Journal of Pure and Applied Agriculture (2025) 10(2): 32-43
ISSN (Print) 2617-8672, ISSN (Online) 2617-8680
http://jpaa.aiou.edu.pk/

Evaluating the effect of iron sulphate on growth, yield, and physiological responses in wheat (*Triticum aestivum* L.)

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Abstract

All creatures require iron (Fe) as a micronutrient. Plants often suffer from iron deficiency because iron has a low bioavailability in aerobic, calcareous, or high-pH soils. Wheat is staple food crop of our country, but its growth is often constrained by deficiencies of essential micronutrients, particularly copper, boron and iron, which require external supplementation for optimal plant development. To cope with this problem, a pot experiment was conducted in the Botanical Garden, Institute of Botany, University of the Punjab, Lahore, Pakistan. The experiment was laid out in a completely randomized design (CRD) under factorial layout with 03 replicates of 02 wheat varieties (Pakistan-2013 and Akbar-2019). Ferrous Sulphate ($FeSO_4.7H_2O$) was applied through soil at concentrations of 10, 20, and 30 ppm, along with control were used. During this experiment, different morpho-physiological parameters, such plant height, leaf length, leaf width, leaf area, spike length, number of spikes per pot, number of spikelets per spike and number of leaves per pot, relative water content, total chlorophyll content and total carotenoids content, were noted. To assess the effect of $FeSO_4 \cdot 7H_2O$, antioxidant enzyme matrix activity, such as glutathione peroxidase (GPX) activity, ascorbate peroxidase (APX) activity, and catalase (CAT) activity were also determined. The result of wheat variety Akbar-2019 showed significantly high vegetative growth at 30 ppm while wheat variety, Pakistan-2013, showed the maximum growth at 20 ppm of $FeSO_4 \cdot 7H_2O$. The physiological parameters were maximum at 30 ppm of $FeSO_4 \cdot 7H_2O$ for wheat variety Akbar-2019. Among the antioxidant enzymes, CAT and GPX exhibited maximal activity at 10 ppm $FeSO_4 \cdot 7H_2O$ in the Akbar-2019 variety, while APX reached its highest activity at 20 ppm in Pakistan-13, indicating varietal differences in Fe responsiveness. However, more investigation is required to carry out extended field studies at the molecular level to comprehend the underlying mechanisms.

Keywords: Antioxidant enzyme activity, Ferrous sulphate, Foliar application, Statistical analysis, Wheat

To cite this article: Khair, K. U., Khalid, Z., Ahmad, M., Aqib, M., Ahmed, R., Azhar, H., Jabeen, S., & Ahsan, A. (2025). Evaluating the effect of iron sulphate on growth, yield, and physiological responses in wheat (*Triticum aestivum* L.). *Journal of Pure and Applied Agriculture, 10*(2), 32-43.

Introduction

Wheat (*Triticum aestivum*) is a daily food crop nurtured on more than 220 million hectares worldwide due to its adaptability and excellent yields (Iqbal et al., 2018; Igrejas & Branlard, 2020). Wheat is critical to the global economy, ensuring food security due to its high caloric and protein content (Mehmood et al., 2020; Alamgeer et al., 2022). In Pakistan, yearly wheat production exceeded 26 million tonnes in 2023 (FAO, 2023), a modest increase from prior years. Wheat planting usually begins at the end of October and lasts through December. Wheat is some nutrient-dense food rich in proteins like prolamin, glutenin, globulin, and albumin, all of which are required for the body's growth and development (Batool et al., 2025). Carbohydrates are significant grain component, coupled with sugars such as maltose and sucrose, contributing to its energy role (Baillière et al., 2022). Furthermore, wheat germ is an essential source of lipid (2-4%) that is particularly rich in fatty acids and vitamin E (Marzocchi et al., 2022). Food demand will rise as the world's population grows from 7.7 billion to approximately 9.6 billion by 2050, resulting in a significant need for food crop production worldwide (Zulfiqar et al., 2020). Wheat is a staple crop grown in many nations across the world (Dinsa & Balcha, 2024). Wheat is a primary food source for the growing population, but despite a variety of tactics, the supply is not increasing sufficiently enough to fulfil human demand (Abbas & Shafique, 2019; Safder et al., 2025). Whilst inorganic fertilizers have been used for many decades to enrich fertilization for many crops, their rising prices have led farmers to switch to alternate fertilization methods (Ahmad & Aslam, 2018; Kumar et al., 2020; Jamilah et al., 2024).

Micronutrient insufficiency in humans is widespread, affecting an estimated three billion individuals globally. Zinc

and iron are the most common micronutrient deficiencies, affecting roughly 17 % and 33 % of the global population, respectively, with infants and gravid women in underdeveloped countries (Kassebaum et al., 2014). Every year, around 500,000 children under the age of five die because of zinc and iron deficiency (Black et al., 2014). Zinc and iron deficiency in humans is thought to be caused mainly by high intake of cereal-based meals with low concentrations and poor bioavailability (Garcia et al., 2018). Iron is a vital component of all creatures. Inadequate nutritional Fe intake has significantly contributed to the incidence of Fe deficiency in humans (Connorton & Balk, 2019). Increasing Fe content and bioavailability in food crops is a significant challenge, as around two billion people worldwide are iron deficient, particularly in areas where staple diets rely on cereal crops like wheat. The term "biofortification" refers to the use of agronomic technologies to improve the nutritional quality of food crops. Wheat is a suitable crop for biofortification because it is the principal cereal crop worldwide, accounting for around 50% of daily calorie intake and serving as a basic dietary supply of proteins and micronutrients in many developing nations (Finkelstein et al., 2017).

