

Efficacy of Iron Fortification to Augment the Nutritional Quality of Some Winter Season Leafy Vegetables

Kshouni Das^{1*}, Ranjit Chatterjee¹ and Trisha Sinha²

¹*Department of Vegetable and Spice Crops, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India.*

²*Department of Botany and Plant Physiology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Author KD investigated the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RC designed the experiment, guided properly to perform the experiment successfully and also contributed his utmost effort on final framing of the manuscript and author TS managed the literature searches and result discussion portion actively. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/CJAST/2020/v39i2330857

Editor(s):

(1) Dr. Md. Hossain Ali, Bangladesh Agricultural University, Bangladesh.

Reviewers:

(1) Luis Rodrigo, University of Oviedo, Spain.

(2) Tahseen Fatima Miano, Sindh Agriculture University, Pakistan.

(3) Shaymaa Abdul Hadi Kadhim Almansoori, Babylon University, Iraq.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/60017>

Original Research Article

Received 05 June 2020

Accepted 11 August 2020

Published 17 August 2020

ABSTRACT

Iron is one of the most important micronutrients essential for human subsistence which is available in our diet through different vegetables (leafy vegetables, leguminous vegetables, cruciferous vegetables, cucurbits, potato, sweet potato, drumstick etc.) but especially the leafy vegetables. The different leafy vegetables are the reservoir of different vitamins and minerals that mostly include calcium, phosphorous and iron. Iron deficiency leads to anaemia is a threat throughout the world, more specifically found in women and children. Enrichment of iron content of these leafy vegetables can be achieved through iron fertilization which may play vital role to alleviate the problem of anaemia. Besides this, less bioavailability of non heme iron content (iron in plants) is also a big challenge. Considering these two factors, an experimental study was conducted in factorial

*Corresponding author: E-mail: kshounidas23@gmail.com;

randomized block design with three replications during the winter season of the year of 2018-19 at UBKV, Pundibari, Cooch Behar to evaluate the status of iron enrichment in ten (10) popular green leafy vegetables (*Amaranthus*, buck wheat leaves, coriander leaves, fenugreek leaves, garden pea leaves, *Malva* leaves, mustard leaves, onion leaves, palak leaves, radish leaves). Ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16% Fe) was applied as a source of iron fertilizer in three different modes (Soil, foliar and combination of soil and foliar) along with control. Application of iron fertilizer significantly increased the leaf iron content and it showed synergistic effect on other quality parameters like ascorbic acid content, vitamin A content, and total chlorophyll content of the leafy vegetables. Highest leaf iron content at first (22.43 mg/100 g), second (21.30 mg/100 g) and third (20.26 mg/100 g) harvesting was found in *Amaranthus* from the treatment of 100% of recommended dose (0.5 g/lit of water) of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16% Fe) through foliar spray at 4 weeks after sowing (T_2L_1). Therefore, iron fortification of leafy vegetables through ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16% Fe) application might be a feasible alternative to mitigate the problem of iron deficiency anaemia as well as to meet the daily needs of iron through consumption of iron rich leafy vegetables.

Keywords: Anaemia; ferrous sulphate; iron fortification; leafy vegetables.

1. INTRODUCTION

Leafy vegetables constitute a major role in balanced human daily diet. They provide dietary fibre, protein, essential vitamins and minerals. Most of the leafy vegetables are rich in carotene (provitamin A), riboflavin (vitamin B₂), ascorbic acid (vitamin C) and minerals like iron, calcium and phosphorous [1,2]. It is estimated that 100g of tropical leafy vegetables can provide 60-140 mg of vitamin C, 100 micro gram of folic acid, 4-7 mg iron and 200-400 mg of calcium [2]. Iron is one of the most important micronutrients essential for human subsistence which is available in our diet through different vegetables and especially the leafy vegetables. The body requires iron for the synthesis of its oxygen transport proteins particularly haemoglobin and myoglobin, and for the formation of heme enzymes and other iron-containing enzymes involved in electron transfer and oxidation-reduction reactions [3,4]. Iron deficiency anaemia is a severe health problem throughout the world especially in women and children. Globally more than 2 billion people are suffering from iron-deficiency that results in anaemia which is associated with symptoms like general fatigue, weakness, pale skin, shortness of breath, dizziness, tongue swelling, reduced productivity cold hands and feet, brittle nails and headaches [5]. Hence, enhancing iron concentration in the crops through iron fortification becomes urgent necessity to overcome the burning worldwide problem. Among different biofortification methods (agronomic, traditional breeding, transgenic biofortification etc), agronomic means of iron fortification is followed in the present experimental study which may eradicate the

emerging need of iron enrichment of leafy vegetables as a solution to mitigate the problem of iron deficiency anaemia.

