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RESEARCH ARTICLE

Effect of different concentrations of soil and foliar applied zinc, boron and iron fertilizers on seedling growth, chlorophyll content and productivity of chickpea seedlings under semi-arid environment

Muhammad Kashif Munir[1], Muhammad Zafar[1], Babar Hussain Babar[2], Nawal Zafar[1], Siraj Ahmed[3], Muhammad Aleem Sarwar[4], Saba Iqbal[5], Asmatullah[5], Naveed Akhtar[6], Muhammad Saeed[7], Muhammad Saqib[8] & Sadam Hussain[9]*

[1]Cereals and Pluses Section, Agronomic Research Institute, Ayub Agricultural Research Institute Faisalabad, Punjab, Pakistan
[2]Vegetable and Oilseeds Section, Agronomic Research Institute, Ayub Agricultural Research Institute Faisalabad, Punjab, Pakistan
[3]Agronomic Research Station, Karor Layyah, Punjab, Pakistan
[4]Soil and Water Testing Laboratory, Ayub Agricultural Research Institute Faisalabad, Pakistan
[5]Agronomic Research Station, Khanewal, Punjab, Pakistan
[6]Agronomic Research Institute, Ayub Agricultural Research Institute Faisalabad, Punjab, Pakistan
[7]Plant Pathology Research Institute Faisalabad, Punjab, Pakistan
[8]Barani Agricultural Research Station, Fateh Jang, Punjab, Pakistan
[9]College of Horticulture, Northwest A&F University, Yangling, China

*Email: ch.sadam423@gmail.com

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Abstract

The effectiveness of zinc (Zn), boron (B) and iron (Fe) is reduced in semi-arid regions, which can lead to a deficiency of these nutrients and inhibit chickpea productivity. In this work, three field experiments were executed over two years where soil and foliar applications of Zn, B and Fe were carried out, including controls (Zn0, B0 and F0), soil application at 4.125 kg/ha (Zn-1, B1 and F1) and 8.25 kg/ha (Zn-2, B2 and F2) and foliar spay at 0.3% at flowering initiating (Zn-3, B3 and F3) and one week after flowering initiation (Zn-4, B4 and F4, respectively). The results indicate that the deficiency of these nutrients inhibited chickpea growth and yield, leading to a reduction in the pigment contents. Nonetheless, soil and foliar application of Zn, B and Fe significantly improved growth, chlorophyll contents and yield, showing a dose-dependent effect. The best results were recorded for Zn-3, B2 and F2 treatments which significantly ($P<0.05$) increased shoot length (20.96-85.19%), root length (42.85-93.65%), shoot fresh (23-76%) and root fresh weight (45-90.32%), compared with the control treatment. Chlorophyll parameters, including chlorophyll a and b contents, showed similar trends. Zn-3, B2 and F2 treatments significantly increased biological and grain yields, which were associated with higher values of the number of pods per plant and the number of seeds per pod. In a nutshell, we suggest that Zn foliar application at 0.3% at flowering initiation and soil application of B and Fe at 25 kg/ha are beneficial for improving the growth, pigment content and overall productivity of chickpea.

Keywords

chickpea; chlorophyll; zinc; yield; soil application; harvest index

Introduction

A limited dietary product based on high calories can cause malnutrition in humans. The deficiency of micronutrients has brought a new challenge of "hidden hunger" to agricultural scientists. According to the report presented by the Food and Agricultural Organization of the United Nations, hidden hunger affects more than 2 billion people worldwide (1). Malnutrition caused by the deficiency of micronutrients (such as Zn, B and Fe) is associated with hidden hunger among populations, leading to various mental, developmental and health disorders (2).

Chickpea is the major crop cultivated in arid to semi-arid regions of Pakistan, thus considered the homeland of chickpea in the country. Chickpea (*Cicer arietinum* L.) ranked 3rd among the other legume crops after peas and beans worldwide (3). It is a rich source of plant protein (22%) for humans (4-5) and is largely consumed in Asian and South Asian countries (6). Also, it is a good source of fiber, vitamins, and minerals essential to human metabolism (7). Due to its ability to thrive with less water, chickpea is typically sown during the winter season in semi-arid regions of Punjab, contributing 76% of the total pulse production in Pakistan (8). However, the arable land under arid or semi-arid climatic conditions in Pakistan is characterized as sandy and calcareous soil, which restricts the availability of micronutrients to plant roots (9).

The production potential of chickpea is quite low in these areas due to low organic matter and poor soil fertility (10). Furthermore, micronutrient (Zn, B, and Fe) uptake also decreases under high soil pH in calcareous soils (9, 11, 12); thus, malnutrition occurs in humans who consume the produce of crops growing in such areas.

Boron plays a key role in various metabolic activities in humans, contributing to the proper functioning of the brain (13). In plants, boron is involved in various cellular functions such as cell wall and membrane functioning (14, 15) and it is essential for fruit setting or seed development (16-18). The unavailability of boron in plants can lead to reduced electron transport chain (ETC) activity, chlorosis and a decrease in the photosynthetic rate (17, 19). Optimal boron availability is crucial for the formation of pollen tubes, grains and their viability (20). Similarly, Zn and Fe deficiencies are observed globally. Zn catalyses different enzymes (RNA-polymerase, SOD etc.), while proteins (hemoglobin and myoglobin) rely on Fe concentration for driving oxygen in the human body. Insufficiency of these micro-nutrients can lead to health disorders and increased mortality rates, making micronutrient deficiency a major challenge to human health worldwide. Fe deficiency affects children (43%) and women (pregnant by 38%, non-pregnant by 29%) with anaemia. It is estimated that more than 2 billion people suffer from Zn deficiency, while anaemia (Fe deficiency) is reported in 7 billion people globally (21). Interestingly, these deficiencies are more prevalent in developing countries (22).

The availability of deficient micronutrients can be increased through biofortification to enhance the nutritional values of crops (23). Among various biofortification techniques, agronomic biofortification has shown a positive response to enriching nutrients in crops (24). In addition, Cakmak (25) reported that soil and foliar applications are the easiest and most economical approaches to increasing the concentration of applied nutrients in the grains of crops (26). Furthermore, an increase in the percentage of nutrients in grains has been observed with foliar application of nutrients (27), leading to yield increases (28). Similarly, nutrient concentration, plant growth and grain yield of crops have been shown to increase with foliar spray compared to soil application of nutrients in low fertile soils (29-32).

