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Effects of Iron Fertilization on Yield and Tissue Micronutrients Concentrations of Different Haricot Bean (Phaseolus Vulgaris L.) Varieties in Southern Ethiopia

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Abstract
Although required in smaller quantity, micronutrients are as essential as macronutrients for optimum growth and yield for beans. This study was conducted under field conditions at Halaba, Butajira and Taba, and under greenhouse conditions with soils collected from the aforementioned locations to evaluate the effect of Fe fertilization on tissue micronutrients (Zn, Fe, Cu and Mn) contents of different haricot bean varieties. The treatments include two factors, haricot bean varieties (Nasir, Ibado, Hawassa Dume, and Sari-1) and levels of foliar-applied iron (Fe) fertilizer (0, 1, 2, and 3% solution). Both the pot and field experiments indicated that yield and tissue micronutrients (Zn, Fe, Cu and Mn) concentrations of haricot bean varied significantly across soils (locations) and among varieties. The highest seed Fe concentration (59.04 mg kg^{-1}) was recorded in Taba soil, whereas the lowest value, 35.63 mg kg^{-1}, was observed in Halaba soil. Hawassa Dume and Nasir produced the highest and equal grain yield, whereas Nasir produced the highest seed Fe concentration (59.02 mg kg^{-1}). The highest leaf Fe concentration (290.19 mg kg^{-1}), was observed with Ibado. Foliar application of $FeSO_4.7H_2O$ did not significantly influence tissue Zn concentration and leaf Cu concentration, but seed Cu, tissue Mn and tissue Fe were significantly affected by Fe fertilization. The application of different levels of Fe fertilizer did not significantly influence yield of haricot bean varieties, but it significantly increased both leaf and seed Fe concentrations. Consequently, Nasir and 3% $FeSO_4.7H_2O$ were found to be the best variety and rate, respectively, for quality production of haricot bean.
Keywords: Manganese, Iron, Copper, Zinc, Concentration and Haricot bean

1. Introduction

Food legumes contain appreciable quantities of iron and forty percent of Fe intake in developing countries is derived from legumes and cereals (Muhamba and Nchimbi-Msolla, 2010). (Nchimbi-Msolla and Tryphone, 2010) also reported that haricot bean (*Phaseolus vulgaris* L.) is an important source of minerals and protein and can supply all of the iron that humans require. Haricot bean is one of the principal food and cash crop grown and consumed throughout the world as a grain and vegetable legume (Muhamba and Nchimbi-Msolla, 2010). It plays an important role in the nutrition of low-income people especially in developing countries, where it is often the most important dietary source of protein, carbohydrate, dietary fiber, and minerals (Prolla et al., 2010). It is also one of the most important cash crops and source of protein for farmers in many lowlands and mid-altitude zones of Ethiopia.

Although required in smaller quantity, micronutrients are as essential as macronutrients for optimum growth and yield for beans. Iron is an essential trace element for all organisms (Aref, 2012). It plays a central role in chlorophyll production, photosynthesis and energy transfer within plant (Singh, 2004**)**. It is as crucial for human health as organic compounds such as carbohydrates, fats, protein and vitamins. High consumption of cereal based foods with low content of Fe causes health hazards in humans. The daily dietary intake of young adult ranges from 10-60 mg but intake less than this value can cause slow physiological processes (Imtiaz, 2010). Rubio et al. (2009) also stated that the estimated daily dietary intake of iron is 13.161mg day^{-1}. Iron is the fourth most abundant element in the earth's crust with a total content of 100 ppm or >10% in the soils and 50 – 250 ppm in the plant (Taber, 2008) with average concentration of 100 ppm (Ronan, 2007). However, it remains the third-most limiting nutrient for plant growth primarily due to the low solubility of the oxidized ferric form in aerobic environments (Aref, 2012). Kalra (1998) reported that Fe content of plant > 500 ppm is toxic. Soil test iron is considered to be very low when DTPA-Fe is less than 2.5 ppm and marginal when DTPA-Fe is between 2.5 and 4.5 ppm. Values above 4.5 ppm indicate low probability of iron deficiency (Wortmann et al., 2012). Deficiencies of iron in soils is caused due to high soil pH (due to an increase of pH in one unit e.g. from 6.5 to 7.5, available Fe decreases 1,000 fold), high free soil $CaCO_3$, poor aeration (excess CO_2) and low organic matter content (Taber, 2008) and high level of phosphorus (Ronan, 2007). Availability of Fe can also be influenced by the presence of other cationic micronutrients such as Zn, Cu and Mn. A soil characterization study carried out in Halaba, Taba, and Butajira soils showed that the DTPA- Fe contents of most of the soils were below 4.5 mg kg^{-1} (Abay et el., 2015), respectively, which were reported to be critical by Aref (2012), (Haque et al., 2000) and Westfall and Bauder (2011) indicating this mineral is deficient in these soils.

