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Research Article

Nutrient Biofortification of microgreens (spinach) to combat hidden hunger

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ABSTRACT

Nutrient deficiency is a major cause of different health disorders in human beings. Malnutrition has become more alarming in under-developed countries and might affect more than 3 billion people in the world. Supplementation and food fortification of vegetable crops with minerals can address the issue of adequate nutrition security. Current study was focused to assess the nutritional status of vegetables after supplementation of different macro and micronutrients. The trial was conducted in growth room, Department of Horticulture, University of Layyah, Layyah. Seed of spinach was procured from a certified agency and directly sown in the trays (2 inches deep). This study employed a total of twenty four treatment conditions, comprising three distinct nutrient treatments (including Fe, Zn, and Ca) as well as a control treatment. Every nutrient was applied in three different concentrations following two application methods, constituting six treatments of individual nutrient. Each treatment was replicated three times, each replication containing 1 tray. The end produce was analyzed for different attributes like morphological parameters, biochemical parameters (Total soluble solids, Vitamin C, acidity, carotenoids, chlorophyll contents, lipophilic antioxidant (LPA), starch contents, amino acids, carotenoids, flavonoids, phenolic contents and ionic contents (Fe, Zn, and Ca**)** . Our results indicated that supplementation of 200ppm Zn, Fe @1.5mM and 200ppm calcium concentration is optimal for maintaining the normal growth of plants and to promote the major Zn, Fe and Ca concentration in the edible part of spinach. Thus, it could be proposed that the growth of spinach under 200ppm Zn, Fe @1.5mM and Ca @200ppm increased the intake of these nutrients and other beneficial compounds for the human health.

Keywords: Micronutrient Deficiency, Phenolics, Vitamin C, Nutrient supplementation, Ionic contents.

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INTRODUCTION

One of the main global challenge to health of human is mineral's deficiency. Demand of consumers for fortified vegetables and bioactive content is rising day by day due to growing interest of peoples in fresh products with premium functional and nutritional quality. Bio fortified vegetables contain essential nutrients such as iron which is essential micronutrient to address hidden hunger, a micronutrient deficiency (Kumari et al., 2022). Probably, malnutrition will become more alarming in under-developed countries and might affect more than 3 billion

people in the world (Carvalho et.al, 2013; Hoekenga et.al, 2014). Hence**,** Process of adding nutrients to food is called bio fortification. Nutrients are important for human being for working and to provide energy. Deficiency of nutrients and vitamins results various negative effects on health and economic so bio fortified crops mitigate negative effects (Prasad, 2015).

Microgreens are versatile crop and it can be grown on small as well as large scale, yet have more concentrations of nutrient elements comparative to their mature form (Mir et al., 2017). This is the reason which makes them best candidates for biofortification studies. Many studies have attempted to improve the profile of microgreen nutrients with success, but these are limited to few nutrients involving iron (Fe), zinc (Zn), selenium (Se), and iodine (I) (Di Gioia et al., 2019; Germ et al., 2019).

Iron and Zinc are the trace elements which are essential for many metabolic processes. Among the top ten risk factors which contributes to disease factors worldwide, Deficiencies regarding these nutrients have been ranked fifth and sixth (Kumar, 2011). However, there are limited effects of calcium element on the different parts of plants like seed, roots and tubers because this element has complex translocation patterns but foliar application of calcium had proven good results in many experiments and it is a good bio fortification tool (Yuan et al., 2018).

Main objectives of the study were to determine the influence of macro and micronutrients on yield and nutrient profile of microgreens and to improve and enhance the phytonutrients through bio fortification of vegetables, it may better serve human health and may prove economical than adding these nutrients in supplements. Another objective was to optimize the application method and dose/rate of nutrients for vegetable biofortification.

MATERIALS AND METHODS

In this experiment, seed of spinach (*Spinacia oleracea* L.) was procured from a certified agency and directly sown in the trays (2 inches deep) which were lined with foil in order to prevent leaching of water and mineral nutrients from the soil. Spinach was grown in growth room with optimal growing temperature (14-18◦C), Department of Horticulture, University of Layyah, Layyah.. The trays were filled with 35% sand, 28% silt and 37% clay with a mean organic matter content. This study employed a total of six treatment conditions, comprising three distinct nutrient treatments (including Fe, Zn and Ca) as well as a control treatment. The details of these treatments are outlined in table 1. Each treatment was replicated three times, each replication containing 1 tray. There were 72 trays (for each foliar and basal) in this experiment. Average five plants from each replication were taken to analyze data. Irrigation was applied as per requirement. Following that, basal and foliar applications were applied on given stages.

