Biological Forum – An International Journal **15(5): 197-204(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Foliar Application of Ferrous Sulphate and its Influence on Growth, Grain Quality and Nutrient Uptake in Soybean (*Glycine max* L.)

Badapmain Makdoh[1], A.P. Singh[1]*, L.T. Longkumer[1], T. Gohain[1], Lanunola Tzudir[1], D. Nongmaithem[1], Rekha Yadav[1] and Damitre Lytan[2]
[1]Department of Agronomy, SASRD, Nagaland University, Medziphema (Nagaland), India.
[2]Department of Entomology, SASRD, Nagaland University, Medziphema (Nagaland), India.

(Corresponding author: A.P. Singh)*
(Received: 24 February 2023; Revised: 15 April 2023; Accepted: 19 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Iron deficiency has been one of the most prevalent micronutrient deficiencies in most part of India particularly the rural Northeast region which has affected children and women. To alleviate iron deficiency which is a major global issue, agronomic biofortification is an easy and sustainable approach that aims at enriching iron content in edible part of the crops. It simultaneously enhances crop performance in terms of yield andiron content of grain. Hence, a study was conducted to see the effect of iron fertilization on soybean being one of the important pulse crops in the tribal communities. A two years pot experiment was conducted during *kharif* season of 2018 and 2019 at the Agronomy farm, SASRD, Nagaland university, Medziphema campus to study the effect of iron sulphate on growth, yield and quality parameters in soybean. Three (3) soybean varieties *viz.*, JS-335, JS-97-52 and local cultivar under six (6) iron (Fe) fertilization treatments laid out in factorial CRD with three replications. The Six iron treatments *viz.*, Fe_0 as Control, Fe_1 (Foliar spray application of $FeSO_4.7H_2O$@ 0.5%), Fe_2 (Foliar spray application of $FeSO_4.7H_2O$@ 1%), Fe_3 (Foliar spray application of $FeSO_4.7H_2O$@ 1.5%), Fe_4 (Foliar spray application of $FeSO_4.7H_2O$@ 2%), Fe_5 (Soil application of $FeSO_4.7H_2O$@ 10 kg ha^{-1}). Results of the two-year experimentation revealed that JS 97-52 was superior in dry matter yield while plant height and leaf area, local cultivar was way superior than the others. Foliar spray application of $FeSO_4.7H_2O$ (ferrous sulphate) @ 1.5% was found most effective in enhancing growth, yield and iron content of soybean. The chlorophyll content was significantly higher in JS-335 at both 30 and 60 DAS. Foliar spray application of 1.5% $FeSO_4.7H_2O$ resulted in significantly higher chlorophyll content at 60 DAS. The highest seed yield was observed with foliar spray application of iron sulphate @ 1.5% (24.89 g pot^{-1}). The highest value of iron content in grain (67.29 mg kg^{-1}) and stover (124.48 mg kg^{-1}) was observed with foliar application of 1.5% iron sulphate along with percent increase of 18.51% and 17.30%, respectively over control. Foliar application of 1.5% iron sulphate significantly enhanced the protein (38.96%) and oil content (18.29%). NPK uptake was found significantly higher in JS 97-52, and among Fe fertilization, Fe_3 significantly improved the N and K uptake only.

Keywords: Soybean, growth, yield, quality, uptake.

INTRODUCTION

Iron and zinc deficiency on human is one of the major concerns of public health particularly developing countries (Welch and Graham 2004). An estimated two billion people across the globe is affected by iron deficiency which is one of the most prevalent micronutrient deficiency (Stoltzfus and Dreyfuss 1998). According to an estimate around 60-70% the world's total population has iron deficiency. Iron deficiency mainly results in anaemia, leading to functional impairments of the human body (Cappellini *et al.*, 2020). The recommended daily allowance of iron is 13 mg per day for children, 17 mg per day for adult. Lack of nutritional diversity in Indian diet has further exacerbated deficiency in micronutrients like iron (Anon., 2009). Apart from the importance on human health and nutrition, iron has many vital roles in plant systems as well. It is a structural component of porphyrin molecules, cytochromes, hematin, ferrichrome and leghaemoglobin involved in oxidation reduction reactions in respiration. Iron is also an important constituent element for the nitrogenase enzyme which plays an important role in N-fixation through the N-fixing bacteria and in the chloroplast for photosynthetic reduction processes.

Soybean shares almost 25% of the global edible oil and contributes almost two-thirds of protein concentrate required for livestock feed production globally. India is the 4th largest producer of soybean in the world. Soybean is also considered to be an important crop in the North-Eastern states of India. This crop has always been an integral part in the culture and farming systems of different ethnic groups and farming communities of North-Eastern India and its uses is diverse depending on tribal communities. To alleviate micronutrient deficiency on human health, many strategies have been executed in recent past where encouraging results were even observed which comprising of iron supplementation, fortification, dietary diversification/ modification and biofortification can be deployed. The approach of 'biofortification' of food crops gradually has gained global attention (Graham *et al*., 2001). Many reports have suggested that foliar application of Fe could improve the quantitative and qualitative production of various crops. For instance, yield and iron concentration in chickpeas has significantly improved with foliar spray of Fe (Pal *et al*., 2019a). Similarly, foliar spray application of Fe resulted in a higher seed yield and grain Fe concentration in wheat crop (Sharma *et al*., 2019). Varieties and strains of any crops have differences in their efficiency to take up iron or zinc from their environment. Iron/zinc efficient ones able to extract more of the micronutrient even under low iron availability and accumulate more dry matter compared to inefficient ones, but may not always produce high iron/zinc concentration (Graham *et al*., 1992). Joshi *et al*. (2010) studied the effect of genotype x environment interaction for variation in Fe and Zn concentrations of wheat grains and reported that micronutrient density in grain is highly variable for different genotypes sown at different locations. Trivedi *et al*. (2011) reported that positive effect of iron and sulphur application was observed on different parameters *viz*., plant height, number of leaves plant^{-1}, root length, chlorophyll content, nitrogen content of leaf, number of pods plant^{-1}, pod length, growth parameters, 100 seed weight and protein content. Kumar *et al*. (2015) indicated that the highest drymatter accumulation and grain yield were recorded with three foliar sprays of 2.0% iron sulphate followed by three foliar sprays of 0.5% iron chelate. They also reported that the highest grain yield was recorded with three (3) foliar sprays of 2.0% iron sulphate followed by three foliar sprays of 0.5% iron chelate. Dhaliwal *et al*. (2022) reported that application of 0.5% $FeSO_4$ application at 30, 60 and 90 DAS resulted in the maximum grain and straw yield (3064 and 9341 kg ha^{-1}, respectively) over the control. Similar results were attained for grain Fe concentration (69.9 mg kg^{-1}) and Fe uptake in grain and straw (214 and 9088 g ha^{-1}, respectively).

