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EFFECTS OF SULFUR AND IRON NANO-FERTILIZERS APPLIED THROUGH FOLIAR SPRAY AND FERTIGATION ON GROWTH AND BIOCHEMICAL CHARACTERISTICS OF LEEK (*ALLIUM AMPELOPRASUM* VAR. *PORRUM* L.)

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Abstract

Nano-fertilizers have emerged as a promising tool for enhancing crop productivity and quality. However, the comparative effects of different nano-nutrient formulations and application methods remain mainly unexplored, particularly in crops such as leek (*Allium ampeloprasum* var. *porrum* L.). The current study addresses this research gap by investigating the response of leek shoots to sulfur (S) and iron (Fe) nano-fertilizers applied individually and in combination using two distinct methods: foliar application and fertigation. We hypothesized that the combined application of sulfur and iron nano-fertilizers would enhance leek growth and biochemical attributes more effectively than individual nano-fertilizer treatments, and that nutrient delivery method would significantly influence plant responses. Despite increasing interest in nano-fertilizers, limited information is available regarding the combined use of sulfur and iron nanoparticles in leek cultivation, particularly under different nutrient delivery systems such as foliar application and fertigation. The experiment was conducted during the 2023-2024 growing season in a dedicated nursery located in the Al-Hay Al-Asri area in the center of Al Diwaniyah Governorate. A two-factor factorial experiment was conducted using a randomized complete block design (RCBD) with three replications. The first factor consisted of four nano-fertilizer treatments (control, nano-S, nano-Fe, and nano-S+Fe), while the second factor comprised four application treatments, namely foliar application once, foliar application twice, fertigation once, and fertigation twice. Key morphological parameters, including plant height, number of leaves per plant, total leaf area, fresh shoot weight, and leaf chlorophyll content, were significantly improved in plants treated with nano-fertilizers compared to the control group. Notably, the combined treatment with foliar sulfur and iron resulted highest plant height (37.26 cm) and leaf number (14.10 leaf plant^{-1}), while fertigation resulted higher plant heights compared to foliar spray. Additionally, twice foliar spray of nano-fertilizer significantly improved fresh shoot weight and chlorophyll content. Biochemical analysis also showed significant improvements in the contents of total protein, soluble sugars, total phenolics, flavonoids, and terpenes. The nano combined (S + Fe) achieved peak levels of total protein (8.56% with twice fertigation) and soluble sugars (14.55% by one fertigation). Total phenolic, flavonoid and terpene contents were significantly higher in nano-fertilizer treated plants compared to control, especially when applied through fertigation. The enhancement of growth, photosynthetic pigments, primary and secondary metabolites suggested that sulfur and iron nano-fertilizers improved both physiological performance and biochemical functionality of leek plants. These responses may be attributed to improved nutrient availability and uptake efficiency associated with nano-scale nutrient delivery. Collectively, these findings demonstrate that sulfur and iron nano-fertilizers can effectively enhance growth performance, physiological status, and biochemical quality of leek, with treatment efficacy varying according to nutrient combination and application method.

Key words: Leek; Shoot; S; Fe; Nano-particles; Nano-fertilizers; Foliar spray; Fertigation

Introduction

Sulfur (S) and iron (Fe) are essential nutrients markedly influencing the growth and quality of allium crops including leek (*Allium ampeloprasum* var. *porrum*). Sulfur is often the fourth major plant nutrient after N, P, and K, and allium vegetables (e.g. onion, garlic, leek) are particularly sulfur-demanding plants. Adequate sulfur is crucial for chlorophyll formation, efficient nitrogen utilization, and the synthesis of defense-related compounds in plants (Arora *et al*., 2024). In sulfur-loving crops like Alliums, S deficiency leads to poor growth, reduced nutrient uptake, and significant decline in yield and quality (Singh *et al*., 2016). For example, severe lack of sulfur during bulb or pseudo-stem development in onion and garlic dramatically diminishes yield and impairs flavor compound formation. Accordingly, leek relies on sulfur for its aromatic organosulfur compounds and nutritional value. Therefore, sufficient S nutrition not only supports growth but also contributes to the health-promoting properties (e.g. anticancer effects) associated with Allium vegetables. Each of these points underscores the critical role of sulfur in leek cultivation (Thangasamy *et al*., 2021; Takahashi *et al*., 2023). Owing to the high sulfur demand of Allium crops, efficient sulfur delivery strategies remain an important research priority for improving crop productivity and quality. This aspect is particularly important in leek because sulfur contributes directly to the synthesis of characteristic organosulfur compounds responsible for flavor, nutritional quality, and several health-promoting properties of Allium vegetables.

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Iron (Fe), is another essential micronutrient for leek and other crops. Iron is a key component of some enzymes and proteins involving in photosynthesis and respiration and is essential for chlorophyll biosynthesis and overall plant metabolism (Arora *et al*., 2024). Iron deficiency is a common nutritional disorder characterized by interveinal chlorosis (yellowing between green veins) in young leaves, because of its immobility in plants (Semenova *et al*., 2024). Even though Fe is abundant in most soils, it often occurs in insoluble forms (especially in calcareous or high-pH soils) and roots cannot uptake it. Consequently, Fe deficiency can severely decrease plant growth and reduce crop yields (Arora *et al*., 2024). Leek plants have the capacity to accumulate significant amounts of Fe in their tissues when it is available. In fact, leeks can contain more Fe than related Alliums like garlic (Golubkina *et al*., 2018). However, despite its abundance in many agricultural soils, limited iron bioavailability remains a major constraint to crop productivity, necessitating the development of more efficient iron delivery systems. Iron nutrition is equally important in leek because vigorous leaf development and chlorophyll accumulation are essential for sustaining photosynthetic activity and biomass production in this leafy vegetable crop.

A combined sulfur and iron nano-fertilizer strategy may provide complementary physiological benefits because both nutrients participate in interconnected metabolic pathways. Sulfur is essential for the synthesis of sulfur-containing amino acids, proteins, and coenzymes, whereas iron plays a critical role in chlorophyll biosynthesis, photosynthetic electron transport, and nitrogen metabolism. Adequate sulfur nutrition can improve protein formation, while sufficient iron availability supports photosynthetic activity and energy production. Therefore, simultaneous delivery of sulfur and iron may enhance nutrient utilization efficiency and plant metabolism more effectively than individual nutrient applications (Narayan *et al*., 2023, Takahashi *et al*., 2023, Arora *et al*., 2024).

