

# Effect of nano NPK fertilizer, cytokinin, and gibberellic acid on vegetative growth and chemical constituents of *Paulownia tomentosa* L plants

Warheel Ali<sup>1\*</sup>, Nizar Mohammed<sup>2</sup>, Zeinab Younis<sup>3</sup>, Pashtivan Zeebaree<sup>1</sup> and Mohammed Qasim<sup>1</sup>

<sup>1</sup>Duhok Polytechnic University, Kurdistan Region, Iraq. <sup>2</sup>Akre University for Applied Sciences, Kurdistan Region, Iraq.

<sup>3</sup>Erbil Polytechnic University, Kurdistan Region, Iraq. \*E-mail: warheel.nadir@dpu.edu.krd

## Abstract

The investigation sought to examine the effects of nano NPK, cytokinin, and GA<sub>3</sub> on the growth and chemical composition of *Paulownia* tree seedlings. The experiment comprised three treatments: Nano NPK at concentrations of 0 and 6 g L<sup>-1</sup>, applied monthly from April to September; cytokinin at concentrations of 0, 100, and 200 mg L<sup>-1</sup>, applied thrice in March, July, and September; and GA<sub>3</sub> at concentrations of 0, 200, and 400 mg L<sup>-1</sup>, applied biannually in May and August. Plant sprayed with 6 g L<sup>-1</sup> Nano NPK increased all vegetative and chemical parameters compared with control. Furthermore, plants sprayed with 200 mg L<sup>-1</sup> cytokinin increase plant height to 96.67 cm, leaves number 31.22 leaf plant<sup>-1</sup>, stem diameter 5.56 cm, leaves area 277.83 cm<sup>2</sup>, nitrogen content in leaves 1.65 %, and total carbohydrate in leaves 8.94 % compared with control. In contrast, the plants sprayed with 400 mg L<sup>-1</sup> GA<sub>3</sub> plant height increased to 92.11 cm, leaves number 29.50 leaf plant<sup>-1</sup>, leaves area 257.90 cm<sup>2</sup>, nitrogen and phosphorus content in leaves 1.77- 2.80 %, and total carbohydrate in leaves 8.30 %. Nonetheless, plants treated with 200 mg L<sup>-1</sup> GA<sub>3</sub> exhibited an increase in total chlorophyll content in leaves to 24.40 mg g<sup>-1</sup> fresh weight in comparison to the control group. Further investigation is necessary to ascertain whether higher concentrations than those employed in this study may produce better outcomes.

**Key words:** *Paulownia* plants, NPK, cytokinin, and gibberellic acid, GA<sub>3</sub>

## Introduction

The empress tree (*Paulownia tomentosa*) is a Chinese native fast-growing tree species, of significant economic and ecological importance. Its timber is prized for being lightweight yet strong and is used in the production of furniture, ornaments and musical instruments (Jakubowski, 2022). This tree has been extensively researched as a bioethanol lignocellulosic feedstock with particular importance in the Southeastern USA (Yadav *et al.*, 2013). Due to the rapid spread and adaptability of the species throughout a variety of climate zones, as well as the suitability of agroforestry systems that demand growth of this species on a large scale throughout the world. However, the dependence of this tree on arable land and invasive potential outside its native habitat are threats that require management and ongoing research (Jakubowski, 2022).

*P. tomentosa* remains one of the driving factors in agroforestry by improving soil stabilization, carbon sequestering and is essential to sustainable agriculture and environmental restoration activities (Woźniak *et al.*, 2018). Because of the potential to grow in less productive soils and different climate ranges, it is a good candidate for reforest and afforestation projects. In addition, by providing proteins, fats and sugars, its leaf can be used as green fertilizer and livestock feed, which are important for sustainable agriculture (Woźniak *et al.*, 2018). This tree has historical importance in many places in East Asia, but it has a strong literary and artistic presence in China and Japan, where it has been referenced since the 19th century.

Recent advances in nanotechnology have opened new frontiers in sustainable agriculture, particularly in the development of nano-formulated fertilizers. These nano-fertilizers, composed of physiologically active metal nanoparticles, are designed to improve nutrient efficiency and plant uptake mechanisms (Solanki *et al.*, 2015; Hayam *et al.*, 2024). Due to their high surface area, enhanced sorption properties, and slow-release kinetics, nano-fertilizers function as “smart delivery systems” that enable targeted nutrient release at specific sites within plant tissues. This precise delivery not only minimizes nutrient loss but also enhances the physiological responses in plants—stimulating seed germination, promoting early seedling vigor, enhancing photosynthetic performance, and improving nitrogen metabolism (Mandal, 2021; Shilpa *et al.*, 2022). Moreover, these nano-nutrients have been shown to support carbohydrate and protein synthesis, ultimately contributing to better vegetative growth and increased biomass accumulation. Within this context, the current study explores the synergistic effects of nano NPK fertilizer in combination with plant growth regulators—cytokinin and gibberellic acid—on the growth dynamics and chemical composition of *P. tomentosa*, a fast-growing, multipurpose tree species known for its ecological and economic importance.

Cytokinins are essential phytohormones that play a crucial role in numerous aspects of plant growth and development (Prasad, 2022). They are particularly important in regulating cell proliferation and facilitating mitotic cell division in shoots. Recent research has shown that cytokinins can have both positive

and negative effects on cell cycle transitions, highlighting their complex role in plant physiology (Prasad, 2022).

Gibberellic acid (GA) markedly stimulates the vegetative growth of Paulownia trees by facilitating cell elongation and division, especially in the stem and internodes. This leads to enhanced stem elongation, enlarged leaves, and superior overall plant vitality. Gibberellin acid promotes subapical meristematic activity, resulting in accelerated shoot growth and elongation. Additionally, it improves nutrient assimilation and photosynthetic efficiency, which supports robust vegetative development (Gupta and Chakrabarty, 2013).

