

## Assessing Yield, Quality and Nutrient Use Efficiency of Rice (*Oryza sativa* L.) with Agronomic Iron Bio-Fortification

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### ABSTRACT

To evaluate the yield, quality and nutrient use efficiency of rice variety SR-4 (*Oryza sativa* L.) under foliar iron higher CGR (23.56 g m<sup>-2</sup> day<sup>-1</sup>), RGR (27.46 mg g<sup>-1</sup> day<sup>-1</sup>), NAR (0.37 g cm<sup>-2</sup> day<sup>-1</sup>), grain nutrition. Field experiment was conducted during *Kharif* 2019 at Crop Research Farm of Division of Agronomy, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Wadura. The experiment comprised of four levels of iron (0.25%, 0.5%, 0.75% and 1%) supplied through three sources (FeSO<sub>4</sub>.7H<sub>2</sub>O, Na-Fe EDTA and Ferric chloride) and a control laid out in RCBD with three replicates. The data revealed that FeSO<sub>4</sub>.7H<sub>2</sub>O @ 1% recorded significantly yield (7.68 t ha<sup>-1</sup>), straw yield (9.87 t ha<sup>-1</sup>), biological yield (17.55 t ha<sup>-1</sup>), iron content (48.96 mg kg<sup>-1</sup>), (205.96 mg kg<sup>-1</sup>), iron uptake (0.38 kg ha<sup>-1</sup>), (1.94 kg ha<sup>-1</sup>) in brown rice and straw respectively, nevertheless iron chelate @ 0.75% recorded highest value of harvest index (43.90%). Among different treatments, iron chelate @ 0.25% recorded higher iron use efficiency (33.33%) whereas highest protein content was observed in FeSO<sub>4</sub>.7H<sub>2</sub>O @ 1%. However, with respect to amylose content a non-significant difference was observed among different treatments. Therefore, it was concluded that FeSO<sub>4</sub>.7H<sub>2</sub>O @ 1% prove to be promising option for agronomic biofortification with iron for enhancing yield and quality of rice under temperate conditions of Kashmir valley.

**Keywords:** Biofortification, chelated iron, Iron sulphate, Iron use efficiency, Rice

### 1 Introduction

Rice (*Oryza sativa* L.) is considered world's utmost paramount and extensively used up principal food for worlds more than half of the population. The estimated population of approximately 3.5 billion depends on rice globally which contribute 20 per cent of their daily calories requirement [1]. Due to rising populations and higher living standards, the demand for rice is still increasing, but the rate of rise in rice production has slowed down. By 2030, it is predicted that 30% more rice would need to be produced [2]. Rice occupies a prime position in the state of Jammu & Kashmir since ancient times and is being cultivated in twelve districts, four of them viz. Anantnag, Jammu, Baramulla and Pulwama are known for their high productivity. The total area under rice production of the respective region is around 0.28 million hectares with a production of 0.55 million tonnes and productivity of 2.1 tonnes hectare<sup>-1</sup> [3]. Unmilled form of rice is a good source of micro as well as macronutrients which after transformation into white rice are mostly removed from rice bran and fat layer by milling [4]. Polished rice, which is mainly consumed, contains only 4-5 mg kg<sup>-1</sup> of iron, whereas the recommended daily allowance of Fe for adults should be in the range of 17-35 milligrams as per ICMR [5]. A low concentration of iron micronutrient in rice grain, leads to increased problems of malnutrition in vast rice consuming populations [6]. Therefore, agronomic biofortification offers a quick and efficient way to increase the concentrations of micronutrients in edible crop products, will slightly improve the nutritional

value of rice to correct iron deficiency and improve human health [7]. Foliar micronutrient feeding, a convenient technique for fortifying cereal grains with micronutrients, is practical and an inexpensive technique for alleviating iron deficiency. Among the various micronutrients, the deficiency of iron is more prevalent in food grains which lead to a reduction in nutritional value and crop yields [8]. The deficiency of iron is mostly accounted for the occurrence of anemia across the globe. Recent studies have linked children with autism spectrum disorder with low maternal Fe intake. Iron is the co-factor of many enzymes in our body needed for performing various basic functions. In developing countries iron deficiency is the sixth leading risk factor for death and disability. Iron deficiency leads to anemia, poor mental growth and disability. Fortifying foods with iron is believed to be cost effective and more economically attractive than supplementation. So, the enrichment of rice with iron fertilization can solve the problem of iron deficiency [9]. Research on the iron fortification of rice in temperate conditions is very rare, so the study to evaluate agronomic iron biofortification of rice (*Oryza sativa* L.) variety SR-4 in temperate conditions was carried out in Kashmir with the objective of finding the improvement in yield, quality and iron content in the rice grain.

## 2 Materials and Methods

### 2.1 Study Location and Experimental Design

Field experiment was conducted for the assessment of iron biofortification on yield, quality and iron use efficiency of rice during *kharif* season of 2019 with rice genotype Shalimar Rice-4 (SR-4) at Crop Research Farm of Division of Agronomy, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Wadura Sopore (34° 34'N latitude and 74°40' East longitude and 1584 m a.m.s.l). The soil of the test field was silty clay loam in texture, with medium organic carbon, with a slightly neutral pH value, normal electrical conductivity and with high iron content. From planting through harvest, a meteorological observatory next to the experimental field kept daily records on the weather. The experimental site is located in a mid-altitude temperate zone with scorching summers and severely cold winters. Annual precipitation at the site averages 812 mm during the past 20 years, with the majority falling between December and April. The cumulative rainfall entertained in the concerned cropping period was 152.5 mm. The minimum temperature exhibited a range of 8.7 °C to 17.8 °C and maximum temperature from 24.2 °C to 32.9 °C and the average maximum relative humidity from 76.4 to 89.0 %, while the average minimum relative humidity ranged from 46.4 to 70.0 % during the crop growing season of *Kharif* 2019. The experimental set up comprised of a Randomized Complete Block Design encircled 13 treatments *viz.* 4 levels of iron (0.25 %, 0.50 %, 0.75 % and 1.0 %) and 3 iron sources (FeSO<sub>4</sub>.7H<sub>2</sub>O, NaFe- EDTA and Iron chloride) with one absolute control replicated thrice.

### 2.2 Growth and Yield observations

Crop growth rate, calculated at 30 days' interval was measured by the increase in plant biomass and computation was performed by the formula defined by Redford [10] and was subsequently expressed as g m<sup>-2</sup> day<sup>-1</sup>

$$\text{CGR (g m}^{-2} \text{ day}^{-1}) = \frac{1}{A} \times \frac{W_2 - W_1}{t_2 - t_1}$$

where, W<sub>1</sub> = Dry matter production per plant (g) at time t<sub>1</sub>

W<sub>2</sub> = Dry matter production per plant (g) at time t<sub>2</sub> and A is the area (spacing).

