



RESEARCH ARTICLE

Effect of iron-zinc-boron on growth and physiological characters of okra (*Abelmoschus esculentus* (L.) Moench)

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Abstract

Essential micronutrients, including iron (Fe), zinc (Zn) and boron (B), play a critical role in regulating plant growth, developmental processes, physiological functions and yield performance in vegetable crops. Deficiencies of these nutrients can adversely affect crop productivity, reduce quality and impair overall plant health. In okra (*Abelmoschus esculentus* (L.) Moench), inadequate micronutrient availability limits growth and physiological efficiency and yield potential. Therefore, this study was undertaken to evaluate the effects of foliar application of Fe, Zn and B on the growth, development and physiological characteristics of okra. A field experiment was conducted during the Navarai season (November–January) using a randomized block design (RBD) with 8 treatments and 3 replications. Different combinations of Fe, Zn and B were applied as foliar sprays during the vegetative, flowering and fruiting stages of crop growth. Observations of growth, yield and physiological parameters were recorded at different stages of crop development and subsequently analysed to assess the effects of micronutrient application. Significant differences among treatments were observed for growth, yield and physiological parameters of okra. T₇ (Zn + Fe + B at 1 %) produced the highest plant height (82.4 cm), fruit length (12.2 cm), fruit weight (18.8 g), leaf area (125.5 cm² plant⁻¹), antioxidant activity (46.2 %), relative water content (RWC) (86.4 %) and chlorophyll content (1.97 mg g⁻¹), while reducing days to first flowering (36 days). The combined foliar application of Fe, Zn and B demonstrated a synergistic effect in enhancing growth performance, physiological efficiency and overall productivity of okra, indicating its potential as an effective nutrient management strategy for improving crop yield and quality.

Keywords: antioxidant activity; boron; growth attributes; iron; micronutrients; okra; physiological traits; zinc

Introduction

Okra (*Abelmoschus esculentus* (L.) Moench), commonly known as lady's finger or bhindi, is an important vegetable crop belonging to the family Malvaceae (1). It is widely cultivated in tropical and subtropical regions for its tender, edible green pods, which are consumed in fresh, cooked, canned and processed forms. Owing to its nutritional and economic significance, okra occupies an important position among vegetable crops. The fruits are rich in carbohydrates, dietary fibre, proteins, minerals and vitamins such as A, B1 and C, making them valuable for human nutrition (2). Fresh okra pods contain approximately 2.1 g protein, 0.2 g fat, 8 g carbohydrates, 1.7 g fibre, 175.2 mg minerals and 88 mL water per 100 g edible portion (3, 4). In addition, the leaves are occasionally utilised as livestock feed and possess nutritional value.

Despite its agronomic and nutritional importance, the productivity of okra in India remains below its potential. One of the major constraints affecting crop performance is inadequate nutrient management, particularly the insufficient availability of essential micronutrients. Micronutrient deficiencies in soil can adversely affect plant growth, reduce physiological efficiency, reduce yield and compromise fruit quality. In severe cases, nutrient deficiency may lead

to substantial yield losses. Micronutrients are involved in several critical biochemical and physiological processes, including chlorophyll formation, enzymatic activation, carbohydrate metabolism, respiration and hormone regulation, thereby influencing overall crop productivity (5).

Among the essential micronutrients, iron (Fe), zinc (Zn) and boron (B) are particularly important for the normal growth and development of vegetable crops. Iron plays a key role in chlorophyll biosynthesis, electron transport during photosynthesis and respiration and activation of enzymes such as catalase, peroxidase and ferredoxin. Iron deficiency often results in interveinal chlorosis in young leaves, reduced photosynthetic activity and poor biomass accumulation. In crops like okra, adequate Fe nutrition supports vigorous vegetative growth and improves pod development. Zinc is another essential micronutrient involved in enzyme activation, chlorophyll synthesis, protein metabolism and auxin production, which collectively contribute to proper plant growth and reproductive development.

