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Zinc nutrition optimization for better cotton productivity on alkaline calcareous soil

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Abstract

Background Zinc (Zn), being the most deficient micronutrient, can largely limit plant growth and development on alkaline calcareous soil. Crop species and varieties within species differently require Zn for optimum productivity. The current study aimed to optimize Zn level and mode of application for better growth, yield, and fiber quality of cotton (*Gossypium hirsutum* L.). The experimental plan comprised a control group with no Zn application, three Zn levels through soil application, i.e. 5 $mg \cdot kg^{-1}$ (SZn5), 10 $mg \cdot kg^{-1}$ (SZn10), and 15 $mg \cdot kg^{-1}$ (SZn15), two levels of foliar application including 0.5% (FZn0.5) and 1% (FZn1) Zn solution, and various combinations of soil plus foliar application. Two cotton cultivars, CIM-663 (Bt) and Cyto-124 (non-Bt) were used, and each treatment was replicated thrice.

Results Zinc nutrition caused a significant ($P \leq 0.05$) improvement in growth, yield, physiological, and fiber quality characteristics of both cotton cultivars. All levels and modes of Zn application were found effective in improving cotton productivity on alkaline calcareous soil. However, integrated soil application and foliar spray showed superiority over sole soil or foliar application. Among different treatments, SZn15 + FZn1 caused the highest improvement in most of the observed growth and yield traits. The said treatment maximally increased the leaf Zn concentration by 270.5% and 218.4% with a subsequent increase in plant height 23.2% and 28.0%, monopodial branches 40.7% and 42.1%, sympodial branches 37.2% and 35.2%, seed cotton yield 32.5% and 36.6%, and lint yield 30.0% and 34.6% in CIM-663 and Cyto-124, respectively, compared with the control. SZn15 + FZn1 also caused the highest increase in relative water contents 32.6% and 22.4%, chlorophyll contents 92.0% and 67.1%, and stomatal conductance 112.8% and 100.8% in CIM-663 and Cyto-124, respectively, compared with the control. Among the fiber quality characteristics, fiber fineness was maximally improved by 19.7% and 15.9% in CIM-663 and Cyto-124, respectively, with SZn15 + FZn1 compared with the control. Leaf Zn concentration was positively correlated with fiber length ($R^2 = 0.717\ 3$), fiber strength ($R^2 = 0.548\ 3$), and fiber fineness ($R^2 = 0.637\ 9$) of both cotton cultivars grown with different levels and application modes of Zn. The benefit-cost ratio was remarkably improved with Zn nutrition, and the highest value of 1.64 was found in CIM-663 at SZn10 + FZn1 and SZn15 + FZn1.

Conclusion The plant growth, physiological, yield, and fiber quality characteristics of cotton cultivars were significantly improved with Zn supply at different levels and modes of application. SZn15 + FZN1 could be recommended to get optimum seed cotton yield and fiber quality of cotton on alkaline calcareous soil.

Keywords Boll weight, Chlorophyll, Fiber quality, Foliar spray, Monopodial branches, Photosynthesis, Seed cotton yield, Sympodial branches

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Background

Cotton (*Gossypium hirsutum* L.) being a major cash crop contributes largely to global economic development and poverty alleviation (Meyer et al. 2024). It has been cultivated globally on an area of 33.55 million hectares with the production of 126.5 million bales during the year 2022–2023 (Food and Agriculture Organization (FAO) 2023). It is the most consumed natural fiber and contributes about 1/4th of total fibers processed in the global textile sector (Hameed et al. 2023). Cotton cultivation contributes significantly to poverty alleviation by providing subsistence to around 100 million households in 75 different countries, and 90% of these are low-income people (Voora et al. 2023). In addition, about 350 million people are involved in the process of cotton ginning, baling, transportation, and storage (Meier et al. 2021). Cotton is a major source of raw materials for the textile industry, and employs about 25 million people worldwide (Khan et al. 2020). In Pakistan, it is grown on an area of 2.42 million hectares with the production of 10.22 million bales in 2023. It contributes about 8.2% to the agriculture sector, and 3.2% to the national income of Pakistan while its share in GDP is around 10% and foreign exchange is about 60% (Pakistan Economic Survey 2024).

Pakistan occupies a prominent position in the world with respect to cotton cultivation and export (Farooq 2024). Average seed cotton yield in Pakistan is far below its potential which might be associated with inadequate plant nutrition, extreme climate, water shortage, and poor plant protection measures (Rana et al. 2020; Ashraf et al. 2024). Adequate plant nutrition is very important for the optimum growth, yield, and fiber quality of cotton because nutrients are required for all growth and yield processes, leading to higher cotton yield and fine fiber quality (Anik et al. 2023). The nutrient management strategy for cotton should comprise the regular addition of macronutrients such as nitrogen (N), phosphorus (P), and potassium (K), and micronutrients including zinc (Zn), iron (Fe), and boron (B) (Ahmed et al. 2020; Abbas et al. 2021).

Zinc, a bluish-white metal, is the 23rd largest element, constituting about 0.02% of the earth's crust (Hussain et al. 2022). Being an essential element, Zn is involved in different growth, metabolic, and reproductive phases of cotton (Anik et al. 2023). It is the structural and functional component of more than 300 enzymes that involved in various metabolic processes in cotton plants (Wang et al. 2020). Zinc is known to affect plant growth and development through its involvement in seed germination (Li et al. 2004), water relations and water use efficiency (Anik et al. 2023), osmolytes synthesis (Hatam et al. 2020), stomatal regulation (Sawar et al. 2022), chlorophyll synthesis and photosynthetic efficiency (More et al. 2018), antioxidant defense system (Cakmak 2000), protein metabolism (Sawan et al. 2001; Lacerda et al. 2018), membrane stabilization (Ghanepour et al. 2015), and reproductive development (Sadeghzadeh 2013).

