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Research Article

Impact of different levels of iron on mitigation of iron chlorosis in varagu CO 3 (*Paspalum scrobiculatum.* L)

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Abstract
Iron (Fe) deficiency is a major nutritional disorder in crops growing in calcareous soils. Varagu crop are more susceptible to (Fe) deficiency in the early stage of growth and the deficiency is exhibited as chlorosis developing interveinally in the new leaves. The objective of the present study was to see the impact of different levels iron on mitigation of chlorosis in varagu, *Paspalum scrobiculatum* under calcareous soil and to investigate the influence of soil and foliar application of iron on growth, physiological and improvement of yield potential of varagu under calcareous soil condition. The varagu variety CO_3 taken for this study The treatments comprised T_1, NPK (44:22:0 kg ha-1) + 12.5 t FYM/ha,T_2, NPK (44:22:30 kg ha-1) +12.5 t FYM/ha, T_3, T_1 + Soil application of $FeSO_4$ (25 kg ha-1), T_4, T_2 + Soil application of $FeSO_4$ (25 kg ha-1), T_5, T_1 + Soil application of $FeSO_4$ (50 kg ha-1), T_6, T_2 + Soil application of $FeSO_4$ (50 kg ha-1), T_7, T_3 + Foliar spray of 0.5% $FeSO_4$, T_8, T_4 + Foliar spray of 0.5% $FeSO_4$, T_9,T_5 + Foliar spray of 0.5% $FeSO_4$, T_{10}, T_6 + Foliar spray of 0.5% $FeSO_4$. During experimentation, morphological characteristics, growth attributes, physiological and biochemical components and biomass traits determined the mitigation of iron chlorosis. The iron deficiency in varagu was effectively controlled by T_{10}, soil treatment 50 kg ha-1 $FeSO_4$ and foliar spray of 0.5% $FeSO_4$ applied on the 30th and 50th days after sowing through maintaining highest growth parameter values, maximum catalase and peroxidase activity and maintaining more chlorophyll content.

Keywords: Chlorosis, Ferrous sulphate iron deficiency, *Paspalum scrobiculatum,* Varagu

INTRODUCTION

Varagu CO 3 (*Paspalum scrobiculatum*), a key minor millet, is widely farmed in India's southern state. Varagu, also known as Kodo millet, is abundant in micronutrients, particularly calcium and iron, as well as dietary fibre, vital amino acids, and a low glycaemic index and hence plays a crucial role in poor people's food and nutritional security (Mal et al., 2010). . Kodo millet is categorized as coarse grain and is mainly grown in India, China, Russia, Japan and Africa. Kodo grains are readily maintained and demonstrated to be a good reserve for starvation. The grains comprise protein 8.35%, fat 1.45%, carbohydrate 65.65% and ash 2.95%. It can be regarded a cereal-nutrient. Kodo millet belongs to the Poaceae family and is also known as cow grass, ditch millet and is cultivated mainly in India and Madhya Pradesh ranks first in its cultivation in the country. It contributes about 50% area and 35% production of total millet in the country (Bhat *et al*., 2017). It is monocot crop and smaller size seeds, 1.5 mm in width, 2 mm in length and light brown to dark gray in

color and it is covered in a husk which is hard to remove (Bunkar et al*.,* 2021). Kodo millet is well known for the highest drought resistance among all minor millets and said to produce good yield with in less growing period i.e. 80–135 days (Saxena et al., 2018). Iron (Fe) insufficiency is a common nutritional problem in crops grown on calcareous soils. The Fe availability ranged from 3.40 to 68.1 parts per million (average of 20.5 ppm).

Interveinal chlorosis, a symptom of iron deficiency in crops, is a worldwide problem. Because iron is immobile in plants and does not translocate from older leaves to newer plant tissues, iron shortage manifests itself first in the younger tissues or leaves. Most crop plants are more vulnerable in their early phases of development, and as a result, they become stunted in the seedling stage. Iron deficiency-induced chlorosis is a common phenomenon in many crops. It was estimated that more than 30% of the crops grown worldwide were threatened by iron deficiency. Iron deficiency is mainly caused by the insoluble ferric hydroxide, the main existing form of iron, in soil, especially calcareous soil. Plants cannot use the insoluble form of iron; thus, the bioavailability is seriously limited. For example, the typical symptom caused by iron deficiency is yellowing leaves with green veins in citrus (Li *et al*., 2021) .

