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The effect of spraying with salicylic acid and humic acid on the physiological and chemical qualities of *Acacia cyanophylla* seedlings

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Abstract. Given the growing environmental challenges and the imperative for successful afforestation initiatives, there is an increasing demand for enhancing seedling growth and quality through natural treatments. This study was conducted to evaluate the effects of salicylic acid and humic acid on the physiological and biochemical attributes of *Acacia cyanophylla* seedlings. The experiment was carried out in the nursery of the tourist village of Nineveh forests, where seedlings were sprayed with salicylic acid (0, 200, 400 mg·L^{-1}) and humic acid (0, 1, 2 g per seedling) to assess their effects on selected physiological and chemical traits. The results showed that spraying with salicylic acid at 200 mg·L^{-1} had a significant impact on carbohydrate content, protein percentage, total chlorophyll content of leaves, and relative moisture content. Similarly, humic acid at 2 g per seedling produced the highest significant increases in these traits. As for the bilateral interaction between salicylic acid and humic acid, the results showed that spraying with salicylic acid at 200 mg·L^{-1} in combination with humic acid at 2 g per seedling resulted in the highest values for most of the studied traits. This combined application significantly enhanced seedling growth and overall physiological vigor. Foliar application of salicylic acid at 200 mg·L^{-1} resulted in a marked increase in chlorophyll content, thereby promoting photosynthetic efficiency, while humic acid

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application markedly improved leaf water retention, substantially augmenting the seedlings' tolerance to environmental stress. Collectively, these results indicate that the integrated use of salicylic acid and humic acid represents a promising and effective approach for enhancing seedling quality and vigor, particularly in the context of afforestation programs

Keywords: foliar spraying; physiological traits; chemical composition; seedling quality; photosynthetic pigments; afforestation

Introduction

Trees are integral to ecosystem stability and serve as a critical defence against environmental degradation by counteracting desertification, attenuating the effects of climate change, curbing soil erosion, and enhancing air quality. Beyond their ecological functions, forest trees supply vital resources – including fuelwood, fodder, medicinal compounds, and materials for construction. As a result, strategically designed afforestation and reforestation initiatives have emerged as central pillars in global strategies to promote environmental sustainability and resilience. The effectiveness of these initiatives hinges critically on the availability of high-quality seedlings characterised by robust physiological performance and heightened tolerance to abiotic stresses – outcomes that can only be achieved through the implementation of evidence-based nursery cultivation and management practices.

According to O. Kosanin *et al.* (2023) the presence of trees in natural forests or their cultivation is one of the most important factors that affect the ecosystem and resist desertification, global warming, dust storms, a source of fuel, medical materials, animal feed, and building materials, prevent soil erosion, and reduce the destructive effects of flood seasons. Well-planned tree planting projects are an important element in global efforts to improve environmental and humanitarian conditions, but tree planting must be in accordance with well-thought-out scientific plans to overcome environmental problems facing the climate and the environment. K.D. Holl & P.H.S. Brancalion (2020) noted that one of the species that is preferred for cultivation is *Acacia cyanophylla*, which belongs to the leguminous family. It is a thornless, evergreen, dangling, fast-growing shrub with branches up to 7 meters high and can reach 10 meters. Its leaves are thick, smooth, waxy and blue-green. The lower leaves are larger than the upper leaves, the flowers are bright golden-coloured, spherical, clustered every 3-5 of them in a cluster.

A. Kheloufi *et al.* (2019) reported that *Acacia cyanophylla* is highly valued for ornamental landscaping, particularly in gardens, urban green spaces, and roadside plantings, due to its attractive yellow inflorescences and persistent evergreen foliage. The authors also emphasised the species' broad geographical distribution, noting its presence in numerous Arab countries. Furthermore, their study indicated that mature plants exhibit moderate tolerance to salinity and drought stress, primarily propagate via seeds, and possess limited capacity for vegetative propagation. F. Marzialetti *et al.* (2019) observed that *Acacia cyanophylla* is native to Australia and is extensively cultivated for multiple purposes, including firewood production and environmental applications such as sand dune stabilisation and windbreak establishment. In efforts to improve afforestation success under field conditions, the application of plant growth-regulating compounds has gained increasing research attention.

