Chemical Composition Of Neem Seedlings (Azadirachta Indica L.) Sprayed With Arginine, Kinetin, And Chelated Iron

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KEYWORDS

Active Ingredients, Amino Acids, Micronutrients, Cytokinin, Meliaceae Family

ABSTRACT

A pot experiment was carried out in the canopy of the Horticulture and Landscape Department - College of Agriculture - University of Kerbala during the 2023-2024 season to study the effect of kinetin (K) (0, 50, 100 mg L-1), arginine (A) (0, 150, 300 mg L-1), and chelated iron (F) (0 and 200 mg L-1) on some qualitative characteristics of neem seedlings. The study was implemented as a factorial experiment with a completely randomized block design with three replications. The majority of chemical features were significantly impacted by kinetin spraying, with the exception of the proportion of nitrogen, potassium, and protein in the leaves. Arginine affected all traits except total chlorophyll, while chelated iron mainly impacted potassium percentage. The research factors had varying effects on the features under investigation in the binary interaction findings. Treatment K1A1 produced the greatest rate of total chlorophyll, measuring 56.19 mg g-1 fresh weight, While the Fe2A2 treatment was superior in increasing the percentage of carbohydrates (13.31 mg g dry weight-1). With an average percentage of 3.308% for nitrogen content and 0.1588% for phosphorus, treatment K0A2 and treatment K1A2 respectively yielded the greatest percentages. However, treatment K0A1 produced a substantially higher percentage of protein, at 20.67%. Only the percentage of phosphorus was significantly affected by the triple intervention; the K1A0Fe2 treatment produced the highest rate of 0.1640%. This study concludes that High kinetin levels may have adverse effects, but when combined with nutrient-rich fertilizers, they can enhance seedling characteristics of trees with medicinal benefits.

1. Introduction

Neem trees, or Azadirachta indica L., are native to Southeast Asia, mainly Bangladesh and India. They thrive in tropical and subtropical regions and are members of the Meliaceae family (Vithalkar, 2023). This tree has garnered attention on a global scale; according to the American Academy of Sciences (Debjit Bhowmik et al., 2010), it has been dubbed the miracle tree and the tree of the twenty-first century. This tree's components are utilized in numerous industrial and agricultural applications, including the production of pharmaceuticals, since they are rich in phytochemicals including phenols, tannins, saponins, flavonoids, alkaloids, terpenoids, and fatty acids (Subapriya et al., 2005). Numerous neem active ingredients, including nimbanal, salannin, gedunin, and azadirachtin, have been demonstrated to have inhibitory functions in a variety of diseases. Many human ailments, including allergies, various skin problems, bleeding, diabetes, high blood pressure, and diuretics, have been treated with them (Chatterjee et al., 2011). The significance of neem tree leaves, branches, and roots in sustainable organic agriculture has been demonstrated by studies and research, since these parts of the tree may supply plants with natural amounts of NPK and microelements. It also contributes to the upkeep of plant health by acting as a powerful pesticide against nematodes, bacteria, fungus, and dangerous insects. For the same reason, it is also utilized as neem seed cake (Biswas et al., 2000 and Datta, 2024).

Plant hormones and growth regulators are non-food organic chemical compounds that are either intentionally supplied to or produced naturally by the plant. When introduced at different phases of growth, these chemicals alter the plant's growth and development (Paridaen, 2009; Al-Khafaji, 2014; Singh et al., 2021). Kinetin is a popular cytokinin that is widely used. It is recognized for its significant function in controlling the growth and differentiation of plant cells (Al-Doori and Hussein 2023), as well as its ability to postpone the aging process (Werner et al., 2001 and Joshi et al., 2019), promote the production of chlorophyll (Ashikari et al., 2005), and facilitate the movement and transfer of nutrients to the treated areas due to the fact that it is highly metabolic (Taiz et al., 2002), in addition to its role in the formation of some enzymes involved in the carbon fixation process (Zhang et al., 2021).

The building block of proteins, amino acids serve a variety of purposes in plants by controlling metabolism and the movement and storage of nitrogen. Because it starts the manufacture of proline, nitric oxide, and polyamines, arginine is the amino acid with the greatest variety of functions in living cells (Liu, 2006). It serves as the primary source of urea, and its ability to withstand environmental conditions including heat, cold, thirst, and salt is its most crucial function. Additionally, it aids in cell division, promotes the growth of roots, and produces chlorophyll (Mansour, 2000; Abdel Hafez, 2006).