Boron is equally vital for plant physiological and reproductive functions, particularly cell division, cell wall formation, sugar translocation, pollen viability, fertilisation and seed development (6).

Deficiency of B may impair flowering, fruit setting and overall crop productivity. Since these micronutrients perform complementary roles in plant metabolism, their combined application may produce synergistic effects on crop growth and yield.

Foliar application has emerged as an effective and efficient approach for supplying micronutrients directly to plants. This method facilitates rapid nutrient absorption, minimise nutrient losses through leaching and fixation in soil and improves nutrient use efficiency. Foliar sprays of nutrients with plant growth regulators can improve production and quality by minimising flower and fruit drop and increasing tolerance to abiotic and biotic stresses (7). Previous studies have also highlighted the beneficial effects of combined micronutrient application. A recent study reported that soil application of FeSO_4 , ZnSO_4 and borax significantly improved plant height, number of nodes per plant and fruit yield in okra. Additionally, the integrated application of Fe, Zn and B was found to enhance the uptake of major nutrients, including nitrogen (N), phosphorus (P) and potassium (K), thereby improving crop performance (8). Although several studies have reported the beneficial effects of Fe, Zn and B on the growth and yield of okra, most of the available information is limited to individual micronutrient applications or soil-based nutrient management practices. Information regarding the comparative effects of foliar application of Fe, Zn and B, applied individually and in combination, on growth and physiological characteristics of okra is limited. Furthermore, the synergistic influence of these micronutrients under local agro-climatic conditions has not been adequately investigated. Therefore, further studies are required to identify the most effective micronutrient combination for improving crop growth and physiological efficiency in okra.

Given the importance of micronutrient management in narrowing the okra yield gap, optimising foliar applications of Fe, Zn and B, both individually and in combination, is essential. Therefore, the present investigation was undertaken to evaluate the effects of foliar application of Zn, Fe and B, applied singly and in combination, on the growth and physiological characteristics of okra to identify the most effective treatment combination to enhance crop growth, physiological efficiency and productivity.

Materials and Methods

A field experiment was conducted on an Inceptisol at the SRM College of Agriculture Sciences farm, Baburanyanpettai, Chengalpattu District, Tamil Nadu, during November 2025 to January 2026 (*Navarai*) to study the effect of Fe-Zn-B on growth and physiological characters of okra (*A. esculentus*). The location is situated between the latitudes of 12.37° N and the longitudes of 79.74° E, with an average altitude of 50 m and a relative humidity of 50–70 %. The average temperature ranged from 23–38 °C during the study period. The experimental soil was classified as an Inceptisol. Prior to sowing, composite soil samples were collected from the experimental field and analysed for physico-chemical properties. The soil had a pH of 7.05, electrical conductivity of 0.3 dS m^{-1} , organic carbon content of 0.79 %, available N of 582 kg ha^{-1} , available P of 10.08 kg ha^{-1} , available K of 23.6 kg ha^{-1} , diethylenetriaminepentaacetic acid (DTPA)-extractable Fe of 32.3 mg kg^{-1} , DTPA-extractable Zn of 4.7 mg kg^{-1} and available B of 1.02 mg kg^{-1} . The experiment was laid out in a randomized block design (RBD) with 8 treatments, consisting of foliar spraying with different percentage combinations of Zn, Fe and B and replicated three times. The treatments included T_1 : Zn at 0.5 % (as

Zinc sulphate heptahydrate, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), T_2 : Fe at 0.2 % (as Ferrous sulphate heptahydrate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), T_3 : B at 0.3 % (as Borax, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), T_4 : Zn + Fe at 0.7 %, T_5 : Zn + Bat 0.8 %, T_6 : Fe + B at 0.5 %, T_7 : Zn + B + Fe at 1.0 % and T_8 : Control.