Haricot bean is sensitive to Fe deficiency (Taber, 2008) and it has high response to applied iron in cases of low soil iron availability (Wortmann et al., 2012). Sensitivity of haricot bean to iron deficiency varies among varieties. Tryphone and Nchimbi-Msolla (2010) reported that variation in response to Fe application was observed among common bean genotypes in Tanazania. Talukder et al. (2009) also indicated that application of iron caused significant differences in concentrations of Fe in haricot bean seeds. Tryphone and Nchimbi-Msolla (2010) likewise reported significant differences in concentrations of iron in seeds and leaves of haricot bean in Tanzania. Improving the nutritional quality of haricot beans such as the micronutrient content is important in sustaining health of poor communities relying on this crop (Ndakidemi et al., 2011). The best way to treat chlorosis in haricot beans is to foliar apply around 1.5% to 2% solution of ferrous sulphate heptahydrate (FeSO4 7H2O) (Ferguson, 2006). Thus, identifying haricot bean varieties with high amounts of Fe and application of Fe containing fertilizers could contribute significantly to improvements of Fe of people depending on the common bean as major component of their diet.

Although haricot bean is important crop at Halaba, Butajira and Taba, the effect of iron fertilization on the concentrations of micronutrients in leaves and seeds of haricot bean varieties grown in these areas was not evaluated. Therefore, this study was conducted to evaluate the effect of application of Fe fertilizer on micronutrients contents in tissues of haricot bean grown at Halaba, Butajira and Taba.

2. Materials and Methods

Experiments were conducted on farmers' fields and in greenhouse conditions. Factor A was Fe fertilizer with four levels (0, 1, 2, and 3% solution) and factor B was haricot bean of four varieties (Nasir, Ibado, Hawassa Dume, and Sari-1). Ferrous sulphate solutions of 1, 2, and 3% were prepared by adding 1, 2, and 3 kg of ferrous sulfate into 100 L of water, respectively (Incitec Pivot, 2003). The experimental sites were located near Halaba (Latitude: 07°20'34.5" and Longitude: 38°06'30.0"), Taba (Latitude: 07°01'01.9" and Longitude: 37°53'57.6"), and Butajira (Latitude: 08°12'25.9" and Longitude: 38°27'33.2"), and soils (0 to 30 cm depth) were also collected from these sites for the greenhouse experiment. The types of soils were: Haplic Luvisols (Humic) in Butajira; Andic Lixisols (Humic) in Halaba; and Haplic Lixisols (Siltic) in Taba. The soil properties of each site are indicated in Table 1.

Table 1. Physico-chemical properties of the experimental soils

Soil properties	Butajira soil	Halaba soil	Taba soil
Textural class	Clay loam	Clay loam	Clay loam
pH (H_2O)	7.4	7.70	7.47
Organic Carbon (%)	2.05	2.35	2.35
DTPA Zn (mg kg^{-1})	0.49	1.33	1.80
DTPA Fe (mg kg^{-1})	1.26	1.60	1.50
DTPA Cu (mg kg^{-1})	1.38	0.26	0.25
DTPA Mn (mg kg^{-1})	4.80	4.50	3.91
Total N (%)	0.34	0.24	0.16
Available P (mg kg^{-1})	12.13	10.00	14.30

The source of Fe was heptahydrated iron sulphate ($FeSO_4.7H_2O$). This was sprayed three times, at 15 day intervals, starting at 15 days after planting at a volume of 100 L ha^{-1} per each application date. Nitrogen and P were equally applied for all treatments. Phosphorus was applied just before planting as TSP at 50 mg P kg^{-1} soil (0.25g P per pot) for the pot experiments and 20 kg P ha^{-1} for the field experiments. Nitrogen was also applied just before planting as urea at 0.045g N per pot band at 18 kg Nha^{-1} for the field experiments. Four haricot bean seeds per pot were sown, thinned to two seedlings at 10 days after sowing. For the field experiments, haricot bean was planted in rows with a spacing of 10 cm between plants and 40 cm between rows with a plot size of 4 × 4 m. The greenhouse experiments were conducted in pots with 5 kg soil per pot. All appropriate management practices were carried out equally for all treatments. Grain yields were collected. Three fully developed leaves at the top of the plant during initial flowering, along with seeds, were collected from each treatment pot and plot, dried in an oven at 70 °C for 24 h, then ground using a rotating sample mill. The ground plant materials were digested and analyzed for Fe content according to the following procedures. A sample of 0.5 g was weighed using five digit sensitive balance into the digestion tube. Six milliliters of nitric acid (HNO_3) was added to each tube. The tubes were placed in a digestion block at 90 °C for 45 min. Five milliliters of hydrogen peroxide (H_2O_2) was added, in two splits (3 ml and 2 ml) while the samples were in the digestion block, and digested for another 65 min. Three milliliters of 6 *M* hydrogen chloride (HCl) was then added and the samples were digested until the solution had turned completely clear (after about 5 minutes). The tubes were removed from the block, cooled for 20 minutes, and shaken using a vortex. The digests were then transferred into dram vials, brought to 25 ml with deionized water, and stored. The concentration of Fe was analyzed using a Microwave Plasma Atomic Emission Spectrometer (MPAES) at 259.940 nm. Analysis of variance (ANOVA) was carried out using Proc GLM procedures in the SAS

9.3 program (SAS Institute Inc., Cary, NC USA) and Least Significant Difference (LSD) test was used for mean separation. All data from the two seasons and three locations were combined.