Although micronutrients are not necessary for plants in large quantities, they are physiologically significant to plant life cycles. One of the elements that is essential for the respiration process is iron (Fe). It is essential for the system of many enzymes, such as catalase, peroxidas, and cytochrome, and plays a crucial role in oxidation and reduction reactions. In these reactions, iron transfers electrons, which is a crucial part of the cell's nutritional metabolism processes (Al-Mariqi, 2005). While not a component of chlorophyll, iron aids in the formation of chlorophyll. It is also vital for the metabolism of nucleic acids and chloroplasts, as well as for maintaining green matter inside plants. It contributes to the production of cytochromes, which are critical for the processes of respiration and photosynthesis (Taiz and Zeiger, 2002).

In addition to being one of the uncommon plants found across Iraq, neem trees also get little local study and research. This study intends to determine how spraying various quantities of growth regulator kinetin, amino acid arginine, and chelated iron, either separately or in combination, affects the chemical composition of neem leaves.

2. Methodology

Between the planting date of July 10, 2023, and June 1, 2024, the study was carried out under the plant canopy at the Department of Horticulture and Landscape Engineering, College of Agriculture, University of Kerbala, with 50% shade provided by saran. Neem seeds were planted in plastic bags of 5 kg of soil, which were filled with a soil mixture with peat moss at a ratio of 2 soil: 1 peat moss. The research was conducted using a factorial experiment utilizing a Randomized Complete Blocks Design (R.C.B.D.) with three replications. In each replication, there were eighteen treatments resulting from the application of three kinetin concentrations (0, 50, and 100 mg L-1), three arginine concentrations (0, 150, and 300 mg L-1), and two chelated iron concentrations (0 and 200 mg L-1). Eight plants (observations) each treatment meant that a total of 432 plants (18 treatments x 8 observations x 3 replicates) were included in the study. When the seedlings were two months old, they received four sprays in autumn and another four in the spring, applied in succession with the research factors. The percentage of nitrogen (Page et al., 1982), phosphorus (Al-Sahhaf, 1989), potassium (Hayness, 1980), and protein (Ibrahim et al., 2000) in the leaves was also determined at the conclusion of the experiment. The total chlorophyll content (mg g-1 fresh weight) was estimated using the method of Chappelle et al. (1992), and the total soluble carbohydrate content (mg g-1 dry weight) according to the method of Herbert et al. (1971).

3. Results and discussion

Total chlorophyll content in leaves (mg g^{-1} FW)

The results presented in Table 1 demonstrate that kinetin has a significant impact on the amount of total chlorophyll in leaves. Specifically, the concentration of 50 mg L^{-1} produced the highest average fresh weight of 49.67 mg L^{-1}, which was not significantly different from the concentration of 100 mg L^{-1}, which produced 45.43 mg L^{-1}, while the comparison treatment produced the lowest average of 42.63 mg g^{-1}. The same table shows that iron and arginine have no discernible influence on the overall amount of chlorophyll in leaves.

Chelated iron	Arginine	Kinetin (K) (mg L^{-1})	Iron x Arginine	Chelated iron

		0 (K0)	50 (K1)	100 (K2)		
0 (Fe1)	0 (A0)	41.81	39.94	41.81	43.34	**47.31**
	150 (A1)	34.64	48.00	34.64	54.09	
	300 (A2)	51.40	46.05	51.40	44.49	
200 (Fe2)	0 (A0)	49.92	39.54	49.92	46.08	**44.52**
	150 (A1)	36.82	52.24	36.82	40.79	
	300 (A2)	37.81	45.94	37.81	46.68	
		LSD. KxAxFe= N.S.			LSD AxFe= 6.723	**LSD. Fe=N.S.**
Averages of Kinetin		42.63	49.67	45.43	**LSD. K=4.754**	
Iron x Kinetin	0 (Fe1)	45.15	51.13	45.64		**Averages of Arginine**
	200 (Fe2)	40.12	48.20	45.23		
Arginine x Kinetin	LSD. Fe × K= N.S.					
	0 (A0)	40.88	48.72	44.53		**44.71**
	150 (A1)	40.13	56.19	45.99		**47.44**
	300 (A2)	46.89	44.08	45.78		**45.59**
	LSD. A× K =8.234					**LSD. A= N.S.**