As per the treatment schedule, the recommended dose of fertilisers was applied as a basal two days before sowing. The seeds were sown in the field by the dibbling method. Two seeds per drill were sown at a spacing of $60 \times 30 \text{ cm}$. Foliar application of Zn (0.5 % as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), Fe (0.2 % as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), B (0.3 % as borax, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), Zn + Fe (0.7 %), Zn + B (0.8 %), Fe + B (0.5 %) and Zn + B + Fe (1.0 %) was carried out at vegetative, flowering and fruiting stages. The spray solution was applied at a spray volume of 1000 L/plant using a knapsack sprayer fitted with a hollow cone nozzle during the early morning hours (08:00–10:00) to ensure maximum foliar absorption and minimise evaporation losses was carried out at the vegetative, flowering and fruiting stages as per the treatment schedule. The data were manually recorded after a week of foliar spraying. Plant protection measures were implemented based on the economic threshold for pests and diseases. All additional recommended management practices were followed for okra cultivation.

Field observation

Biometric observation consisted of plant height and stem diameter. Plant height was determined by measuring the height of the primary stem of a random sample of plants in each treatment from the base at the soil surface to the apex, using a metric ruler. The growth rate of okra plants was determined from height measurements and expressed as absolute growth rate (AGR), based upon increments of measured plant heights between sampling dates (i.e. height at day 2–height at day 1) over the time period (number of days) between measurement dates.

Post-harvest data included seed pod⁻¹ fruit length (cm) and fruit weight (g), total leaf area ($\text{cm}^2 \text{ plant}^{-1}$) and days to first flowering (days) from 5 random samples from each treatment plot. A graduated metric ruler was used to measure fruit length and fruit weight was determined by weighing sample fruits per treatment from each harvest, using a calibrated electronic balance. Total leaf area was measured using the leaf length-breadth (non-destructive) method. Fully expanded leaves from each tagged plant were selected and leaf length (L) and maximum breadth (B) were recorded using a digital measuring scale. Leaf area was estimated using the formula:

$$\text{Leaf area} = L \times B \times \text{correction factor } (0.62) \quad (9)$$

The total leaf area per plant was obtained by summing the leaf area of all leaves and expressed as square centimetre per plant. Days to first flowering were recorded as the number of days taken from sowing to the appearance of the first flower bud opening in each treatment plot. Observations were made daily and the date of first visible flowering was noted for tagged plants. The duration was calculated using:

$$\text{Days to first flowering} = \text{Date of first flowering} - \text{Date of sowing}$$

The mean value was expressed in days.

Physiological observations

Relative water content (RWC) was estimated following the procedure of Barrs and Weatherley (1962). Leaf discs of known fresh weight (FW) were floated on distilled water for 4 hr at room temperature under diffused light to obtain the turgid weight (TW). The turgid leaf discs were then oven-dried at 70 °C to constant

weight to obtain the dry weight (DW). Relative water content was calculated using the formula:

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

Total chlorophyll content was estimated following the method of Amon (1949) (10). Fresh leaf tissue (0.5 g) was homogenised in 80 % acetone containing a pinch of MgCO_3 and filtered through Whatman No. 1 filter paper. The extract volume was made up to 100 mL with 80 % acetone. Absorbance was recorded at 663 and 645 nm using a UV-visible spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll contents were calculated based on standard procedure and expressed as milligram per gram fresh weight (10).

Total phenol content was determined by the Folin-Ciocalteu method as described by Bray and Thorpe (11). Fresh leaf tissue (0.5 g) was extracted with 80 % ethanol and centrifuged at 10000 rpm for 15 min. The supernatant was reacted with the Folin-Ciocalteu reagent and 20 % sodium carbonate solution. After incubation in the dark for 30 min, absorbance was measured at 650 nm. Phenol content was estimated using a gallic acid standard curve and expressed as milligram per gallic acid equivalents per gram fresh weight.

