RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (5) 2232-2239, 2025
http://doi.org/10.54910/sabrao2025.57.5.44
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

EFFECT OF HUMIC ACID AND CHELATED IRON ON THE GROWTH AND NUTRITIONAL CONTENT OF APPLE (*MALUS DOMESTICA* L.)

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SUMMARY

Various processes can boost growth traits and the nutritional value of fruits. One such technique is the application of chelating substances for enhancing crop growth and productivity. The following study aimed to determine the effect of humic acid and chelated iron on the growth and nutritional content of apple (*Malus domestica* L.) cv. Sharabi, carried out in 2022 at the University of Kufa, Iraq, The experimental layout was in a randomized complete block design with three replications. The experiment included nine fertilizer combinations of the humic acid and chelated iron in different ratios. Results showed the fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) was superior in the apple seedling height, number of branches, number of leaves, leaf area, dry weight of shoots, carbohydrate content, and nitrogen content, with an increased average values (39.33 cm, 7.84 branches $seedling^{-1}$, 295.07 leaves $seedling^{-1}$, 33.01 cm^2, 43.46 g, 8.81 mg g^{-1}, and 2.42%, respectively). The fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (0 mg L^{-1}) excelled in the fresh weight of shoots and the chlorophyll content (99.18 g and 36.10 mg g^{-1}, respectively). For phosphorus content, the combination of humic acid (2.5 ml L^{-1}) and chelated iron (15 mg L^{-1}) was leading with an average of 0.57%, while for potassium content, the combination of excelled humic acid (5 ml L^{-1}) and chelated iron (15 mg L^{-1}) showed the highest average (3.90%) in the apple cultivar Sharabi.

Keywords: Apple (*M. domestica* L.), humic acid, chelated iron, growth and yield traits, nutritional content

Key findings: In apple (*M. domestica* L.), the fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) proved superior in most traits, such as seedling height, the number of branches and leaves, leaf area, dry weight of shoots, and carbohydrate and nitrogen contents.

Communicating Editor: Dr. A.N. Farhood

Manuscript received: April 21, 2024; Accepted: October 08, 2024.

Citation: Neamah SS, Naser SM, Almosawi AMM (2025). Effect of humic acid and chelated iron on the growth and nutritional content of apple (*Malus domestica* L.). *SABRAO J. Breed. Genet.* 57(5): 2232-2239. http://doi.org/10.54910/sabrao2025.57.5.44.

INTRODUCTION

Apple (*Malus domestica* L.) belongs to the genus *Malus* and family Rosaceae, which includes more than 55 genera and 15,555 wild and cultivated species. Apples, known since ancient times, had their original home in the western slopes of the Himalayas, Central Asia, and the Caucasus, where wild apple forests prevail (Cornille *et al.,* 2019). The apple is one of the important fruit trees because of its economical and biological characteristics. Apple has many varieties and occupies larger areas in most parts of the world.

Apple production ranks fourth worldwide behind bananas, oranges, and grapes. China leads its global production with 15%, followed by the US with 81.8 million tons (FAOSTAT, 2021). Recently, liquid organic fertilizers have become alternatives over mineral fertilizers to improve plant strength, produce clean, pollution-free fruits, and reduce the residual effect of nitrates and nitrites in fruit juice, especially nitrogen fertilization (Al-Alaf, 2017).

Humic acid (polymeric polyhydroxy acid) is one of the essential organic fertilizers used in the field. It is one of the organic acids produced naturally and a compound of humic matter resulting from the decomposition of organic matter. Humic acid composition comprised carbon, hydrogen, nitrogen, and oxygen in varying proportions, which results in the formation of compounds with differentiated molecular weight (Al-Salmani and Al-Douri, 2024). Adding humic acid to the soil helps increase absorption of nutrients by the crop plant. It also acts as a medium for transferring nutrients from the soil to the plants, especially when exposed to drought. Similarly, it plays a better role of enriching the growth and strength of root crops by enhancing lateral branches of roots and the dry and wet weight of plants. It raises the plant's content of proteins and boost the population of beneficial microorganisms in the soil (Fathy *et al.,* 2010).

