63	6.78
T ₂ - Soil + Mango leaf scrap 100 g+ humic acid 1500 ppm	1.40	2.97	5.73	8.67	11.13
T ₃ - Soil + Mango leaf scrap 100 g+ humic acid 2000 ppm	2.02	3.67	7.06	10.23	13.22
T ₄ - Soil + Mango leaf scrap 200 g+ humic acid 1500 ppm	1.43	2.83	6.27	6.77	7.43
T ₅ - Soil + Mango leaf scrap 200 g+ humic acid 2000 ppm	1.95	3.51	5.83	7.50	9.72
T ₆ - Soil + Sapota leaf scrap 100 g+ humic acid 1500 ppm	2.01	3.43	6.82	9.40	12.23
T ₇ - Soil + Sapota leaf scrap 100 g+ humic acid 2000 ppm	1.63	3.15	6.13	9.23	11.22
T ₈ - Soil + Sapota leaf scrap 200 g+ humic acid 1500 ppm	1.85	3.03	6.57	8.07	9.52
T ₉ - Soil + Sapota leaf scrap 200 g+ humic acid 2000 ppm	1.91	3.50	6.77	9.31	11.37
T ₁₀ - Soil + Karonda leaf scrap 100 g+ humic acid 1500 ppm	1.27	3.47	5.03	7.04	9.07
T ₁₁ - Soil + Karonda leaf scrap 100 g+ humic acid 2000 ppm	1.10	3.49	5.53	7.01	8.08
T ₁₂ - Soil + Karonda leaf scrap 200 g+ humic acid 1500 ppm	1.97	2.90	6.61	7.15	7.77
T ₁₃ - Soil + Karonda leaf scrap 200 g+ humic acid 2000 ppm	1.90	3.37	6.20	8.59	10.07
SEM ±	0.12	0.16	0.16	0.12	0.26
CD @ 5%	0.34	0.47	0.46	0.35	0.75
CV	12.24	8.77	4.44	2.59	4.51

3.3 Stem Girth

Stem girth, an important indicator of seedling sturdiness and field establishment potential, was significantly affected by the treatments. The maximum stem girth (1.86 mm) was recorded in seedlings treated with mango leaf scrap (100 g) and humic acid at 2000 ppm, compared to 1.23 mm in the control. The increase in stem girth under integrated treatments indicates improved structural development of seedlings.

The improvement in stem thickness can be linked to enhanced translocation of assimilates and better vascular tissue development under humic acid application. Humic substances are known to stimulate enzymatic activity and secondary growth, leading to stronger seedling stems. These observations are in agreement with earlier reports highlighting the positive role of humic acid in improving stem diameter in fruit crops (Canellas *et al.*, 2002)^[3].

Table 3: Effect of leaf scrap and humic acid on stem girth (mm) (30, 60, 90, 120, 150 DAS)

Treatment	30DAS	60DAS	90DAS	120DAS	150DAS
T ₁ - Control	0.46	0.84	1.12	1.18	1.23
T ₂ - Soil + Mango leaf scrap 100 g+ humic acid 1500 ppm	0.65	1.21	1.18	1.46	1.60
T ₃ - Soil + Mango leaf scrap 100 g+ humic acid 2000 ppm	0.88	1.25	1.49	1.63	1.86
T ₄ - Soil + Mango leaf scrap 200 g+ humic acid 1500 ppm	0.60	1.23	1.35	1.42	1.58
T ₅ - Soil + Mango leaf scrap 200 g+ humic acid 2000 ppm	0.65	1.07	1.34	1.41	1.46
T ₆ - Soil + Sapota leaf scrap 100 g+ humic acid 1500 ppm	0.87	1.25	1.35	1.49	1.63
T ₇ - Soil + Sapota leaf scrap 100 g+ humic acid 2000 ppm	0.73	1.15	1.17	1.43	1.45
T ₈ - Soil + Sapota leaf scrap 200 g+ humic acid 1500 ppm	0.65	1.15	1.20	1.44	1.45
T ₉ - Soil + Sapota leaf scrap 200 g+ humic acid 2000 ppm	0.82	1.09	1.24	1.27	1.48
T ₁₀ - Soil + Karonda leaf scrap 100 g+ humic acid 1500 ppm	0.55	1.12	1.16	1.23	1.26
T ₁₁ - Soil + Karonda leaf scrap 100 g+ humic acid 2000 ppm	0.44	1.04	1.22	1.29	1.30
T ₁₂ - Soil + Karonda leaf scrap 200 g+ humic acid 1500 ppm	0.52	1.02	1.13	1.26	1.27
T ₁₃ - Soil + Karonda leaf scrap 200 g+ humic acid 2000 ppm	0.65	1.21	1.19	1.25	1.26
SEM ±	0.03	0.06	0.05	0.06	0.06
CD @ 5%	0.10	0.17	0.15	0.16	0.18
CV	8.90	9.02	6.98	7.12	7.27