Very little information has been reported on agronomic biofortification of iron on soybean and pulse crops especially in soils under iron sufficiency range. In the past, agronomic biofortification programmes were mainly focussed on major cereal crops like wheat, maize and rice enhancing micronutrient density in grain. The idea of applying the concept of agronomic biofortification of iron in soybean being a commonly cultivated crop in the region can be considered as a sustainable approach. Hence, this study was conducted with the goal to evaluate the effect of iron on growth, yield and enhancing grain quality in soybean grain.

MATERIALS AND METHODS

The pot experiment was conducted at School of Agricultural Sciences and Rural Development (SASRD), Nagaland University, Medziphema, Nagaland, situated at a latitude of 25°45′43″ North and longitude 95°53′04″ East with altitude of 310 meters above mean sea level (MSL). The experimental farm lies in a humid subtropical region with annual rainfall ranging from 2000-2500mm. The soil of the experimental field was sandy loam, pH of the soil 4.90 (1: 2.5 soil and water ratio), with organic carbon 1.51% (Walkley and Black's rapid titration method given by Jackson 1973). The soil had 526.7 kg ha^{-1} alkaline permanganate oxidizable N, 32.8 kg ha^{-1} available P (Bray's method), 264.82 kg ha^{-1} ammonium acetate exchangeable K and available Fe extract by diethylene triamine penta acetic acid (DTPA) method developed by Lindsay and Norvell (1978) in soil was 78.43 mg kg^{-1} of soil.

Soil from the top 15 cm was collected randomly from experimental field weighing 12 kg which then filled cement pots of 20 L capacity each. This pot experiment was conducted to study the biofortification of iron in soybean with three soybean varieties *viz*., JS-335, JS-97-52 and local cultivar under six iron fertilization treatments laid out in Factorial CRD replicated thrice. Six (6) iron treatments *viz*., Fe_0 as Control, Fe_1 (Foliar spray application of $FeSO_4.7H_2O$@ 0.5%), Fe_2 (Foliar spray application of $FeSO_4.7H_2O$@ 1%), Fe_3 (Foliar spray application of $FeSO_4.7H_2O$@ 1.5%), Fe_4 (Foliar spray application of $FeSO_4.7H_2O$@ 2%), Fe_5 (Soil application of $FeSO_4.7H_2O$@ 10 kg ha^{-1}). Each treatment was given to the pots in triplicate and the experiment was laid out in Factorial Completely Randomized Design (FCRD). Two foliar applications of each foliar treatments were applied at pre-flowering stage of the crop. The crop was sown on second week of July 2018 and 2019 by maintaining five plants each pot for initial crop establishment. Recommended NPK (RDF) dose of 20:60:40 kg ha^{-1} NPK (in the form of Urea, SSP and MOP) was applied along with FYM @ 10 t ha^{-1} as general dose to all the pots irrespective of treatments on soil weight basis where seed treatment with rhizobium culture was done. In pots with foliar application treatments accordingly accurate amount of ferrous sulphate heptahydrate containing 32.8% iron was used where two foliar spays was executed during pre-flowering and flowering stage. Lime supernatant was used as spray solution to avoid any salt injury to the foliage.

Data on plant height, number of branches plant^{-1}, dry matter yield, leaf area, grain and stover yield were recorded as per the standard procedures. The procedure developed by Witham *et al*. (1971) was followed for estimation of chlorophyll content of leaves at 30 and 60 DAS (days after sowing). Oven-dried plant samples were grounded to a fine powder using a mechanical grinder. A representative grounded straw and grain sample of 0.5 g were digested using a di-acid mixture (HNO_3:$HClO_4$, 3:1) on an electric hot plate procedure as described by Prasad *et al*. (2006). For N content, seed samples were analysed by Kjeldahl method. Phosphorus in grain and stover samples were determined in diacid (HNO_3, $HClO_4$) extract by advocating standard procedure (Jackson, 1973). K content (%) in the plant sample was determined by flame photometer (Jackson, 1973). The Fe content was estimated from the digested plant extracts through ICP Spectrophotometer model: iCAP 7000 series in mg kg^{-1}.

Macronutrient uptake (g pot^{-1}) =

$$\frac{\text{Nutrient content (\%)} \times \text{yield (g pot}^{-1})}{100}$$

All the replicated data obtained from the experiment were statistically analysed using the F test as per the procedure given by Gomez and Gomez (1984). Critical difference values at P = 0.05 were used to determine the significance of differences between treatment means.

RESULTS AND DISCUSSION

Growth parameters

Plant height. Varieties differed significantly in plant height at all crop stages (Table 1). At harvest, local cultivar (72.07 cm) registered the highest plant height while the lowest value observed in JS-335 (49.30 cm). Among the varieties, local cultivar found to be much superior in plant height being a traditionally taller cultivar when compared to short duration high yielding improved varieties. The main reason for this could be due to varietal and genotypic differences in morphology which is characteristics of the variety. Iron fertilization failed to result any significant difference on plant height of soybean at all crop stages during both the years of study. Although, no significant effect upon Fe fertilization on plant height, however Fe_3 (Foliar spray application of $FeSO_4.7H_2O$@ 1.5% at pre-flowering stage) has numerical superiority (63.22 cm) over the rest. The increase in the availability of iron might have stimulated the metabolic and enzymatic activities, thereby increasing the growth of the plant reported by (Trivedi *et al*., 2011). Fe treated plants have better uptake of plant nutrients which might resulted in enhancement of more photosynthesis in turn resulted to more leaves, leaf area and dry matter accumulation and ultimately on plant height. Balachandar *et al*. (2003); Kobraee *et al*. (2011); Dhaliwal *et al*. (2013); Soni and Kushwaha (2020) also reported similar findings with respect to effect of Fe on plant height.