Iraqi soils greatly suffer from insufficient microelements, including iron, which is a low-mobility element within plants, absorbed in the form of Fe^{+3}. Iron also plays an intermediate role in forming the chlorophyll pigment. Likewise, it contributes in the synthesis of cytochrome and participates in creating the peroxidase enzyme (Khudher and Alkhaled, 2024). Therefore, this study sought to determine the effect of humic acid and chelated iron on vegetative growth indicators and nutritional content of apple cv. Sharabi.

MATERIALS AND METHODS

The practical experiment commenced in 2022 in the field affiliated with the College of Agriculture, University of Kufa, Najaf Governorate, Iraq. It aimed to determine the effect of humic acid and chelated iron on vegetative growth indicators and nutritional content of the apple (*M. domestica* L.) cultivar Sharabi. The field soil preparation succeeded after applying several agricultural practices, including soil plowing, smoothing, and leveling. Soil samples' random collection ensued, with the soil physical and chemical properties analyzed (Table 1).

The apple cultivar Sharabi's homogeneous seedlings were at two years of age when planted in lines, with a 3-m distance between lines and a 2-m distance between seedlings. All field service operations, including irrigation and weeding, proceeded, as per recommended package. The experiment, carried out in a randomized complete block design (RCBD), had three replications. The experiment included nine different fertilizer combinations, as follows: control, humic acid (2.5 ml L^{-1}) + chelated iron (0 mg L^{-1}), humic acid (5 ml L^{-1}) + chelated iron (0 mg L^{-1}), humic acid (0 ml L^{-1}) + chelated iron (15 mg L^{-1}), humic acid (2.5 ml L^{-1}) + chelated iron (15 mg L^{-1}), humic acid (5 ml L^{-1}) + chelated iron (15 mg L^{-1}), humic acid (0 ml L^{-1}) + chelated iron (30 mg L^{-1}), humic acid (2.5 ml L^{-1}) + chelated iron (30 mg L^{-1}), and humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}). Adding the humic fertilizer and chelated iron to the apple seedlings in the field used the feeding method, with treatments repeated monthly for each line.

Table 1. Physical and chemical properties of the experimental soil.

Properties	Unit	Values
Electrical conductivity (E.C)	dS m^{-1}	3.56
Soil acidity (pH)		7.6
Nitrogen	mg L^{-1}	39.6
Phosphorus	mg L^{-1}	8.2
Potassium ion	mmol L^{-1}	5.41
Sodium ion	mmol L^{-1}	3.4
Magnesium ion	mmol L^{-1}	9.5
Sulfate ion	mmol L^{-1}	5.2
Bicarbonate	mmol L^{-1}	0.87
Organic matter	%	1.51
Iron	mg L^{-1}	5.19
Boron	mg L^{-1}	0.26
Clay	g kg^{-1}	88
Silt	g kg^{-1}	204
Sand	g kg^{-1}	708
Soil texture	Sandy loam	

Data recorded

The data recorded comprised seedling height, the number of leaves and branches, leaf area, fresh and dry weight of shoots, and the chlorophyll, carbohydrates, nitrogen, phosphorus, and potassium contents in leaves.

Statistical analysis

All the data underwent the analysis of variance (ANOVA) as per the RCBD. Furthermore, the treatment means' comparison and separation used the least significant difference ($LSD_{0.05}$) test (Al-Rawi and Khalaf-Allah, 2000).

RESULTS AND DISCUSSION

Seedling height

Results showed significant differences among the combined fertilization for the plant height in apple (*M. domestica* L.) cultivar Sharabi (Table 2). The fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) excelled by giving the tallest average plant height (39.33 cm) compared with the control treatment (26.41 cm). Humic acid helps synthesize tryptophan, which is the building block for auxin, the plant hormone located in the developing apex and responsible for plant height. It also promotes cell division and elongation, which increases growth and apple seedling height (Zhang *et al*., 2013). Aside from the positive role of the iron element in supporting vertical plant growth, it enhances the plant's ability to absorb more water and nutrients from the soil, contributing to seedling growth. Likewise, it activates many enzymes that are important in various vital processes, including synthesis of hormones and proteins necessary for plant growth, which enhances apples' plant growth and development (Dehghanipoodeh *et al.,* 2018).