1st Application of nutrients (foliar and basal) was provided on germination stage

2nd Application of nutrients (foliar and basal) was provided one week after germination.

3rd Application of nutrients (foliar and basal) was applied one week before harvesting.

Table1. Treatment details of nutrients applied to spinach

Nutrient	Application method	Concentration
No nutrient	Foliar & Basal	Control (distilled water)
Fe	Foliar & Basal	Fe_1 (1mM)
		Fe_2 (1.5mM)
		Fe_3 (2mM)
Zn	Foliar & Basal	Zn_1 (100ppm)
		Zn_2 (150ppm)
		Zn_3 (200ppm)
Ca	Foliar & Basal	Ca_1 (100ppm)
		Ca_2 (150ppm)
		Ca_3 (200ppm)

Plant analysis of fresh samples of spinach

Shoot length and root length were measured in centimeters with the help of measuring scale.

Fresh and dry weight of spinach plant was assessed by placing the plant on weighing balance (PA-413 manufactured by Chaus Corporation, USA). Number of leaves were counted and averaged. Leaf area was measured with the help of measuring scale by following formula;

$$\text{Leaf area (cm}^2\text{)} = \text{Length of leaf (cm)} \times \text{width of leaf (cm)}$$

Total soluble solid (TSS) were determined through digital refractometer, placing a few drops of spinach juice on the prism in the specimen chamber of the refractometer. The ascorbic acid concentration was measured using titration method of 2, 6-dichlorophenol-indophenol (Horwitz, 1975; SAPRC, 1986a). Oxalic acid was used to dilute the juice and filter aliquot was titrated with the help of indophenol dye to the light pink end point coloration.

$$\text{Vitamin C} = R_1 \times V \times 100/R \times W \times V_1$$

R_1= flask/burette value, V= volume of aliquot made by adding 0.4% Oxalic acid, V_1= ml of aliquot used after filtration for titration, W= juice taken (ml), R= standard reading

To measure total carotenoid contents, a grounded leaf sample were centrifuged with 80% acetone (10 mL) at 12000 rpm for 5 min. The absorbance of carotenoids were recorded in nm in UV-VIS spectrophotometer (WE6000). The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample with the help of UV-Vis spectrophotometer (WE6000). The concentration of total flavonoid content in the test samples was calculated from the calibration plot ($Y = 0.0162x + 0.0044$, $R2 = 0.999$). To measure total phenolic compounds, sample and standard readings were made using a spectrophotometer (WE6000) at 765 nm.

Amino acids (proline contents) were determined with the help of ninhydrin reagent. Absorbance was recorded with help of UV-VIS spectrophotometer (WE6000).

A sample of 0.5 g grounded leaf was centrifuged with 80% acetone (10 mL) at 12000 rpm for 5 min. The absorbance of Chlorophyll ‘‘a” and ‘‘b” was recorded at 645 nm and 663 nm in UV-VIS spectrophotometer (WE6000).

Plant analysis of Dry samples of spinach

The lipophilic antioxidant activity (LAA) was measured with the 2,20 -azinobis 3-ethylbenzothiazoline-6-sulfonic acid ABTS method by UV–Vis spectrophotometry. After measuring absorbance at 734nm for 30mints, ABTS % were calculated by using formula

ABTS %= (AB-AA/AB) X 100

- AB =absorbance of ABTS radical + methanol
- AA= absorbance of ABTS radical + sample extract

To determine the Fe concentration in plants Sample and standard readings were made using a UV-Vis Spectrophotometer (WE6000). The Zn and Ca contents in digested fluid were determined by using atomic absorption spectrophotometer method (Wilson, 1966).

