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Technology to increase the yield and grain Fe content of rice

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Abstract
An experiment was conducted at the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore during 2019 in *kharif* season (July to October) to evaluate the foliar application of $FeSO_4$ on rice yield, grain Fe content and uptake of CO 51 rice cultivar. The experiment was laid out in a factorial completely randomized design (FCRD) with two factors *viz.*, five levels of $FeSO_4$ (Control (water spray), 0.5%, 0.75%, 1.0% and 1.5% $FeSO_4$ along with 0.1% citric acid) and 14 different foliar application stages of rice crop (T_1-Panicle initiation, T_2-Flowering, T_3-Milking, T_4-Dough, T_5-Panicle initiation + Flowering, T_6-Panicle initiation + Milking, T_7-Panicle initiation + Dough, T_8-Flowering + Milking, T_9- Flowering + Dough, T_{10}-Milking + Dough, T_{11}-Panicle initiation + Flowering + Milking, T_{12}-Panicle initiation + Flowering+ Milking, T_{13}-Panicle initiation + Milking + Dough, T_{14}-Panicle initiation+ Flowering + Milking+ Dough). The effect was assessed on grain and straw yields and content and uptake of Fe in rice grain. The results revealed that foliar application of 1.0% $FeSO_4$ + 0.1% CA during Flowering + Milking stages recorded highest grain yield (23.54 g pot^{-1}), straw yield (32.96 g pot^{-1}), grain Fe content (170 mg kg^{-1}) and uptake (40.25 µg pot^{-1}). The per cent increase over control was 30.78, 48.70, 25.90 and 64.65 in grain, straw yield, grain Fe content and uptake respectively.

Keywords: rice, Fe, $FeSO_4$, iron and foliar spray of $FeSO_4$

Introduction
Rice is a life-saving cereal and widely cultivated crop around the world. It is grown under diverse climatic conditions. It is a potent energy source for the global population and feeds 50% of the people. Rice crop is grown in an area of about 163.62 million hectares, with the production of 499.89 million tones globally. India covers the area of about 43.99 million hectares, with a production of 109.7 million tons (WAP, 2018) [9]. It gives about 21% of the dietary calories for more than half of the global world populations (Fitzgerald *et al.*, 2009) [1]. Among the factors limiting rice productivity, micronutrient deficiency is most critical, specifically Fe deficiency. The deficiency of iron in human beings causes the death of about 0.8 million people around the globe. These deficiencies can be corrected by enriching the cereal with micronutrients (Sudha and Stalin, 2015) [8]. Foliar spray of $FeSO_4$ is the most widely used method in crops for alleviating Fe deficiency. Foliar sprays of $FeSO_4$ at different growth stages of rice crop (maximum tillering, pre-anthesis and post-anthesis) increased the growth and yield attributes. The present study undertaken to enrich the rice grain iron content and yield at different levels of foliar $FeSO_4$ at different growth stages of CO 51 rice variety.

Materials and Methods
A pot experiment conducted during 2019 in kharif season (July to October) with rice ruling variety CO 51 at the Department of soil science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore. The experimental soil was sandy clay loam in texture with low available nitrogen (190.4 kg ha^{-1}), phosphorous (10.5 kg ha^{-1}) and organic carbon (0.33%) and high in available potassium (346 kg ha^{-1}) status. The STCR recommended dose of NPK was applied as 191, 110, and 26 kg ha^{-1} of N (urea), P (single super phosphate), and K (muriate of potash). The nitrogen and potassium were applied in three splits v*iz.*, basal, active tillering and flowering stages, and full dose phosphorous was applied as basal. The nursery was raised, and 21 days old seedling was transplanted three hills per pot. The experimental design was factorial completely randomized design (FCRD). Foliar dose of $FeSO_4$ (control (water spray), 0.5% $FeSO_4$, 0.75% $FeSO_4$, 1.0% $FeSO_4$ and 1.5% $FeSO_4$ along with 0.1% citric acid) was applied at various growth stages of crop plants *i.e.*, T_1-Panicle initiation, T_2-Flowering, T_3-Milking, T_4-Dough, T_5-Panicle initiation + Flowering, T_6-Panicle initiation + Milking, T_7-Panicle initiation + Dough, T_8-Flowering + Milking, T_9-Flowering + Dough,

