# Vegetative growth of neem seedlings (*Azadirachta indica* L.) as affected by kinetin, arginine, and chelated iron

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## Abstract

A pot experiment was carried out in the canopy of the Horticulture and Landscape Department - College of Agriculture - University of Kerbala For the period from July 2023 to June 2024. The study was carried out as a factorial experiment with three replications in a randomized complete block design to study the effect of kinetin (0, 50, and 100 mg $L^{-1}$) (K0, K1, K2, respectively), and arginine (0, 150, and 300 mg $L^{-1}$) (A0, A1, A2, respectively), and chelated iron (0 and 200 mg $L^{-1}$) (Fe1, Fe2, respectively) in the vegetative growth of neem seedlings. The results showed the varying effect of adding the study factors individually, as kinetin had a significant effect on most of the vegetative traits under study, while adding arginine had no significant effect on most of the vegetative traits, except for stem diameter, which was significantly affected, while chelated iron had a significant effect on leaf area and plant height. The results of the binary interaction varied between the study factors on the studied traits. The triple intervention had a significant effect on the plant height, as the treatment (Fe2K2A2) gave the highest rate of plant height, reaching (46.00 cm), and the highest rate of stem diameter, reaching (6.337 mm), While the treatment (Fe1K2A2) gave the highest average leaf area, reaching (1123 $cm^2$). It can be concluded from this study that spraying arginine with chelated iron had an effective role in improving the early vegetative growth of seedlings, but the use of high concentrations of kinetin must be taken into account, as the results reflected the negative effects of high concentrations on most of the traits studied.

**Keywords** Medicinal plants, Meliaceae, Azadirachtin, cytokinins, Amino acids.

## Introduction

Medicinal plants and herbs have been used as therapeutic treatments for thousands of years and have become commonly used, due to their availability, ease of obtaining, cheap price, and lack of toxicity. Among these medicinal herbs is the neem tree (*Azadirachta indica* L.), which belongs to the family (Meliaceae) and is native to India as a tropical tree. It tolerates arid conditions, temperatures up to 50°C, and living in poor soils. Neem is an evergreen tree with alternately pinnate leaves with a bitter taste and produces white, fragrant flowers that give white oval fruits that turn golden yellow at maturity and contain a single seed [1, 2]. The neem tree occupies an important place in folk medicine, agriculture, and various industries, and there are many references and studies that touched on the benefits and uses of all parts of this tree [3, 4]. All parts of the neem tree contain, but in varying proportions, many effective and biologically active compounds that have given it great importance in the pharmaceutical industry, such as (Nimbin), (Azadirachtin), (Nimblid), (Limonoids) and other substances that have been tested as anti-cancer and anti-diabetic agents. It can also increase the body's immune strength, and can act as antibacterial, antifungal, and antiviral agents, and can be used in the manufacture of cosmetics, skin care, and teeth [5, 6]. Its products have recently been found to be used in sterilization materials to reduce the Corona pandemic and encourage testing of the compounds found in this plant to treat this virus [7, 8]. Neem products have been used in agriculture as natural pesticides or soil amendments, and as plant growth stimulants as alternatives to synthetic chemicals in sustainable agriculture [9, 10, 11] .

The hormonal balance within the plant is very important to help the plant reach optimal production, so it has become common to treat plants with auxins, gibberellins, cytokinins, and abscisic acid, which play important and different roles within the plant, whether by treating the seeds or spraying them on the shoots [12]. Kinetin is one of the cytokinins that is produced naturally within plants, but it can be added externally to plants to play an important and fundamental role in cell division, building nucleic acids (DNA) and (RNA), vascular tissue differentiation, flower formation, delaying aging, and increasing chlorophyll content [13]. All of these indicators are reflected in increased vegetative and root growth and improved production quality. This has been confirmed by previous studies and research on various horticultural crops [14, 15, 16].

Amino acids play an important and major role in increasing vegetative growth, increasing productivity and improving the quality of horticultural plants because they are considered the basic structure for building proteins and they directly affect physiological activities through their role in building the basic organic compounds for building protoplasm and indirectly through building enzymes and as an energy store [17]. Arginine is one of the most important amino acids in the vital activities of plants because it contains the highest ratio of nitrogen to carbon, which makes it a storehouse of organic nitrogen necessary for plant growth and the construction of nucleic acids and proteins [18]. The physiological role of arginine is demonstrated by its stimulation of physiological processes that lead to increased carbohydrate synthesis, reduced aging hormone synthesis, increased synthesis of gibberellins and auxins, and thus increased cell division, and this has been proven by previous studies and research [19, 20, 21,22]. In addition, arginine plays a role in plant resistance to various environmental stresses because it is the basic substance for the synthesis of proline, polyamines, and nitric oxide [23, 24].