However, it is a pre-requisite to optimize the quantity of micronutrients (foliar or soil) before field evaluation. To address micronutrient deficiencies in the food chain, cereals such as rice (*Oryza sativa*) and wheat (*Triticum aestivum*) have been continuously studied by researchers to increase the bioavailability of zinc, iron, and boron. Unfortunately, legumes, mainly chickpea (*Cicer arietinum* L.), have not received much attention for biofortification. Literature indicates that there have been no studies published on optimizing the rate of Zn, B and Fe nutrient application (foliar and soil) to address micronutrient deficiency in chickpeas growing under semi-arid conditions in Punjab, Pakistan. Thus, a two-year research trial was conducted to evaluate the effects of different rates of foliar and soil-applied Zn, B and Fe on the quality grain production of chickpea in semi-arid conditions. We hypothesized that soil-applied Zn and foliar application of B and Fe would effectively improve chickpea growth, physiology and yield under semi-arid conditions.

Materials and Methods

Experimental details

A two-year research trial, during the growth seasons of 2019 and 2020, was conducted to evaluate the performance of soil and foliar (w/v %age) applied Zn, B and Fe on chickpea (cv. Noor 91) grain quality and yield production at the Cereals and Pulses Section, Agronomic Research Institute, Faisalabad (73°74 E, 30°31.5 N, with an elevation of 184 m). It was three different trials with three nutrients individually under a randomized complete block design (RCBD) with three replications. Treatments included soil and foliar applications of Zn, B and Fe, including controls (Zn0, B0 and F0), soil application at 4.125 kg/ha (Zn-1, B1 and F1) and 8.25 kg/ha (Zn-2, B2 and F2), and foliar spray at 0.3% at flowering initiating (Zn-3, B3 and F3) and one week after flowering initiation (Zn-4, B4 and F4, respectively).

Crop husbandry

The field was cultivated twice, followed by planking at field capacity to prepare the fine seedbed. Proper row spacing of 60 cm was maintained for chickpea sowing with a hand drill while the fertilizers were applied at 23:58:30 NPK, respectively, using urea, DAP and SOP. All P & K doses were given as a basal dose to the soil whereas nitrogen was applied in three splits, i.e., basal, at flowering and one-month after flowering. Furthermore, micronutrients were applied using 300-liter water on a hectare basis according to set treatments. Moreover, the soil profile was studied with standard protocols and the results are given in Table 1.

Table 1. Soil properties in the experimental field

Year	Soil depth (cm)	pH	N (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})	B (mg kg^{-1})
2019	1-6	7.7	0.039	3.5	103.3	0.63	8.8	0.13
	7-12	7.9	0.032	1.16	94	0.25	5.8	0.14
2020	1-6	8.2	0.031	10	260	0.67	1.65	0.43
	7-12	8.2	0.034	11.4	240	0.87	2.29	0.52

Climatic conditions during the research

Climatic conditions were collected from the meteorological observatory of Agronomic Research Institute Faisalabad as given in Fig. 1.

Data collection

A truly representative sample (five plants) from every plot was collected at physiological maturity to take the number of observations; plant height was measured with a meter rod starting from the soil to the tip of the selected plants, and primary branches and their pod numbers were counted and then averaged. The pod length of five randomly selected pods from every plant was measured with a measuring scale and averaged. The grain yield from the harvest crop was attained at harvesting maturity. Three sub-samples were collected with a weighing balance and then averaged to get a 1000-grain weight. Moreover, for quality analysis, seeds were subjected to oven drying at 60°C, grounded, and passed through a 1 mm sieve prior to use in the process of wet digestion to measure seed Zn and Fe contents on Atomic Absorption Spectrophotometer (33), according to the set protocol explained by Estefan et al. (34). On the other hand, dry ashing was used to quantify the seed boron contents (35) and subsequent determination was done by colorimetry using Azomethine-H (36).

Physiological traits

The chlorophyll contents i.e., chlorophyll a, and b were determined using fresh leaves samples. Firstly, about 0.20 g of fresh leaves were extracted in 20 mL 80% acetone solution at 25°C for 24 h in the dark. Later, by using the supernatant, the absorbance was measured at 470 and 646 nm using a UV-visible spectrophotometer. The 80% acetone solution was considered as a blank control.

Statistical analysis

The recorded observation was analyzed with Fisher's analysis of variance technique (ANOVA) and their means were compared with Tuckey's Honestly Significant Difference (HSD) test (37).

Results

Effects of zinc (Zn) application on the growth and physiological traits of Chickpea

Recorded data revealed that Zn application significantly improved seedling length and their fresh weight during both study years (Figs. 2, 3). Among the application rates, maximum shoot and root length and their fresh weights were depicted for Zn-3 treatment, which increased shoot length by 20.96 and 22.12%, root length by 61.11 and 42.85%, shoot fresh weight by 83.34 and 85.19% and root fresh weight by 42.85 and 43.65% in 2019 and 2020, respectively, compared with control treatment without Zn application. Overall, these treatments were ordered as Zn-3>Zn-4>Zn-1>Zn-1>Zn0.

Data regarding the chlorophyll content revealed that Zn application showed a significant influence during both study years. Among the treatments, Zn-3 depicted significantly higher values than other treatments whereas, the Ck treatment depicted significantly lower values than other treatments. As compared to the control, T4 increased chlorophyll a content by 78.11 and 82.2% and chlorophyll b content by 67.25 and 75.23% in 2019 and 2020, respectively (Fig. 4). Overall, for chlorophyll contents, these treatments were ordered as Zn-3>Zn-4>Zn-2>Zn-1>Zn0.