The estimation of relative growth rate was accomplished by the formula proposed by Blackman [11] and is marked as the rate of dry weight gain per unit dry weight. The results were put across in terms of mg g<sup>-1</sup> day<sup>-1</sup>.

$$\text{RGR (mg g}^{-1}\text{day}^{-1}) = \frac{\log W_2 - \log W_1}{t_2 - t_1}$$

$W_1$  and  $W_2$  are dry matter production of plants at time  $t_1$  and  $t_2$  respectively

The net assimilation rate was worked out in line with the formula put forward by Evans [12] and is defined as the rate of increase in the overall dry weight of plant per unit leaf area per unit time conveyed as gram per square centimeter per day. Furthermore, the calculation was carried out according to the formula:

$$\text{NAR (g cm}^{-2}\text{ day}^{-1}) = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log L_2 - \log L_1}{L_2 - L_1}$$

$W_1$  and  $W_2$  represent the total dry weight of plants at time  $t_1$  and  $t_2$  respectively.  $L_1$  and  $L_2$  represent total leaf area of plants at time  $t_1$  and  $t_2$  respectively.

The numbers of panicles residing in quadrant 0.25 m<sup>2</sup> were enumerated from individual plots prior to harvesting followed by its conversion to m<sup>2</sup>. The panicle weight estimate was made from randomly labeled plants from each plot of each replication, the average of which was expressed in grams. From each panicle number of grains were calculated, the mean value of which was conveyed as grains panicle<sup>-1</sup>. After the harvest and subsequent sun drying for 3-4 days the bundle weight of each net plot was recorded on an electronic balance and estimated in tonnes per hectare. The produce from each plot was sun dried following its proper threshing and cleaning. From each plot the yield was measured respectively as kg per plot followed by its conversion to tonnes per hectare. By deducting the grain yield from respective biological yield, the straw yield of each plot was worked out and expressed in tonnes per hectare. The calculation of harvest index was carried out in line with the formula as:

$$\text{H.I} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

### 2.3 Estimation of Protein and Amylose content

The calculation of protein content was performed Juliano [13] by multiplying nitrogen concentration with a coefficient factor of 5.77 and then expressed in terms of percentage. For calculating amylose content powdered sample of rice was made by crushing 1 g milled rice grain in pestle and mortar and then storing it at uniform 12 % moisture. 100 mg sample was weighed and transferred to a volumetric flask (100 ml). To soak the flour, 10 ml of 1N NaOH was pipetted into an Erlenmeyer flask and then ethanol at the rate of 1 ml was added and mixed uniformly, the sample suspension was heated in a water bath for 10 minutes after 1 hour of gelatinization. A volume of 100 ml of distilled water was prepared, and an aliquot of 2.5 ml was placed in a 50 ml volumetric flask with around 20 ml of water added. The addition of three drops of phenolphthalein indicator was well mixed. By gradually adding 0.1 N HCL, the contents were acidified until the pink colour vanished. The volume was then increased to 50 ml and 1 ml of iodine reagent was added for the development of blue colour. The spectrophotometer was employed to measure the absorbance at 590 nm. On the basis of the absorbance values of known concentrations of pure amylose, a standard curve was generated. Pure amylose (range 0.2-1 mg) was used to generate the standard curve, which was used to compare the sample's amylose content to a blank created by diluting 1 ml of iodine reagent to 50 ml of distilled water [14].

Calculation:

Absorbance corresponds to 2.5 ml of test solution = 'x' mg amylose in test solution. 100 ml contains =  $\frac{x}{2.5} \times 100\%$  amylose

### 2.4 Plant Iron Content and Uptake

Grain and straw iron content of rice was estimated by the method delineated by Prasad et al. [15] employing Atomic Absorption Spectrophotometer (AAS) and calculated as mg kg<sup>-1</sup>. The

determination of iron uptake was done by multiplying iron content of grain and straw with their respective yields and was calculated in terms of  $\text{kg ha}^{-1}$ .

### 2.5 Soil Nutrient Studies

For Soil studies after harvesting the crop, soil samples were gathered from individual plots up to 15 cm depth and were shade-dried label wise for a few days on plane white paper. After drying, soil samples were sieved through a 2 mm mesh and from each representative sample, a composite sample was taken for further laboratory studies. Rapid titration method described by Walkley and Black [16] was executed for the determination of soil organic carbon (%). The pH was measured by a 1: 2.5 ratio suspension of soil water and was read by “Blackman's glass electrode pH meter” and electrical conductivity was measured by solubridge conductivity meter as described by [17]. The determination of available nitrogen for each soil sample was carried out by alkaline permanganate method given by [18]. For each treatment the determination of available phosphorous in soil samples was performed using 0.5 N  $\text{NaHCO}_3$  at pH 8.5 [19]. The determination of available potassium was done by 1 N ammonium acetate extraction method at pH 7 [17]. However, the determination of available iron in soil samples from each treatment was done in accordance with the procedure defined by Lindsay and Norvell [20] using Atomic Absorption Spectrophotometer.

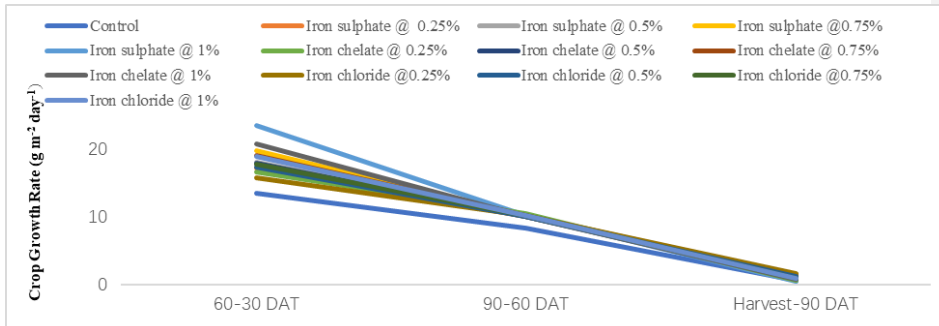
### 2.5 Statistical Analysis

The association between growth and yield attributes with yield was worked out using correlation analysis. Employing the Statistical Package for Social Science, regression analysis of yield was fitted to evaluate the response of yield explained by growth and yield features (SPSS).

## 3 Result

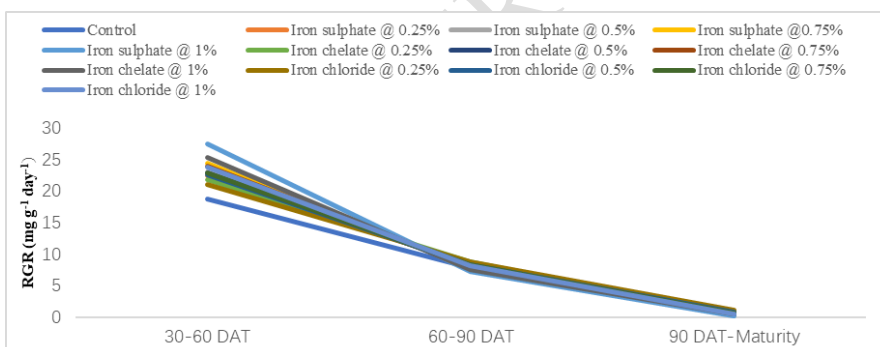
### 3.1 Crop Growth Rate, Relative Growth Rate and Net Assimilation Rate