Iron plays a key role in many plant functions. These functions include energy exchange within the plant, respiration, photosynthesis, chlorophyll production, a part of various enzymes and proteins, and nitrogen fixation (Eskandari, 2011). Although wheat plants need just trace levels of Fe, multiple studies have shown that administering Fe alone or in conjunction with other minerals via foliar spraying improves wheat crop growth and production characteristics (Rawashdeh and Flori, 2015). Iron insufficiency is a major nutritional issue that damages crops, especially those grown on calcareous soils. It results in lower vegetative growth and considerable reductions in production and quality (Abadía et al., 2011). In recent decades, Fe toxicity has become an increasingly common hazard in crop production due to industrialization and increased agricultural and mining activities (Lapaz et al., 2022). For example, frequent Fe mining practices pollute the surrounding soils and aquatic environment, resulting in a decline in the utilisation rate of arable land (da Silva Lopes et al., 2021; Li et al., 2024). However, excess Fe induces Fenton's reaction, which enhances the production and accumulation of reactive oxygen species (ROS), thus causing oxidative damage to plant cells (Lapaz et al., 2022). Research findings indicate that the Fe content in plants is approximately 30–300 μg/g dry weight (DW), and Fe contents > 400 μg/g DW can cause phytotoxicity (Delias et al., 2022; Tisarum et al., 2023).

As the world's population increase and impacts of environmental stress and climate change worsen, agricultural scientists have a daunting task in combating Fe deficiency. As a result, Fe bio-fortification of cereal crops, particularly wheat, shows promise as a lifetime and practical solution for upgrade public health and super scribe nutritional pledge. Supplementation, Fe foliar spray and dietary variety are important strategies for reducing the prevalence of Fe deficiency (Silva et al., 2019). An economical way to raise Fe levels without altering consumers' eating patterns is to fortify staple foods like wheat flour. Populations that consume large amounts of wheat-based goods benefit from bio-fortified wheat (Field et al., 2021). Micronutrients are applied to plants using several methods, including broadcast, foliar feeding, and seed priming. Furthermore, feeding micronutrients through soil and seed priming could help plants through preliminary significant growth stages, resulting in yield increment (Mikula et al., 2024).

Biofortification through agronomic measures are a highly encouraging and cost-effective strategy for beating malnutrition. Zayed et al. (2011) found that foliar spray is more successful than seed priming in rice because the crop responds immediately. Although the same protein families are involved in several Fe and Zn transport stages, their physiological activities differ across plant tissues (Borrill et al., 2014). It is evident from the information above that applying macro and micronutrients, such as iron, is necessary for the growth of wheat crops. To boost crop output and promote soil fertility, these essential nutrients should be applied in the proper amounts. The objective of the current study was to evaluate the effect of different iron sulphate ($FeSO_4 \cdot 7H_2O$) concentrations on various morpho-physiological, enzymatic activity and yield-related parameters of wheat.

Materials and Methods

Experimental setup

Certified wheat seeds were obtained from the registered seed centre of the University of Punjab, Lahore. For the experimental setup, seeds of similar sizes and good health were used. The Botanical Garden at the University of Punjab, Lahore served as the experimental setup for studying the soil application of Fe on wheat. The experimental work fled from November 10, 2021, to March 20, 2022. Before being used in experiments, wheat seeds were surface sterilized for ten minutes in a 5% sodium hypochlorite solution following the method described by Sauer & Burroughs (1986). Three healthy wheat seeds were sown in each 12 cm diameter plastic pot containing 1kg soil. For research, the soil was analysed at the Soil and Water Testing Laboratory and the soil's physiochemical parameters are shown in Table 1. Completely randomized design (CRD) under factorial with three replicates were used to set up the experiment. Three different levels of iron sulphate ($FeSO_4 \cdot 7H_2O$) were used as a treatment: 10, 20, and 30 ppm (designated as T_1, T_2, and T_3, respectively), in addition to control (T_0). Two wheat varieties including Pakistan-13 and Akbar-19 were evaluated under these experimental treatments. The wire house was 28+2 degrees, with enough sunlight for the pots. Regular plant irrigation was carried out.

Table 1 Soil physiochemical evaluation

Sr. No.	Name parameters	Garden soil
1	EC (dS m^{-1})	2.30
2	pH	8.3
3	Organic matter (%)	0.3
4	Saturation	24
5	Texture	Sandy loam
6	Available phosphorous (mg/kg)	62
7	Zn (mg/kg)	11.10
8	Fe (mg/kg)	4.3

Morphological data collection

After twenty-five days following germination, plants were harvested to record morphological data. The plants were meticulously cleaned of dust and debris using deionized water shortly after harvest. The morphological parameters such as plant height (cm), number of leaves per pot, leaf area (cm^2), leaf width (cm), and leaf length (cm) were measured. The plant height and leaf length were measured using a vernier caliper. The number of leaves was calculated by taking a count of leaves in each pot. Wheat leaf area was calculated using the Quarrie and Jones equation:

$$\text{Leaf Area} = \text{Leaflength} \times \text{leaf width} \times 0.75$$

The relative water content (RWC, %) was determined using the Sade et al. (2015) methodology. This involved weighing fresh leaves, immersing them in 10 millilitres of deionized water for a full day, and determining their turgid weights. The sample was dried at 70 °C, and the dry weight was determined using the following formula:

$$\text{RCW \%} = \frac{(\text{Fresh weight} - \text{Dry Weight})}{(\text{Turgid Weight} - \text{Dry Weight}) \times 100}$$

Chloroplast pigments and total soluble protein

Fresh leaves were used to obtain the pigments found in chloroplasts. Each plant's weighed mass of 0.1g leaves was submerged in 10ml of (DMSO) dimethyl sulfoxide in falcon tubes. The leaves were incubated for 48 hours at 25°C in the dark. After collecting the supernatant, the solution was incubated for 48 hours at room temperature in the dark. We then used a spectrophotometer to analyse the extracts at wavelengths of 663nm, 645nm, and 480nm. The carotenoid and chlorophyll contents were measured using Arnon's (1949) and Wellburn's (1994) methods, respectively:

$$\text{Total Chlorophyll Content (mg g}^{-1}\text{ Fresh weight)} = \frac{[(0.00802)(D663)] + [(0.0202)(D645)][\text{ml solvent}]}{\text{Fresh Weight (g)}}$$

