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Interactive Effects of Nano-Iron and Spent Mushroom Extract on Growth and Nutrient Uptake of *Citrus trifoliata* Rootstock Saplings

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Abstract

Citrus trifoliata rootstock production is critical for the successful establishment of citrus orchards because sapling vigor strongly influences orchard performance. An experiment was conducted under 50% shade to evaluate two factors: foliar application of nanoiron at three concentrations (0, 10, and 20 mg/L) and an organic amendment, spent mushroom extract (SME), applied via irrigation water at three concentrations (0, 50, and 100 ml/L), on the growth and nutrient status of *C. trifoliata* saplings. The combined treatment of nanoiron at 20 mg/L plus SME at 100 ml/L increased plant height by 130%, stem diameter by 180%, and branch number per plant by 350% over the control, along with concurrent increases in leaf area, chlorophyll content, and carbohydrate accumulation. Foliar application of nanoiron integrated with SME provides a long-lasting, sustainable nutrient source that markedly improves sapling health and growth. This integrated approach can be considered an effective and practical strategy for enhancing nursery management and supporting sustainable citrus production.

Keywords: citrus rootstocks, nano-nutrients, organic fertilizers

Introduction

Citrus rootstocks are fundamentally responsible for orchard vigor, adaptability, and sustainability; thus, their successful propagation becomes a core priority in all major citrus-growing countries, including Iraq (Chhikara et al., 2018; Li et al., 2025). The commercial nurseries urgently require the rapid production of healthy seedlings of trifoliate orange or *Citrus trifoliata/Poncirus trifoliata* due to its high tolerance towards low temperatures; excellent compatibility with most scion varieties; and well-known contribution graft vigor as well as resistance against different stresses (El-Gioushy et al., 2021; Morales et al., 2023; Ollitrault & Navarro, 2011). However, the slow growth rate observed in *C. trifoliata* seedling development becomes one limitation factor for nurseries aiming at supplying increasing demands on uniform vigorous rootstocks suitable for orchard establishment.

Studies on the uses of chemical fertilizers on citrus growth have been reported. Recently, considerable attention has been paid to the use of safe, environmentally friendly, organic plant-based extracts as an alternative growth regulator. This is because of their non-toxic biological nature, containing micronutrients, amino acids, vitamins, and other bioactive compounds that improve plant physiology by different mechanisms such as enhanced nutrient uptake, stimulation of enzymatic activity, and support for carbohydrate metabolism (Abbas et al., 2020; Al-Budairy & Al-Taweel, 2025a). Spent mushroom extract (SME) is highly valued as an effective organic amendment due to its already decomposed lignocellulosic components, as well as its nutrient richness, which can enhance both root development and metabolic activity (Ahmed & Jebur, 2025). Therefore, SME could be a good option for sustainable horticultural systems that promote seedling growth.

Iron is an essential micronutrient that

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directly supports plant vigor and biomass accumulation through chlorophyll synthesis, the electron transport chain, and carbohydrate formation. The process becomes available to farmers in most parts of Iraq where such soils prevail; hence, deficiency restricts photosynthesis and seedling development (Zhou et al., 2025). A very recent advanced solution to this problem is by using nano-iron fertilizers because they are highly soluble with a large surface area due to small particle size, thus mobility plus penetration through plant tissues results in better photosynthetic efficiency, nutrient transport and metabolic activity (Hagagg et al., 2018; Qureshi et al., 2018). Nano-iron has also been reported to improve fruit quality and nutrient uptake in several horticultural crops (Lu et al., 2025; Wu et al., 2025); therefore, it could be used in nurseries as well.

Many studies have been conducted on nano-fertilizers or organic extracts, but few studies deal with their combined effects on citrus rootstocks, particularly *C. trifoliata*. The suggested interaction between nano-iron and organic extracts, such as SME, may maximize benefits by addressing both chlorophyll formation (iron-dependent) and carbohydrate accumulation (organic-extract–enhanced), thereby improving physiological efficiency. This practice could be an important step toward sustainable nursery practices under the dual constraints of resource limitations and alkalized soil conditions, where micronutrient availability is limited.