T_{10}-Milking + Dough, T_{11}-Panicle initiation + Flowering + Milking, T_{12}-Panicle initiation + Flowering + Milking, T_{13}-Panicle initiation + Milking + Dough, T_{14}-Panicle initiation+ Flowering + Milking + Dough. Grain and straw yield were recorded at harvest. The grain samples were powdered and triple acid digestion was done to estimate iron content through AAS. Iron uptake in grain was computed as per the treatment by multiplying respective grain yield with corresponding iron content. The data were subjected to statistical analysis using SPSS (16.0 version) software. The difference among the treatments was compared using Duncan at a 5% probability level.

Result

Grain yield

The effect of foliar application of $FeSO_4$ in various concentrations sprayed at different growth stages of rice caused significant improvement in grain yield over control (Table 1). The grain yield ranged from 17.65 to 24.95 g pot^{-1}.

The grain yield increased due to different levels of foliar spray of $FeSO_4$ along with 0.1% CA ranged from 17.86 to 21.60 g pot^{-1}. The highest grain yield (21.60 g pot^{-1}) recorded in 1.5% $FeSO_4$ + 0.1% CA. However, it was on par with 1.0% $FeSO_4$ + 0.1% CA (21.33 g pot^{-1}) but superior to 0.75%, 0.5% and water spray (control).

With respect to spray stages, grain yield ranged from 17.91 to 22.16 g pot^{-1}. The grain yield was recorded highest (22.16 g pot^{-1}) when applied four times *viz.*, Panicle initiation + Flowering + Milking + Dough (T_{14}) and it was on par with three times (22.07 g pot^{-1}) application during Panicle initiation + Flowering + Milking (T_{11}) and two times (22.16 gpot^{-1}) sprayed treatment Flowering + Milking (T_8) but higher to rest of the treatments. The lowest grain yield (17.91 g pot^{-1}) was noticed in single spray at Dough stage (T_4).

The interaction between levels of $FeSO_4$ and spraying at different growth stages was significant on grain yield. The foliar spray of $FeSO_4$ @ 1.5% + 0.1% CA sprayed at four times Panicle initiation + Flowering + Milking + Dough (T_{14}) was recorded maximum grain yield (24.95 g pot^{-1}) and it was comparable with $FeSO_4$ @ 1.0% at two times (23.54 g pot^{-1}) spray Flowering + Milking (T_8) but notable to rest of the treatments. The lowest grain yield was registered in water spray treatment. The per cent increase in grain yield at 1.0% spray at two times was 30.78 over control.

Table 1: Effect of foliar nutrition of $FeSO_4$ on rice (CO 51) grain yield (g pot^{-1})

Spray at different growth stages of rice crop		**Levels of $FeSO_4$ (%)**					**Mean**
Treatments	**Stages of spray**	**Water spray**	**0.5**	**0.75**	**1.0**	**1.5**	
T_1	Panicle initiation	17.65	17.55	18.00	18.15	18.25	17.92
T_2	Flowering	17.69	18.56	20.06	20.56	20.86	19.55
T_3	Milking	17.84	19.57	21.07	21.57	21.87	20.38
T_4	Dough	18.00	17.54	17.69	18.14	18.19	17.91
T_5	Panicle initiation + Flowering	17.82	18.83	18.98	21.43	21.86	19.78
T_6	Panicle initiation + Milking	17.87	19.00	20.79	21.54	21.97	20.23
T_7	Panicle initiation + Dough	17.91	17.94	18.36	18.79	18.91	18.38
T_8	Flowering + Milking	18.00	19.84	22.54	23.54	24.24	21.63
T_9	Flowering + Dough	17.73	18.63	18.94	21.14	21.23	19.53
T_{10}	Milking + Dough	17.79	18.71	20.28	21.26	21.38	19.88
T_{11}	Panicle initiation + Flowering + Milking	17.82	19.92	23.28	24.43	24.92	22.07
T_{12}	Panicle initiation + Flowering+ Dough	18.07	19.14	20.94	21.73	21.79	20.33
T_{13}	Panicle initiation + Milking + Dough	17.94	19.21	21.21	21.85	22.00	20.44
T_{14}	Panicle initiation + Flowering + Milking + Dough.	17.88	20.11	23.33	24.53	24.95	22.16
Mean		**17.86**	**18.90**	**20.39**	**21.33**	**21.60**	
		$FeSO_4$ Levels		**Spray Stages**		**Levels x stages**	
SE_d		0.227		0.335		0.795	
CD @ 5%		0.455		0.710		1.590	