Iron is one of the essential nutrients for all living organisms, including plants, and although they require it in small quantities and it falls within the group of micronutrients, its deficiency causes physiological disturbances in plant growth, which may cause its death and crop failure, due to its physiological importance within the plant through its role in building DNA. RNA, chlorophyll, catalyzing many enzymes, respiration, and redox reactions [25]. Iron is also involved in the construction and effectiveness of chloroplasts and is involved in the synthesis of cytochromes, which play an important role in transferring electrons during the respiration process. Iron is also a cofactor for many enzymes necessary for the synthesis of plant hormones such as ethylene and abscisic acid in response to environmental stresses to which the plant is exposed [26, 27]. Many studies and research have demonstrated the positive effects that iron can have when added to horticultural plants [28, 29, 30]. There are also some studies that have shown the harmful effects of iron deficiency and its toxicity on plants [31, 32, 33].

## Materials and Methods

The study was conducted in the plant canopy covered with saran with 50% shading at the Horticulture and Landscape Department / College of Agriculture / University of Kerbala for the period from planting date 10/7/2023 to 1/6/2024. 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 study was implemented as a factorial experiment with a Randomized Complete Blocks Design (R.C.B.D.) with three replications, where each replication contained 18 treatments resulting from the use of three concentrations of kinetin (0, 50, and 100 mg $L^{-1}$) and three concentrations of arginine (0, 150, and 300 mg $L^{-1}$) with two concentrations of chelated iron (0 and 200 mg $L^{-1}$). There were 8 plants (observations) in each treatment, so the number of plants included in the study was (18 treatments x 8 observations x 3 replicates = 432 seedlings). The seedlings were sprayed with the study factors successively when the seedlings were two months old, four treatments in the autumn and four in the spring. At the end of the experiment, vegetative measurements were taken, such as plant height (cm), stem diameter (mm), number of leaves (leaf $plant^{-1}$), and leaf area ($cm^2$ $plant^{-1}$) (the leaf area was calculated using the Image program in the Windows 7 operating system and by multiplying the area of one leaf by the number of leaves per plant, the leaf area of the plant was calculated.

## Results and Discussion

### 1- Plant height (cm)

The results of Table (1) indicate that there are significant differences in plant height as a result of spraying with kinetin, where the spray concentration (100 mg $L^{-1}$) achieved the highest average of (41.10 cm), while the treatment (50 mg $L^{-1}$) gave the lowest average of (37.72 cm). The same table shows that there are no significant differences for arginine treatments in plant height. There is a significant effect of chelated iron on plant height, as the concentration (200 mg $L^{-1}$) gave the highest average for this trait, reaching (40.09 cm) compared to the comparison treatment, which gave (38.90 cm). The same table indicates that the double interference treatments had a significant effect on the above trait, as the interference treatment (K1A2) excelled, giving the highest average plant height of (42.55 cm), while the treatment (K2A1) gave the lowest average of (36.21 cm). The triple intervention had a significant effect on plant height, as it showed superiority to the intervention treatment (Fe2K0A2) by giving the highest average plant height, which reached (46.00 cm), compared to the comparison treatment, which gave the lowest average plant height, which reached (36.25 cm).

**Table (1):** Effect of spraying with kinetin, arginine, and chelated iron on plant height (cm) 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) | 40.11 | 36.57 | 37.03 | 38.44 | 38.90 |
| | 150 (A1) | 41.31 | 41.50 | 41.51 | 38.14 | |
| | 300 (A2) | 36.25 | 42.94 | 38.29 | 40.13 | |
| 200 (Fe2) | 0 (A0) | 36.41 | 39.05 | 33.37 | 40.06 | 40.09 |
| | 150 (A1) | 38.94 | 40.68 | 39.09 | 41.24 | |
| | 300 (A2) | 46.00 | 39.84 | 42.02 | 38.97 | |
| | | LSD. KxAxFe= 5.560 | | | LSD AxFe=N.S | LSD. Fe=1.853 |
| Averages of Kinetin | | 39.67 | 37.72 | 41.10 | LSD. K=2.270 | |
| Iron x Kinetin | 0 (Fe1) | 39.55 | 37.86 | 39.29 | | Averages of Arginine |
| | 200 (Fe2) | 39.80 | 37.57 | 42.90 | | |
| Arginine x Kinetin | LSD. Fe × K= N.S. | | | | | |
| | 0 (A0) | 38.34 | 39.17 | 41.51 | | 39.25 |
| | 150 (A1) | 39.60 | 37.35 | 36.21 | | 39.69 |
| | 300 (A2) | 39.81 | 42.55 | 40.93 | | 39.55 |
| | LSD. A× K = 1.935 | | | | | LSD. A= N.S. |