Accelerating the production cycle of citrus rootstocks in local nurseries requires sustainable alternatives to traditional fertilizers, whose efficiency is often reduced due to leaching, adsorption, and precipitation (Chaitra et al., 2021). This highlights the need for novel nutrient approaches that integrate modern nutrient delivery methods to enhance rootstock vigor effectively. Therefore, this experiment aimed to determine the optimal concentration of nano-iron combined with the spent mushroom extract (SME) organic extract, applied via foliar spray, on *C. trifoliata* saplings. The goal was to identify a treatment level that promotes rapid growth while producing healthy seedlings capable of permanent field establishment within a short timeframe.

Materials and Methods

The experiment was conducted at the University of Kufa agricultural research station in Najaf from March 15 to November 15, 2024. The response of trifoliate orange seedlings to spraying with nano-iron and mushroom extract was investigated. One hundred and eight seedlings were selected from the planting beds in the certified citrus seedling production nursery. The seedlings were of uniform growth and as similar in size as possible from six-month-old trifoliate orange seedlings (*Citrus trifolata*), with a height of 30-35 cm. Each seedling was planted in a 10 kg plastic pot containing a mixture of river sand and peat moss (2:1 v/v) from which potting mixture samples were taken and subjected to soil analysis to determine physical and chemical properties (Page, 1982) (Table 1). The seedlings were maintained under a wire shade covered with Saran, and all planting services were applied evenly across all treatments, including irrigation, weeding, and pest control.

The experiment was set up as a randomized complete block design. Three replicates per treatment were used, with each replicate comprising four plants. Randomization within blocks was done by random-number assignment to eliminate any possible positional bias. Chlorophyll content was determined by using a spectrophotometer. At the same time, standard methods were employed for determination of leaf area and carbohydrates so that the results could be comparable with those obtained in other studies employing similar methodologies, 50% shade house average temperature (25-30 °C), relative humidity (55%-65%), and light intensity (450-550 $\mu mol \cdot m^{-2} \cdot s^{-1}$) conditions under which optimal growth response occurred have been stated.

Experimental Design and Treatments

The factorial experiment was conducted using a completely randomized block design

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with three blocks (Hoshmand, 2006) and two factors: spraying with nano-iron at three levels (0, 10, and 20 mg/L) and spraying with mushroom extract at three levels (0, 50, and 100 ml/L). Nine treatments with three replicates were used, 27 experimental units, and four seedlings per experimental unit.

Preparation of Organic Compost Extract

Spent mushroom remains were collected from the Najaf Mushroom Cultivation Project National Local Project/Kufa, Najaf, Iraq. Four kilograms of spent mushrooms were soaked in four liters of distilled water and left for two days with occasional shaking. The mixture was filtered through cheesecloth and filter paper to obtain the stock solution (100%). The other concentration 50% was prepared by adding 500 ml of stock to 500 ml of DW. The chemical properties of the organic fertilizer extract were estimated using Black's (1965) method.

Study Measurements

Rate of increase in seedling height and primary stem diameter, leaf area, leaf chlorophyll content (mg/100 g FW fresh weight), and leaf carbohydrate content (mg/g dry weight). The stem diameter was measured using a Vernier caliper at a height of 5 cm above the crown. Likewise, the increase in the number of branches and leaves was determined. Measurements also included the actual values of sapling leaf area for three fully developed leaves from each experimental unit (Al-Zaidi, 2016), using a scanner and ImageJ software (Sadik et al., 2011). The leaf content of chlorophyll was also determined using a spectrophotometer at wavelengths 645 and 663 nm, and the total chlorophyll content was calculated (mg/100 g FW) (Ranganna, 1977). In addition to the carbohydrate content estimated (mg/g dry weight) using the phenol-sulfuric acid method, a spectrophotometer was used at 490 nm (Herbert et al., 1971; Mohamadipoor et al., 2013).

Statistical Analysis

The experiment was finalized by taking samples for data analysis, and statistical analyses and ANOVA (analysis of variance) were performed using GenStat. The differences among treatments means were compared

Table 1

Soil Physical and Chemical Properties at Planting

Soil particles	Unit	Value
Clay	g/kg	88
Silt		204
Sand		708
Soil texture	Sand-loam	
Property analysis	Unit	Value
EC	dS/m	3.62
Ph	-	7.4
Available N	mg/L	37.2
Available P		7.9
SO_4^{-2}	mM/L	5.2
CO_3^{-2}		Nil
HCO_3^{-}		0.87
Organic matter	%	1.51
Fe	mg/L	5.19

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according to the least significant difference (LSD) for the actual values and based on Duncan's multiple range test compared with the control at the probability level (0.05).