2. MATERIALS AND METHODS

The present experiment was conducted at the Instructional Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar (26°19' 86" N latitude and 89° 23'53"E longitude, at an elevation of 43 meter above the mean sea level), West Bengal, India during the winter season of 2018-19. The soil of the experimental site is sandy loam in nature having a pH of 5.78 with 0.89% organic carbon, 117.6 kg/ha available nitrogen, 15.0 kg/ha available phosphorus and 104.23 kg/ha available potassium. This region comes under subtropical humid region with an average minimum temperature ranging from 7 to 8°C to and maximum of 24 to 33.2°C during the experimental period (November- March).

The experiment was laid out in factorial randomized block design with three replications. Treatments consist of ten winter season leafy vegetables [L₁- *Amaranthus* leaves (*Amaranthus tricolor*), L₂- Buck wheat leaves (*Fagopyrum esculentum*), L₃- Coriander leaves (*Coriandrum sativum*), L₄- Fenugreek leaves (*Trigonella foenum-graecum*), L₅- Garden pea leaves (*Pisum sativum* var. *hortense*), L₆- *Malva* leaves (*Malva verticillata*), L₇- Mustard leaves (*Brassica campestris*), L₈- Onion leaves (*Allium cepa*), L₉- Palak leaves (*Beta vulgaris* var. *bengalensis*), L₁₀- Radish leaves (*Raphanus sativus*)] with three different modes of iron fertilizer, ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16% Fe) application along with control. The treatments

were T₁- 100% of recommended dose (2.5 kg/ha) through soil application, T₂ -100% of recommended dose (0.5 g/lit of water) through foliar spray at 4 weeks after sowing, T₃ -50% of recommended dose through soil application (1.25 kg/ha) + 50% of recommended dose through foliar spray (0.25 g/lit of water) at 4 weeks after sowing and T₄- Control plot. Seeds of different leafy vegetables were sown in the field in plot size of 1.5m x 1.5 m maintaining a spacing of 30 cm x 15 cm. The crops were raised by following standard package of practices. Sowing was done in middle of November and first harvesting was started in middle of December at 35 days after sowing and continued till middle of January. Farmyard manure @ 20 t /ha and a common fertilizer dose of N: P₂O₅: K₂O @ 50:25:25 kg/ha was applied to each plots irrespective of treatment application. Estimation of different quality parameters namely iron content of leaves, ascorbic acid content, vitamin A content and chlorophyll content of fresh leaves at different harvesting stages were determined. For estimation of leaves iron content, dried leaf sample (0.5g) was digested in tri-acid mixture (HNO₃: H₂SO₄: HClO₄ : : 9 : 4 : 1) on hot plate and after digestion filtration was done through Whatman No. 42 filter paper and volume make up was made in 50 mL volumetric flask and reading was taken in Atomic Absorption Spectrophotometer (AAS). Ascorbic acid content was estimated by following standard biochemical methods (AOAC, 1990) [6] and vitamin A content was determined by spectrophotometric method (Davies, 1976) [7]. Chlorophyll A, B and total Chlorophyll content were estimated as per the method suggested by Sadasivam and Manickam (1996) [8]. The data obtained of different quality parameters were subjected to statistical analysis by the Analysis of Variance method (Gomez and Gomez, 1984) [9] and the significance of different sources of variations were tested by Error Mean Square by Fisher and Snedecor's 'F' test at probability level 0.05. For determination of critical difference at 5% level of significance, Fisher and Yates' table was consulted.

3. RESULTS

3.1 Iron Content in Leaves (mg/100 g)

The data obtained on iron content of leaves (Table 1) indicated that among the different modes of iron fertilizer applications, different crops and their interaction effects have significant differences in terms of iron content of leaves. Among the different modes of ferrous

sulphate heptahydrate (FeSO₄, 7H₂O, 16% Fe) applications, the maximum iron content of leaves at first (13.38 mg/100 g), second (12.61 mg/100 g) and third harvesting (12.00 mg/100 g) were obtained from the treatment of 50% of recommended dose through soil application (1.25 Kg/ha) + 50% of recommended dose through foliar spray (0.25g/lit of water) at 4 weeks after sowing (T₃).

The leaf iron content varied significantly among the different leafy vegetables and the highest leaf iron content was recorded by *Amaranthus* (L₁) at first (21.28 mg/100 g), second (20.12 mg/100 g) and third harvesting (19.21 mg/100 g) followed by radish leaves at first (18.87 mg/100 g), second (17.89 mg/100 g) and third (16.96 mg/100 g) harvesting and palak at first (17.88 mg/100g), second (16.63 mg/100 g) and third (15.75 mg/100 g) harvesting.

The interaction effects, showed that the highest leaf iron content was obtained from *Amaranthus* leaves through the use of 100% of recommended dose (0.5 g/lit of water) of ferrous sulphate heptahydrate(FeSO₄, 7H₂O , 16% Fe) through foliar spray at 4 weeks after sowing (T₂L₁) for the first (22.43 mg/100 g), second (21.30 mg/100 g) and third (20.26 mg/100g) harvesting respectively. The results are in conformity with the former findings of Prasad et al., (2017) [10] on *Amaranthus*.