In green plants, there is generally a strong link between Fe supply and chlorophyll concentration, with plants that are well supplied with Fe having a high chlorophyll content (Jacobson and Oertli, 1956; Dekock et al., 1960). Fe fertilizers are either applied to the soil or delivered to the foliage to control Fe deficiency, and the use of Fe fertilization is increasing. Soil applications, trunk and branch injections, and foliar sprays are all used to fertilize trees with iron. Increasing the crop-available quantity of Fe has traditionally been applied to soils, irrigation water, plant seeds, roots, shoots, and foliage through the use of Fe fertilizers The goal of this study was to see how varying quantities of iron levels affected chlorosis mitigation in Varagu, *Paspalum scrobiculatum.* L.

MATERIALS AND METHODS

The present study was conducted under field conditions in the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. The varagu variety CO3 was taken for this study. The experiment was laid out in a randomized block design (RBD), and ten treatments consisted of three replications. As per the treatment schedule, potassium in the form of muriate potash and ferrous sulfate were applied to the soil on the 30th and 50th days after sowing (DAS). Foliar spray of ferrous sulfate was given on the 30th and 50th DAS, coinciding with the tillering and vegetative stages of the crop, respectively. The treatment details are mentioned in Table 1.

Observations of the morphological characteristics, physiological components and biochemical constituents, iron content of the leaf and biometric traits were recorded at various stages based on observations of fixed levels of iron for the mitigation of chlorosis in Varagu. Plant height (cm), root length (cm), number of tillers, leaf number, leaf area (cm^2), leaf area index, leaf area duration (days) , and specific leaf weight (mg cm^{-2}) were all measured morphologically. Physiological and biochemical traits were chlorophyll content, soluble protein content, nitrate reductase, proline content, catalase, peroxidase content, and days to 50 % flowering. These observations were taken after treatment application.

Statistical analysis

Statistical analyses of the data were performed by analysis of variance (ANOVA). The significant differences between the means of treatments were determined by the least significant difference (LSD) test in AGRESS software.

RESULTS AND DISCUSSION

Plant height, leaf area, and chlorophyll content are all key factors to consider when attempting to mitigate chlorosis in Varagu CO 3 (*P. scrobiculatum.* L). The promotion of growth in terms of increase in plant height has been thought to be by altering the plasticity of the cell wall. Plasticity changes are mainly contributed by the hydrolysis of starch to sugars which lowers the water potential of the cell, resulting in the entry of water into the cell, and causing elongation (Faldu, *et al*., 2018).

In the present investigation, iron nutrition, in addition to the recommended dose of fertilizers, showed significant enhancement in plant growth, and the treatment soil application of 50 kg $FeSO_4$ ha^{-1} along with NPK

Table 1. Treatment details in the present study

T_1	NPK (44:22:0 kg ha-1) + 12.5 t FYM/ha
T_2	NPK (44:22:30 kg ha-1) +12.5 t FYM/ha
T_3	T_1 + Soil application of $FeSO_4$ (25 kg ha-1)
T_4	T_2 + Soil application of $FeSO_4$ (25 kg ha-1)
T_5	T_1 + Soil application of $FeSO_4$ (50 kg ha-1)
T_6	T_2 + Soil application of $FeSO_4$ (50 kg ha-1)
T_7	T_3 + Foliar spray of 0.5% $FeSO_4$
T_8	T_4 + Foliar spray of 0.5% $FeSO_4$
T_9	T_5 + Foliar spray of 0.5% $FeSO_4$
T_{10}	T_6 + Foliar spray of 0.5% $FeSO_4$

(44:22:30 kg ha^{-1}) was the most effective in enhancing the plant height (102 cm) to the highest level (Table 2). This finding is in agreement with Ali *et al.* (2021) reported that soil application and foliar spray of Fe alone or in combination with other micronutrients increased the plant height of wheat.