Straw yield

The data furnished in (Table 2) underlined that significant influence of different levels of $FeSO_4$ applied at different stages on straw yield over control (water spray). The straw yield ranged from 26.48 to 42.00 g pot^{-1}.

The straw yield increased due to foliar spray of $FeSO_4$ at various concentrations along with 0.1% citric acid ranged from 26.79 to 36.66 g pot^{-1}. The straw yield (36.66 g pot^{-1}) was noticed higher in 1.5% $FeSO_4$ + 0.1% CA and it was comparable with 1.0% spray (36.28 g pot^{-1}), but superior to 0.75%, 0.5% and water spray (control).

Pertaining to spray stages, straw yield ranged from 29.73 to 36.87 g pot^{-1}. The straw yield was maximum (36.87 g pot^{-1}) when foliar spray of $FeSO_4$ four times *viz.*, Panicle initiation + Flowering + Milking + Dough (T_{14}). However it was comparable with three times spray (36.71 g pot^{-1}) at Panicle initiation + Flowering + Milking (T_{11}) and two times (36.08 g pot^{-1}) during Flowering + Milking stages (T_8) but higher to remaining treatments.

The interaction between levels of $FeSO_4$ and spraying at different stages was significant on straw yield. Irrespective of time of spray, the foliar spray of $FeSO_4$ @ 1.5% + 0.1% CA (42.00 g pot^{-1}) sprayed at four times *viz.*, Panicle initiation + Flowering + Milking + Dough (T_{14}) was recorded the maximum straw yield and it was comparable with $FeSO_4$ @ 1.0% at two times spray (*i.e.*) Flowering + Milking (40.15 g pot^{-1}). The straw yield was lowest at water spray treatment.

Table 2: Effect of foliar nutrition of $FeSO_4$ on rice (CO 51) straw yield (g pot^{-1})

Spray at different growth stages of rice crop		Levels of $FeSO_4$ (%)					Mean
Treatments	Stages of spray	Water spray	0.5	0.75	1.0	1.5	
T_1	Panicle initiation	26.48	29.84	30.60	30.86	31.03	29.76
T_2	Flowering	26.54	31.55	34.10	34.95	35.46	32.52
T_3	Milking	26.76	33.27	35.82	36.67	37.18	33.94
T_4	Dough	27.00	29.82	30.07	30.84	30.92	29.73
T_5	Panicle initiation + Flowering	26.73	32.01	32.27	36.43	37.16	32.92
T_6	Panicle initiation + Milking	26.81	32.30	35.34	36.62	37.35	33.68
T_7	Panicle initiation + Dough	26.87	30.50	31.21	31.94	32.15	30.53
T_8	Flowering + Milking	27.00	33.73	38.32	40.15	41.21	36.08
T_9	Flowering + Dough	26.60	31.67	32.20	35.94	36.09	32.50
T_{10}	Milking + Dough	26.69	31.81	34.48	36.14	36.35	33.09
T_{11}	Panicle initiation + Flowering + Milking	26.73	33.86	39.58	41.53	41.86	36.71
T_{12}	Panicle initiation + Flowering+ Dough	27.11	32.54	35.60	36.94	37.04	33.85
T_{13}	Panicle initiation + Milking + Dough	26.91	32.66	36.06	37.15	37.40	34.03
T_{14}	Panicle initiation + Flowering + Milking + Dough.	26.82	34.19	39.66	41.70	42.00	36.87
Mean		**26.79**	**32.12**	**34.66**	**36.28**	**36.66**	
		$FeSO_4$ Levels		**Spray Stages**		**Levels x stages**	
SE_d		0.266		0.446		0.997	
CD @ 5%		0.527		0.882		1.973	