3.2 Ascorbic Acid Content of Leaves (mg/100 g)

The results (Table 1) revealed that ferrous sulphate heptahydrate (FeSO₄, 7H₂O, 16% Fe) application in all the modes (soil, foliar and combination of soil and foliar) increased the ascorbic acid content of the leaves significantly. The maximum ascorbic acid content of leaves was found from the treatment of 50% of recommended dose through soil application (1.25 Kg/ha) + 50% of recommended dose through foliar spray (0.5 g/lit of water) at 4 weeks after sowing (T₃) for the first (61.06 mg/100 g), second (63.00 mg/100 g) and third (65.53 mg/100g) harvesting.

Among the different leafy vegetables, highest ascorbic acid content was recorded for coriander leave at first (139.30 mg/100 g), second (143.48 mg/100 g) and third (144.42 mg/100 g) harvesting followed by radish leaves at first (109.06 mg/100 g), second (113.39 mg/100 g) and third (114.08 mg/100 g) harvesting.

The results of interaction effects revealed that maximum ascorbic acid content was found in the coriander leaves raised with 100% of recommended dose (2.5 kg/ha) of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16 % Fe) through soil application (T_1L_3) for the first (143.44 mg/100 g), second (148.22 mg/100 g) and third (148.00 mg/100 g) harvesting. Application of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16% Fe) increased the leaf iron content and it was found to establish synergistic effect on ascorbic acid content of leaves so that ultimately ascorbic acid content of leaves was also increased. This finding was also supported by Sakthidharan (2013) [11].

3.3 Vitamin A Content of Leaves (IU)

The observation of vitamin A content of fresh leaves (Table 1) showed significant variation due to different modes of iron fertilizer application. Highest vitamin A content was recorded in the treatment containing 50% of recommended dose of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16% Fe) through soil application (1.25 Kg/ha) + 50% of recommended dose through foliar spray (0.25 g/lit of water) at 4 weeks after sowing (T_3) for the first (3800.11 IU), second (3893.30 IU) and third (3951.23 IU) harvesting.

Variation in vitamin A content of leaves of the different leafy vegetables may be attributed to differences in genetic constitution of the crops. Highest values of vitamin A content was recorded in *Amaranthus* at first (9111.97 IU), second (9357.10 IU) and third (9515.13 IU) harvesting followed by palak at first (8038.90 IU), second (8251.62 IU) and third (8387.36 IU) harvesting. The lowest amount of vitamin A content was recorded in radish leaves at first (556.45 IU), second (564.56 IU) and third (565.80 IU) harvesting.

The interaction effects showed that maximum vitamin A content was found in the leaves of *Amaranthus* grown with the application of 50% of recommended dose of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16% Fe) through soil application (1.25 Kg/ha) + 50% of recommended dose through foliar spray (0.25g/lit of water) at 4 weeks after sowing (T_3L_1) at first (9195.03 IU), second (9438.14 IU) and third (9600.22 IU) harvesting. Improvement of vitamin A content of leaves through use of iron fertilizer either soil or foliar or by both approaches were also previously reported by Sakthidharan (2013) [11].

3.4 Chlorophyll A, Chlorophyll B and Total Chlorophyll Content of Leaves

Data presented in Table 2 indicated that iron fertilizer application significantly increased the chlorophyll A, chlorophyll B and total chlorophyll content of leaves. Highest chlorophyll A content was estimated for the treatment containing 50% of recommended dose of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16%Fe) through soil application (1.25 Kg/ha) + 50% of recommended dose through foliar spray (0.25g/lit of water) at 4 weeks after sowing (T_3) at first (221.42 mg/100 g), second (236.77 mg/100 g) and third (227.21 mg/100 g) harvesting, whereas, highest amount of chlorophyll B content for the first (89.83 mg/100 g), second (95.02 mg/100 g), third (89.50 mg/100 g) harvesting as well as total chlorophyll content was found to be maximum at first (310.62 mg/100 g), second (331.04 mg/100 g) and third (316.02 mg/100 g) harvesting respectively when 100% of recommended dose (0.5 g/lit of water) of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16%Fe) was applied through foliar spray at 4 weeks after sowing (T_2). Significant increase in chlorophyll content of leaves due to iron application might be as a result of active involvement of iron in chlorophyll synthesis which was also supported by Prasad et al., (2017) [10] in case of *Amaranthus* and Tavakoli et al.,(2015) [12] in lettuce.