Salicylic acid – a naturally occurring plant hormone present in trace amounts in plant tissues – plays a critical role in plant defense against pathogens and in adaptation to various abiotic stresses, including drought, heavy metal toxicity, extreme temperatures, and osmotic stress. As demonstrated by N.N. Parihar & V. Shelar (2023), salicylic acid functions as an effective growth regulator, positively influencing a range of physiological and biochemical processes such as seed germination, respiration, vegetative growth, flowering, and photosynthetic activity. A. Dauletbay *et al.* (2024) examined the properties, structure, and agricultural uses of humic substances and found that these compounds substantially enhance soil fertility by improving soil structure, boosting cation exchange capacity, modulating soil pH, and promoting the activity of beneficial soil microorganisms. L.P. Canellas *et al.* (2020) reported that humic substances function as chemical priming agents in plants, affecting cellular metabolism – particularly in cell walls, membranes, and the cytoplasm. Their research demonstrated that humic acids increase photosynthetic efficiency, respiration rates, and protein synthesis through hormone-like effects. Additionally, K. Ampong *et al.* (2022) confirmed that humic acids indirectly promote plant growth by enhancing nutrient availability and uptake, primarily by stimulating microbial activity in the rhizosphere, thereby facilitating the absorption of essential mineral nutrients.

Based on the importance of this plant species in forests as well as gardens or in the afforestation of roads, the impact of both salicylic acid and humic acid on the vitality and productivity of *Acacia cyanophylla* seedlings was decided to be investigated in this study. The goal of this study was to evaluate the effects of salicylic acid and humic acid on the physiological and biochemical attributes of *Acacia cyanophylla* seedlings, particularly focusing on their influence on seedling growth, stress tolerance, and overall vigor.

Materials and Methods

The experiment was carried out in the area of the tourist village inside the forests of Nineveh at the intersection of a latitude of (36°22’42’’) north, a longitude of (43°70’60’’) east, and at an altitude of 219 m above sea level. For the period from (September 2024 to September 2025), laboratory analyses were conducted within the Development Laboratory of the Forestry Science Department. The study site was chosen to establish a young tree on an area of 2 dunums within the tourist village, where cleaning operations were carried out for the study site of tree waste, jungle and wood pieces scattered within the site and regular modification and levelling of the land. The jungle was controlled with specialised pesticides to eliminate it. Planting sites for seedlings and the drilling corps were planned within the site at equal distances and depths of more than 50 cm according to the field experiment scheme. An organised distillation network was extended on the drilling. Distillation nozzles were placed at each hole and the distillation system was connected with the water source (artesian well) within the site. Planting of seedlings was carried out at the beginning of the ninth month and regular maintenance and watering operations continued throughout the study period.

The seedlings were brought from the Umm Al-Rubaieen nursery of the Department of Horticulture and Garden Engineering in the Farms District, which is one of the government nurseries affiliated with the Ministry of Agriculture. The seedlings were one year old with consistent and equal lengths and diameters and were planted in black polyethylene bags with a diameter of 10 cm and a length of 30 cm per bag. The height of the seedlings inside the bag was about 70 cm. The seedlings were planted from seeds sourced from a number of growing trees inside the nursery. The seedlings were brought to the study site with a number of (324 seedlings), and the seedlings were planted inside the pits designated for them. Watering and cleaning operations for the bush were carried out on a regular basis throughout the study period.

Experimental factors:

Factor I: Salicylic acid (400,200,0) $mg \cdot L^{-1}$;

Factor II: Humic acid (2,1,0) $g \cdot L^{-1}$.

The experiment comprised nine treatment combinations, each including six seedlings, arranged in three replications according to a randomised complete block design (RCBD). Statistical analysis was performed using SAS software (version 9.0), and treatment means were compared using Duncan's multiple range test at a significance level of $P \leqslant 0.05$ (Dafaallah, 2019). The evaluation of seedling response to the applied treatments was based on the assessment of selected physiological and biochemical parameters related to water status, photosynthetic capacity, and metabolic activity. Accordingly, relative leaf water content, carbohydrate concentration, total protein percentage, and total chlorophyll content were determined using standard analytical methods.