By examining the effects of nano NPK fertilizer, cytokinin, and gibberellic acid on *P. tomentosa*, this study aims to contribute to our understanding of how these treatments can influence both the vegetative growth and chemical composition of this valuable tree species. The results may have implications for improving cultivation practices and enhancing the production of beneficial phytochemicals in *P. tomentosa*.

## Materials and methods

This experiment was conducted at the nursery of Akre Technical College, Kurdistan region of Iraq. The Paulownia seedlings with length 7 cm and 0.3 cm diameter were planted in the medium that include (one part of sand + two parts of river soil + two parts of peat moss) in plastic pots with diameter 54 cm. The experiment was conducted to examine three factors, with the first factor involving the application of nano NPK fertilizer at concentrations of 0 and 6 g L<sup>-1</sup>. This fertilizer, sourced from Khazra Company, was applied monthly, beginning on 1<sup>st</sup> April and continuing until 1<sup>st</sup> September. The second factor after one week of planting seedlings in pots, the seedlings were sprayed with three different concentrations (0, 100, and 200 mg L<sup>-1</sup>) of cytokinin, obtained from Oxford Lab Chm Company, start from March 25<sup>th</sup>, July 15<sup>th</sup>, and September 15<sup>th</sup>. The last factor involved the application of gibberellic acid (GA<sub>3</sub>) to the plants at different concentrations (0, 200, and 400 mg L<sup>-1</sup>) sourced from Bio World Company. This treatment was carried out twice, on May 20<sup>th</sup> and August 20<sup>th</sup>. The study involved three factors 2 Nano NPK x 3 cytokinin x 3 GA<sub>3</sub> = 18 treatments with three replicates and 3 plants for each one. The RCBD design was used to carry out the factorial experiment. Data were analyzed using the SAS program, and mean comparisons were performed using Duncan's Multiple Range Test at a 5% significance level (SAS, 2010).

At the end of the experiment, the following parameters were recorded: plant height (cm), number of leaves (leaf plant<sup>-1</sup>), stem diameter (cm), leaf area (cm<sup>2</sup>), nitrogen percentage in leaves (%) according to Horneck and Miller (1998), phosphorus percentage in leaves (%) described by John (1970), potassium percentage in leaves (%) by Richards (1954), and total chlorophyll content in leaves (mg g<sup>-1</sup> fresh weight) according to Bruinsma (1963). And total carbohydrate percentage (%) according to Dubois *et al.* (1956).

## Results

**Plant height (cm):** Paulownia plants treated with a concentration of 6 g L<sup>-1</sup> demonstrated a notable enhancement in height, attaining

94.19 cm in contrast to the control (Table 1). Furthermore, elevated cytokinin concentrations markedly improved plant height, with 200 mg L<sup>-1</sup> yielding a height of 96.67 cm. The plant height increased significantly when sprayed with 400 mg L<sup>-1</sup> of gibberellic acid, reaching 92.11 cm compared to the control, which measured 80.28 cm.

Table 1. Effect of nano NPK fertilizers, cytokinin and gibberellic acid on the plants height (cm) of Paulownia tree plants

Nano NPK (g L <sup>-1</sup> )	Cytokinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* Nano Cytokinin NPK
		0	200	400	
0	0	82.00g-h	94.67cd	89.00d-f	88.56bc
	100	79.00h	83.00f-h	91.67de	84.56d
	200	94.00cd	86.67e-g	86.67e-g	89.11bc
6	0	87.00e-g	80.67g-h	92.67de	86.78cd
	100	81.67g-h	101.67b	91.33de	91.56ab
	200	112.00a	99.33bc	101.33b	104.22a
Nano NPK* GA <sub>3</sub> 6	0	85.00c	88.11bc	89.11b	Cytokinin
	100	93.56a	93.89a	95.11a	
	200	84.50de	87.67cd	90.83bc	87.67b
Cytokinin* GA <sub>3</sub>	0	80.33e	92.33b	91.50bc	88.06b
	100	103.00a	93.00b	94.00b	96.67a
	200	89.28b	91.00ab	92.11a	
Effect GA <sub>3</sub> acid		89.28b	91.00ab	92.11a	

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The combination of Nano NPK fertilizers and cytokinin had a significant impact on plant height. The tallest Paulownia trees, attaining a height of 104.22 cm, were those subjected to a treatment of 6 g L<sup>-1</sup> Nano NPK fertilizer and 200 mg L<sup>-1</sup> cytokinin. The shortest trees, measuring 84.56 cm, were those that received no Nano NPK fertilizer (0 g L<sup>-1</sup>) but were administered a dose of 100 mg L<sup>-1</sup> cytokinin. Despite the application of cytokinin, these trees exhibited a notable 23.24% increase in height relative to the baseline; however, the fertilizer had a significant impact.

The interaction among the three factors revealed that plants treated with 6 g L<sup>-1</sup> Nano NPK fertilizers, 200 mg L<sup>-1</sup> Cytokinin, and 0 mg L<sup>-1</sup> GA<sub>3</sub> had the greatest height, reaching 112 cm. This treatment resulted in a 41.77% increase in height than the control.

**Number of leaves:** The findings presented in Table 2 showed that applying Nano NPK to Paulownia trees at a concentration of 6 g L<sup>-1</sup> led to a significant rise in the number of leaves, which increased to 31.04 leaves per plant compared to 26.42 leaves per plant for the control group. Additionally, elevating the concentrations of cytokinin on the plants resulted in a statistically significant enhancement of this trait, with counts rising from 26.28 to 28.67 and 31.22 leaves per plant for concentrations of 0, 100, and 200 mg/L, respectively. Meanwhile, treating the Paulownia trees with GA<sub>3</sub> at a concentration of 400 mg/L also significantly improved this characteristic, resulting in 29.50 leaves per plant compared to the control value of 27.50 leaves per plant.

