

The effect of spraying nano iron and chitosan on the growth characteristics and yield of apple trees *Malus domestica* Borkh

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Abstract

This research was carried out in one of the private orchards located in Al Khalis District, Diyala Governorate for the agricultural season 2020–2021 on apple trees (Ibrahimi cultivar), which were newly planted in the region. The experiment included the study of two factors. The first factor included three levels of nano-iron Fe (0, 15, 30) mg.L⁻¹ and the second factor included three levels of chitosan (0, 250, 500) mg.L⁻¹. A factorial experiment with three replications was conducted using a randomized complete block design (RCBD). The averages were compared using Duncan's polynomial test at a probability level of 0.05 after the findings were statistically processed using the statistical program (SAS,2003). The study's findings demonstrated that nano iron therapy at a concentration of 15 and 30 mg.L⁻¹ was significantly in leaf area, total chlorophyll, nutrient level (N P K) in leaves, fruit set, total number of fruits, fruit weight, percentage of total soluble solids, and total yield per plant compared to the control treatment. The results also showed a significant superiority of the chitosan treatment at a concentration of 250 and 500 mg.L⁻¹ in all studied characteristics compared with control treatment. The interaction treatment between nano iron at a concentration of 30 mg.L⁻¹ and chitosan at a concentration of 500 mg.L⁻¹ led to a significant superiority in all characteristics compared with the other treatments.

Keywords: Nano iron, chitosan, apple trees.

Introduction

tons, followed by the United States of America (4 million tons), and Poland (13). The date of ripening of the fruits of the Ibrahimi apple cultivar starts from mid-June until the beginning of July, and it is one of the best varieties grown in the central region of Iraq. Foliar feeding has become an important agricultural process in plants' feeding and providing them with nutrients at the present time, it includes spraying nutrients in the form of solutions on the plant's vegetative system in a way that is easily absorbed by the leaves, as the leaves are a major center for many metabolic

Apple *Malus domestica* Borkh belongs to the Rosaceae family, it is cultivated in various temperate regions of the world (40), the original homeland of the apple extends from the Caucasus Mountains, Central Asia, India, Pakistan, and western China (22). The apple fruit has great economic importance, especially in countries with high productivity, as it constitutes an economic resource in exporting countries, and its fruits are rich in pectin, sugars,). China is one of the most apple-producing countries in the world, with a production rate of approximately 40,500 million



Recently, researchers have resorted to using biopolymer-based materials to control plant diseases and pests and increase crop production as complementary materials to the use of chemicals, whether fertilizers or pesticides, these substances showed sufficient activity against plant pathogens as well as their ability to increase the productivity of many agricultural crops and avoid the use of large quantities of chemical fertilizers (25). Chitosan, a positively charged biopolymer that is the second-largest biomaterial in nature after cellulose and is found mostly in the exoskeletons of crustaceans, insects, and the cell walls of fungi, is one of these substances. Chitosan consists of glucose amine units linked together by glycoside bonds (β 1-4 linkage), and it has ability to create ionic and hydrogen interactions with other molecules, including lipids and proteins, because to its free hydroxyl and amine groups (9), and it is characterized by its non-toxicity and biodegradation, and it has no local or general effects on living tissues (27).

Due to the lack of studies in this direction, the research was conducted for the care of apple trees and improvement of their growth and yield by foliar feeding with nano iron and chitosan, and determining the optimal concentration.

Materials and Methods

The study was carried out in one of the private orchards located in Al Khalis District, Diyala Governorate for the agricultural season 2020–2021 on apple trees (Ibrahimi cultivar), which were newly planted in the region. Two factors were investigated as part of the experiment. Three levels of nano-iron Fe (0, 15, and 30 mg.L⁻¹) were included in the first factor, while three levels of chitosan (0, 250, and 500 mg.L⁻¹) were added in the second. Two trees were used for each experimental unit in a factorial experiment with three replications using a randomized complete block design (RCBD). The averages were

activities and have the ability to absorb nutrients like the roots (31). Iron is a chemical element of metals, it is the oldest metal in terms of discovery and is symbolized by Fe, its atomic number is 26 and its molecular weight is 55.84. It is found in nature in the form of oxides (7). Iron is a basic element for plant growth, chlorophyll synthesis, respiration, and redox reactions within plant tissues, as well as for the production of crucial cytokinins for photosynthesis (36).

