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Iron Fortification of Chickpea through foliar application reduces Iron deficiency and improves grain yield and quality

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Abstract

Chickpea (*Cicer arietinum* L.) is widely grown as a major food legume for its rich nutritional profile and serves as a primary source of protein in developing nations. To overcome hidden hunger linked with Fe deficiency, a field experiment on agronomic biofortification of chickpea through foliar application was conducted at the research farm of MNS-University of Agriculture, Multan, Pakistan, during the Rabi season, 2020-21. The experiment was laid out under Randomized Complete Block Design (RCBD) with three replications. FeSO₄ was used as the Fe source for foliar application; control, water spray, and 0.5% and 1% FeSO₄ were applied. The foliar application of Fe at 1% FeSO₄ resulted in a significant 21% increase in yield and quality attributes, including a 17% increase in grain Fe content, compared with the control. In conclusion, Fe foliar feeding

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of chickpea served as an effective, economical, and easy approach to enrich chickpea with Fe to address hidden hunger linked with Fe deficiency.

Keywords: Micronutrients, Hidden Hunger, Fe deficiency, Yield

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Introduction

Malnutrition is a serious threat to human health, which is affecting billions of people around the world (Webb et al., 2018). Malnutrition refers to any deficiency, excess, or imbalance in food intake and includes both under- and over-nutrition (Ntenda, 2019). Malnutrition is a great concern that is affecting people of all ages in Pakistan. Malnutrition has been a serious problem in countries like Pakistan over the last decade, particularly in protein, vitamin, and mineral (iron) deficiencies (Asim & Nawaz, 2018).

Fe is an important element for humans and plants, required in small quantities (Kobayashi & Nishizawa, 2012). It is essential for a healthy lifestyle as it plays an important role in the normal growth and development of the human body (Beard & Connor, 2003). Iron deficiency is the most common, affecting more than 2 billion people globally (WHO, 2011). Its deficiency has an impact on the immune system. For humans, the recommended daily Fe intake is 8 mg for men and 18 mg for women, and insufficient intake prevents the production of biologically essential molecules, most notably haeme, leading to anemia (Trumbo et al., 2001). As an element, Fe also plays a significant role in crops, as it is vital for many enzymes, including cytochrome, which is involved in chlorophyll synthesis, enzymatic activity, maintenance of chloroplast structure, and the electron transport chain. Fe deficiency in agricultural lands negatively impacts crop productivity and nutritional quality (Cakmak, 2009). In high-pH calcareous soils, Fe deficiency is a common problem. The lack of Fe in soils retard crop growth and results in low crop yield, thereby necessitating the use of remedial procedures to overcome this deficiency (Alvarez-Fernandez et al., 2004).

Fe deficiency can be assessed through artificial supplementation or intake, but it is costly and difficult to manage regularly, especially in underdeveloped countries (Best et al., 2011). Fe deficiency can be alleviated by increasing the bioavailable Fe content in food crops through Fe fortification (Rana et al., 2012). Biofortification is a long-term, effective method for overcoming Fe deficiency in the human race. It is the most cost-effective, sustainable, and feasible strategy for poor people worldwide (Garcia-Banuelos et al., 2014).

Pulses provide important minerals, fiber, and vitamins, in addition to their high protein content. Fe in pulses is non-heme Fe, which has lower bioavailability than heme Fe (Sulaiman et al., 2021). A major challenge in pulses is to increase Fe bioavailability and concentration. Despite being an important protein source in poor countries, pulses such as chickpea have received less attention. After dry beans and peas, chickpea is the world's third most significant pulse crop. Chickpea is a primary source of protein for most people who become vegetarians due to economic constraints or by choice. Although iron foliar application is beneficial, little research has been conducted on the biofortification of pulses, particularly chickpea, to standardize the FeSO₄ foliar dose and compare it with water spray and a control to assess their roles in increasing chickpea yield and quality.