RESULTS

Vegetative parameters

Statistically results are significant regarding plant fresh and dry weight in response to iron supplementation in spinach which were compared by Tuckey test at 1% probability level. Maximum average plant fresh weight (29.25g), plant dry weight (6.71g), plant longest shoot and root length (11.80cm and 7.06cm), number of leaves (6.50) and leaf area (15.04cm^2) in spinach were noticed when Fe was applied @ 1.5mM concentration (Table 2). Interaction effect between treatment and method is illustrated in table 3. Comparison of treatment means showed that Zn concentration 200ppm performed best among all the concentrations. Maximum average plant fresh weight (33.09g) and maximum average plant dry weight (7.71g), longest shoot and root length (11.30cm and 7.10cm), number of leaves (6.50) and leaf area (16.63cm^2) were assessed in Zn @200ppm concentration (Table 4). Treatment and method interaction is given in Table 5. In the current study, Comparison of treatment means showed that Ca (200ppm) performed best among all the concentrations. Maximum average fresh weight (25.81g) and maximum average dry weight (6.71g), longest shoot and root length (10.81cm and 5.46cm), number of leaves (6.33) and leaf area (15.13cm^2) were recorded in Ca @ 200ppm concentration (Table 6). However interaction effect is mentioned in Table 7. Foliar application method gave maximum results in contrast to basal application method in all the parameters of iron, zinc and calcium but longest root length was assessed in basal application method.

Table 2. Effect of different Iron treatments on spinach vegetative and biochemical analysis

Parameters	Treatment means			
	(Control)	T_1(1mM)	T_2(1.5mM)	T_3(2mM)
Fresh weight (g)	17.86 (c)	22.96 (b)	29.25 (a)	21.96 (b)
Dry weight (g)	4.50 (c)	5.55 (b)	6.71(a)	5.04 (bc)
Shoot length (cm)	6.76 (c)	8.08 (b)	11.80 (a)	8.33 (b)
Root length (cm)	4.76 (c)	5.56 (b)	7.06 (a)	5.30(bc)
No. of leaves	3.66 (d)	5.66 (b)	6.50 (a)	4.50(d)
Leaf area (cm^2)	7.017 (b)	10.50 (b)	15.04 (a)	9.38 (b)
TSS (◦Brix)	6.73 (c)	11.38 (a)	9.51 (b)	7.51 (c)
Ascorbic acid (mg)	8.33 (c)	11.66 (b)	13.33 (a)	8.5 (c)
Phenols (mg /g FW)	32.66 (a)	28.5 (b)	24.66 (c)	21.66 (c)
LAA (µM trolox g^{-1} FW)	21.76 (b)	27.16 (b)	30.16 (a)	20.33 (b)
Amino acids (nmol/g)	21 (bc)	24.16(b)	28.50 (a)	18.66 (c)
Flavonoids (mg)	9.93 (b)	10.31 (b)	13.43 (a)	8.73 (b)
Carotenoids (µg $g^{-1)}$	4.01(d)	4.89 (b)	5.14 (a)	4.47 (c)
Chlorophyll a (mg/g/FW)	0.32(d)	0.85 (a)	0.62 (b)	0.58(c)
Chlorophyll b (mg/g/FW)	0.26(d)	0.56 (a)	0.43 (b)	0.35 (c)

Table 3. Effect of treatment and method interaction in response to iron supplementation on spinach vegetative and biochemical analysis

Parameter	Foliar				Basal			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Fresh weight(g)	17.86c	27.40b	35.8a	25.16b	17.74c	18.5c	22.6bc	18.7c
Dry weight (g)	4.50c	6.43ab	7.76a	5.30bc	4.44c	4.66c	5.66bc	4.77bc
Shoot length (cm)	6.76c	9.23b	12.1a	8.33bc	6.65c	6.93c	11.4a	8.33bc
Root length (cm)	4.76	6.06	6.86	5.20	4.70	5.06	7.26	5.40
No. of leaves	3.66	6	7	5	3.33	5.33	6	4
Leaf area (cm^2)	7.01	11.16	15.5	9.49	7.01	9.8	14.5	9.26
TSS (◦Brix)	6.73 d	12.7a	10.7b	7.96 cd	6.70d	10 b	8.33c	7.06cd
Ascorbic acid (mg)	8.33d	13ab	15.3ab	9 cd	8.30d	10.3bcd	11.3bc	8 d
Phenols (mg /g f.w)	32.6	29.6	26.3	23	32.4	27.3	23	20.3
LAA (µM trolox g^{-1} f.w.)	21.76c	30ab	35.3a	21c	21.66c	24.3bc	25bc	19.6c
Amino acids (nmol/g)	21	25.3	30.3	20.6	20.5	23	26.6	16.6
Flavonoids (mg)	9.93bc	11.3b	15.6a	10.13bc	9.1bc	9.30bc	11.2b	7.33c
Carotenoids (µg $g^{-1)}$	4.01 d	5.55a	5.83a	4.37bc	4. d	4.22 cd	4.44bc	4.57b
Chlorophyll a (mg/g/FW)	0.32 e	1.17 a	0.77b	0.61c	0.31 e	0.54cd	0.46d	0.42de
Chlorophyll b (mg/g/FW)	0.26d	0.71a	0.54b	0.36cd	0.24d	0.41c	0.33cd	0.34cd