CGR, RGR and NAR are the important tools for estimating growth of the crop. The data depicted in Fig.1 revealed that as the crop reaches upto maturity and closer to harvest, its crop growth rate is slowed down. Among the different iron treatments, numerically higher crop growth rate was recorded in  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 1% ( $23.56 \text{ g m}^{-2} \text{ day}^{-1}$ ) treatment which, statistically, was comparable with iron chelate @ 1% ( $20.79 \text{ g m}^{-2} \text{ day}^{-1}$ ) at 60-30 DAT and at 90-60 DAT, Iron chelate @ 0.25 % ( $10.46 \text{ g m}^{-2} \text{ day}^{-1}$ ) recorded highest crop growth rate which was statistically at par with  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 1% ( $10.22 \text{ g m}^{-2} \text{ day}^{-1}$ ), iron chloride @ 1% ( $10.22 \text{ g m}^{-2} \text{ day}^{-1}$ ) and iron chloride @ 0.75% ( $10.22 \text{ g m}^{-2} \text{ day}^{-1}$ ). Further, Iron chloride @ 0.25 % recorded highest crop growth rate ( $1.58 \text{ g m}^{-2} \text{ day}^{-1}$ ) at maturity-90 DAT which statistically was comparable to  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 0.5% ( $1.39 \text{ g m}^{-2} \text{ day}^{-1}$ ) followed by  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 0.25% ( $1.23 \text{ g m}^{-2} \text{ day}^{-1}$ ), and iron chloride @ 0.5 % ( $1.23 \text{ g m}^{-2} \text{ day}^{-1}$ ). However, the control treatment recorded lowest crop growth rate of 13.53, 8.38 and 0.57 at 60-30 DAT, 90-60 DAT and at maturity to 90 DAT, respectively.



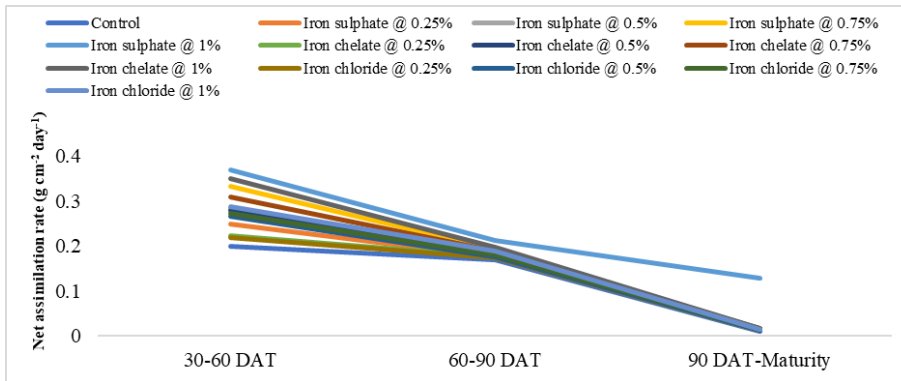
**Fig 1: Effect of different sources and levels of iron on crop growth rate ( $\text{g m}^{-2}\text{day}^{-1}$ ) of rice.**

Relative growth rate as presented in Fig. 2 suggested that as the crop ages, the relative growth rate decreases significantly. Among the different iron treatments, higher relative growth rate was recorded in  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 1% ( $27.46 \text{ mg g}^{-1} \text{ day}^{-1}$ ) followed by iron chelate @ 1% ( $25.27 \text{ mg g}^{-1} \text{ day}^{-1}$ ) at 30-60 DAT. At 60-90 DAT Iron chelate @ 0.25% ( $8.82 \text{ mg g}^{-1} \text{ day}^{-1}$ ) recorded highest relative growth rate followed by iron chloride @ 0.25% ( $8.77 \text{ mg g}^{-1} \text{ day}^{-1}$ ) and at 90 DAT to maturity Iron chloride @ 0.25 % ( $1.18 \text{ mg g}^{-1} \text{ day}^{-1}$ ) recorded highest relative growth rate. However, at different periodic intervals the lowest relative growth rate was documented in control treatment.



**Fig 2: Effect of different sources and levels of iron on relative growth rate ( $\text{mg g}^{-1} \text{ day}^{-1}$ ) of rice.**

Furthermore, Fig. 3 illustrated that the net assimilation rate decreased with the advancement of crop age and showed a declining trend. Among different levels and sources of iron  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 1% recorded highest net assimilation rate ( $0.37 \text{ g cm}^{-2} \text{ day}^{-1}$ ) at 30-60 DAT and at 60-90 DAT ( $0.21 \text{ g cm}^{-2} \text{ day}^{-1}$ ) in rice which were statistically at par with iron chelate @ 1% which recorded values of  $0.35 \text{ g cm}^{-2} \text{ day}^{-1}$  and  $0.19 \text{ g cm}^{-2} \text{ day}^{-1}$  at 30-60 DAT, and at 60-90 DAT, respectively and  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 0.75% with values of  $0.33 \text{ g cm}^{-2} \text{ day}^{-1}$  and  $0.19 \text{ g cm}^{-2} \text{ day}^{-1}$  at different intervals with control recording lowest net assimilation rate at 30-60 DAT ( $0.20 \text{ g cm}^{-2} \text{ day}^{-1}$ ), and at 60-90 DAT ( $0.17 \text{ g cm}^{-2} \text{ day}^{-1}$ ). Different levels and sources of iron with respect to net assimilation rate showed non-significant result at 90 DAT to maturity. Among different sources and levels of iron, iron sulphate @ 1% recorded highest CGR, RGR and NAR.



**Fig 3. Panicle Density ( $m^{-2}$ ), Panicle Weight (g) and Grains Panicle $^{-1}$**

Different treatments of iron had significant effect on the panicle density which is one of the most important yield attribute in rice (Table 1). The Iron sulphate @ 1% treatment recorded maximum Panicle  $m^{-2}$  (365.20) but was statistically at par with iron chelate @ 1% (365.87),  $FeSO_4 \cdot 7H_2O$  @ 0.75 % (365.20) and iron chelate @ 0.75 % with value of 364.87, respectively. Moreover, the control treatment recorded lowest panicle  $m^{-2}$  with value of 344.87. Analysis of data showed a significant effect of various treatments for panicle weight (Table 1). Iron sulphate @ 1% treatment recorded maximum panicle weight (2.97g) and was statistically at par with iron chelate @ 1%, iron sulphate @ 0.75 % which recorded panicle weight of 2.80g and 2.77g respectively. The lowest panicle weight was recorded for the control treatment (1.83 g). Grains per panicle (Table 1) varied significantly under different iron concentrations. Iron chloride @ 1% recorded maximum number of grains Panicle $^{-1}$  (113.6) followed by iron chloride @ 0.25% (110.73). However,  $FeSO_4 \cdot 7H_2O$  @ 1% recorded highest number of filled grains per panicle and lowest number of grains per panicle (106.53) were recorded in control treatment.