Materials and Methods

The present study was carried out in the Research area of Muhammad Nawaz Shareef University of Agriculture, Multan, during the 2020-21 growing season to fortify

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chickpea with Fe through foliar application of iron sulfate. The study was laid out under a randomized complete block design (RCBD) with three replications and a net plot size of 20 m² (5 m × 4 m). The experiment consisted of a control, in which no treatment was applied; water spray, where plants received a foliar spray of water only; 0.5% FeSO₄, in which plants were treated with a 0.5% ferrous sulfate (FeSO₄) foliar spray; and 1% FeSO₄, where plants received a foliar spray of 1% ferrous sulfate (FeSO₄).

The analytical findings indicated that the experimental field's soil had a sandy loam texture. The seedbed was prepared by plowing, followed by leveling. For optimal growth and development during the growing season, two irrigations were provided at the pre-flowering (45 days) and pod-filling (75 days) stages after sowing. Fertilizers containing phosphorus and nitrogen were applied at the rate of 32 and 85 kg ha⁻¹, respectively. Nitrogen was applied in two splits: the first as a basal dose, and the second at 45 days after sowing. However, all phosphatic fertilizers were applied as a basal dose. Throughout the experiment, all agronomic operations were maintained at normal levels and even. Plant protection methods were implemented to keep the crop free of weeds, diseases, and insects. Weed control was established through manual weeding at early stages. To control gram pod borer, Emamectin was sprayed. Fungicide was applied to control Ascochyta blight.

In this experiment, different concentrations of Fe were applied as FeSO₄ solutions. FeSO₄ was applied to chickpea leaves. For the preparation of 0.5% and 1% foliar solutions of Fe, the required amount of Fe was prepared by dissolving 5g and 10 g of Fe sulfate (as per treatment) in 1 liter of water. All management practices, except for the treatment under study, were kept the same across all experimental units.

Randomly selected plants were used to record plant height, 100-grain weight, chlorophyll contents, and grain yield. After winnowing, the seed yield per plot was weighed. The collected data were then analyzed by using Fisher's analysis of variance. Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at 0.05 probability (Steel et al., 1997) using Statistix 8.1 software.

Weather data

The climate of this region is typically arid, with extreme temperatures in summer and winter. During summer, the temperature may reach as high as 48- 50 °C. The weather data (maximum and minimum temperatures, sunshine hours, and rainfall) for the crop season were obtained from the Agronomy Department, MNS University of Agriculture, Multan. Temperature remained at a minimum during January and gradually increased as the growing season progressed to maturity. In March, rainfall was received, while all other months of the growing season were dry.

Results and Discussion

Plant height

Analysis of Variance showed a significant impact of FeSO₄ on plant height and yield-associated parameters (such as branches per plant, chlorophyll content, pods per plant, number of grains per pod, 100-grain weight, grain yield, and harvest index) and quality parameters (such as grain protein and iron contents) of kabuli chickpea. The tallest plants were observed in plots where 1% FeSO₄ was applied to chickpea foliage, which was statistically at par with 0.5% FeSO₄, whereas the lowest were observed in the water-spray treatment, which was statistically at par with the control (Fig. 1A). Higher plant height

was recorded with the application of Fe, which might be due to the role of Fe in starch formation and protein synthesis (Rout & Sahoo, 2015) as well as maintenance and synthesis of chlorophyll in plants (Masoud et al., 2012). The increase in the availability of Fe to the plant might have stimulated the metabolic and enzymatic activities, thereby increasing the growth and ultimately the height of the plant. These results agree with the findings of Pingoliya et al. (2014), who reported that chickpea plant height increased with the application of Fe.