Number of branches. The number of branches significantly varied at 60 and 90 DAS (days after sowing). At 60 DAS, JS-97-52 (3.70) was found superior although statistically at par with JS-335 (3.48). However, with progress of crop stage till harvest, the local cultivar was at par in the number of branches per plant with the other varieties (Table 1). The number of branches significantly increased with increasing dose of ferrous sulphate. Fe fertilization did not significantly influence on the number of branches in both the years. Numerically, we could observe an increasing trend with increasing dose of ferrous sulphate but limited up to 1.5% concentration. The increase in the number of branches and dry matter upon application of ferrous sulphate might be due adequate supply of nutrients at crop critical stages which resulted in better photosynthesis rate, assimilation and translocation of photosynthates from source to sink (Muthal *et al*., 2016). These results were in conformity to the findings reported by Kumar *et al.* (2009); Farhan and Al-Dulaemi (2011); Abdel *et al.* (2014).

Leaf area. With respect to leaf area there is significant variations among varieties at all crop stages and local cultivar was found superior in this parameter (Table 1). There was significant difference of Fe fertilization on leaf area at 60 DAS only. At 60 DAS, foliar spray application of ferrous sulphate @ 1.5% (Fe_3) recorded the highest leaf area (1401.02 cm^2 $plant^{-1}$) which was followed by Fe_2 (1351.27 cm^2 $plant^{-1}$) and soil application of $FeSO_4.7H_2O$ @ 10 kg ha^{-1} (1280.10 cm^2 $plant^{-1}$). The least value of leaf area was recorded in control (1116.99 cm^2 $plant^{-1}$). At 90 DAS, leaf area remained non-significant with Fe treatments as the two varieties achieved senescence stage where leaf fall resulted in drastic decline in leaf area except for local cultivar where the leaf area was progressing (2068.44 cm^2 $plant^{-1}$). The enhanced leaf area upon Fe fertilization might be due to iron being the component of the photosynthetic apparatus as well as its rate and formation of pigment chlorophyll. Iron applied acts as an important catalyst in the enzymatic reaction of metabolism. This ultimately would have helped in larger biosynthesis of photoassymilates, thereby enhancing vegetative growth of plant. It was also reported that enhanced photosynthesis and respiration rates, more crop growth, and improved physiological and biochemical processes were observed by the application of iron (Zeidan *et al.*, 2010) which could have increased leaf area of crop. These results are in accordance with earlier findings that reported by Farhan and Al-Dulaemi (2011); Trivedi *et al*. (2011); Kamble *et al*. (2021).

Dry matter yield (g $plant^{-1}$). There was significant variation in dry matter accumulation (DMA) among varieties at all crop stages (Table 1). At harvest, the variety JS 97-52 (16.63 g $plant^{-1}$) was statistically at par with local cultivar (16.18 g $plant^{-1}$) and least was in JS-335 (12.19 g $plant^{-1}$). Application of ferrous sulphate, Fe_3 resulted in maximum value (16.20 g $plant^{-1}$) followed by Fe_2 (15.79 g $plant^{-1}$) and the least was in control (13.91 g $plant^{-1}$). The positive effect of Fe nutrition in enhancing DMA might be due to the balanced application of plant nutrients that ultimately enhanced growth and development of the crop. Iron tends to increase the synthesis of enzymes like IAA production and protein synthesis, which helps promote vegetative growth. The results of this investigation are in accordance with the findings of Meena *et al*. (2006); Kamble *et al*. (2021).

Chlorophyll content. The results presented in Table 2 shows that chlorophyll content varied significantly among varieties at all crop stages. At 60 DAS, the same trend was observed with respect to varietal difference in chlorophyll content. Chlorophyll "a", "b" and total chlorophyll content was significantly higher in JS-335 at both 30 and 60 DAS and was at par with JS 97-52 and the least was in local cultivar. The difference among the varieties on the chlorophyll content was mainly due to genotypic difference which is supported by another study given by Mahmoudi *et al*. (2007) who reported in chickpea. Fe fertilization significantly influenced chlorophyll content of soybean at all crop stages. With increasing spray concentration of $FeSO_4.7H_2O$ at 60 DAS there was incremental enhancement of chlorophyll content and up to 1.5% $FeSO_4.7H_2O$. Foliar spray application of 1.5% $FeSO_4.7H_2O$ resulted in significantly higher chlorophyll "a" content (0.92, mg g^{-1}) over the other treatments although at par with Fe_2 (0.91, 0.88 mg g^{-1}). Similar trend was also seen in chlorophyll "b" and total chlorophyll content. Percentage increase with foliar spray of $FeSO_4.7H_2O$ @ 1.5%, 1.0%, 0.5% over control (11.28 %), (6.02%) and (6.02%), respectively. Iron is known as the main factor for chlorophyll formation and photosynthesis and its vital role plant enzyme systems and respiration (Halvin *et al*., 1999). The increase value of chlorophyll content under Fe treatments was likely because of its role or involvement in the biosynthesis pathway of chlorophyll and haeme (Baele, 1999). According to the study of Chereskin and Castelfrance (1982), iron is the metabolic constituent of caproporphyrinogen oxidase which is part of the biosynthesis of δ-aminolevulinic acid (ALA). It is well concluded that Fe involves in chlorophyll synthesis indirectly by affecting its precursor ALA. Iron also plays a major role in the structure porphyrin of chlorophyll as well as a component of chloroplasts (Rout and Sahoo, 2015). Iron sulphate application plays an important role in synthesis of chlorophyll and plant growth regulator (Jin *et al*., 2008). With application of $FeSO_4.7H_2O$ either to soil or foliar there is possible increase in the level of ferrous ion (Fe^{2+}) uptake by the plant leaves which enhances absorption and translocation of iron. This might help the cellular activity and directly or indirectly take part in the formation of chlorophyll. This finding was also in agreement with the previous studies reported by Shukla and Shukla (1999) and Kumawat *et al*. (2006).