3. Results and Discussions

Pot experiments

The pot experiment indicated that yield and tissue Fe concentrations of haricot bean varied significantly across soils (Table 2). The highest seed Fe concentration (59.04 mg kg^{-1}) was recorded in Taba soil, whereas the lowest value, 35.63 mg kg^{-1}, was observed in Halaba soil. However, highest values of grain yield (16.44a $kgha^{-1}$) and leaf Fe concentration (302.97 mg kg^{-1}) were obtained in Halaba soil. The lowest value of leaf Fe concentration (224.37 mg kg^{-1}) was observed in Butajira soil. Growing soils significantly affected accumulation of Zn, Cu and Mn in the tissues of haricot bean (Table 2). Both leaf and seed Zn concentrations in haricot bean grown in Butajira soils were significantly higher than the concentrations in tissues of the crop grown in Halaba and Taba soils. Haricot bean grown in Taba soil produced significantly higher leaf and seed Cu concentrations, which could be attributed to the possibility of reduced Fe and Zn absorption, which otherwise could affect Cu absorption, caused by high P availability. The concentrations of Cu accumulated in tissues of haricot bean grown in Butajira soils were significantly higher than the values produced in the tissues of the crop grown in Halaba soils, which could be attributed to the higher contents of Cu in Butajira soil. Both leaf and seed Mn concentrations were significantly higher in the tissues of the crop grown in Butajira soils than Halaba and Taba soils, which could be attributed to the highest Mn content of Butajira soil. Tissue Mn was higher in the crop grown in Halaba soil than the one grown in Taba soil. The highest and lowest leaf and seed Mn concentrations were 219.22 and 28.46 mg kg^{-1}, respectively, whereas the lowest values were 91.72 and 20.30 mg kg^{-1}, respectively.

Table 2 micronutrients contents of tissue of haricot bean varieties as influenced by soil

Soil	Grain yield (g/pot)	Leaf Fe (mg kg^{-1})	Seed Fe (mg kg^{-1})	Leaf Zn (mg kg^{-1})	Seed Zn (mg kg^{-1})	Leaf Cu (mg kg^{-1})	Seed Cu (mg kg^{-1})	Leaf Mn (mg kg^{-1})	Seed Mn(mg kg^{-1})
Butajira soil	16.29a	262.40b	59.04a	19.09a	27.34a	4.60b	5.60b	219.22a	28.46a
Taba soil	11.15b	224.37c	54.51b	13.46c	20.50b	8.03a	9.71a	91.72c	20.30c
Halaba soil	16.44a	302.97a	35.63c	17.60b	16.76c	4.33b	4.86c	157.75b	21.77b
CV (%)	22.81	14.69	14.09	13.43	5.78	15.45	9.00	12.74	10.64
LSD (5%)	1.34	15.67	2.84	0.91	0.50	0.35	0.25	8.07	1.01

Yield and tissue Fe concentrations also varied significantly among haricot bean varieties (Table 3) indicating haricot bean varieties vary in yield potential and Fe accumulating capability. This is in agreement with Gebre-Egziabher et al. (2014), who reported that haricot bean varieties varied significantly in grain yield in northern Ethiopia. Bhargava et al. (2008) reported that the accumulation of Fe varies greatly among crop varieties. The highest leaf Fe concentration (290.19 mg kg^{-1}), was observed with Ibado. Hawassa Dume produced the highest grain yield (15.85 $kgha^{-1}$), whereas Nasir produced the highest seed Fe concentration (59.02 mg kg^{-1}). Though Hawassa Dume yielded the highest grain (15.85 g per pot), it is almost equal to the yield of Nasir (15.75 g per pot). The lowest leaf and seed Fe, 235.68 and 44.56 mg kg^{-1}, respectively, were recorded with Hawassa Dume. Moraghan et al. (2002) similarly reported in pot experiment that yield and tissue Fe concentrations varied among haricot bean varieties in the Netherlands. Tryphone and Nchimbi-Msolla (2010) likewise reported that Fe concentrations in leaf and seed varied among haricot bean genotypes. They rated genotypes with leaf Fe concentrations ranging from 163.7 to 270 mg kg^{-1} as low, 271 to 310 mg kg^{-1} as moderate, and 311 to 485.6 mg kg^{-1} as high; and rated seed iron content ranging from 23.6 to 42.0 mg kg^{-1} as low, 43.0 to 59.6 mg kg^{-1} as moderate, and 60.4 to 105.5 mg kg^{-1} as high. The findings of this experiment using soils collected in Ethiopia revealed leaf and seed Fe concentrations consistent with the low to moderate ratings. Thus, Hawassa Dume had low and moderate leaf and seed Fe concentrations, respectively, whereas Ibado and Nasir had moderate leaf and seed Fe concentrations, respectively. Therefore, varieties Nasir and Hawassa Dume as seed, and Ibado as leaf are recommended to be consumed for better Fe supply. Oyelude et al. (2012) reported that consumption of *Phaseolus vulgaris* leaf could assist to meet part of the daily requirement of iron in the diet. The authors also reported that the phytates level in *Phaseolus vulgaris* leaf does not hinder availability of iron.