Results and Discussion

The results indicate that all individual and combined treatments of nano-iron and SME extract resulted in sapling height increases higher than those recorded in the untreated control (Figure 1A). The properties of the SME extract is in Table 2. A slight effect was observed with spraying nano-iron NFe at a concentration of 10 mg/L, whereas increasing the concentration to 20 mg/L consistently resulted in significant increases in all indicators compared to the lower concentration. Plant height increased significantly with the spent mushroom extract (SME) over the control. A 50 ml per sapling treatment resulted in a 43% increase in plant height compared to the control. However, a nearly similar effect was observed for nano-iron and SME extract on stem diameter, which increased by approximately 40% and 80%, respectively, at NFe concentrations of 10 and 20 mg/L (Figure 1B). A relatively low effect was also observed for SME at a concentration of 50 ml per sapling, while it increased to over 100% in the 100 ml per sapling SME treatment. The most significant increase in stem diameter was observed in the 20 NFe × 100 SME treatment.

In contrast to the relatively low effect of NFe on plant height, it was observed that nano-iron had a higher impact than SME on the rate of increase in the number of branches (Figure 1C). The 10 mg NFe treatment achieved half the effect recorded in the 20 mg NFe treatment, which, in turn, recorded an increase in the number of branches (140%) higher than that recorded in 100 SME. It was also observed that the 20 NFe treatment recorded a higher increase in the number of branches than the interaction of 10 NFe and 50 SME. In general, the highest increase was in the combination treatment with the highest concentrations of both factors.

The combination treatments also excelled in the nutritional content of the vegetative group, especially 20 mg per plant nano iron + 100 ml per plant extract, which recorded the highest total chlorophyll content in the leaves (Figure 2 A), reaching (104.09 mg.100 g fresh weight) and the highest carbohydrate content (35.20 mg/100 g dry weight) (Figure 2 B), compared to the control treatment, with lower values recorded (77.04 mg/100 g fresh weight) and (17.74 mg/100 g dry weight), respectively.

The results showed that the combination of foliar fertilizer with nano-iron and organic extract of spent mushroom improved the growth parameters of trifoliate orange saplings, increased sapling height, stem diameter, number of branches, and leaf area, as well as improved the nutritional status of the saplings compared to

Table 2

Components and Properties of the Spent Mushroom Extract (100%) Used in the Experiment

Properties	Value or %
EC (dS/m)	1.3
pH	6.2
Humic acid (mg/g)	65.43
Fulvic acid (mg/g)	87.61
Nitrogen g/kg	16.2
Phosphorus g/kg	0.835
Potassium g/kg	10.215
Fe mg/L	0. 952

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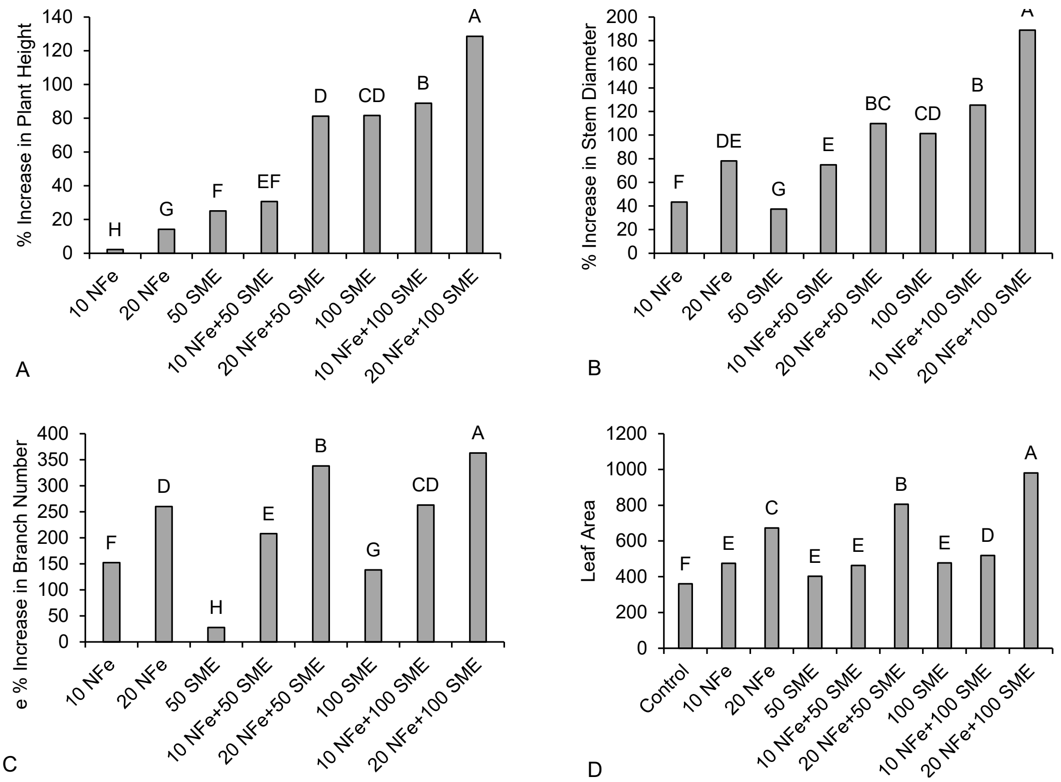
Figure 1