This might be ascribed to the increased synthesis

Yield

Seed yield. Seed yield varied significantly among the varieties (Table 2) where JS-97-52 registered the highest value (29.04 g pot^{-1}) followed by JS-335 (20.76 g pot^{-1}) and local cultivar recorded the lowest (17.36 g pot^{-1}). Improved variety like JS 97-52 markedly surpassed over the other two in yield potentials over the two years of experimentation irrespective of the treatments imposed mainly attributed to the effect of Genotype x Environment interaction. Foliar application of iron sulphate significantly enhanced seed yield per pot as depicted in Table 2. Foliar spray application of ferrous sulphate @ 1.5% +recorded the highest value of 24.89 (g pot^{-1}) and the lowest in control (19.50 g pot^{-1}). The per cent increase in seed yield with two foliar applications of $FeSO_4.7H_2O$ @ 1.5% was to the tune of 27.60% over control. The increase in yield due to Fe application was attributed to better performance in the growth and yield parameters which could have influenced the physiological processes and photosynthates build up under adequate availability of major and micro nutrients in soil as explained by Tabassum *et al*. (2013). It can also be explained by the fact that Fe is part of ferrodoxin and cytochrome structures which are electron carrier and plays vital role in various metabolic processes *viz.*, hormone production, nitrogen fixation, chlorophyll construction, photosynthesis, respiration, DNA synthesis (Vaghar *et al*., 2020). Thus, significant increase in yield of soybean might be due to the improved leaf and stem nutrition and intensification of photosynthesis due to foliar application of Fe (Rai *et al*., 2021). Iron application might have increased number of enzymatic activities of Fe-containing enzymes which cumulatively have positive effect on crop yield. These results were in concordance with the results obtained from Ghasemian *et al*. (2010), Kobraee *et al*. (2011) and Kumar *et al*. (2016).

Stover yield. JS 97-52 recorded the highest stover yield (29.68 g pot^{-1}) which was significantly higher than JS-335 (20.89 g pot^{-1}) and local cultivar (19.69 g pot^{-1}). The highest value of stover yield was observed in Fe_3 (26.22, 26.33 g pot^{-1}). Application of 1.5% $FeSO_4.7H_2O$ foliar spray enhanced the stover yield by (28.13 %) over the control. Iron known to improves photosynthesis and assimilates transportation to sinks which then finally increases stover yield of crop. Similar effect of foliar spray of iron was observed in cowpea in sandy loam soil of Kerala by Anitha *et al*. (2005); Yadav *et al*. (2013) in mungbean.

Quality parameters

Iron content (mg kg^{-1} DM) in grain and stover. Varieties significantly differed from each other in grain Fe concentration. The highest value of grain Fe content was found in cultivar JS-335 (67.50 mg kg^{-1}) which was statistically at par with JS 97-52 (65.13 mg kg^{-1}) followed by statistically inferior local cultivar (56.78 mg kg^{-1}). The variation of grain Fe content among the varieties could be explained by the varietal difference in efficiency level with respect to Fe acquisition which was supported by the findings of Rengel and Graham (1996); Graham *et al*. (1997); Dhaliwal *et al*. (2013). With application of $FeSO_4.7H_2O$ there was positive effect on the Fe biofortification and enhancement in grain Fe concentration. Foliar spray application of ferrous sulphate @1.5% recorded maximum grain Fe content (67.29 mg kg^{-1}) (Table 3). The per cent increase in grain Fe content upon iron fertilization over the control was (18.51 %). Similar results have also been reported by Dhaliwal *et al*. (2010). In the latest study reported by Dhaliwal *et al*. (2022) in soybean, it was indicated that maximum increase in Fe density can be achieved through either 2-3 foliar sprays of 0.5% $FeSO_4.7H_2O$. Their results substantiated the findings of our experiment, where foliar application of iron sulphate at 1.5%, 1% and 0.5% resulted higher value of iron content on grain. Foliar application of $FeSO_4.7H_2O$ was found superior over soil application as corroborated by the findings of Nayyar and Takkar (1989); Pal *et al*. (2019b). Varieties did not show any significant difference on the iron content of stover, although Fe significant difference observed in the pooled data only. The percent increase of Fe content in stover with 1.5% iron sulphate spray application was 17.30 % over the control pot.

Protein content in grain. The protein content was significantly higher in JS-335 (37.94%) which was statistically at par with JS 97-52 (37.73%) and the least value in local cultivar (36.71%) (Fig. 1). Fe fertilization observed to markedly influenced the protein content of grain where highest value observed with foliar spray application of ferrous sulphate @ 1.5% (38.96%) that was significantly higher than the rest of the treatments (Table 3). With foliar application of $FeSO_4.7H_2O$ @1.5% the percent increase of protein was 8.50 % over control. The increase in protein content might be due to the role of iron as vital element of structure of enzymes involved in amino acids synthesis and ultimately protein synthesis. Hence, with application and assimilation of external applied Fe via iron sulphate might have enhanced the protein content. It can also be explained by the fact that iron is an essential component for nitrogen fixation and this might have resulted in better availability of nitrogen and its absorption hence leads to increase in protein content in grain of soybean. Enhancement in grain protein content increased the storage capacity for Fe and Zn (Cakmak *et al*., 2010) which supports the idea that grain protein largely influences the grain capacity to accumulate Fe (Gomez-Becerra *et al*., 2010). Similar results on the effect of ferrous sulphate (Fe) on grain protein is supported by the findings of Yadav *et al.* (2002); Khattak *et al.* (2015).

Oil content in grain. The data revealed that there was significant variation among varieties in oil content and the highest value observed in JS 97-52 (18.08 %) which was statistically similar with JS-335 (17.87%). The least value was observed in local cultivar (17.21%). Oil content did not vary significantly upon Fe application,yet numerically higher value was observed in foliar spray application of $FeSO_4.7H_2O$ @ 1.5% (18.29%)(Table 3).Ferrous sulphate also contains sulphur in addition to iron and being one of the important secondary nutrients required by the crops. Sulphur and iron might have helped to obtain a higher oil yield of soybean as sulphur resulted in better biosynthesis of oil in soybean. These findings corroborated the ones reported upon by Janakiraman *et al.* (2005); Ravi *et al*. (2008), Abdel *et al.* (2014) on groundnut, safflower and soybean, respectively.