Among the ten different leafy vegetables, highest chlorophyll A content at first (347.27 mg/100 g), second (370.96 mg/100 g), third (356.01 mg/100 g) harvesting and the highest chlorophyll B content at first (234.07 mg/100 g), second (249.28 mg/100 g), third (237.52) harvesting as well as the total chlorophyll content at first (580.18 mg/100 g), second (619.01 mg/100 g) and third (592.35 mg/100 g) harvesting were observed in palak leaves. Similar findings were also reported by Aslam et al., (2016) [13] for total chlorophyll content of spinach. Second highest chlorophyll A content at first (271.94 mg/100 g), second (290.85 mg/100 g) and third (279.20 mg/100 g) harvesting, chlorophyll B content at first (100.55 mg/100 g), second (106.19 mg/100 g) and third (100.43 mg/100 g) harvesting and total chlorophyll content at first (371.85 mg/100 g), second (396.36 mg/100 g) and third (378.98 mg/100 g) harvesting were found in radish leaves. Variation in vitamin A content among the different leafy vegetables might be due to different genetic constitution of the crops.

Table 1. Response of iron fertilizer on Iron content, ascorbic acid content and vitamin A content of different leafy vegetables

	Iron content in leaves(mg/100 g)			Ascorbic acid content in leaves (mg/100 g)			Vitamin A content in leaves (IU)		
	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest
	Mode			Mode			Mode		
T ₁	12.89	12.22	11.60	60.38	62.54	63.97	3771.74	3863.59	3919.77
T ₂	13.03	12.33	11.68	60.81	62.96	65.47	3788.76	3883.04	3939.47
T ₃	13.38	12.61	12.00	61.06	63.00	65.53	3800.11	3893.30	3951.23
T ₄	12.15	11.52	10.93	57.84	59.96	60.67	3741.76	3828.20	3881.24
SEm±	0.06	0.06	0.06	0.24	0.27	0.29	4.17	3.99	4.11
CD at 5%	0.16	0.18	0.16	0.66	0.76	0.81	11.74	11.23	11.57
	Crops			Crops			Crops		
L ₁	21.28	20.12	19.21	102.50	106.07	107.00	9111.97	9357.10	9515.13
L ₂	12.03	11.46	10.86	25.55	26.43	29.00	3018.64	3081.44	3121.61
L ₃	1.87	1.76	1.66	139.30	143.48	144.42	6661.94	6837.52	6944.89
L ₄	16.20	15.38	14.59	53.83	56.25	57.00	3797.95	3883.71	3938.41
L ₅	8.36	7.95	7.53	31.64	32.59	35.67	643.57	648.97	652.35
L ₆	7.53	7.16	6.78	15.08	15.71	17.25	1968.53	2007.02	2029.32
L ₇	16.74	15.88	15.09	33.44	34.11	36.08	2963.94	3030.12	3064.80
L ₈	7.87	7.46	7.09	17.35	17.94	21.42	994.06	1008.24	1009.59
L ₉	17.88	16.63	15.75	72.50	75.18	77.17	8038.90	8251.62	8387.36
L ₁₀	18.87	17.89	16.96	109.06	113.39	114.08	556.45	564.56	565.80
SEm±	0.09	0.09	0.09	0.37	0.43	0.46	6.59	6.31	6.50
CD at 5%	0.26	0.28	0.25	1.04	1.20	1.28	18.56	17.76	18.30
	Interaction			Interaction			Interaction		
T ₁ L ₁	20.93	19.64	18.74	100.94	105.71	105.67	9081.58	9332.79	9492.17
T ₁ L ₂	12.24	11.83	11.16	25.00	26.07	27.33	3033.50	3098.33	3136.14
T ₁ L ₃	2.02	1.89	1.78	143.44	148.22	148.00	6650.46	6826.04	6928.69
T ₁ L ₄	16.44	15.61	14.79	53.75	56.79	57.67	3789.84	3873.58	3924.91
T ₁ L ₅	8.40	8.04	7.59	31.88	32.14	34.33	640.19	645.60	653.70
T ₁ L ₆	7.34	7.03	6.64	15.31	15.71	17.00	1955.70	1996.21	2017.83
T ₁ L ₇	17.74	16.81	16.00	33.75	33.93	35.33	2971.37	3036.20	3074.01
T ₁ L ₈	8.16	7.74	7.36	18.13	18.57	24.33	1002.16	1015.67	1021.07
T ₁ L ₉	17.10	16.02	15.39	71.88	74.29	75.67	8030.79	8244.19	8379.25
T ₁ L ₁₀	18.52	17.54	16.57	109.69	113.93	114.33	561.86	567.26	569.96
T ₂ L ₁	22.43	21.30	20.26	106.57	108.93	111.33	9141.01	9392.22	9548.89
T ₂ L ₂	11.81	11.19	10.62	26.25	27.14	30.00	3009.19	3071.31	3110.43
T ₂ L ₃	1.83	1.73	1.64	137.50	143.93	146.33	6699.08	6874.66	6982.71
T ₂ L ₄	15.91	15.10	14.33	54.38	56.07	56.33	3806.05	3900.59	3954.62
T ₂ L ₅	8.33	7.90	7.49	32.50	33.57	39.33	659.10	669.91	669.91
T ₂ L ₆	7.63	7.24	6.87	15.63	16.43	19.00	1998.92	2042.14	2071.96
T ₂ L ₇	16.14	15.32	14.54	33.13	34.64	38.33	2957.86	3022.69	3052.40
T ₂ L ₈	7.81	7.41	7.04	17.19	17.85	20.00	1021.07	1029.17	1037.28
T ₂ L ₉	19.35	18.04	16.79	74.06	76.07	78.33	8047.00	8268.50	8403.57
T ₂ L ₁₀	19.06	18.09	17.17	110.94	115.00	115.67	548.35	559.16	562.88
T ₃ L ₁	21.51	20.32	19.61	104.69	107.86	108.67	9195.03	9438.14	9600.22
T ₃ L ₂	12.87	12.17	11.55	26.56	27.14	33.00	3057.81	3125.34	3168.56
T ₃ L ₃	1.92	1.82	1.71	141.56	143.57	143.67	6672.07	6850.35	6963.80
T ₃ L ₄	17.15	16.28	15.44	55.31	57.86	59.33	3827.66	3916.80	3976.23
T ₃ L ₅	8.67	8.23	7.81	32.19	33.21	36.67	653.70	659.10	661.80
T ₃ L ₆	7.94	7.54	7.15	15.31	16.07	17.67	1980.01	2017.83	2039.38
T ₃ L ₇	17.41	16.53	15.71	34.06	34.64	36.67	2990.28	3052.41	3092.92
T ₃ L ₈	8.00	7.59	7.19	17.19	17.86	23.00	980.55	999.46	996.76
T ₃ L ₉	18.45	16.82	15.99	73.44	76.43	80.33	8068.61	8284.71	8422.47
T ₃ L ₁₀	19.86	18.85	17.89	110.31	115.36	116.33	575.36	588.87	590.11
T ₄ L ₁	20.23	19.20	18.23	97.81	101.79	102.33	9030.26	9265.26	9419.24
T ₄ L ₂	11.20	10.63	10.09	24.38	25.36	25.67	2974.07	3030.79	3071.31