Relative moisture content of leaves (%). The moisture content of the leaves was estimated according to M.R.B. Siddique *et al.* (2000), where 20 leaves were weighed for each seedling in the experimental unit, and they were directly wet with a sensitive scale with a sensitivity of 0.01. Their wet weight was recorded, then they are immersed in distilled water for 16-18 hours at room temperature 23-25°C and under low lighting conditions in order to saturate the leaves with distilled water. Their swell weight is recorded in the case of saturation Turgid Weight, then the leaves are dried in the oven at a temperature of $70^{\circ} \pm 1$ and until the weight was fixed and the dry weight was recorded, then the relative moisture content of the leaves was calculated for each transaction A.G. Konings *et al.* (2021) according to the following mathematical relationship:

$$\text{relative water content of the leaves} = \\ = \frac{\text{wet weight} - \text{dry weight of the leaf}}{\text{turgid weight} - \text{weight of the leaf}} \times 100. \quad (1)$$

Estimation of carbohydrate content of leaves ($mg \cdot g^{-1}$ dry weight). The content of carbohydrates in the leaves was estimated according to the method of M. Dubois *et al.* (1956) by taking a weight of 0.1 g of dry leaves, where they were crushed in a ceramic mortar and added 10 ml of distilled water, then the solution is taken and placed in a test tube and placed in a centrifuge for 15 minutes at a speed of 3,000 cycles/minute, after which the filtrate is taken and a distilled water mechanism is added for the purpose of completing the volume to 10 ml, then it was taken 1 ml of the solution and add 1 ml of phenol at a concentration of 5%, then add 5 ml of concentrated sulfuric acid H_2SO_4 and leave the sample for (15) minutes, then put in a water bath at a temperature of (25-30)° for a period of 20 minutes , after which the absorption is read using a spectrophotometer at a wavelength of 488 nm, and the readings are dropped on the standard curve of glucose sugar to obtain the concentration of carbohydrate as mentioned by D. Herbert *et al.* (1971):

$$\text{Carbohydrates (mg/g)} = \\ = \frac{\text{Volume of extract used} \times \text{Total extract volume} \times \text{Read device}}{\text{Sample weight} \times 1000}. \quad (2)$$

Estimating the percentage of total protein in the leaves. Total protein content in the leaves was determined by weighing 0.1 g of dried leaf material, which was finely ground using a ceramic mortar. The sample was mixed with 10 mL of distilled water and centrifuged for 15 min at 3,000 rpm. The supernatant was collected, and distilled water was added to adjust the final volume to 10 mL. From this extract, 1 mL was taken and mixed with 20 µL of a ready-to-use analysis kit (Fortress Diagnostics, UK).

Protein determination was based on the Biuret method, which relies on the formation of a violet-coloured complex resulting from the reaction between proteins and copper ions in an alkaline medium. The intensity of the colour, proportional to protein concentration, was measured spectrophotometrically at a wavelength of 550 nm, following the method described by C.A. Burtis & E.R. Ashwood (1999). The reagents used included a Biuret reagent composed of sodium hydroxide (200 mmol · L^{-1}), copper sulphate (18 mmol · L^{-1}), and sodium potassium tartrate (32 mmol · L^{-1}), as well as a standard protein solution prepared at a concentration of 6 g per 100 mL. Total protein concentration was calculated according to the following equation:

$$\text{Total protein (g dL}^{-1}\text{)} = \text{Total protein (g/dl)} \frac{\text{A sample}}{\text{Astandra}} \times \text{standard concentration}, \quad (3)$$

where *A* represents absorbance intensity and 1 dL = 100 mL.

Estimation of total chlorophyll content in leaves (mg · g^{-1} soft vegetable fabric). Chlorophyll a and b and total chlorophyll were estimated based on the Mackinney. Leaves were taken from the developing top of three samples of *Acacia cyanophyll* seedlings and then cut to facilitate the estimation process. Then a weight of 0.2 g of healthy soft green leaves was taken. The leaves were crushed by a ceramic mortar and then acetone 12 ml was added to the concentration (80%). After that, they were placed in the centrifuge for 5 minutes at a speed of 3,000 cycles/minute for the purpose of separating the leachate from the precipitate. The leachate was then taken and the absorbency was read at wavelengths 645, 663 nm by the spectrophotometer. The following equations were used to calculate the amount of chlorophyll in the leaves calculated on the basis of (mg · g^{-1} wet weight).

$$\text{Total Chlorophyll} = [20.2\,(\text{D645}) + 8.02\,(\text{D663})] \times \text{V}/1000 \text{ x W}, \quad (4)$$

where V = final volume of leachate after completion of separation by centrifuge, D = read the photodensity (absorptivity) of the extracted chlorophyll, W = mild weight in g. All research procedures involving plant material were conducted in compliance with international ethical standards and relevant regulations, in accordance with the principles of the Convention on Biological Diversity (1992).

