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Effects of Zinc Fertilization on Yield and Tissue Concentrations of Manganese, Copper, Iron and Zinc in Leaves and Seeds 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 with adequate tissue concentrations of generally below 100 parts per million (ppm). The experiment was executed in a quadruple factorial design on farmers' fields and in a greenhouse to evaluate the effect of Zn fertilization on the yield and concentrations of Zn, Fe, Cu and Mn in tissues of different haricot bean varieties grown in soils of Halaba, Butajira and Taba. The treatments include zinc fertilizer (0, 0.5, 1, or 1.5%) and haricot bean variety (Nasir, Ibado, Hawassa Dume, or Sari-1). The results indicated that yield and tissue micronutrients contents of haricot bean varied significantly among the different locations (soils) the highest yields under greenhouse (17.62 g/pot) and at field (3553.6 kg ha^{-1}) being obtained in Butajira soils. Variety Nasir was the best both in yield and Zn and Fe concentrations of seeds. Yield of haricot beans and tissue concentrations of Fe, Cu and Mn were not significantly affected by Zn fertilization, but both leaf and seed Zn concentrations were significantly increased. The highest leaf Zn and seed Zn, 50.71 and 20.53 mg kg^{-1}, respectively, were obtained at applications of 3 and 2% $ZnSO_4.7H_2O$, respectively. We can, therefore, conclude that foliar Zn fertilization is vital to increase the grain Zn content of haricot bean which in turn can contribute to alleviated Zn deficiency of people who depend on haricot bean diet for their Zn source. Consequently, foliar application of 2% $ZnSO_4.7H_2O$ is recommended for significant increase of grain Zn content of haricot bean.

Keywords: Manganese, Iron, Copper, Zinc, Concentration and Haricot bean

1. Introduction

Micronutrients are essential trace elements for plants, animals, and humans (Ai-qing et al., 2013). They include zinc (Zn), iron (Fe), manganese (Mn), boron (B), chlorine (Cl), copper (Cu), and molybdenum(Mo).They are needed in very small amounts with adequate concentrations in plants of generally below 100 parts per million (ppm) (Lohry, 2007). Although required in smaller quantity, micronutrients are as essential as macronutrients for optimum growth and yield for beans (Wortmann et al., 2008). Zinc is essential for activating plants' enzymatic systems, protein synthesis (Hafeez et al., 2013), photosynthesis, reproduction of genetic material (DNA) during cell division (Singh, 2004), and the synthesis of chlorophyll and carbohydrates (Kobraee et al., 2011). Zinc deficiency not only retards the growth and yield of plants, but also affects human beings. Its deficiency is the fifth most important risk factor associated with illness and death in the developing world, and affects about one-third of the world's population (Ai-qing et al., 2013), particularly in areas where the population is heavily dependent on an unvaried diet of cereal-based foods. The optimum dietary intake for adults is 15 mg Zn per day (Hafeez et al., 2013). Iron is required for the formation of chlorophyll in plant cells. It serves as an activator for biochemical processes such as respiration, photosynthesis and symbiotic nitrogen fixation (Lohry, 2007). According to this author, copper is an activator of several enzyme systems in plants and functions in electron transport and energy capture by oxidative proteins and enzymes, and plays a role in vitamin A production. Manganese also serves as an activator for enzymes in growth processes and assists iron in chlorophyll formation.

Phaseolus vulgaris L. is an important leguminous crop of great nutritional status to poor communities in African countries. It is nicknamed "poor man's protein" due to its potential role in the daily diet of the poor who cannot afford expensive animal protein (Ndakidemi *et al.,* 2011). It can provide 15% of the Zn requirements (Beebe et al., 2000); however, it is recognized as being highly susceptible (sensitive) to zinc deficiency (Goh and Karamanos, 2003; Singh, 2004; Imtiaz et al., 2010), which reduces its yield and nutritional value (Kobraee et al., 2011). In general, zinc may be needed for haricot bean where the soil is calcareous (pH greater than 7.3 because of excess free lime), the topsoil has been removed by erosion, land has been leveled or terraced, and soils are very sandy with low organic matter content (Wortmann et al., 2008).