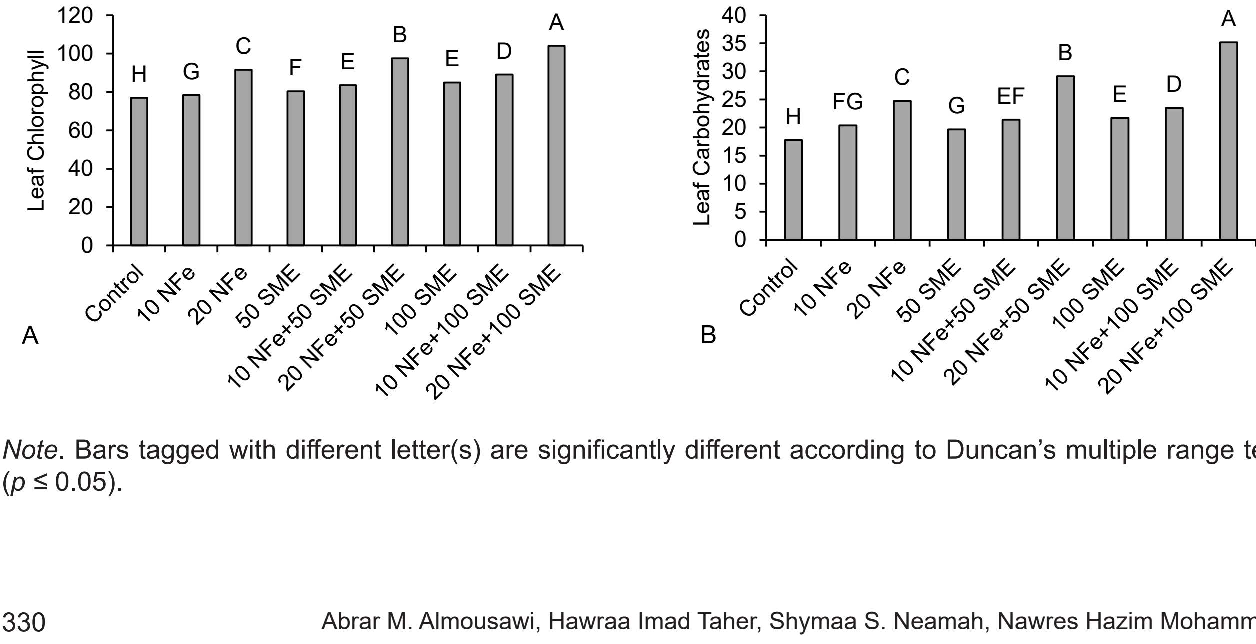
Effect of Foliar Spray with Nano-Iron and Spent Mushroom Extract SME on Plant Height, Stem Diameter, Branch Number, and Leaf Area of Trifoliate Orange Saplings



Note. Bars tagged with different letter(s) are significantly different according to Duncan's multiple range test (*p ≤ 0.05*).

Figure 2

Effect of Foliar Spray with Nano-Iron and Spent Mushroom Extract on Nutritional Status of Trifoliate Orange Saplings



Note. Bars tagged with different letter(s) are significantly different according to Duncan's multiple range test ($p \le 0.05$).

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the control treatment (sprayed with distilled water only). Simultaneous application of both factors provides the soil with the necessary nutrients in a readily available form, enhancing sapling root absorption of nutrients, especially the significant elements involved in most physiological activities, as well as their role in reducing soil pH (Gamble et al., 2014).