NPK uptake by grain and stover. The grain N uptake differed significantly among varieties for both the years of study (Table 4). Significantly and higher NPK uptake by grain was observed in JS 97-52 (1.758, 0.108, 0.411 g pot^{-1}), respectively followed by JS-335 (1.262, 0.074, 0.286 g pot^{-1}). Foliar spray application of ferrous sulphate @ 1.5% at pre-flowering stage (1.553, 0.088, 0.357 g pot^{-1}) registered the highest N, P and K uptake by grain with the least value in control (1.118, 0.078, 0.272 g pot^{-1}). However, the P uptake by grain and stover was found non-significant. N, P and K uptake by stover of soybean varied significantly among the varieties where JS 97-52 registered the highest value (0.581, 0.088, 0.664 g pot^{-1}) followed by JS-335 (0.437, 0.059, 0.467 g pot^{-1}) respectively. The N and K uptake by stover as influenced by iron fertilization was found to be significantly higher in Foliar application of $FeSO_4.7H_2O$ @ 1.5% (0.564, 0.603 g pot^{-1}) while P uptake was non-significantly affected. The significantly higher value of grain and stover yield in certain varieties and iron fertilization have cumulatively added to overall significantly higher value in N and K uptake except for P due to antagonistic effect of P and Fe content (Mundra and Bhati 1991). Although there was drastic reduction in phosphorus content in grain upon application of iron sulphate yet the P uptake was found to be higher under Fe fertilization although not significant. The reason was due to significant enhancement in grain yield which might have offset the negative effect of Fe over P content. Similar findings have also been reported by Sahu *et al.* (2008); Yadav *et al*. (2002). Iron nutrition in plants helps in number of enzymatic and physiological processes which are directly linked to photosynthetic activities and accumulation of photosynthates in grain which leads to improvement in overall grain yield and thus to the nutrient uptake like nitrogen and other nutrients. The results corroborated by the findings of Singh *et al.* (2004); Abbas *et al*. (2012). Our result on improvement of K uptake upon Fe application was in confirmation to the findings of Kumawat *et al.* (2006); Sahu *et al.* (2008).

Table 1: Effect of varieties and iron fertilization on growth parameters of soybean.

Treatments	Plant height (cm)				No. of branches plant^{-1}				Leaf area (cm^2)			Dry matter yield (g plant^{-1})			
	30 DAS	60 DAS	90 DAS	harvest	30 DAS	60 DAS	90 DAS	harvest	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	harvest
Varieties															
V_1	30.91	49.20	51.64	49.30	0.12	3.48	3.96	4.11	343.60	1089.69	688.78	2.17	9.42	11.80	12.19
V_2	34.28	56.39	58.47	56.66	0.13	3.70	4.20	4.14	481.39	1109.73	617.57	2.64	10.25	15.88	16.63
V_3	33.93	66.25	71.19	72.07	0.19	3.19	3.87	3.97	471.64	1629.21	2068.44	3.02	9.20	14.42	16.18
SEm±	*0.55*	*0.98*	*1.10*	*1.29*	*0.04*	*0.08*	*0.07*	*0.09*	*8.57*	*15.72*	*22.55*	*0.06*	*0.13*	*0.18*	*0.32*
CD at 5%	*1.54*	*2.75*	*3.10*	*3.62*	*NS*	*0.22*	*0.21*	*NS*	*24.15*	*44.31*	*63.58*	*0.16*	*0.37*	*0.51*	*0.89*
Iron fertilization															
Fe_0	31.91	53.08	55.96	55.01	0.11	3.30	3.72	3.92	430.15	1116.99	1058.82	2.54	9.26	13.04	13.91
Fe_1	32.47	56.16	59.60	58.37	0.20	3.39	3.94	3.94	434.51	1267.14	1188.53	2.55	9.72	14.01	15.08
Fe_2	32.29	58.55	62.65	60.54	0.24	3.52	4.17	4.19	348.11	1351.27	1102.18	2.57	9.87	14.26	15.79
Fe_3	33.38	59.88	63.22	60.85	0.13	3.61	4.33	4.25	424.74	1401.02	1159.81	2.57	10.16	15.28	16.20
Fe_4	33.96	57.84	59.57	59.35	0.07	3.44	3.89	4.06	424.41	1240.73	1117.29	2.68	9.26	13.45	14.30
Fe_5	34.21	58.18	61.58	61.94	0.13	3.48	4.02	4.08	450.63	1280.10	1122.98	2.76	9.46	14.18	14.72
SEm±	*0.77*	*1.38*	*1.55*	*1.82*	*0.06*	*0.11*	*0.11*	*0.13*	*12.12*	*22.23*	*31.90*	*0.08*	*0.19*	*0.26*	*0.45*
CD at 5%	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.30*	*NS*	*NS*	*62.66*	*NS*	*NS*	*NS*	*0.72*	*1.26*

Table 2: Effect of variety and iron application chlorophyll content of soybean (pooled of 2 years).

Treatments	30 DAS			60 DAS		
	Chlorophyll "a"	Chlorophyll "b"	Total Chlorophyll	Chlorophyll "a"	Chlorophyll "b"	Total Chlorophyll
Varieties						
JS-335	0.852	0.52	1.37	0.92	0.56	1.47
JS 97-52	0.822	0.50	1.32	0.90	0.55	1.45
Local	0.779	0.48	1.26	0.80	0.49	1.28
SEm±	***0.009***	***0.01***	***0.01***	***0.01***	***0.00***	***0.01***
CD (P=0.05)	***0.024***	***0.02***	***0.04***	***0.02***	***0.01***	***0.03***
Iron fertilization						
Fe_0	0.812	0.49	1.30	0.83	0.51	1.33
Fe_1	0.816	0.5	1.32	0.88	0.53	1.41
Fe_2	0.817	0.5	1.32	0.88	0.54	1.41
Fe_3	0.827	0.51	1.33	0.92	0.56	1.48
Fe_4	0.820	0.51	1.33	0.86	0.53	1.38
Fe_5	0.813	0.50	1.31	0.86	0.53	1.4
SEm ±	***0.012***	***0.01***	***0.02***	***0.01***	***0.01***	***0.02***
CD (P=0.05)	***NS***	***NS***	***NS***	***0.03***	***0.02***	***0.05***

Table 3: Effect of varieties and iron application on yield and quality parameters (pooled of two years).