Zinc fertilization is one method to prevent zinc deficiency and increase its concentration in grains (Blair et al., 2009). As zinc is taken up by plants in small amounts, crop demands for zinc can be met through foliar sprays (Pivot, 2003). Ai-qing et al. (2013) reported that foliar Zn application increased grain Zn concentration in wheat more effectively than Zn application to soil. Foliar Zn application also reduced the grain [phytic acid]/ [Zn] ratio and increased the estimated Zn absorption more than did soil Zn application. Foliar application is the most efficient method of supplying Zn, which is needed only in a small quantity and may become unavailable if applied to the soil (FAO, 2000) as it does not directly contact the soil, avoiding losses through fixation (Nasri et al., 2011). However, the narrow difference between phytotoxicity and deficiency requires that appropriate application rates be determined. Further, crop responses to applied zinc vary depending on the soil type, crop, variety, available nutrient status, severity of deficiency, etc. Tryphone and Nchimbi-Msolla (2010) reported that haricot bean genotypes varied in response to the application of Zn in Tanzania. Talukder et al. (2009) indicated that application of zinc caused significant differences in concentrations of Zn in haricot bean seeds. Tryphone and Nchimbi-Msolla (2010) also reported significant differences in concentrations of zinc in seeds and leaves of haricot bean in Tanzania. The critical level of zinc in bean tissue is 15 to 20 ppm. Levels greater than 120 ppm can decrease yields. The average concentration of Fe in tissue is 100 ppm (Ronan, 2007). 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).

Abay et al. (2015) reported that the DTPA Zn contents of most of the soils at Halaba, Taba, and Butajira were below 1.5 mg kg^{-1}, 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. Besides, the effect of zinc on the concentrations of Zn, Fe, Cu and Mn in leaves and seeds of haricot bean was not evaluated. Therefore, this study was conducted to evaluate the effect of Zn fertilization on the yield and concentrations of the aforementioned micronutrients in tissues of different haricot bean varieties grown at Halaba, Butajira and Taba.

2. Materials and methods

The experiment was executed in a quadruple factorial design on farmers' fields and in a greenhouse. The two factors were the concentration of zinc fertilizer (0, 0.5, 1, or 1.5%) and the haricot bean variety (Nasir, Ibado, Hawassa Dume, or Sari-1). Zinc sulfate solutions of 0.5, 1, and 1.5% were prepared by adding 0.5, 1, and 1.5 kg, respectively, of zinc sulfate into 100 L of water (Pivot, 2003). The experimental sites were Halaba, Taba, and Butajira, and soils (0–30 cm depth) were also collected from these sites for the greenhouse experiments. The types of soils included Haplic Luvisols (Humic) in Butajira, Andic Lixisols (Humic) and Andic Cambisols (Humic) in Halaba, and Haplic Lixisols (Siltic) and Haplic Lixisols (Humic) 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 zinc was heptahydrated zinc sulfate ($ZnSO_4{\cdot}7H_2O$), which contains 21% Zn (Tryphone and Nchimbi-Msolla, 2010). The zinc sulfate was sprayed at a volume of 100 L/ha on the plants three weeks and six weeks after the sowing date (Abdel-Mawgoud et al., 2011). Nitrogen and phosphorus (P) were applied equally for all treatments. Phosphorus was applied just before planting as TSP at 50 mg P kg^{-1} soil (0.25 g P/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 0.045 g N/pot and at 18 kg N ha^{-1} for the field experiments. Four haricot bean seeds/pot were sown and two of the seedlings were thinned at 10 days after sowing.

For the field experiments, haricot beans were planted in rows with a spacing of 10 cm between plants and 40 cm between rows in 3 × 4 m plots, while the greenhouse experiments were conducted in pots with 5 kg of soil per pot. All appropriate management practices were carried out equally for all treatments.

Grain yields were recorded. Three fully developed leaves from the top of the plant during initial flowering and some seeds were collected from each treatment pot and plot, dried in an oven at 70 °C for 24 hours, and ground using a sample rotating mill. The ground plant materials were digested and analyzed for their iron contents with the following procedures. A sample of 0.5 g was weighed on a balance sensitive to the nearest 0.00001g and put into a digestion tube. Six milliliters of HNO_3 was added to each tube and placed in a digestion block at 90 °C for 45 minutes. Then, 5 ml of H_2O_2 was added in two splits (3 ml and 2 ml) while the samples were in the digestion block and digestion continued for another 65 minutes. Finally, 3 ml of 6 M HCl was added and the samples were digested until the solution had turned completely clear (about 5 minutes). Then, the tubes were removed from the block and cooled for 20 minutes, shaken using a vortex, and the digests were transferred from digestion tubes to dram vials and stored after the solution was brought to a volume of 25 ml with deionized water. The concentrations of Zn were analyzed using a microwave plasma atomic emission spectrometer (MPAES) at 213.857 nm. Data were analyzed using SAS computer software.