Tissue Zn concentration varied among haricot bean varieties (Table 3). Both the highest leaf Zn (17.47 mg kg^{-1}) and seed Zn (22.86 mg kg-1) were produced from Sari-1; however, they are not significantly different from the values observed with variety Nasir. Leaf and seed Zn concentrations in Nasir and Sari-1 were significantly higher than the values in Ibado and Hawassa Dume. The lowest leaf Zn (16.30 mg kg^{-1}) and seed Zn (19.91 mg kg^{-1}) were observed with variety Ibado, but they were not significantly lower than the values obtained from variety Hawassa Dume. These results indicate that varieties Nasir and Sari-1 are better varieties in accumulating tissue Zn suggesting consuming these varieties can contribute to alleviate Zn deficiency of people who consume the crop as a major component of their diet. Similar to that of Cu, the seed Zn concentration was higher than the leaf Zn concentration. This difference indicates accumulation of minerals vary between plant tissues.

Leaf and seed Cu concentrations were significantly varied among haricot bean varieties (Table 3). Ibado and Sari-1 yielded statistically equal leaf and seed Cu concentrations but significantly higher than Nasir and Hawassa Dume varieties indicating that Ibado and Sari-1 are better varieties in accumulating Cu. The highest leaf and seed Cu were 6.06 and 6.92 mg kg^{-1}, respectively, whereas the lowest values were 5.21 and 6.43 mg kg^{-1}, respectively. There was also significant difference among the haricot bean varieties in terms of tissue Mn concentrations. Nasir, Ibado and Sari-1 produced statistically equal and significantly higher leaf Mn than Hawassa Dume. Nasir produced significantly the highest seed Mn (25.71 mg kg^{-1}), whereas Ibado gave significantly the lowest seed Mn (19.77 mg kg^{-1}). On the other hand Hawassa Dume and Sari-1 yielded statistically equal seed Mn. In all varieties seed Cu concentration was higher than leaf Cu concentration. Reversely, leaf Mn accumulation was by far greater than the accumulation in the seed having similarity with Fe. The difference accumulations in the seeds and leaves indicate that different tissues accumulate different concentrations of minerals.

Table 3 Tissue micronutrients concentrations of different haricot bean varieties under green house conditions

Variety	Grain yield (g/pot)	Leaf Fe (mg kg^{-1})	Seed Fe (mg kg^{-1})	Leaf Zn (mg kg^{-1})	Seed Zn (mg kg^{-1})	Leaf Cu (mg kg^{-1})	Seed Cu (mg kg^{-1})	Leaf Mn (mg kg^{-1})	Seed Mn (mg kg^{-1})
Nasir	15.75a	270.21b	59.02a	16.73ab	22.79a	5.21b	6.63b	154.40ab	25.71a
Ibado	12.65c	290.19a	44.78c	16.30b	19.91c	6.06a	6.92a	160.41a	19.77c
H.D.	15.85a	235.68c	44.56c	16.36b	20.57b	5.44b	6.43b	147.63b	24.35b
Sari-1	14.24b	256.90b	50.55b	17.47a	22.86a	5.91a	6.92a	162.48a	24.21b
CV (%)	22.81	14.69	14.09	13.43	5.78	15.45	9.00	12.74	10.64
LSD (5%)	1.55	18.09	3.28	1.05	0.58	0.41	0.28	9.32	1.17
		p-value							
*V	0.0005	<.0001	<.0001	0.1095	<.0001	0.0001	0.0015	0.0094	<.0001
Fe	0.6308	<.0001	0.0004	0.0914	0.0060	0.3853	0.0363	<.0001	<.0001
V × Fe	0.1307	0.0109	<.0001	0.2091	<.0001	0.9143	0.1599	0.0058	0.0021
Location	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Means followed by the same letter(s) within a column are not significantly different at $P \leq 0.05$ according to least significant difference (LSD) test. *V= variety; H.D.= Hawassa Dume