Table 4. Effect of different Zinc treatments and application methods on spinach vegetative and biochemical analysis

Parameters (Vegetative)	Treatment means			
	(Control)	T_1(100ppm)	T_2(150ppm)	T_3(200ppm)
Fresh weight (g)	17.86 (c)	18.36 (c)	27.31 (b)	33.09(a)
Dry weight (g)	4.50 (c)	4.65 (c)	5.53 (b)	7.71(a)
Shoot length (cm)	6.76 (c)	7.05(c)	8.58 (b)	11.30 (a)
Root length (cm)	4.76 (c)	5.73(b)	6.10 (b)	7.10 (a)
No. of leaves	3.66 (b)	4.33 (b)	5.66 (a)	6.50(a)
Leaf area (cm^2)	7.017 (b)	13.47 (a)	15.55 (a)	16.63 (a)
TSS (◦Brix)	6.73 (c)	7.63 (c)	9.51 (b)	11.23 (a)
Ascorbic acid (mg)	8.33 (c)	10.58 (b)	11.43 (b)	14.00 (a)
Phenols (mg /g f.w)	32.66 ab	28.83 (b)	33.00 (ab)	37.33 (a)
LAA (µM trolox g^{-1} f.w.)	21.76 (b)	23.16 (b)	25.33 (ab)	28.66 (a)
Amino acids (nmol/g)	21.00 (c)	21.67 (c)	27 (b)	31.33 (a)
Flavonoids (mg)	9.93 (c)	11 (c)	14.33 (b)	15.66 (a)
Carotenoids (µg $g^{-1)}$	4.01(d)	4.42(c)	4.68 (b)	5.15(a)
Chlorophyll a (mg/g/F. wt)	0.32(d)	0.50 (c)	0.66 (b)	0.97(a)
Chlorophyll b (mg/g/F. wt)	0.26(b)	0.34 (b)	0.47 (a)	0.55 (a)

Table 5. Effect of treatment and method interaction in response to Zinc supplementation on spinach vegetative and biochemical analysis

Parameters	Foliar				Basal			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Fresh weight (g)	17.86	18.48	30.1	35.7	17.74	18.2	24.5	30.4
Dry weight (g)	4.50c	4.70d	6.36bc	8.46a	4.44c	4.60d	5.30cd	6.96b
Shoot length (cm)	6.76	7.20	9.0	12.03	6.65	6.90	8.16	10.5
Root length (cm)	4.76	5.63	5.90	6.96	4.70	5.83	6.30	7.23
No. of leaves	3.66	4.66	6	7	3.33	4	5.33	6
Leaf area (cm^2)	7.01	14.18	16.5	17.7	7.01	12.7	14.6	15.5
TSS (◦Brix)	6.73 d	8.56c	11 b	12.9a	6.70d	6.70d	8.03cd	9.53bc
Ascorbic acid (mg)	8.33d	12.3bc	13.5ab	16a	8.30 d	8.83de	9.33cde	12 bcd
Phenols (mg /g f.w)	32.6a	33.6b	36.6b	42.3a	32.4a	24 c	29.3bc	32.3b
LAA (µM trolox g^{-1} f.w.)	21.76	24.3	27.6	30.6	21.66	22	23	26.6
Amino acids (nmol/g)	21	23.6	28.3	33.6	20.5	19.6	25.6	29
Flavonoids (mg)	9.93bc	12 d	17 a	15.3ab	9.1bc	10 e	14.3b	13.3cd
Carotenoids (µg $g^{-1)}$	4.01 d	4.55c	5.87 a	4.86 b	4. d	4.29 cd	4.43 c	4.51 c
Chlorophyll a (mg/g/F. wt)	0.32 e	0.61cd	0.81b	1.23 a	0.31 e	0.39e	0.51 d	0.71bc
Chlorophyll b (mg/g/F. wt)	0.26c	0.40c	0.58ab	0.68a	0.24 c	0.29c	0.36c	0.42bc