Number of branches

For the number of branches in apple cultivar Sharabi, notable alterations were evident among fertilization combinations (Table 2). The fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) showed the most average number of branches (7.84 branches $seedling^{-1}$) compared with the control treatment (3.05 branches $seedling^{-1}$). Humic acid is an essential mineral necessary for activating several enzymes that help synthesize proteins and, eventually, plant growth. It also works as a growth regulator in the synthesis of auxin, which is a plant hormone critical for plant growth and development, by stimulating the formation of new shoots, causing an increased number of

Table 2. Effect of fertilizer combinations on the vegetative growth indicators in apples.

Fertilizer combinations	Seedling height (cm)	Branches seedling^{-1}	Leaves seedling^{-1}	Leaf area (cm^2)
Control	26.41	3.05	206.17	20.01
Humic acid (2.5 ml L^{-1}) + Chelated iron (0 mg L^{-1})	29.55	4.15	243.26	22.11
Humic acid (5 ml L^{-1}) + Chelated iron (0 mg L^{-1})	36.36	5.26	261.19	24.88
Humic acid (0 ml L^{-1}) + Chelated iron (15 mg L^{-1})	29.64	3.98	274.37	22.12
Humic acid (2.5 ml L^{-1}) + Chelated iron (15 mg L^{-1})	36.40	4.85	280.40	23.11
Humic acid (5 ml L^{-1}) + Chelated iron (15 mg L^{-1})	40.95	5.34	173.50	25.88
Humic acid (0 ml L^{-1}) + Chelated iron (30 mg L^{-1})	34.10	6.19	208.65	28.88
Humic acid (2.5 ml L^{-1}) + Chelated iron (30 mg L^{-1})	38.30	7.21	269.94	31.11
Humic acid (5 ml L^{-1}) + Chelated iron (30 mg L^{-1})	39.33	7.84	295.07	33.01
$LSD_{0.05}$	1.03	0.28	3.41	2.19

branches in apple trees (Al-Salmani and Al-Douri, 2024). For chlorophyll formation, iron is also necessary as a vital component of photosynthesis. The photosynthesis process is necessary to provide the energy for plant growth and development, including the production of branches in apples and pears (Temple *et al.,* 2004).

Number of leaves

Study findings revealed considerable differences among fertilization treatments for the number of leaves in apples (Table 2). The fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) provided the most number of leaves (295.07 leaves seedling^{-1}) versus the control treatment, giving an average of 206.17 leaves seedling^{-1}. Humic acid and iron are crucial in promoting the overall plant growth, including increasing the number of leaves. Humic acid can play as a cofactor in activating various enzymes necessary for protein synthesis and cellular growth. Similarly, humic acid and iron help in the synthesis of auxin, a plant hormone promoting vegetative growth, causing an increased number of leaves in apple seedlings (Asaad, 2014).

Leaf area

The outcomes indicated substantial variances among fertilization treatments for the leaf area in apple cultivar Sharabi (Table 2). The broadest average leaf area (33.01 cm^2) resulted in the fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) compared with the control treatment (20.01 cm^2). This widened leaf area could be due to the positive role of humic acid in the synthesis of the amino acid tryptophan, critical in cell division and elongation, as well as nucleic acids (RNA and DNA), necessary for cell division. Consequently, it increases the activity of absorbing water and nutrients, positively reflecting in a broader leaf area in the apple (Al-Mehemdi and Al-Ani, 2021). Iron is also necessary for the chlorophyll formation, contributing directly to an efficient photosynthesis process. In turn, it leads to heightened efficiency of dry matter accumulation and thus, promotes the growth and expansion of leaves in apple (*M. domestica* Borkh.) (Avestan *et al.,* 2018).

Shoot fresh weight

Results detailed significant differences among the fertilization treatments for the shoot fresh weight in apple cultivar Sharabi (Table 3). The premier shoot fresh weight (99.18 g) came from the fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (0 mg L^{-1}) compared with the control treatment (73.80 g). The ensuing increase may be attributable to positive effects of the added fertilizers, as the increased level of humic acid promotes vegetative growth, in general. Moreover, the readiness of nutrients boosted their absorption by the plants, containing macro- and micronutrients necessary to carry out vital processes. This type of fertilizer also has an influential effect and a considered relative rich source, as it contains a high concentration of nutrients (Zhang *et al.,* 2013). The addition of

Table 3. Effect of fertilizer combinations on the growth and quality traits in apples.