	Iron content in leaves(mg/100 g)			Ascorbic acid content in leaves (mg/100 g)			Vitamin A content in leaves (IU)		
	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest
T ₄ L ₃	1.70	1.61	1.52	134.69	138.21	139.67	6626.15	6799.03	6904.38
T ₄ L ₄	15.31	14.54	13.79	51.87	54.28	54.67	3768.23	3843.87	3897.89
T ₄ L ₅	8.05	7.64	7.24	30.00	31.43	32.33	621.29	621.28	623.98
T ₄ L ₆	7.20	6.83	6.48	14.06	14.64	15.33	1939.49	1971.91	1988.12
T ₄ L ₇	15.66	14.87	14.12	32.81	33.21	34.00	2936.25	3009.18	3039.87
T ₄ L ₈	7.50	7.11	6.75	16.88	17.50	18.33	972.45	988.65	983.25
T ₄ L ₉	16.60	15.63	14.83	70.63	73.93	74.33	8009.19	8209.07	8344.14
T ₄ L ₁₀	18.04	17.09	16.21	105.31	109.29	110.00	540.25	542.95	540.25
SEm±	0.18	0.22	0.18	0.74	0.85	0.90	13.19	12.61	13.00
CD at 5%	0.52	0.61	0.51	2.08	2.40	2.54	37.12	35.51	36.60

Table 2. Response of iron fertilizer on chlorophyll A, chlorophyll B and total chlorophyll content of different leafy vegetables