The interaction between Nano NPK fertilizers and Cytokinins had a significant impact on the number of leaves per plant. The highest leaf count observed was 32.89 leaves per plant with a treatment of 6 g L<sup>-1</sup> Nano NPK and 200 mg L<sup>-1</sup> cytokinin was applied, while the control group exhibited the lowest count at 24.22 leaves per plant. Additionally, plants treated with 6 g L<sup>-1</sup> Nano NPK and 200 mg L<sup>-1</sup> GA<sub>3</sub> displayed an impressive leaf count of 32.67 leaves per plant, in contrast to the lowest count of 25.67 leaves per plant

recorded for those treated with 0 g L<sup>-1</sup> Nano NPK and 200 mg L<sup>-1</sup> GA<sub>3</sub>. The third dual interaction between cytokinin and GA<sub>3</sub> showed that the best significant interaction was for 200 mg L<sup>-1</sup> cytokinin and 400 mg L<sup>-1</sup> GA<sub>3</sub>, which gave an average of 33.17 leaves per plant compared to the lowest value of 25.33 leaves per plant for both cytokinin and GA<sub>3</sub> at a concentration of 0 mg L<sup>-1</sup>.

Table 2. Effect of nano NPK fertilizers, cytokinin and gibberellic acid on the number of leaves (leaf plant<sup>-1</sup>) of Paulownia tree plants

Nano NPK (g L <sup>-1</sup> )	Cytokinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* Cytokinin	Nano NPK
		0	200	400		
0	0	22.67 <sup>i</sup>	25.00g <sup>i</sup>	25.00g <sup>i</sup>	24.22c	26.41b
	100	27.00e <sup>-h</sup>	24.00h <sup>-i</sup>	25.33f <sup>-i</sup>	25.44c	
	200	28.67c <sup>-g</sup>	28.00d <sup>-g</sup>	32.00a <sup>-c</sup>	29.56b	
6	0	28.00d <sup>-g</sup>	27.67d <sup>-h</sup>	29.33c <sup>-e</sup>	28.33b	31.04a
	100	29.67c <sup>-e</sup>	35.00a	31.00b <sup>-d</sup>	31.89a	
	200	29.00c <sup>-f</sup>	35.33a	34.33ab	32.89a	
Nano NPK* GA <sub>3</sub>	0	26.11c	25.67c	27.44bc	Cytokinin	
	6	28.89b	32.67a	31.56a		
	0	25.33e	26.33de	27.17c <sup>-e</sup>		
GA <sub>3</sub>	100	28.33cd	29.50bc	28.17cd	28.67b	
	200	28.83cd	31.67ab	33.17a	31.22a	
Effect GA <sub>3</sub> acid		27.50b	29.17a	29.50a		

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The interaction among the NPK fertilizer concentration, cytokinins and GA<sub>3</sub> revealed that plants treated with 6 g L<sup>-1</sup> of Nano NPK, along with 200 mg L<sup>-1</sup> of both cytokinin and GA<sub>3</sub>, produced the highest number of leaves, reaching an average of 35.33 leaves per plant. In contrast, the control group only achieved an average of 22.67 leaves per plant.

**Stem diameter (cm):** Application of Nano NPK at a concentration of 6 g L<sup>-1</sup> on Paulownia trees resulted in a significant increase in stem diameter, reaching 5.67 cm compared to the control group (Table 3). In contrast, applying cytokinin at concentrations of 100 mg L<sup>-1</sup> and 200 mg L<sup>-1</sup> also significantly enhanced stem diameter to 5.50 cm and 5.56 cm, respectively, compared to the control, which had a diameter of 4.27 cm, reflecting an increase of 30.21%. However, treatments with any concentration of GA<sub>3</sub> did not lead to significant changes in stem diameters.

Table 3. Effect of nano NPK fertilizer s, cytokinin and gibberellic acid on the stem diameter (cm) of Paulownia tree plants

Nano NPK (g L <sup>-1</sup> )	Cytokinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* Cytokinin	Nano NPK
		0	200	400		
0	0	4.07c	4.53c	4.00c	4.20b	4.55b
	100	4.67bc	5.33a <sup>-c</sup>	4.33c	4.78b	
	200	4.67bc	4.33c	5.00a <sup>-c</sup>	4.67b	
6	0	3.33c	4.33c	5.33a <sup>-c</sup>	4.33b	5.67a
	100	5.67a <sup>-c</sup>	5.67a <sup>-c</sup>	7.33a	6.22a	
	200	7.00ab	7.00ab	5.33a <sup>-c</sup>	6.44a	
Nano NPK* GA <sub>3</sub>	0	4.47b	4.73ab	4.44b	Cytokinin	
	6	5.33ab	5.67ab	6.00a		
	0	3.70b	4.43ab	4.67ab		
GA <sub>3</sub>	100	5.17ab	5.50a	5.83a	5.50a	
	200	5.83a	5.67a	5.17ab	5.56a	
Effect GA <sub>3</sub> acid		4.90a	5.20a	5.22a		

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The study on the interaction between Nano NPK fertilizers and cytokinin concentrations demonstrated that the stem diameter increased to 6.44 cm with a treatment of 6 g L<sup>-1</sup> of Nano NPK fertilizers combined with 200 mg L<sup>-1</sup> of cytokinin, while the control group exhibited the smallest stem diameter at 4.20 cm. In considering the interaction between Nano NPK fertilizers and GA<sub>3</sub>, the maximum stem diameter of 6.0 cm was attained with the application of 6 g L<sup>-1</sup> Nano NPK and 400 mg L<sup>-1</sup> GA<sub>3</sub>, in contrast to the minimum stem diameter of 4.44 cm recorded in plants treated with 0 g L<sup>-1</sup> of Nano NPK fertilizers and 400 mg L<sup>-1</sup> GA<sub>3</sub>. Furthermore, the dual interaction between cytokinin and GA<sub>3</sub> revealed that the most significant results were obtained with 100 mg L<sup>-1</sup> cytokinin combined with 400 mg L<sup>-1</sup> GA<sub>3</sub>, as well as with 200 mg L<sup>-1</sup> cytokinin paired with 0 mg L<sup>-1</sup> GA<sub>3</sub>, both resulting in a stem diameter of 5.83 cm. In contrast, the control group recorded the lowest stem diameter at 3.70 cm.