Leaf area is the fundamental determinant of the rate of photosynthesis of any plant, and optimum leaf area development aids in the effective interception of light energy and facilitates higher dry matter production. In the present investigation, iron application significantly affected the leaf area (Fig. 1). Soil application of 50 kg $FeSO_4$ ha^{-1} along with NPK (44:22:30 kg ha^{-1}) combined with 0.5% foliar spray of $FeSO_4$ was the most effective treatment in improving the leaf area 116.4 cm^2 (Table 2). This result was supported by Saini et al. (2015), who indicated that iron is an essential component of dehydrogenase, proteinase, peptidase and that increased photosynthesis and food material translocation resulted in increased leaf area, which was strongly connected with cell multiplication, cell division, and cell differentiation.

The basic roles of photosynthetic pigments, which are made up of chlorophyll a, b, and total, are to intercept and store light energy via inductive resonance via antenna complexes and then to transport electrons via Photosystem II (Taiz and Zeiger, 2002). The efficiency with which leaves create assimilates and how long they last are largely determined by photosynthetic pigments. The most important is leaf chlorophyll concentration, which is linked to an increase in PSII photochemistry photosynthate generation and dry matter accumulation. As a result, measuring chlorophyll explains the efficiency of photosynthesis and the production of photosynthates in an indirect manner.

In the present investigation, the contents of chlorophyll pigments (a, b and total) were found to show an increasing trend up to the fruit development stage, indicating its contribution to the better development of reproductive structures as a result of the iron application. The data recorded on the total chlorophyll content of the leaf indicated a trend similar to that of chlorophyll a and b contents. All the treatments significantly (0.05 level) differed at all the stages of growth. T_{10} exhibited its superiority in maintaining the highest total chlorophyll content at all stages of growth. At the panicle initiation stage, T10 recorded the highest value of 1.90 mg/g over the control (Fig. 2). Similar result was observed by (Eskandari, 2011).). Fe is a crucial nutrient in crops because it is required for chlorophyll synthesis as well as the activation of numerous critical enzymes, including cytochrome, which is involved in the electron

Table 2. Effect of iron application on growth parameters at the grain maturation stage of varagu (Values are means of three observations)

Treatments	PH (cm)	RL (cm)	NT	LN/plant	LA (cm^2)	LAI (m^2)	LAD days	SLW (mg cm^{-2})
T1	98.8	10.5	3.6	10.3	94.9	0.422	9.2	13.83
T2	101.5	12.3	3.8	10.3	102.9	0.457	10.31	15.92
T3	97.2	12.0	3.6	10.2	114.7*	0.510	11.21	14.17
T4	95.9	13.8	3.6	10.9	101.2	0.540	12.11	16.08
T5	101.8	14.6*	3.8	11.8*	117.4*	0.698*	12.53	18.71*
T6	101.6	14.0	4.2*	10.9	104.6	0.687*	13.12	16.41
T7	100.9	14.7*	4.2*	11.3	112.3	0.712*	13.21	17.60
T8	103.8	14.9*	3.8	11.4	105.1	0.667*	13.52	18.49*
T9	97.4	15.4*	4.0	11.7*	120.5*	0.764*	15.82*	17.51
T10	102.0	15.6*	4.6*	11.9*	116.4*	0.862*	17.40*	19.62*
Mean	101.72	13.72	3.90	11.03	107.45	0.6274	13.130	17.37
S.Ed	1.49	0.22	0.08	0.20	2.77	0.0101	0.330	0.396
CD (P=0.05)	3.14	0.47	0.18	0.43	5.83	0.0211	0.694	0.832

T_1. NPK (44:22:0 kg ha-1) + 12.5 t FYM/ha, T_2. NPK (44:22:30 kg ha-1) +12.5 t FYM/ha, T_3. T_1 + Soil application of $FeSO_4$ (25 kg ha-1), T_4. T_2 + Soil application of $FeSO_4$ (25 kg ha-1), T_5. T_1 + Soil application of $FeSO_4$ (50 kg ha-1), T_6. T_2 + Soil application of $FeSO_4$ (50 kg ha-1), T_7. T_3 + Foliar spray of 0.5% $FeSO_4$. T_8. T_4 + Foliar spray of 0.5% $FeSO_4$, T_9. T_5 + Foliar spray of 0.5% $FeSO_4$, T_{10}. T_6 + Foliar spray of 0.5% $FeSO_4$; *Significant at 0.05 level; PH- Plant height ; LAI-Leaf Area Index; RT-Root Length;LAD-Leaf Area Duration; NT-Number of Tillers; SLW-Specific Leaf Weight;N-Leaf Number; LA-leaf Area

transport chain and hence in the photosynthetic process.