Grain iron content

Grain iron content was significantly influenced by foliar application of $FeSO_4$ at different concentrations at various crop growth stages over control (Table 3). The iron content ranged from 134 to 180 mg kg^{-1}

Concerning to different levels of foliar $FeSO_4$, the grain iron content varied from 137 to 169 mg kg^{-1}. Irrespective of stages of spray, the highest concentration of iron in rice grain (169 mg kg^{-1}) was noticed in 1.5% $FeSO_4$ + 0.1 % CA. However, it was at par with 1.0% spray (168 mg kg^{-1}) but superior to other levels.

As regard to different growth stages of rice crop, the grain iron content ranged from 154 to 169 mg kg^{-1}. Among the treatments, the highest grain iron content was marked in four times application (169 mg kg^{-1}) at Panicle initiation + Flowering + Milking + Dough (T_{14}) and it was on par with two times spray (168 mg kg^{-1}) during Flowering + Milking (T_8) and lowest grain iron content was noticed in one time spray specifically at Dough stage (T_4).

Interaction effect between levels of foliar spray of $FeSO_4$ and time of application was significant on grain Fe content The foliar spray of $FeSO_4$ @ 1.5 % + 0.1 % CA sprayed at four times (180 mg kg^{-1}) during Panicle initiation + Flowering + Milking + Dough stages (T_{14}) were recorded maximum grain iron content and it was comparable with $FeSO_4$ @ 1.0 % spray (175 mg kg^{-1}) at two times application (*i.e.*) Flowering + Milking stages (T_8) but superior to rest of the treatments and lowest at water spray. The per cent increase in grain iron content at 1.0% spray at two times was 25.90 over control.

Table 3: Effect of foliar nutrition of $FeSO_4$ on rice (CO 51) grain Fe content (mg kg^{-1})

Spray at different growth stages of rice crop		Levels of $FeSO_4$ (%)					Mean
Treatments	Stages of spray	Water spray	0.5	0.75	1.0	1.5	
T_1	Panicle initiation	136	156	158	161	162	155
T_2	Flowering	137	158	160	163	164	156
T_3	Milking	134	160	162	165	166	157
T_4	Dough	136	155	157	160	160	154
T_5	Panicle initiation + Flowering	138	163	165	168	170	161
T_6	Panicle initiation + Milking	135	165	168	170	171	162
T_7	Panicle initiation + Dough	137	157	160	162	162	156
T_8	Flowering + Milking	139	167	172	175	177	166
T_9	Flowering + Dough	138	162	164	167	167	160
T_{10}	Milking + Dough	135	164	167	169	169	161
T_{11}	Panicle initiation + Flowering + Milking	141	169	174	177	179	168
T_{12}	Panicle initiation + Flowering+ Dough	137	166	169	171	171	163
T_{13}	Panicle initiation + Milking + Dough	134	167	171	173	173	164
T_{14}	Panicle initiation + Flowering + Milking + Dough.	140	170	176	178	180	169
Mean		**137**	**163**	**166**	**168**	**169**	
		$FeSO_4$ Levels		**Spray Stages**		**Levels x stages**	
SE_d		0.835		2.398		3.126	
CD @ 5%		1.654		3.767		6.187	

Grain iron uptake

Scrutiny of analytical data depicted in (Table 4) revealed that a significant influence on time of application of different concentration of $FeSO_4$ on grain iron uptake over control. The grain iron uptake ranged from 23.91 to 44.45 µg pot^{-1}.