**Table 1:** Effect of different sources and levels of iron on yield attributes of rice

Treatment	Panicle $m^{-2}$	Panicle weight (g)	Grains panicle $^{-1}$
Control	344.87	1.83	106.53
Iron sulphate @ 0.25%	350.53	2.27	109.66
Iron sulphate @ 0.5%	358.20	2.43	109.73
Iron sulphate @ 0.75%	365.20	2.77	109.80
Iron sulphate @ 1%	367.20	2.97	108.73
Iron chelate @ 0.25%	349.53	2.17	110.27
Iron chelate @ 0.5%	358.20	2.33	109.34
Iron chelate @ 0.75%	364.87	2.48	106.93
Iron chelate @ 1%	365.87	2.80	110.00
Iron chloride @ 0.25%	348.20	2.13	110.73
Iron chloride @ 0.5%	356.53	2.27	109.20
Iron chloride @ 0.75%	356.87	2.33	108.47
Iron chloride @ 1%	361.20	2.43	113.60
<b>SEm <math>\pm</math></b>	5.24	0.13	0.15
<b>C.D(p<math>\leq</math>0.05)</b>	15.72	0.40	0.42

### 3.3 Yield ( $t\ ha^{-1}$ ) and Harvest Index (%)

Significant enhancement in the yield of rice was found with application of different iron treatments with varying levels and sources. The highest grain yield ( $7.68\ t\ ha^{-1}$ ), straw yield ( $9.87\ t\ ha^{-1}$ ) and biological yield ( $17.55\ t\ ha^{-1}$ ) were obtained with Iron sulphate @ 1%, while the lowest yields were obtained with the control (Table 2). However, the yield obtained with iron sulphate @ 1% was statistically at par with iron chelate @ 1% indicating that the two sources equally suitable for foliar application of iron in rice.

Significant impact of various iron treatments was observed on harvest index. The highest harvest index was recorded with application of iron chelate @ 0.75% treatment recording a value of 43.90% but was statistically at par with iron sulphate @ 0.75% treatment with value of (43.88%) and iron sulphate @ 1% (43.76%). The control treatment showed lowest value for harvest index (43.08%). The function of grain yield to the total biological yield (grain + straw) is known as harvest index. Different levels and sources of iron produced significant impact on harvest index. Iron chelate @ 0.75 % recorded highest harvest index with lowest values recorded in control treatment (Table 2).

**Table 2:** Effect of different sources and levels of iron on grain, straw, biological yield and harvest index of rice.

Treatment	Grain yield ( $t\ ha^{-1}$ )	Straw yield ( $t\ ha^{-1}$ )	Biological yield ( $t\ ha^{-1}$ )	Harvest index (%)
Control	5.70	7.53	13.23	43.08
Iron sulphate @ 0.25%	6.87	8.95	15.82	43.42
Iron sulphate @ 0.5%	7.06	9.21	16.27	43.39
Iron sulphate @ 0.75%	7.32	9.36	16.68	43.88
Iron sulphate @ 1%	7.68	9.87	17.55	43.76
Iron chelate @ 0.25%	6.81	8.94	15.75	43.23
Iron chelate @ 0.5%	7.04	9.06	16.10	43.72
Iron chelate @ 0.75%	7.27	9.29	16.56	43.90
Iron chelate @ 1%	7.34	9.49	16.83	43.61
Iron chloride @ 0.25%	6.71	8.76	15.47	43.37
Iron chloride @ 0.5%	6.91	8.99	15.90	43.45
Iron chloride @ 0.75%	6.95	9.02	15.97	43.51
Iron chloride @ 1%	7.16	9.21	16.37	43.73
<b>SEm <math>\pm</math></b>	0.12	0.17	0.31	0.10
<b>C.D(p<math>\leq</math>0.05)</b>	0.36	0.52	0.90	0.31

### 3.4 Iron content, uptake in grain and straw and iron use efficiency

Different treatments of iron varied significantly in terms of iron content and its uptake in grain and straw (Table 3). The foliar application of iron sulphate @ 1% significantly increases its content ( $48.96\ mg\ kg^{-1}$ ), ( $205.96\ mg\ kg^{-1}$ ) and uptake ( $0.38\ kg\ ha^{-1}$ ), ( $1.94\ kg\ ha^{-1}$ ) in brown rice and straw respectively. The Fe uptake in rice straw increased significantly by Fe fertilization and with foliar sprays of 1%  $FeSO_4 \cdot 7H_2O$  application was highest as compared to other treatments. Among different sources and levels of iron, the highest iron use efficiency (33.33%) was observed with iron chelate @ 0.25% and lowest (4.36%) was recorded in iron chloride @ 1% (Table 3).

**Table 3:** Effect of different sources and levels of iron on iron content, uptake in rice grain and straw and iron use efficiency.

Treatment	Grain iron content (mg/kg)	Grain uptake (kg/ha)	Straw iron content (mg/kg)	Straw uptake (kg/ha)	Iron use efficiency (%)
Control	27.00	0.15	176.33	1.35	—
Iron sulphate @ 0.25%	43.66	0.30	200.66	1.73	26.32
Iron sulphate @ 0.5%	45.80	0.32	202.80	1.78	14.91
Iron sulphate @ 0.75%	47.70	0.35	204.70	1.84	11.70
Iron sulphate @ 1%	48.96	0.38	205.96	1.94	10.09
Iron chelate @ 0.25%	43.03	0.29	200.03	1.71	33.33
Iron chelate @ 0.5%	45.30	0.32	202.30	1.77	20.24
Iron chelate @ 0.75%	47.46	0.35	204.46	1.80	15.87
Iron chelate @ 1%	48.66	0.36	205.66	1.84	12.50
Iron chloride @ 0.25%	42.66	0.29	199.66	1.67	13.57
Iron chloride @ 0.5%	44.63	0.31	201.63	1.75	7.75
Iron chloride @ 0.75%	44.96	0.31	201.96	1.76	5.18
Iron chloride @ 1%	46.23	0.33	203.23	1.79	4.36
<b>SEm ±</b>	1.86	18.96	2.57	30.00	1.61
<b>C.D(p≤0.05)</b>	5.47	55.69	7.56	88.08	4.83

### 3.5 Protein and Amylose content

The protein content varied significantly with different sources and levels of iron. However, amylose content does not show any notable variations between the different treatments, but numerically the higher amylose content values were recorded lower iron application rates. Highest protein content (6.45%) was recorded in FeSO<sub>4</sub>.7H<sub>2</sub>O @ 1% whereas the lowest protein content (5.93%) was found in control treatment (Table 4).