	Chlorophyll A content in leaves(mg/100 g)			Chlorophyll B content in leaves(mg/100 g)			Total chlorophyll content in leaves (mg/100 g)		
	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest
	Mode			Mode			Mode		
T ₁	218.84	233.95	224.46	85.81	90.74	85.61	304.12	324.12	309.53
T ₂	221.34	236.59	227.07	89.83	95.02	89.50	310.62	331.04	316.02
T ₃	221.42	236.77	227.21	89.61	94.56	89.20	310.49	330.75	315.85
T ₄	216.67	231.63	222.25	82.12	86.87	81.89	298.27	317.96	303.62
SEm±	0.24	0.28	0.26	0.27	0.28	0.25	0.44	0.45	0.41
CD at 5%	0.69	0.78	0.74	0.77	0.78	0.69	1.24	1.26	1.14
	Crops			Crops			Crops		
L ₁	195.48	209.16	200.59	69.97	73.15	68.72	265.00	281.83	268.86
L ₂	187.87	200.87	192.83	66.10	69.45	64.82	253.54	269.85	257.22
L ₃	186.75	199.62	191.58	58.66	61.77	57.61	245.00	260.96	248.78
L ₄	170.73	182.46	175.00	59.99	63.34	59.52	230.33	245.39	234.11
L ₅	262.07	280.27	268.92	82.35	86.70	81.65	343.85	366.36	349.99
L ₆	175.04	187.04	179.50	61.78	65.86	61.79	236.42	252.47	240.87
L ₇	242.59	259.42	248.98	76.82	81.13	76.29	318.88	339.99	324.74
L ₈	155.95	166.70	159.86	58.13	61.11	57.14	213.71	227.42	216.63
L ₉	347.27	370.96	356.01	234.07	249.28	237.52	580.18	619.01	592.35
L ₁₀	271.94	290.85	279.20	100.55	106.19	100.43	371.85	396.36	378.98
SEm±	0.39	0.44	0.42	0.43	0.44	0.39	0.70	0.71	0.64
CD at 5%	1.09	1.23	1.17	1.22	1.23	1.09	1.96	1.99	1.80
	Interaction			Interaction			Interaction		
T ₁ L ₁	194.58	208.14	199.49	67.19	70.97	66.85	261.33	278.64	265.89
T ₁ L ₂	186.78	199.83	191.86	64.59	67.45	62.71	250.94	266.83	254.15
T ₁ L ₃	185.84	198.63	190.57	56.89	60.22	56.24	242.33	258.42	246.41
T ₁ L ₄	169.64	181.47	174.00	58.47	61.43	57.60	227.73	242.48	231.21
T ₁ L ₅	263.16	281.53	270.12	84.32	88.51	83.58	346.90	369.43	353.11
T ₁ L ₆	176.01	188.08	180.44	63.07	67.40	63.26	238.67	255.04	243.29
T ₁ L ₇	241.56	258.11	247.65	74.73	78.87	74.68	315.77	336.43	321.80
T ₁ L ₈	154.77	165.41	158.58	57.37	60.03	55.97	211.78	225.05	214.18
T ₁ L ₉	345.02	368.42	353.59	232.89	248.03	235.95	576.76	615.22	588.37
T ₁ L ₁₀	271.05	289.84	278.28	98.59	104.46	99.22	369.01	393.62	376.85
T ₂ L ₁	198.53	212.34	203.77	75.24	78.56	73.67	273.29	290.39	276.97
T ₂ L ₂	188.67	201.59	193.66	67.54	71.55	66.44	255.77	272.67	259.65
T ₂ L ₃	189.74	202.75	194.78	63.49	66.74	62.00	252.81	269.04	256.35
T ₂ L ₄	171.78	183.56	176.10	60.97	64.31	60.48	232.36	247.45	236.18
T ₂ L ₅	264.63	282.91	271.59	87.43	92.39	86.33	351.47	374.68	357.33

	Chlorophyll A content in leaves(mg/100 g)			Chlorophyll B content in leaves(mg/100 g)			Total chlorophyll content in leaves (mg/100 g)		
	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest	First harvest	Second harvest	Third harvest
	Mode			Mode			Mode		
T ₂ L ₆	173.74	185.60	178.13	59.89	64.29	60.46	233.23	249.46	238.19
T ₂ L ₇	243.24	260.34	249.96	77.77	82.43	77.49	320.48	342.20	326.91
T ₂ L ₈	156.74	167.55	160.72	59.57	62.53	58.47	215.94	229.69	218.81
T ₂ L ₉	351.50	375.40	360.01	240.01	256.05	244.18	590.33	630.20	602.99
T ₂ L ₁₀	274.82	293.91	282.01	106.34	111.37	105.52	380.50	404.57	386.86
T ₃ L ₁	196.21	209.94	201.34	72.44	74.69	70.18	268.19	284.15	271.06
T ₃ L ₂	190.73	203.90	195.72	70.81	74.36	69.70	261.08	277.78	264.96
T ₃ L ₃	187.47	200.48	192.47	60.31	63.56	59.20	247.37	263.59	251.25
T ₃ L ₄	173.84	185.58	178.11	64.23	67.95	64.12	237.67	253.10	241.82
T ₃ L ₅	261.36	279.77	268.13	80.60	84.41	79.93	341.39	363.59	347.49
T ₃ L ₆	177.94	190.27	182.68	65.65	69.51	65.00	243.17	259.33	247.25
T ₃ L ₇	245.80	262.94	252.40	81.94	86.23	80.98	327.19	348.59	332.82
T ₃ L ₈	158.93	169.87	163.04	61.69	65.33	61.27	220.24	234.79	223.92
T ₃ L ₉	349.32	373.31	358.27	236.06	251.34	239.34	584.21	623.41	596.42
T ₃ L ₁₀	272.64	291.64	279.96	102.39	108.19	102.26	374.38	399.14	381.55
T ₄ L ₁	192.61	206.21	197.77	64.99	68.39	64.19	257.17	274.13	261.52
T ₄ L ₂	185.31	198.15	190.09	61.47	64.42	60.44	246.37	262.13	250.11
T ₄ L ₃	183.95	196.62	188.52	53.93	56.57	52.98	237.49	252.78	241.10
T ₄ L ₄	167.66	179.23	171.77	56.27	59.69	55.86	223.56	238.52	227.25
T ₄ L ₅	259.13	276.87	265.85	77.03	81.46	76.75	335.61	357.74	342.05
T ₄ L ₆	172.47	184.21	176.74	58.53	62.24	58.41	230.61	246.04	234.76
T ₄ L ₇	239.75	256.30	245.93	72.84	76.98	72.03	312.08	332.73	317.44
T ₄ L ₈	153.35	163.99	157.11	53.88	56.54	52.86	206.87	220.15	209.61
T ₄ L ₉	343.23	366.71	352.17	227.34	241.71	230.62	569.44	607.22	581.64
T ₄ L ₁₀	269.25	288.03	276.56	94.87	100.74	94.74	363.50	388.11	370.67
SEm±	0.77	0.87	0.83	0.87	0.87	0.78	1.39	1.41	1.28
CD at 5%	2.18	2.45	2.34	2.44	2.46	2.19	3.92	3.98	3.60