The total soluble protein was assessed according to the protocol of Rekowski et al. (2021). The extract's absorbance was measured at 480nm using a Shimadzu UV-1800 spectrophotometer to determine its carotenoid content. Carotenoids were calculated using the Wellborn (1994) formula:

$$\text{Carotenoids Content} = \frac{[(1000 * 480A - 1.29 * \text{Chlorophyll A})(D - 663) - (52.78 * \text{Chlorophyll B})(D - 645)]}{220}$$

Enzymatic antioxidants activity

Weigh the 0.250g of tissue from leaf samples, then fill a mortar to the brim with liquid nitrogen. Pound the tissue with a mortar and pestle until it is ground into a powder and the liquid nitrogen has almost completely evaporated. Add 1 millilitre of the cold extraction solution (150 millimetres of phosphate buffer, pH = 7) to the powdered tissue and mill for a few minutes. If the mortar is too cold from the liquid nitrogen, the ground tissue will freeze once the buffer is added and this needs to be cleared out before grinding. Keep the mortar on ice as you grind centrifuge homogenate for 20 minutes at 12000g (4 times if possible). Catalase (CAT) activity was measured Spectrophotometrically at 240nm monitoring the decomposition of H_2O_2, following the improved UV-kinetic method described by Hadwan et al. (2024). The activity of ascorbate peroxidase (APX) was determined following the method of Nakano and Asada (1981) with minor modifications. The reaction mixture contained 3.75mL of sodium acetate buffer (pH 7.0), 50µL of EDTA, 50µL of hydrogen peroxide (H_2O_2), 50µL of ascorbic acid, and 100µL of the enzyme extract. The decrease in absorbance due to the oxidation of ascorbic acid was recorded at 290nm every 10 seconds for one minute using a Shimadzu UV-1800 spectrophotometer. The rate of decrease in absorbance was used to calculate APX activity, where a change of 0.01 per minute was defined as one unit of enzyme activity. Peroxidase (GPX) activity was determined spectrophotometrically at 470nm following the method of Sakharov and Ardila (1999). The Superoxide dismutase (SOD) activity was determined following the method of Chen and Pan (1996). To measure the

guaiacol peroxidase activity, a reaction mixture including 1.25ml of guaiacol, 263ml of ethanol solution, 2.5ml of H_2O_2, 1.7grams of Hac-NaAc pH 5, and 100µl of extracted enzyme solution was prepared. With a Shimadzu UV-1800 spectrophotometer, the reduction in absorbance was measured at 390nm every 10 seconds for 60 to 80 seconds. A change in absorbance of 0.01 per minute is considered one unit of enzyme activity.

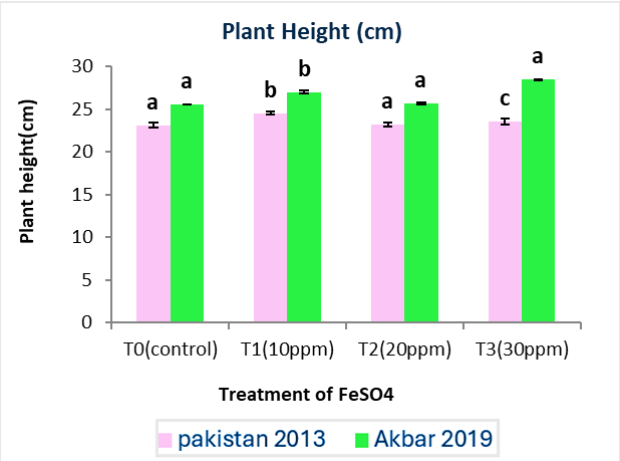
Statistical analysis

The data were analysed statistically using a version of the Statistix (ver. 12.0) software. The data were analysed using Two-way analysis of variance (ANOVA) (Steel et al., 1997) and post-hoc testing to find significant differences ($p<0.05$) between varieties and treatments. The treatments were ranked using Duncan Multiple Range (DMR) Test (Gomez and Gomez, 1984). The data analysis considered replications as random factors, while treatments, verities, and the interaction between treatment and variety were fixed factors.