Iron uptake in rice grain increased due to different levels of $FeSO_4$ ranged from 24.45 to 36.67 µg pot^{-1}. The highest uptake of iron in rice grain (36.67 µg pot^{-1}) was noticed with 1.5 % $FeSO_4$ + 0.1% CA and it was on par with 1.0 % (36.03

μg pot^{-1}) but significantly superior to 0.75%, 0.5% and water spray.

Grain iron uptake in rice increased due to application of $FeSO_4$ at different stages ranged from 27.51 to 37.68 μg pot^{-1}. The maximum iron uptake in rice grain (37.68 μg pot^{-1}) was recorded, when foliar spray of $FeSO_4$ four times *viz.*, Panicle initiation + Flowering + Milking + Dough (T_{14}). However, it was on par with two times (36.20 μg pot^{-1}) application at Flowering+ Milking (T_8) and lowest uptake was noticed in Dough stage (T_4) treatment.

Interaction effect between levels of foliar spray of $FeSO_4$ and time of application was significant on grain iron uptake. The foliar spray of $FeSO_4$ @ 1.5% + 0.1% CA sprayed at four times (44.45 μg pot^{-1}) Panicle initiation + Flowering + Milking + Dough (T_{14}) and it was comparable with $FeSO_4$ @ 1.0% spray at two times spray at Flowering + Milking (41.20 μg pot^{-1}) but superior to rest of the treatments. The per cent increase in grain iron uptake at 1.0% spray at two times was 64.65 over control.

Table 4: Effect of foliar nutrition of $FeSO_4$ on rice (CO 51) grain Fe uptake (μg pot^{-1})

Spray at different growth stages of rice crop		Levels of $FeSO_4$ (%)					
Treatments	**Stages of spray**	**Water spray**	**0.5**	**0.75**	**1.0**	**1.5**	**Mean**
T_1	Panicle initiation	24.00	27.38	28.44	29.22	29.57	27.72
T_2	Flowering	24.24	29.32	32.10	33.51	34.21	30.68
T_3	Milking	23.91	31.31	34.13	35.59	36.30	32.25
T_4	Dough	24.48	27.19	27.77	29.02	29.10	27.51
T_5	Panicle initiation + Flowering	24.59	30.69	31.32	36.00	37.16	31.95
T_6	Panicle initiation + Milking	24.12	31.35	34.93	36.62	37.57	32.92
T_7	Panicle initiation + Dough	24.54	28.17	29.38	30.44	30.63	28.63
T_8	Flowering + Milking	25.02	33.13	38.77	41.20	42.90	36.20
T_9	Flowering + Dough	24.47	30.18	31.06	35.30	35.45	31.29
T_{10}	Milking + Dough	24.02	30.68	33.87	35.72	36.35	32.13
T_{11}	Panicle initiation + Flowering + Milking	25.13	33.66	40.51	43.24	44.32	37.37
T_{12}	Panicle initiation + Flowering+ Dough	24.76	31.77	35.39	37.16	37.26	33.27
T_{13}	Panicle initiation + Milking + Dough	24.04	32.08	36.27	37.80	38.06	33.65
T_{14}	Panicle initiation + Flowering + Milking + Dough.	25.03	34.19	41.06	43.66	44.45	37.68
Mean		**24.45**	**30.79**	**33.93**	**36.03**	**36.67**	
		$FeSO_4$ Levels		**Spray Stages**	**Levels x stages**		
SE_d		0.342		0.573	1.118		
CD @ 5%		0.679		1.151	2.236		