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay following Blois (1958)(12). Leaf extract was mixed with 0.1 mM DPPH solution in methanol and incubated in the dark for 30 min. Absorbance was recorded at 517 nm and antioxidant activity was calculated as:

$$\text{DPPH scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extract.

Statistical analysis

The analysis of variance (ANOVA) for RBD was used to statistically assess the data obtained from each replication and the analysis was performed using R statistical software 2023 (version 4.3.1). At a 5 % probability level ($p < 0.05$), the significance of treatment differences was examined. In cases where the F -test was significant, treatment means were compared using critical difference (CD) values (13–14).

Results and Discussion

The experiment was conducted to study the effect of Fe-Zn-B on the growth and physiological characters of okra using a RBD. The data were statistically analysed and the results are presented in Table 1.

Biometric observations

Plant height

Plant height differed significantly among treatments. The highest plant height was recorded in T_7 (82.4 cm), followed by T_6 (74.4 cm), whereas the lowest value was observed in T_8 control (42.3 cm). T_7 exceeded the control by 40.1 cm, indicating a statistically significant improvement. Individual micronutrient sprays produced intermediate responses, with T_3 (B, 62.6 cm) and T_2 (Fe, 58.6 cm) outperforming T_1 (Zn, 55.4 cm), while combined treatments were generally superior to single-nutrient applications. The superior plant height under T_7 indicates that combined foliar application of Zn, B and Fe promoted vegetative growth more effectively than single or dual combinations. Similar responses have been reported in okra where combined micronutrient application increased plant height over control and single-element treatments (15–17). The Zinc- and B-based combinations have repeatedly produced taller plants in okra, including Zn + B foliar sprays and soil-applied Zn + B combinations (17–19). Iron also contributes to improved vegetative growth in okra when included in multi-nutrient sprays and Fe-containing combinations have been associated with higher plant height and overall crop vigour (20–22).

Number of seeds per pod

Number of seeds per pod varied significantly among treatments. The maximum number of seeds per pod was obtained in T_7 (55), followed by T_3 (51) and T_1 (49), while the minimum was recorded in the control T_8 (30). The increase in T_7 over control was 25 seeds pod⁻¹, far exceeding the CD of 1.82, confirming statistical significance. Iron alone (T_2) resulted in the lowest seed number among micronutrient treatments, suggesting that Fe by itself was less effective than B-containing treatments for this reproductive trait (37).

The marked increase in seeds per pod under T_7 is consistent with the established role of B and Zn in reproductive development, pollen function, fertilisation and seed setting in okra (19, 23). In okra, combined boron and Zn foliar sprays significantly improved the number of filled seeds per fruit, with the best seed response reported at 0.2 % borax + 0.2 % ZnSO_4 (20). Evidence is somewhat more mixed across cultivars for seed traits than for vegetative growth, since one cultivar-based study found improved germination and pod set after micronutrient sprays but not a universal increase in seeds per pod across all genotypes (21). Even so, the present advantage of T_7 over all other treatments strongly supports a beneficial interaction among Zn, B and Fe for seed formation (Table 1).

Fruit traits

Fruit length and fruit weight were both significantly influenced by micronutrient treatments. The longest fruits were recorded in T_7

Table 1. Effect of iron-zinc-boron (Fe-Zn-B) on growth characters of okra

Treatment	Plant height (cm)	Seeds pod ⁻¹	Total leaf area (cm ² plant ⁻¹)	Day to first flowering (days)
T1	55.4	49	88.0	41
T2	58.6	37	86.9	42
T3	62.6	51	93.7	43
T4	60.4	42	105.0	39
T5	52.4	45	110.1	43
T6	74.4	44	116.8	40
T7	82.4	55	125.5	36
T8	42.3	30	76.8	46
S.Ed.	4.4	0.84	1.8	0.85
CD	9.4	1.82	3.9	1.82