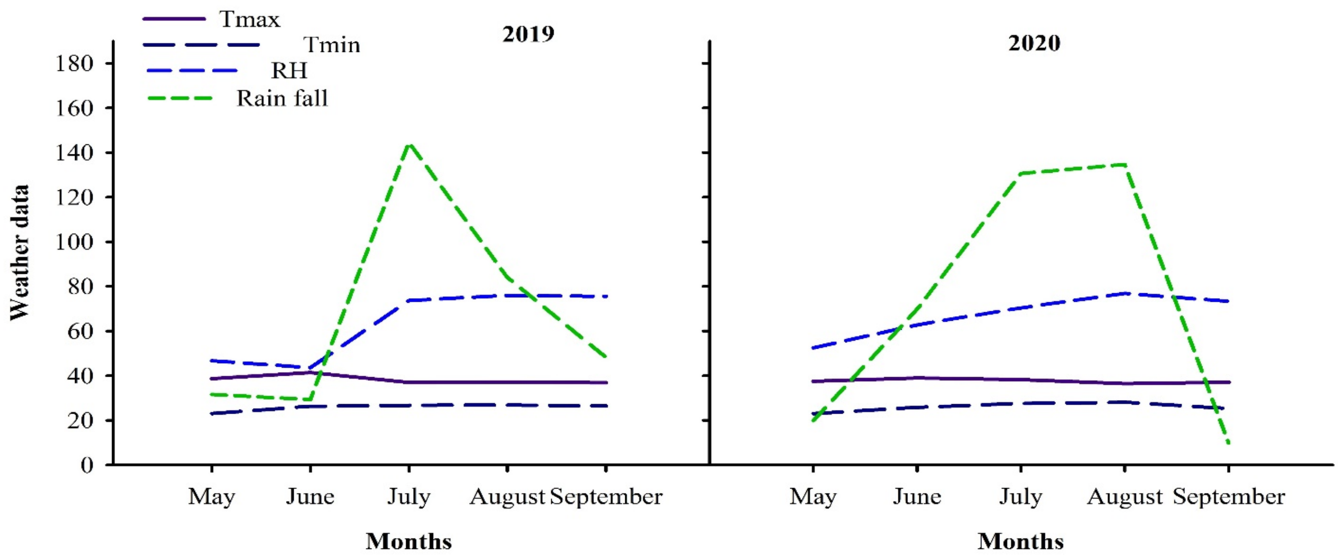

Fig. 1. Climatic data during 2019 and 2020.

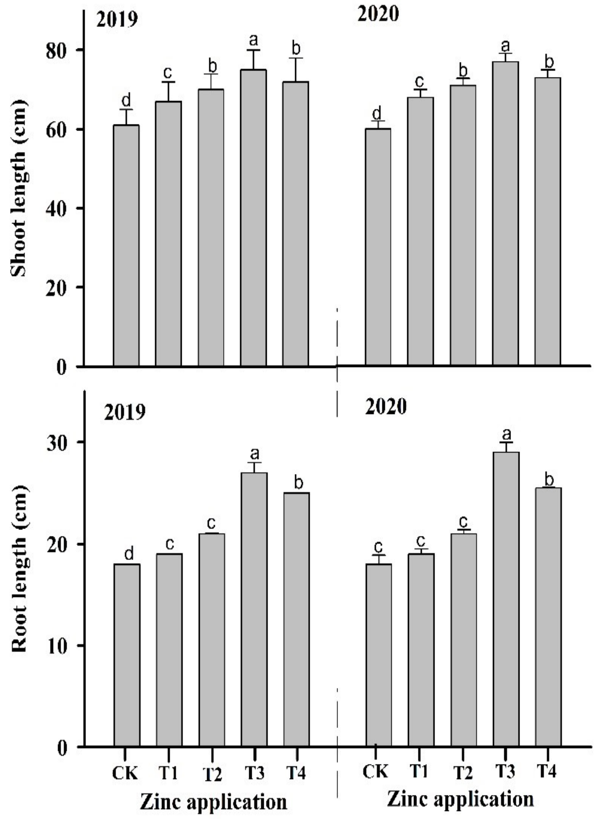

Fig. 2. Influence of zinc (Zn) application treatments such as control (Zn-0, Ck), soil application at 4.125 kg/ha (Zn-1, T1) and 8.25 kg/ha (Zn-2, T2) and foliar spay at 0.3 % at flowering initiating (Zn-3, T3) and one week after flowering initiation (Zn-4, T4) on shoot and root length in chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

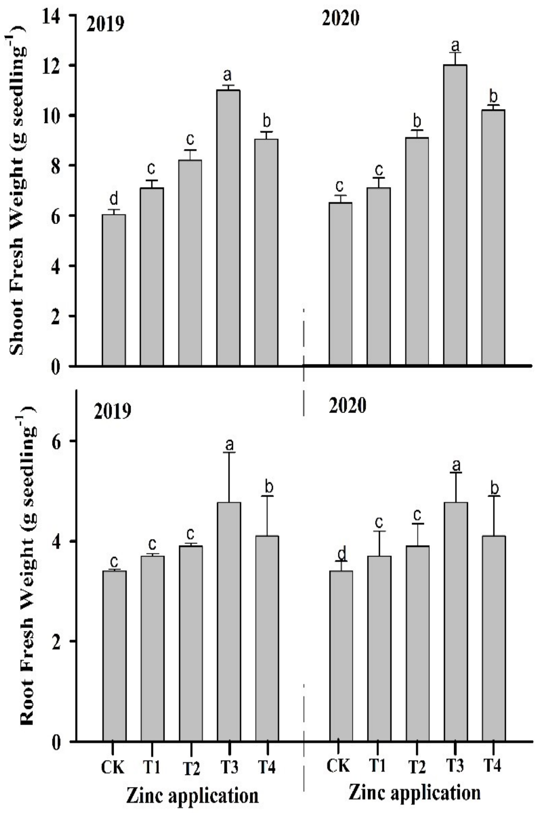

Fig. 3. Influence of zinc (Zn) application treatments such as control (Zn-0, Ck), soil application at 4.125 kg/ha (Zn-1, T1) and 8.25 kg/ha (Zn-2, T2) and foliar spay at 0.3 % at flowering initiating (Zn-3, T3) and one week after flowering initiation (Zn-4, T4) on shoot fresh weight and root fresh weight of Chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