The low Zn addition coupled with poor utilization efficiency, particularly on alkaline-calcareous soils, might be an important factor responsible for low cotton productivity in Pakistan and many other countries (Najafi-Ghiri et al. 2013; Kulkarni et al. 2020). The problem of poor Zn utilization efficiency can be overcome by foliar application of Zn on alkaline calcareous soils (Doolette et al. 2018). According to Ahmed et al. (2020), foliar application may be more efficient for improving cotton growth and yield on soils having high Zn adsorption capacity. However, a high evaporation rate under warm climatic conditions not only reduces the Zn utilization efficiency of foliar application but also cause its toxicity (Hussein et al. 2018). On the other hand, Hatam et al. (2020) preferred soil application over foliar spray for improving plant growth as it ensured long and continuous Zn supply. Raj et al. (2019) reported that seed treatment with Zn might be an economical and efficient method for improving the growth and yield of cotton.

Adequate regulation of Zn is critical for the optimum growth, yield, and fiber quality of cotton (Cecen et al. 2022). However, Bt and non-Bt cotton cultivars may respond differently to various levels and modes of Zn application depending upon genetic makeup, internal Zn requirements, yield potential, and environmental conditions (Abbas et al. 2020; Sarwar et al. 2021). The current study was planned to optimize the level and mode of Zn application for improving the growth, yield, and fiber quality of CIM-663 (Bt) and Cyto-124 (non-Bt) cotton cultivars under alkaline calcareous conditions. The study was based on the hypothesis that a higher level of Zn through soil addition plus foliar spray pattern would be more efficient to get better cotton productivity on alkaline calcareous soil.

Materials and methods

Description of experimental site

A pot experiment was done in the warehouse having GPS coordinates 30.10° N latitude, 71.25° E longitude, and 128.3 m altitude. During the experimental period, the minimum monthly temperature ranged from 18.5–28.3 °C while the maximum was 35.8–44.6 °C. The average monthly rainfall was 14.5–26.4 mm and evapotranspiration was 3.4–8.9 mm. The wind speed was recorded in the range of 0.79–2.69 $m \cdot s^{-1}$ and sunshine 8.4–10.6 $h \cdot d^{-1}$. The experimental soil was Loamy Hyperthermic Typic Torricalcids. The soil was collected from the cultivated field at a depth of 0–10 cm under a cotton-wheat cropping system. The soil was analyzed for various physico-chemical properties using standard procedures, and presented in Table 1.

Experimental plan

The experimental plan comprised twelve treatments, i.e., control (No Zn application); SZn5: Zn at 5 mg·kg^{-1} through soil application; SZn10: Zn at 10 mg·kg^{-1} through soil application; SZn15: Zn at 15 mg·kg^{-1} through soil application; FZn0.5: 0.5% Zn solution foliar spray; FZn1: 1.0% Zn solution foliar spray; SZn5 + FZn0.5: Zn at 5 mg·kg^{-1} through soil application plus 0.5% Zn solution foliar spray; SZn5 + FZn1: Zn at 5 mg·kg^{-1} through soil application plus 1% Zn solution foliar spray; SZn10 + FZn0.5: Zn at 10 mg·kg^{-1} through soil application plus 0.5% Zn solution foliar spray; SZn10 + FZn1: Zn at 10 mg·kg^{-1} through soil application plus 1% Zn solution foliar spray; SZn15 + FZn0.5: Zn at 15 mg·kg^{-1} through soil application plus 0.5% Zn solution foliar spray; SZn15 + FZn1: Zn at 15 mg·kg^{-1} through soil application plus 1% Zn solution foliar spray. Zinc sulfate ($ZnSO_4$) was used as a Zn source. Soil application of Zn was done before sowing by incorporating the required amount of $ZnSO_4$ according to the treatment plan. The foliar spray was made at 25 and 50 days after seedling emergence using 30 mL solution for each plant per spray. The experiment was planned according to a completely randomized design. Each treatment was replicated thrice. Each replication consisted of one pot having two plants.

Crop sowing and growth conditions

The pots were lined with polythene sheets, and 20 kg of soil was filled in each pot. Two cotton cultivars CIM-663 (Bt) and Cyto-124 (non-Bt) developed by Central Cotton Research Institute, Multan, Pakistan were used in the experiment. Eight seeds were sown in each pot. After two weeks of seedling emergence, plants were thinned to one plant per pot. Fertilizers were applied at N 80 mg·kg^{-1} as urea, P_2O_5 50 mg·kg^{-1} as single superphosphate, and K_2O 50 mg·kg^{-1} as potassium sulfate. The whole of P_2O_5, K_2O, and 1/3 N were added before sowing while remaining N at 30 and 60 days after seedling emergence in two equal splits. To protect the plants against different insects and pests, Novastar, Bifenthrin, Acephate, and Pyriproxyfen were sprayed after pest scouting.