The results of the dual interaction between the study factors show that there was a significant effect on the trait mentioned above. Specifically, the intervention treatment K1A1 outperformed the comparison treatment, giving the highest rate of total chlorophyll, amounting to 56.19 mg g^{-1}, while the comparison treatment gave the lowest rate, amounting to 40.88 mg g^{-1}. Additionally, there was a significant effect from the dual interaction between arginine and chelated iron; the Fe1A1 treatment gave a rate of 54.09 mg g^{-1}, while the comparison treatment gave a rate of 43.34 mg g^{-1}. There was no discernible impact of the triple interaction of the parameters under investigation on the chlorophyll characteristic (Table 1).

Table 1 Effect of spraying with kinetin, arginine, and chelated iron on the total chlorophyll content (mg g^{-1} fresh weight) in the leaves of neem seedlings

Total carbohydrates in leaves (mg g DW^{-1})

The substantial impact of kinetin treatments on the leaves' total carbohydrate content is seen in Table 2. When compared to the control treatment, which recorded the lowest average of 9.23 mg g dry weight-1, the kinetin treatment with a dosage of 100 mg L-1 was superior, recording 11.33 mg g dry weight-1. This difference did not vary significantly from the treatment with a concentration of 50 mg L-1. It was evident that arginine had a major impact because at a concentration of 300 mg L-1, it produced the highest average amount of carbohydrates 11.66 mg g dry weight-1 compared to the lowest average amount 8.26 mg g dry weight-1 recorded by the comparison treatment. The aforementioned attribute was not significantly affected by iron treatments, according to the same data.

The dual interaction between the factors is also evident in Table 2, where the Fe2A2 interaction treatment outperformed the other and produced the highest rate of total carbohydrates at 13.31 mg g dry weight-1, while the Fe2A0 treatment produced the lowest rates at 7.62 mg g dry weight-1. No discernible variations exist in the total quantity of carbohydrates in the leaves of neem seedlings, as indicated by the three-way interaction among the research variables.

Table 2 Effect of spraying with kinetin, arginine, and chelated iron on the total carbohydrate content (mg g^{-1} dry weight) in the leaves of neem seedlings.

Chelated iron (Fe) (mg L^{-1})	Arginine (A) (mg L^{-1})	Kinetin (K) (mg L^{-1})			Iron x Arginine	**Chelated iron averages**
		0 (K0)	50 (K1)	100 (K2)		
0 (Fe1)	0 (A0)	6.67	7.67	10.20	8.90	**10.05**
	150 (A1)	8.59	9.38	12.83	11.45	
	300 (A2)	5.99	6.49	11.51	9.80	
200 (Fe2)	0 (A0)	11.22	9.27	13.48	7.62	**10.09**
	150 (A1)	14.05	8.71	12.65	9.33	
	300 (A2)	8.18	10.75	13.63	13.31	
		LSD. KxAxFe= N.S.			LSD AxFe=2.356	**LSD. Fe=N.S.**
Averages of Kinetin		9.23	9.66	11.33	**LSD. K=1.666**	
Iron x Kinetin	0 (Fe1)	8.75	8.92	12.48		**Averages of Arginine**
	200 (Fe2)	9.70	10.40	10.17		
Arginine x Kinetin	LSD. Fe × K= N.S					
	0 (A0)	7.17	6.24	11.38		**8.26**
	150 (A1)	9.40	11.36	10.41		**10.39**
	300 (A2)	11.11	11.37	12.19		**11.56**
	LSD. A× K =N.S.					**LSD. A= 1.666**

Leaf content of Nitrogen %

The results presented in Table 3 indicate that the percentage of nitrogen in the leaves was not significantly affected by either kinetin or iron treatments alone. However, arginine spraying had a significant effect on this trait, as the treatment with a concentration of 150 mg L^{-1} excelled and recorded the highest average of 2.989%, which did not differ significantly from the 300 mg L^{-1} concentration treatment recorded 2.765%, while the comparison treatment recorded the lowest average of 2.474%.