Results

Relative moisture content of leaves (%). The results of Table 1 regarding the relative moisture content of the leaves showed significant differences between the concentrations of humic acid according to Duncan's test. Where the concentration was recorded 2 g · L^{-1} The highest moral increase in this characteristic amounted to 74.74% while the concentration recorded 0 g · L^{-1} The lowest moral value reached 72.21% with a moral difference of 2.53%. As for the concentrations of salicylic acid, Duncan's test showed significant differences between the concentrations and gave the concentration 400 mg · L^{-1}. The highest intangible value of 76.99% compared to the concentration of 0 mg · L^{-1}, which gave the lowest rate of 71.63% with a moral difference of 5.36%.

Table 1. The effect of spraying with salicylic acid and humic acid and the overlaps between them in the relative moisture content of the leaves (%)

		Salicylic acid concentrations mg · L^{-1}			Effect of humic acid mg · L^{-1}
		0	200	400	
Humic acid concentrations mg · L^{-1}	0	69.73 i	75.58 d	77.35 b	72.21 b
	1	71.64 h	77.23 c	73.88 e	74.24 a
	2	73.55 f	78.18 a	72.52 g	74.74 a

Table 1, Continued

	Salicylic acid concentrations mg · L^{-1}			Effect of humic acid mg · L^{-1}
	0	200	400	
Effect of salicylic acid mg·L^{-1}	71.64 c	76.99 a	74.58 b	

Note: numbers with similar letters for individual factors and their overlaps do not differ significantly between them according to Duncan's polynomial test at a probability level of 0.05

Source: developed by the authors

As the bilateral overlap between the concentrations of salicylic acid and the concentrations of humic acid showed, Duncan's test indicated significant differences between the concentrations in this characteristic, and between the overlap between salicylic acid 200 mg · L^{-1} and humic acid 2 g · L^{-1} has the highest significant value among the overlaps of the factors and gave the highest significant value of 78.18% compared to the treatment of the overlap of salicylic acid 0 mg · L^{-1} and humic acid (0 g · L^{-1}, which recorded the lowest rate of 69.73% with a moral difference of 8.45%.

Estimation of the carbohydrate content of leaves (mg·g^{-1} dry weight). The results of Table 2 showed the effect of humic acid concentrations, as they differed significantly in the effect on the leaf content of carbohydrates and reached the highest rate at the concentration of 1 g · L^{-1}, which amounted to 25.00 mg · L^{-1} dry weight for the lowest intangible value found at the concentration 0mg · L^{-1} dry weight, which amounted to 24.07 mg · L^{-1} dry weight with a moral difference of 0.73%.

Table 2. The effect of spraying with salicylic acid and humic acid and the overlaps between them in the characterisation of the leaf content of carbohydrates (mg · g^{-1} dry weight)

		Salicylic acid concentrations mg · L^{-1}			Effect of humic acid g · L^{-1}
		0	200	400	
Humic acid concentrations g · L^{-1}	0	21.84 i	24.77 e	25.62 c	24.07 b
	1	22.60 h	25.75 b	24.82 d	24.39 b
	2	23.33 g	26.96 a	24.73 f	25.00 a
Effect of salicylic acid mg · L^{-1}		22.59 c	25.82 a	25.05 b	

Note: numbers with similar letters for individual factors and their overlaps do not differ significantly between them according to Duncan's polynomial test at a probability level of 0.05