(12.2 cm), followed by T_6 (10.9 cm) and T_5 (10.8 cm), while the shortest fruits occurred in T_2 (8.1 cm). Fruit weight also reached its maximum in T_7 (18.8 g), followed by T_5 (17.2 g) and T_3 (16.7 g), whereas the control produced the lightest fruits (9.4 g). Since the differences between T_7 and control exceeded the respective CD values for fruit length (0.4 cm) and fruit weight (0.6 g), these improvements were significant. The superiority of T_7 for fruit length and fruit weight agrees with several okra studies showing that combined micronutrient treatments improve pod size and individual fruit mass (7, 15, 16). Zn + B combinations have been especially effective in increasing fruit length and weight in okra, with higher values reported under combined foliar treatments than under control or single nutrient sprays (17, 23, 24). A similar trend was also observed where Zn + B treatment increased pod length to 15.63 cm and pod weight to 24.98 g in okra under swat conditions (19). Increased fruit size under micronutrient application has been linked to improved leaf area and chlorophyll status, which support greater assimilate production and partitioning to developing fruits (16, 25) (Fig. 1).

Leaf area

Total leaf area per plant was significantly affected by treatment. The maximum leaf area was recorded in T_7 (125.5 cm² plant⁻¹), followed by T_6 (116.8 cm²) and T_5 (110.1 cm²), whereas the minimum was found in the control T_8 (76.8 cm²). The difference between T_7 and control (48.7 cm²) was much greater than the CD of 3.9 cm², confirming significance. Dual combinations generally produced greater leaf area than single nutrient sprays, indicating a cumulative benefit of micronutrient interaction on canopy development. This result is consistent with earlier okra studies in which foliar micronutrients significantly enhanced leaf area, especially when nutrients were applied in combination (12, 13, 25). Combined Zn + B treatments and broader micronutrient mixtures have been associated with larger assimilatory surface and improved growth efficiency in okra (16, 26, 27). The likely explanation is that micronutrients improved chlorophyll formation, metabolic activity and leaf expansion, which together increased photosynthetic area and supported stronger sink development in fruits (15, 20, 25).

Days to first flowering

Days to first flowering showed significant variation among treatments and lower values indicated earlier flowering. The earliest flowering was observed in T_7 (36 days), followed by T_4 (39 days) and T_6 (40 days), whereas the control T_8 took the longest time (46 days). The reduction of 10 days in T_7 compared with control exceeded the CD of 1.82 days, indicating a significant earliness response. Among single nutrient treatments, Zn (T_1 , 41 days) was slightly earlier than Fe (T_2 , 42 days) and B (T_3 , 43 days). Earlier flowering under combined micronutrient application agrees with published okra evidence showing that B- and micronutrient-based treatments reduce time to flowering and harvest (15, 28, 29). The Zn + B combinations have also reduced days to flowering and first picking in okra under field conditions (19, 21, 30). This response appears to reflect improved nutritional status and faster transition from vegetative to reproductive growth, which supports earlier bud differentiation and fruit initiation (15, 23).

Total antioxidant

Total antioxidant activity differed significantly among treatments because the treatment difference between T_7 and control was 32.7 percentage points, which is far greater than the CD of 1.5. The highest total antioxidant activity was recorded in T_7 (46.2 %), followed by T_6 (40.8 %) and T_5 (36.5 %), whereas the lowest value was observed in T_8 (13.5 %). Single nutrient sprays were less effective than combined treatments, indicating a strong positive interaction among Zn, B and Fe for antioxidant improvement.

The superior antioxidant activity under T_7 is consistent with evidence that foliar micronutrient supply improves biochemical quality in okra, including chlorophyll and vitamin-linked quality attributes (7, 15). Okra pods are naturally rich in antioxidant-related phytochemicals, including phenolics and pigments, so treatments that improve plant nutrition can reasonably elevate antioxidant capacity (31, 32). In okra under stress, foliar-applied compounds that strengthen antioxidant systems also improve fruit biochemical status, showing that enhancement of oxidative defence is a relevant pathway for better fruit quality (33) (Table 2).