Iron fertilization did not significantly influence tissue Zn concentration of haricot bean; however, decreasing and increasing trends of leaf and seed Zn, respectively, were observed (Table 4). The highest and the lowest leaf Zn concentrations, 17.49 and 16.18 mg kg^{-1}, respectively, were observed with the applications of 3% and zero $FeSO_4.7H_2O$, respectively. Contrariwise, the highest and lowest seed Zn concentrations, 21.86 and 20.89 mg kg^{-1}, respectively, were produced with application of zero and 2% $FeSO_4.7H_2O$, respectively. Foliar application of $FeSO_4.7H_2O$ did not significantly influence leaf Cu concentration, but seed Cu was significantly affected by Fe fertilization (Table 4). The seed Cu concentration produced by application of 2% $FeSO_4.7H_2O$ was significantly higher than the value obtained by application of 3% $FeSO_4.7H_2O$ though it was not significantly different from the values obtained by application of zero and 1% $FeSO_4.7H_2O$. The highest values of both leaf and seed Cu concentrations were obtained by application of 2% $FeSO_4.7H_2O$. Application of 3% $FeSO_4.7H_2O$ reduced both leaf and seed Cu concentrations indicating applying more than 2% $FeSO_4.7H_2O$ can cause antagonistic effect on Cu absorption. Therefore, application of $FeSO4.7H_2O$ up to 3% did not significantly influence tissue Cu concentrations of haricot bean. However, for sound conclusion, further research by including $FeSO_4.7H_2O$ above 3% should be conducted. Iron fertilization could not reverse the plant tissues where higher and lower Cu concentrations could accumulate, i.e. higher Cu accumulation was observed in the seed than in the leaves. Foliar application of $FeSO_4.7H_2O$ increased both leaf and seed Mn concentration. Application of 3% of $FeSO_4.7H_2O$ significantly resulted in higher leaf and seed Mn concentrations than the rest of the treatments (Table 4). There was no significant tissue Cu concentrations were observed between application of zero and 1% $FeSO_4.7H_2O$; however, application of 2% gave significantly higher leaf Cu but not seed Cu. Like that of Cu, the tissues for higher and lower Mn accumulation were not changed due to Fe fertilization, i.e. higher Mn was accumulated in leaves than in seeds. The highest leaf and seed Mn were 180.38 and 25.88 mg kg^{-1}, respectively.

The application of different levels of Fe fertilizer did not significantly influence yield of haricot bean varieties, but it significantly increased both leaf and seed Fe concentrations (Table 4) indicating that the varieties are relatively nonresponsive, irrespective of Fe deficiencies, as suggested above (Hawassa Dume had low leaf Fe concentrations). Anu et al. (2014) reported that foliar application of Fe increased Fe content in linseed shoot. The highest concentrations of both leaf (339.50 mg kg^{-1}) and seed Fe (59.46 mg kg^{-1}) were observed at application of the highest level of Fe fertilizer (3% $FeSO_4.7H_2O$). A similar finding was reported by Zhao et al. (2011) for wheat. In the Ethiopian haricot bean experiment, the highest seed Fe concentration was 59.46 mg kg^{-1}, which is enough for 4.5 days per person (Rubio et al. 2009), i.e. 1 kg of haricot bean produced at application of 3% $FeSO_4.7H_2O$ can supply a single person's Fe needs for 4.5 days.

Table 4. Tissue micronutrients concentrations of haricot bean varieties as influenced by Fe fertilization under greenhouse conditions

$FeSO_4.7H_2O$ (%)	Grain yield (g/pot)	Leaf Fe (mg kg^{-1})	Seed Fe (mg kg^{-1})	Leaf Zn (mg kg^{-1})	Seed Zn (mg kg^{-1})	Leaf Cu (mg kg^{-1})	Seed Cu (mg kg^{-1})	Leaf Mn (mg kg^{-1})	Seed Mn(mg kg^{-1})
0	14.94a	183.04d	46.23c	16.18ab	21.86a	5.71a	6.68ab	137.31c	22.43b
1%	13.99a	240.17c	48.81bc	16.49ab	21.71a	5.45a	6.71ab	144.94c	22.29b
2%	14.34a	290.27b	50.41ab	16.70ab	20.89b	5.80a	6.96a	162.29b	23.43b
3%	14.71a	339.50a	53.46a	17.49a	21.67a	5.66a	6.54b	180.38a	25.88a
CV (%)	22.81	14.69	14.09	13.43	5.78	15.45	9.00	12.74	10.64
LSD (5%)	1.55	18.09	3.28	1.05	0.58	0.41	0.28	9.32	1.17

The interaction between $FeSO_4.7H_2O$ and varieties significantly influenced leaf Fe, seed Fe, seed Zn, leaf Mn and seed Mn (Fig 1). The interaction between variety and levels of Fe fertilizer significantly ($p<0.01$) influenced concentrations of Fe in tissues of haricot bean indicating varieties vary in Fe fertilizer requirements to accumulate the same amount of Fe in their tissues. The highest tissue Fe concentrations of Nasir and Sari-1 were obtained at application of 3% $FeSO_4.7H_2O$, while the lowest values were observed with no Fe fertilizer. On the other hand, Ibado and Hawassa Dume produced the highest leaf and seed Fe concentrations at applications of 3 and 2% $FeSO_4.7H_2O$, respectively, while the lowest values were observed with no Fe fertilizer. Therefore, application of 3% $FeSO_4.7H_2O$ is recommended to produce quality product from Nasir and Sari-1.On the other hand, application of FeSO4.7H_2O at 3% (if leaf is considered) and 2% (if seed is considered) are recommended for quality production from Ibado and Hawassa Dume, respectively. The concentrations of Fe in leaves were by far higher than that of seeds in all varieties, locations, and at different levels of Fe fertilizers suggesting that concentrations of plant minerals vary between plant tissues (Nchimbi-Msolla and Tryphone, 2010). Nchimbi-Msolla and Tryphone (2010) likewise reported that a significant difference in Fe concentration was observed among cultivars of haricot bean between locations in Tanzania.