Physiological characteristics

Physiological characteristics of both cotton cultivars in terms of chlorophyll contents, relative water content (RWC), and membrane stability index (MSI) were measured at 60 days after seedling emergence. Chlorophyll contents were determined using the 3rd topmost leaf of each plant in accordance with the method of Arnon (1949) using a spectrophotometer (DU730 UV–Vis Spectrophotometer, Beckman Coulter, USA). Leaf RWC was also measured using the 3rd topmost leaves of both cotton cultivars. After measuring fresh weight (FW), leaf segments were soaked in distilled water for 4 h, and reweighed to measure turgid weight (TW). After recording TW, leaf segments were air-dried and then oven-dried at 70 °C for constant weight in an oven (EYELA WFO-600ND, Tokyo Rikaikai Co., Ltd, Tokyo, Japan) to record oven-dried weight (DW). The equation proposed by Barrs et al. (1962) was used to calculate RWC.

$$\text{Leaf RWC} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

Table 1 Pre-sowing physico-chemical characteristics of experimental soil

Soil characteristic	Type/values	Reference
Soil textural class	Loam	Gee et al. (1986)
pH	8.09	Bigham (1996)
Electrical conductivity /(dS·m $^{-1}$)	1.10	Bigham (1996)
Sodium adsorption ratio /(mmol·L^{-1})$^{1/2}$	6.42	Richards (1953)
Cation exchange capacity /(Cmol (+) kg $^{-1}$)	11.92	Thomas (1982)
Calcium carbonate /%	10.58	Black (1965)
Organic matter /%	0.61	Nelson et al. (1982)
Saturation percentage /%	29.42	Wilcox (1951)
Total nitrogen /(g·kg $^{-1}$)	0.052	Nelson et al. (1982)
Available phosphorus /(mg·kg $^{-1}$)	7.82	Olsen et al. (1954)
Extractable potassium /(mg·kg $^{-1}$)	156.00	Soltanpour et al. (1979)
DTPA extractable zinc /(mg·kg $^{-1}$)	0.29	Lindsay et al. (1978)

For the determination of MSI, the leaf was cut into small pieces and put into two sets of test tubes, each having a 100 mg leaf sample and 10 mL distilled water. One set of test tubes was heated for 30 min at 40 °C in a water bath (DIN 12876–3-K1, Memmert GmbH & Co. KG-Schwabach, Germany), and EC of suspension was measured as C1 using an EC meter (CM183EC-TDS analyzer, Elico, India). The 2nd set of test tubes was heated at 100 °C for 20 min in a water bath, and EC was recorded as C2. Following formula was used to measure MSI in accordance with Blum et al. (1981).

$$\mathrm{MSI} = [1 - (\mathrm{C1}/\mathrm{C2})] \times 100$$

Gas exchange characteristics
The determination for gas exchange characteristics in terms of photosynthetic rate, transpiration rate, and stomatal conductance was made on the fully expanded 4th topmost leaf of each plant using an open system portable infrared gas analyzer (CI–340 hand–held Photosynthesis meter, ADC Bioscientific, Hoddesdon, UK) during a sunny day at 10 am to 11 am.

Leaf zinc concentration
Sixty days after seedling emergence, leaf samples were collected from each plant, and washed with distilled water. The leaf samples were first air-dried and then oven-dried at 70 °C for 48 h in an oven (SLN 32, POL-EKO-APARATURA, Poland). After oven drying, leaf samples were ground to 40 mesh using a stainless steel plant grinder (MF 10 IKA–WERKE, GMBH & Co. KG, Germany). The ground leaf samples (0.5 g) were digested in a di-acid mixture of HNO_3 and $HClO_4$ (2:1, v/v) at 250 °C using a hot plate (CE Hot Plate SH-5A/SH-5C, Faithful, China) according to the method described by Miller (1998). Zinc concentration was measured using an atomic absorption spectrophotometer (Z-8200 Polarized Zeeman AAS, Hitachi, Japan).

Growth, yield, and quality characteristics
At maturity, plant growth and yield characteristics in terms of plant height, monopodial branches per plant, and sympodial branches per plant were recorded. Plant height was measured with a meter rod while, branches were counted manually. The yield attributes including the number of bolls per plant were counted manually, boll size was measured with the help of the vernier caliper. Seed cotton yield was measured after picking while lint yield after ginning. Fiber length was measured using a Fibrograph (ASTM 1994a), fiber strength was measured by a Pressley Fiber Bundle Tester (ASTM 1994b), and fiber fineness was measured with a Micronaire Tester (ASTM 1994c).

Statistical analysis
The effect of treatments on growth, physiological, yield, and fiber quality characteristics of both cotton cultivars were determined by ANOVA on Statistics 8.1 software (IBM, USA). Treatment effectiveness was determined by means, and the differences were measured by the least significant difference test at a 5% probability level (Steel et al. 1997).

Results
Cotton productivity in terms of growth, yield, physiological, gas exchange, and fiber quality characteristics of CIM-663 and Cyto-124 were significantly ($P \leq 0.05$) improved with varying levels and modes of Zn application. When comparing among different treatments, SZn15 + FZn1 maximally improved most of the observed traits of both cotton cultivars. While, application modes were ranked in order of integrated use of soil plus foliar application > soil application > foliar spray.

Physiological attributes
Physiological attributes of cotton such as chlorophyll contents, RWC, and MSI were significantly ($P \leq 0.05$) improved with different levels and modes of Zn application (Table 2). Chlorophyll contents were maximally improved by 92.1% in CIM-663 and 67.1% in Cyto-124 at SZn15 + FZn1 compared with the control. The highest RWC in both cotton cultivars was found at SZn15 + FZn1. This set of treatments also increased the RWC by 32.6% in CIM-663 and 22.4% in Cyto-124 compared with the control. The membrane stability index was increased maximally by 34.5% in CIM-663 at SZn15 + FZn0.5, and 21.2% in Cyto-124 at SZn15 + FZn1 compared with the control.