Treatment	Yield (g pot^{-1})		Fe content (mg kg^{-1})		Protein content in grain (%)	Oil content in grain (%)
	Grain	stover	grain	stover		
JS-335	20.76	20.89	67.50	113.76	37.94	17.87
JS 97-52	29.04	29.68	65.13	117.95	37.73	18.08
Local	17.36	19.69	56.78	119.50	36.71	17.21
SEm±	*0.36*	*0.53*	*1.07*	*2.15*	*0.19*	*0.16*
CD (P=0.05)	*1.00*	*1.49*	*3.02*	*NS*	*0.58*	*0.45*
Fe_0	19.50	20.51	56.78	106.12	35.91	17.21
Fe_1	22.66	22.94	64.24	115.21	37.29	17.91
Fe_2	21.87	23.91	65.94	119.70	37.71	17.85
Fe_3	24.89	26.28	67.29	124.48	38.96	18.29
Fe_4	22.44	23.31	61.94	119.47	37.19	17.57
Fe_5	22.96	23.55	62.63	117.45	37.70	17.49
SEm ±	*0.50*	*0.75*	*1.52*	*3.04*	*0.33*	*0.23*
CD (P=0.05)	*1.42*	*2.11*	*4.27*	*8.58*	*0.94*	*NS*

Table 4: Effect of variety and iron application on N, P and K uptake (pooled of 2 years).

Treatment	N uptake		P uptake		K uptake	
	grain	stover	grain	stover	grain	stover
Varieties						
JS-335	1.262	0.437	0.074	0.059	0.286	0.467
JS 97-52	1.758	0.581	0.108	0.088	0.411	0.664
Local	1.02	0.372	0.067	0.063	0.251	0.443
SEm±	*0.023*	*0.013*	*0.002*	*0.002*	*0.006*	*0.016*
CD (P=0.05)	*0.065*	*0.038*	*0.006*	*0.006*	*0.017*	*0.046*
Iron fertilization						
Control (no Fe)	1.118	0.388	0.078	0.065	0.272	0.449
Foliar application of $FeSO_4.7H_2O$ @ 0.5%	1.356	0.441	0.086	0.069	0.319	0.507
Foliar application of $FeSO_4.7H_2O$ @ 1%	1.323	0.471	0.08	0.070	0.307	0.531
Foliar application of $FeSO_4.7H_2O$ @ 1.5%	1.553	0.564	0.088	0.074	0.357	0.603
Foliar application of $FeSO_4.7H_2O$ @ 2%	1.339	0.464	0.08	0.067	0.315	0.517
Soil application of $FeSO_4.7H_2O$ @ 10 kg ha^{-1}	1.390	0.452	0.086	0.074	0.326	0.541
SEm ±	*0.033*	*0.019*	*0.003*	*0.003*	*0.009*	*0.023*
CD (P=0.05)	*0.092*	*0.053*	*NS*	*NS*	*0.024*	*0.064*

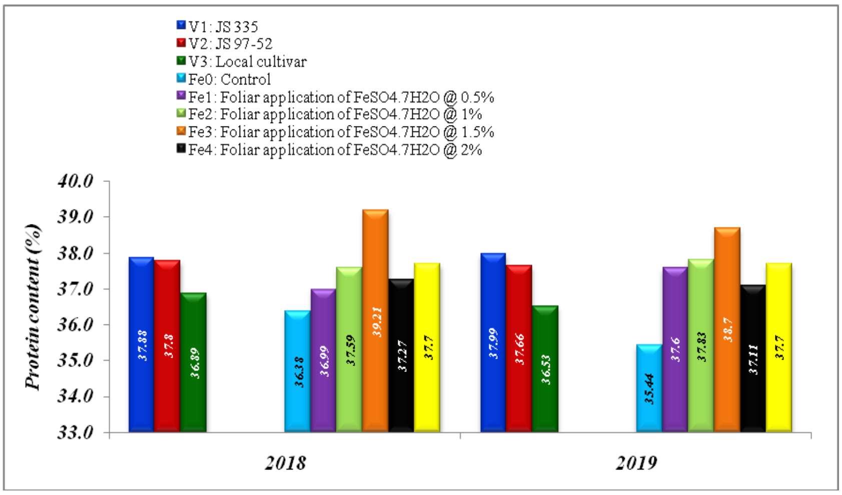

Fig. 1. Effect of varieties and Fe fertilization on protein content of soybean.

CONCLUSIONS

Based on the two years of investigation, it can be concluded that local cultivar was found superior in most of the growth parameter followed by JS 97-52. Whereas in respect to yield, oil content and NPK uptake, JS 97-52 was found to have higher value. For achieving higher crop growth, grain yield and enrichment of grain quality, two foliar applications of iron sulphate @ 1.5% at pre-flowering and flowering stage can be considered as an effective alternative means that can be recommended as iron biofortification strategy for soybean in the region.

FUTURE SCOPE

Not ably as realized from the above findings, there exists varietal difference in response to iron application. Hence, in the future it becomes imperative to take up more intensive studies in the field of identifying a micronutrient or iron efficient cultivar with higher bioavailable iron in soybean or related crops which is specifically indigenous to the region. More studies on finding the best spray concentration, number of foliar applications and right crop stage will further help future agronomic biofortification programmes.

Acknowledgement. The first author is highly grateful to the Director of ICAR research complex for NEH region, Umiam for the permission to pursue the Ph.D programme and also to Department of Agronomy, SASRD, Nagaland University for providing all the necessary supports and facilities for conducting the investigation.
Conflict of Interest. None.

REFERENCES

Abbas, G., Hussain, F., Anwar, Z., Khattak, J. Z. K. and Ishaque, M. (2012). Effects of iron on the wheat crop (*Triticum aestivum* L.) by uptake of nitrogen, phosphorus and potassium. *Asian Journal of Agricultural Sciences*, *4*(3), 229-235.

Abdel, E., Latif, M. A. and Haggan, E. L. (2014). Effect of micronutrients foliar application on yield and quality traits of soybean cultivars. *International Journal of Agriculture and Crop Science*, ***7***(11), 908-914.

Anitha, S., Sreenivasan, E. and Purushothaman, S. M. (2005). Response of cowpea {*Vigna unguiculata* (L.) Walp.} to foliar nutrition of zinc and iron in the oxisols of Kerala. *Legume Research-An International Journal*, *28*(4), 294-296.

Anonymous (2009). Nutrient requirement and recommended dietary allowances for Indians. National Institute of Nutrition, ICMR, Hyderabad. 331-332.

Baele, S. I. 1999. Enzymes of chlorophyll biosynthesis. *Photosynthesis Research*, *60*, 43-73.

Balachandar, D., Nagarajan, P. and Gunasekaran, S. (2003). Effect of organic amendments and micronutrients on nodulation and yield of blackgram in acid soil. *Legume Research-An International Journal*, *26*(3), 192-195.