Source: developed by the authors

With regard to the effect of salicylic acid, the data of Table 2 of the Duncan's test showed significant differences between the concentrations and the concentration of 400 mg · L^{-1}, the highest moral increase of 25.02 mg · L^{-1} dry weight compared to the comparison treatment with a concentration of 0 mg · L^{-1} dry weight, which gave the lowest rate of 22.59 mg · L^{-1} dry weight with a moral difference of 2.43%. Table 2 of the Duncan's test between the concentrations of salicylic acid and the concentrations of humic acid showed that there were significant differences between the concentrations in this interference ,and between the interference between salicylic acid 200 mg · L^{-1} and humic acid 2 g · L^{-1} recorded the highest moral increase among the interactions of the factors, which amounted to 26.96 mg · L^{-1} dry weight compared to interferometric treatment salicylic acid 0 mg · L^{-1} and humic acid 0 g · L^{-1}, which recorded the lowest rate of increase, amounting to 21.84 mg · L^{-1} of dry weight with a moral difference of 5.12%.

Estimating the leaf content of total proteins (%). Table 3 indicates that there are significant differences between the concentrations of humic acid in the character of the leaf content of proteins according to the Duncan' test. Where the concentration was recorded 1 g · L^{-1} The highest moral increase in this characteristic amounted to 6.40% while the concentration recorded 0 g · L^{-1} The lowest moral value was 6.08% with a moral difference of 0.32%. As for the concentrations of salicylic acid, Duncan's test showed Table 3 that the concentration is 200 mg · L^{-1} and concentration 400 mg · L^{-1} recorded the highest rates, respectively 6.44 and 6.45%, but they did not differ significantly between them, but they outperformed the comparison treatment with a concentration of 0 mg · L^{-1}, which gave the lowest increase of 5.17% with a moral difference of 1.27 and 1.28%.

Table 3. The effect of spraying with salicylic acid and humic acid and the overlaps between them in the character of the leaf content of total proteins (%)

		Salicylic acid concentrations mg · L^{-1}			Effect of humic acid g · L^{-1}
		0	200	400	
Humic acid concentrations g · L^{-1}	0	5.65 h	6.18 e	6.39 c	6.08 b
	1	5.36 i	6.24 d	6.77 b	6.12 a
	2	6.11 g	6.92 a	6.17 f	6.40 a
Effect of salicylic acid mg · L^{-1}		5.17 b	6.45 a	6.44 a	

Note: numbers with similar letters for individual factors and their overlaps do not differ significantly between them according to Duncan's polynomial test at a probability level of 0.05
Source: developed by the authors

Between the bilateral overlap between the concentrations of salicylic acid and the concentrations of humic acid, the Duncan's test Table 3 showed significant differences between the concentrations in this characteristic ,and the overlap between salicylic acid 200 mg · L^{-1} and humic acid 2 g · L^{-1} has the highest significant value among the overlaps of the factors, which amounted to 6.92% compared to the treatment of the overlap with salicylic acid 0 mg · L^{-1} and humic acid 0 g · L^{-1}, which recorded the lowest rate of 5.65% with a moral difference of 1.27%.

Estimation of the total chlorophyll content of leaves (mg·g^{-1} soft weight). The results of Table 4 regarding the total chlorophyll content of the leaves indicated that there are significant differences between the concentrations of humic acid according to the Duncan's test, where the concentration was recorded 1 g · L^{-1}. The highest moral increase in this characteristic amounted to 1.48 g · L^{-1} g soft weight while the concentration was recorded 0 g · L^{-1} with a minimum intangible value of 1.30 g · L^{-1} of soft weight with a significant difference of 0.18%.

Table 4. The effect of spraying with salicylic acid and humic acid and the overlaps between them in the character of the total chlorophyll content of the leaves (mg · g^{-1} soft weight)

		Salicylic acid concentrations mg · L^{-1}			Effect of humic acid mg · L^{-1}
		0	200	400	
Humic acid concentrations g · L^{-1}	0	1.22 i	1.38 f	1.46 b	1.35 c
	1	1.33 h	1.46 c	1.45 d	1.41 b
	2	1.34 g	1.60 a	1.44 e	1.46 a

Table 4, Continued

0	Salicylic acid concentrations mg · L^{-1}			Effect of humic acid mg · L^{-1}
	200	400		
Effect of salicylic acid mg · L^{-1}	1.30 b	1.48 a	1.45 a	

Note: numbers with similar letters for individual factors and their overlaps do not differ significantly between them according to Duncan's polynomial test at a probability level of 0.05
Source: developed by the authors