Gas exchange characteristics
The highest photosynthetic rate in both cotton cultivars was observed at SZn10 + FZn1 where the improvement was 92.6% in CIM-663 and 72.0% in Cyto-124 compared with the control (Table 3). The highest transpiration rate was observed in both cotton cultivars at SZn15 + FZn1. The said set of treatments increased the transpiration rate by 69.9% in CIM-663 and 50.2% in Cyto-124 compared with the control (Table 3). The highest increase in stomatal conductance of 112.8% was observed in CIM-663 at SZn15 + FZn1 while, 100.8% in Cyto-124 at SZn10 + FZn1 compared with the control (Table 3). CIM-663 showed relatively higher photosynthesis, transpiration, and stomatal conductance compared with Cyto-124 regardless of treatments.

Table 2 Physiological characteristics of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions

Treatments	Chlorophyll contents in FW /(mg·g^{-1})		Membrane stability index /%		Relative water content /%	
	CIM-663	Cyto-124	CIM-663	Cyto-124	CIM-663	Cyto-124
Control	0.63f	0.70e	58.00f	57.10f	61.29ef	59.80f
SZn5	0.78de	0.81d	62.22de	60.42e	69.13cd	65.29de
SZn10	0.86d	0.92c	64.18d	63.34de	71.54c	67.52d
SZn15	0.97c	1.07b	65.44d	64.19d	73.18bc	70.80c
FZn0.5	1.10b	0.83d	59.15ef	58.25f	67.23d	61.91e
FZn1	0.71e	0.88cd	62.80de	60.10e	68.14cd	63.65e
SZn5 + FZn0.5	0.76de	0.93c	68.90c	63.37de	68.21cd	65.19de
SZn5 + FZn1	0.90cd	1.01bc	73.28b	64.12d	69.34abcd	66.18d
SZn10 + FZn0.5	1.04b	1.10b	77.86a	65.24d	74.47bc	67.33d
SZn10 + FZn1	1.13ab	1.16a	77.68a	65.28d	76.36b	68.85cd
SZn15 + FZn0.5	1.11ab	1.13ab	78.00a	66.26cd	78.17ab	71.67c
SZn15 + FZn1	1.21a	1.17a	68.13c	69.18c	81.28a	73.18bc

Values are means of three replicates ($n=3$). Values with the same letters in a column do not differ significantly at $P \leq 0.05$

Table 3 Gas exchange characteristics of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions

Treatments	Photosynthetic rate /(μmol CO_2·m^{-2}·s^{-1})		Stomatal conductance /(mol·m^{-2}·s^{-1})		Transpiration rate /(mg H_2O·m^{-2}·s^{-1})	
	CIM-663	Cyto-124	CIM-663	Cyto-124	CIM-663	Cyto-124
Control	5.93fg	5.78g	0.133de	0.126e	3.36de	3.29e
SZn5	6.97ef	6.37f	0.163d	0.153d	4.17cd	3.78d
SZn10	8.52d	6.70f	0.193c	0.183c	4.68bc	4.27c
SZn15	9.29c	8.10de	0.197c	0.190c	5.57a	4.29c
FZn0.5	6.74ef	6.17fg	0.156d	0.153d	4.17cd	4.10cd
FZn1	7.70de	6.98ef	0.173cd	0.213bc	4.60bc	4.46c
SZn5 + FZn0.5	8.78cd	7.36e	0.193c	0.180cd	4.97b	4.24c
SZn5 + FZn1	9.18cd	8.43d	0.220bc	0.226b	4.98b	4.72bc
SZn10 + FZn0.5	9.63bc	8.34d	0.216bc	0.206bc	5.20ab	4.83b
SZn10 + FZn1	11.42a	9.94bc	0.256ab	0.253ab	5.37ab	4.86b
SZn15 + FZn0.5	10.57ab	9.29c	0.230b	0.216bc	5.28ab	4.75bc
SZn15 + FZn1	10.46b	9.88bc	0.283a	0.243b	5.71a	4.94b

Values are means of three replicates ($n=3$). Values with the same letters in a column do not differ significantly at $P \leq 0.05$

Leaf Zn concentration

Leaf Zn concentration in both cotton cultivars was remarkably improved with all levels and modes of Zn application (Fig. 1). However, the highest improvement in leaf Zn concentration was found at 270.5% in CIM-663 and 218.4% in Cyto-124 at SZn15 + FZn1 compared with the control. Leaf Zn concentration was positively correlated with photosynthetic rate ($R^2=0.7\,817$).

Plant growth characterization

Plant growth characteristics of both cotton cultivars in terms of plant height, monopodial branches, and sympodial branches were significantly ($P \leq 0.05$) improved by Zn nutrition. In both cultivars, SZn15 + FZn1 produced the tallest plants. It increased the plant height by 23.2% in CIM-663 and 28.0% in Cyto-124 compared with the control (Fig. 2). The highest sympodial branches per plant were also found in both cultivars at SZn15 + FZn1 where the increase was 37.2% in CIM-663 and 35.2% in Cyto-124 compared with the control (Fig. 3). Likewise, monopodial branches per plant were maximally increased in both cultivars at SZn15 + FZn1 where the increase was 40.7% in CIM-663