The same data shows that the binary intervention treatments had a considerable impact since, at 3.308%, treatment K0A1 produced the greatest average nitrogen content, much higher than that of the comparative treatment, which came in at 2.438%. The preceding table indicates that the three-way interaction among the research components does not have any noteworthy impacts.

Table 3 Effect of spraying with kinetin, arginine, and chelated iron on the total Nitrogen content (%) in the leaves of neem seedlings.

Chelated iron (Fe) (mg L^{-1})	**Arginine (A) (mg L^{-1})**	**Kinetin (K) (mg L^{-1})**			**Iron x Arginine**	**Chelated iron averages**
		0 (K0)	50 (K1)	100 (K2)		
0 (Fe1)	0 (A0)	2.483	2.392	3.115	2.231	
	150 (A1)	3.500	2.917	2.800	3.133	**2.712**
	300 (A2)	1.867	2.800	2.900	2.772	
200 (Fe2)	0 (A0)	2.117	3.225	2.915	2.717	
	150 (A1)	2.342	2.960	3.383	2.844	**2.773**
	300 (A2)	2.917	2.175	2.558	2.758	
		LSD. KxAxFe= N.S.			LSD AxFe=N.S	**LSD. Fe=N.S.**
Averages of Kinetin		2.868	2.637	2.723	**LSD. K=1.666**	
Iron x Kinetin	0 (Fe1)	2.838	2.664	2.633		**Averages of Arginine**
	200 (Fe2)	2.897	2.611	2.812		
Arginine x Kinetin	LSD. Fe × K= N.S					
	0 (A0)	2.438	2.333	2.651		**2.474**
	150 (A1)	3.308	2.508	3.150		**2.989**
	300 (A2)	2.858	3.070	2.367		**2.765**
	LSD. A× K = 0.5681					**LSD. A=0.3280**

Leaf content of Phosphorus %

The percentage of phosphorus in the leaves is shown to be significantly affected by kinetin treatments in Table 4. The treatment with a concentration of 50 mg L-1 performed best, recording the highest average of 0.1467%. This treatment did not differ significantly from the treatment with a concentration of 100 mg L-1 when compared to the control treatment, which recorded the lowest average of 0.1467% for phosphorus. 0.1338%. The table also shows that the arginine treatments had a significant impact on the characteristic in question, as evidenced by the highest average percentage of phosphorus found at a concentration of 300 mg L^{-1}, or 0.1497%, which was not significantly different from the concentration of 150 mg L^{-1}, or 0.1427%, while the comparison treatment obtained data on Phosphorus had the lowest average, at 0.1316%. The proportion of phosphorus in leaves was not significantly impacted by chelated iron applications.

Table 4. Effect of spraying with kinetin, arginine, and chelated iron on the total Phosphorous content (%) in the leaves of neem seedlings.

Chelated iron (Fe) (mg L^{-1})	Arginine (A) (mg L^{-1})	Kinetin (K) (mg L^{-1})			Iron x Arginine	**Chelated iron averages**
		0 (K0)	50 (K1)	100 (K2)		
0 (Fe1)	0 (A0)	0.1217	0.1190	0.1390	0.1319	**0.1409**
	150 (A1)	0.1413	0.1390	0.1430	0.1419	
	300 (A2)	0.1330	0.1350	0.1483	0.1490	
200 (Fe2)	0 (A0)	0.1463	0.1640	0.1537	0.1313	**0.1417**
	150 (A1)	0.1410	0.1400	0.1383	0.1434	
	300 (A2)	0.1427	0.1440	0.1547	0.1504	
		LSD. KxAxFe= 0.01297			LSD AxFe=N.S	**LSD. Fe=N.S**
Averages of Kinetin		0.1338	0.1467	0.1434	**LSD. K=0.000529**	
Iron x Kinetin	0 (Fe1)	0.1332	0.1484	0.1411		**Averages of Arginine**
	200 (Fe2)	0.1344	0.1450	0.1458		
Arginine x Kinetin	LSD. Fe × K= 0.00749					
	0 (A0)	0.1203	0.1340	0.1405		**0.1316**
	150 (A1)	0.1402	0.1473	0.1405		**0.1427**
	300 (A2)	0.1410	0.1588	0.1493		**0.1497**
	LSD. A× K = 0.00917					**LSD. A=0.4967**