The interaction among the three factors studied revealed that the plants treated with 6 g L<sup>-1</sup> Nano NPK fertilizers, 100 mg L<sup>-1</sup> Cytokinin, and 400 mg L<sup>-1</sup> GA<sub>3</sub> achieved the greatest stem diameter of 7.33 cm. In contrast, the lowest stem diameter recorded was 3.33 cm for the plants that received 6 g L<sup>-1</sup> Nano NPK, but no cytokinin or GA<sub>3</sub>.

**Leaves area (cm<sup>2</sup>):** The application of 6 g L<sup>-1</sup> Nano NPK on Paulownia trees resulted in a significant increase in leaf area, measuring 291.37 cm<sup>2</sup>, in contrast to the control's 201.04 cm<sup>2</sup>. Elevating cytokinin concentration to 200 mg/L further augmented leaf area to 277.83 cm<sup>2</sup>, representing a percentage increase of 24.74% relative to controls, which measured 222.72 cm<sup>2</sup>. The application of GA<sub>3</sub> at 400 mg L<sup>-1</sup> also significantly boosted leaf area to 257.9 cm<sup>2</sup>, compared to the control's 231.5 cm<sup>2</sup>.

The interaction between Nano NPK fertilizers and cytokinin showed that leaf area increased to an impressive 359.33 cm<sup>2</sup> when combining 6 g L<sup>-1</sup> Nano NPK with 200 mg L<sup>-1</sup> cytokinin, while the lowest measurement was just 196.33 cm<sup>2</sup> for plants treated with no Nano NPK but with cytokinin at 200 mg L<sup>-1</sup>. On the other hand, a combination of Nano NPK with GA<sub>3</sub> produced leaf area (314.67 cm<sup>2</sup>) which was significantly more than the control (193.22 cm<sup>2</sup>). The suitable interaction between cytokinin and GA<sub>3</sub> had also occurred, so that in the combination of 200 mg L<sup>-1</sup> cytokinin and 400 mg L<sup>-1</sup> GA<sub>3</sub> it generated the largest leaf area of 297.50 cm<sup>2</sup> versus the lowest control value 206.67 cm<sup>2</sup>.

Table 4. Effect of nano NPK fertilizers, cytokinin and gibberellic acid on the leaves area (cm<sup>2</sup>) of Paulownia tree plants

Nano NPK (g L <sup>-1</sup> )	Cyto-kinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* Cytokinin	Nano NPK
		0	200	400		
0	0	196.67ij	230.00g <sup>-i</sup>	200.67ij	209.11d	201.04b
	100	200.00ij	193.67j	199.33ij	197.67d	
	200	183.00j	202.67ij	203.33ij	196.33d	
6	0	216.67h <sup>-j</sup>	237.33f <sup>-h</sup>	255.00e <sup>-g</sup>	236.33c	291.37a
	100	266.33ef	271.67de	297.33cd	278.44b	
	200	326.33c	360.00b	391.67a	359.33a	
Nano NPK* GA <sub>3</sub>	0	193.22d	208.78d	201.11d	Cytokinin	
	6	269.78b	289.67b	314.67a		
	0	206.67d	233.67bc	227.83c		
GA <sub>3</sub>	100	233.17bc	232.67bc	248.33bc	238.06b	
	200	254.67b	281.33a	297.50a	277.83a	
effect GA <sub>3</sub> acid		231.5b	249.2a	257.9a		

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The interaction of the three factors indicated that the integrated application of 6 g L<sup>-1</sup> Nano NPK, 200 mg L<sup>-1</sup> cytokinin, and 400 mg L<sup>-1</sup> GA<sub>3</sub> recorded the maximum leaf area of 391.67 cm<sup>2</sup>, compared to 183.00 cm<sup>2</sup> for plants not treated with Nano NPK but treated with cytokinin and without application of GA<sub>3</sub>. This study demonstrates that a particular combination of fertilizers and plant hormones could boost stem diameter and leaf area in Paulownia trees and therefore represents a vital step towards developing strategies to improve growth in other similar plants through targeted nutrient management practices.

**Nitrogen in leaves (%):** The findings in Table 5 show that treating Paulownia trees with 6 g L<sup>-1</sup> Nano NPK fertilizers significantly increased nitrogen content in leaves to 1.70%, compared to 1.41% in the control group. Similarly, applying 200 mg L<sup>-1</sup> cytokinins raised nitrogen levels to 1.65%, while the lowest recorded value was 1.47%. The use of 400 mg L<sup>-1</sup> GA<sub>3</sub> also boosted nitrogen content to 1.63%, compared to 1.49% in untreated plants.

Interactions between Nano NPK fertilizers and cytokinins revealed that combining 6 g L<sup>-1</sup> Nano NPK fertilizers with 200 mg L<sup>-1</sup> cytokinins resulted in the highest nitrogen content of 1.93%, whereas the lowest value of 1.36% was observed in plants treated with no Nano NPK fertilizers and 100 mg L<sup>-1</sup> cytokinins. Similarly, the interaction between Nano NPK fertilizers and GA<sub>3</sub> showed that nitrogen levels increased to 1.88% when treated with 6 g L<sup>-1</sup> Nano NPK fertilizers and 400 mg L<sup>-1</sup> GA<sub>3</sub>, while the control group recorded the lowest level at 1.32%.

Table 5. Effect of nano NPK fertilizers, cytokinin and gibberellic acid on the Nitrogen in leaves (%) of Paulownia tree plants.