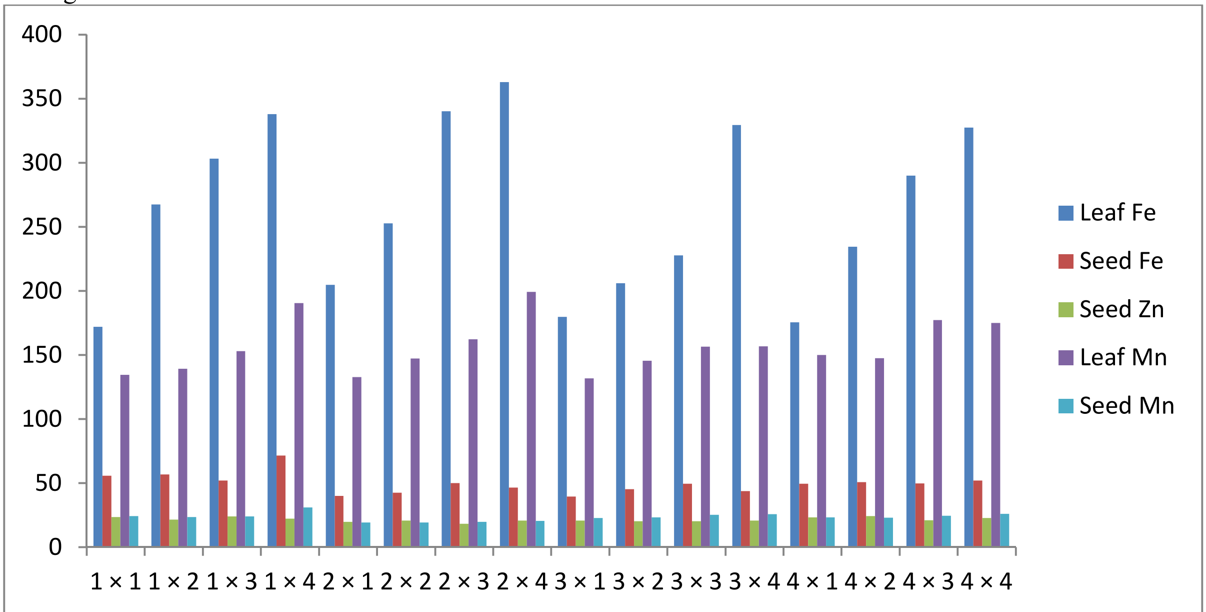

Fig. 1. Leaf and seed micronutrients concentrations as influenced by interaction between haricot bean varieties and different levels of Fe fertilizer

Field experiments

Like the pot experiments, the field experiments showed significant variation between haricot bean varieties in yield and tissue micronutrients concentrations (Table 5). Sebuwufu (2013) reported similar finding on yield and yield components of haricot bean. Ghanbari et al. (2013) also reported that haricot bean genotypes varied in tissue Fe concentration. The highest grain yield (3099.55 kg ha-1) was observed with Hawassa Dume, which is almost equal to the yield of Nasir (3068.67 kg ha^{-1}), but significantly higher than that of Ibado and Sari-1. The highest leaf Fe (167.12 mg kg^{-1}) and seed Fe (86.46 mg kg^{-1}) were observed with Ibado and Nasir, respectively. Similar to the pot experiment, tissue Zn concentrations were significantly varied among varieties (Table 5). The highest leaf and seed Zn were produced by variety Nasir followed by Sari-1, but there was no significant difference in leaf Zn between Nasir and Sari-1. Nasir yielded significantly higher tissue Zn concentrations than the rest of the varieties. The least leaf Zn concentration was observed with variety Hawassa Dume, while the least seed Zn was obtained from variety Ibado indicating that Ibado accumulates the least seed Zn concentrations. Like leaf Zn, leaf Cu was also not significantly different between Nasir and Sari-1. Nasir produced significantly higher tissue Cu than the rest of the other varieties. The highest leaf and seed Cu were 6.95 and 7.15 mg kg^{-1}, respectively, whereas the lowest values were produced by Hawassa Dume and Ibado, respectively. Like that of the pot experiments, the field experiments also revealed that both seed Zn and seed Cu were higher than leaf Zn and leaf Cu. Therefore, Zn and Cu have similar characteristics in terms of their accumulations in haricot bean varieties. Varieties Nasir, Hawassa Dume and Sari-1 produced statistically equal and significantly higher leaf Mn than variety Ibado (Table 5). Similar trends were observed with seed Mn. The seed Mn concentrations produced by Nasir and Sari-1 are statistically equal, though Nasir yielded higher than Sari-1. The highest leaf and seed Mn concentrations, 127.24 and 38.11 mg kg^{-1}, respectively, were observed with varieties Sari-1 and Nasir, respectively. The lowest leaf and seed Mn concentrations, 110.35 and 31.35 mg kg^{-1}, respectively, were obtained from variety Ibado indicating it is weaker in accumulating Mn.