Foliar spraying with nano-fertilizers is one of the modern agricultural processes that has been used in the field of plant nutrition, and nanomaterials are elements with micro-particles with dimensions of between (0.1-100) nanometers, which is one thousand-millionth of a meter, and these materials behave completely differently from the usual materials, which possess large molecules in their chemical and physical properties (8). Al-Dosari (3) showed that the direction of the ultra-fine nanoparticles inside the plant and the way to control them at the interaction led to an increase in plant growth. Nano foliar fertilizers can achieve the fastest plant response, especially with problems in soils, high pH, and incompetent root growth (38).

compared using Duncan's polynomial test at a probability level of 0.05 and the findings were statistically analyzed using SAS 2003.

Studied traits

Leaf area (cm²)

The method of discs with known area and fresh weight was used according to Dvornic (10), where eight leaves were taken from each treatment, then weighed using a sensitive scale, and pieces of leaves with known area were weighed, and the leaf area was calculated on the basis of the fresh weight according to the following equation:

$$\text{Leaf area (cm}^2\text{)} = \frac{\text{Fresh leaf weight (g)} \times \text{disk area (cm}^2\text{)}}{\text{Fresh disks weight (g)}} \div 8$$

Total chlorophyll content (mg. g⁻¹ fresh weight)

In order to quantify the amount of chlorophyll in the leaves, 0.2 g of leaves were crushed and 20 ml of acetone (80%) was added, and the optical absorbance was estimated using a

spectrophotometer at two wavelengths of 663 and 645 nanometers, and the following equation was used to determine the amount of chlorophyll in the leaves:

$$\text{Total chlorophyll content (mg. g}^{-1}\text{)} = \frac{\text{Volume of solution} \times (663 A \ 8.02 + 645 A \ 20.2)}{\text{Sample weight} \times 1000}$$

Where: A= wavelength (17)

The nutrient content (N P K) in leaves

The dry leaves were crushed with an electric mill, 0.2 g was taken and digested using concentrated sulfuric and perchloric acid at a ratio of 1:4, respectively, according to **Johnson and Ulrich (20)**. The nitrogen content in leaves (%) was estimated using a spectrophotometer according to the method of (30). The phosphorous content in leaves (%) was estimated using a spectrophotometer (19). The potassium

content in leaves (%) was estimated using a flame photometer (39).

Fruit set (%)

Four branches in different Directions of the tree were selected and marked with numbers and according to the fruit set in the marked branches after the plants reached the stage of full flowering at the beginning of March, and the proportion of fruit set was determined using the following formula based on the fruit set on the same marked branches during the first week of April:

$$\text{Fruit set (\%)} = \frac{\text{The number of the fruit set in the branches}}{\text{The total number of flowers in the branches}} \times 100 \quad (41)$$

Total number of fruits per tree (one fruit per plant)

Total number of fruits was calculated at harvest per experimental unit.

Fruit weight (g)

Fruit weight was measured using a sensitive electronic scale.

Total soluble solids TSS %

$$\text{The yield of one tree (kg)} = \frac{\text{The average weight of one fruit (g)} \times \text{the number of fruits remaining on the plant}}{1000}$$

Total soluble solids were calculated using a device Hand Refractometer.

Yield per tree (kg)

The yield was calculated using the average fruit weight and the quantity of fruits in the experimental unit at harvest to calculate the yield

Results and discussion

The results of both Tables (1 and 2) show a significant superiority of spraying with nano iron15 and 30 mg compared with control (spraying with distilled water) in characteristics of leaf area of 15.26 and 16.81 cm, the percentage of total chlorophyll 12.47 and 14.26 mg.g⁻¹, the percentage of nitrogen in the leaves 1.56 and 1.74%, the percentage of phosphorous 0.18 and

0.22%, and the percentage of potassium 1.13 and 1.17%, as well as the fruitful characteristics such as percentage of fruit set of 8.38 and 9.80%, the number of fruits 460.80 and 628.11, the weight of one fruit 29.80 and 33.18 g, the percentage of total soluble solids 10.17 and 10.47%, and the yield of one tree 13.70 and 20.86 kg, compared with the control treatment, which gave the lowest percentages of the mentioned traits.