Conventional fertilization is inefficient because large proportions of applied nutrients are lost before plant uptake via leaching, volatilization, or fixation. Nano-fertilizers nutrients (engineered at the 1–100 nm scale) address these limitations by enabling controlled release, targeted delivery and better aligning nutrient availability with plant demand. Their high surface area and small size improve soil–plant transfer, tissue penetration, and uptake compared to bulk fertilizers. Studies have shown that nano-fertilizers can enhance nutrient use efficiency and growth of plants, allow lower application rates of fertilizers and reduce environmental impact, making them an attractive next-generation fertilizer technology (Ding *et al*., 2023; Seleiman *et al*., 2025). These inefficiencies waste resources and lead to environmental problems such as groundwater contamination and eutrophication. Consequently, nano-fertilizers have attracted increasing attention as an alternative nutrient delivery system capable of improving nutrient use efficiency while reducing environmental losses.

In the context of sulfur nutrition, elemental sulfur nanoparticles (“nano-sulfur”) have demonstrated strong benefits for crops. Among various nano-nutrients, nano-sulfur stands out for its ability to enhance plant growth, improve tolerance to stresses, and boost the nutritional quality of edible tissues (Sun *et al*., 2024). Nanoscale elemental sulfur is substantially more reactive and bioavailable than bulk S amendments and its application to sulfur deficient soils markedly increase its use efficiency and faster translocation from vegetative tissues to developing grains. Moreover, sulfur nanoparticles act as growth stimulants and stress mitigators (for example increasing resistance to pathogens such as Fusarium), enhancing biomass production and nutritional quality of plants edible tissues. Additionally, nano-sulfur has been associated with reduced plant uptake of certain toxic metals, offering potential benefits for food safety and environmental impact (Arora *et al*., 2024. Furthermore, evidence regarding their effectiveness in leek cultivation is still scarce.

Iron-based nano-fertilizers are designed to address the challenge of low Fe bioavailability in soils. By delivering iron in nano-particulate form (such as nano-sized Fe oxides or Fe sulfide particles), the pool of plant-accessible iron in soil can be increased. Nanoscale iron particles (typically <100 nm in size) are small enough to penetrate the soil matrix and can be uptaken by plant roots more efficiently than larger size conventional Fe sources (Yadav *et al*., 2023). Several studies demonstrate that iron nano-fertilizers can substantially improve plant Fe status, growth and yield when applied at appropriate doses. For example, foliar iron nano-chelates increased soybean chlorophyll by ~10%, foliar iron nanoparticles raised cowpea seed weight by ~7%, and low doses of Fe_2O_3 nanoparticles (~50 mg L^{-1}) stimulated rice seedling growth while high doses (~500 mg L^{-1}) were phytotoxic and reduced root development. In a greenhouse trial, soybean treated with moderate superparamagnetic Fe_3O_4 nano-particles (~45 mg L^{-1}) exhibited higher leaf chlorophyll than plants receiving an equivalent Fe dose as Fe-EDTA, implying superior uptake or sustained release from the nano-particles. These findings emphasize a clear dose-dependent window of efficacy and toxicity for nano-Fe (Arora *et al*., 2024). Previous studies demonstrated that application method can substantially influence nutrient availability, uptake efficiency, and plant response. However, direct comparisons between foliar application and fertigation for sulfur and iron nano-fertilizers remain insufficiently investigated. Iron nutrition is equally important in leek because vigorous leaf development and chlorophyll accumulation are essential for sustaining photosynthetic activity and biomass production in this leafy vegetable crop.

Beyond fertilizer formulation, application method and timing critically determine nutrient use by plants. Nano-fertilizers applied foliar or to the soil (including via fertigation: injecting fertilizers into the irrigation system) differ markedly in availability, uptake pathway and crop response (Seleiman *et al*., 2025). Foliar sprays deposit nanoparticle suspensions on leaf surfaces and stomata, often at lower doses providing rapid correction of tissue deficiencies, and reduce leaching compared with soil application. However, foliar feeding especially is useful for immobile micronutrients such as iron (Ding *et al*., 2023). Soil-based delivery, including fertigation, deposits nutrients in the root zone for uptake by root hairs and can create a longer-lasting nutrient reservoir that is readily synchronized with irrigation schedules. Supplying sulfur nanoparticles via soil or fertigation places elemental S where microbes can oxidize it to plant-available sulfate, and potentially improving S availability and uptake over time. Fertigation is particularly compatible with drip irrigation and timed nutrient programs (Seleiman *et al*., 2025).

However, soil application raises specific questions about nanoparticle transport, agglomeration and interactions with soil biota that may reduce bioavailability. Few studies have directly compared foliar versus soil nano-fertilizer delivery for S and Fe in the same system (Seleiman *et al*., 2025). Consequently, foliar and soil routes likely offer complementary advantages: foliar feeding gives rapid tissue responses while soil/fertigation better supports sustained root uptake. Optimizing nano-fertilizer efficacy requires matching route, formulation, dose and timing to crop needs and soil processes.

Nevertheless, neither application method is universally superior. Foliar application provides rapid nutrient delivery and is particularly effective for correcting micronutrient deficiencies; however, nutrient absorption may be influenced by leaf surface characteristics, environmental conditions, and application timing. In contrast, fertigation promotes nutrient availability within the root zone and may support prolonged nutrient uptake, although nanoparticle interactions with soil constituents can potentially reduce nutrient mobility and bioavailability. These contrasting mechanisms indicate that plant responses may differ depending on the nutrient source, crop species, and growth conditions, highlighting the need for direct comparisons under specific production systems.

Split applications and careful timing of fertilizer deliveries can improve nutrient use by plants and reduce leaching (Kumar *et al*., 2023). In general, both application route (foliar spray versus soil fertigation) and application frequency (single versus repeated doses) can markedly influence the efficacy of sulfur and iron nano fertilizers in leek by altering nutrient availability, uptake dynamics, translocation, and ultimately growth, physiology, and yield (Seleiman *et al*., 2025). However, comprehensive investigations of their interactive effects remain limited, and empirical data on responses to nanoparticulate S and Fe under differing delivery strategies are rather sparse. To fill this gap, this study compares nano-fertilizers as a first factor, foliar spray or fertigation by S or Fe or both nanoparticles, each applied once or twice as a second factor, and the interaction between them, evaluates the effects on leek growth and nutritional status.

Previous studies have demonstrated the beneficial effects of sulfur nanoparticles on plant growth, photosynthetic performance, and nutrient utilization in several horticultural crops (Salem *et al*., 2016; Najafi *et al*., 2020; Sun *et al*., 2024). Similarly, iron-based nano-fertilizers have been reported to improve chlorophyll synthesis, biomass accumulation, and crop productivity in a range of crop species (Sheykhbaglou *et al*., 2010; El-Desouky *et al*., 2021; Arora *et al*., 2024).

Despite these advances, most available studies have evaluated sulfur and iron nanoparticles separately. Moreover, information regarding the response of leek to combined sulfur and iron nano-fertilization under different nutrient delivery systems is scarce. Comparative evaluations of foliar application and fertigation for nano-fertilizer delivery are also rarely reported.