**Table 4:** Effect of different levels and sources of iron on grain quality parameters of rice.

Treatment	Protein content (%)	Amylose content (%)
Control	5.93	13.80
Iron sulphate @ 0.25%	6.10	13.76
Iron sulphate @ 0.5%	6.14	13.63
Iron sulphate @ 0.75%	6.31	13.83
Iron sulphate @ 1%	6.45	13.84
Iron chelate @ 0.25%	6.20	13.60
Iron chelate @ 0.5%	6.14	13.64

Iron chelate @ 0.75%	6.20	13.83
Iron chelate @ 1%	6.37	13.66
Iron chloride @ 0.25%	6.08	13.72
Iron chloride @ 0.5%	6.12	13.76
Iron chloride @ 0.75%	6.14	13.77
Iron chloride @ 1%	6.16	13.79
<b>SEm ±</b>	0.06	0.063
<b>C.D(p≤0.05)</b>	0.19	NS

### 3.6 Soil Nutrient Studies

Soil nutrient status after the harvest of the crop is an indication of the nutrient mobilization and utilization by the crop. Iron sources and levels did not reveal any significant differences with respect to pH, EC, organic carbon, P and K at harvest, however, the lowest soil available N (244.22 kg ha<sup>-1</sup>) was found in iron sulphate @ 1% whereas, highest soil available N (264.73 kg ha<sup>-1</sup>) was recorded under control treatment (Table 5). This indicates that iron sulphate @ 1% has utilized N efficiently in comparison to other treatments. The results of our study revealed that the iron present in soil varied significantly with different sources and levels of iron.

**Table 5:** Nutrient status of soil at harvest as affected by different sources and levels of iron.

Treatment	N (kg ha <sup>-1</sup> )	P (kg ha <sup>-1</sup> )	K (kg ha <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Organic carbon (%)	pH	EC (ds m <sup>-1</sup> )
Control	264.73	16.35	179.88	54.66	0.87	6.50	0.85
Iron sulphate @ 0.25%	253.62	16.10	173.12	57.53	0.85	6.45	0.83
Iron sulphate @ 0.5%	249.41	16.01	169.28	59.13	0.84	6.43	0.84
Iron sulphate @ 0.75%	246.47	15.91	166.34	61.30	0.84	6.40	0.81
Iron sulphate @ 1%	244.22	15.72	164.70	63.60	0.84	6.41	0.86
Iron chelate @ 0.25%	254.10	16.14	173.18	57.36	0.87	6.42	0.82
Iron chelate @ 0.5%	249.51	16.03	171.09	59.03	0.85	6.37	0.86
Iron chelate @ 0.75%	247.34	15.94	168.21	60.46	0.85	6.35	0.81
Iron chelate @ 1%	245.13	15.90	165.94	62.10	0.84	6.52	0.83
Iron chloride @ 0.25%	255.14	16.23	174.90	56.83	0.86	6.48	0.84
Iron chloride @ 0.5%	251.93	16.8	171.55	58.36	0.85	6.33	0.85
Iron chloride @ 0.75%	250.88	16.07	171.30	59.00	0.84	6.39	0.83
Iron chloride @ 1%	248.62	15.95	168.63	60.10	0.84	6.46	0.84
<b>SEm ±</b>	3.03	0.43	1.83	0.56	0.007	0.09	0.08
<b>C.D(p≤0.05)</b>	9.08	NS	NS	1.66	NS	NS	NS

### 3.7 Correlation studies

The correlation studied (table 6) depicted a positive and significant correlation of panicle length with panicle weight (0.96), test weight (0.37), grain yield (0.82), straw yield (0.80), biological yield (0.81), harvest index (0.74), grain iron uptake (0.78) and straw iron uptake (0.77). A significant and positive relationship of panicles  $m^2$  with panicle weight (0.92), grain yield (0.87), straw yield (0.83), biological yield (0.85), harvest index (0.88), grain iron uptake (0.85) and straw iron uptake (0.83) was marked. From the perusal of data, panicle weight was marked to exhibit a positive and significant correlation with grain yield (0.91), straw yield (0.89), biological yield (0.90), harvest index (0.76), grain iron uptake (0.87) and straw iron uptake (0.87). The results also revealed a positive and significant relationship of grain yield with biological yield (0.99), harvest index (0.81), grain iron uptake (0.99) and straw iron uptake (0.99). A positive and significant correlation of straw yield with biological yield (0.99), harvest index (0.74), grain iron uptake (0.98) and straw iron uptake (0.99) was also observed. The findings further revealed a positive as well as significant relationship of biological yield with harvest index (0.78), grain iron uptake (0.99) and straw iron uptake (0.99). Additionally, a positive and significant correlation was exhibited by harvest index with grain iron uptake (0.80) and straw iron uptake (0.76). A positive and significant correlation was observed between grain iron uptake and straw iron uptake (0.99). The data dispensed in Table 7 elucidated a positive and significant correlation of plant height with leaf area index (0.96), dry matter accumulation (0.87), grain yield (0.84) and straw yield (0.80). The analysis of data marked a significant positive relationship of leaf area index with dry matter accumulation (0.93), grain yield (0.93) and straw yield (0.90). Dry matter accumulation was marked to exhibit a significant and positive association with grain yield (0.98) and straw yield (0.98).

Table 6: Correlation studies of yield and yield parameters with grain and straw iron uptake

	<b>Panicle length</b>	<b>Panicle m<sup>-2</sup></b>	<b>Panicle weight (g)</b>	<b>Grains panicle<sup>-1</sup></b>	<b>Test weight</b>	<b>Grain yield (t ha<sup>-1</sup>)</b>	<b>Straw yield (t ha<sup>-1</sup>)</b>	<b>Biological yield</b>	<b>Harvest index (%)</b>	<b>Grain iron uptake (kg/ha)</b>	<b>Straw iron uptake (kg/ha)</b>
<b>Panicle length</b>	1										
<b>Panicle m<sup>-2</sup></b>	0.93**	1									
<b>Panicle weight (g)</b>	0.96**	0.92**	1								
<b>Grains panicle<sup>-1</sup></b>	0.10	0.09	0.16	1							
<b>Test Weight</b>	0.37	0.41	0.32	0.12	1						
<b>Grain yield (t ha<sup>-1</sup>)</b>	0.82**	0.87**	0.91**	0.31	0.20	1					
<b>Straw yield (t ha<sup>-1</sup>)</b>	0.80**	0.83**	0.89**	0.34	0.15	0.99**	1				
<b>Biological yield</b>	0.81**	0.85**	0.90**	0.33	0.17	0.99**	0.99**	1			
<b>Harvest index (%)</b>	0.74**	0.88**	0.76**	0.13	0.45	0.81**	0.74**	0.78**	1		
<b>Grain uptake (kg/ha)</b>	0.78**	0.85**	0.87**	0.33	0.20	0.99**	0.98**	0.99**	0.80**	1	
<b>Straw uptake (kg/ha)</b>	0.77**	0.83**	0.87**	0.35	0.17	0.99**	0.99**	0.99**	0.76**	0.99**	1