The Duncan's test (Table 4) showed that the concentration is 200 mg · L^{-1} and concentration 400 mg · L^{-1} recorded the highest rates, respectively 1.44 and 1.48 mg · L^{-1} of soft weight, but they did not differ significantly between them, but they outperformed the comparison treatment with a concentration of 0 mg · L^{-1}, which gave the lowest increase of 1.30 mg · L^{-1} of soft weight and a significant difference of 0.14, 0.18, respectively. The bilateral overlap between the concentrations of salicylic acid and the concentrations of humic acid in the Dunkin's test Table 4 showed that there are significant differences between the entries in this trait ,as the overlap between salicylic acid 200 mg · L^{-1} and humic acid 2 g · L^{-1} gave the highest significant value between the overlaps of the factors and gave the highest significant value of 1.60 mg · L^{-1} soft weight compared to interferometric treatment salicylic acid 0 mg · L^{-1} and humic acid 0 g · L^{-1}, which recorded the lowest rate of 1.22 mg · L^{-1} of soft weight with a significant difference of 0.38%.

The results showed that spraying with humic acid had a significant impact on the physiological and chemical qualities of *Acacia cyanophylla* seedlings as clear differences were recorded between the transactions, indicating that the seedlings were affected by spraying with humic acid. It was found that the spraying was at a concentration of 2 g · L^{-1} led to a significant increase in the rates of the studied traits (relative moisture content, carbohydrate percentage, total protein percentage, leaf content of LeRovell). The data also showed that the spray was 0 mg · L^{-1} led to a decrease in the rates of the studied traits. This is attributed to the fertilisation processes with humic acid in plants, which increases chlorophyll in the leaves and encourages growth, increases the size of cells, and vegetative properties. The effect of humic acid due to its containment of oxygen, hydrogen, carbon, and nitrogen in varying proportions and with light molecular weights when used for plants, plays an essential role in feeding plants, which is reflected in photosynthesis, increased proteins, increased carbohydrates, and respiration. In addition, humic substances contribute to raising the internal levels of plant hormones, such as oxines and gibrilins, by stimulating their formation or preventing their decomposition. The reason behind the increase in vegetative growth indicators and plant chemical content may be that humic substances increase the proportion of oxygen-rich solid compounds in cells. The results are consistent with those reported by F.S. Al-Marsoumi (2019), who investigated the foliar application of three concentrations of humic acid (0, 1, and 2 g · L^{-1}) on seedlings of the 'Qalb al-Thawr' mango cultivar (*Mangifera indica* L.). The findings revealed that the 2 g · L^{-1} concentration significantly outperformed other treatments across two consecutive growing seasons, yielding the highest values for plant height increase (47.9 and 39.1 cm), stem diameter (4.68 and 9.24 mm), leaf number (65.8 and 89.3 leaves · plant^{-1}), and branch number (4.33 and 7.83 branches), while the control treatment produced the lowest values for all measured traits. These findings also align with the study conducted by F.S. Al-Marsoumi & M.E.A. Al-Hadethi (2020) on one-year-old mango seedlings *Mangifera indica* L., in which foliar application of humic acid at concentrations of 0, 1, and 2 $mL \cdot L^{-1}$ significantly enhanced leaf nutrient content. Specifically, the 2 $mL \cdot L^{-1}$ treatment resulted in the highest leaf nitrogen content (1.403% and 1.743%), potassium content (0.520% and 0.463%), iron content (190.6 and 235.3 ppm), and zinc content (44.46 and 51.38 ppm) during the two consecutive seasons, respectively.

Furthermore, the current results are in agreement with N. Vikram *et al.* (2022), who described humic acids as complex organic compounds derived from the decomposition of organic matter in soil. These substances play a pivotal role in regulating various biological and environmental processes. They improve soil structure, enhance water and nutrient retention capacity, stimulate plant growth and beneficial microbial activity, and effectively modulate nitrogen and carbon cycling – thereby enhancing the overall efficiency and sustainability of agricultural ecosystems. The findings also corroborate those of H.M. Ibraheem (2021), who evaluated the effect of organic fertiliser application on the growth of *Pinus pinea* L. seedlings. In that study, seedlings were foliar-sprayed with four concentrations (0, 1, 2, and 3 $g \cdot L^{-1}$) of organic fertiliser. The results demonstrated that all treated seedlings exhibited significant improvements in all measured growth parameters compared to the control. Notably, the 2 $g \cdot L^{-1}$ concentration was statistically superior across all evaluated traits relative to the untreated control.