Therefore, a significant knowledge gap exists regarding the interaction between sulfur and iron nano-fertilizers and the extent to which application method influences their effectiveness in leek production. The novelty of the present study lies in simultaneously evaluating sulfur and iron nanoparticles, individually and in combination, under contrasting application methods and frequencies. Accordingly, the objectives of this study were to evaluate the effects of sulfur and iron nano-fertilizers on the growth and biochemical characteristics of leek, compare foliar application and fertigation as nutrient delivery systems, and determine whether repeated applications provide additional benefits. It was hypothesized that combined nano-S and nano-Fe application would outperform individual treatments and that plant responses would vary according to nutrient delivery strategy.

Therefore, the objectives of this study were to:

(i) Evaluate the effects of sulfur and iron nano-fertilizers applied individually and in combination on the growth and biochemical characteristics of leek (*Allium ampeloprasum* var. porrum L.);

(ii) compare foliar application and fertigation as nutrient delivery systems for nano-fertilizers; and

(iii) Determine whether repeated applications provide additional benefits compared with single applications. Therefore, the objectives of this study were to evaluate the effects of sulfur and iron nano-fertilizers applied individually and in combination on the growth and biochemical characteristics of leek, compare foliar application and fertigation as nutrient delivery systems, and determine whether repeated applications provide additional benefits. Particular attention was given to assessing whether the combined application of sulfur and iron nanoparticles could provide greater improvements than individual nano-fertilizer treatments and whether plant responses varied according to nutrient delivery strategy.

Materials and Methods

Description of the experimental site: The experiment was conducted during the 2023–2024 growing season in a dedicated experimental nursery located in the Al-Hay Al-Asri area, Al-Diwaniyah Governorate, Iraq. Al-Diwaniyah Governorate is characterized by a hot arid climate with high solar irradiance, low annual rainfall, and pronounced seasonal temperature fluctuations. Summer temperatures frequently exceed 45°C, whereas winter temperatures typically range between 8 and 12°C. Relative humidity is generally low during the growing season, contributing to high evapotranspiration rates. The nursery was established specifically for conducting controlled pot experiments under local environmental conditions. All experimental units were maintained under the prevailing climatic conditions of the region, and routine agronomic practices were applied throughout the study period. Pots were irrigated regularly to maintain adequate soil moisture and avoid water stress during plant growth. Irrigation was managed regularly throughout the experiment to maintain suitable soil moisture conditions for plant growth.

Preparation of experimental determinants: Black plastic pots with a size of 24 × 22 cm were filled with 10 kg of soil. Some soil physicochemical properties were determined in the central laboratory of the College of Science/ University of Baghdad (Table 1) (Estefan *et al*., 2013).

Nano-fertilizers used in this research was purchased from US Research Nanomaterials, Inc. Houston, TX USA (https://www.us-nano.com/home).

The physicochemical characteristics of the sulfur and iron nanoparticles, including particle size and purity, were based on the specifications provided by the manufacturer (US Research Nanomaterials Inc., Houston, TX, USA). Independent characterization of nanoparticle morphology, crystallinity, and surface properties was not performed in the present study, as the primary objective was to evaluate the agronomic response of leek to commercially available nano-fertilizer formulations.

Sulphur (S) Nanoparticles/ Nano powder (S, 47 nm, Laser Gas Phase Synthesis, Orthorhombic). Iron (Fe) Nano powder/Fe Nanoparticles (Fe, 99.5+%, 35-45 nm, metal basis).

Table 1. Some physicochemical properties of soil used in the experiment.

Trait		Value	Unit of measurement
pH		6.8	-
Electrical conductivity (ECe))		4.46	ds m^{-1}
Cation exchange capacity (CEC)		34.89	centimole^{+} kg^{-1}
Carbonate Minerals		35.22	g kg^{-1}
Organic matter		8.7	%
Available nitrogen		19.10	g kg^{-1}
Available phosphorus		10.85	mg kg^{-1}
Available potassium		350.00	mg kg^{-1}
Bulk density		1.1559	g cm^{-3}
Soil components	Sand	449	g kg^{-1}
	Silt	351	g kg^{-1}
	Clay	200	g kg^{-1}
Soil texture		Loam	

Experimental design and implementation: The experiment was arranged as a two-factor factorial experiment in a randomized complete block design (RCBD) with three replications. (Steel *et al*., 1997). Factor A consisted of 4 nano-fertilizer treatments: control (water), nano-S, nano-Fe, and nano-S+Fe. Factor B consisted of 4 application treatments: foliar application once, foliar application twice, fertigation once, and fertigation twice. The factorial arrangement generated 16 treatment combinations (4 × 4), each replicated three times.

Nano-fertilizer solutions were prepared at the designated concentration and applied uniformly to the foliage using a hand-held sprayer. Foliar applications were performed during the morning hours to minimize evaporation losses and maximize nutrient absorption. No surfactant was added to the spray solutions. Foliar applications were performed during the morning hours to minimize evaporation losses and maximize nutrient absorption. The exact timing of foliar applications was not recorded and therefore could not be reported in detail.

The selected concentration (1 mg L^{-1}) was chosen based on previous studies reporting beneficial physiological and growth responses to sulfur and iron nanoparticles at relatively low concentrations while minimizing the possibility of phytotoxic effects associated with excessive nanoparticle application (Najafi *et al*., 2020; Arora *et al*., 2024). Nano-fertilizer solutions were prepared at the designated concentration and applied uniformly to the foliage using a hand-held sprayer until adequate leaf coverage was achieved.

Studied indicators

Growth parameters

Plant height: The plant height was measured using a metric measuring tape, starting from the soil surface of the pot to the highest peak of the plant. This done for all plants from each treatment at all replicates.

Number of leaves per plant: The number of leaves was calculated for three plants in each replicate for all treatments.

Total leaf area: The leaf area was calculated for three leaves taken randomly from each pot by measuring the maximum length and width of each leaf using a measuring tape for thin-leaved plants (Liang *et al*., 1973). The total leaf area of the plant was calculated by multiplying the area of one leaf by the number of leaves on the plant.

$$\text{Leaf area (cm}^2\text{)} = [\text{Leaf length (cm)} \times \text{Leaf width (cm)} \times 0.75]$$

Shoot fresh weight: The fresh weight of the plant shoot was calculated after collecting the plants, cleaning, and washing them from the remnants of the attached soil. Then, they were weighed using a sensitive electronic scale (Metler HK 160, Swiss).