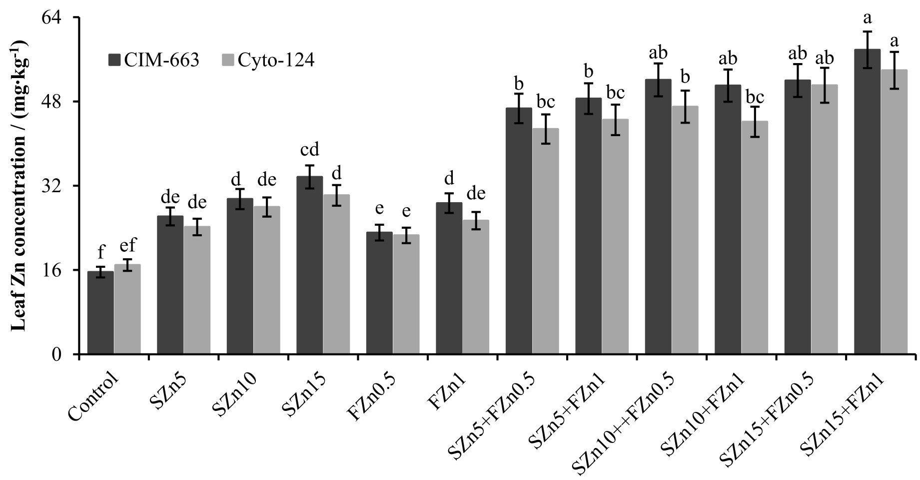

Fig. 1 Leaf Zn concentration of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions. Values are means of three replicates ($n=3$). The bars having the same letters do not differ significantly at $P \leq 0.05$

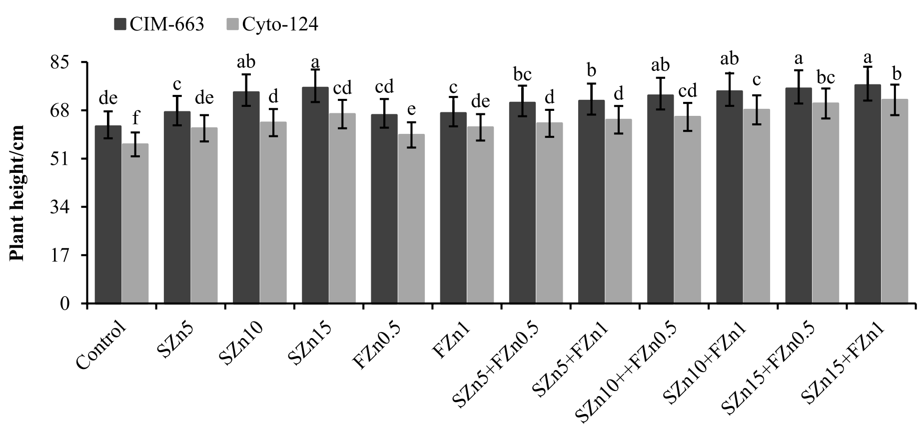

Fig. 2 Average plant height of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions. Values are means of three replicates ($n = 3$). The bars having the same letters do not differ significantly at $P \leq 0.05$

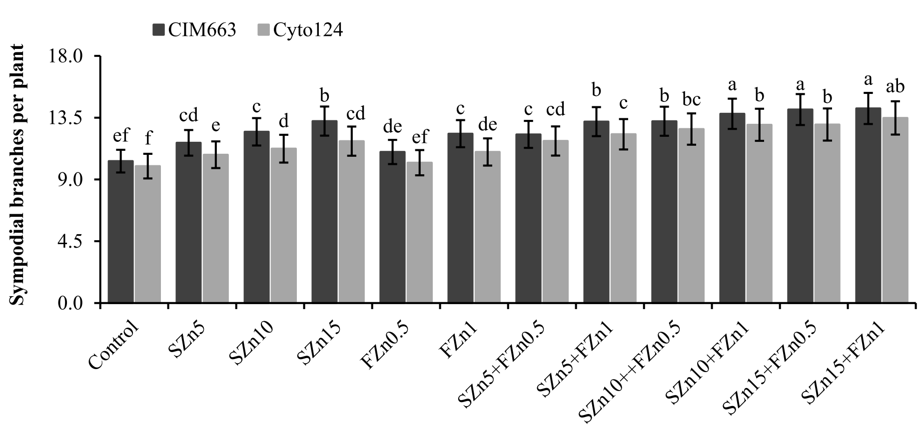

Fig. 3 Average number of sympodial branches per plant of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions. Values are means of three replicates ($n=3$). The bars having the same letters do not differ significantly at $P \leq 0.05$

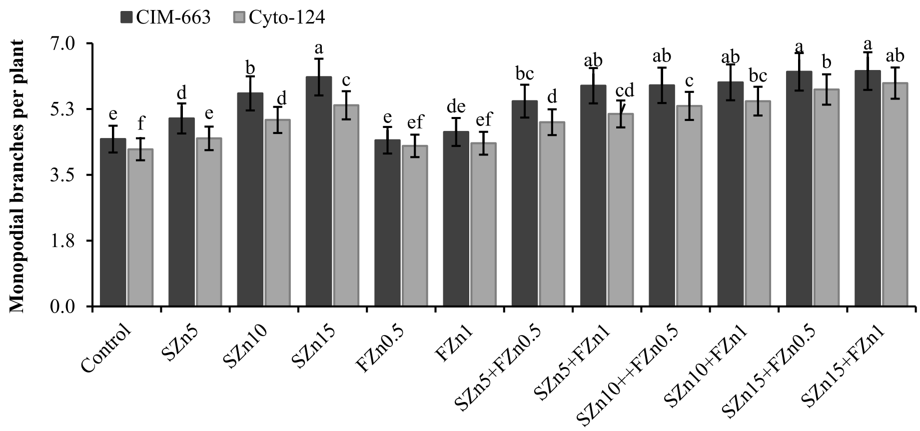

Fig. 4 Average number of monopodial branches per plant of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions. Values are means of three replicates ($n = 3$). The bars having the same letters do not differ significantly at $P \leq 0.05$

and 42.1% in Cyto-124 compared with the control (Fig. 4).