Nano NPK (g L <sup>-1</sup> )	Cytokinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* cytokinin	Effect Nano NPK
		0	200	400		
0	0	1.25fg	1.64b-fi	1.57c-g	1.49bc	1.41b
	100	1.40e-g	1.47d-g	1.20g	1.36c	
	200	1.30e-g	1.45d-g	1.37e-g	1.37c	
6	0	1.83a-d	1.34e-g	1.60b-f	1.59b	1.70a
	100	1.47d-g	1.43e-g	1.87a-c	1.59b	
	200	1.67b-e	1.96ab	2.17a	1.93a	
Nano NPK* 0	GA <sub>3</sub>	1.32d	1.52b-d	1.38cd	Cytokinin	
Cytokinin* 0	GA <sub>3</sub>	1.66b	1.58bc	1.88a		
GA <sub>3</sub>	0	1.54ab	1.49b	1.59ab	1.54ab	
	100	1.44b	1.45b	1.54ab	1.47b	
	200	1.49b	1.70ab	1.77a	1.65a	
Effect GA <sub>3</sub> acid		1.49b	1.55ab	1.63a		

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The interaction between cytokinin and GA<sub>3</sub> demonstrated that the combination of 200 mg L<sup>-1</sup> cytokinins and 400 mg L<sup>-1</sup> GA<sub>3</sub> yielded a nitrogen content of 1.77%, compared to the lowest value of 1.44%, which was recorded for plants treated with 100 mg L<sup>-1</sup> cytokinins without GA<sub>3</sub>.

Finally, the triple interaction among Nano NPK fertilizers, cytokinins, and GA<sub>3</sub> revealed that plants treated with 6 g L<sup>-1</sup> Nano NPK fertilizers, 200 mg L<sup>-1</sup> cytokinins, and 400 mg L<sup>-1</sup> GA<sub>3</sub> achieved the highest nitrogen content in leaves at 2.17%, while the lowest nitrogen content of 1.20% was observed in plants receiving no Nano NPK fertilizers, cytokinins, or GA<sub>3</sub>. These results highlight the synergistic effects of combining Nano NPK fertilizers, cytokinins, and GA<sub>3</sub> on nitrogen uptake and assimilation in Paulownia trees.

**Phosphorus in leaves (%):** The findings presented in Table 6 indicate that treating Paulownia tree plants with a Nano NPK solution at a concentration of 6 g L<sup>-1</sup> significantly increased phosphorus levels in the leaves to 2.30%, compared to 1.95% in the control group. Conversely, the application of different concentrations of cytokinins did not yield any significant alterations in leaf phosphorus content. Nonetheless, the application of 400 mg L<sup>-1</sup> of GA<sub>3</sub> to the plants resulted in a substantial elevation of phosphorus levels, attaining 2.80%, in contrast to 1.19% observed in the control group.

The interaction results indicated that the maximum leaf phosphorus content, at 2.81%, was observed in plants treated with 6 g L<sup>-1</sup> of Nano NPK and 200 mg L<sup>-1</sup> of cytokinin, demonstrating a significant difference from all other treatments. The interaction between Nano NPK fertilizers and GA<sub>3</sub> indicated that phosphorus concentrations in the leaves attained 3.05% for plants treated with 6 g L<sup>-1</sup> of Nano NPK and 400 mg L<sup>-1</sup> of GA<sub>3</sub>, whereas the control group exhibited the lowest phosphorus concentration at merely 0.91%. The interaction between cytokinin and GA<sub>3</sub> revealed that the most pronounced effect was observed with 200 mg L<sup>-1</sup> of cytokinin in conjunction with 400 mg L<sup>-1</sup> of GA<sub>3</sub>, yielding a phosphorus concentration of 3.00%, in contrast to the minimum value of 1.05% for plants treated with 200 mg L<sup>-1</sup> of cytokinin alone.

Table 6. Effect of nano NPK fertilizers, cytokinin and gibberellic acid on the phosphorus in leaves (%) of Paulownia tree plants.

Nano NPK (g L <sup>-1</sup> )	Cytokinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* Cytokinin	Nano NPK
		0	200	400		
0	0	0.64g	2.46b-e	3.23b	2.11b	1.95b
	100	1.06fg	2.61b-d	2.56b-e	2.08b	
	200	1.04fg	2.05d-e	1.85d-f	1.65b	
6	0	1.63ef	2.28c-e	1.86d-f	1.92b	2.30a
	100	1.70d-f	1.65ef	3.13bc	2.16b	
	200	1.05fg	3.24b	4.16a	2.81a	
Nano NPK* 0	GA <sub>3</sub>	0.91d	2.38b	2.55b		
Cytokinin *GA <sub>3</sub>	0	1.46c	2.39b	3.05a	Cytokinin	
Effect GA <sub>3</sub> acid	0	1.14c	2.37ab	2.55ab	2.02a	
	100	1.38c	2.13b	2.85a	2.12a	
	200	1.05c	2.65ab	3.00a	2.23a	
Effect GA <sub>3</sub> acid		1.19c	2.38b	2.80a		

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The interaction among the three examined factors demonstrated that plants subjected to 6 g L<sup>-1</sup> of Nano NPK fertilizers, 200 mg L<sup>-1</sup> of cytokinin, and 400 mg L<sup>-1</sup> of GA<sub>3</sub> displayed the highest phosphorus concentration in their foliage, attaining 4.16%, whereas control plants exhibited a markedly lower phosphorus level of merely 0.64%.

**Potassium concentration in foliage:** Table 7 demonstrates that the application of a Nano NPK solution at a concentration of 6 g L<sup>-1</sup> to Paulownia trees resulted in a significant elevation of potassium levels in the leaves, attaining 2.05%, in contrast to the 1.75% recorded in the control group. Additionally, applying any concentrations of cytokinins and GA<sub>3</sub> did not result in significant changes in leaf potassium content.

The interaction results indicated that the highest potassium

concentration in leaves was 2.31% for plants treated with 6 g L<sup>-1</sup> of Nano NPK combined with 200 mg L<sup>-1</sup> of cytokinin. The lowest concentration, 1.64%, was recorded in plants that did not receive Nano NPK fertilizer but were administered 200 mg L<sup>-1</sup> of cytokinin. The interaction between Nano NPK fertilizers and GA<sub>3</sub> indicated that the potassium content in leaves was 2.09% for plants treated with 6 g L<sup>-1</sup> of Nano NPK without GA<sub>3</sub>, whereas the lowest concentration was 1.65% for plants that received no Nano NPK and 200 mg L<sup>-1</sup> of GA<sub>3</sub>.