Biochemical parameters

Chlorophyll *a*: Chlorophyll *a* content was determined according to the method of Nagata & Yamashita (1992). Accordingly, 1 g of the fresh leaves' material was homogenated with 10 ml of the acetone-hexane mixture (2:3 v/v) using electric blender (Toshiba Al-Arabi/ Egypt) for 2 min. The homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The absorbance of supernatants was measured at 645 and 663 nm using a UV/VIS (Cary 50 Scan spectrophotometer, UK). The content of Chl *a* was calculated using the following equations:

$$[\text{Chlorophyll } a \text{ (mg g}^{-1}\text{ FW)} = 0.999\ A_{663} - 0.0989\ A_{645}].$$

Total protein: The protein percentage was estimated by multiplying the N (%) (estimated according to the method of Cresser & Parsons (1979) by a constant factor (6.25) according to AOAC (2000) using following equation:

$$[\text{Protein (\%)} = \text{Nitrogen (\%)} \times 6.25]$$

Soluble sugars: The percentage of soluble sugars was estimated according to the method of Herbert *et al*., (1971). In order to, 0.1 g of the leaves dry and powdered material was dissolved in 10 ml of boiling water for 10 min, and then centrifuged (Hettich EBA 35, Germany) at 5000 rpm for 5 min. The residue was re-extracted in the same way and the supernatants (first and second) were collected. The phenol – sulfuric acid method was used for estimation of soluble sugar content. The absorbance of samples was determined at 540 nm using a spectrophotometer (Bichrom-Libra S22-UK 2005). At the same time, the standard curve was prepared using different concentrations of glucose. Finally, the soluble sugar percentage was estimated according to the following equation:

[Soluble sugars (%) = 100 × glucose (mg)/ Test sample volume]

Total phenol content: The dry powder was extracted using 60% ethanol (1:2 w/v) at room temperature for 72 hours with occasional shaking. The extract was filtered, and the precipitate was re-extracted until the solvent was exhausted. The ethanol was removed under reduced pressure at 4°C. For analysis, 1 mL of the sample was mixed with 5 mL of Folin-Cioalto reagent and 4 mL of 7% sodium bicarbonate (w/v). The mixture was incubated in the dark at room temperature for 2 hours, and the absorbance was measured at 725 nm. Total phenols were calculated using a standard curve prepared with gallic acid and expressed in mg of gallic acid equivalents per gram of dry weight (GAE g^{-1} DW) (Pothitirat *et al*., 2009).

Total flavonoid content: The extract previously prepared and stored for total phenol determination was used for total flavonoid analysis. 2 ml of the preserved extract was mixed with 2 ml of 2% $AlCl_3$ solution and incubate at room temperature for 30 minutes. Finally, the absorbance of samples was recovered at 415 nm against a blank lacking $AlCl_3$. Total flavonoids content was calculated using a quercetin standard curve and expresse as mg quercetin equivalents per g dry weight (mg QE g^{-1} DW) (Miliauskas *et al*., 2004).

Terpenes content: Frozen leaf tissue (500 mg fresh weight) was pulverized in liquid nitrogen and stored at −80°C until analysis. For extraction, frozen tissue was homogenized with chilled 95% methanol using tungsten-carbide beads in a bead mill and further macerated with an iced mortar and pestle. Beads were removed and the combined extract was incubated in the dark at room temperature for approximately 48 h. Samples were centrifuged and the supernatant was collected. For colorimetric determination, 200 µL of extract (treat linalool standards identically) was mixed with 1.5 mL chloroform and 100 µL concentrated H_2SO_4, incubated in the dark for 1.5–2 h to allow color development, then the liquid phase was decanted and the precipitate was dissolved in 1.5 mL 95% methanol. Absorbance was measured at 538 nm and total terpenes were calculated as linalool equivalents from a blank-corrected standard curve. All measurements were performed in at least technical triplicate and results are reported as linalool equivalents per gram fresh weight (Ghorai *et al*., 2012).

Statistical analysis: Data obtained from the experiment were subjected to analysis of variance (ANOVA) appropriate for a two-factor factorial experiment arranged in a randomized complete block design (RCBD). Prior to analysis, data were screened for outliers and examined for compliance with the assumptions of ANOVA. The effects of nano-fertilizer treatments, application methods, and their interactions were evaluated. Treatment means were compared using the Least Significant Difference (LSD) test at the 5% probability level ($p \leq 0.05$). Statistical analyses were performed using IBM SPSS 26. Significant differences among treatment means were determined based on the calculated LSD values at $p \leq 0.05$.

Results

Plant height: Nano-fertilizer application significantly increased plant height compared with the untreated control (Table 2). Among the fertilizer treatments, the combined S + Fe treatment produced the greatest plant height, whereas control plants exhibited the lowest values. Application method also influenced plant growth, with fertigation generally resulting in taller plants than foliar spraying, particularly when applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied through twice fertigation producing the maximum plant height. The superiority of the combined S + Fe treatment was consistently observed across application methods, indicating a positive combined effect of the two nano-fertilizers on vegetative growth.

The combined S + Fe treatment increased approximately 26% leaf number compared with the control (Fig. 1).

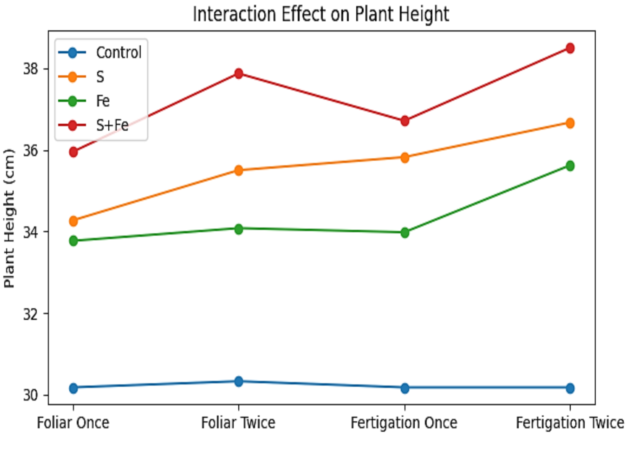

Fig. 1. Interaction effect of nano-fertilizer treatments and application methods on plant height of leek.

Leaves number: Nano-fertilizer application significantly increased the number of leaves per plant compared with the untreated control (Table 3). Among the fertilizer treatments, the combined S + Fe application produced the greatest increase in leaf number, whereas control plants recorded the lowest values. Foliar application generally resulted in a higher number of leaves than fertigation, particularly when treatments were applied twice. The interaction between fertilizer type and application method was significant, with the combined S + Fe treatment applied twice as a foliar spray producing the highest leaf number observed in the experiment. The enhanced leaf production under the combined S + Fe treatment suggested improved vegetative development when both nutrients were supplied simultaneously. The combined S + Fe treatment increased approximately 26% leaf number compared with the control.

Table 2. Influence of S and Fe nanofertilizers and application method on *Allium ampeloprasum* var. *porrum* plant height (cm).