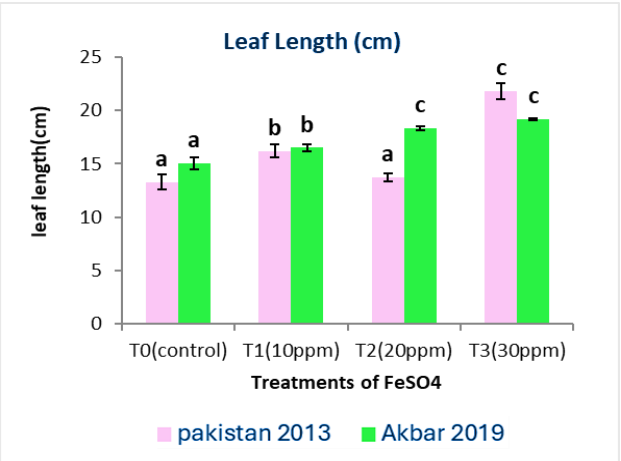
Results

Effect of $FeSO_4 \cdot 7H_2O$ on morphological traits

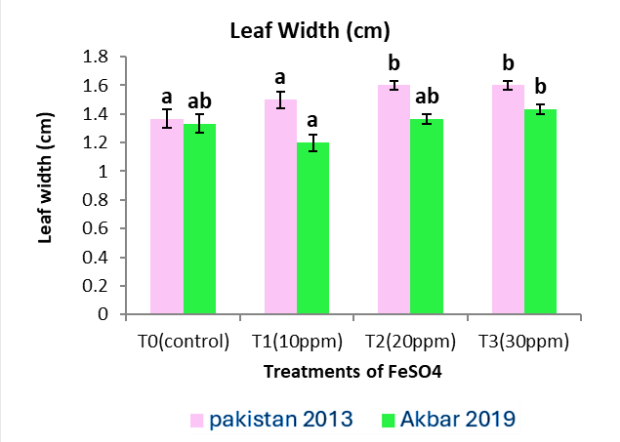
Data revealed the growth parameters in two wheat varieties grown in alkaline soil with a pH of 8.3, at different concentrations of $FeSO_4 \cdot 7H_2O$ (10, 20 and 30 ppm). The current findings showed that all morphological parameters (plant height, leaf length, leaf width leaf area and number of leaf) increased in all cultivars as $FeSO_4 \cdot 7H_2O$ levels increased when compared to the control. Akbar-2019 wheat variety shows maximum growth by the value of (28.46 cm) at 30 ppm of $FeSO_4 \cdot 7H_2O$ as compared to Pakistan-2013 wheat variety which showed maximum growth that is (24.53 cm) at 10 ppm solution of $FeSO_4 \cdot 7H_2O$ (Fig. 1). Plant height (Fig. 1a) is a vital indicator of vegetative growth and overall plant vigour. The application of $FeSO_4 \cdot 7H_2O$ significantly enhances plant height, with the best response at 20 ppm concentration. Taller plants generally capture more sunlight and nutrients, leading to improved productivity. The Akbar variety shows slightly better growth compared to Pakistan. Leaf length (Fig. 1b) determines the photosynthetic efficiency and energy production of the plant. $FeSO_4 \cdot 7H_2O$ treatments increase leaf length, with the maximum observed at 20 ppm. Longer leaves enhance light interception and contribute to biomass accumulation. Akbar consistently shows greater leaf elongation than Pakistan. Leaf width reflects the leaf's surface area available for photosynthesis (Fig.1c). While $FeSO_4 \cdot 7H_2O$ treatment caused only slight changes, higher doses still showed modest improvement. Wider leaves are beneficial for maximizing light capture and gas exchange. Both varieties showed similar patterns, with minimal differences. Leaf area is crucial for determining the total photosynthetic potential of a plant. $FeSO_4 \cdot 7H_2O$ significantly increases leaf area (Fig. 1d), especially at 20 ppm, enhancing growth efficiency. A larger surface area supports greater carbohydrate synthesis and plant vigour. The Akbar variety displayed a stronger response compared to Pakistan. The highest level of $FeSO_4 \cdot 7H_2O$ (30 ppm) also produced significantly high results in all other parameters (spike length (Fig. 1e), spike number, spikelet number, and number of leaves per pot). Additionally, at all $FeSO_4 \cdot 7H_2O$ levels in the alkaline soil, the results showed that Akbar-2019 produced more significant results for the iron treatment than the other variety, Pakistan-2013. On all levels, however, there was no discernible difference between the number of spikes (Fig. 1f) and number of spikelets per spike (Fig. 1g) in Pakistan in 2013 (14 spikelets) and Akbar-19 (13 spikelets). Spike length (Fig. 1e) is directly linked with reproductive success and yield potential. $FeSO_4.7H_2O$ improves spike elongation, with the best outcome observed at 20 ppm. Longer spikes can bear more spikelet, thus increasing grain yield. The Akbar variety performed slightly better under $FeSO_4.7H_2O$ application. The number of spikes per pot indicates the tillering capacity of the crop (Fig. 1f). $FeSO_4.7H_2O$ treatments significantly enhance spike production, especially at 20 ppm. More spikes translate into higher yield potential per plant. Both varieties responded positively, with Pakistan showing greater variability. Number of spikelets per spike determines the grain-bearing ability of the spike (Fig. 1g). $FeSO_4.7H_2O$ treatment boosts spikelet development, with the maximum effect at 20 ppm. More spikelets directly contribute to increased grain yield. Both varieties showed similar improvements, confirming $FeSO_4.7H_2O$ role in reproductive growth. Number per leaves per pot reflects overall vegetative growth and vigour. $FeSO_4.7H_2O$ application enhances leaf production. Maximum number of leaves per pot were observed in Akbar-19 at 10 ppm followed by Pakistan-13 at 20 ppm (Fig. 1h). More leaves increase photosynthetic activity, improving biomass and productivity. The Akbar variety demonstrated stronger vegetative growth compared to Pakistan.


Plant Height (cm)
Plant height(cm)
30
25
20
15
10
5
0
a a b b a a c a
T0(control) T1(10ppm) T2(20ppm) T3(30ppm)
Treatment of FeSO4
pakistan 2013
Akbar 2019

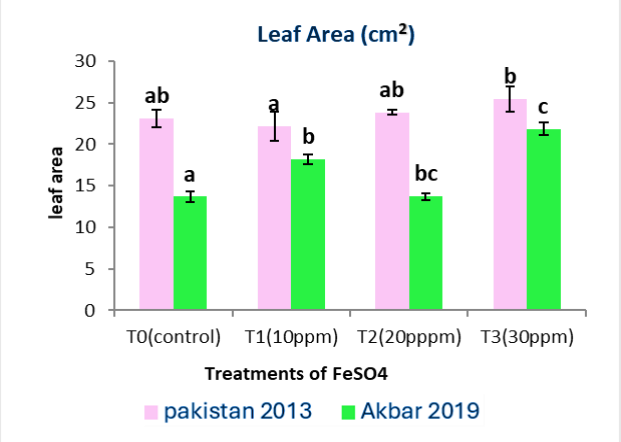
(a)


Leaf Length (cm)
leaf length(cm)
25
20
15
10
5
0
a a b b a c c c
T0(control) T1(10ppm) T2(20ppm) T3(30ppm)
Treatments of FeSO4
pakistan 2013
Akbar 2019