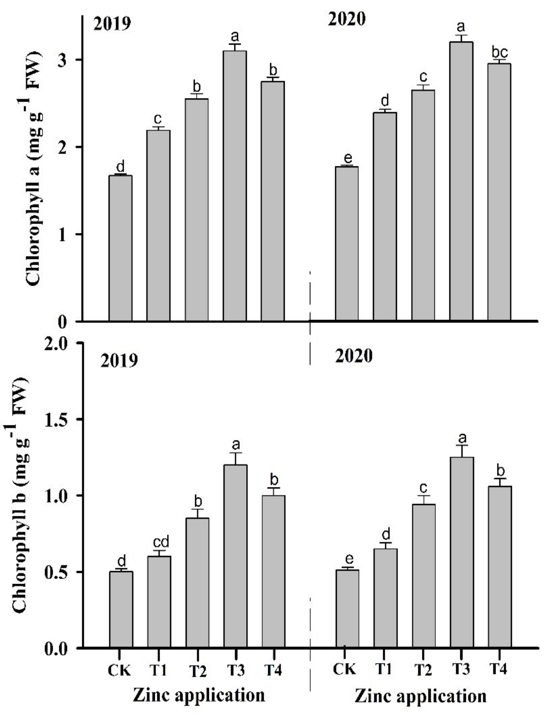

Fig. 4. Influence of zinc (Zn) application treatments such as control (Zn-0, Ck), soil application at 4.125 kg/ha (Zn-1, T1) and 8.25 kg/ha (Zn-2, T2) and foliar spay at 0.3 % at flowering initiating (Zn-3, T3) and one week after flowering initiation (Zn-4, T4) on chlorophyll a and b contents in Chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

Effects of Boron (B) application on the growth and physiological traits of Chickpea

Boron application, both soil application and foliar spray, significantly influence seedling growth and their fresh weights during both years (Figs. 5, 6). Among different treatments, maximum shoot and root length and their fresh weights were depicted for the B2 treatment, whereas minimum values were recorded for the control treatment without B application. As compared to B0, B2 treatment shoot length by 28.14 and 29.43%, root length by 67.44 and 75.55%, shoot fresh weight by 73.45 and 77.85% and root fresh weight by 87.15 and 90.12% in 2019 and 2020, respectively. Overall, for increasing seedling growth and fresh weights, these treatments were ordered as B2>B1>B3>B4>B0.

Data regarding the influence of B on chlorophyll content revealed that its application showed a significant and positive effect on these traits during both study years. Among the treatments, T2 depicted significantly higher values than other treatments, whereas B0 treatment without B application depicted significantly lower values than other treatments. As compared to the control, B2 treatment increased chlorophyll a content by 76.99 and 80.82% and chlorophyll b content by 78.29 and 81.33% in 2019 and 2020, respectively (Fig. 7). Overall, for chlorophyll contents, these treatments were ordered as B2>B1>B3>B4>B0.

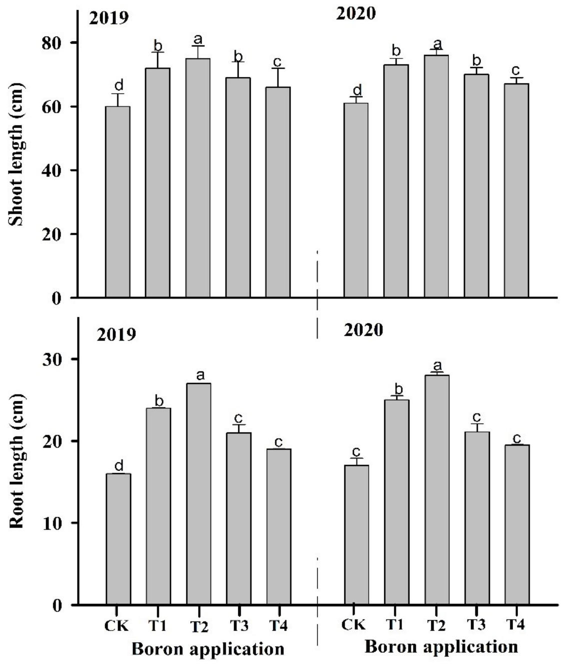

Fig. 5. Influence soil and foliar applications of B including control (B0, Ck), soil application at 4.125 kg/ha (B1, T1) and 8.25 kg/ha (B2, T2) and foliar spray at 0.3 % at flowering initiating (B3, T3) and one week after flowering initiation (B4, T4) on shoot length and root length in chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

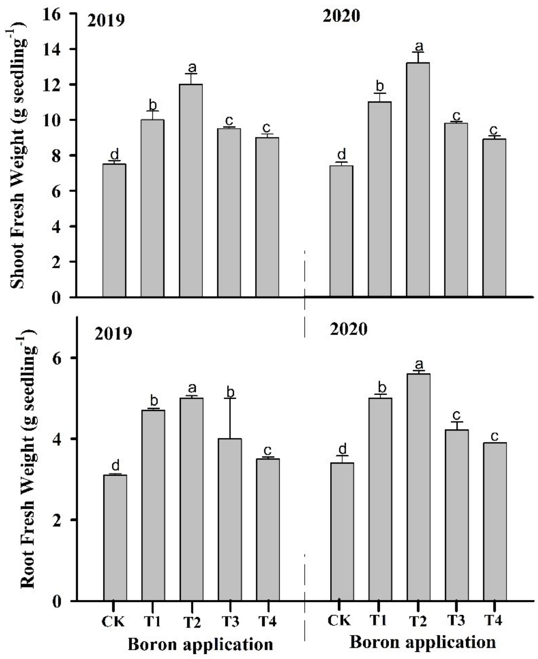

Fig. 6. Influence soil and foliar applications of B including control (B0, Ck), soil application at 4.125 kg/ha (B1, T1) and 8.25 kg/ha (B2, T2) and foliar spray at 0.3 % at flowering initiating (B3, T3) and one week after flowering initiation (B4, T4) on shoot fresh weight and root fresh weight in chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

Effects of iron (Fe) application on the growth and physiological traits of Chickpea

Iron application through both soil application and foliar spray significantly influenced the seedling growth in terms of shoot and root length and their fresh weights in 2019 and 2020 (Figs. 8, 9). Among different treatments, maximum shoot and root length and their fresh weights were depicted for the F2 treatment, whereas minimum values were recorded for the control treatment without Fe application. For that, F2 treatment shoot length by 38.28 and 42.29%, root length by 88.45 and 92.32%, shoot fresh weight by 122.23 and 125.56% and root fresh weight by 52.34 and 56.12% in 2019 and 2020, respectively, as compared to Ck. Overall, for increasing seedling growth and fresh weights, these treatments were ordered as F2>F1>F3>F4>F0.