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	30.18	30.33	30.18	30.18	30.22
S (1 mg L^{-1})	34.27	35.50	35.82	36.67	35.57
Fe (1 mg L^{-1})	33.77	34.08	33.98	35.62	34.36
S + Fe	35.95	37.87	36.71	38.50	37.26
Mean of application method	33.54	34.45	34.17	35.24	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 1.05; Factor 2: Application method = 0.94; Interaction = 1.72

Table 3. Number of Leaves per Plant in Leek (*Allium ampeloprasum* var. *porrum*) under sulfur and iron nanofertilizer treatments (leaf $plant^{-1}$).

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	10.89	10.95	10.95	10.95	10.94
S (1 mg L^{-1})	12.50	13.00	12.00	12.24	12.44
Fe (1 mg L^{-1})	11.28	12.10	11.50	11.77	11.66
S + Fe	13.90	14.10	13.09	14.00	13.77
Mean of application method	12.14	12.54	11.89	12.24	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 0.65; Factor 2: Application method = 0.87; Interaction = 1.18

Table 4. Response of leek total leaf area (cm^2 $plant^{-1}$) to S and Fe nanofertilizers applied by different methods.

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	135.00	136.80	136.80	136.80	136.35
S (1 mg L^{-1})	171.18	177.00	167.00	169.47	171.16
Fe (1 mg L^{-1})	162.29	168.99	157.30	160.07	162.16
S + Fe	179.09	185.11	170.12	175.44	177.44
Mean of application method	161.89	166.98	157.81	160.45	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 1.95; Factor 2: Application method = 1.09; Interaction = 2.33

Table 5. Leek shoot fresh weight (g $plant^{-1}$) response to S and Fe nanofertilizer application methods.

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	11.00	11.50	11.50	11.50	11.38
S (1 mg L^{-1})	14.28	15.10	13.14	14.05	14.14
Fe (1 mg L^{-1})	13.00	13.70	12.34	12.87	12.98
S + Fe	15.87	16.22	14.07	14.20	15.09
Mean of application method	13.54	14.13	12.76	13.16	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 0.23; Factor 2: Application method = 0.09; Interaction = 0.64

Table 6. Changes in chlorophyll a content (mg g^{-1} FW) in leek after S and Fe nanofertilizer application.

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	1.30	1.33	1.33	1.33	1.32
S (1 mg L^{-1})	1.36	1.42	1.44	1.51	1.43
Fe (1 mg L^{-1})	1.40	1.44	1.31	1.37	1.38
S + Fe	1.50	1.54	1.45	1.48	1.49
Mean of application method	1.39	1.43	1.38	1.42	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 0.08; Factor 2: Application method = 0.12; Interaction = 0.17

Total leaf area: Nano-fertilizer application significantly increased total leaf area compared with the untreated control (Table 4). Among the fertilizer treatments, the combined S + Fe application produced the largest leaf area, whereas control plants exhibited the lowest values. Foliar application was generally more effective than fertigation in promoting leaf expansion, particularly when treatments were applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied twice as a foliar spray producing the greatest leaf area recorded in the experiment.

Total leaf area was increased by approximately 30% under the combined S + Fe treatment relative to the control.

Shoot fresh weight: Nano-fertilizer application significantly increased shoot fresh weight compared with the untreated control (Table 5). Among the fertilizer treatments, the combined S + Fe application produced the greatest shoot biomass, whereas control plants recorded the lowest values. Foliar application was generally more effective than fertigation in enhancing shoot fresh weight, particularly when treatments were applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied twice as a foliar spray producing the highest shoot fresh weight was recorded (Fig. 2).

Chlorophyll *a* content: Nano-fertilizer application significantly increased chlorophyll a content compared with the untreated control (Table 6). Among the fertilizer treatments, the combined S + Fe application produced the highest chlorophyll a concentration, whereas control plants exhibited the lowest values. Foliar application generally resulted in slightly greater chlorophyll accumulation than fertigation, particularly when treatments were applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied twice as a foliar spray producing the highest chlorophyll a content.

Total protein: Nano-fertilizer application significantly increased total protein content compared with the untreated control (Table 7). Among the fertilizer treatments, the combined S + Fe application produced the highest protein concentration, whereas the lowest values were recorded in control plants. Fertigation was generally more effective than foliar application in enhancing protein accumulation, particularly when treatments were applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied twice through fertigation producing the highest protein content.

Soluble sugars: Nano-fertilizer application significantly increased total soluble sugar content compared with the untreated control (Table 8). Among the fertilizer treatments, the combined S + Fe application produced the highest soluble sugar concentration, whereas control plants recorded the lowest values. Fertigation generally resulted in greater sugar accumulation than foliar application, particularly when treatments were applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied twice through fertigation producing the highest total soluble sugar content recorded in the experiment.

Total phenol content: Nano-fertilizer application significantly increased total phenol content compared with the untreated control (Table 9). Among the fertilizer treatments, the combined S + Fe application produced the greatest phenolic accumulation, whereas control plants exhibited the lowest values. Fertigation generally resulted in higher phenol content than foliar application, particularly when treatments were applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied twice through fertigation producing the highest total phenol content recorded in the experiment (Fig. 3).

Total flavonoid content: Nano-fertilizer application significantly increased total flavonoid content compared with the untreated control (Table 10). Among the fertilizer treatments, the combined S + Fe application resulted in the greatest flavonoid accumulation, whereas in control plants the lowest values were recorded. Fertigation generally promoted higher flavonoid content than foliar application, particularly when treatments were applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied twice through fertigation producing the highest flavonoid content recorded in the experiment.

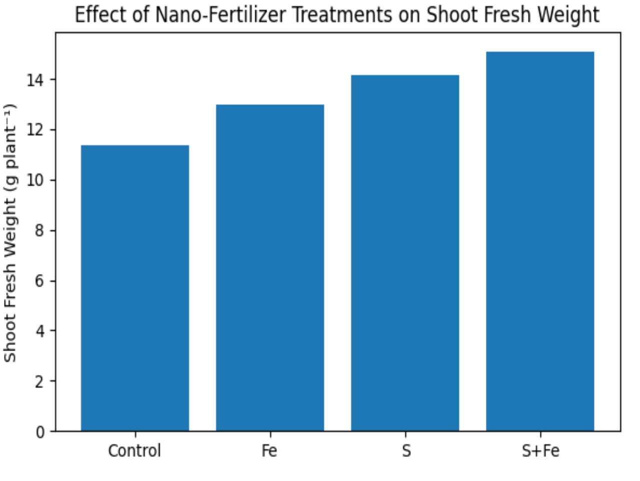

Fig. 2. Effect of nano-fertilizer treatments on shoot fresh weight of leek plants.