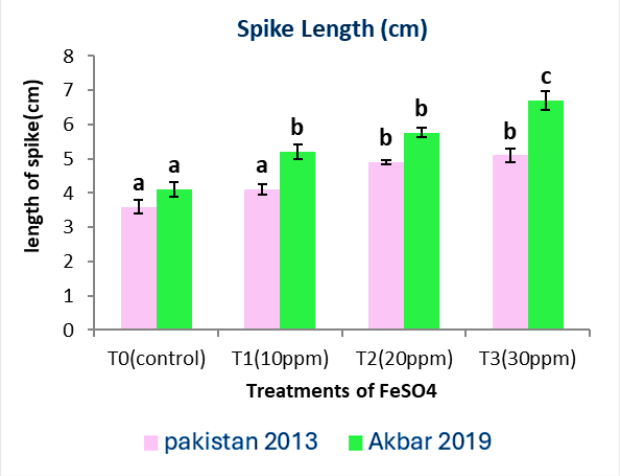
(b)


Leaf Width (cm)
Leaf width (cm)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a ab a a b ab b b
T0(control) T1(10ppm) T2(20ppm) T3(30ppm)
Treatments of FeSO4
pakistan 2013
Akbar 2019

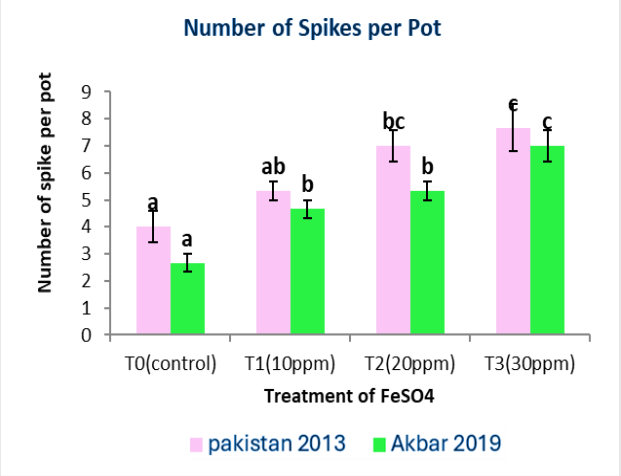
(c)


Leaf Area (cm²)
leaf area
30
25
20
15
10
5
0
ab a a b ab bc b c
T0(control) T1(10ppm) T2(20pppm) T3(30ppm)
Treatments of FeSO4
pakistan 2013
Akbar 2019

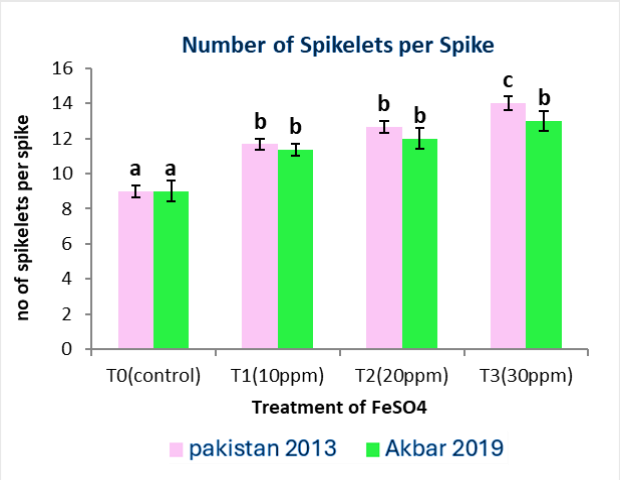
(d)


Spike Length (cm)
length of spike(cm)
8
7
6
5
4
3
2
1
0
a a a b b b b c
T0(control) T1(10ppm) T2(20ppm) T3(30ppm)
Treatments of FeSO4
pakistan 2013
Akbar 2019

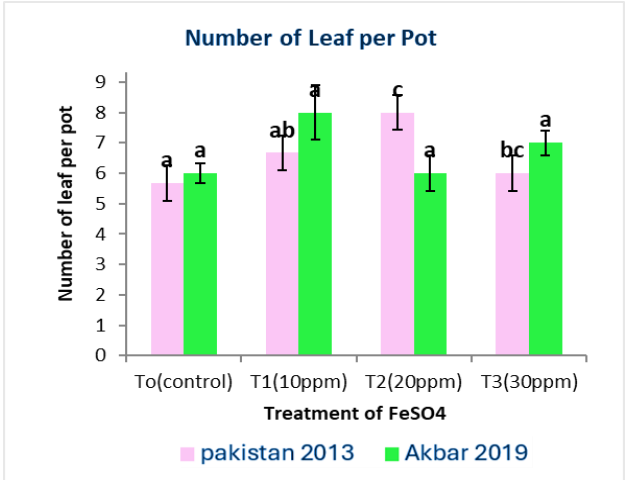
(e)


Number of Spikes per Pot
Number of spike per pot
9
8
7
6
5
4
3
2
1
0
a a ab b bc b c c
T0(control) T1(10ppm) T2(20ppm) T3(30ppm)
Treatment of FeSO4
pakistan 2013
Akbar 2019

(f)



(g)



(h)