3. Results and Discussion

Pot experiment

The pot experiment indicated that haricot bean production varied significantly among soils of the different locations (Table 2). Tissue micronutrients contents significantly varied among soils. Both yield and tissue Zn concentrations were significantly influenced by location soils. The highest value of grain yield (17.62 g/pot) was obtained in Butajira, but the lowest value was observed in Halaba soil. The highest leaf Zn (42.10 mg kg^{-1}) and seed Zn (20.94 mg kg^{-1}) were observed in Taba, while the lowest values, 32.22 and 15.74 mg kg^{-1}, respectively, were obtained in Butajira soil. This indicates that Butajira and Taba are better locations for haricot bean production in terms of quantity and quality, respectively. The significantly higher seed Zn concentration observed in bean grown in Halaba soil than in Butajira could be attributed to the dilution effect by higher yield at Butajira and higher soil Zn content in Halaba soil. Nchimbi-Msolla and Tryphone (2010) reported that bean leaf and seed Zn concentrations were varied among location soils due to the environments in which the crop was grown. Both leaf and seed Fe were higher in Butajira and Taba soils than Halaba soil, which might be attributed to the higher pH value in Halaba soil to reduce Fe uptake. Similarly, tissue Cu contents also significantly varied among soils. The highest tissue Cu contents were observed in Butajira soils, which could be attributed to the higher Cu contents in the soil than Taba and Halaba soils. The highest leaf and seed Fe, were 8.22 and 9.37 mg kg^{-1}, respectively, while the lowest values were 4.58 and 4.36 mg kg^{-1}, respectively, was observed in haricot bean grown at Halaba soils. Tissue Mn concentrations significantly varied across locations, the highest leaf Mn (237.47 mg kg^{-1}) and seed Mn (32.10 mg kg^{-1}) being obtained at Taba soil followed by Halaba soil. The lowest Mn concentrations were observed at Butajira.

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})
Halaba soil	13.74b	108.54b	40.98b	33.13b	20.18b	4.58c	4.36c	119.79b	23.45b
Butajira soil	17.62a	126.88a	52.53a	32.22b	15.74c	8.22a	9.73a	79.14c	20.44c
Taba soil	14.51b	133.99a	45.47ab	42.20a	20.94a	5.25b	6.87b	237.47a	32.10a
CV (%)	21.31	15.87	15.27	18.76	6.22	13.68	6.30	19.83	7.23
LSD (5%)	1.32	7.92	10.38	2.73	0.48	0.33	0.18	11.69	0.74

Means followed by the same letter(s) within a row are not significantly different at $P \leq 0.05$.

Haricot bean production varied significantly among varieties in terms of yield and tissue micronutrients concentrations (Table 3). The lowest leaf Zn (32.39 mg kg^{-1}), and highest grain yield (16.78 g/pot) were recorded with Nasir. Sari-1 produced the highest leaf Zn (39.40 mg kg^{-1}). Nasir and Sari-1 had the highest seed Zn, 20.13 and 20.14 mg kg^{-1}, respectively. Although the highest grain yield was observed with Nasir, it was not significantly different from the value for Hawassa Dume. Both leaf and seed Fe significantly varied among varieties. Variety Ibado produced significantly higher leaf Fe than the other varieties. Varieties Nasir, Hawassa Dume and Sari-1 yielded statistically equal leaf Fe. Variety Ibado produced 133.1 mg kg^{-1}of leaf Fe, whereas Nasir yielded the lowest value (117.27 mg kg^{-1}). The highest seed Fe (55.28 mg kg^{-1}) was obtained from variety Nasir, and it is significantly higher than the value produced by Ibado and Hawassa Dume, but it is statistically equal with the seed Fe produced by variety Sari-1. Leaf and seed Cu also significantly varied among haricot bean varieties. The highest leaf Cu (6.27 mg kg^{-1}) and seed Cu (7.22 mg kg^{-1}) were produced from Ibado and Sari-1, respectively, whereas the lowest values, 5.68 mg kg^{-1} and 6.70 mg kg^{-1}, respectively, were observed with varieties Nasir and Hawassa Dume, respectively. There was no statistical difference was observed between varieties Nasir and Sari-1 in terms of their seed Cu contents. Similarly, Ibado and Hawassa Dume produced statistically equal seed Cu indicating that these varieties have similar characteristics in terms of accumulating seed Cu. The contents of Fe, Zn and Cu in leaves of Ibado were higher than the value in the other varieties, but contrarily leaf Mn of Ibado was the lowest value.