The two-way interaction between the study factors has significant effects, as Table 4 shows. The Fe2K2 intervention treatment performed better than the comparison treatment, delivering the highest percentage of phosphorus (0.1458%) compared to 0.1332%. The K1A2 intervention treatment produced a percentage of 0.1588% compared to the comparison treatment, which resulted in the lowest percentage (0.1203%). Significant differences can be seen from the triple interaction between the factors; the Fe2K1A0 intervention treatment had the highest average percentage of phosphorus, measuring 0.1640%, while the Fe1K1A0 treatment produced the lowest averages, measuring 0.1190% (Table 4).

Leaf content of Potassium %

According to Table 5, arginine had a significant impact on the potassium content of the leaves, with the treatment at a concentration of 300 mg L-1 recording the highest average of 1.739% compared to the control treatment's lowest average of 1.481%. However, kinetin did not significantly affect the potassium content of the leaves. Chelated iron also had a substantial impact; at 200 mg L-1 of concentration, it produced the greatest rate of 1.708% as opposed to the lowest rate of 1.472% for the no-spray treatment. The aforementioned attribute was not significantly affected by the two- or three-way interactions between the research components.

Table 5 Effect of spraying with kinetin, arginine, and chelated iron on the total Potassium content (%) in the leaves of neem seedlings.

Chelated iron (Fe) (mg L^{-1})	**Arginine (A) (mg L^{-1})**	**Kinetin (K) (mg L^{-1})**			**Iron x Arginine**	**Chelated iron averages**
		0 (K0)	50 (K1)	100 (K2)		
0 (Fe1)	0 (A0)	2.162	1.408	1.736	1.556	**1.472**
	150 (A1)	1.636	1.314	1.927	1.437	
	300 (A2)	1.164	1.661	1.094	1.423	
200 (Fe2)	0 (A0)	1.249	1.536	1.864	1.563	**1.708**
	150 (A1)	1.341	1.621	1.479	1.504	
	300 (A2)	1.628	1.419	2.374	2.055	
		LSD. KxAxFe= N.S.			LSD AxFe=N.S	**LSD. Fe=0.2885**
Averages of Kinetin		1.697	1.428	1.644	**LSD. K=N.S.**	
Iron x Kinetin	0 (Fe1)	1.737	1.265	1.413		**Averages of Arginine**
	200 (Fe2)	1.657	1.592	1.874		
Arginine x Kinetin	LSD. Fe × K= N.S					
	0 (A0)	1.785	1.413	1.481		**1.560**
	150 (A1)	1.686	1.172	1.554		**1.471**
	300 (A2)	1.621	1.700	1.897		**1.739**
	LSD. A× K = N.S.					**LSD. A=0.3533**

Leaf content of total protein %

The results presented in Table 6 indicate that the application of kinetin and chelated iron alone did not significantly impact the total protein content in the leaves. However, arginine spraying did have a significant effect on the percentage of total protein in the leaves, with the concentration of 150 mg L-1 exhibiting the highest total protein content (18.68%) in comparison to the comparison treatment, which resulted in the lowest average of 15.46% for the same characteristic.

The results of the binary interactions between the research factors demonstrated the significant impact of arginine and kinetin. Specifically, the K0A1 interaction treatment outperformed the comparison treatment, recording the highest average percentage of total protein in the leaves (20.67%), while the comparison treatment gave 15.23%. There are no discernible impacts on the percentage of total protein in the leaves, according to the three-way interaction of the research variables.

Table (6) Effect of spraying with kinetin, arginine, and chelated iron on the total Protein content (%) in the leaves of neem seedlings.