The third interaction between cytokinin and GA<sub>3</sub> demonstrated that the most pronounced effect was observed at 100 mg L<sup>-1</sup> of cytokinin without GA<sub>3</sub>, yielding a potassium concentration of 2.21%, significantly exceeding the control group's minimum value of 1.58%.

Table 7. Effect of nano NPK fertilizers, cytokinin and gibberellic acid on the Potassium in leaves (%) of Paulownia tree plants

Nano NPK (g L <sup>-1</sup> )	Cytokinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* Cytokinin	Nano NPK
		0	200	400		
0	0	1.69bc	1.85a-c	1.91a-c	1.82bc	
	100	1.83a-c	1.67bc	1.84a-c	1.78bc	1.75b
	200	1.72bc	1.44c	1.77bc	1.64c	
6	0	1.48c	1.89a-c	1.93a-c	1.76bc	
	100	2.59a	1.92a-c	1.72bc	2.08ab	2.05a
	200	2.19a-c	2.29ab	2.45ab	2.31a	
Nano NPK* GA <sub>3</sub>		01.75ab	1.65b	1.84ab	Cytokinin	
		62.09a	2.03ab	2.03ab		
Cytokinin* GA <sub>3</sub>	0	1.58b	1.87ab	1.92ab	1.79a	
	100	2.21a	1.80ab	1.78ab	1.93a	
	200	1.95ab	1.87ab	2.11ab	1.98a	
Effect GA <sub>3</sub> acid		1.92a	1.84a	1.94a		

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The interaction of the three factors demonstrated that plants receiving 6 g L<sup>-1</sup> of Nano NPK fertilizers, 100 mg L<sup>-1</sup> of cytokinin, and no GA<sub>3</sub> had the highest potassium concentration in their leaves (2.59%), whereas the control group exhibited a markedly lower potassium level.

#### Total chlorophyll content in leaves (mg g<sup>-1</sup> fresh weight):

The results in Table 8 indicate that the application of Nano NPK at a concentration of 6 g L<sup>-1</sup> on Paulownia trees significantly elevated potassium levels in the leaves to 24.44 mg g<sup>-1</sup> of fresh weight, in contrast to 21.23 mg g<sup>-1</sup> of fresh weight observed in the control group. Moreover, no significant differences were observed in the total chlorophyll content of the leaves when plants were subjected to different concentrations of cytokinin. A notable increase in total chlorophyll content was recorded when the plants were treated with 200 mg L<sup>-1</sup> of GA<sub>3</sub>, resulting in 24.5 mg g<sup>-1</sup> of fresh weight, compared to 21.3 mg g<sup>-1</sup> of fresh weight in the control group.

The results of the interaction studies indicated that leaves treated with 6 g L<sup>-1</sup> Nano NPK and 100 mg L<sup>-1</sup> cytokinin had a total chlorophyll content of 26.42 mg g<sup>-1</sup> fresh weight. Additionally, the combination of Nano NPK fertilizers with GA<sub>3</sub> demonstrated a higher chlorophyll content of 27.59 mg g<sup>-1</sup> fresh weight when

plants were sprayed with 6 g L<sup>-1</sup> Nano NPK and 200 mg L<sup>-1</sup> of GA<sub>3</sub>, whereas the lowest chlorophyll content recorded was 20.99 mg g<sup>-1</sup> fresh weight for the treatment with 0 g L<sup>-1</sup> Nano NPK and 400 mg L<sup>-1</sup> GA<sub>3</sub>. Furthermore, the interaction between cytokinin and GA<sub>3</sub> revealed that the optimal combination was 100 mg L<sup>-1</sup> cytokinin with 200 mg L<sup>-1</sup> GA<sub>3</sub>, resulting in a chlorophyll content of 25.21 mg g<sup>-1</sup> fresh weight, compared to the control, which had a lower value of 20.63 mg g<sup>-1</sup> fresh weight.

Table 8. Effect of nano NPK fertilizers, cytokinin and gibberellic acid on the Total chlorophyll content in leaves (mg g<sup>-1</sup> fresh weight) of Paulownia tree plants

Nano NPK (g L <sup>-1</sup> )	Cytokinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* Cytokinin	Nano NPK
		0	200	400		
0	0	19.87c	21.74c	20.75c	20.79bc	
	100	20.66c	21.54c	19.46c	20.55c	21.23b
	200	23.08bc	21.20c	22.76bc	22.35bc	
6	0	21.40c	24.87a-c	22.83bc	23.03bc	
	100	22.53bc	28.89a	27.85ab	26.42a	24.44a
	200	20.24c	29.01a	22.36bc	23.87ab	
Nano NPK* GA <sub>3</sub>	0	21.20c	21.49bc	20.99c	Cytokinin	
	6	21.39bc	27.59a	24.35b		
	0	20.63b	23.30ab	21.79ab	21.91a	
Cytokinin* GA <sub>3</sub>	0	20.63b	23.30ab	21.79ab	21.91a	
	100	21.60ab	25.21a	23.65ab	23.49a	
	200	21.66ab	25.10a	22.56ab	23.11a	
Effect GA <sub>3</sub> acid		21.3b	24.5a	22.7ab		

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The interaction among the three factors studied revealed that plants treated with 6 g L<sup>-1</sup> Nano NPK fertilizers, along with 200 mg L<sup>-1</sup> cytokinin and 200 mg L<sup>-1</sup> GA<sub>3</sub>, exhibited the highest total chlorophyll content in their leaves, measuring 29.01 mg g<sup>-1</sup> of fresh weight. In contrast, the lowest total chlorophyll content, which was 19.46 mg g<sup>-1</sup> of fresh weight, was observed in plants that received no Nano NPK 0 g L<sup>-1</sup>, along with 100 mg L<sup>-1</sup> cytokinin and 400 mg L<sup>-1</sup> GA<sub>3</sub>.