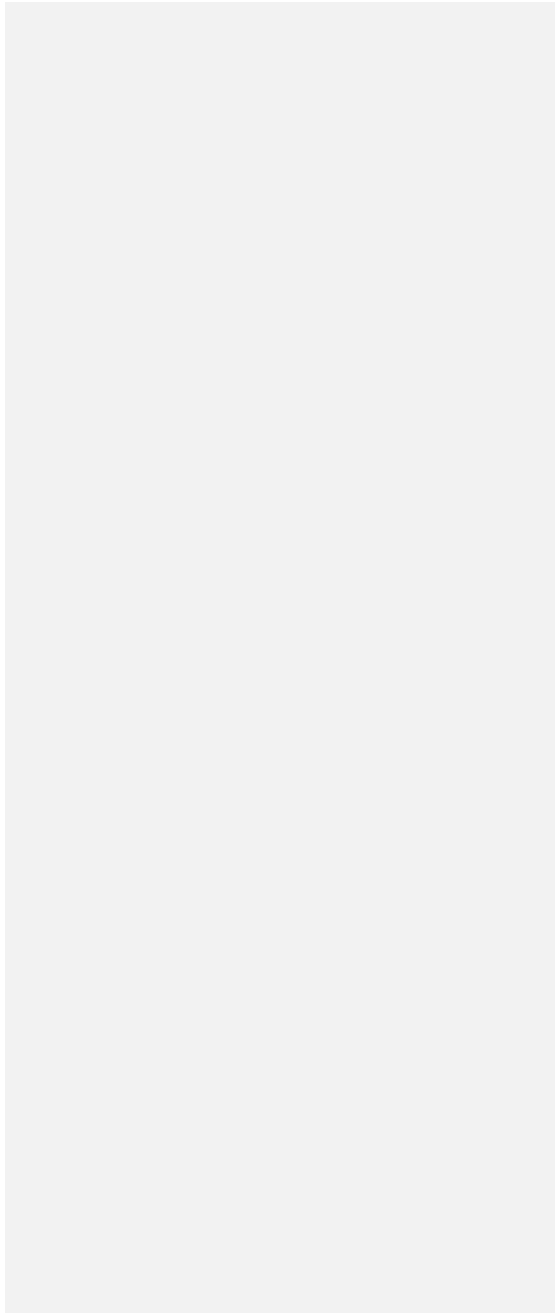
\*\* . Correlation is significant at the 0.01 level (1-tailed).

Table 7: Correlation studies of Growth parameters with yield

	<b>Plant Height</b>	<b>Leaf Area Index</b>	<b>Dry Matter Accumulation</b>	<b>Grain Yield</b>	<b>Straw Yield</b>
<b>Plant Height</b>	1				
<b>Leaf area index</b>	0.96**	1			
<b>Dry matter accumulation</b>	0.87**	0.93**	1		
<b>Grain Yield</b>	0.84**	0.93**	0.98**	1	
<b>Straw Yield</b>	0.80**	0.90**	0.98**	0.99**	1

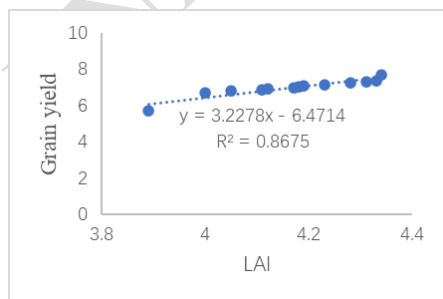
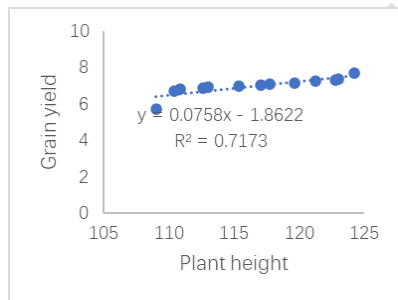
\*\* . Correlation is significant at the 0.01 level (1-tailed).

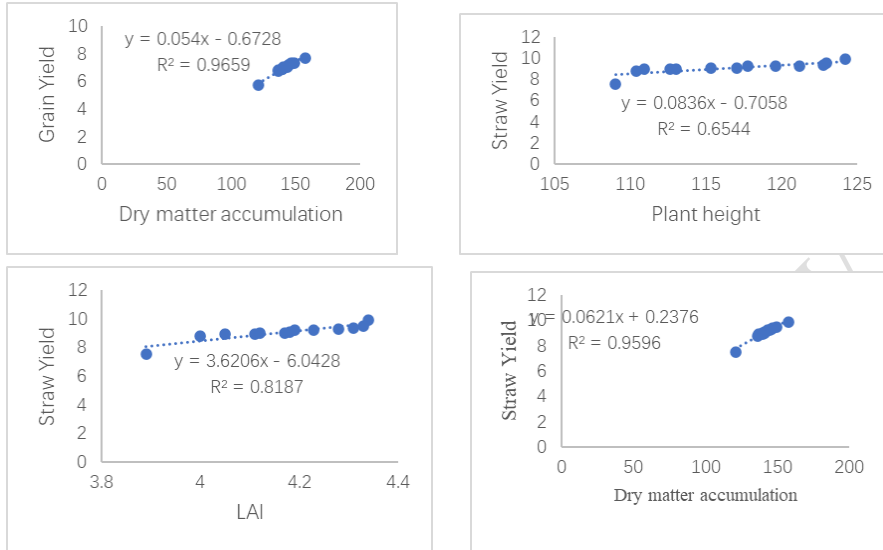
UNDER PEER REVIEW



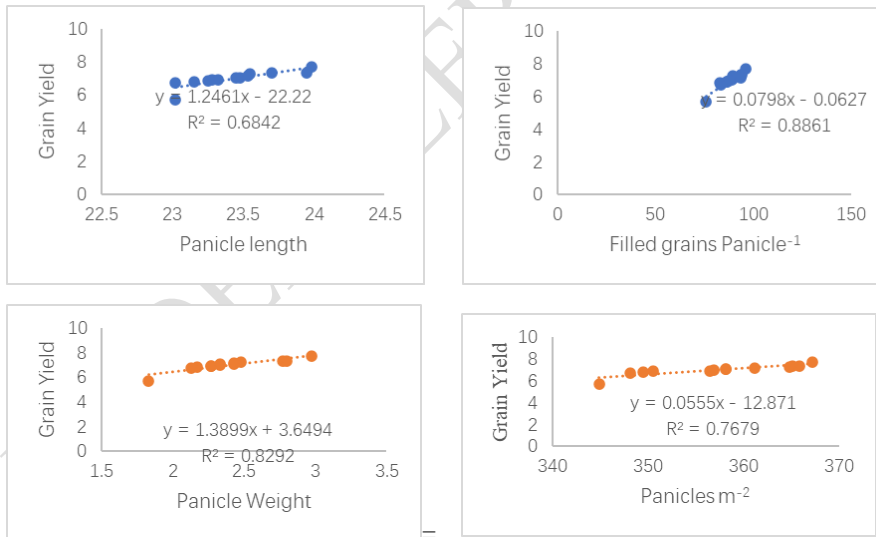
### 3.8 Regression Between Growth and Yield Attributes with Grain Yield