Table 3. Micronutrients contents in tissue of haricot bean varieties

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	16.78a	117.27b	55.28a	39.40a	20.13a	5.68b	7.16a	150.61ab	26.06a
Ibado	14.39b	133.10a	41.35b	36.30ab	17.51b	6.27a	6.86b	137.24b	22.73b
Hawassa Dume	16.27a	123.28b	39.08b	35.31bc	18.04b	5.98ab	6.70b	140.37ab	26.00a
Sari-1	13.73b	118.89b	49.59ab	32.39c	20.14a	6.14a	7.22a	153.63a	26.51a
CV (%)	21.31	15.88	15.27	18.76	6.22	13.69	6.30	19.83	7.23
LSD (5%)	1.53	9.15	11.98	3.15	0.55	0.39	0.21	13.5	0.86
		P- value							
*V	0.0002	0.0039	0.0313	0.0004	<.0001	0.0215	<.0001	0.0506	<.0001
Zn	0.0025	0.5268	0.8181	<.0001	<.0001	0.5167	<.0001	0.0206	0.0078
V × Zn	0.4966	0.4455	0.4467	0.0061	0.0051	0.3811	<.0001	0.0006	0.0747
Location	<.0001	<.0001	0.0891	<.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

The application of Zn fertilizers significantly increased tissue Zn concentrations (Table 4). A similar finding was reported by Itamar et al. (2004), who found that foliar Zn fertilization significantly affected plant height. Kobraee et al. (2011) and Kumar and Babel (2011) also reported that the application of increasing levels of Zn fertilizer significantly increased both leaf and seed Zn concentrations. The highest leaf and seed Zn concentrations, 50.71 and 20.53 mg kg^{-1}, were observed at applications of 1.5 and 1% zinc sulfate, respectively, indicating that the levels of Zn fertilizer yielding the highest concentrations of Zn in leaves and seeds may differ. It also suggests that the amount of Zn fertilizer to be applied may depend on the purpose of production. The lowest concentrations of both leaf and seed Zn, 19.83 and 17.55 mg kg^{-1}, respectively, were observed with the control (no Zn fertilizer). Therefore, the application of 1.5 and 1% of Zn sulfate resulted in concentrations of Zn in leaves and seeds that were 155.72 and 16.98% higher, respectively, than the control (no Zn). The concentrations of Zn in leaves and seeds were different (higher in leaves), which are expected, suggesting that plant mineral concentrations may vary among plant tissues. Narwal et al. (2010) also reported similar findings on wheat in Australia. The grain yield was not significantly different among different levels of Zn fertilizer application and the control. Ai-qing et al. (2013) also found that the application of Zn fertilizer usually had no significant effect on grain yield in wheat. Application of Zn fertilizer did not significantly influence leaf and seed Fe concentrations of haricot bean varieties (Table 4). However, tissue Fe concentrations decreased with application of Zn fertilizer, which could be attributed to the antagonistic relation between the two minerals. Leaf Cu was also not significantly affected by the application of Zn fertilizers, while seed Cu was significantly influenced the highest value (7.39 mg kg^{-1}) being obtained from application of 2% $ZnSO_4.7H_2O$ (%). Tissue Mn was also significantly affected by the application of Zn fertilizer. The highest leaf Mn (154.59 mg kg^{-1}) was observed with application of zero Zn, while the lowest (133.34 mg kg^{-1}) was obtained at application of 2% Zn fertilizer. The highest seed Mn (25.76 mg kg^{-1}) was observed at the application of 2% Zn fertilizer. Generally Zn fertilization increased seed Mn concentration, but decreased leaf Mn concentration.