3.4 Biomass Accumulation (Shoot and Root)

Fresh and dry biomass accumulation of both shoots and roots was significantly influenced by the treatments. The highest fresh shoot weight (260.33 mg) and dry root weight (63.33 mg) were recorded in the mango leaf scrap (100 g) + humic acid (2000 ppm) treatment. In contrast, the control treatment recorded substantially lower dry root weight (9.23 mg).

Greater biomass accumulation under integrated treatments suggests improved photosynthate production and efficient partitioning towards both above- and below-ground parts. Humic acid application has been reported to stimulate root growth by enhancing lateral root formation and root hair development, thereby increasing the absorptive surface area (Zandonadi *et al.*, 2007)^[8]. Enhanced root growth, in turn, supports shoot biomass accumulation through improved water and nutrient uptake.

3.5 Leaf Area Index (LAI)

Leaf Area Index differed significantly among treatments at 150

DAS. The maximum LAI (2.09) was observed in seedlings grown with mango leaf scrap (100 g) combined with humic acid at 2000 ppm, while the control recorded the lowest value (0.71). Higher LAI indicates better canopy development and greater photosynthetic surface area.

The increase in LAI may be attributed to enhanced leaf expansion and increased leaf number under humic acid treatment. Humic substances are known to improve chlorophyll content and photosynthetic efficiency, resulting in larger and more functional leaves. The gradual nutrient release from mango leaf litter might have further contributed to sustained leaf growth, as also reported by Adelani *et al.* (2020)^[2] in organic residue-amended systems.

3.6 Leaf Area Duration (LAD)

Leaf Area Duration, which represents the persistence of photosynthetically active leaf area over time, was significantly higher in seedlings treated with mango leaf scrap (100 g) and humic acid at 2000 ppm (32.43 cm² days) compared to the

control (11.10 cm² days). Higher LAD values indicate prolonged photosynthetic activity and better assimilate production.

The improved LAD observed in the present study suggests delayed senescence and sustained leaf functionality under integrated organic and humic acid treatments. This may be due to improved nutrient availability and enhanced physiological stability of leaves, leading to extended photosynthetic duration. Similar trends have been reported in fruit seedlings receiving humic substances and organic amendments (Mosa *et al.*, 2020) [4].

3.7 Root–Shoot Ratio

The root–shoot ratio was significantly influenced by the treatments, with the highest value (2.45) recorded in seedlings treated with mango leaf scrap (100 g) and humic acid at 2000 ppm. The control recorded a comparatively lower ratio (1.01), indicating relatively poor root development.

A higher root–shoot ratio reflects a well-developed root system capable of supporting shoot growth and improving seedling resilience under field conditions. Humic acid–induced stimulation of root growth, combined with improved soil

physical conditions due to leaf litter decomposition, likely contributed to this balanced growth pattern. These findings align with previous studies reporting enhanced root development under humic substance application (Canellas *et al.*, 2002; Zandonadi *et al.*, 2007) [3, 8].