As for the effect of salicylic acid concentrations on some of the vegetative traits studied for pine protein seedlings, the results of Tables 1-4 indicate that there are differences between the concentrations and it has a significant impact, especially at the concentration of 400 $mg \cdot L^{-1}$ compared to the comparison treatment. The reason may be that salicylic acid plays an important role in regulating physiological and biological processes (Song *et al.,* 2023), and is considered one of the most important compounds responsible for promoting plant health and resistance to harmful environmental conditions. Promotes cell division and elongation, which contributes to increased vegetative growth, stimulates metabolic reactions such as protein and carbohydrate synthesis, and increases chlorophyll levels in leaves and leaf water content, improving photosynthetic efficiency and energy production. Contributes to the improvement of gas exchange by regulating the opening and closing of stomata, as noted by J.D. Vashi (2023). It also reduces the harmful effects of water shortage by regulating osmotic pressure and increasing drought resistance, and contributes to protecting plants from high temperatures by promoting an antioxidant system that promotes the movement of nutrients such as nitrogen, phosphorus, and potassium towards the aerial parts of the plant, improving growth and productivity and interacting with other plant hormones (such as oxines and gibellins) to regulate vital processes These results also consistent with those reported by A.H. Alalaf & M.M. Ibrahim (2020), who investigated the foliar application of salicylic acid at three concentrations (0, 200, and 400 $mg \cdot L^{-1}$) on papaya (*Carica papaya* L.) seedlings. The results demonstrated that the 200 $mg \cdot L^{-1}$ treatment significantly enhanced seedling height increase, while the 400 $mg \cdot L^{-1}$ concentration yielded the highest values for vegetative growth parameters and leaf content of nitrogen, potassium, and phosphorus. In contrast, the control treatment (0 $mg \cdot L^{-1}$) recorded the lowest significant values across all evaluated traits.

These results also align with the study by S.A. Hussein & H.S. Kanber (2024), who examined the effect of foliar-applied salicylic acid at concentrations of 0, 100, and 200 $mg \cdot L^{-1}$ on fig (*Ficus carica* L.) seedlings. Their findings indicated that the 200 $mg \cdot L^{-1}$ treatment produced the greatest plant height, leaf area, and leaf number, suggesting a pronounced growth-promoting effect of salicylic acid on seedling development. Furthermore, the present outcomes are in agreement with J.A. Linné *et al.* (2025), who studied the response of seedlings of the endangered species *Cedrela fissilis* Vell. to foliar applications of salicylic acid at 100, 200, and 300 $mg \cdot L^{-1}$. Their results showed that the 200 $mg \cdot L^{-1}$ concentration induced the most favourable vegetative and root growth responses, significantly outperforming the other concentrations, which yielded comparatively lower values for the measured parameters. Additionally, the results corroborate those of L.C. Saracho *et al.* (2021), who evaluated the impact of salicylic acid on *Schinus terebinthifolia* seedlings subjected to varying irrigation regimes. Using concentrations of 0, 50, 100, and 200 $mg \cdot L^{-1}$, they found that the 200 $mg \cdot L^{-1}$ treatment produced the highest stem length (20.74 cm) and leaf area (116.04 cm^2), along with the greatest dry mass accumulation in stems, leaves, and roots. These parameters were significantly superior to those observed under lower concentrations or the untreated control.

The results of this study highlight the significant role of salicylic acid and humic acid in enhancing the physiological and biochemical properties of seedlings. These compounds, through their effects on growth regulation and stress tolerance, improve seedling vigor and resilience under challenging environmental conditions. The observed interactions between salicylic acid and humic acid further emphasise their synergistic potential in promoting plant health. Overall, these findings suggest that the application of these biostimulants can be an effective strategy for improving seedling establishment and growth in afforestation projects.