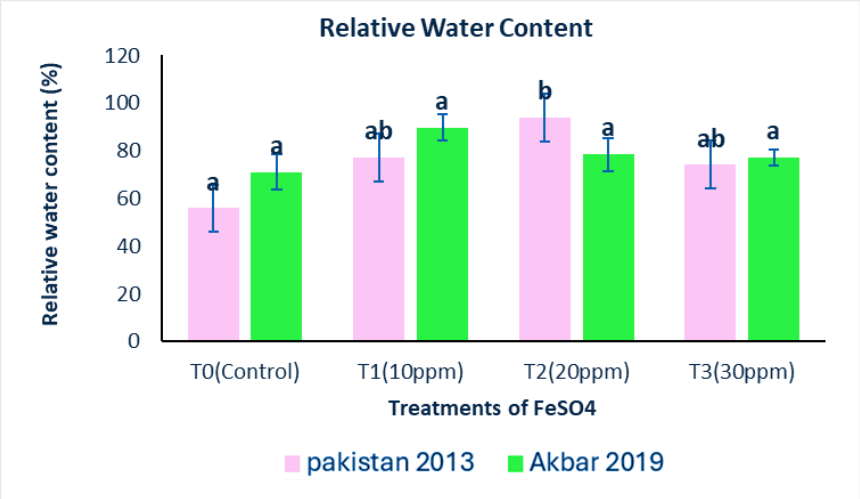
Fig. 1 Impact of varying $FeSO_4 \cdot 7H_2O$ concentrations (0, 10, 20, and 30 ppm) on the morphological traits of *Triticum aestivum* cultivars, specifically the **(a)** plant height **(b)** leaf length **(c)** leaf width **(d)** leaf area **(e)** length of spike **(f)** number of spike per pot **(g)** number of spikelet per spike **(h)** number of leaves per pot

Influence of $FeSO_4 \cdot 7H_2O$ on biochemical traits

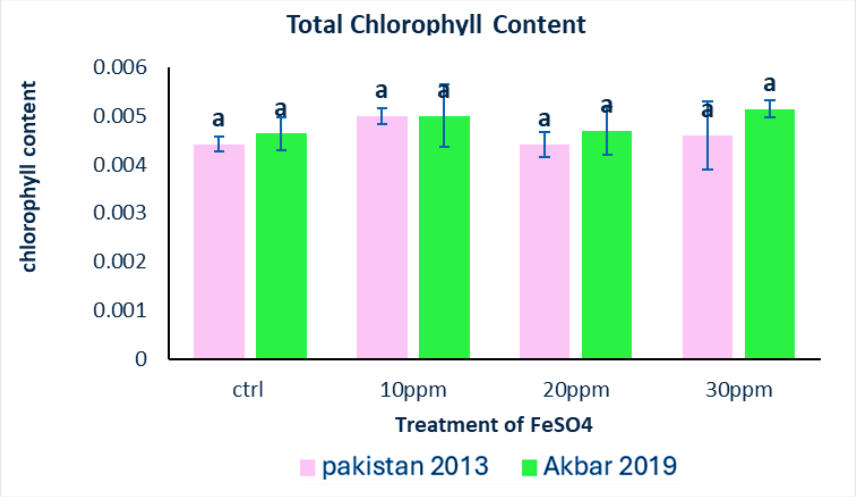
The relative water content, total chlorophyll content, and total carotenoid content of two wheat varieties at different $FeSO_4 \cdot 7H_2O$ concentrations as given in Fig. 2 (a, b, c, respectively). The increase in photosynthetic pigments ($p<0.05$) was induced by increasing $FeSO_4 \cdot 7H_2O$ concertation, according to our findings. In comparison to their relative control, the relative water content peaked at 20 ppm and 10 ppm, with 93.8 and 89.70 in Akbar-2019 and Pakistan-2013, respectively (Fig. 2a). Similarly, among all $FeSO_4 \cdot 7H_2O$ levels, the highest levels of 30 ppm produced significant high carotenoid content in Akbar-2019. At the level of 30 ppm, chlorophyll was increased by (0.00054) in iron-treated plants while in Pakistan-2013 increases were seen at the level of 10 ppm (Fig. 2b). Furthermore, carotenoids in Pakistan-2013 were significantly low in comparison to Akbar (Fig. 2c). The graph (Fig. 2a) shows that relative water content increased with $FeSO_4.7H_2O$ treatment compared to the control, with the highest value observed at 20 ppm. This suggests that moderate Fe supplementation supports better water retention in wheat. Excessive concentration (30ppm) did not improve water content significantly, indicating an optimal range for efficiency. Total chlorophyll content was enhanced by $FeSO_4.7H_2O$ application, with significant improvement at 10 ppm across both cultivars. This indicates that Fe supplementation promotes photosynthetic pigment synthesis, essential for plant growth. However, higher concentration (30 ppm) did not further increase chlorophyll, showing diminishing returns (Fig. 2b). Carotenoid content followed a similar trend, with maximum values at 20 ppm treatment, highlighting Fe's role in pigment accumulation. Carotenoids are vital for photo protection and ant oxidative defence. Excess Fe (30 ppm) reduced content, suggesting potential stress effects at higher doses.