Fertilizer combinations	Fresh weight (g)	Dry weight (g)	Chlorophyll content (mg g^{-1})	Carbohydrate content (mg g^{-1})
Control	73.80	31.00	24.36	4.18
Humic acid (2.5 ml L^{-1}) + Chelated iron (0 mg L^{-1})	79.42	31.33	26.77	5.55
Humic acid (5 ml L^{-1}) + Chelated iron (0 mg L^{-1})	99.18	31.67	36.10	8.36
Humic acid (0 ml L^{-1}) + Chelated iron (15 mg L^{-1})	75.38	33.00	25.10	4.67
Humic acid (2.5 ml L^{-1}) + Chelated iron (15 mg L^{-1})	87.41	35.90	28.80	6.60
Humic acid (5 ml L^{-1}) + Chelated iron (15 mg L^{-1})	90.40	37.09	31.30	8.02
Humic acid (0 ml L^{-1}) + Chelated iron (30 mg L^{-1})	86.80	38.19	27.45	5.89
Humic acid (2.5 ml L^{-1}) + Chelated iron (30 mg L^{-1})	89.55	41.04	33.29	7.02
Humic acid (5 ml L^{-1}) + Chelated iron (30 mg L^{-1})	92.45	43.46	35.90	8.81
L.S.D $_{0.05}$	1.39	1.09	0.57	0.27

humic acid, as characterized by its ability to provide an acidic environment and chelating with positive ions to form a chelating complex, is vital for micronutrients. Additionally, humic acid is a source of nitrogen, thus, increasing available nutrients to the plant, which has a positive impact on the vegetative growth of *M. xiaojinensis* plants (Zha *et al.,* 2014).

Shoot dry weight

For apple shoot dry weight, marked variations appeared among fertilization treatments (Table 3). The topmost average shoot dry weight (43.46 g) resulted in the fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) versus the control treatment (31.00 g). The abundance of nutrients can contribute to raising the level of efficiency of growth and development processes. Therefore, the increase in dry shoot weight can refer to an increase in the plant height, the number of branches, and leaves, as well as the plant fresh weight. Past studies revealed that foliar application of humic acid boost the growth characteristics in apple (*M. domestica* Borkh) (Khalaf, 2023).

Chlorophyll content

Regarding leaf chlorophyll content in apples, significant differences existed among fertilization treatments (Table 3). The fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (0 mg L^{-1}) provided the highest average of chlorophyll content (36.10 mg g^{-1}) compared with the control treatment (24.36 mg g^{-1}). The rate of chlorophyll may have improved by adding humic acid, especially when the plant suffers from its deficiency. Apple (*M. domestica*) trees deplete large amounts of nutrients, hence, adding humic acid can affect the rate of chlorophyll by activating the enzymes associated with it (Zhang *et al.,* 2013). Iron is also necessary for the chlorophyll formation in pear plants and for photosynthesis, and thus, contributes to raising the level of vital processes (Hamouda *et al.,* 2015).

Carbohydrate content

For carbohydrate content in apple cultivar Sharabi, large differences were noticeable among fertilization treatments (Table 3). The fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) showed the utmost average of carbohydrate content (8.81 mg g^{-1}), as compared with the control treatment, with an average of 4.18 mg g^{-1}. Humic acid is considerably an important element involved as a structural component in a wide range of enzymes and proteins in numerous biological and chemical pathways. These are responsible for carbohydrate metabolism in photosynthesis and the conversion of complex sugars into starch and protein metabolism and auxin regulation. On regulating the process of photosynthesis in plants, it comes with activating enzymes associated with converting carbon dioxide (CO_2) and water (H_2O) into carbohydrates. In turn, these become transported during the stages of the metabolic process, and then, accumulated in the leaves of Anna apple trees (Mosa *et al.,* 2015). Additionally, the beneficial iron element enhances the plant's carbohydrate content by improving metabolic processes that affect the transport and storage of carbohydrates within the plant. Metabolic enzymes also need iron to influence the conversion of sugars into starch in Anna apple trees (El-Shazly and Dris, 2004).