Cakmak, I., Kalayci, M., Kaya, Y., Torun, A.A., Aydin, N., Wang, Y., Arisoy, Z., Erdem, H., Yazici, A., Gokmen, O., Ozturk, L. and Horst, W. J. (2010). Biofortification and localization of zinc in wheat grain. *Journal of Agricultural and Food Chemistry*, *58*, 9092-9102.

Cappellini, M. D., Musallam, K. M. and Taher, A. T. (2020). Iron deficiency anaemia revisited. *Journal of internal medicine*, *287*(2), 153-170.

Chereskin, B. M. and Castelfranco, P. A. (1982). Effects of iron and oxygen on chlorophyll biosynthesis: II. Observations on the biosynthetic pathway in isolated etiochloroplasts. *Plant Physiology*, *69*(1), 112-116.

Dhaliwal, S. S., Sadana, U. S., Khurana, M. P. S., Dhadli, H. S. and Manchanda, J. S. (2010). Enrichment of rice grains with zinc and iron through ferti-fortification. *Indian Journal of Fertilisers*, *6*(7), 28-35.

Dhaliwal, S. S., Sadana, U. S., Manchanda, J. S., Khurana, M. P. S. and Shukla, A. K. (2013). Differential response of maize cultivars to iron applied through ferti-fortification. *Indian Journal of Fertilisers, 9*(8), 52-57.

Dhaliwal, S.S., Sharma, V., Shukla, A.K., Kaur, J., Verma, V., Kaur, M., Singh, P., Kaur, L., Verma, G., Singh, J. and Gaber, A. (2022). Biofortification of Soybean (*Glycine max* L.) through $FeSO_4.7H_2O$ to Enhance Yield, Iron Nutrition and Economic Outcomes in Sandy Loam Soils of India. *Agriculture*, *12*(5), 586.

Farhan, H. N. and Al-Dulaemi, T. M. B. (2011). The effect of foliar application of some microelements on growth and productivity of Wheat (*Triticum aestivum* L.) in Jordan. *Journal of Agriculture Science*, *7*(1), 105-118.

Ghasemian, V., Ghalavand, A., Zadeh, A. S. and Pirzad, A. (2010). The effect of iron, zinc and manganese on quality and quantity of soybean seed. *Journal Phytologist*, *2*(11), 73-79.

Gomez, K. A. and Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John Wiley & sons.

Gomez-Becerra, H.F., Erdem, H., Yazici, A., Tutus, Y., Torun, B., Ozturk, L., Cakmak, I. (2010). Grain concentrations of protein and mineral nutrients in a large collection of spelt wheat grown under different environments, *Journal of Cereal Science*, *52*, 342-34.

Graham, R.D., Ascher, J.S. and Hynes, S.C. (1992). Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant and Soil*, *146*, 241-250.

Graham, R.D., Senadhira, D. and Ortiz-Monasterio, I. (1997). A strategy for breeding staple-food crops with high micro-nutrient density. *Soil Science and Plant Nutrition, 43*, 1153-1157.

Graham, R.D., Welch, R.M., Bouis. H.E. (2001). Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: Principles, perspectives, and knowledge gaps. *Advances in Agronomy*, *70*, 77-142.

Havlin, J. L., Beaton, J. D., Tisdale, S. L. and W. L. Nelson. (1999). Soil Fertility and Fertilizers: An Introduction to Nutrient Management. *Prentice-Hall, Inc., London*, pp.406-425.

Jackson, M.L. (1973). Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi, India.

Janakiraman, N., Venkataravana, P. and Seenapa, C. (2005). Effect of iron, zinc and boron on yield and seed quality of groundnut (*Arachis hypogaea* L.). *Mysore Journal of Agricultural Sciences*, *39*(2), 286-288.

Jin, Z., Wang, M., Wu, J. and Shi, C. (2008). Impacts of combination of foliar iron and boron application on iron biofortification and nutritional quality of rice grain. *Journal of Plant Nutrition*, *31*, 1599-1611.

Joshi, A.K., Crassa, J., Arun, B., Chand, R., Trethowan, R., Vargas, M. and Ortiz-Monsterio. I. (2010). Genotype x environment interaction for zinc and iron concentration of wheat grain in eastern Gangetic plains of India. *Field Crops Research*,*116*, 268-77.

Kamble, B. M., Meena, R. and Gajbhiye, P. N. (2021). Effect of Iron Nutrition on Growth, Quality and Yield of Soybean (*Glycine Max* L.) Grown on Problematic Inceptisol. *Journal of Experimental Agriculture International*, *43*(12), 20-28.

Khattak, S. G., Dominy, P. J. and Ahmad, W. (2015). Effect of Zn as soil addition and foliar application on yield and protein content of wheat in alkaline soil. *Journal of National Science Foundation of Sri Lanka, 43*(4), 303-312.

Kobraee, S., NoorMohamadi, G., HeidariSharifabad, H., DarvishKajori, F. and Delkhosh, B. (2011). Influence of micronutrient fertilizer on soybean nutrient composition. *Indian Journal of Science and Technology*, *4*(7), 763-769.

Kumar, M., Jha, A. K., Hazarika, S. and Verma, B. C. (2016). Micronutrients (B, Zn, Mo) for Improving Crop Production on Acidic Soils of Northeast India. *The National Academy of Sciences, 39*(2), 85-89.

Kumar, V., Dwivedi, V. N. and Tiwari, D. D. (2009). Effect of phosphorus and iron on yield and mineral nutrition in chickpea. *Annals of Plant Soil Research*, *11*,16-18.

Kumar, V., Kumar, D., Singh, Y. V. and Raj, R. (2015). Effect of iron fertilization on dry-matter production, yield and economics of aerobic rice (*Oryza sativa*). *Indian Journal of Agronomy*, *60*(4), 547-553.

Kumawat, R. N., Rathore, P. S., Nathawat, N. S. and Mahatma, M. (2006). Effect of sulfur and iron on enzymatic activity and chlorophyll content of mungbean. *Journal of plant nutrition*, *29*(8), 1451-1467.

Lindsay, W. L. and Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, *42,* 421-428.

Meena, M. C., Patel, K. P. and Rathod, D. D. (2006). Effect of Zn and Fe enriched FYM on mustard yield and micronutrient availability in loamy sand soil (Typic Haplustept) of Anand. *Journal of the Indian Society of Soil Science*, *54*(4), 495-499.