Yield characteristics

Seed cotton yield and yield characteristics such as boll size and the number of bolls per plant were significantly ($P \leq 0.05$) improved with Zn nutrition. The highest increase in seed cotton yield was found at SZn15 + FZn1 where the increase was 32.5% in CIM-663 and 36.6% in Cyto-124 compared with the control (Fig. 5). Lint yield was maximally increased by 30.0% in CIM-663 and 34.6% in Cyto-124 at SZn15 + FZn1 compared with the control (Fig. 6). The highest number of bolls per plant were observed at SZn15 + FZn0.5 where the increase was 34.3% in CIM-663 and 29.4% in Cyto-124 compared with the control (Fig. 7). Boll size was maximally increased at SZn15 + FZn0.5 where the increase was 31.4% in CIM-663 and 29.6% in Cyto-124 compared with the control (Fig. 8). When comparing among the cultivars, CIM-663 produced relatively higher seed cotton yield and yield characteristics compared with Cyto-124, regardless of the treatments.

Fiber quality characteristics

Fiber quality characteristics in terms of fiber length, strength, and fineness were significantly ($P \leq 0.05$) affected by Zn nutrition (Table 4). The treatment SZn15 + FZn1 proved the most efficient in improving the fiber quality of both cultivars under alkaline calcareous conditions. It was found that fiber length was maximally increased by 14.2% in CIM-663 at SZn15 + FZn1, and 9.9% in Cyto-124 at SZn10 + FZn1 compared with the control. The highest increase in fiber strength was 11.9% in CIM-663 and 10.4% in Cyto-124 at SZn10 + FZn1 compared with the control. Fiber fineness was maximally increased in both cotton cultivars at SZn15 + FZn1 where the increase was 19.7% in CIM-663 and 15.9% in Cyto-124 compared with the control. There was a positive correlation between leaf Zn concentration and fiber quality characteristics in terms of fiber length ($R^2 = 0.717\ 3$), fiber strength ($R^2 = 0.548\ 3$), and fiber fineness ($R^2 = 0.637\ 9$) in both cotton cultivars grown at different levels and modes of Zn application under alkaline calcareous conditions.

Benefit-cost ratio

The benefit-cost ratio varied greatly for both cotton cultivars grown at different levels and application modes of Zn under alkaline calcareous conditions. It ranged from 1.51 to 1.64 for CIM-663 while, 1.09 to 1.21 for Cyto-124. The highest benefit-cost ratio was 1.64 for CIM-663 at SZn10 + FZn1 and SZn15 + FZn1, and 1.21 for Cyto-164 at SZn10 + FZn0.5, respectively (Table 5).

Discussion

Zinc-mediated improvement in physiological and gas exchange characteristics of both cotton cultivars CIM-663 and Cyto-124 was attributed to its involvement in the synthesis of chlorophyll, stabilization of cell membrane, and higher water retention in plants. Zinc being the important constituent of chloroplast could improve chlorophyll synthesis and photosynthesis as evidenced by a positive correlation between leaf Zn concentration and photosynthetic rate (Lacerda et al. 2018; More et al. 2018; Hassan et al. 2020). Improvement in RWC and MSI at

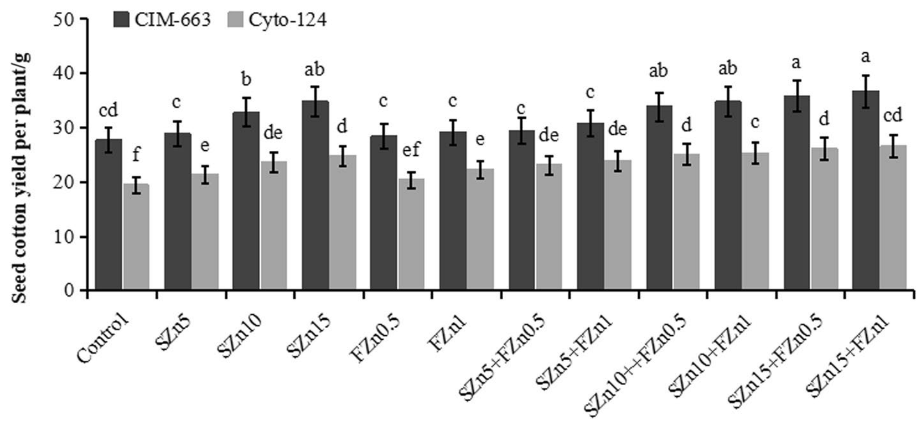

Fig. 5 Seed cotton yield per plant of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions. Values are means of three replicates ($n=3$). The bars having the same letters do not differ significantly at $P \le 0.05$

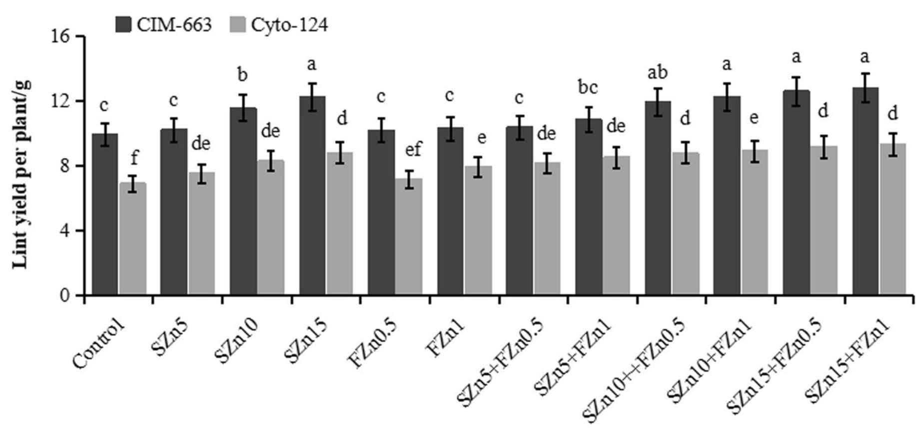

Fig. 6 Lint yield per plant of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions. Values are means of three replicates ($n=3$). The bars having the same letters do not differ significantly at $P \le 0.05$