The results of interaction effect showed that highest amount of chlorophyll A content at first (351.50 mg/100 g), second (375.40 mg/100 g), third (360.01 mg/100 g) harvesting, chlorophyll B content at first (240.01 mg/100 g), second (256.05 mg/100g), third (244.18 mg/100 g) harvesting and total chlorophyll content at first(590.33 mg/100 g), second (630.20 mg/100 g) and third (602.99 mg/100 g) harvesting were also found in palak leaves when it was raised following the treatment of 100% of recommended dose (0.5 g/lit of water) of ferrous sulphate heptahydrate (FeSO₄, 7H₂O, 16%Fe) through foliar spray at 4 weeks after sowing (T₂L₉).

4. DISCUSSION

Iron is available in two forms, heme iron and nonheme iron, in our daily diet. Heme iron is found in non-vegetarian diets such as meat, poultry and seafood which is well absorbed whereas mostly non-heme iron is found to be present in plant foods like whole grains, nuts, seeds, legumes, leafy vegetables and also found

in animal flesh (as animals consume plant foods containing non heme iron) which are not absorbed properly [14]. The vegetarian people require 1.8 times more nonheme iron compared to non-vegetarian people as they have to totally depend upon non-heme iron diet to fulfil the iron requirement [14]. Therefore, selection of iron-rich diet and better bioavailability techniques for enrichment of the foods are essential to meet the demand of daily consumption of iron rich leafy vegetables as well as to shrunken the chances of iron deficiency anaemia. Iron status and its bioavailability can be increased in the leafy vegetables through supplementation of iron-containing fertilizer. In this experiment, ferrous sulphate heptahydrate (FeSO₄, 7H₂O, 16%Fe) was applied as iron enhancer to fortify the leafy vegetables in a logistic manner. Iron content in leaves (Table 1) was significantly influenced by different modes (soil, foliar and combination of soil and foliar) of iron fertilizer, ferrous sulphate heptahydrate (FeSO₄, 7H₂O, 16%Fe) application. Leaf iron content was found to be highest in *Amaranthus* leaves due to application of 100% of

recommended dose (0.5 g/lit of water) of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16 % Fe) through foliar spray at 4 weeks after sowing (T_{2L1}) at the first (22.43 mg/100 g), second (21.30 mg/100 g) and third (20.26 mg/100 g) harvesting respectively. The activity of iron in both soils as well as in plant is a complex phenomenon. After application of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16%Fe) in the soil ferrous (Fe^{2+}) compounds react with soil air and get oxidised to ferric form (Fe^{3+}) and this ferric compounds (Fe^{3+}) is then transported across the plasma membrane of root epidermal cells by an iron regulated transporter (IRT1) [15,16]. In case of foliar application also ferrous (Fe^{2+}) compounds on contact with air get oxidised and transformed to ferric form (Fe^{3+}) and penetration of ferric compound (Fe^{3+}) would occur via the cuticle through cuticular cracks and imperfections and through stomata, leaf hairs, and other specialized epidermal cells [17]. Smooth foliage penetration of iron through stomata requires the help of surfactants or external pressure [18]. After absorption of iron inside the plant body, it will increase the concentration of iron in leaves, shoots and roots and it will play significant role in basic biological processes such as photosynthesis, chlorophyll synthesis, respiration, nitrogen fixation, uptake mechanisms [19]. Enhanced activities of all these biological mechanisms increase chlorophyll content, improve the plant growth and ultimately increase the economic yield of the crops.