Proline is thought to protect plant tissues from stress by acting as a nitrogen store molecule, an osmoregulator, and a protector for enzymes and cellular structure. Proline functions as an osmoprotectant, membrane stabilizer, and reactive oxygen species (ROS) scavenger, as well as protecting subcellular structures and enzymes and boosting cellular osmolarity (turgor pressure), which provides the turgor required for cell growth under stress (Reddy et al., 2004). In the present study, the proline content was increased significantly due to the application of iron. A similar effect of iron on proline was reported by Khattab (2004) and further explained that the application of iron under stress conditions might have helped the plant utilize the mechanisms, which eventually resulted in the production of antioxidants, more effectively and slightly increasing the amount of proline in plants.

Borowski (2013) observed that the free proline content in leaves was increased by foliar application of Fe in spinach. Pourgholam (2013) also reported that foliar application of zinc and iron (0.5%) each significantly increased the proline content of the leaf to the maximum under drought stress. In tomatoes grown under salinity stress conditions, the combination of KNO3 + FeSO4 + Borax treatment significantly increased the proline content of the leaves, as observed by Nandhitha and Sivakumar (2016). The proline content of the leaves was recorded at the vegetative stage and grain development stage (Table 3), and the proline content was not significantly influenced by iron content. At the vegetative stage, among the treatments, the highest proline content was recorded at T6 (3.2 mg g^{-1}).The detoxification of active oxygen species, particularly hydrogen peroxide, is carried out by the enzyme catalase. Increased catalase activity works as a damage control system, protecting cells from oxidative stress, which may otherwise result in membrane peroxidation, cell organelle destruction, and inhibition of photosynthesis and other enzyme activities (Sairam, 1994). In the current study, a 50 kg ha-1 soil treatment of FeSO4 com-

Table 3. Effect of iron application on proline content at various stages of varagu growth (values are means of three observations)

Treatments	Proline content (mg g^{-1})	
	Vegetative stage	Grain development Stage
T1 (NPK (44:22:0 kg ha-1) + 12.5 t FYM/ha)	2.183	1.937
T2 (NPK (44:22:30 kg ha-1) +12.5 t FYM/ha)	2.255	1.960
T3 (T_1 + Soil application of $FeSO_4$ (25 kg ha-1))	2.575*	2.204*
T4 (T_2 + Soil application of $FeSO_4$ (25 kg ha-1))	2.669*	2.110*
T5 (T_1 + Soil application of $FeSO_4$ (50 kg ha-1))	2.610*	1.651
T6 (T_2 + Soil application of $FeSO_4$ (50 kg ha-1))	3.265	2.967*
T7 (T_3 + Foliar spray of 0.5% $FeSO_4$)	1.920	1.867
T8 (T_4 + Foliar spray of 0.5% $FeSO_4$)	2.118	2.075*
T9 (T_5 + Foliar spray of 0.5% $FeSO_4$)	2.248	1.388
T10 (T_6 + Foliar spray of 0.5% $FeSO_4$)	2.373	1.398
Mean	2.41	1.9557
S.Ed	0.0458	0.0498
CD (P=0.05)	0.0963	0.1014

* Significant at 0.05 level

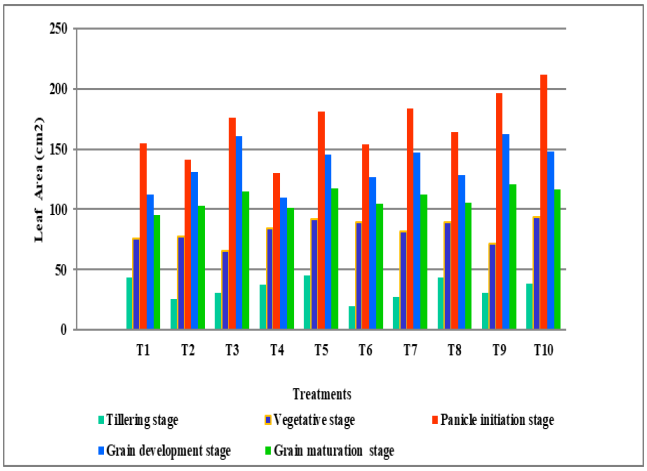

Fig. 1. *Effect of different levels of iron on leaf area of varagu at different stages*