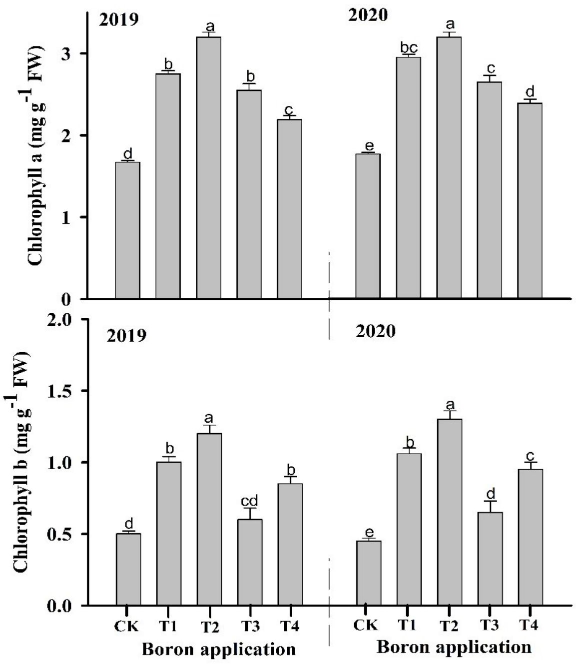

Fig. 7. Influence soil and foliar applications of B including control (B0, Ck), soil application at 4.125 kg/ha (B1, T1) and 8.25 kg/ha (B2, T2) and foliar spray at 0.3 % at flowering initiating (B3, T3) and one week after flowering initiation (B4, T4) on chlorophyll a and b contents in chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

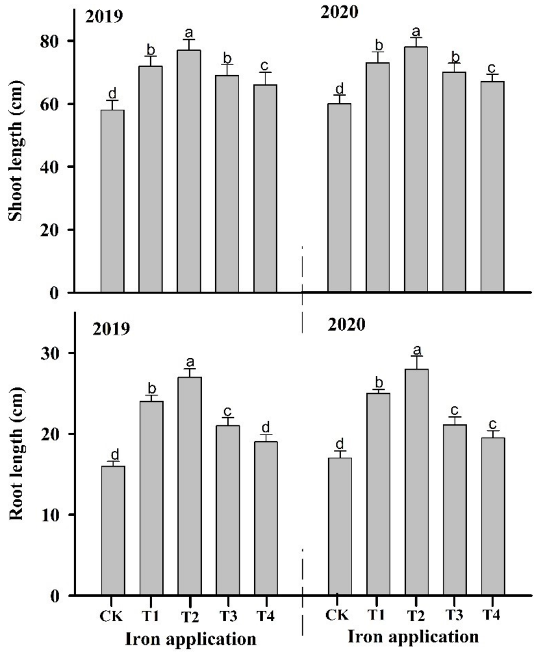

Fig. 8. Influence of iron (Fe) application treatments such as soil and foliar applications of Fe including control (F0, Ck), soil application at 4.125 kg/ha (F1, T1) and 8.25 kg/ha (F2, T2) and foliar spray at 0.3 % at flowering initiating (F3, T3) and one week after flowering initiation (F4, T4) on shoot length and shoot length in chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

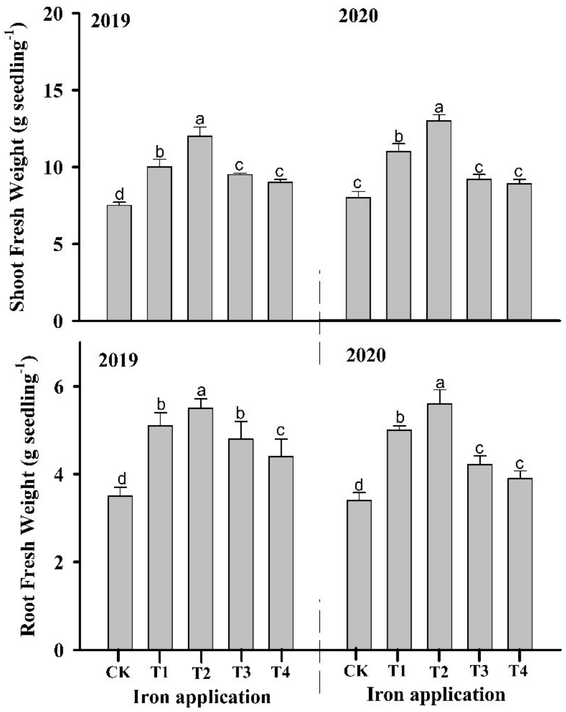

Fig. 9. Influence of iron (Fe) application treatments such as soil and foliar applications of Fe including control (F0, Ck), soil application at 4.125 kg/ha (F1, T1) and 8.25 kg/ha (F2, T2) and foliar spay at 0.3 % at flowering initiating (F3, T3) and one week after flowering initiation (F4, T4) on shoot and root fresh weights in chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

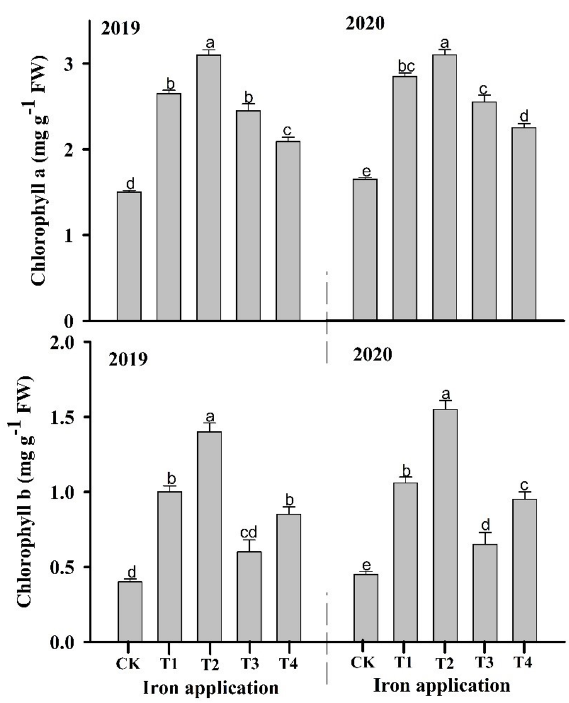

Fig. 10. Influence of iron (Fe) application treatments such as soil and foliar applications of Fe including control (F0, Ck), soil application at 4.125 kg/ha (F1, T1) and 8.25 kg/ha (F2, T2) and foliar spay at 0.3 % at flowering initiating (F3, T3) and one week after flowering initiation (F4, T4) on chlorophyll a and b contents in chickpea in 2019 and 2020. Different lower-case letters above bars showed significant differences at 5 % probability level.