Discussion

Grain and straw yield

The foliar application of mineral fertilizers revealed that efficient approach to increase the Fe content in cereal crops (Fang *et al.*, 2008; Wei *et al.*, 2012; Yuan *et al.*, 2012) [3, 10, 11]. In the present study, the highest grain and straw yield was reported in 1.5% $FeSO_4$ spray along with 0.1% citric acid and it was on par with 1.0% spray. The thinkable reasons may be due to foliar $FeSO_4$ application increases the chlorophyll content, antioxidant enzymes, and their activities and finally increasing the yield parameters resulted in higher grain yield. Pertaining to different growth stages, four times *viz.*, Panicle initiation + Flowering + Milking + Dough (T_{14}) was recorded maximum grain yield (22.16 g pot^{-1}) and straw yield (36.87 g pot^{-1}) and it was comparable with $FeSO_4$ @ 1.0 % two times spray during Flowering + Milking (T_8) (21.63 g pot^{-1} and 36.08 g pot^{-1}) stage of grain and straw yield. The plausible reason that grain and straw yield increased at Flowering and Milking stage. This might be due to improvement in enzymatic activities and the process of photosynthesis results in increasing the straw yield. These findings were supported by Mathpal *et al.* (2018) [5]. The foliar spray of $FeSO_4$ at single spray is responded but it is not effective compare to number of times of spray. The foliar feeding of nutrient is slow in response because probably that is the reason we have to spray number of times. The interaction between the exogenous foliar spray of $FeSO_4$ at various growth stages of rice crop was found to be significant. It was comparable with previous study by Singh *et al.* (2013) [7]; Shaygany *et al.* (2012) [6] they stated that foliar spray of Fe showing effective translocation to economic parts and increased the yield.

Grain iron content and uptake

Iron concentration at different growth stages have been increased with foliar application of $FeSO_4$ at maximum tillering, pre-anthesis, post-anthesis and maturity stages (Singh *et al.*, 2013) [7] it was comparable with the present study. Higher grain iron content and uptake was noticed in the foliar spray of $FeSO_4$ @ 1.5 % + 0.1% CA and it was on par with 1.0% spray. This might be due to foliar-applied $FeSO_4$ penetrate into the inner layer of starchy endosperm, the leaf tissue translocates the iron into vascular phloem sap which helps in transfer the iron into grains.

With respect to different growth stages of rice crop, four times sprayed during Panicle initiation + Flowering + Milking + Dough (T_{14}) and it was comparable with 1.0 % spray at two times application during Flowering + Milking stage (T_8). This is because leaf tissue translocate the Fe into vascular sap which is helpful for transferring the Fe into grains. The increased concentration and uptake of Fe nutrients in grain due to the synthesis of photosynthetic products with specific absorption and transport sites and it also increases the plant biomass. Negatively charged citric acid helps to induce the phytochemical reduction of Fe^{3+}, which act as pre requisite for uptake of Fe. Foliar applied $FeSO_4$ plus citric acid treatments contains higher active iron content is due to absorption and translocation of iron in its citrate form for two reason, one reason that applied citric acid helps to maintain the iron in soluble forms within the plants, other reason was formation of Fe-citrate complex is move faster through the xylem. The similar results were suggested by Zhang *et al.* (2009) [12]. $FeSO_4$ could enrich the nutritive value of rice grains and also increasing the Fe content with the help of conducting pot culture experiment on rice crop. These findings were tandem with Singh *et al.* (2013) [7] foliar spray of $FeSO_4$ at different growth stages of rice crop results in increasing the uptake of Fe in grains. Dhaliwal *et al.* (2010) [2] reported that Fe spray improve the grain uptake in rice grains. Foliar applied $FeSO_4$ alone became inefficient due to poor translocation within the plants. Therefore, foliar application of $FeSO_4$ along with citric acid helps for better translocation. Foliar spray of iron acidified by citric acid as the low pH of the spray solution improving the absorption and translocation of Fe within the plants.

Conclusion

The foliar method of application of nutrients makes it to absorb and translocate quickly within the plants and its timely application result its accumulation in edible parts. Thus, a pot culture experiment was conducted to assess the effects of $FeSO_4$ to enrich the rice grain iron content and yield. The results revealed that foliar spray of 1.0 % $FeSO_4$ + 0.1% CA spray at two times application during Flowering + Milking stages registered the best performing treatment by enriching the grain Fe content to the tune of 25.90 % over control. Based on this finding, $FeSO_4$ along with CA as foliar spray could be used as an effective technology for improving the Fe content in rice grains to overcome the iron malnutrition.

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