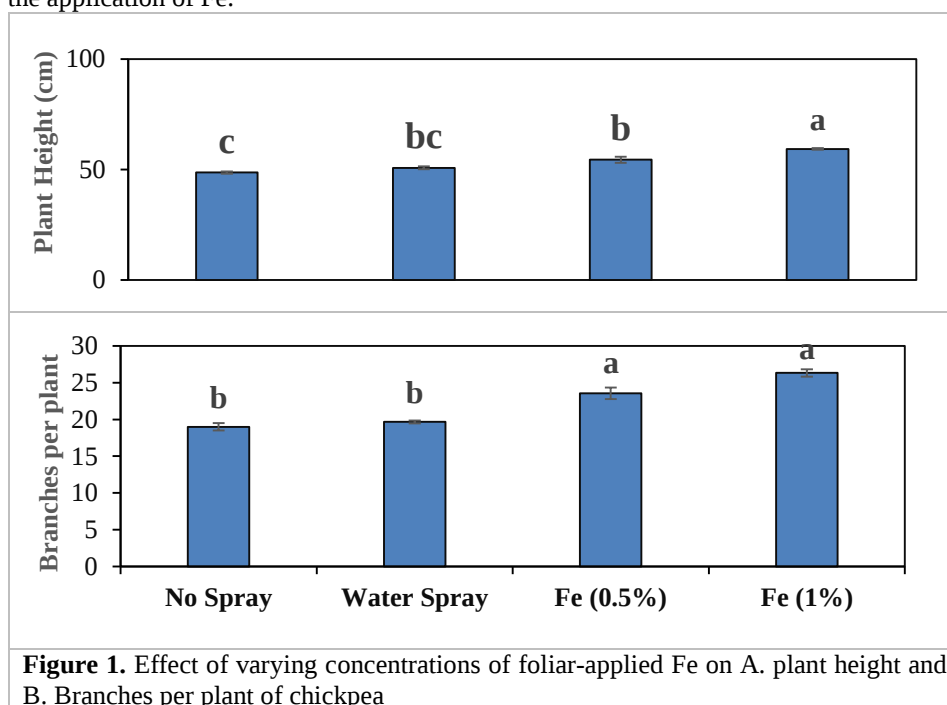


Figure 1. Effect of varying concentrations of foliar-applied Fe on A. plant height and B. Branches per plant of chickpea

Branches per plant

The highest number of branches per plant was observed in plots where chickpea foliage was sprayed with 1% FeSO₄, which was statistically at par with 0.5% FeSO₄. In contrast, the lowest was observed in the water spray, which was statistically at par with the control (Figure 3). A higher number of branches with Fe application might be due to Fe's role in chlorophyll synthesis and chloroplast development (Masoud et al., 2012). Higher availability of Fe to crop plants might have stimulated the enzymatic activities responsible for increased numbers of primary and secondary branches per plant. Patel et al. (2011) reported a higher number of branches plant⁻¹ in cowpea with foliar spray of FeSO₄.

It absorbs solar radiation for photosynthesis and is an important parameter in the regulation of photosynthesis. Fe plays an important role in physiological processes in plants, such as water and nutrient uptake, nucleic acid synthesis, and IAA synthesis (El-Fouly et al., 2011). Fe plays an important role in the synthesis and maintenance of chlorophyll in plants. It has a role in nucleic acid metabolism and activates several enzymes (Kumar et al., 2019).

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The application of Fe increased the chlorophyll content in cowpea (Kumawat et al., 2006) and in soybean (Trivedi et al., 2011).

Chlorophyll content

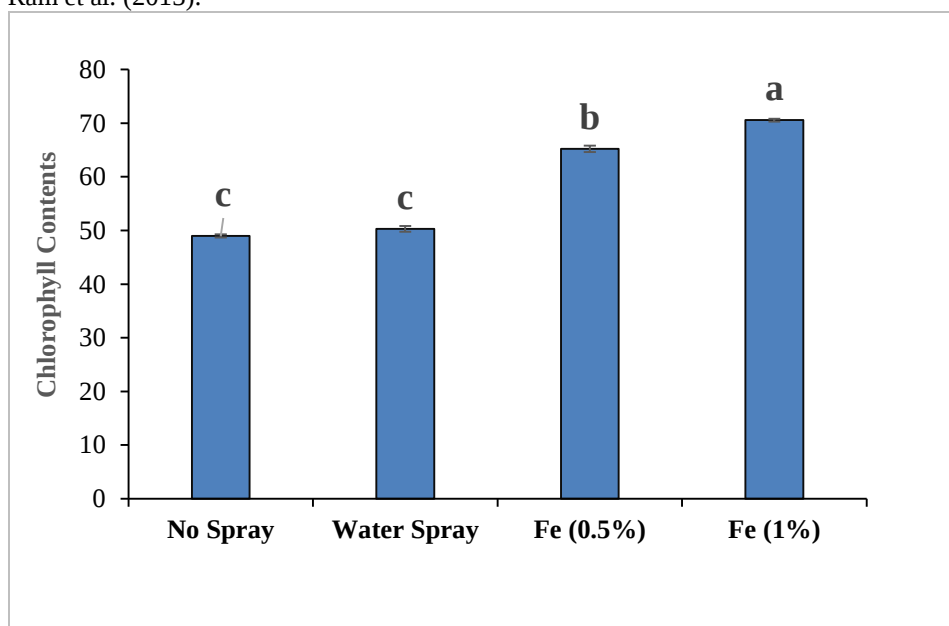
The highest chlorophyll content was observed with FeSO₄ at 1% concentration, which is statistically at par with FeSO₄ at 0.5%. In contrast, the lowest was observed in water spray, which is statistically at par with control (Figure 2A).