Mahmoudi, H., Labidi, N., Ksouri, R., Gharsalli, M. and Abdelly, C. (2007). Differential tolerance to iron deficiency of chickpea varieties and Fe resupply effects. *Comptes Rendus Biologies,330*, 237-246.

Mundra, S. L. and Bhati, D. S. (1991). Effect of iron, manganese and Rhizobium inoculation on nutrient content and uptake by cowpea (*Vigna unguiculata*). *Indian Journal of Agronomy,36*, 294-296.

Muthal, Y. C., Deshmukh, S. L., Sagvekar, V. V. and Shinde, J. B. (2016). Response of foliar application of macro and micronutrients on growth, yield on quality of kharif greengram (*Vigna radiata* L.). *International Journal of Tropical Agriculture*, *34*(7), 2137-2141.

Nayyar, V. K. and Takkar, P. N. (1989). Combating iron deficiency in rice grown in sandy soils of Punjab. In: *International Symposium on Managing Sandy Soils, CAZRI, Jodhpur*, 379-384.

Pal, V., Singh, G. and Dhaliwal, S. S. (2019a). Yield enhancement and biofortification of chickpea (*Cicer arietinum* L.) grain with iron and zinc through foliar application of ferrous sulfate and urea. *Journal of Plant Nutrition*, *42*(15), 1789-1802.

Prasad, R. (2006). Zinc in soils and in plants, human & animal nutrition. Indian Journal of Fertilizers, *2*(9), 103-119.

Pal, V., Singh, G. and Dhaliwal, S. S. (2019b). Agronomic biofortification of chickpea with zinc and iron through application of zinc and urea. *Communications in Soil Science and Plant Analysis, 50*(15), 1864-1877.

Rai, S., Singh, P. K., Mankotia, S., Swain, J. and Satbhai, S. B. (2021). Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese, *Plant Stress, 1*, 100008.

Ravi, S., Channal, H. T., Hebsur, N. S., Patil, B. N. and Dharmatti, P. R. (2008). Effect of sulphur, zinc and iron nutrition on growth, yield, nutrient uptake and quality of safflower. *Karnataka Journal Agricultural Sciences*, *21*(3): 382-385.

Rengel, Z. and Graham, R. D. (1996). Uptake of zinc from chelate-buffered nutrient solutions by wheat genotypes differing in zinc efficiency. *Journal of Experimental Botany*,*47*, 217-219.

Rout, G. R. and Sahoo, S. (2015). Role of iron in plant growth and metabolism. *Reviews in Agricultural Science*, *3*, 1-24.

Sahu, S., Lidder, R. S. and Singh, P. K. (2008). Effect of micronutrient and biofertilizars on growth, yield and nutrient uptake by chickea (*Cicer aeritinum* L.) in vertisols of Madhya Pradesh. *Advances in plant sciences*, *21*, 501-503.

Sharma, S., Chandra, S., Kumar, A., Bindraban, P., Saxena, A.K., Pande, V. and Pandey, R. (2019). Foliar application of iron fortified bacteriosiderophore improves growth and grain Fe concentration in wheat and soybean. *Indian journal of microbiology*, *59*, 344-350.

Shukla, V., and Shukla, I. C. (1999). Effect of Fe, Mo, Zn and P on symbiotic nitrogen fixation of chick pea (*Cicer arietinum* L.*). Indian Journal of Agricultural Chemistry*, *32*(3), 118-123.

Singh, M., Chaudhary, S. R., Sharma, S. R. and Rathore, M. S. (2004). Effect of some micronutrients on content and uptake by chickpea (*Cicer arietinum*). *Agriculture Science Digest*, *24*(4), 268-270.

Soni, J. and Kushwaha, H. S. (2020). Effect of foliar spray of zinc and iron on productivity of mungbean (*Vigna radiata* (L.) Wilczeck). *Journal of Pharmacognosy and Phytochemistry*, *9*(1), 108-111.

Stoltzfus, R. J. and Dreyfuss, M. L. (1998). Guidelines for the use of iron supplements to prevent and treat iron deficiency Anemia. International Nutritional Anemia Consultative Group (INACG). ILSI Press. Washington DC, USA.

Tabassum, S., Khamparia, R. S. and Singh, S. (2013). Effect of zinc and organic manures on yield attributes and yield of rice. *Bioinfolet-A Quarterly Journal of Life Sciences*, *10*(3a), 879-881.

Trivedi, A.K., Hemantaranjan, A. and Pandey, S. K. (2011). Iron and sulphur application may improve growth and yield of soybean. *Indian Journal of plant physiology*, *16*(3/4), 309-313.

Vaghar, M. S., Sayfzadeh, S., Zakerin, H. R., Kobraee, S. and Valadabadi, S. A. (2020). Foliar application of iron, zinc, and manganese nano-chelates improves physiological indicators and soybean yield under water deficit stress. *Journal of Plant Nutrition*, *43*(18), 2740-2756.

Welch, R. M. and Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, *55***,** 353-364.

Yadav, G. S., Dinesh, K. and Subhash, B. (2013). Enhancing iron density and uptake in grain and straw of aerobic rice through mulching and rhizo-foliar fertilization of iron. *Global Journal of Agricultural Research and Reviews*, *8*(44), 5447-5454.

Yadav, P. S., Kameriya, P. R. and Rathore, S. (2002). Effect of phosphorus and iron fertilization on yield, protein content and nutrient uptake in mungbean on loamy sand soil. *Journal of the Indian Society of Soil Science*, *50*(2), 225-226.

Zeidan, M. S., Mohamed, M. F. and Hamouda, H. A. (2010). Effect of foliar fertilization of Fe, Mn and Zn on wheat yield and quality in low sandy soils fertility. *World Journal of Agriculture and Soil Science*, *6*(6), 696-699.

How to cite this article: Badapmain Makdoh, A.P. Singh, L.T. Longkumer, T. Gohain, Lanunola Tzudir, D. Nongmaithem, Rekha Yadav and Damitre Lytan (2023). Foliar Application of Ferrous Sulphate and its Influence on Growth, Grain Quality and Nutrient Uptake in Soybean (*Glycine max* L.). *Biological Forum – An International Journal*, *15*(5): 197-204.