Data regarding the influence of Fe on chlorophyll contents, i.e., chlorophyll a and b, revealed that its application showed a significant and positive effect on these traits during both study years. Among the treatments, F2 depicted significantly higher values than other treatments, whereas F0 treatment without Fe application depicted significantly lower values than other treatments. As compared to the control, F2 treatment increased chlorophyll a content by 82.09 and 84.18% and chlorophyll b content by 88.77 and 91.13% in 2019 and 2020, respectively (Fig. 10). Overall, for chlorophyll contents, these treatments were ordered as F2>F1>F3>F4>F0.

Effects of zinc (Zn) application on yield performance of Chickpea

The data presented in Table 2 showed that Zn application had a significant impact on growth and yield. The maximum plant height (71.53 cm and 70.0 cm) and number of primary branches (4.3 and 4.47) were observed for Zn-2, followed by Zn-1 in both years, whereas the lowest plant height (3.50 and 3.26 cm) and primary branches (63 and 58.66) were counted for Zn0 in both years (Table 2). Likewise, maximum number of pods/plant (32.50 and 31.76), seeds/pod (2.00 and 1.93), test weight (29.80 and 29.00 g), grain yield (1.98 and 1.96 t ha^{-1}), biological yield (4.80 and 4.75 t ha^{-1}) and harvest index (42.73 and 42.42%) were recorded with Zn-3 followed by Zn-4, whereas pods/plant and biological yield in Zn-3 was statistically similar to Zn-4 treatments followed by Zn-2 treatment in both years. However, the lowest value of these parameters was recorded in Zn0 during both years (Table 2).

Effects of boron (B) application on yield performance of Chickpea

The observations of B application on chickpea crops presented in Table 3 showed a significant effect on growth, yield, and yield-related parameters. B2 significantly increased the primary branches up to 4.00 in both years, which was on par with the B1 treatment, while the minimum number of primary branches (3.00 and 2.96) were counted from the B0 treatment in both years (Table 3). Similarly, the plant height was also increased up to 68.28 cm in B2 in 2019, although it was observed non-significantly in 2020 (Table 3). Moreover, the highest number of pods/plant (32.17 and 31.67), seeds/pod (1.91 and 1.99), test weight (28.80 and 28.90 g), grain yield (1.94 and 1.96 t ha^{-1}), biological yield (5.20 and 4.97 t ha^{-1}) and harvest index (39.29 and 39.40%) were recorded for B3, which was statistically similar to B4, followed by B2 and the least value of these parameters was noted for B0 in both years (Table 3). However, the value of pods/plant and seeds/pod obtained from B3 treatment in 2020 was found to be statistically similar ($p \leq 0.05$) to B4 treatment in 2019. On the contrary, the biological yield and harvest index recorded from the B3 plot in 2019 were statistically similar ($p \leq 0.05$) to B4 in 2020 (Table 3).

Effects of iron (Fe) application on the performance of Chickpea

The F2 resulted in an increase in the number of primary branches (4.06 and 4.09), which was at par with F1 in both years. Additionally, the increased plant height (67.40 cm) was found statistically similar ($p \leq 0.05$) to F1, followed by F3 in 2020. Conversely, the least number of primary branches, up to 3.56, was observed in both years for F0

Table 2. Effects of Zn application on the performance of chickpea

Treatments	2019	2020	2019	2020
	Primary branches		Plant height (cm)	
Control (no application)	3.50 C	3.26 C	63.00 D	58.66 D
S.A of Zn at 12.5 kg/ha	4.23 A	4.26 A	68.00 B	67.67 B
S.A of Zn at 25 kg/ha	4.30 A	4.47 A	71.53 A	70.00 A
F.A of Zn at 0.3 % at F.I	3.89 B	3.93 B	66.00 BC	66.00 B
F.A of Zn at 0.3 % at one week after F.I	3.62 C	3.70 B	65.60 C	62.00 C
Tukey's HSD at p≤0.05	0.19	0.31	2.02	2.27
	Pods/plant		Seeds/pod	
Control	27.00 D	27.14 D	1.62 D	1.72 C
S.A of Zn at 12.5 kg/ha	29.60 C	29.00 C	1.75 C	1.76 C
S.A of Zn at 25 kg/ha	30.46 BC	30.91 B	1.81 B	1.84 B
F.A of Zn at 0.3 % at F.I	31.76 A	32.50 A	1.93 A	2.00 A
F.A of Zn at 0.3 % at one week after F.I	31.12 AB	31.74 AB	1.84 B	1.88 B
Tukey's HSD at p≤0.05	0.87	0.94	0.35	0.05
	Test weight (g)		Grain yield (t ha^{-1})	
Control	26.50 E	27.55 E	1.56 E	1.61 C
S.A of Zn at 12.5 kg/ha	27.06 D	28.28 D	1.60 D	1.71 B
S.A of Zn at 25 kg/ha	28.53 C	28.91 C	1.70 C	1.74 B
F.A of Zn at 0.3 % at F.I	29.00 A	29.80 A	1.96 A	1.98 A
F.A of Zn at 0.3 % at one week after F.I	28.80 B	29.33 B	1.72 B	1.95 A
Tukey's HSD at p≤0.05	0.15	0.36	0.02	0.03
	Biological yield (t ha^{-1})		Harvest index (%)	
Control	4.27 C	4.41 C	36.42 D	36.58 D
S.A of Zn at 12.5 kg/ha	4.35 C	4.64 B	37.82 C	38.00 C
S.A of Zn at 25 kg/ha	4.53 B	4.70 AB	38.03 C	38.83 C
F.A of Zn at 0.3 % at F.I	4.75 A	4.80 A	42.42 A	42.73 A
F.A of Zn at 0.3 % at one week after F.I	4.63 AB	4.75 AB	39.71 B	40.20 B
Tukey's HSD at p≤0.05	0.17	0.11	1.19	0.94

S.A = Soil Application, F.A = Foliar application, F.I= Flowering initiation; Means not sharing a letter in common differs significantly at a 5 % probability level.