Chelated iron (Fe) (mg L^{-1})	**Arginine (A) (mg L^{-1})**	**Kinetin (K) (mg L^{-1})**			**Iron x Arginine**	**Chelated iron averages**
		0 (K0)	50 (K1)	100 (K2)		
0 (Fe1)	0 (A0)	15.52	14.95	19.47	13.94	**16.95**
	150 (A1)	21.87	18.23	17.50	19.58	
	300 (A2)	11.67	17.50	18.12	17.33	
200 (Fe2)	0 (A0)	13.23	20.16	18.22	16.98	**17.33**
	150 (A1)	14.64	18.50	21.15	17.78	
	300 (A2)	18.23	13.59	15.99	17.24	
		LSD. KxAxFe= N.S.			LSD AxFe=N.S	**LSD. Fe=N.S**

Averages of Kinetin		17.02	16.48	17.92	**LSD. K=N.S**	
Iron x Kinetin	0 (Fe1)	16.46	16.65	17.74		**Averages of Arginine**
	200 (Fe2)	17.57	16.32	18.11		
Arginine x Kinetin	LSD. Fe × K= N.S					
	0 (A0)	15.23	14.58	16.57		**15.46**
	150 (A1)	20.67	15.68	19.69		**18.68**
	300 (A2)	17.86	19.19	14.79		**17.28**
	LSD. A× K =3.551					**LSD. A=2.050**

The distinct and combined significant effects of kinetin, arginine, and iron on various chemical properties of neem seedling leaves (total chlorophyll content, total carbohydrate content, percentage of phosphorus, nitrogen, phosphorus, potassium, and total protein) were displayed in Tables 1, 2, 3, 4, 5, and 6.

According to Al-Halabi (2012), kinetin has a significant role in extending the pace and duration of carbon absorption, which is represented by proteins, phenols, and carbohydrates. This explains why spraying kinetin improves several chemical qualities. Moreover, kinetin promotes the activity of enzymes that convert and transport carbohydrates as well as those that produce certain essential enzymes, including nitrate reductase (NR) enzymes. with relation to the process of carbon absorption (Al-Dosqi, 2008). According to Al-Shahat (2000) and Joshi et al (2019), kinetin has a crucial function in raising nitrogen build-up in old leaf sites and lowering it in new leaf sites, which promotes the synthesis of amino acids and delays the senescence of leaves. This result aligned with findings from studies conducted on olive seedlings by Al-Isaw and Al-Janabi (2021), guava (Psidium guajava) seedlings by Alkalabi and Bajlan (2023), and lettuce (Lactuca sativa L.) seedlings by Urbutis et al. (2024).

The plants that were treated with arginine exhibited superior chemical properties. This is because amino acids play a crucial role in the production of proteins and enzymes, which in turn leads to an increase in the number of leaves and overall vegetative growth. Ultimately, this efficiency boosts the process of photosynthesis, which in turn increases the amount of carbohydrates found in leaves (Hassan et al., 2014). This was also demonstrated by EL-Naggar et al. (2013) and Abd-Elkader et al. (2020).

Iron, while not being a component of chlorophyll, is crucial for its synthesis, and treating neem seedlings with chelated iron increased the amount of chlorophyll in their leaves through interactions with kinetin and arginine. The reason for this is because iron acts as an activator and catalyst, guiding a sequence of chemical events that result in the production of chlorophyll molecules, which raise the chlorophyll content of leaves (Mengel et al., 1987). These outcomes agree with the orange tree study conducted by Al-Mmureib (2008). Additionally, it was shown that neem seedlings sprayed with chelated iron combined with arginine and kinetin showed an increase in the amount of carbohydrates in the leaves. The explanation for this is that iron is involved in the formation of cytochromes, which play a crucial role in the processes of photosynthesis and respiration by accepting and transferring electrons. Additionally, iron is found in chloroplasts, which accounts for 70% of its total iron content, which makes iron essential for photosynthesis and increases the amount of carbohydrates in leaves (Al-Naimi, 2000). The outcomes of the research conducted on apple, orange, and guava trees by El-Shazly et al. (2004), Al-Mmureib (2008), and Al-Tamimi et al. (2012) are in agreement with these findings.

4. Conclusion and future scope

This study suggests that the chemical properties of tree seedlings with potential medicinal advantages can be enhanced by combining growth regulators with nutrient-rich fertilizers. The outcomes moreover shown that the combination of these variables produced more noteworthy outcomes than did any one of them alone. Given certain chemical properties, it is advised against using excessive quantities of the growth regulator (Kinetin) since this might have negative effects on the plant, Considering the age of the seedling during application of the treatment.

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