Conclusions

The present study conclusively demonstrates that foliar application of salicylic acid and humic acid significantly enhances the physiological and biochemical attributes of *Acacia cyanophylla* seedlings under nursery conditions. The experimental findings revealed that both growth-regulating compounds, either individually or in combination, exerted a pronounced positive influence on key indicators of seedling vigor, including relative leaf water content, carbohydrate accumulation, total protein synthesis, and chlorophyll concentration. Notably, the optimal response was consistently observed when seedlings were treated with 200 $mg \cdot L^{-1}$ of salicylic acid in conjunction with 2 $g \cdot L^{-1}$ of humic acid, underscoring the existence of a synergistic interaction between these two biostimulants. This combination maximised the expression of all measured traits, suggesting that integrated application strategies may offer superior benefits compared to the use of either compound alone.

The improvement in relative leaf water content reflects enhanced cellular hydration and osmotic adjustment, which are critical for drought resilience particularly relevant given *A. cyanophylla* intended use in arid and semi-arid afforestation projects. The significant increases in carbohydrate and protein content further indicate elevated metabolic activity and improved photosynthetic efficiency, likely mediated through salicylic acid's role in modulating stress-responsive pathways and humic acid's capacity to stimulate enzymatic and hormonal activity. Moreover, the elevated chlorophyll levels observed in treated seedlings correlate with greater photosynthetic potential and overall plant health, aligning with established mechanisms by which humic substances enhance nutrient uptake and chloroplast function.

These results collectively affirm that exogenous application of salicylic and humic acids serves as an effective cultural practice to bolster seedling quality prior to out planting. Given the species' ecological and ornamental value particularly in urban greening, roadside planting, and soil stabilisation initiatives such pre-planting interventions can significantly improve establishment success in challenging environments. Therefore, it is recommended that nursery protocols for *A. cyanophylla* incorporate foliar sprays of 200 mg·L^{-1} salicylic acid and 2 g·L^{-1} humic acid as a standard practice to optimise seedling performance. Future research should explore the carry-over effects of these treatments post-transplantation and under field stress conditions to validate their long-term efficacy in real-world afforestation programs.

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Conflict of Interest

None.

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Вплив обробки саліциловою кислотою та гуміновою кислотою на фізіологічні та хімічні якості сіянців *Acacia cyanophylla*

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Анотація. З огляду на зростаючі екологічні виклики та необхідність успішних ініціатив з озеленення, зростає попит на покращення росту та якості сіянців за допомогою природних обробок. Це дослідження було проведено для оцінки впливу саліцилової кислоти та гумінової кислоти на фізіологічні та біохімічні характеристики сіянців *Acacia cyanophylla*. Експеримент проводився в розсаднику туристичного селища лісів Ніневії, де сіянці обробляли саліциловою кислотою (0, 200, 400 мг · л$^{-1}$) та гуміновою кислотою (0, 1, 2 г на сіянця) для оцінки їхнього впливу на обрані фізіологічні та хімічні ознаки. Результати показали, що обробка саліциловою кислотою при концентрації 200 мг · л$^{-1}$ мала значний вплив на вміст вуглеводів, відсоток білка, загальний вміст хлорофілу в листках та відносний вміст вологи. Аналогічно, гумінова кислота при дозі 2 г на сіянця призвела до найвищих значних збільшень цих характеристик. Щодо взаємодії саліцилової кислоти та гумінової кислоти, результати показали, що обробка саліциловою кислотою при концентрації 200 мг · л$^{-1}$ у поєднанні з гуміновою кислотою 2 г на сіянця призвела до найвищих значень для більшості досліджених ознак. Така комбінована обробка значно покращила ріст сіянців та загальний фізіологічний потенціал. Листова обробка саліциловою кислотою при концентрації 200 мг · л$^{-1}$ спричинила помітне збільшення вмісту хлорофілу, що сприяло підвищенню фотосинтетичної ефективності, тоді як обробка гуміновою кислотою значно покращила утримання води в листках, значно підвищуючи стійкість сіянців до екологічного стресу. Загалом, ці результати свідчать, що інтегроване використання саліцилової кислоти та гумінової кислоти є перспективним та ефективним підходом для покращення якості та життєздатності сіянців, особливо в контексті програм озеленення

Ключові слова: листова обробка; фізіологічні характеристики; хімічний склад; якість сіянців; фотосинтетичні пігменти; озеленення