Pods per plant

The highest number of pods per plant was observed with FeSO₄ at 1%, while the lowest was observed with water spray, which is statistically at par with the control (Figure 2B). A higher number of pods per plant might be due to foliar applications of Fe, which are readily absorbed by aerial plant parts and transported to growth sites. This stimulates auxin activity at growing points of crops and improves shoot development (Verma et al., 2017).

Grains per pod

The maximum number of grains per pod was observed with FeSO₄ at 1%, while the lowest was observed in the water spray treatment, which was statistically at par with the control (Figure 3A). Grains per plant may be due to a Fe spray, which makes it easier for assimilates to move from the plant to the soil. Similar observations were obtained by Ram et al. (2013).



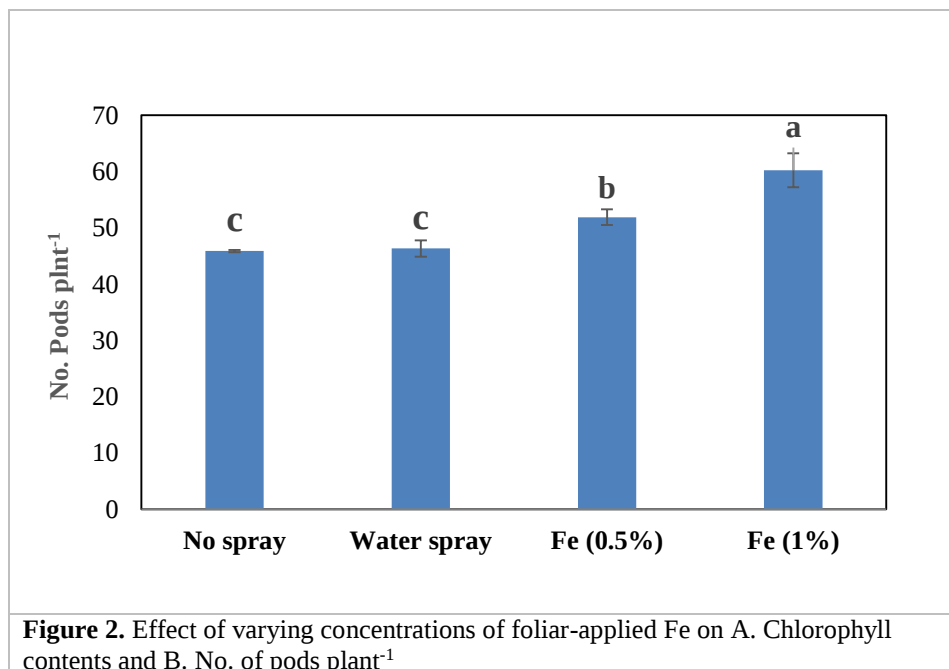
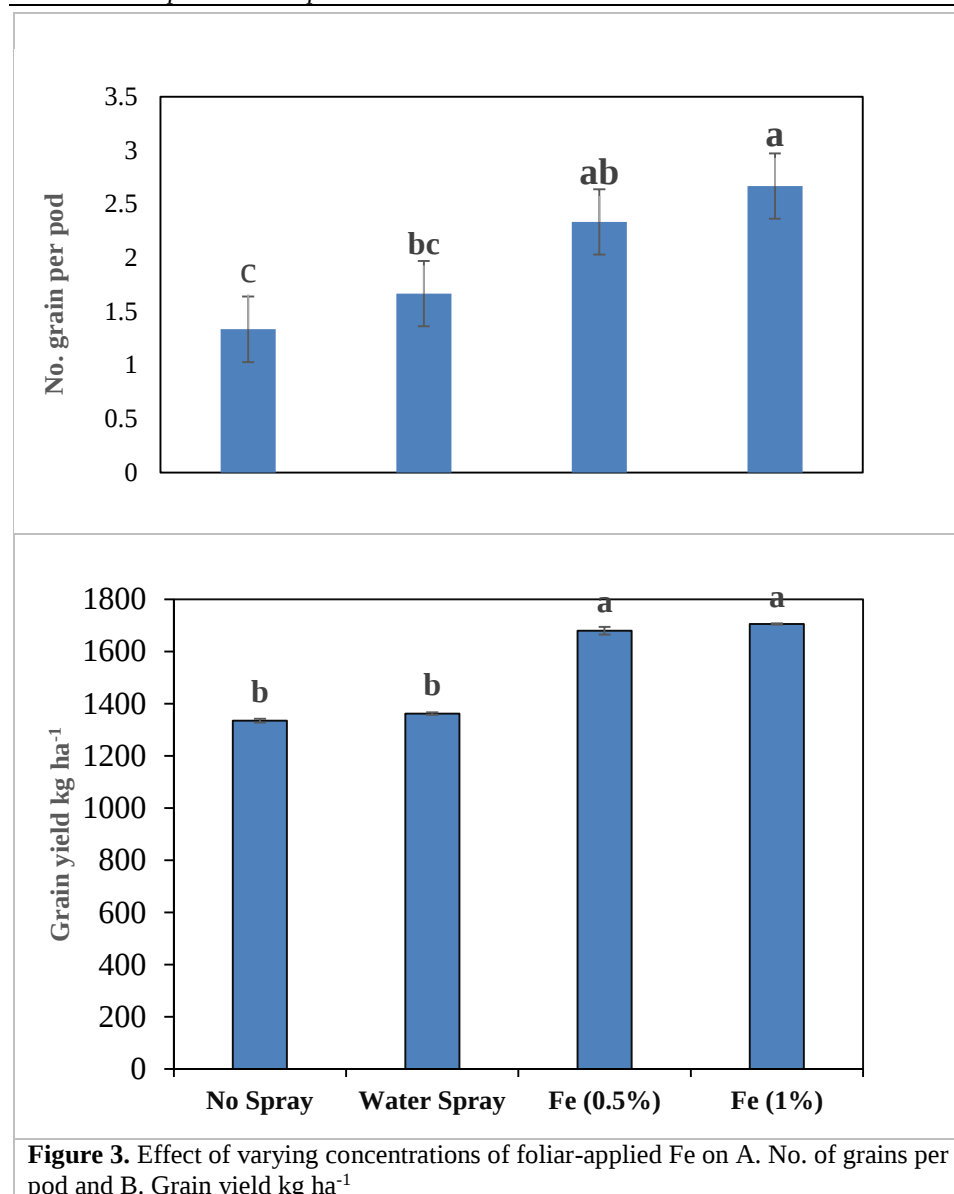


Figure 2. Effect of varying concentrations of foliar-applied Fe on A. Chlorophyll contents and B. No. of pods plant⁻¹

Grain yield (kg ha⁻¹)