Table 3. Effects of B application on the performance of chickpea

Treatments	2019	2020	2019	2020
	Primary branches		Plant height (cm)	
Control (no application)	3.00 C	2.96 C	62.00 D	64.00
S.A of B at 12.5 kg/ha	4.28 A	4.30 A	66.20 B	66.60
S.A of B at 25 kg/ha	4.40 A	4.40 A	68.28 A	67.23
F.A of B at 0.3 % at F.I	3.62 B	3.60 B	65.50 BC	66.83
F.A of B at 0.3 % at one week after F.I	3.35 BC	3.34 B	64.00 C	65.00
Tukey's HSD at p≤0.05	0.36	0.38	1.57	NS
	Pods/plant		Seeds/pod	
Control	28.70 D	28.90 C	1.65 D	1.65 D
S.A of B at 12.5 kg/ha	29.26 CD	29.03 C	1.73 CD	1.74 C
S.A of B at 25 kg/ha	29.83 C	29.65 BC	1.75 BC	1.76 C
F.A of B at 0.3 % at F.I	32.17 A	31.67 A	1.91 A	1.99 A
F.A of B at 0.3 % at one week after F.I	30.87 B	30.53 AB	1.82 AB	1.91 B
Tukey's HSD at p≤0.05	0.83	1.36	0.08	0.05
	Test weight (g)		Grain yield (t ha^{-1})	
Control	25.66 D	25.54 E	1.76 C	1.74 D
S.A of B at 12.5 kg/ha	26.37 C	26.45 D	1.79 BC	1.79 C
S.A of B at 25 kg/ha	26.76 B	26.86 C	1.84 B	1.83 B
F.A of B at 0.3 % at F.I	28.80 A	28.90 A	1.94 A	1.96 A
F.A of B at 0.3 % at one week after F.I	28.60 A	28.53 C	1.93 A	1.94 A
Tukey's HSD at p≤0.05	0.25	0.29	0.05	0.03
	Biological yield (t ha^{-1})		Harvest index (%)	
Control	4.48 C	4.49 C	36.57 C	36.51 C
S.A of B at 12.5 kg/ha	4.78 B	4.75 B	38.01 B	37.67 B
S.A of B at 25 kg/ha	4.81 B	4.78 B	38.03 B	38.20 B
F.A of B at 0.3 % at F.I	5.20 A	4.97 A	39.29 A	39.40 A
F.A of B at 0.3 % at one week after F.I	5.00 AB	4.95 A	38.56 AB	39.00 A
Tukey's HSD at p≤0.05	0.23	0.08	1.22	0.53

S.A = Soil Application, F.A = Foliar Application, F.I= Flowering Initiation; Means not sharing a letter in common differ significantly at a 5 % probability level.

(Table 4). On the contrary, the yield components, including pods/plant (30.33 and 31.82), seeds/plant, test weight (28.23 and 28.67 g), grain yield (1.82 and 1.93 t ha^{-1}), biological yield (5.02 and 5.17 t ha^{-1}) and harvest index (37.13 and 37.36%), were recorded for F3, followed by the F4 treatment in both years, respectively (Table 4). Moreover, the least values of pods/plant (28.06 and 29.75), seeds/pod (1.64 and 1.66), test weight (25.56 and 26.00 g), grain yield (1.68 and 170 t ha^{-1}) and biological yield (4.53 and 4.3 t ha^{-1}) were quantified from the control F0 treatment in both years (Table 4). Interestingly, the harvest index (36.83%) from the control plot (F0) was found at par ($p \leq 0.05$) with F3 in 2020, whereas the minimum value of the harvest index (35.45%) was recorded from the F1 treatment (Table 4).

Discussion

Boron, zinc, and iron applications significantly improved growth, physiological and yield traits, supporting our hypothesis. Boron is reported to have positive consequences for crop growth, physiological and yield characteristics (38, 39). Similar results were recorded in this experiment, showing that B supplementation significantly increased chickpea growth, chlorophyll content and yield- and yield-related traits. Boron serves as a structural component of the cell wall and plays numerous essential roles in membrane integrity (17). There is increasing evidence that it facilitates chlorophyll pigment formation and increases carotenoid contents, thus improving growth and photosynthetic rate (40). Similar findings were reported by Mehboob et al. (41), who demonstrated that increased photosynthesis under B application was associated with higher production of light-harvesting pigments, including chlorophyll and carotene contents. Boron facilitates carbohydrate metabolism and increases phenolic acid production, thus improving plant growth and development. Similarly, Wang et al. (42) established that B is also an important component in acetic acid metabolism, which works as an important growth regulator. According to Xu et al. (43), B application enhances chlorophyll pigments and photosynthesis; higher assimilate production can lead to enhanced dry matter production and seed yield. In addition, several published reports have also established that B application significantly increased yield and related traits in different field crops, including chickpea (41, 44). Similar results were recorded in this study, showing that B application significantly improved yield and related traits in chickpeas. The higher productivity of chickpeas under the B application was mainly attributed to higher values of leaf area index, leaf area duration and crop growth rates (41). B application was also reported to improve yield, which was attributed to enhancing physiological processes in plants, such as pollen germination, elongation of pollen tubes, retention of flowers and fruit development, ultimately leading to improved seed set, pod formation and overall seed yield.