## Stem diameter (mm)

Table (2) shows the significant effect of kinetin treatments on stem diameter, as the treatment (100 mg $L^{-1}$) was superior, which gave the highest rate of (5.983 mm), which did not differ significantly from the treatment with a concentration of (50 mg $L^{-1}$), which recorded (5.672 mm), compared to the control treatment, which recorded the lowest average stem diameter of (5.447 mm). Arginine had a significant effect, as the concentration exceeded (300 mg $L^{-1}$) and gave the highest average stem diameter, reaching (5.854 mm) compared to the comparison treatment, which recorded the lowest average stem diameter (5.484 mm). The table indicates that the iron treatments had no significant effect on the trait referred to, while it is noted from the three-way interaction between the study factors that there are significant differences, as the treatment (F2K2A2) excelled, which gave the highest rate of (6.337 mm), which differs significantly from the comparison treatment, which gave the lowest rate (5.083 mm).

**Table (2):** Effect of spraying with kinetin, arginine, and chelated iron on stem diameter (mm) 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) | 5.083 | 5.520 | 5.673 | 5.453 | 5.679 |
| | 150 (A1) | 4.940 | 5.383 | 6.080 | 5.814 | |
| | 300 (A2) | 5.560 | 5.583 | 5.660 | 5.770 | |
| 200 (Fe2) | 0 (A0) | 6.090 | 5.737 | 5.400 | 5.516 | 5.721 |
| | 150 (A1) | 5.717 | 5.443 | 6.110 | 5.710 | |
| | 300 (A2) | 6.100 | 6.190 | 6.337 | 5.939 | |
| | | 1.2166LSD. KxAxFe= | | | LSD AxFe=N.S | LSD. Fe=N.S |
| Averages of Kinetin | | 5.447 | 5.672 | 5.983 | 0.4967LSD. K= | |
| Iron x Kinetin | 0 (Fe1) | 5.380 | 5.652 | 6.006 | | Averages of Arginine |
| | 200 (Fe2) | 5.513 | 5.691 | 5.960 | | |
| Arginine x Kinetin | LSD. Fe × K= N.S. | | | | | |
| | 0 (A0) | 5.302 | 5.572 | 5.580 | | 5.484 |
| | 150 (A1) | 5.307 | 5.875 | 6.105 | | 5.762 |
| | 300 (A2) | 5.732 | 5.568 | 6.265 | | 5.854 |
| | LSD. A× K = N.S. | | | | | LSD. A= 0.4967 |

**Number of leaves (leaf plant$^{-1}$)**

Kinetin treatments had a significant effect on the number of leaves, and the treatment with a concentration of (100 mg $L^{-1}$) was superior, and recorded the highest average of (28.32 leaves plant$^{-1}$), which did not differ significantly from the concentration treatment (50 mg $L^{-1}$), which recorded (23.01 leaves plant$^{-1}$), while the control treatment recorded the lowest average (20.23 leaves plant$^{-1}$) (Table 3). In the same table, spraying with arginine and iron did not have a significant effect on the number of leaves. The same table indicates that there is a significant effect for the double interference treatments, as the interference treatment (Fe2A2) gave the highest average for the number of leaves, amounting to (27.87 leaves plant$^{-1}$), while the interference treatment (Fe1A2) gave the lowest average, amounting to (21.42 leaves plant$^{-1}$). The aforementioned table shows that there are no significant effects of the three-way interaction between the study factors.

**Table (3):** Effect of spraying with kinetin, arginine, and chelated iron on the number of leaves (leaf plant$^{-1}$) 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) | 22.13 | 17.43 | 21.73 | 22.89 | 23.39 |
| | 150 (A1) | 20.80 | 17.60 | 21.67 | 25.87 | |
| | 300 (A2) | 20.80 | 23.20 | 24.07 | 21.42 | |
| 200 (Fe2) | 0 (A0) | 21.13 | 22.67 | 26.20 | 23.61 | 24.31 |
| | 150 (A1) | 25.73 | 30.20 | 31.80 | 21.47 | |
| | 300 (A2) | 22.47 | 24.00 | 35.73 | 27.87 | |
| | | LSD. KxAxFe=N.S | | | LSD AxFe=4.923 | LSD. Fe=N.S |
| Averages of Kinetin | | 20.23 | 23.01 | 28.32 | LSD. K=3.481 | |
| Iron x Kinetin | 0 (Fe1) | 20.49 | 22.51 | 27.18 | | Averages of Arginine |
| | 200 (Fe2) | 19.97 | 23.51 | 29.47 | | |
| | LSD. Fe × K= N.S. | | | | | |
| Arginine x Kinetin | 0 (A0) | 19.78 | 22.00 | 27.97 | | 23.25 |
| | 150 (A1) | 21.27 | 22.60 | 27.13 | | 23.67 |
| | 300 (A2) | 19.63 | 24.43 | 29.87 | | 24.64 |
| | LSD. A× K = N.S | | | | | N.S. LSD. A= |