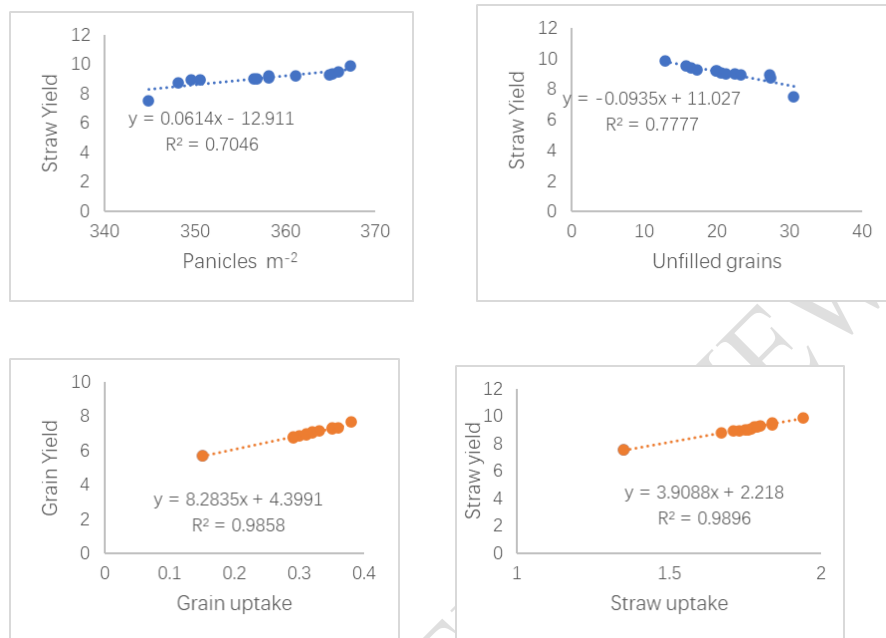
Regression analysis depicted the relationship between growth parameters and yield. A positive relationship between plant height at harvest and grain yield ( $R^2= 0.71$ ) depicted a 71% accountability of plant height to grain yield. Leaf area index was marked to exhibit a positive association with grain yield ( $R^2= 0.86$ ), accounting for 86% of grain yield. A positive affiliation of dry matter accumulation at harvest with grain yield was noted ( $R^2= 0.96$ ). The analysis reflected a positive relationship of plant height ( $R^2= 0.65$ ), leaf area index at flowering ( $R^2= 0.81$ ) and dry matter accumulation at harvest ( $R^2= 0.95$ ) with straw yield. The regression analysis reflected an association between yield, yield attributes (Panicles  $m^{-2}$ , panicle weight and grains panicle $^{-1}$ ). A positive association between grain yield and panicles per sq. m ( $R^2= 0.76$ ) reflected that the panicles per sq. m accounted for 76% of the grain yield. A positive relationship between grain yield and panicle weight ( $R^2= 0.82$ ) indicated 82% accountability of panicle weight to grain yield. A positive relationship of panicle length ( $R^2= 0.68$ ) and filled grains per panicle ( $R^2= 0.88$ ) with grain yield was noted. Analysis of data revealed a positive association of panicle length ( $R^2= 0.65$ ), panicle weight ( $R^2= 0.80$ ), panicles per square meter ( $R^2= 0.70$ ) and unfilled grains per panicle ( $R^2= 0.77$ ) with straw yield. A positive relationship of grain yield with grain iron uptake ( $R^2= 0.98$ ) revealed that grain iron uptake accounted for 98% of the grain yield. However, a positive association of straw yield with straw iron uptake ( $R^2= 0.98$ ) marked a 98 % accountability of straw iron uptake to grain yield.





**Fig.4:** Regression analysis of different growth parameters and yield.





**Fig.5:** Regression analysis of different yield attributes, yield and iron uptake.

#### 4 Discussion

CGR, RGR and NAR are the important tools for estimating growth of the crop. The data depicted that as the crop reaches upto maturity and closer to harvest, its crop growth rate is slowed down whereas the relative growth rate and net assimilation rate decreased significantly with the advancement of crop age. Among the different iron treatments, higher growth rates were recorded in  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 1% treatment which, statistically, was comparable with iron chelate @ 1%. Among different sources and levels of iron, iron sulphate @ 1% recorded highest CGR, RGR and NAR. A sufficient supply of iron helps the plants to improve various metabolic processes by increasing the uptake and availability of other essential nutrients. Due to these reasons the crop growth will be enhanced resulting in higher yield attributes. External application of Fe helps to increase photosynthesis, net assimilation and relative growth in rice as it is essential for metabolism of chlorophylls. The findings of Iqbal et al. [21] also found a significant effect of iron on forage crops.

The iron sulphate @ 1% and iron chelate @ 1% recorded maximum Panicle m<sup>-2</sup>, panicle weight and grains per panicle whereas the control treatment recorded lowest value of yield attributes. The superior yield attributes in rice are manifested as iron increases and stimulates the crop growth and enhances the production of tillers. The reason for increase in number of panicles m<sup>-2</sup> is that iron application increases the availability of many

essential nutrients resulting in the overall growth of crop plants. The results of Kumar et al. [7] also depict a favorable effect of iron on rice. During the post flowering phase, the weight of panicles and grains increased due to enhanced photosynthesis and transfer of assimilates. The efficiency of plant activities such as membrane integrity, regulation of stomata, formation of chlorophyll and utilization of energy during early stages of plant growth resulted in increased size and weight of grains at later stages on foliar spray of iron which lead to increase in panicle weight [22,23]. The increase in grains panicle<sup>-1</sup> on application of different iron treatments is because iron aids the accessibility of other nutrients, enhances the nutrient uptake and transfers photosynthates from source to sink, thereby increasing the fertility rate of the panicles and results in producing more number of grains panicle<sup>-1</sup> [24,25].

The data showed that foliar application of iron at higher rate (1%) through iron sulphate and iron chelate had significantly improved grain, straw and biological yield of rice. The higher grain yield in any crop plant is attributed to its vegetative growth and accumulation of dry matter. The higher the vegetative growth of plant, the higher the dry matter which results in higher grain yield. However, grain yield is the sum of all the yield attributing parameters. The higher grain yield in rice on foliar application of iron is due to the fact that iron enhances the panicles m<sup>-2</sup>, grains panicle<sup>-1</sup>. Another reason is that iron plays a key role in initiation of primordial reproductive organs and in biosynthesis of Indole Acetic Acid (IAA) also the higher straw yield for iron applications compared to control can also be attributed due to the fact that iron supports the entire vegetative growth of crops by making other nutrients available and supporting their uptake. Kumar et al. [8] also reported increased yield of rice with iron fortification. The function of grain yield to the total biological yield (grain + straw) is known as harvest index. Iron chelate @ 0.75 % recorded highest harvest index which might be the result of improved carbohydrate translocation from source to sink [8].

Foliar application of iron sulphate (1%) improved the grain and straw iron content and uptake significantly. The mobilization of iron sulphate is high in plants that might be the reason for increase in iron content in brown rice. Due to its high mobilization the iron is readily available to plants, helps in quick absorption, easily translocate to different parts of plants. The components of yield vary proportionately with foliar spray of micronutrients which then enrich the flour effectively in rice. The crop yield showed a positive correlation with the iron uptake in the grain. The nutrient uptake is high with foliar application of iron due to its continuous supply and easy availability of other nutrients proportionately. Moreover, the iron content in straw was found more as compared to grain. This may be due to increased availability, absorption and limited mobility of iron in different parts of plants. The Fe uptake in rice straw increased significantly by Fe fertilization and with foliar sprays of 1% FeSO<sub>4</sub>.7H<sub>2</sub>O application was highest as compared to other treatments. The reason may be that micronutrients and rice yield from this treatment are high. However, the iron use efficiency decreased with higher rates of iron application and was found maximum in chelated iron because the

chelated form of iron is taken by the plant effectively Iron use efficiency decreases when content of a particular source or level of iron is increased [26].