The highest grain yield was observed with FeSO₄ at 1% concentration, while the lowest was observed in the water spray treatment, which was statistically at par with the control (Figure 3B). Fe application enhances photosynthesis and promotes higher growth and yield in the crop (Balachandar et al., 2003). It helps achieve a higher photosynthesis rate and dry matter assimilation in the sink, increasing the final yield of the crop. Foliar application of Fe increases photosynthesis and assimilate transport from source to sink, thereby increasing grain and stover yields in mungbean (Saini & Singh, 2017). Similar results were reported in chickpea with soil application of Fe at 5.0 kg ha⁻¹ (Pingoliya et al., 2014) and in cowpea with foliar spray of FeSO₄ at 0.5% at 25 and 45 days (Anitha et al., 2005). Fe is a component of ferredoxin and cytochromes involved in photosynthesis; therefore, an increased grain yield with foliar application of Fe may be due to increased synthesis of carbohydrates and proteins, and their transport to the site of grain formation. Fe is required for the synthesis of growth factors, including proteins, nucleic acids, and auxins, as well as for seed maturation (Rout & Sahoo, 2015). Fe is essential for the production of chlorophyll (Jin et al., 2008). Thus, foliar application of Fe during the flowering and pod formation stages may have accelerated photosynthesis and mobilized photosynthate to sinks, thereby increasing grain yield (Balachandar et al., 2003).

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100 grain weight (g)

The highest value of 100 seed weight was observed in the case of FeSO₄ at 1%, while the lowest was observed in water spray, which is statistically at par with the control (Figure 4A). Fe also helps in absorption of other nutrients (Kuldeep et al., 2017). Higher nitrogen fixation rates and nutrient absorption of other ions might have increased photosynthetic efficiency, which helped increase carbohydrate loading to the sink and was

reflected in higher 100-grain weight. Similar findings were reported by Sharma et al. (2010).

Harvest index (%)

The highest harvest index was observed with FeSO₄ at 1%, while the lowest was observed with water spray, which was not statistically different from the control (Figure 4B). Harvest index is due to higher accumulation of photosynthates and translocation of nutrients to the economic part of the crop. Similar results were observed by Nandan et al. (2018).

Protein content (%)

The highest grain protein content was observed with FeSO₄ at 1%, while the lowest was observed with water spray, which is statistically at par with the control (Figure 4C). Higher Protein content might be due to the stimulating effect of foliar Fe spray on several dehydrogenase, proteinase, and peptidase enzymes, as Fe is an important constituent of these enzymes and in the synthesis of many amino acids. Similar findings were also reported by Wasmatkar et al. (2002).

Grain Fe content (mg kg⁻¹)

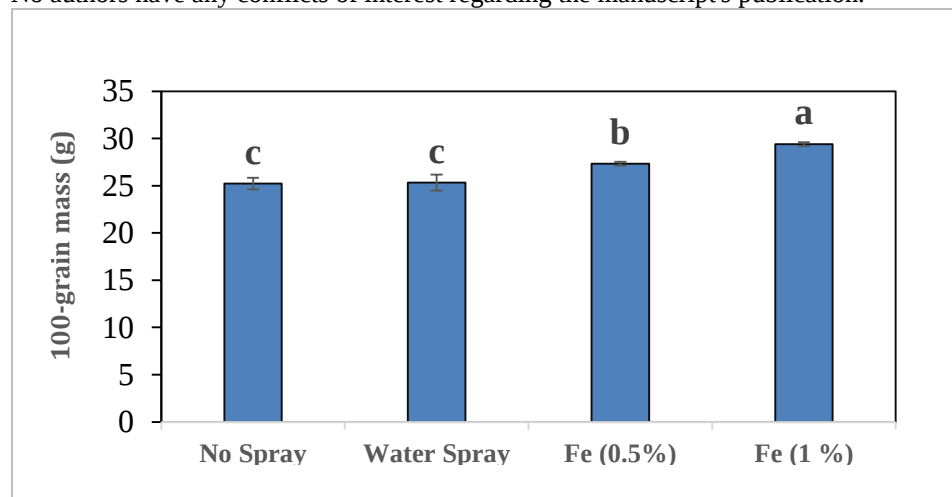
The highest value of grain protein content was observed in the case of FeSO₄ at 1%, while the lowest was observed in water spray, which is statistically at par with the control (Figure 4D). Fe content in chickpeas was higher after foliar application of 1% FeSO₄ than in other treatments. The most efficient method for increasing nutrient concentrations in plants' edible parts, such as grains, is thought to be foliar nutrient spraying.

Conclusion

Iron foliar application increased grain Fe because of biofortification. Moreover, farmers can obtain a 27% increase in grain yield over no spray, with better quality of chickpea grains through agronomic biofortification of 1% FeSO₄ foliar spray.