**Total carbohydrate in leaves (%):** The findings presented in Table 9 showed that applying Nano NPK at a concentration of 6 g L<sup>-1</sup> to Paulownia trees resulted in a notable increase in the total carbohydrate content in the leaves, measuring at 9.16 % compared to the control group. Conversely, the application of 200 mg L<sup>-1</sup> of cytokinins and GA<sub>3</sub> also led to significant differences in the total carbohydrate levels in the leaves, which reached 8.94 % and 8.30 %, respectively, when compared to the control.

The study showed that the interaction between Nano NPK fertilizers and cytokinin concentration led to a significant increase in total carbohydrates in the leaves. Specifically, the leaves of plants treated with 6 g L<sup>-1</sup> Nano NPK fertilizers and 200 mg L<sup>-1</sup> cytokinin had a total carbohydrate content of 11.14 %, while the control group had the lowest value at 6.15 %. Moreover, the combination of Nano NPK fertilizers and GA<sub>3</sub> also resulted in higher carbohydrate levels, with leaves of plants treated with 6 g L<sup>-1</sup> Nano NPK and 200 mg L<sup>-1</sup> GA<sub>3</sub> reaching 9.78 %, compared to just 6.00 % in the control group. Additionally, the interaction of cytokinin and GA<sub>3</sub> revealed that plants treated with 200 mg L<sup>-1</sup> cytokinin and 400 mg L<sup>-1</sup> GA<sub>3</sub> achieved a carbohydrate level of 9.66 %, whereas the control group's carbohydrate content was only 5.17 %.

Table 9. Effect of nano NPK fertilizers, cytokinin and gibberellic acid on the Total carbohydrate in leaves (%) of Paulownia tree plants

Nano NPK (g L <sup>-1</sup> )	Cytokinin (mg/L)	Gibberellic acid (mg L <sup>-1</sup> )			Nano NPK* Cytokinin	Nano NPK
		0	200	400		
0	0	5.67e-g	4.95g	7.83c-f	6.15c	6.56b
	100	6.92d-g	8.07c-f	5.33f-g	6.77c	
	200	5.40f-g	7.44d-g	7.40d-g	6.75c	
6	0	4.68g	8.58b-d	8.48b-e	7.25c	9.16a
	100	8.74b-d	9.76a-d	8.82b-d	9.11b	
	200	10.49a-c	11.01ab	11.91a	11.14a	
Nano NPK* GA <sub>3</sub>	0	6.00c	6.82bc	6.85bc	Cytokinin	
	6	7.97b	9.78a	9.74a		
	200	5.17d	6.76cd	8.15a-c	6.70b	
Cytokinin* GA <sub>3</sub>	0	5.17d	6.76cd	8.15a-c	6.70b	
	100	7.83a-c	8.91ab	7.08bc	7.94a	
	200	7.95a-c	9.23a	9.66a	8.94a	
Effect GA <sub>3</sub> acid		6.98b	8.30a	8.30a		

Means with the same letter for every component and interaction do not differ significantly at the 5% level.

The triple interaction among the three-factor studied showed that the plants which sprayed 6 g L<sup>-1</sup> Nano NPK fertilizers, 200 mg L<sup>-1</sup> cytokinin and 400 mg L<sup>-1</sup> GA<sub>3</sub> gave the highest value of total carbohydrate content 11.14 % when compared with the lowest value 4.68 % for plants sprayed 6 g L<sup>-1</sup> Nano NPK fertilizer and 0 mg L<sup>-1</sup> for both cytokinin and GA<sub>3</sub>.

## Discussion

The study examined the impact of Nano NPK fertilizers, cytokinin, and GA<sub>3</sub> on the growth of Paulownia seedlings. Nano NPK fertilizers at 6 g L<sup>-1</sup> markedly enhanced several growth parameters, such as plant height, leaf count, stem diameter, and leaf area. This enhancement is ascribed to the fertilizers' extensive surface area and gradual nutrient release, which optimize absorption and foster robust growth (Saied, 2018; Sajyan *et al.*, 2020). Nano NPK fertilizers enhanced stem diameter by supplying vital nutrients that promote cell division and expansion. NPK uptake in leaves was enhanced, presumably due to increased bioavailability and efficient absorption (Veronica *et al.*, 2015; Solanki *et al.*, 2015). The fertilizers increased total chlorophyll content by swiftly supplying nutrients via stomata, thereby accelerating pigment synthesis (Al-Mohammad *et al.*, 2021).

Cytokinin at 200 mg L<sup>-1</sup> increased branch number, plant height, stem diameter, leaf area, and nitrogen content in leaves. This is due to cytokinin's role in stimulating protein production, regulating the cell cycle, and encouraging chloroplast maturation (George *et al.*, 2008; Mohamad *et al.*, 2022). GA<sub>3</sub> at 400 mg L<sup>-1</sup> markedly enhanced branch count, plant height, leaf area, nitrogen and phosphorus levels, total chlorophyll, and carbohydrate percentage in leaves. The effects of GA<sub>3</sub> are ascribed to its capacity to elevate auxin levels, stimulate cell elongation and division, and initiate the synthesis of hydrolytic enzymes (Nelissen *et al.*, 2012; Baliah *et al.*, 2018).

The integration of Nano NPK fertilizers, cytokinin, and GA<sub>3</sub> produced synergistic effects on the growth of Paulownia seedlings. This comprehensive strategy resulted in improved nutrient absorption, increased photosynthetic efficacy, and overall enhanced plant growth.

The results indicate that a judicious application of Nano NPK fertilizers, cytokinin, and GA<sub>3</sub> can markedly improve the growth and development of Paulownia seedlings. The research underscores the efficacy of combining these treatments to enhance plant growth metrics and nutrient composition in horticultural and forestry contexts (Majid and Abbas, 2019; Al-Rawi *et al.*, 2016).