Table 5. Micronutrients contents in tissues of haricot bean varieties under field conditions

Variety	Grain yield (kg ha^{-1})	Leaf Fe (mg kg^{-1})	Seed Fe (mg kg^{-1})	Leaf Zn (mg kg^{-1})	Seed Zn (mg kg^{-1})	Leaf Cu (mg kg^{-1})	Seed Cu (mg kg^{-1})	Leaf Mn (mg kg^{-1})	Seed Mn(mg kg^{-1})
Nasir	3068.67a	156.12a	79.27a	19.95a	25.26a	6.95a	7.15a	124.08a	38.11a
Ibado	2709.72b	152.54a	86.46a	19.12bc	22.56c	6.56bc	6.40b	110.35b	31.65c
H. Dume	3099.55a	148.32a	84.69a	19.05c	23.93b	6.41c	6.45b	121.00a	35.76b
Sari-1	2359.34c	155.80a	80.69a	19.75ab	24.10b	6.70ab	6.70b	127.24a	36.07ab
CV (%)	20.29	21.35	16.41	12.62	10.50	15.22	22.9	28.87	20.95
LSD (5%)	160.48	9.29	20.32	0.70	0.72	0.29	0.43	9.90	2.1
					p-value				
*V	<.0001	0.3164	0.8880	0.0224	<.0001	0.0024	0.0031	0.0058	<.0001
Fe	0.5874	0.0112	0.0453	0.2219	0.4038	0.5229	0.6213	0.6642	0.3769
V × Fe	0.6749	0.9867	0.3958	0.5405	0.6648	0.3923	0.5659	0.8367	0.1069
Location	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Means followed by the same letter(s) within a column are not significantly different at $P \leq 0.05$ according to least significant difference (LSD) test.

*V= variety; H.Dume = Hawassa Dume

Application of different levels of Fe significantly increased tissue Fe concentrations, but did not influence grain yield (Table 6). The highest leaf and seed Fe concentrations, 195.60 and 89.18 mg kg^{-1}, respectively, were observed at 3% $FeSO_4.7H_2O$, while the lowest values, 149.89 and 28.72 mg kg^{-1}, respectively, were obtained at zero Fe fertilizer. Similarly, Yadav et al. (2013) reported a significantly increased fruit yield by application of Fe fertilizer to peach trees in India, and Kobraee et al. (2011) reported increased yield and tissue Fe concentration by application of Fe fertilizer to soybean in Iran. In contrast, Imakumbili et al. (2010) reported application of Fe fertilizer did not significantly affect seed Fe concentration of bean in Tanzania. Tissue micronutrients concentrations, except Fe, were not significantly influenced by iron fertilization (Table 6).

Growing locations significantly affected tissue micronutrients concentrations in haricot bean (Table 7). The highest leaf Zn and seed Mn were observed at Taba, whereas the lowest values were observed at Butajira, which could be attributed to low soil Zn at Butajira. The highest seed Zn was observed at Halaba, while the lowest was observed at Taba. Like the pot experiments, seed Zn was higher than leaf Zn. The highest leaf and seed Cu concentrations, 9.78 and 9.63 mg kg^{-1}, respectively, were observed at Butajira and these values were significantly higher than the values observed at the other locations, which could be attributed to the higher value of Cu at Butajira. The highest leaf Mn content was observed at Halaba and it was significantly higher than the value observed at the other locations. The interaction between haricot bean varieties and Fe fertilizer did not significantly affect tissue micronutrients concentrations of haricot bean.

Table 6. Tissue micronutrients contents of haricot bean varieties as influenced by Fe fertilization under field conditions

$FeSO_4.7H_2O$ (%)	Grain yield (kg ha^{-1})	Leaf Fe (mg kg^{-1})	Seed Fe (mg kg^{-1})	Leaf Zn (mg kg^{-1})	Seed Zn (mg kg^{-1})	Leaf Cu (mg kg^{-1})	Seed Cu (mg kg^{-1})	Leaf Mn (mg kg^{-1})	Seed Mn(mg kg^{-1})
0	2792.93a	149.89a	84.72a	19.25a	23.61a	6.70a	6.64a	118.78a	36.40a
1%	2754.04a	155.71a	79.53a	19.38a	24.06a	6.53a	6.60a	122.43a	35.55a
2%	2744.2a	152.58a	77.67a	19.92a	24.20a	6.74a	6.85a	123.31a	34.61a
3%	2846.12a	154.60a	89.18a	19.32a	23.99a	6.65a	6.60a	118.15a	35.03a
CV (%)	20.29	21.35	16.41	12.62	10.50	15.22	22.9	28.87	20.95
LSD (5%)	160.48	9.29	20.32	0.70	0.72	0.29	0.43	9.90	2.1