Impact of $FeSO_4 \cdot 7H_2O$ on antioxidant enzymes

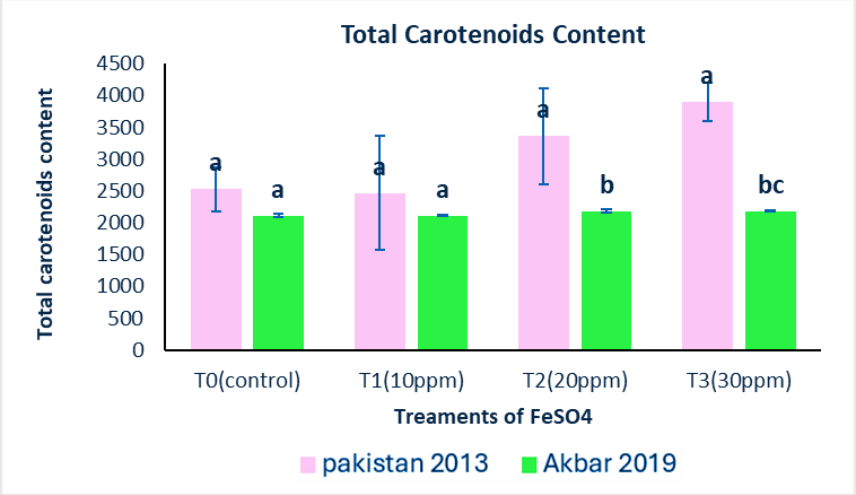
In the current study, we additionally evaluated the antioxidant enzymes in the leaves of all cultivars cultivated in alkaline soil with $FeSO_4 \cdot 7H_2O$. Fig. 3, 4 & 5 depicted the results of antioxidants such as glutathione peroxidase (GPX), catalase (CAT) and ascorbate peroxidase (APX), respectively, extracted from *T. aestivum* cultivar leaves. The study found that adding 10 ppm of $FeSO_4 \cdot 7H_2O$ significantly increased GPX activity in Akbar-2019 leaves by 0.108 (Fig. 3). As GPX activity grew in Akbar-2019, so did all other antioxidant activities, including APX (0.499) and CAT (0.0766) in Pakistan-2013 in leaves at 20 and 30 ppm. Catalase activity was significantly elevated in plants treated with $FeSO_4 \cdot 7H_2O$, particularly at 10 ppm, compared to the control (Fig. 4). This reflects improved oxidative stress management since CAT breaks down harmful hydrogen peroxide. However, excessive $FeSO_4 \cdot 7H_2O$ (30 ppm) led to reduced activity, showing a threshold limit. Glutathione peroxidase activity increased notably with Fe supplementation, peaking at 10 ppm treatment. This demonstrates enhanced detoxification of peroxides, indicating that Fe boosts anti oxidative capacity. A decline at 30 ppm suggests over-supply may impair enzymatic balance. Ascorbate peroxidase activity also improved under Fe treatment, again highest at 20 ppm in Akbar-2019 (Fig. 5). This highlights $FeSO_4 \cdot 7H_2O$ contribution to the ascorbate–glutathione cycle for reactive oxygen species (ROS) detoxification. The activity decreased at 30 ppm, indicating reduced effectiveness beyond the optimal level.



(a)



(b)



(c)

Fig. 2 Effects of varying $FeSO4 \cdot 7H_2O$ concentrations (0, 10, 20, and 30 ppm) on *T. aestivum* (cultivars) photosynthetic pigment **(a)** relative water content, **(b)** total chlorophyll content and **(c)** total carotenoids content

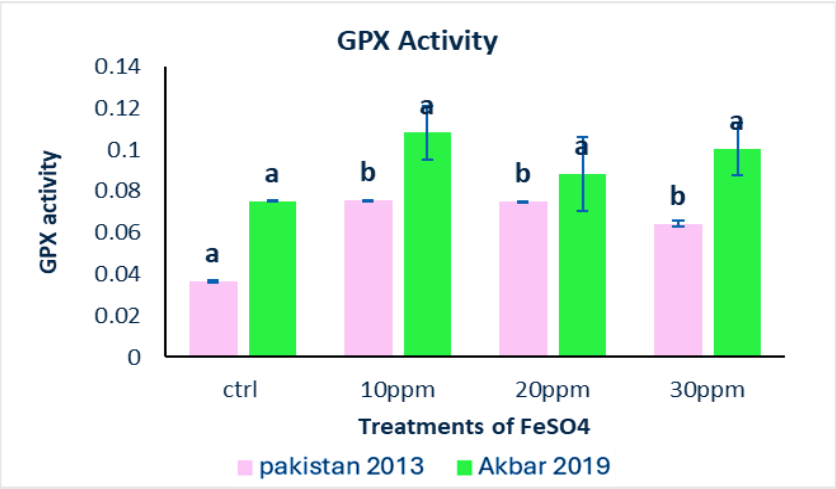


Fig. 3 Impact of varying $FeSO_4 \cdot 7H_2O$ concentrations (0, 10, 20, and 30 ppm) on the antioxidant enzymes. GPX activity found in *T. aestivum* (cultivar) leaves

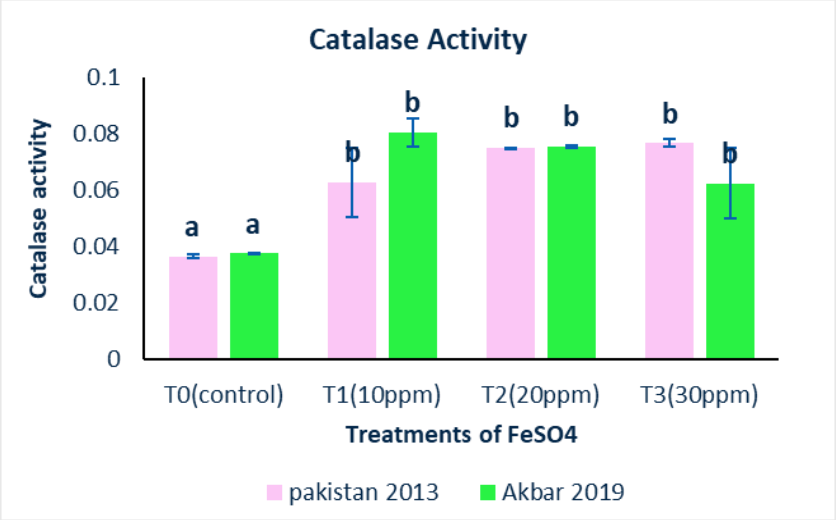


Fig. 4 Impact of varying $FeSO_4 \cdot 7H_2O$ concentrations (0, 10, 20, and 30 ppm) on the catalyse (CAT) activity found in *T. aestivum* (cultivar) leaves