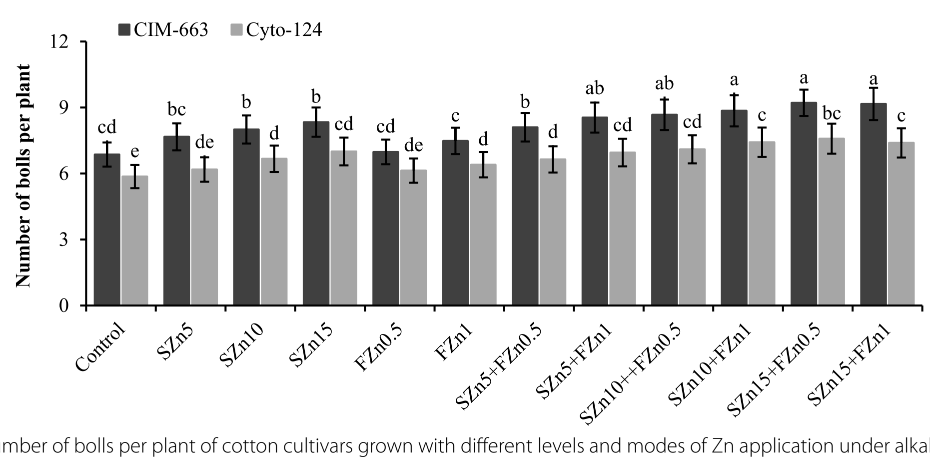

Fig. 7 Average number of bolls per plant of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions. Values are means of three replicates ($n=3$). The bars having the same letters do not differ significantly at $P \le 0.05$

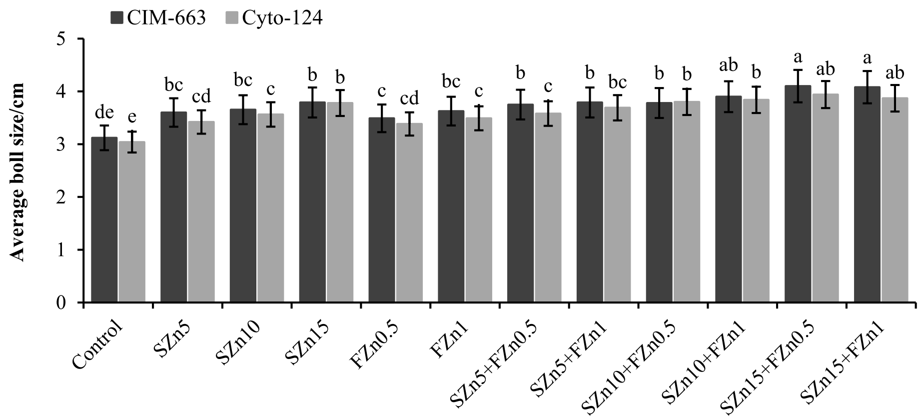

Fig. 8 Average boll size of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions. Values are means of three replicates ($n=3$). The bars having same letters do not differ significantly at $P \le 0.05$

various levels of Zn supply was associated with its role in the development and functionality of vascular tissues, stomatal regulation, and metabolic activities (Hatam et al. 2020; Sarwar et al. 2022). Anik et al. (2023) reported that Zn-induced improvement in chlorophyll synthesis, photosynthesis, plant water status, membrane stabilization, and enzymatic activities could be the primary mechanisms for improving cotton growth and yield, particularly under drought stress. The combined use of soil addition and foliar spray ensured a quick and persistent Zn supply to plants, leading to higher improvement in the physiological and gas exchange characteristics of cotton cultivars. Nayak et al. (2023) also found that the combined use of soil addition and foliar spray of Zn could more efficiently improve wheat growth, yield, and physiological characteristics compared with the individual use either through soil addition or foliar spray.

The improvement in leaf Zn concentration of both cotton cultivars at different levels of Zn supply was associated with adequate Zn replenishment through soil addition and foliar spray. The highest leaf Zn concentration with the combined use of soil and foliar application was linked to quick Zn supply by foliar spray and consistent availability by soil application (Zhao et al. 2020). In the present study, alkaline soil pH, low organic matter, and high calcium carbonate contents were the major factors to restrict the solubility and bioavailability of Zn in soil, resulting in low leaf Zn concentration. It could be overcome by foliar application avoiding Zn precipitation in soil (Ahmed et al. 2022). However, due to hot and arid

Table 4 Fiber quality characteristics of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions

Treatments	Fiber length /mm		Fiber strength /tppsi		Fiber fineness /(μg·inch^{-1})	
	CIM-663	Cyto-124	CIM-663	Cyto-124	CIM-663	Cyto-124
Control	26.90de	26.80e	100.28d	96.78e	4.36de	4.28e
SZn5	27.45d	27.65d	102.32cd	98.56de	4.57cd	4.46d
SZn10	28.60c	27.94cd	105.40bc	103.28c	4.72c	4.69c
SZn15	29.10bc	28.12cd	108.46ab	105.53bc	4.93b	4.91b
FZn0.5	27.20de	27.20de	101.52cd	97.48de	4.60cd	4.39d
FZn1	27.50d	27.44d	103.00c	101.56cd	4.51cd	4.42d
SZn5 + FZn0.5	27.94cd	28.20cd	106.22bc	100.37d	4.56cd	4.67c
SZn5 + FZn1	28.42c	29.10bc	108.31b	102.45cd	4.80bc	4.83bc
SZn10 + FZn0.5	28.95bc	28.35c	106.27bc	104.50bc	4.98b	4.72c
SZn10 + FZn1	29.84ab	29.45b	112.21a	106.82b	5.18a	4.86b
SZn15 + FZn0.5	29.80ab	28.62c	111.10a	105.17bc	4.88b	4.77bc
SZn15 + FZn1	30.72a	29.28b	107.53b	106.24bc	5.22a	4.96b