Table 6. Effect of Calcium supplementation on spinach vegetative and biochemical analysis

Parameters (Vegetative)	Treatment means			
	(Control)	T_1(1mM)	T_2(1.5mM)	T_3(2mM)
Fresh weight (g)	17.86 b	16.95 b	23.33 a	25.81 a
Dry weight (g)	4.50 c	4.46 c	5.66 b	6.71 a
Shoot length (cm)	6.76 c	6.36 c	8.46 b	10.81 a
Root length (cm)	4.76 bc	4.40 c	5.10 ab	5.46 a
No. of leaves	3.66 bc	3.33 c	4.83 b	6.33a
Leaf area (cm^2)	7.017 c	9.93 b	14 a	15.13 a
TSS (◦Brix)	6.73 c	7.11 c	8.83 b	10.01 a
Ascorbic acid (mg)	8.33 c	8.66 b	10.50 b	12 a
Phenols (mg /g f.w)	32.66 b	31.33 b	33.33 ab	38 a
LAA (µM trolox g^{-1}f.w.)	21.76 b	22.50 b	24.83 ab	28 a
Amino acids (nmol/g)	21 c	20.66 c	24.50 b	30.67 a
Flavonoids (mg)	9.93 a	8.83 a	8.98 a	9.60 a
Carotenoids (µg $g^{-1)}$	4.01c	4.06 c	4.35 b	4.63 a
Chlorophyll a (mg/g/F. wt)	0.32c	0.54 b	0.62 b	0.99 a
Chlorophyll b (mg/g/F. wt)	0.26c	0.33 c	0.41 b	0.54 a

Table 7. Effect of treatment and method interaction in response to calcium supplementation on spinach vegetative and biochemical analysis

Parameters	Foliar				Basal			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Fresh weight (g)	17.86	17.45	23.8	27.26	17.74	16.4	22.80	24.36
Dry weight (g)	4.50c	4.63de	6bc	7.16a	4.44c	4.30e	5.33cd	6.26b
Shoot length (cm)	6.76	6.56d	8.93	11.66	6.65	6.16	8	9.96
Root length (cm)	4.76	4.26	5	5.33	4.70	4.53	5.20	5.60
No. of leaves	3.66	3.33	5	6.66	3.33	3.33	4.66	6
Leaf area (cm^2)	7.01b	12.92a	14.84a	16.63a	7.01b	6.93b	13.17a	13.63a
TSS (◦Brix)	6.73 d	7.93c	10.43b	12.23a	6.70d	6.30d	7.23cd	7.80c
Ascorbic acid (mg)	8.33d	9.33c	12b	14.33a	8.30 d	8c	9c	9.66c
Phenols (mg /g f.w)	32.6a	35.33b	36.66ab	43.6a	32.4a	27.33c	30bc	32.3bc
LAA (µM trolox g^{-1} f.w.)	21.76	23.66	26.6	30	21.66	21.3	23	26
Amino acids (nmol/g)	21 d	23bcd	27b	35.3a	20.5d	18.3d	22cd	26bc
Flavonoids (mg)	9.93	9.66	9.83	9.98	9.1	8	8.13	9.23
Carotenoids (µg $g^{-1)}$	4.01	4.11	4.47	4.81	4.0	4.02	4.23	4.45
Chlorophyll a (mg/g/F. wt)	0.32 d	0.71b	0.77b	1.33a	0.31 d	0.37d	0.47cd	0.65bc
Chlorophyll b (mg/g/F. wt)	0.26c	0.37bc	0.50ab	.63a	0.24c	0.28c	0.33c	0.46b

Biochemical indices

Comparison of treatment means showed that Fe @1mM concentration performed best regarding TSS (11.38 ◦Brix) while Fe @ 1.5mM gave maximum ascorbic acid (13.33 mg/100 g). Maximum phenolic contents (32.66 mg/g FW) were noted in control treatment and LAA (30.16 µM trolox g^{-1} FW) were recorded in 1.5mM concentration of Iron. Fe @1.5mM concentration performed best regarding amino acids (28.50 nmol g^{-1}), flavonoids (13.43 mg) and carotenoids (5.14 µg g^{-1}). Fe concentration @1mM concentration performed best regarding chlorophyll a (0.85 mg/g/FW) and chlorophyll b (0.563 mg/g/FW) given in Table 2. Interaction effect between treatment and method is illustrated in Table 3.