All yield and tissue Fe concentrations varied significantly between locations (Table 7). The highest values of grain yield (3014.49 kg ha^{-1}) and seed Fe concentration (119.79 mg kg^{-1}) were observed at Butajira, whereas the lowest values, 2620.01 kg ha^{-1} and 55.94 mg kg^{-1}, respectively, were observed at Halaba. Contrary to the field experiment, the highest seed Fe concentration was observed in Taba soil in the pot experiment. This could be because Fe absorption in Taba fields might be more affected by environmental factors than in Butajira. However, under controlled conditions, where environmental factors have less effect, Fe absorption was higher in Taba than in Butajira that could be attributed to the higher soil Fe content of Taba soils. The highest leaf Fe concentration

(217 mg kg^{-1}) was also observed at Halaba, which was significantly higher than that at Butajira and Taba, while the lowest (91.24 mg kg^{-1}) value was observed at Taba. Generally, addition of Fe resulted in best haricot bean production at Butajira, in terms of both quantity and quality, which might be attributable to lower available Fe and higher total nitrogen in the experimental soil as compared to the other locations. The concentrations of Fe were by far higher in leaves than in seeds of all varieties and levels of Fe fertilizers, suggesting that concentrations of plant minerals vary between plant tissues.

According to Govindaraj et al. (2011), sufficiency range of Fe and Zn concentrations in critical stages of pulses, are 50-250 and 20-40 mg kg^{-1}, respectively. Hodges (2010) also reported that deficiency of Fe in most plant species occurred when the Fe content of leaves was below 10- 80 mg kg^{-1}. Munson (1998) reported that the sufficiency range of Mn in mature leaf tissue is between 20 and 300 mg kg^{-1}. Accordingly, Fe, and Mn concentrations in the leaves of haricot bean with all treatments in all locations were sufficient. But Zn was not sufficient in all varieties, haricot beans grown with all treatments, and at Butajira, except at Taba and Halaba. Leaf Cu concentration of plants grown in soils of all locations were above 2 mg kg^{-1} and sufficient, according to Kabata-Pendias and Pendias (1992), who reported that Cu tissue levels below 2 mg kg^{-1} are generally inadequate for plants. Therefore, Zn fertilization is required for haricot bean production at Butajira.

Table 7. Micronutrients contents of tissue of haricot bean varieties as influenced by location

Location	Grain yield (kg ha^{-1})	Leaf Fe (mg kg^{-1})	Seed Fe (mg kg^{-1})	Leaf Zn (mg kg^{-1})	Seed Zn (mg kg^{-1})	Leaf Cu (mg kg^{-1})	Seed Cu (mg kg^{-1})	Leaf Mn (mg kg^{-1})	Seed Mn(mg kg^{-1})
Butajira	3014.5a	151.35b	119.79a	17.06c	23.64b	9.78a	9.63a	81.05c	32.50b
Taba	2718.5b	91.24c	72.61b	20.98a	23.33b	5.14b	4.95c	109.77b	44.70a
Halaba	2620.01b	217.00a	55.94b	20.36b	24.92a	5.05b	5.45b	171.17a	28.99c
CV (%)	20.29	21.35	16.41	12.62	10.50	15.22	22.9	28.87	20.95
LSD (5%)	138.98	8.05	17.60	0.60	0.62	0.25	0.38	8.57	1.82

4. Conclusions

Both pot and field experiments revealed that yield and tissue micronutrients concentrations varied significantly across soils (locations). Generally, Fe fertilization resulted in best haricot bean production at Butajira both in quantity and quality, which could be attributed to lower available Fe and higher total nitrogen in the experimental soil as compared to the other locations. Yield and micronutrients concentrations also varied significantly among haricot bean varieties. Nasir produced the highest grain yield and seed Fe concentration and was found to be best variety both in quality and quantity. Tissue micronutrients concentrations, except Fe, and yield of haricot bean varieties were not significantly influenced by iron fertilization, but it significantly increased tissue Fe concentrations, the highest values being observed at 3% $FeSO_4.7H_2O$.

This study revealed that tissue Fe concentration varies among haricot bean varieties, and Fe fertilization enriches haricot bean with the element and hence improves its quality. Consequently, Nasir and 3% $FeSO_4.7H_2O$ were found to be the best variety and rate, respectively, for quality production of haricot bean. Therefore, growing Nasir at 3% $FeSO_4.7H_2O$ can contribute to alleviate the deficiency of Fe in people who consume the crop as a major component of their diet. The study also revealed that Zn fertilization is required for haricot bean production at Butajira.

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