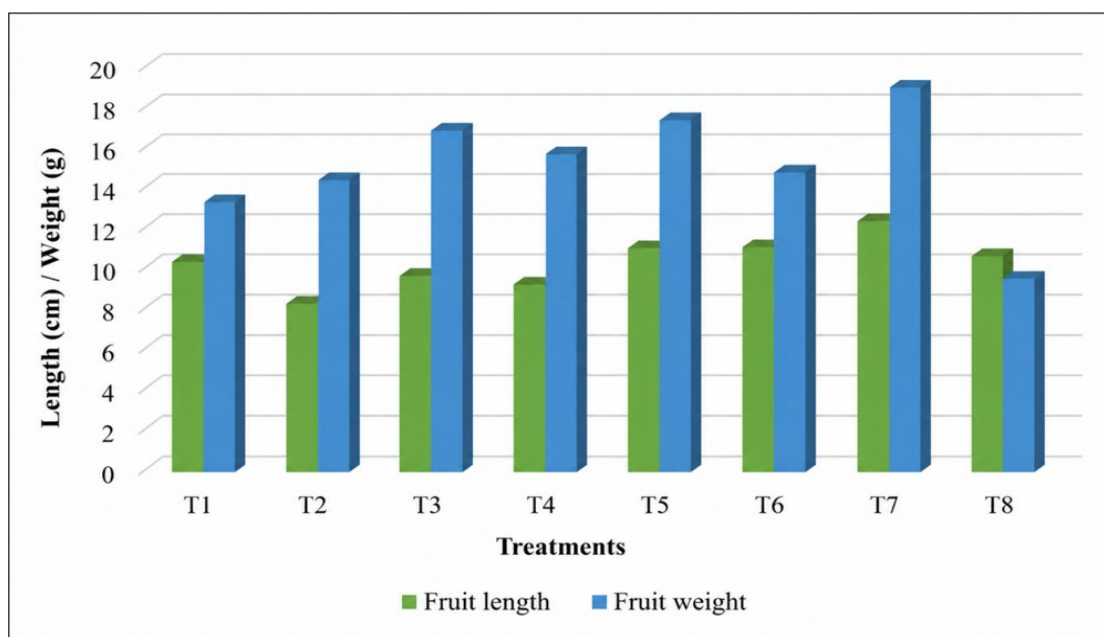


Fig. 1. Effect of iron-zinc-boron (Fe-Zn-B) on fruit length and weight of okra.

Table 2. Effect of iron-zinc-boron (Fe-Zn-B) on physiological characters of okra

Treatment	Total antioxidant (%)	Phenol content (GAE/100 g)	Relative water content (%)	Total chlorophyll (mg/g)
T ₁	20.7	1.1	75.0	1.45
T ₂	23.4	1.2	72.2	1.52
T ₃	26.2	1.4	79.0	1.57
T ₄	32.1	1.5	82.1	1.60
T ₅	36.5	1.6	82.9	1.68
T ₆	40.8	1.6	81.3	1.60
T ₇	46.2	1.8	86.4	1.97
T ₈	13.5	1.0	69.7	1.12
S.Ed.	0.8	0.03	1.8	0.02
CD	1.5	0.06	3.8	0.05

Phenol content

Phenol content also varied significantly, since the maximum difference between treatments exceeded the CD value of 0.06 by a wide margin. T₇ recorded the highest phenol content (1.8 GAE/100 g), followed by T₅ and T₆ (1.6 GAE/100 g), while T₈ had the lowest value (1.0 GAE/100 g). Among the single micronutrients, B (T₃) performed better than Zn (T₁) and Fe (T₂), but combined application produced a larger response than any individual nutrient.