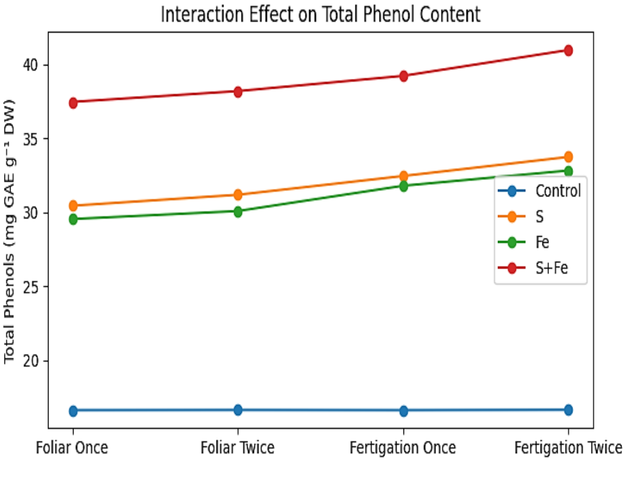

Fig. 3. Interaction effect of nano-fertilizer treatments and application methods on total phenol content of leek.

Table 7. Effect of sulfur and iron nanofertilizers on total protein (%) in leek.

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	4.88	5.18	5.56	5.90	5.38
S (1 mg L^{-1})	7.71	8.09	8.11	8.12	8.01
Fe (1 mg L^{-1})	6.99	7.33	7.37	7.41	7.27
S + Fe	8.23	8.28	8.38	8.56	8.36
Mean of application method	6.95	7.22	7.36	7.50	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 0.28; Factor 2: Application method = 0.86; Interaction = 1.07

Table 8. Soluble sugars (%) in leek after application of S and Fe nanofertilizers (%).

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	12.39	12.57	12.70	12.78	12.61
S (1 mg L^{-1})	13.20	13.44	13.67	13.76	13.52
Fe (1 mg L^{-1})	13.10	13.24	13.39	13.48	13.30
S + Fe	14.21	14.33	14.49	14.55	14.40
Mean of application method	13.22	13.39	13.56	13.64	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 0.58; Factor 2: Application method = 0.67; Interaction = 0.89

Table 9. Effect of sulfur and iron nanofertilizers on total phenols in leek (mg GAE g^{-1} DW).

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	16.62	16.63	16.62	16.64	16.63
S (1 mg L^{-1})	30.44	31.18	32.45	33.74	31.95
Fe (1 mg L^{-1})	29.54	30.08	31.79	32.82	31.06
S + Fe	37.46	38.19	39.22	40.97	38.96
Mean of application method	28.52	29.02	30.02	31.04	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 1.18; Factor 2: Application method = 1.24; Interaction = 2.35

Table 10. Total flavonoid content of *Allium ampeloprasum* var. *porrum* as affected by S and Fe nanofertilizers (mg QE g^{-1} DW).

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	1.00	1.01	1.03	1.04	1.02
S (1 mg L^{-1})	3.45	3.68	3.74	3.91	3.70
Fe (1 mg L^{-1})	2.76	2.81	2.89	2.96	2.86
S + Fe	4.18	4.55	4.80	4.95	4.62
Mean of application method	2.85	3.01	3.12	3.22	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 0.49; Factor 2: Application method = 0.75; Interaction = 1.01

Table 11. Terpene content (mg LnE g^{-1} DW) in leek following S and Fe nanofertilizer applications by method.

Nano-fertilizer	Application method				Mean of nano-fertilizer
	Foliar spray		Soil (Fertigation)		
	One-time	Twice	One-time	Twice	
Control (water)	0.10	0.12	0.13	0.13	0.12
S (1 mg L^{-1})	1.12	1.42	1.49	1.56	1.40
Fe (1 mg L^{-1})	0.90	0.95	1.11	1.24	1.05
S + Fe	2.14	2.31	2.42	2.53	2.35
Mean of application method	1.07	1.20	1.29	1.37	

LSD ($p \leq 0.05$) Factor 1: Nano-fertilizer = 0.01; Factor 2: Application method = 0.02; Interaction = 0.03

Terpenes content: Nano-fertilizer application significantly increased total terpene content compared with the untreated control (Table 11). Among the fertilizer treatments, the combined S + Fe application produced the greatest terpene accumulation, whereas control plants exhibited the lowest values. Fertigation generally resulted in higher terpene content than foliar application, particularly when treatments were applied twice. A significant interaction between fertilizer type and application method was observed, with the combined S + Fe treatment applied twice through fertigation producing the highest terpene content recorded in the experiment.

Discussion

The evaluated growth, physiological, and biochemical parameters provide important indicators of plant performance and nutrient responsiveness. These traits were selected to assess the early effects of sulfur and iron nano-fertilizers on leek development under controlled experimental conditions.

Growth and physiological parameters

Growth: Both sulfur (S) and iron (Fe) are essential nutrients and play pivotal roles in plant growth and development (Narayan *et al*., 2023). The application of these elements in nano-particle form has been observed to significantly enhance vegetative growth parameters in leek (Kaur *et al*., 2025). Similar to the findings of Almoussawi & Jameel (2022), in this research leeks treated with sulfur nano-fertilizer showed markedly increase in plant height, number of leaves, and leaf area compared to controls. Adequate sulfur nutrition is known to stimulate foliage growth, boost of leaf development and yield in plants in *Allium* species including onion and garlic (Majid, 2017). Fe nano-fertilizer treatments showed positive effects on leek growth. Fe is crucial for chloroplast development and many metabolic enzymes (El-Desouky *et al*., 2021), so improved Fe availability can stimulate vegetative growth. Although specific data on leeks with Fe nano-fertilizer are limited, results from other crops support this finding. Sheykhbaglou *et al*., (2010) demonstrated that foliar nano-Fe increased soybean height and biomass yield (48%), compared to control. Likewise, Jaberzadeh *et al*., (2013) observed about a 23% increase in wheat grain yield with nano-Fe sprays, and Delfani *et al*., (2014) recorded enhanced growth and plasma membrane stability in cowpea with nano-Fe application.

Leek plants are susceptible to Fe deficiency in higher pH soils. Field studies have noted no phytotoxicity from proper doses of Fe nano-particles and indeed report that they “increased chlorophyll levels, with no trace of toxicity”, leading to vigorous, green plant growth (Pieter & Morteza, 2013). Thus, it is plausible that the Fe nano-particle treated leeks in our study achieved greater height, leaf area, and fresh weight due to improved iron nutrition supporting overall biomass production. Overall, the enhancement in plant stature and biomass with S and Fe nano-fertilizers was consistent with the fundamental roles of these nutrients. Sulfur is required for the synthesis of amino acids (cysteine, methionine) and coenzymes that build plant tissue, while iron is required for cellular respiration and DNA synthesis in growing tissues (Pieter & Morteza, 2013).