Table 4. Micronutrients contents of tissue of haricot bean varieties as influenced by Zn fertilization

$ZnSO_4.7H_2O$ (%)	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	16.29a	121.01a	43.99a	19.83d	17.55d1	6.02a	6.70c	154.59a	24.40b
1%	15.18a	126.74a	49.53a	32.19c	18.41c	6.18a	7.17b	147.97a	25.63a
2%	13.60a	124.01a	46.55a	40.66b	20.53a	5.91a	7.39a	133.34b	25.76a
3%	16.10a	120.78a	45.24a	50.71a	19.32b	5.96a	6.68c	145.97ab	25.53a
CV (%)	21.31	15.87	15.27	18.76	6.22	13.68	6.30	19.83	7.23
LSD (5%)	1.53	9.15	11.98	3.15	0.55	0.39	0.21	13.5	0.86

Means followed by the same letter(s) within a row are not significantly different at $P \leq 0.05$.

Significant differences in the concentrations of Zn in leaves (p = 0.0061) and seeds (p = 0.0051) were observed due to the interactions between varieties and levels of Zn fertilizer (Fig. 1). All varieties produced the highest leaf Zn concentrations with the highest level of Zn fertilizer (1.5% $ZnSO_4 \cdot 7H_2O$), but they produced the highest seed Zn concentrations at the application of 1% zinc sulfate except for Hawassa Dume, which produced highest seed Zn at 1.5%. However, the highest leaf and seed Zn concentrations differed among the varieties. The highest leaf Zn concentrations of Nasir, Ibado, Hawassa Dume, and Sari-1 were 43.65, 56.69, 49.62, and 52.90 mg kg^{-1}, respectively, whereas the lowest values, 20.24, 20.86, 18.58, and 19.64 mg kg^{-1}, respectively, were observed with no zinc fertilizer. On the other hand, the highest values of seed Zn in the above varieties were

22.47, 19.07, 18.90, and 22.00 mg kg^{-1}, respectively. These indicate that all varieties accumulated Zn in their tissues similarly except for Hawassa Dume, which differed in how much was accumulated in seeds. Therefore, the same amount of zinc fertilizer is recommended to be applied for all varieties. The interaction between haricot bean varieties and Zn fertilizers significantly affected seed Cu and leaf Mn (Fig. 1).

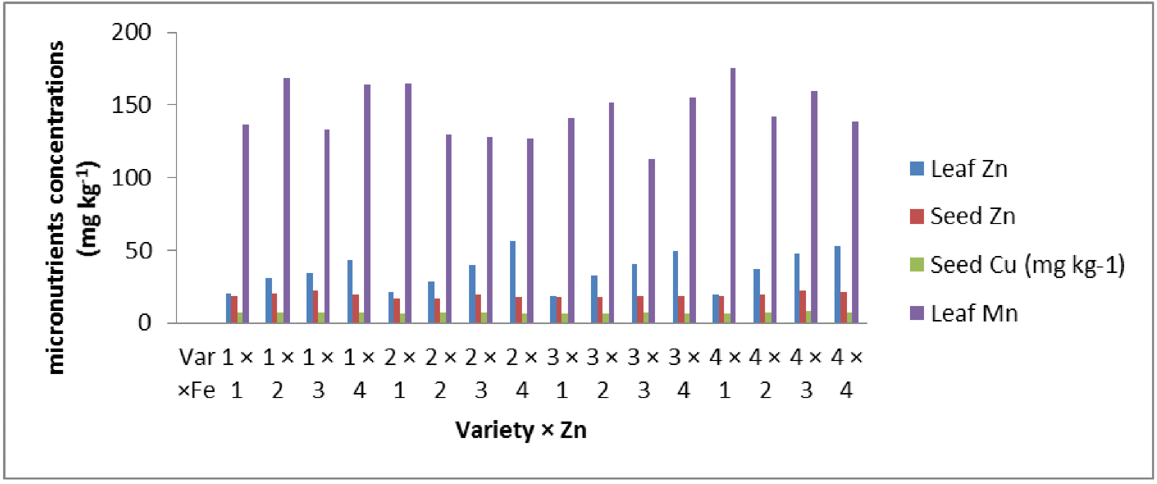

Fig.1 Tissue micronutrients as influenced by haricot bean variety and Zn fertilizer interaction