Table 4. Effects of Fe application on the performance of chickpea

Treatments	2019	2020	2019	2020
	Primary branches		Plant height (cm)	
Control (no application)	3.56 C	3.56 C	64.33	64.36 C
S.A of Fe at 12.5 kg/ha	3.98 A	4.04 A	66.41	67.15 A
S.A of Fe at 25 kg/ha	4.06 A	4.09 A	68.36	67.40 A
F.A of Fe at 0.3 % at F.I	3.85 B	3.84 B	66.21	65.37 B
F.A of Fe at 0.3 % at one week after F.I	3.82 B	3.82 B	66.00	65.06 B
Tukey's HSD at p≤0.05	0.10	0.09	NS	0.60
	Pods/plant		Seeds/pod	
Control	28.06 D	29.75 D	1.64 E	1.66 D
S.A of Fe at 12.5 kg/ha	29.20 C	30.69 C	1.68 D	1.71 C
S.A of Fe at 25 kg/ha	29.39 C	30.82 C	1.73 C	1.72 C
F.A of Fe at 0.3 % at F.I	30.33 A	31.82 A	1.82 A	1.94 A
F.A of Fe at 0.3 % at one week after F.I	29.99 B	31.40 B	1.78 B	1.90 B
Tukey's HSD at p≤0.05	0.32	0.22	0.02	0.02
	Test weight (g)		Grain yield (t ha^{-1})	
Control	25.56 D	26.00 D	1.68 D	1.70 D
S.A of Fe at 12.5 kg/ha	26.60 C	27.53 C	1.73 C	1.73 D
S.A of Fe at 25 kg/ha	26.66 C	27.73 C	1.74 C	1.76 C
F.A of Fe at 0.3 % at F.I	28.23 A	28.67 A	1.82 A	1.93 A
F.A of Fe at 0.3 % at one week after F.I	27.85 B	28.38 B	1.79 B	1.82 B
Tukey's HSD at p≤0.05	0.31	0.26	0.02	0.02
	Biological yield (t ha^{-1})		Harvest index (%)	
Control	4.53 C	4.63 D	34.83 C	36.83 AB
S.A of Fe at 12.5 kg/ha	4.77 B	4.88 C	35.52 BC	35.45 C
S.A of Fe at 25 kg/ha	4.84 B	4.90 C	35.95 B	35.96 C
F.A of Fe at 0.3 % at F.I	5.02 A	5.17 A	37.13 A	37.36 A
F.A of Fe at 0.3 % at one week after F.I	4.98 A	5.05 B	36.35 AB	36.17 BC
Tukey's HSD at p≤0.05	0.14	0.06	1.11	0.83

S.A = Soil Application, F.A = Foliar Application, F.I= Flowering Initiation; Means not sharing a letter in common differ significantly at 5 % probability level.

Zinc, one of the major essential micronutrients for plants, plays a fundamental role in growth and developmental processes. It facilitates nucleic acid as well as protein synthesis. Zinc application also enhances the uptake of other nutrients such as phosphorus and nitrogen, thereby aiding in seed formation. Being a fundamental element, zinc acts as a structural component and serves as a reaction site in numerous proteins involved in the growth and developmental processes of plants. It is well established that the exogenous application of Zn facilitates plant growth, development, and the yield of different crops. For example, Ullaha et al. (45) demonstrated that Zn application improved the leaf area index and dry matter production in chickpeas. Similarly, Ullah et al. (46) reported that Zn treatments improved crop stand, seedling dry weight and nutrient uptake in desi and kabuli chickpeas. In another study with the same crop, Ullah et al. (47) stated that Zn supplementation increased seedling vigor, growth and seed yield of chickpeas under rainfed conditions. Likewise, Zn application, both through soil application and foliar spray, improves crop growth, physiological traits and yield of chickpeas under field conditions. Zinc is an essential component of leg-hemoglobin biosynthesis. Thus, increased growth under Zn application is associated with a greater nodule number and symbiotic N fixation in chickpeas (47). Improved nutrient uptake under the Zn application also facilitates better plant growth and seed yield formation. In this work, a high grain yield of chickpea plants treated with Zn was associated with more pods per plant and seeds per pod. Similar results were reported by Ullah et al. (47), who noted a significant increase in seed yield (>40%) under Zn application, which was associated with a greater number of pods per plant and a higher number of seeds per pod. Under Zn application, a strong and positive association between grain yield and crop stand, chlorophyll pigments, and leaf photosynthesis was also reported previously (46, 47). Similar findings were recorded in this work. Some studies have also stated that improved grain yield under Zn application was attributed to high photosynthetic activity, improving nutrient uptake, and facilitating the development of reproductive organs. The above-mentioned mechanisms help optimise crop growth and overall productivity, resulting in higher biomass and grain yield.

Furthermore, our results demonstrated that Fe application significantly improved the growth, physiological and yield attributes of chickpea. Iron, a crucial micronutrient for plants, improves leaf chlorophyll and carotenoid contents, photosynthesis and the formation of reducing sugar, ultimately leading to enhanced seed yield (48). Increased photosynthesis and respiration under Fe application have been well reported by (49). Fe supplements facilitate the structural functioning of photosynthetic organs by increasing the formation of chlorophyll and carotenoid pigments. This improved photosynthesis and reduced leaf senescence have been demonstrated to retard plant growth and improve crop yield (50). Similarly, we recorded that Fe application, particularly through foliar spray, significantly increased seedling fresh and dry weights, chlorophyll content and yield traits. Consistent with our results, foliar application of Fe has been reported to improve the growth and yield of field crops. Foliar spray can increase Fe concentrations in plant tissues, subsequently improving stomatal density, the thickness of the cuticle layer, the density of epidermal hairs and the nutritional status of the plants. It is also reported that Fe improves seed yield by promoting chlorophyll content, enabling electron transfer in photosynthesis and respiration and improving enzyme activities for better nutrient uptake and metabolism. Moreover, it contributes to improving crop performance for optimal plant growth and development.

Conclusion

The present study provides strong evidence that the deficiency of zinc, iron and boron inhibits chickpea growth, physiology and yield. We found that soil and foliar application of these nutrients significantly improved the growth, pigment content and yield of chickpeas. The best results were recorded with the Zn-3, B2 and F2 treatments, which significantly increased seedling length, fresh weight, chlorophyll parameters and biological and grain yields. Higher values of biological and grain yield were associated with higher values of the number of pods per plant and the number of seeds per pod. This work provides evidence of growth, physiological and yield improvements. However, further studies are needed to clarify the underlying molecular mechanisms of soil and foliar application of these nutrients for chickpea.

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Authors' contributions

MKM, MZ, BHB and NA prepared the research plan and analysis. MAS, M. Saeed and MS provided guidelines for the research work and research methodology. NZ, SA, SI and A performed the experiment and conducted the analyses. SH helped with the writing and proofreading of the manuscript.

Compliance with ethical standards

Conflict of interest: The Authors declare that there is no conflict of interest.

Ethical issues: None.

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