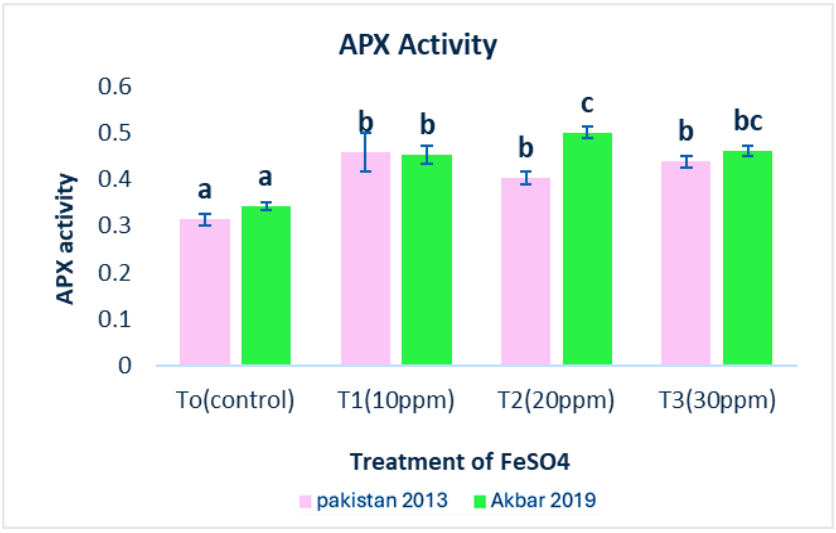


Fig. 5 Impact of varying $FeSO_4 \cdot 7H_2O$ concentrations (0, 10, 20, and 30 ppm) on the APX activity found in *T. aestivum* (cultivar) leaves

Discussion

The present study demonstrated that the application of $FeSO_4 \cdot 7H_2O$ significantly enhanced the morphological and physiological performance of both wheat cultivars, with Akbar-2019 showing superior responses compared to Pakistan-2013. Enhanced leaf length, width, and area at 30 ppm $FeSO_4 \cdot 7H_2O$ in Akbar-2019 and at 20 ppm in Pakistan-2013 indicate varietal differences in iron uptake and utilization efficiency. The reason for this may be that some varieties have a genetic tendency to do better than others in alkaline soils where high pH, free calcium carbonate and bicarbonates make iron bioavailability low (Rengel, 2015; Akhtar et al., 2019). Although these soils bear witness to this fact, that micronutrient deficiencies in these soils are worsening day by day due to the excessive use of macronutrient fertilizers (Salim & Raza, 2020).

In conformity with these previous studies, supplementary iron considerably improved vegetative and reproductive growth traits such as spike length, number of spikes and spikelets per spike (Rawashdeh and Florin, 2015; Zulfiqar et al., 2020). Under calcareous conditions, $FeSO_4 \cdot 7H_2O$ treatment at 30 ppm particularly promoted spike elongation and tiller formation in Akbar-2019, indicating an optimal concentration for soil application under calcareous conditions. Comparable improvements in grain Fe concentration from iron fertilizer app have been made as well reported by Taskin and Gunes (2022) who pointed to $FeSO_4.7H_2O$+urea as the best source for increasing grain Fe content. The positive influence of Fe on photosynthetic pigments further supports its role in chlorophyll biosynthesis and maintenance of chloroplast integrity. Increased chlorophyll a and b contents under Fe treatments align with the observations of Ghafari and Razmjoo (2013), suggesting that Fe facilitates enhanced light capture and energy conversion efficiency. Similarly, carotenoid accumulation, particularly in Akbar-2019 at 30 ppm, indicates improved antioxidative protection of photosystems. Variations between cultivars may reflect differential regulatory control of pigment synthesis under Fe availability (Hussain et al., 2020).

The antioxidant enzyme activities also exhibited significant variation across treatments. Enhanced catalase (CAT), ascorbate peroxidase (APX), and peroxidase (GPX) activities at moderate Fe concentrations indicate strengthened defense mechanisms against oxidative stress induced by Fe imbalance. In Akbar-2019, higher CAT and APX activities at 10–20 ppm suggest efficient detoxification of reactive oxygen species (ROS), while excessive Fe (30 ppm) slightly suppressed enzyme activity possibly due to ROS overaccumulation or enzyme inactivation. These results are consistent with reports showing that Fe amendments, especially in nano or chelated forms, elevate antioxidant defense up to an optimum threshold (Ghafari and Razmjoo, 2013; Rizwan et al., 2019). Overall, the findings confirm that moderate to high $FeSO_4 \cdot 7H_2O$ levels (20–30 ppm) improve growth, pigment composition, and antioxidant activity in wheat, particularly in Akbar-2019, by mitigating Fe deficiency typical of alkaline soils. The observed varietal differences emphasize the need to consider genotype-specific Fe tolerance and uptake capacity when designing biofortification or soil-amelioration strategies. Future studies should integrate molecular and field-scale analyses to elucidate Fe transport, assimilation pathways, and long-term yield responses under variable agro-ecological conditions.

Conclusion

The negative effects of Fe-deficient alkaline soils were reduced by applying $FeSO_4 \cdot 7H_2O$ to the soil. Data showed that adding $FeSO_4 \cdot 7H_2O$ to ferrous deficient alkaline soils promoted plant growth and improved the activities of enzymatic antioxidants, thereby reducing oxidative damage. Compared to other rates, its application at a high concentration of 30 ppm proved to be more effective. Akbar-2019 performed better than the other cultivars among those that were tested. It is crucial to note that excessive Fe levels harm wheat seedlings by interfering with physiological and biochemical functions, decreasing nutrient absorption, and causing cellular damage and slower crop growth. Further research is necessary to clarify the precise mechanisms through which elevated levels of iron have a detrimental impact on crop seedlings. Long-term molecular field studies concentrating on plant growth and Fe uptake patterns are recommended to gain a deeper understanding of the underlying mechanism. In conclusion, our findings showed that applying $FeSO_4 \cdot 7H_2O$ to the soil can effectively address Fe deficiency, improving plant growth and antioxidant activity.

Authors contribution statement: KUK and MAQ contributed to the conceptualization, literature survey, and initial manuscript drafting. MAH and AAH supervised the study, provided critical revisions, and approved the final version for submission. ZK and MAH managed data compilation and prepared figures and tables. RAA contributed to the methodological framework and reviewed agronomic content. HAZ and SJ were responsible for literature organization, formatting, and reference management. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no competing interests

Data availability: The data presented in this study are available from the corresponding authors upon reasonable request.

Funding source: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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