This may be due to the important characteristics of nano-fertilizers, such as their small size, as well as an increase in their surface area, which increases the absorption surface and the possibility of direct entry into plant cells (35). Nanoparticles or their aggregates whose diameter is less than the size of the pathway in the cell wall can easily enter through those walls and reach the plasma membrane (29). Since iron is required for the synthesis of the chlorophyll molecule and because green leaves contain 29–35% of the world's iron, it plays a crucial part in the development of the enzymes and other substances that do so (5). Due to its significant participation in numerous biological processes in plants that are connected to improving the features of vegetative growth, nano iron's function in enhancing the aforementioned traits may be explained, because it plays a part in the representation of nucleic acids and enzymes in the plastids that promote an increase in cell divisions and elongation, which results in an increase in the content of chlorophyll, which then increases photosynthesis efficiency and improves the characteristics of vegetative and fruit growth (1).

Numerous essential plant functions, such as the creation of food, the promotion of the synthesis of amino acids and enzymes that boost the activity of antioxidant enzymes, and cell division, all benefit from the presence of nano iron (23). Nano iron has a role in increasing root growth, represented by its dry weight, which is a site for the production of cytokinins and their export to the vegetative part (26). This is agreed with Rui et al. (34) that the increase in the dry weight of the plant by the effect of spraying with nano iron is due to an increase in the representation of CO₂ and the absorption of nutrients present in the soil and an increase in the synthesis of carbohydrates (16, 33). This study's findings on plants are consistent with those of (28, 32, 37).

The results of both Tables (1 and 2) showed that spraying of chitosan at 250 and 500 mg.L⁻¹ was significantly superior in the vegetative

characteristics, which included the leaf area of 15.37 and 15.85 cm², the percentage of total chlorophyll 12.81 and 13.13 mg.g⁻¹, the percentage of nitrogen in the leaves 1.59 and 1.64%, the percentage of phosphorus 0.18 and 0.19%, and the percentage of potassium 1.14%, as well as the fruiting characteristics, such as fruit set 7.81 and 8.15%, the yield of one tree at 15.12 and 16.49 kg, compared to the control treatment (spraying with distilled water only), which produced the lowest percentages for the aforementioned traits. The number of fruits was 502.88 and 527.78, the weight of one fruit was 29.61 and 30.70g, the percentage of total soluble solids was 10.13 and 10.39%, and the yield per tree was 15.12 16.49 kg.

Chitosan's ability to improve vegetative growth features like leaf area and chlorophyll content in leaves can be ascribed to its involvement in promoting auxin and gibberellin production in the plant (18), because it includes the nitrogen required for the synthesis of growth-promoting compounds. As a result, it rises in the plant, which causes a rise in cell division and elongation. It participates in the synthesis of tryptophan, the essential component for the production of IAA, as well as the entry into the purine ring, the building block for the production of cytokinins .

Chitosan's beneficial effects on the plant's ability to produce more vegetative matter may be the cause of the rise in the mineral elements such as nitrogen, phosphorus, and potassium found in the leaves as a result of its application. In order to attain equilibrium, the root total grows due to an increase in the number of root hairs and branches, which increases the root's surface area and promotes mineral absorption and accumulation in the leaves. These outcomes are in line with what Dzung et al. (11) discovered that after spraying coffee plants with various concentrations of chitosan (6, 15), and this was amply demonstrated by an improvement in the efficiency of carbon metabolism, the accumulation of carbohydrates and nutrients in the leaves and their transportation



to the other plant parts, as well as the stimulation of the main enzymes' activities (2), which led to an increase in the products of the photosynthesis process such as carbohydrates and the provision of building compounds and energy needed to form flowers and fruits set and increase the weight of the fruit, thus increasing the total yield of the plant. The lack of nutrition is one of the reasons why fruit set and the process of turning a flower into a fruit fails since these processes require the

division and growth of cells (14), these results agree with the findings (12) when spraying grape plants (Thompson Seedless cultivar) with chitosan at a concentration of 500 mg. liter⁻¹.

For all the features examined in the study across all treatments, the results of Tables 1 and 2 demonstrated a significant superiority of the interaction treatment between spraying with nano iron at a concentration of 30 mg.L⁻¹ and spraying with chitosan at a concentration of 500 mg.L⁻¹.