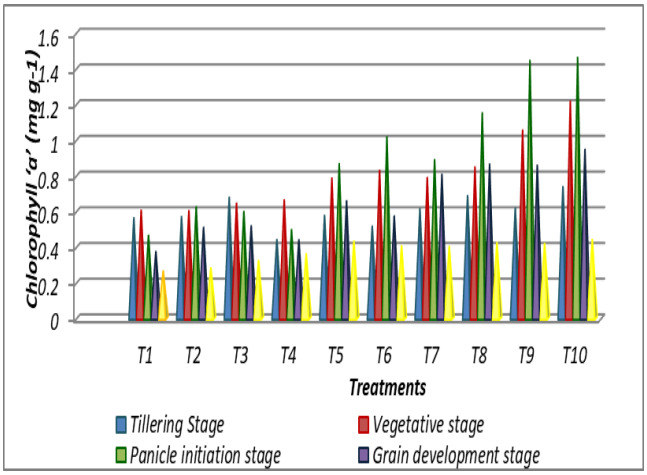

Fig. 2. *Effect of different levels of iron on the total chlorophyll content of varagu at different stages*

bined with a 0.5% FeSO4 foliar spray resulted in a 5-fold increase in catalase activity compared to the control during the grain development stage. El-Wahab and Mohamad backed up this finding (2008).

Catalase has iron in its structure and catalyses the conversion of hydrogen peroxide to oxygen and water, according to Yu and Rengel (1999). CAT protects cells from damage caused by oxidative processes by reducing hydrogen peroxide with reduced glutathione Weisany *et al (2012)*. The time trend of catalase activity revealed an interesting trend .The enzyme activity decreased from the tillering to panicle initiation stage of the crop, followed by an increase up to the final stage. All the treatments significantly altered catalase activity

Table 4. Effect of iron application on catalase activity at various stages of varagu growth (Values are means of three observations)

Treatments	Catalase activity (µg H_2O_2 reduced g^{-1} min^{-1})				
	Tillering Stage	Vegetative stage	Panicle initiation stage	Grain development Stage	Grain maturation stage
T1	7.8	10.72	9.66	10.09	11.69
T2	7.2	10.63	9.56	9.03	11.19
T3	7.1	10.49	9.44	9.03	9.57
T4	6.3	8.50	8.56	8.50	9.43
T5	5.2	9.56*	7.44	4.25	6.38*
T6	4.4*	9.06*	7.41	7.44	8.50
T7	3.5*	7.44*	6.38*	4.25*	5.32*
T8	3.2*	7.32*	6.35*	4.25*	5.30*
T9	3.0*	7.14*	5.31*	3.19*	4.25*
T10	2.0*	6.38*	4.25*	2.13*	4.15*
Mean	5.01	8.92	7.75	6.32	7.76
S.Ed	0.112	0.224	0.202	0.138	0.158
CD (P=0.05)	0.235	0.471	0.425	0.291	0.332

* Significant at 0.05 level; T_1. NPK (44:22:0 kg ha-1) + 12.5 t FYM/ha, T_2. NPK (44:22:30 kg ha-1) +12.5 t FYM/ha, T_3. T_1 + Soil application of $FeSO_4$ (25 kg ha-1), T_4. T_2 + Soil application of $FeSO_4$ (25 kg ha-1), T_5. T_1 + Soil application of $FeSO_4$ (50 kg ha-1), T_6. T_2 + Soil application of $FeSO_4$ (50 kg ha-1), T_7. T_3 + Foliar spray of 0.5% $FeSO_4$. T_8. T_4 + Foliar spray of 0.5% $FeSO_4$, T_9. T_5 + Foliar spray of 0.5% $FeSO_4$, T_{10}. T_6 + Foliar spray of 0.5% $FeSO_4$

Table 5. Effect of iron application on peroxidase activity at various stages of varagu growth (Values are means of three observations)