Nitrogen (%)

Significant differences were prominent among fertilization treatments for nitrogen content (Table 4). The fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (30 mg L^{-1}) excelled by giving the highest average of nitrogen (2.42%), as compared with the control treatment (1.25%). The lack of humic acid in the soil indicates the difficulty of absorbing many nutrients with a low pH, and thus, neutral nitrogen becomes ineffective even if it is available in sufficient quantities in the soil. Therefore, it is preferable to add humic acid and improve its availability because it enhances the plants' ability to use optimum nitrogen. Hence, humic acid became a part of enzymes that contribute to regulating and activating the process of absorbing nitrogen available in the soil and using it in plants. This can ultimately lead to increased growth and development of plant parts, increasing the polarization of nutrients, including nitrogen (Mohammed and Mahmood, 2023). Moreover, iron has an important role in the formation and activation of some enzymes vital in the nitrogen fixation cycle within the plant, aside from improving vital processes and increasing nutrient absorption ability, including nitrogen from the soil (Amiri and Fallahi, 2009).

Phosphorus (%)

For phosphorus content in apple cultivar Sharabi, remarkable variances were evident among fertilization treatments (Table 4). The fertilizer combination of humic acid (2.5 ml L^{-1}) + chelated iron (15 mg L^{-1}) showed the maximum average content of phosphorus (0.57%) versus the control treatment that revealed an average of 0.21%. The increased percentage of phosphorus in apple leaves could be due to the positive effect of elevated availability of nutrients, including available nitrogen and potassium, in the soil. Therefore, humic acid and iron are critical in increasing the absorption of phosphorus, which is a considered slow-release element in the apple field (Khan *et al.,* 2018). Aside from iron indirectly improving the plant's ability to absorb and use phosphorus by enhancing root activity, it is essential for the growth and development of apple roots, causing better absorption of nutrients, including phosphorus (Dehghanipoodeh *et al.,* 2018).

Potassium (%)

Notable differences emerged among fertilizer treatments for the potassium content in apple cultivar Sharabi (Table 4). The fertilizer combination of humic acid (5 ml L^{-1}) + chelated iron (15 mg L^{-1}) demonstrated the supreme average of potassium content (3.90%), while the control treatment gave an average of 1.07%. This increase could refer to the favorable role of humic acid in activating many enzymes involved in stimulating vital processes like respiration and photosynthesis.

Table 4. Effect of fertilizer combinations on quality traits in apples.

Fertilizer combinations	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Control	1.25	0.21	1.07
Humic acid (2.5 ml L^{-1}) + Chelated iron (0 mg L^{-1})	1.75	0.33	1.35
Humic acid (5 ml L^{-1}) + Chelated iron (0 mg L^{-1})	1.84	0.55	3.71
Humic acid (0 ml L^{-1}) + Chelated iron (15 mg L^{-1})	1.61	0.28	1.08
Humic acid (2.5 ml L^{-1}) + Chelated iron (15 mg L^{-1})	1.78	0.57	2.61
Humic acid (5 ml L^{-1}) + Chelated iron (15 mg L^{-1})	2.20	0.40	3.90
Humic acid (0 ml L^{-1}) + Chelated iron (30 mg L^{-1})	1.65	0.36	2.15
Humic acid (2.5 ml L^{-1}) + Chelated iron (30 mg L^{-1})	1.80	0.46	3.15
Humic acid (5 ml L^{-1}) + Chelated iron (30 mg L^{-1})	2.42	0.51	3.45
$L.S.D_{0.05}$	0.05	0.02	0.18

The raised percentage of potassium in various parts of the apple plant can be attributable to the dynamic role of humic acid in raising the availability of nutrients, including potassium (Zhang *et al.,* 2013). Iron also positively regulates the ions' balance within the plant, influencing the availability and absorption of potassium, aside from its role in the plant's resistance to various environmental stresses, such as drought and salinity, modifying nutrient absorption. Therefore, it can provide an environment that better allows greater utilization of potassium available in the soil by the apple trees (Sun *et al.,* 2021).

CONCLUSIONS

The addition of humic acid and chelated iron has contributed to enhancing the availability of nutrients and the ease of their absorption by the apple plant. It also provides an ideal environment for growth, as reflected with an increase in vegetative growth indicators, as well as, in qualitative and nutritional gauges of plant trees.

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