Iron being an important cofactor and plays a tremendous role to enhance the nitrite and nitrate reductase enzymes could be the reason for higher protein content due to foliar application with iron. The presence of iron in chloroplast aids in the metabolism of Sulphur and nitrogen, and its role in the constitution of heme and non-heme proteins is another reason for increase in protein content [27].

Iron sources and levels did not reveal any significant differences with respect to pH, EC, organic carbon, P and K at harvest, however, the lowest soil available N was found in iron sulphate @ 1% whereas, highest soil available N was recorded under control treatment. This indicates that iron sulphate @ 1% has utilized N efficiently in comparison to other treatments. The results of our study revealed that the iron present in soil varied significantly with different sources and levels of iron. Application of iron sulphate @ 1% significantly increased the available iron in soil at harvest [28].

Foliar Fe fertilization is an effective agricultural approach for enhancing yield, iron content, bioavailability Fe and protein content of rice. From this experiment, it was concluded that application of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  @ 1% was more effective among various sources and levels of iron in enhancing crop growth rate, relative growth rate, net assimilation rate, yield, protein content, iron content and its uptake in brown rice and straw besides increasing soil iron content and decreasing soil nitrogen at harvest however, iron chelate @ 0.25% recorded highest iron use efficiency, therefore making it a convenient source for agronomic fortification of rice with iron under temperate conditions of Kashmir.

## References

1. Maclean, J., Hardy, B., Hettel, G. (2013). *Rice Almanac: Source book for one of the most important economic activities on earth*. IRRI.
2. CD/FAO (2018). "OECD-FAO agricultural outlook 2018–2027". *Pneumologie*, 44, p.107.
3. DES (2018). *Agricultural Statistics at a Glance*. Directorate of Economics and Statistics, Ministry of Agriculture, Government of India. <https://desagri.gov.in/en/document-report/agricultural-statistics-at-a-glance-2018/>.
4. Steinger, G., N., Muller-Fischer, H., Cori, B. Conde-Petit (2014). Fortification of rice: technologies and nutrients. *Annals of the New York Academy of Sciences*, 1324 (1), 29-39.

5. Alloxances, R. D. (2009). Nutrient requirements and recommended dietary allowances for Indians. *National Institute of Nutrition, Indian Council of Medical Research: Hyderabad, India.*
6. Sharma, A., A., Yadav, V., Baig, M., Swarnkar, R., Singh, Kumar, S. (2015). Malnutrition and associated risk factors among under five childrens. *Indian Journal of Community Health, 27 (3), 311-319.*
7. Kumar, D., Dhaliwal, S. S., Naresh, R. K., Salaria, A. (2018a). Agronomic biofortification of paddy through nitrogen, zinc and iron fertilization: A review. *International Journal of Current Microbiology and Applied Sciences, 7, 2942-2953.*
8. Kumar, V., D. Kumar, Y.V. Singh, R. Raj, N. Singh (2018b). Effect of iron nutrition on plant growth and yield of aerobic rice. *International Journal of Chemical Studies, 6(4), 999-1004.*
9. Nandan, B., B.C. Sharma, G. Chand, K., Bazgalia, R. Kumar, Banotra, M. (2018). Agronomic fortification of zinc and iron in chickpea an emerging tool for nutritional security-A global perspective. *ACTA Scientific Nutritional Health, 2(4), 12-19.*
10. Redford, P.J. (1967). Growth analysis formulae, their use and abuse. *Crop Science, 7, 171-175.*
11. Blakman, V. H. (1919). The compound interest law and plant growth. *Annals of botany, 33 (131), 353-360.*
12. Evans, J. R. (1983). Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant physiology, 72 (2), 297-302.*
13. Juliano, B.O. (1990). Chemical basis of rice grain quality. In: *Proceedings of Workshop on Chemical Aspects of Rice Grain Quality*, International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines. pp. 69-70.
14. Thimmiah, S.K. (1999). *Determination of amylose.* In: *Standard Method of Biochemical Analysis.* Kalyani Publishers. pp. 60-61.
15. Prasad, R., Y.S. Shivay, D. Kumar and S.N. Sharma (2006). *Learning by Doing Exercises in Soil Fertility (A Practical manual for soil fertility)*, Division of Agronomy, Indian Agricultural Research Institute, New Delhi, India. p. 68.
16. Walkley, A., Black, I., A. (1934). An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science, 37, 29– 37.*

17. Jackson, M. L. (1973). *Soil Chemical Analysis*. Prentice Hall of India Private Limited, New Delhi 15, 13-22.
18. Subbiah, B. V. and G.L. Asija (1956). A rapid procedure for the estimation of available nitrogen in soil. *Current Science*, 25, 259-60.
19. Olse n, S. R. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate* (No. 939). US Department of Agriculture.
20. Lindsay, W., L., W., A., Norvell (1978). Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of American Journal*, 42, 421-428.
21. Iqbal, M., A., H., Abdul, H., S., Muzammil, H., Imtiaz, A., Tanveer, I., Saira, A., Anser (2019). A Meta-analysis of the impact of foliar feeding of micronutrients on productivity and revenue generation of forage crops. *Planta Daninha*, 37, 234-256.
22. Aziz, M. Z., M., Yaseen, T., Abbas, M., Naveed, A., Mustafa, Y., Hamid, Q., Saeed, X., U., Ming-gang (2019). Foliar application of micronutrients enhances crop stand, yield and the biofortification essential for human health of different wheat cultivars. *Journal of Integrative Agriculture*, 18 (6), 1369-1378.
23. Vikash, K., Dinesh, K., Singh, Y., V., Raj, R. (2015). Effect of iron fertilization on dry matter production, yield and economics of aerobic rice (*Oryza sativa* L.). *Indian Journal of Agronomy*, 60 (4), 547-553.
24. Dehaghi, M., A., Agahi, D., Talei, M., Seddighi (2015). Morpho-agronomical response of rice (*Oryza sativa* L.) to foliar iron. *Bulgarian Journal of Agricultural Science*, 21 (6), 1229-1234.
25. Dhanalakshmi, M., Prabhakaran, J., Senthil, K., Thiyageshwari, S., Sathyamoorthy, N., K. (2019). Effect of chelated micronutrients on the yield attributes and yield of rice. *International Journal of Chemical Studies*, 7(3), 3040-3042.
26. Kandil, E., E., A., O., M., Eman (2017). Response of some wheat cultivars to nano mineral fertilizers and amino acids foliar application. *Alexandria science exchange journal*, 38(1), 53-68.
27. Rana, D., S., B., Singh, K., Gupta, A., K., Dhaka, S., Arya (2013). Response of fodder sorghum [*Sorghum bicolor* (L.)] to zinc and iron. *Journal of Forage Research*, 39, 5-7.
28. Choudhary, S., K., M., K., Jat, A., K., Mathur (2017). Effect of micronutrient on yield and nutrient uptake in sorghum. *Journal of Pharmacognosy and Phytochemistry*, 6 (2), 105-108.