In this study, Comparison of treatment means showed that 200ppm Zn concentration gave maximum TSS (11.23◦Brix), ascorbic acid (14 mg/100 g), phenolic contents (37.33 mg/g FW), LAA (28.66 µM trolox g^{-1} FW), flavonoids, carotenoids and amino acids (15.66 mg, 5.15 µg g^{-1} and 31.33 nmol g^{-1}), chlorophyll a (0.975 mg/g/FW) and chlorophyll b (0.55 mg/g/FW) contents (Table 4). Treatment and method interaction is given in Table 5.

In the current work, treatment means showed that 200ppm Ca concentration performed best regarding TSS (10.01◦Brix), ascorbic acid (12 mg), maximum phenolic contents (38 mg /g FW) and LAA (28 µM trolox g^{-1} FW), amino acids (30.66 nmol g^{-1}), flavonoids (9.93 mg) and carotenoids (4.63 µg g^{-1}), chlorophyll a and chlorophyll b contents (Table 6). However interaction effect is mentioned in Table 7. Maximum results in all the nutrients regarding all the biochemical parameters were noted in foliar application method.

Nutrient analysis

Our results demonstrated that maximum iron concentration (2.49mg in shoots in foliar and 2.59mg in roots in basal application) was measured in Fe @2mM concentration (Figure 1). Furthermore, Zn @200ppm concentration depicted maximum zinc contents (252.47mg in shoots and 230.80mg in roots) in spinach (Figure 2). Our results also illustrated that Ca @200ppm concentration assessed maximum calcium contents (83.73mg in shoots and 64.07mg in roots) in foliar and basal application method (Figure 3), respectively.

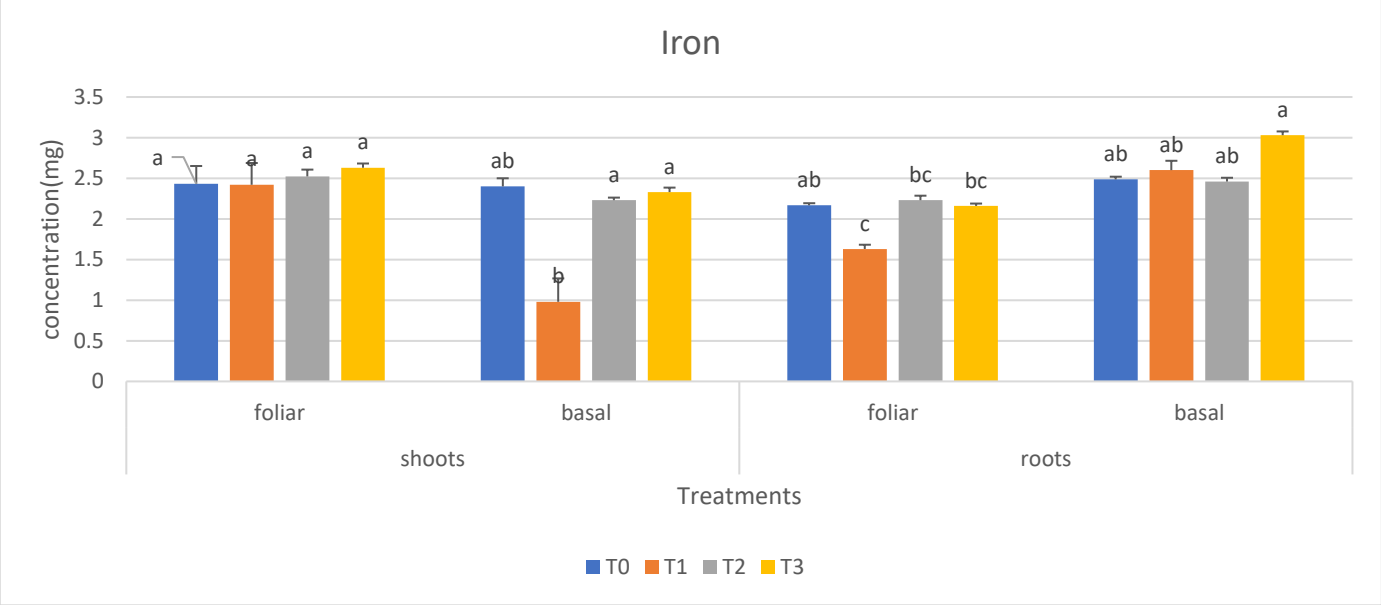

Figure 1. Iron concentration in shoots and roots of spinach plants, when supplemented through foliar and basal application methods