3.8 Seedling Vigour Indices and Survival Percentage

Seedling Vigour Index I and II were significantly higher in treatments receiving humic acid and leaf litter amendments, with the maximum values recorded in mango leaf scrap (100 g) + humic acid (2000 ppm). This treatment also recorded the highest survival percentage (81.00%), whereas the control showed the lowest survival (58.33%).

Improved vigour indices and survival percentage are a cumulative outcome of enhanced morphological growth, physiological efficiency, and root system development. The integrated application of organic leaf litter and humic acid likely improved overall seedling health and adaptability, resulting in better survival. Similar improvements in seedling vigour under humic acid application have been reported in citrus and other perennial crops (Nardi *et al.*, 2002) [6].

Table 4: Effect of leaf scrap and humic acid on seedling vigour index I, seedling vigour index II and survival percentage

Treatment	Root: Shoot ratio	LAI @ 120DAS	LAI @150DAS	LAD (cm ² /day)	SVI I	SVI II	Root: Shoot ratio	Survival %
T ₁ – Control	1.01	0.52	0.71	11.10	615.57	2399.4	1.01	58.33
T ₂ - Soil + Mango leaf scrap 100 g+ humic acid 1500 ppm	1.60	0.97	1.35	21.16	1899.09	7609.63	1.60	65.33
T ₃ - Soil + Mango leaf scrap 100 g+ humic acid 2000 ppm	2.45	1.50	2.09	32.43	2931.85	19096.23	2.45	81.0
T ₄ - Soil + Mango leaf scrap 200 g+ humic acid 1500 ppm	1.11	0.56	0.67	10.62	779.44	3861.93	1.11	64.67
T ₅ - Soil + Mango leaf scrap 200 g+ humic acid 2000 ppm	1.20	0.70	1.33	20.67	777.58	4074.93	1.20	64.33
T ₆ - Soil + Sapota leaf scrap 100 g+ humic acid 1500 ppm	1.65	1.08	1.97	30.65	1499.17	11431.93	1.65	73.0
T ₇ - Soil + Sapota leaf scrap 100 g+ humic acid 2000 ppm	1.12	1.04	1.80	28.51	1182.37	4455.19	1.12	64.33
T ₈ - Soil + Sapota leaf scrap 200 g+ humic acid 1500 ppm	1.11	0.59	1.54	23.62	752.7	6775.11	1.11	68.33
T ₉ - Soil + Sapota leaf scrap 200 g+ humic acid 2000 ppm	1.42	0.87	1.39	21.77	1172.14	9295.36	1.42	73.0
T ₁₀ - Soil + Karonda leaf scrap 100 g+ humic acid 1500 ppm	1.09	0.83	1.18	18.59	863.53	4353.91	1.09	65.33
T ₁₁ - Soil + Karonda leaf scrap 100 g+ humic acid 2000 ppm	1.09	0.45	1.12	17.32	771.84	7249.0	1.09	64.67
T ₁₂ - Soil + Karonda leaf scrap 200 g+ humic acid 1500 ppm	1.03	0.55	1.06	16.38	783.64	4333.91	1.03	66.67
T ₁₃ - Soil + Karonda leaf scrap 200 g+ humic acid 2000 ppm	1.05	0.74	1.01	15.89	1011.6	6382.93	1.05	68.0
SE ±	0.05	0.03	0.06	0.85	50.68	47.04	0.05	2.96
CD @ 5%	0.15	0.08	0.17	2.49	147.91	137.30	0.15	8.65
CV	6.99	5.87	7.50	7.14	7.59	1.16	6.99	7.61

4. Conclusion

The results of the present investigation clearly indicate that soil amendment with mango leaf scrap at 100 g combined with humic acid seed priming at 2000 ppm creates a favourable physiological and nutritional environment for acid lime seedlings. This integrated treatment significantly improves morphological growth, root system development, photosynthetic efficiency, and survival percentage under nursery conditions. Adoption of this practice may help in producing high-quality planting material for acid lime cultivation. Further studies under different soil types and environmental conditions are suggested to validate these findings.

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