Treatments	Peroxidase activity (µmol min^{-1} mg^{-1})				
	Tillering Stage	Vegetative stage	Panicle initiation stage	Grain development stage	Grain maturation stage
T1	15.03	18.60	28.10	43.60	12.10
T2	16.31	29.05	29.40	45.20	15.35
T3	18.68*	28.63	35.15	48.35	16.60
T4	19.50*	36.78	47.05*	49.95	16.90
T5	18.91*	27.45	33.05	50.35	18.40
T6	14.00	44.80*	39.80*	74.70*	21.10
T7	18.11*	45.50*	40.35*	62.60	20.10
T8	14.74	49.68*	34.05	73.65*	25.30*
T9	17.82*	36.93	39.55*	89.25*	37.00*
T10	20.03*	43.80*	38.65*	85.05*	39.40*
Mean	17.00	37.39	35.96	60.70	21.05
S.Ed	0.382	0.840	0.777	0.997	0.651
CD (P=0.05)	0.804	1.76	1.633	2.09	1.36

* Significant at 0.05 level; T_1. NPK (44:22:0 kg ha-1) + 12.5 t FYM/ha, T_2. NPK (44:22:30 kg ha-1) +12.5 t FYM/ha, T_3. T_1 + Soil application of $FeSO_4$ (25 kg ha-1), T_4. T_2 + Soil application of $FeSO_4$ (25 kg ha-1), T_5. T_1 + Soil application of $FeSO_4$ (50 kg ha-1), T_6. T_2 + Soil application of $FeSO_4$ (50 kg ha-1), T_7. T_3 + Foliar spray of 0.5% $FeSO_4$. T_8. T_4 + Foliar spray of 0.5% $FeSO_4$, T_9. T_5 + Foliar spray of 0.5% $FeSO_4$, T_{10}. T_6 + Foliar spray of 0.5% $FeSO_4$

(Table 4). At the grain development stage, T10 (2.13 µg H_2O_2 min^{-1} g^{-1}) and T9 (3.19 µg H_2O_2 min^{-1} g^{-1}), followed by T_2 (NPK (44:22:30 kg ha-1) +12.5 t FYM/ha) , T_3 (T_1 + Soil application of $FeSO_4$ (25 kg ha-1)) , T_4 (T_2 + Soil application of $FeSO_4$ (25 kg ha-1)), and T_6 (T_2 + Soil application of $FeSO_4$ (50 kg ha-1)), had the lowest catalase activity compared with the control (10.09 µg H_2O_2 min^{-1} g^{-1}).

Another enzyme involved in the detoxification of reactive oxygen species is peroxidase. In this research, iron application induced peroxidase activity higher than the control, and soil application of 50 kg ha-1 FeSO4 with 0.5% foliar spray caused a double-fold increase in the activity of the enzyme. This finding was corroborated with the earlier findings of Agarwala et al. (1964) in radish, in which iron supply increases peroxidase activity. Peroxidase activity was twofold higher than that of the control at grain development was observed in soil and foliar application of 50 kg ha-1 FeSO4 with 0.5% FeSO4. In contrast to this finding, both iron deficiency and toxicity in the Gimmieza wheat cultivar enhanced peroxidase enzyme activity when compared to normal iron content (Ragaee et al., 2006). The effects of iron fertilization through soil and foliage on the peroxidase activity of varagu were recorded at various stages. Iron concentration significantly increased peroxidase activity from the tillering stage to the grain development stage, and peroxidase activity declined at the grain maturation stage (Table 5). The data showed higher peroxidase activity at grain development stages T_9 (89.25 µmol min^{-1} mg^{-1}) and T_{10} (85.05 µmol min^{-1} mg^{-1}).

Conclusion

The study concluded that for alleviating iron chlorosis in varagu (*Paspalum scrobiculatum*), the application of (T_{10}) $FeSO_4$ @ 50 kg ha^{-1} through the soil at 30 and 50 days after sowing combined with foliar spray of 0.5% $FeSO_4$ at 30 and 50 days after sowing effectively alleviated iron chlorosis in varagu. Fe deficiency is a major nutritional disorder in crops growing in calcareous soils. Varagu crops are more susceptible to Fe deficiency in the early stage of growth. The deficiency is exhibited as chlorosis developing interveinally in the new leaves. It caused 40-50% yield reduction under cultivating calcareous soil, while application of (T_{10}) $FeSO_4$ @ 50 kg ha^{-1} through the soil combined with foliar spray of 0.5% $FeSO_4$ at 30 and 50 days after sowing effectively alleviated iron chlorosis and 40 % yield improvement in varagu under calcareous soil.

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Conflict of interest

The authors declare that they have no conflict of interest.

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