The experiment showed that Paulownia tree seedlings responded positively to NPK fertilizers, cytokinin, and GA<sub>3</sub>. The treatment with 6 g L<sup>-1</sup> Nano NPK, and 200 mg L<sup>-1</sup> cytokinin and 400 mg L<sup>-1</sup> GA<sub>3</sub> resulted in improved vegetative and chemical parameters, while sprayed plant with 200 mg L<sup>-1</sup> GA<sub>3</sub>, showed increased total chlorophyll content in leaves. The optimal levels of these treatments were found to promote growth and development of the Paulownia tree. Further research is needed to explore the effects of higher concentrations.

## Reference

- Al-Mohammad, M.H., T.F. Sachet and Z.S. Al-Dulaimi, 2021. Effect of phenylalanine, jasmonic acid and biofertilizer on growth, yield and anthocyanin pigments of Roselle Calyces. *IOP Conf. Ser.: Earth Environ. Sci.*, 910: 012077.
- Al-Rawi, W.A.A., M.E.A. Al-Hadethi and A.A. Abdul-Kareem, 2016. Effect of foliar application of gibberellic acid and seaweed extract spray on growth and leaf mineral content on peach trees. *Iraqi J. Agric. Sci.*, 47(7-special issue): 98-105.
- Baliah, N.T., P.C. Sheeba and S. Mallika, 2018. Encouraging effect of gibberellic acid on the growth and biochemical characters of green gram (*Vigna radiata* L.). *J. Glob. Biosci.*, 7(8): 5522-5529.
- Brunsmas, J. 1963. The quantitative analysis of chlorophylls a and b in plant extracts. *Photochem. Photobiol.*, 2(2): 241-249.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28(3): 350-356.
- George, E.F., M.A. Hall and G.D. Klerk, 2008. *Plant Propagation by Tissue Culture*. Dordrecht: Springer.
- Gupta, R. and S.K. Chakrabarty, 2013. Gibberellic acid in plant: Still a mystery unresolved. *Plant Signal Behav.*, 8(9): e25504.
- Horneck, D.A. and R.O. Miller, 1998. Determination of Total Nitrogen in Plant Tissue. In: *Handbook of Reference Methods for Plant Analysis*, edited by Y.P. Kalra, Taylor & Francis Group LLC., pp: 73.
- Hayam, A.A. Mahdy, A.S. Tantawy, A.M.R. Abdel-Mawgoud and Z.F. Fawzy, 2024. Physiological and growth attributes of salt-stressed tomato plants in response to foliar application of nano phosphorus or potassium. *Journal of Applied Horticulture*, 26(1): 68-72. <https://doi.org/10.37855/jah.2024.v26i01.13>.
- Jakubowski, M. 2022. Cultivation potential and uses of Paulownia wood: A review. *Forests*, 13(5): 668.
- John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Sci.*, 109(4): 214-220.
- Majid, I. and N. Abbas, 2019. Signal transduction in leaf senescence: An Overview. In: *Senescence Signaling and Control in Plants*, M. Sarwat and N. Tuteja (ed.), Elsevier Inc.
- Mandal, D., 2021. Nano fertilizer and its application in horticulture. *J. Appl. Hort.*, 23(1): 70-77.
- Mohamad, M.E., A.A. Awad, A. Majrashi, O.A. Abd Esadek, M.T. El-Saadony, A.M. Saad and A.S. Gendy, 2022. *In vitro* study on the effect of cytokinins and auxins addition to growth medium on the micropropagation and rooting of Paulownia species (*Paulownia hybrid* and *Paulownia tomentosa*). *Saudi J. Biol. Sci.*, 29(3): 1598-1603.
- Nelissen, H., B. Rymen, Y. Jikumaru, K. Demuyneck, M. Van Lijsebettens, Y. Kamiya and G.T. Beemster, 2012. A local maximum in gibberellin levels regulates maize leaf growth by spatial control of cell division. *Curr. Biol.*, 22(13): 1183-1187.

- Prasad, R. 2022. Cytokinin and its key role to enrich the plant nutrients and growth under adverse conditions—An update. *Front. Genet.*, 13: 883924.
- Richards, L.A., 1954. Diagnosis and Improvement of Saline and Alkali Soils. U.S.D.A Agriculture Handbook No. 60, U.S Government Printing Office, Washington.
- Saied, H.H., 2018. Response of Keite mango trees to spraying nano NPK Mg fertilizers. *Researcher*, 10: 1-5.
- Sajyan, T.K., S.M. Alturki and Y.N. Sassine, 2020. Nano fertilizers and their impact on vegetables: contribution of nano-chelate Super Plus Zfm and Lithovit standard to improve salt tolerance of pepper. *Annals Agric Sci.*, 65(2): 200-208.
- SAS Institute Inc., 2010. Statistical Analysis System. Cary, NC: SAS Institute Inc.
- Shilpa, R.S., C. Kant and N. Prashar, 2022. Role of Nano Fertilizers in Horticulture. *Pharma Innov. J.*, 11(6): 831-836.
- Solanki, P., A. Bhargava, H. Chhipa, N. Jain and J. Panwar, 2015. Nano Fertilizers and Their Smart Delivery System. In *Nanotechnologies Food Agric.*, 81-101.
- Veronica, N., T. Guru, R. Thatikunta and S. Narender Reddy, 2015. Role of nano fertilizers in agricultural farming. *Int J. Environ Sci Technol.*, 1(1): 1-3.
- Woźniak, M., A. Gałązka and M. Frąć, 2018. *Paulownia? Szybko Rosnące, Wielofunkcyjne Drzewo Bioenergetyczne. Kosmos*, 67(4): 781-789.
- Yadav, N.K., B.N. Vaidya, K. Henderson, J.F. Lee, W.M. Stewart, S.A. Dhekney and N. Joshee, 2013. A review of paulownia biotechnology: A short rotation, fast growing multipurpose bioenergy tree. *Am. J Plant Sci.*, 4(11): 2070.

---

Received: January, 2025; Revised: February, 2025; Accepted: February, 2025