Conflict of Interests

No authors have any conflicts of interest regarding the manuscript's publication.



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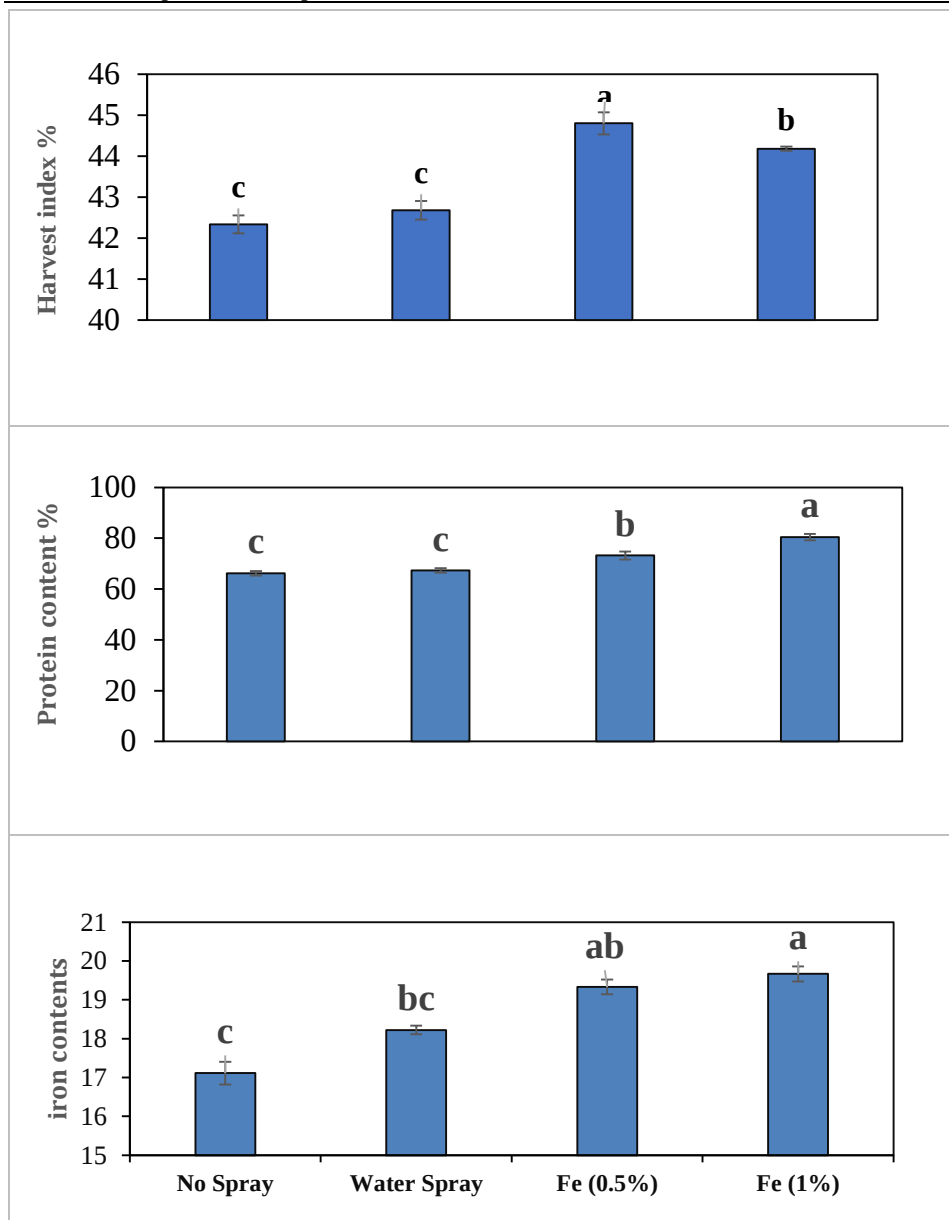


Figure 4. Effect of varying concentrations of foliar-applied Fe on A. 100-grain mass, B. Harvest index, C. Protein content, and D. Iron contents

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