## Leaf area (cm$^2$ plant$^{-1}$)

The results of Table (4) show that there are significant effects on the leaf area of the plant as a result of kinetin treatments, as the concentration (100 mg L$^{-1}$) was superior, giving the highest average leaf area of (956 cm$^2$ plant$^{-1}$), which did not differ significantly from the concentration (50 mg L$^{-1}$), which recorded (855 cm$^2$ plants$^{-1}$) compared to the comparison treatment, which recorded the lowest average of (743 cm$^2$ plants$^{-1}$). There was no significant effect of spraying with arginine and chelated iron on this trait. The binary interactions between the treatments show a significant effect, as the Fe2A0 treatment, which gave the highest average (988 cm$^2$ plant$^{-1}$), outperformed the comparison treatment, which gave (771 cm$^2$ plant$^{-1}$). In the triple interaction between the study factors, the treatment (Fe1A1K2) excelled, giving the highest average leaf area of (1123 cm$^2$ plant$^{-1}$), compared to the treatment (Fe2A0K0), which recorded the lowest average of (605 cm$^2$ plant$^{-1}$).

**Table (4):** Effect of spraying with kinetin, arginine, and chelated iron on leaf area (cm2 plant$^{-1}$) 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) | 692 | 924 | 686 | 771 | 827 |
| | 150 (A1) | 835 | 636 | 683 | 962 | |
| | 300 (A2) | 852 | 999 | 1079 | 747 | |
| 200 (Fe2) | 0 (A0) | 605 | 672 | 926 | 988 | 876 |
| | 150 (A1) | 770 | 1040 | 1123 | 800 | |
| | 300 (A2) | 959 | 934 | 911 | 840 | |
| | | LSD. KxAxFe=360.4 | | | LSD AxFe=208.1 | LSD. Fe=120.1 |
| Averages of Kinetin | | 743 | 855 | 956 | LSD. K=147.1 | |
| Iron x Kinetin | 0 (Fe1) | 671 | 867 | 942 | | Averages of Arginine |
| | 200 (Fe2) | 814 | 843 | 970 | | |
| | LSD. Fe × K= N.S. | | | | | |
| Arginine x Kinetin | 0 (A0) | 808 | 925 | 905 | | 880 |
| | 150 (A1) | 760 | 842 | 1041 | | 881 |
| | 300 (A2) | 659 | 799 | 923 | | 794 |
| | LSD. A× K = N.S. | | | | | LSD. A= N.S. |

The results obtained in Tables (1, 2, 3, and 4) showed that there are significant effects of kinetin on all vegetative traits. The reason for this is attributed to the role of kinetin in cell division, differentiation, growth of lateral shoots, increase in leaf area, development of plastids, and it preserves the chlorophyll pigment due to its role in drawing Nutrients from the soil to the leaves and growing tops stimulate the formation of chlorophyll and prevent its loss, and these are the reasons that made it have a positive effect on the studied traits [34].

As for the amino acid arginine, it had a significant effect in improving some of the characteristics of vegetative growth when added alone or in combination with kinetin and iron. The reason is that the amino acid works to manufacture gibberellins and enzymes that are important in biological processes, proteins, and the transport and storage of nitrogen [35]. It encourages vital activities, especially the processes of division and expansion of plant cells. It also plays a role in increasing the activity of enzymes that decompose organic compounds and works to liberate elements from them, increasing their readiness and increasing plant growth rates [36,37].

The effect of iron on some of the mentioned characteristics is due to its important role in many vital processes in plants that are related to increasing the characteristics of vegetative growth, which is involved in the metabolism of plastids, nucleic acids, and enzymes, which work to encourage increased cell divisions and cell elongation, which has led to increased efficiency of the process of photosynthesis, thus increasing the characteristics of vegetative growth [38]. Iron also participates in oxidation and reduction reactions in the process of respiration and photosynthesis and is involved in the synthesis of chloroplasts, which encourages an increase in the products of the photosynthesis process and thus increases the rate of vegetative growth [39].

The results of this study showed that all treatments containing arginine and chelated iron had an effective role in improving the early vegetative growth of seedlings, and this was parallel to increasing concentrations. On the other hand, caution must be taken and consideration must be given to the use of high concentrations of kinetin because the results reflected the negative effects of high concentrations on most of the traits studied. This may be because the high concentrations used are not appropriate for the young age of the seedlings.

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