The superior performance observed under the combined sulfur and iron treatment may be attributed to the complementary physiological functions of these nutrients. Sulfur contributes directly to amino acid and protein synthesis, whereas iron is essential for chlorophyll formation and electron transport processes. Simultaneous improvement of these metabolic pathways may enhance photosynthetic efficiency, nutrient assimilation, and biomass accumulation, thereby producing stronger growth responses than individual nutrient applications.

The observed improvements in vegetative growth and chlorophyll accumulation are biologically important because they indicate enhanced photosynthetic capacity and biomass production potential. Increased leaf development and leaf area can improve light interception and carbon assimilation, thereby supporting overall plant productivity. An important observation in the present study was that different application methods favored different plant traits. Foliar application generally promoted greater leaf development, leaf area expansion, shoot biomass accumulation, and chlorophyll content. This response may be attributed to the rapid absorption of nano-sized nutrients through leaf surfaces and their direct delivery to photosynthetically active tissues. Consequently, nutrients become immediately available for chlorophyll synthesis and vegetative growth processes.

By delivering S and Fe more effectively, nano-fertilizers prevent growth-limiting nutrient deficiencies. Results of our experiment on leek plants verified that nano-fertilized plants were visibly more vigorous, with more abundant and larger foliage, compared to control plants. Similar growth stimulation by S or Fe nano-particles has been reported in other species such as tomato (Salem *et al*., 2016) and soybean (Dola *et al*., 2022). This evidence strongly supports the notion that S and Fe nano-fertilizers act as effective growth promoters in leek when applied at appropriate dosages.

Photosynthetic pigments (Chlorophyll): The application of nano-fertilizers containing iron (Fe) and sulfur (S) nanoparticles resulted in a significant increase in photosynthetic pigment content in leeks, as evidenced by the greening of the leaves and elevated chlorophyll levels. Fe is a specific micronutrient for chlorophyll synthesis, acting as a cofactor for protochlorophyllid reductase and as a component of chloroplast cytochromes. Therefore, the application of nano-iron alleviates iron deficiency-induced yellowing of the leaves and promotes chlorophyll accumulation. The uptake of magnetic iron oxide (Fe_3O_4) nanoparticles has been shown to increase chlorophyll content in soybean leaves (Ghafariyan *et al*., 2013). Furthermore, the application of Fe_3O_4 to the leaves increases the chlorophyll content of soybeans (*Glycine max*) under both adequate irrigation and drought conditions (Dola *et al*., 2022).

Nano-sulfur fertilization resulted in a significant improvement in chlorophyll a (up to 1.43 mg g^{-1} FW), providing readily absorbable sulfur for chloroplast protein synthesis and chlorophyll synthesis via amino acid and ferridoxine pathways. Bio-synthesized sulfur nanoparticles improved chlorophyll and photosynthetic performance in lettuce (Najafi *et al*., 2020), and adequate sulfur uptake is crucial for sustaining chlorophyll synthesis and garlic crop growth (Przygocka-Cyna *et al*., 2020; Almousawi & Jamil, 2022). Combined nano-treatments with sulfur and iron indicate efficient nutrient delivery and uptake, enhancing photosynthetic capacity and potentially increasing carbohydrate production by improving light uptake. These benefits are achieved within optimal dosage ranges, while excessive concentrations of nanoparticles may cause plant stress. The observed safety and effectiveness are consistent with previous reports (Pieter & Morteza, 2013; Dola *et al*., 2022).

Soluble protein and sugar contents: The nano-fertilizer applications led to an increase in leaf protein and soluble sugars contents in leek plants.

Leek plants treated with sulfur and iron nano-particles had higher crude protein levels in the leaves compared to the control. This can be attributed to two main factors: (1) better nitrogen and S utilization, (2) direct role of S and Fe in protein biosynthesis. Sulfur is a constituent of the amino acids' cysteine and methionine, and is required for the formation of many co-enzymes and proteins. If sulfur is deficient, plants cannot fully convert absorbed nitrogen into protein, leading to accumulation of non-protein forms of N. Therefore, according to our results by supplying sulfur adequately (via nano-fertilizer), the leeks were able to synthesize more protein from the available nitrogen. Similarly, Abd El-Aziz & Balbaa (2007) reported that foliar application of micronutrients such as Fe and Zn increased nitrogen percentage and protein content in plant tissues (*Salvia farinacea*).

Iron, is a vital component of enzymes involved in nitrogen metabolism such as nitrate reductase and ferredoxin-dependent glutamate synthase (Rashid *et al*., 2022). Adequate iron facilitates the reduction of nitrate to ammonia and its incorporation into amino acids. Foliar iron application has been observed to raise protein levels in seeds and leaves of crops suffering Fe deficiency (Mahmoud *et al*., 2022). Baral *et al*., (2022) reported that spraying of Fe_3O_4 nanoparticles increased soybean seed protein content by 7.3% under well-watered conditions. Furthermore, combining Fe with Zn nano-fertilizer yielded the highest bean seed protein in another study, reflecting the synergy of micronutrients in enzyme activation. Similarly, in our experiment Fe leek plants treated with nano-particle showed a notable protein increase. This suggested that iron nano-fertilizer improved the efficiency of nitrogen assimilation in leek leaves, allowing the formation of more amino acids and proteins than in Fe-limited plants. Sheykhbaglou *et al*., (2018) similarly reported that foliar nano-Fe fertilizer boosted protein content in soybean, and related it to the role of Fe in amino acid biosynthetic enzymes.

Leek leaves accumulated higher concentrations of soluble sugars under S and Fe nano-fertilizer treatments. An increase in soluble sugar content is a common response to enhanced photosynthesis. In our experiment, the chlorophyll-rich, vigorously growing plants (by nano-S and nano-Fe application) presumably had higher photosynthetic rates, and produced more sugars. Some of these sugars were utilized for growth, but an excess might be stored in the leaves, detected as higher soluble sugar content. Najafi *et al*., (2020) reported that lettuce plants treated with an optimal dose of sulfur nano-particles significantly raised the soluble sugar level in leaves, and attributed this to a better photosynthetic capacity. The boost in protein and soluble sugars in leek leaves reflects a heightened metabolic activity due to nano-fertilizer supplementation. These changes are beneficial from both an agronomic and a nutritional standpoint.

In contrast to the vegetative traits, biochemical parameters such as proteins and soluble sugars generally responded more strongly to fertigation. A possible explanation is that nutrient delivery through the root zone provides a more sustained nutrient supply over time, supporting continuous nutrient uptake and metabolic activity. This prolonged nutrient availability may favor nitrogen assimilation, protein biosynthesis, and carbohydrate accumulation, resulting in greater concentrations of primary metabolites.