Values are means of three replicates ($n=3$). Values with the same letters in a column do not differ significantly at $P \le 0.05$

Table 5 Benefit-cost ratio of cotton cultivars grown with different levels and modes of Zn application under alkaline calcareous conditions

Treatments	CIM-663			Cyto-124		
	Benefit per hectare /PKR	Cost per hectare /PKR	B/C ratio	Benefit per hectare /PKR	Cost per hectare /PKR	B/C ratio
Control	227 750	145 850	1.56	159 310	145 850	1.09
SZn5	237 445	156 388	1.52	175 495	156 388	1.12
SZn10	269 241	166 921	1.61	194 146	166 921	1.16
SZn15	286 084	177 459	1.61	203 841	177 459	1.15
FZn0.5	234 158	149 135	1.57	166 951	149 135	1.12
FZn1	239 252	152 420	1.57	182 397	152 420	1.20
SZn5 + FZn0.5	241 306	159 670	1.51	189 709	159 670	1.19
SZn5 + FZn1	252 891	162 958	1.55	195 953	162 958	1.20
SZn10 + FZn0.5	277 703	170 206	1.63	205 813	170 206	1.21
SZn10 + FZn1	285 344	173 491	1.64	207 702	173 491	1.20
SZn15 + FZn0.5	294 300	180 740	1.63	214 522	180 740	1.19
SZn15 + FZn1	301 283	184 029	1.64	217 972	184 029	1.18

climatic conditions, water evaporated quickly to reduce the absorption of foliar spray of Zn. It necessitated the combined application of soil addition and foliar spray as a promising option to attain adequate Zn concentration in plant tissues (Khalid et al. 2022).

Zinc-mediated improvement in plant growth and yield characteristics of both cotton cultivars might be attributed to its role in chlorophyll synthesis, photosynthetic activities, stomatal conductance, membrane stabilization, and plant water status (Hatam et al. 2020; Anik et al. 2023). Current study found that adequate Zn nutrition through soil addition and foliar spray improved photosynthesis and other physiological characteristics, leading to an increase in plant height, the number of sympodial branches and monopodial branches per plant. Sakya et al. (2018) reported that Zn increased plant leaf area which resulted in a higher photosynthetic rate, and improvement in growth characteristics. Zinc might help to improve the translocation of photosynthates from leaves to bolls, resulting in bolls with higher size, and subsequently higher seed cotton yield (Abbas et al. 2020).

The improvement in fiber quality characteristics of cotton in terms of fiber length, strength. and fineness at adequate Zn supply was attributed to its role in physiological and reproductive development. According to Raj et al. (2019), Zn-induced mechanisms responsible for improving fiber quality of cotton might include improved photosynthesis, fertilization, flower, and boll setting as well as mobilization of photosynthates from leaves to bolls. However, genetic makeup and environmental conditions could also be important to control fiber quality of cotton (Grimes et al. 1990). The combined use of soil addition and foliar spray ensured adequate Zn concentration in plant tissues required for fiber maturity, resulting in long and strong fiber (Mehran et al. 2023). It was confirmed by a positive correlation between leaf Zn concentration and fiber quality characteristics of both cotton cultivars.

Cotton is grown in many countries of the world for fiber, oil, and feed. Being an important cash crop, it can play a vital role in employment generation and poverty alleviation. Recent changes in climate have made the cotton crop more vulnerable to different abiotic and biotic stress factors and necessitate the development of plant tolerance mechanisms against stress conditions. In this regard, adequate plant nutrition, particularly with micronutrients can provide a promising strategy for improving plant tolerance/resistance to stress conditions. Zinc is the most widespread deficient microelement under alkaline calcareous conditions, and there is a dire need to regulate its supply for optimum plant growth and development. The current research suggested valuable recommendations regarding the adequate level and application mode of Zn for optimum growth, physiological, yield, and fiber quality characteristics of Bt and non-Bt cotton cultivars under alkaline calcareous conditions. These findings could strategically direct future planning in plant nutrition and soil fertility development for cotton. An understanding of total soil Zn distribution into different fractions under alkaline calcareous conditions is a key factor in determining Zn solubility and phytoavailability. Future research should, therefore, be based on the determination of different Zn fractions in soil and the effect of Zn application at different growth stages on the productivity of Bt and non-Bt cotton cultivars.

Conclusions

The plant growth, physiological, yield, and fiber quality characteristics of cotton cultivars were significantly improved with Zn supply at different levels and modes of application. However, SZn15 + FZn1 maximally improved most of the observed growth, yield, and fiber quality traits under alkaline calcareous conditions. Zinc-mediated increases in chlorophyll synthesis, photosynthesis, stomatal conductance, and boll development were the major factors responsible for improving cotton growth and yield. SZn15 + FZn1 could be recommended for optimum cotton productivity under alkaline calcareous conditions. However, these results need to be verified under field conditions.

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Authors' contributions
Ashraf M. and Qamar F designed and executed the experiment, and wrote the manuscript. Mehran M and Masood S helped in the execution of experiment. Shahzad SM, Javed MS, and Azhar MT helped in analytical work and manuscript writing.

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