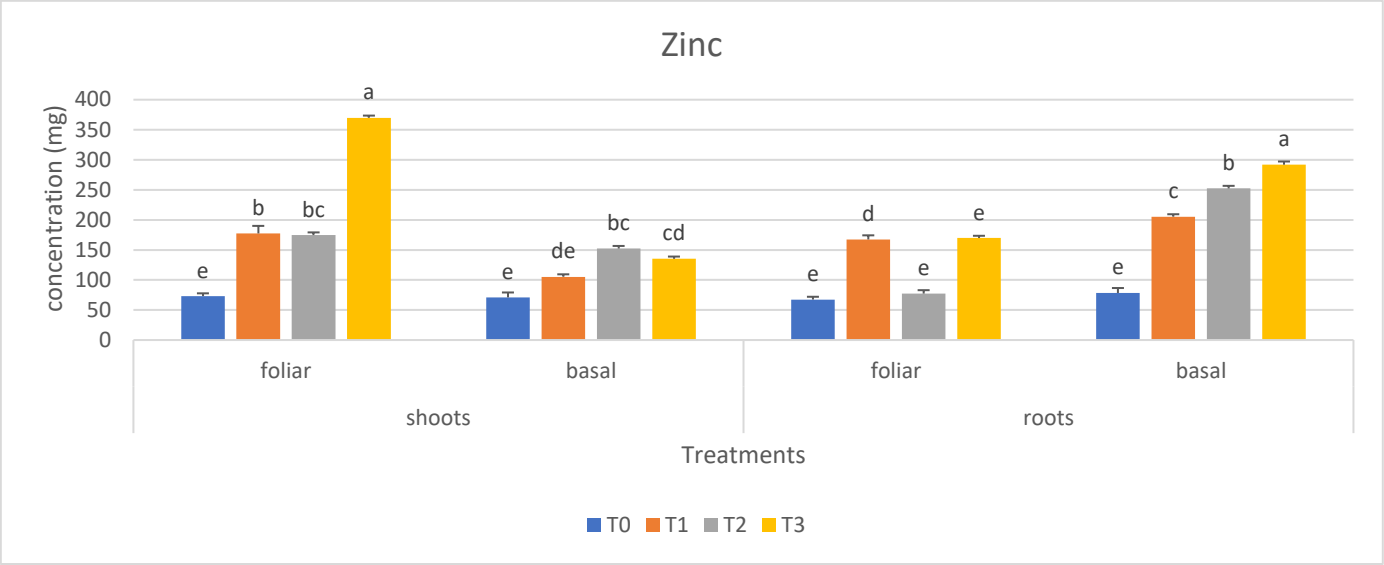

Figure 2. Zinc concentration in shoots and roots of spinach plants, when supplemented through foliar and basal application methods