5. CONCLUSION

The findings of the present study revealed that iron fortification increased the leaf iron content as well as improved different quality parameters like ascorbic acid content, vitamin A content, chlorophyll A, chlorophyll B and total chlorophyll content. Considering the iron content of leaves at first, second and third harvesting the treatment consisted of 100% of recommended dose (2.5 kg/ha) of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16%Fe) through soil application (T_1) was emerged best for coriander leaves, mustard leaves and onion leaves, the treatment comprised of 100% of recommended dose (0.5 g/lit of water) through foliar spray at 4 weeks after sowing (T_2) was found to be superior in *Amaranthus* and palak and the treatment consisted of 50% of recommended dose through soil application + 50% of recommended dose through foliar spray at 4 weeks after sowing (T_3) was found best for the buck wheat leaves, fenugreek leaves, garden pea leaves, *Malva*

leaves and radish leaves. All the modes of iron fertilizer application improved the other quality parameters (ascorbic acid content, vitamin A content, chlorophyll A, chlorophyll B and total chlorophyll content) in all the crops under study. In general, for improvement of iron content of leafy vegetables, application of 100% recommended dose (0.5 g/lit) of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16%Fe) of water through foliar spray at 4 weeks after sowing (T_2) and 50% of recommended dose through soil application + 50% of recommended dose through foliar spray at 4 weeks after sowing (T_3) may be adopted for large scale cultivation. Inclusion of ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 16% Fe) as source of iron fertilizer in leafy vegetables can be recommended to reduce the world wide health problem of anaemia and to enhance the quality of the leafy vegetables in an upright manner.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dhaliwal MS. Leafy and Salad Vegetables. In: Dhaliwal MS, editor. Handbook of vegetable crops. 3rd ed. New Delhi: Kalyani Publishers; 2017.
2. Thamburaj S, Singh N. Vegetables, tuber crops and spices. New Delhi: ICAR; 2001.
3. Hurrell RF. Bioavailability of iron. European Journal of Clinical Nutrition. 1997;51(1):S4–S8.
4. McDowell LR. Minerals in animal and human nutrition. 2nd ed. Amsterdam: Elsevier Science; 2003.
5. Yang XE, Chen WR, Feng Y. Improving human micronutrient nutrition through biofortification in the soil-plant system: China as a case study. Environmental Geochemistry and Health. 2007;29:413–428.
6. Helrich K. Official methods of analysis of the association of official analytical chemists. 15th edition. Arlington: Association of official analytical chemists, Inc; 1990.
7. Davies BH. Carotenoids. In: Chemistry and biochemistry of plant pigments. London: Academic Press; 1976.
8. Sadasivam S, Manickam A. Biochemical methods. 2nd ed. New Delhi: New Age International Publisher; 1996.

9. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd ed. Newyork: Jhon Wiley and Sons;1984.
10. Prasad JG, Vadodaria JR, Karthick K, Varsatvabik A. Effect of zinc and iron on quality of Amaranth (*amaranthus spp*) cv pusa kiran. International Journal of Agriculture Sciences. 2017;9(32):4476-4478.
11. Sakthidharan A. Iron and zinc fortification in amaranthus (*Amaranthus tricolor*) through bioaugmentation. M.Sc. Thesis. Kerala Agricultural University, Thiruvananthapuram, Kerala; 2013.
12. Tavakoli MM, Bagheri V, Roosta HR. Comparison of efficiency of different Fe sources on growth and physiological characteristics of lettuce under alkaline conditions in hydroponic system. Journal of Science and Technology of Greenhouse Culture. 2015;5(20):41-49.
13. Aslam M, Sultana B, Anwar F, Munir H. Foliar spray of selected plant growth regulators affected the biochemical and antioxidant attributes of spinach in a field experiment. Turkish Journal of Agriculture and Forestry. 2016;40:136-145.
14. Peter A. Vegeterian foods that are loaded with iron. Healthline. 2017;4:5. Accessed 14June 2020. Available:<https://www.healthline.com/nutrition/iron-rich-plant-foods>.
15. Silver J. The chemistry of iron. In: Silver J, editor. Introduction to Fe chemistry. Glasgow: Blackie Academic and Professional, Champan and Hall; 1993.
16. Cotton FA, Wilkinson G, Murillo CA, Bochmann M. The elements of the first transition series: Iron. In: Advanced inorganic chemistry. New York: John Willey and Sons; 1999.
17. Tukey HB, Wittwer SH, Bukovac MJ. Absorption of radionuclides by aboveground plant parts and movement within the plant. Agriculture, Food and Chemistry. 1961;9:106–112.
18. Fernandez V, Ebert G. Foliar iron fertilization: A critical review. Journal of Plant Nutrition. 2005; 28:2113-2124.
19. Kim J, Rees DC. Structural models for the metal centers in the nitrogenous molybdenum-iron protein. Science. 1992; 257:1677-1682.

© 2020 Das et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/60017>