The enhancement of proteins, soluble sugars, phenolic compounds, flavonoids, and terpenes suggested that sulfur and iron nano-fertilizers influenced not only plant growth but also metabolic activity and biochemical quality. These compounds contribute to nutritional value, stress tolerance, and the characteristic quality attributes of leek.

Phenolics, flavonoids, and terpenes: Nanoferries containing sulfur (S) and iron (Fe) increased the total phenolic compounds, flavonoids, and terpenes in leeks, which were essential compounds for plant defense, antioxidant capacity, and flavor/aromatic expression. Nanofertilization appears to meet nutritional needs and act as a mild catalyst for secondary metabolism, consistent with reports that nanomaterials can stimulate antioxidant biosynthesis. Najafi *et al*., (2020) reported that lettuce treated with environmental friendly, sulfur nanoparticles (S-NPs) (1 mg/ml) exhibited significantly higher levels of total phenolic compounds, flavonoids, anthocyanins, and tannins, demonstrating dose-dependent responses. Mechanistically, sulfur nanoparticles likely activate the phenylpropanoid pathway by upregulating the enzyme phenylalanine ammonia lyase (PAL), as demonstrated by Sobhy *et al*., (2025) in lotus callus cultures, as discussed by Almousawi & Jamil (2022).

Similarly, iron nanoparticles increased phenol and terpene levels. Khanizadeh *et al*., (2024) reported that Fe_2O_3-NPs increased total phenols and essential oil (volatile terpene) production in *Dracocephalum kotschyi*, suggesting a role for signaling mediation by reactive oxygen species.

In our experiments on leeks, the nanoparticle dosages were within the effective range, resulting in higher levels of phenols, flavonoids, and terpenes without any stress-induced damage. These results indicated that S and Fe nanoparticle fertilizers acted as dual-action agents—nutrient sources and biostimulants with their beneficial effects being dose-dependent, as excessive concentrations caused oxidative damage (Najafi *et al*., 2020; Khanizadeh *et al*., 2024).

The enhancement of phenolic compounds, flavonoids, and terpenes is biologically significant because these metabolites contribute to antioxidant capacity, stress tolerance, defense responses, and product quality. Increased accumulation of these compounds suggested that sulfur and iron nano-fertilizers influenced not only plant growth but also secondary metabolic pathways associated with nutritional and functional quality attributes of leek.

Although the combined sulfur and iron treatment consistently produced superior responses relative to individual nutrient applications, the present study did not directly quantify synergistic interactions using formal synergy assessment approaches. Therefore, the observed responses are more appropriately interpreted as enhanced effects of combined nutrient application rather than confirmed synergistic interactions. Future studies involving dose-response analyses and quantitative interaction modeling would be valuable for clarifying the nature of sulfur–iron interactions in leek.

Significant improvements were observed in vegetative growth and biochemical composition, additional agronomic indicators such as marketable yield, pseudostem diameter, harvest index, nutrient-use efficiency, and economic performance were not assessed in the present study. Evaluation of these parameters would provide a more comprehensive assessment of the practical value of sulfur and iron nano-fertilizers under commercial production conditions.

The observed responses demonstrate the potential benefits of sulfur and iron nano-fertilizers for improving growth and biochemical characteristics of leek, the present study was conducted under controlled pot conditions at a single location and growing season. Therefore, caution should be exercised when extrapolating these findings to commercial production systems. Further field-scale investigations across diverse environmental conditions are required to validate the consistency and practical applicability of the observed responses.

It should be noted that only a single nano-fertilizer concentration (1 mg L^{-1}) was evaluated in the present study. Therefore, the possibility that lower or higher concentrations may induce different physiological, biochemical, or growth responses cannot be ruled out. Future investigations should examine a wider range of nano-fertilizer concentrations to establish dose–response relationships and determine optimum application rates for leek production. The exact spray volume applied per plant was not recorded and therefore could not be quantitatively reported.

An additional limitation of the study is that the physicochemical properties of the nanoparticles were not independently characterized prior to application. Therefore, the observed plant responses were interpreted based on the manufacturer-reported particle specifications. Future studies should incorporate detailed nanoparticle characterization to better understand the relationship between nanoparticle properties and plant performance.

Several limitations of the present study should be acknowledged. The experiment was conducted under controlled pot conditions at a single location and growing season. In addition, only one nano-fertilizer concentration (1 mg L^{-1}) was evaluated, and conventional sulfur and iron fertilizer treatments were not included in the experimental design. Consequently, the observed responses should be interpreted relative to the untreated control rather than as evidence of superiority over conventional fertilizer sources. Future investigations should incorporate multiple nano-fertilizer concentrations, conventional fertilizer comparisons, and field-scale validation to establish optimum application strategies for leek production.

Although treatment responses exhibited relatively consistent patterns, the experiment was conducted under controlled pot conditions, which may have reduced environmental variability relative to open-field production systems. Future studies should extend these findings by evaluating marketable yield, nutrient-use efficiency, and other agronomic performance indicators under field conditions to better quantify the agricultural benefits of nano-fertilizer application.

Conclusions

The present study demonstrated that sulfur and iron nano-fertilizers positively influenced vegetative growth, chlorophyll accumulation, protein content, soluble sugars, phenolic compounds, flavonoids, and terpenes in leek plants grown under controlled pot conditions. The combined application of sulfur and iron nanoparticles generally exhibited better responses than individual nutrient applications, indicating the beneficial effects of supplying both nutrients simultaneously.

The results further demonstrated that nutrient delivery method influenced plant performance. Foliar application was generally more effective in promoting vegetative growth and chlorophyll accumulation, whereas fertigation favored the accumulation of proteins, soluble sugars, and secondary metabolites. These findings highlight the importance of nutrient application strategy in determining plant physiological and biochemical responses.

The observed improvements in growth and metabolic characteristics suggest that sulfur and iron nano-fertilizers have potential to improve leek performance under controlled conditions. However, the present study was conducted as a pot experiment at a single nano-fertilizer concentration and did not include conventional sulfur or iron fertilizer treatments. Furthermore, agronomic indicators such as marketable yield, nutrient-use efficiency, harvest index, and economic performance were not evaluated.

Therefore, the findings should be considered preliminary and interpreted within the limitations of the experimental design. Future studies should investigate multiple nano-fertilizer concentrations, compare nano-fertilizers with conventional fertilizer sources, and validate the observed responses under field conditions. Additional research should also assess marketable yield, nutrient-use efficiency, and economic feasibility to determine the practical applicability of sulfur and iron nano-fertilizers for leek production.

A limitation of the present study is that environmental variables such as temperature, relative humidity, and solar radiation were not continuously monitored. Therefore, future studies should incorporate detailed environmental measurements to further evaluate the interaction between nano-fertilizer treatments and growing conditions.

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