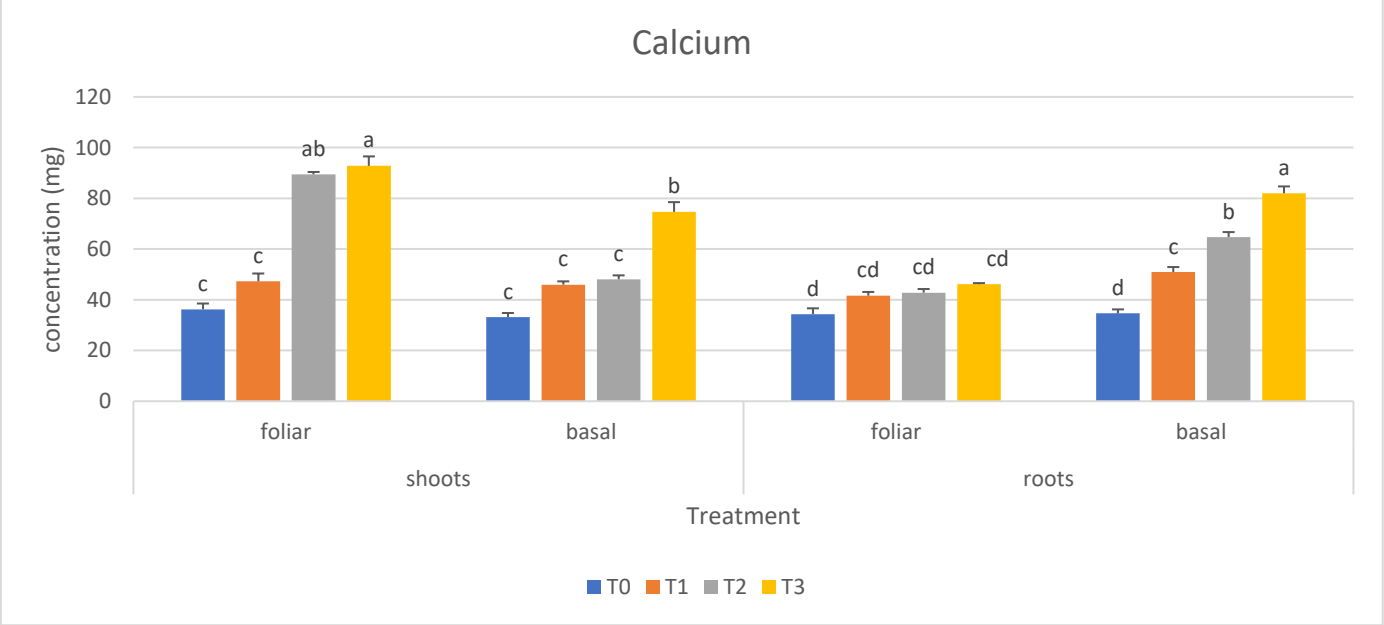

Figure 3. Calcium concentration in shoots and roots of spinach plants, when supplemented through foliar and basal application methods

DISCUSSION

In the current work, foliar application of zinc (200ppm) enhanced the spinach plant biomass as it accumulates in the vegetative tissues. Present study also confirms the findings of (Zou and Zhang, 2011) that foliar supplementation of zinc was mobile in the phloem tissues of plant which further transferred to developing grains. It may be due to no interaction of zinc with soil and this foliar application method prevented it from fixation. In vegetable crops as well as in a crop like wheat, foliar application of Zn has been found to be superior in increasing grain Zn concentration comparative to soil application. In basal applications iron concentrations did not performed well comparative to foliar this might be because iron is only be slightly mobile in plants (Tiffin, 1970). Minimum chlorophyll contents were recorded in control treatment in this experiment results confirms the studies of (Broadley, 2012) that lack/ deficiency of iron results in chlorosis of young leaves of herbaceous plants and chlorophyll deficiency. Moreover, in the current investigation Ca @200ppm concentration significantly increased plant fresh weight, number of leaves, leaf area and biochemical indices. There were no negative effects of calcium biofortificaton on spinach plants and these results are in accordance with findings of (Imperio et al., 2016) that Ca biofortification @200mgL^{-1} in baby leaf vegetables

caused a significant enhancement of Ca and it doesn't affected the vegetable growth but boosted the vegetative mass of plant, biochemical contents and marketable quality.

CONCLUSION

The application of Fe @1.5mM concentration illustrated maximum results regarding vegetative parameters (plant fresh weight, plant dry weight, shoot and root length, number of leaves and leaf area) and some biochemical parameters including ascorbic acid, lipophilic antioxidants, amino acids, flavonoids and carotenoids while TSS and chlorophyll contents gave maximum results in Fe @1mM concentration. However phenolic contents were maximum in control treatment. Foliar application method was better in contrast to basal application method in all the morphological and analytical parameters. Spinach plants growing in Zn basal and foliar applications with various concentrations (100ppm, 150ppm and 200ppm) increased the plant biomass and biochemical indices with Zn @200ppm concentration in foliar application method. Overall, non-significant differences were assessed in Zn @100ppm and Zn@150ppm concentration. Ca @200ppm concentration indicated maximum results in all morphological and analytical properties. Calcium concentrations also did not affected flavonoids contents. Longest root length and shoot length was observed in basal application method while highest results in all other parameters were evaluated in foliar application method.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors declare that the research was carried without any commercial or financial relationships that could be construed as a potential conflict of interest.

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