This rise in phenol content under combined micronutrient spray agrees with broader crop evidence showing that Zn and B foliar application can increase phenolics and antioxidant capacity in plant tissues (34). In okra itself, fruits contain substantial phenolic compounds, with quercetin derivatives reported as major phenolics and these compounds contribute directly to antioxidant potential (31). Additional okra evidence shows treatment-dependent variation in total phenolics and total antioxidant capacity, confirming that crop management practices can materially alter phenolic accumulation in pods (35) (Table 2).

Relative water content

Relative water content ranged from 69.7 % in T₈ to 86.4 % in T₇ and the difference of 16.7 % points was much higher than the CD of 3.8, confirming significant treatment effects. T₇ gave the highest RWC, followed by T₅ (82.9 %), T₄ (82.1 %) and T₆ (81.3 %), while Fe alone (T₂) was less effective than Zn alone (T₁) or B alone (T₃). The results indicate that micronutrient combinations improved plant water status more effectively than single nutrient sprays.

The higher RWC under T₇ is supported by okra evidence that Zn improves plant water relations and alleviates osmotic stress. Under saline conditions, exogenous Zn increased RWC and membrane stability in okra, indicating a direct role in maintaining tissue hydration and physiological balance (36). Foliar antioxidant-related treatments in okra also improved ionic balance and reduced oxidative damage while sustaining better physiological performance (33). Together, these findings support the interpretation that Zn-, B- and Fe-based combinations improved leaf hydration by strengthening metabolic activity and reducing physiological stress (Table 2).

Total chlorophyll

Total chlorophyll was significantly influenced by treatments, with values increasing from 1.12 mg g⁻¹ in T₈ to 1.97 mg g⁻¹ in T₇; this difference exceeded the CD of 0.05 by a large margin. T₇ produced the maximum chlorophyll content, followed by T₅ (1.68 mg g⁻¹), T₄ and T₆ (1.60 mg g⁻¹), whereas the control showed the minimum chlorophyll level. Among the individual nutrients, B, Zn and Fe all improved chlorophyll over control, but their combined application was clearly superior.

This trend closely matches previous okra studies where combined micronutrient treatments produced the highest chlorophyll content. In Kashmir, the full micronutrient combination gave the maximum leaf chlorophyll content in okra and omission of Fe, Zn, or other elements reduced chlorophyll levels (15). Another okra study reported the highest leaf chlorophyll and fruit chlorophyll under the most complete foliar micronutrient treatment, while control plants had the lowest values (16). A separate okra trial also found that the all-micronutrient treatment recorded the highest chlorophyll content of 1.80 mg g⁻¹ along with superior fruit quality (7) (Table 2).

Conclusion

The findings of the present study revealed that T₇ (zinc (Zn) + iron (Fe) + boron (B) at 1.0 %) consistently exhibited superior performance in growth, fruit attributes and physiological parameters of okra. The combined application of Fe, Zn and B enhanced plant growth, physiological activity and overall crop performance compared with individual micronutrient applications and the control. Improvements in key physiological attributes, including relative water content (RWC), phenol content, antioxidant activity and chlorophyll concentration, contributed significantly to enhanced growth performance and yield characteristics. Therefore, the combined foliar application of Zn, Fe and B at Zn + B + Fe at 1.0 % (T₇) may be considered an effective nutrient management strategy for improving physiological efficiency, growth and overall crop productivity in okra for the Inceptisol soils of Chengalpattu district, Tamil Nadu. Further studies across different soil types, seasons and agro-climatic conditions are required to validate the consistency of the observed responses.

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Authors' contributions

TV conducted the field experiment, collected data, performed laboratory analyses and prepared the first draft of the manuscript. RAS conceived and designed the study, supervised the research work, interpreted the results and critically revised the manuscript.

RRTS assisted in planning the experiment, data interpretation and manuscript review. CP contributed to the statistical analysis, interpretation of results and critical revision of the manuscript. VS assisted in laboratory analyses, data compilation and manuscript editing. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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