Conclusion

In summary, foliar application of nano iron and chitosan markedly enhanced growth and productivity of apple trees (Ibrahimi cultivar) under Diyala conditions. Relative to the control, nano iron at 15–30 mg L⁻¹ improved leaf area, total chlorophyll, and leaf N, P, and K, and translated these gains into higher fruit set, fruit number, fruit weight, total soluble solids, and yield. Chitosan at 250–500 mg L⁻¹ produced comparable

improvements in both vegetative and reproductive traits. Notably, the combined treatment of 30 mg L⁻¹ nano iron with 500 mg L⁻¹ chitosan delivered the greatest increases across all measured parameters, indicating a synergistic effect. These findings support integrated foliar nutrition with nano iron and chitosan as a practical strategy to improve apple tree performance and fruit quality in similar agro-ecological environments

Table 1. The impact of spraying nano iron and chitosan on apple trees' vegetative growth traits

Treatments	Iron	Leaf area	Chlorophyll (mg.g ⁻¹)	Nitrogen (%)	Phosphorous (%)	Potassium (%)	
Nano iron (Fe)	0	14.27 C	11.67 C	1.49 C	0.14 C	1.10 C	
	15	15.26 B	12.47 B	1.56 B	0.18 B	1.13 B	
	30	16.81 A	14.26 A	1.74 A	0.22 A	1.17 A	
Chitosan (C)	0	15.09 C	12.46 C	1.56 C	0.19 C	1.12 B	
	250	15.37 B	12.81 B	1.59 B	0.18 B	1.14 A	
	500	15.85 A	13.13 A	1.64 A	0.19 A	1.14 A	
Nano iron (Fe) * Chitosan (C)	Fe1	C1	14.12 f	11.32 i	1.48 g	0.13 H	1.07 c
		C2	14.26 f	11.74 h	1.49 g	0.14 g	1.12 b
		C3	14.43 fc	11.94 g	1.50 gf	0.14 g	1.12 b

	Fe2	C1	14.71 de	12.23 f	1.53 ef	0.15 f	1.12 b
		C2	15.06 d	12.49 e	1.54 e	0.17 e	1.12 b
		C3	15.90 d	12.68 d	1.60 d	0.20 d	1.13 b
	Fe3	C1	16.44 b	13.83 d	1.67 d	0.21 c	1.16 a
		C2	16.79 ab	14.20 b	1.73 b	0.22 b	1.17 a
		C3	17.21 a	14.75 a	1.82 a	0.22 a	1.17 a

Table 2. The impact of nano iron and Chitosan spraying on apple tree yield characteristics

Treatments	Iron	Fruit set (%)	Number of fruits	Fruit weight	TSS (%)	Yield (kg)	
Nano iron (Fe)	0	5.14 C	402 C	26.54 C	10.47 C	10.67 C	
	15	8.38 B	460.80 B	29.80 B	10.17 B	13.70 B	
	30	9.80 A	628.11 A	33.18 A	10.47 A	20.86 A	
Chitosan (C)	0	7.37 C	460.22 C	29.18 B	9.83 C	13.66 C	
	250	7.81 B	502.88 B	29.61 B	10.13 B	15.12 B	
	500	8.15 A	527.78 A	30.70 A	10.39 A	16.49 A	
Nano iron (Fe) * Chitosan (C)	Fe1	C1	4.44 f	369.00 e	26.46 f	9.36 g	9.76 g
		C2	5.35 e	404.33 d	26.54 f	9.84 f	10.73 gf
		C3	5.64 e	432.67 d	26.62 f	9.93 f	11.52 ef
	Fe2	C1	8.14 d	414.33 d	28.68 e	9.85 f	11.88 e
		C2	8.31 cd	478.00 c	29.61 d	10.15 e	14.15 d
		C3	8.70 c	490.00 c	31.01 c	10.51 b	14.15 d
	Fe3	C1	9.53 b	597.33 b	32.36 b	10.28 d	19.33 c
		C2	9.77 ab	626.33 b	32.68 b	10.40 c	20.47 b
		C3	10.10 a	660.67 a	34.48 a	10.73 a	22.77 a

Conflict of interest

The authors declare no conflict of interest.

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