Foliar spraying with nano-iron showed an apparent effect on the vegetative and nutritional growth characteristics of trifoliate orange seedlings. Iron plays a role in increasing the rate of photosynthetic products used in various growth processes, as iron is a component of the nitrogenase and aconitase enzymes, which have essential functions in plants (Phogat, 2016). This is consistent with previous results (Al-Alaf et al., 2020) on grapefruit seedlings grafted onto orange rootstock. The increased chlorophyll content in leaves with increasing nano-iron concentration indicates an indirect role of iron in chlorophyll synthesis, as it is involved in the synthesis of various cytochromes important in electron transport (Souri & Hatamian, 2019). Iron is also involved in the synthesis of the enzyme coproporphyrinogen oxidase, which participates in the sixth step of porphyrin metabolism and is essential for the synthesis of α-aminolevulinic acid, the raw material for chlorophyll synthesis (Al-Budairy & Al-Taweel, 2025b; Baker & Stratton, 2015). An increase in the proportion of carbohydrates in leaves was also observed with increasing nano-iron foliar spray concentration. This may be attributed to the increased chlorophyll content and leaf area of seedlings sprayed with nano-iron, which subsequently increases nutrient availability (Eldin, 2015).

Spent mushroom extract SME is a low-cost fertilizer source compared to mineral fertilizers, suitable for various plant applications with no harm to the environment (Chen et al., 2022). The microelements, especially iron, copper, and zinc, in the SME have a positive impact on vegetative indicators by increasing photosynthesis and carbohydrate accumulation in the leaves, which stimulates growth and cell division (El-Salama et al., 2017). Additionally, they help reduce the impacts of heavy metals (Chen et al., 2022; Pereira & Santos et al., 2025).

The improved saplings' growth traits can also be attributed to the SM extract's content of growth hormones (cytokinins and auxins), carbohydrates, proteins, amino acids, and various vitamins (Lou et al., 2017; Velusami et al., 2021). Generally, SME contains plant hormones that stimulate cell division and promote rapid root growth, increasing stem thickness and photosynthetic efficiency due to the vitamins and enzymes it activates, thereby increasing root and vegetative growth of the plant (Souri & Hatamian, 2019). The potassium content in SME facilitates the absorption of mineral elements, particularly nitrogen, and contributes to the synthesis of sugars and the transport of carbohydrates within the plant. Potassium also participates in the formation and transfer of proteins and plant hormones. It activates plant enzymes and increases cell division, encouraging the growth of meristematic tissues and the development of the vegetative and root systems (Naseem et al., 2019; Pintarič et al., 2024).

The organic preparation of SME increased the percentage of total chlorophyll and total soluble carbohydrates in the vegetative system and improved vegetative growth characteristics (Almousawi, 2023). This allowed the plant to be exposed to more light, which is necessary for higher metabolic activity and greater carbohydrate accumulation, and subsequently for the formation of other vital compounds. This is mainly attributed to the increased nitrogen content of the fertilizer, which, in turn, enters the structure of the chlorophyll molecule and positively affects photosynthetic efficiency, leading to the accumulation of total soluble carbohydrates (Al-Akaishi, 2018; Igual, 2024; Naseem et al., 2019; Yin et al., 2025).

Conclusions

Trifoliate orange seedlings responded strongly to nano-iron foliar spray at 20 mg/L, increasing height, stem diameter, branch number, leaf area, chlorophyll, and carbohydrate content. Spent mushroom extract (SME) showed similar but lesser effects, while their combination

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outperformed individual treatments. This approach enhances seedling vigor, shortens nursery time, and supports sustainable citrus production by reducing reliance on traditional fertilizers while promoting environmental safety. Apply nano-iron at ~20 mg/L during active growth to boost vegetative parameters and chlorophyll synthesis, monitoring iron and chlorophyll levels to avoid toxicity. Integrate SME via irrigation to improve soil microbes, nutrient retention, and vigor. Test long-term field effects across soil types and climates for commercial scalability. High pH and bicarbonates in alkaline soils limit iron availability, stunting growth. Nano-iron enhances vegetative growth, chlorophyll content, nutrient uptake, and photosynthetic efficiency under such conditions, though higher doses may slightly reduce zinc and manganese levels. Foliar iron (nano, chelated, or conventional) stimulates enzymatic activity, chlorophyll synthesis, and carbohydrate production in citrus, optimizing nursery and field performance.

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