Field experiments

Like the pot experiment, the field experiments showed that haricot bean production varied significantly among locations (Table 5). The highest values for yields were observed at Butajira. The highest grain yield was 3553.6 kg ha^{-1} while the lowest, from Taba, was 2250.4 kg ha^{-1}. The highest concentrations of both leaf and seed Zn, 26.93 and 29.52 mg kg^{-1}, respectively, were observed at Halaba, while the lowest values were obtained at Taba. This could be because Zn absorption in Taba fields might be more affected by environmental factors than in Halaba. However, under controlled conditions, where environmental factors have less effect, Zn absorption was higher in Taba than in Halaba that could be attributed to the higher soil Zn content of Taba soils (Table 1). Goh and Karamanos (2003) also found that yield and tissue Zn concentrations varied among locations. Similar findings were reported by Ai-qing et al. (2013) on wheat in China. All tissue micronutrients varied across locations (Table 5). Leaf Fe and Mn were significantly higher in Halaba than the other locations, while seed Fe and Mn were significantly higher in Taba than the other locations. The highest leaf Fe and Mn were, 174.29 and 158.53 mg kg^{-1}, respectively, whereas the highest seed F and Mn were 111.67 and 51.08 mg kg^{-1}, respectively. Both leaf and seed Cu were significantly higher in Butajira than the other locations, which could be attributed to the higher soil Cu content in Butajira than Taba and Halaba soil. This indicates tissue Cu concentration increase with increasing its availability in the soil.

Similar to the pot experiment, the field experiments also indicated that haricot bean production, both in terms of yield and tissue Zn concentrations, varied among varieties (Table 6). Similar findings were reported by McKenzie et al. (2001) on haricot bean in Canada. The lowest grain yield (2503.4 kg ha^{-1}) was observed with Ibado. Hawassa Dume had the highest grain yield (3149.3 kg ha^{-1}), and it was significantly higher than that of Ibado and Sari-1, but not significantly different from the yield of Nasir. Nasir produced the highest leaf Zn (23.69 mg kg^{-1}), and seed Zn (25.79 mg kg^{-1}).

Table 5. 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	3553.6a	142.03b	83.01b	21.45b	22.06b	9.43a	9.57a	80.85c	25.19b
Taba	2250.4b	123.26c	111.67a	19.08c	22.03b	4.70c	4.49c	139.62b	51.08a
Halaba	2467.8b	174.29a	77.75b	26.93a	29.52a	5.16b	5.56b	158.53a	24.74b
CV (%)	40.92	24.91	54.40	23.45	24.39	19.04	16.71	19.02	38.35
LSD (5%)	277.6	8.98	12.15	1.30	1.47	0.30	0.27	6.00	3.18

Means followed by the same letter(s) within a row are not significantly different at $P \leq 0.05$.

Therefore, it was found to be the best variety both in terms of quantity and quality. Muhamba and Nchimbi-Msolla (2010) also reported that leaf and seed Zn concentrations varied among haricot bean varieties in Tanzania, ranging from 15.7 to 78.3 mg kg^{-1} and from 19.00 to 56.13 mg kg^{-1}, respectively. Ai-Qing et al. (2013) reported that the grain Zn concentration varied among wheat varieties in China. Unlike the pot export, the leaf and seed Fe concentrations did not vary among varieties, but the trend was similar. The highest leaf Fe was obtained from Ibado and the lowest was obtained from Hawassa Dume. Reversely, the highest seed Fe was obtained from Hawassa Dume and the lowest was obtained from Nasir. Tissue Cu also was not significantly affected among varieties; however, the highest leaf was obtained from variety Nasir, and the highest seed Cu was obtained from Hawassa Dume. Significantly varied leaf and seed Mn concentrations were observed among haricot bean varieties. Ibado produced significantly lower leaf Mn than the other three varieties, whereas Nasir and Hawassa Dume yielded almost equal and significantly higher seed Mn than Ibado and Nasir.

Table 6. Micronutrients contents of tissue of different haricot bean varieties

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	2870.4a	148.09a	87.24a	23.69a	25.79a	6.55a	6.51a	131.56a	36.74a
Ibado	2503.4b	149.92a	89.34a	22.75ab	23.87b	6.35a	6.38a	119.12b	29.91b
H. Dume	3149.3a	141.91a	97.95a	21.97b	24.08b	6.35a	6.67a	128.12a	35.93a
Sari-1	2506.1b	146.20a	88.71a	21.54b	24.40ab	6.48a	6.60a	126.54a	32.10b
CV (%)	40.92	24.91	24.40	23.45	24.39	19.04	16.71	19.02	38.35
LSD (5%)	320.54	10.37	14.03	1.50	1.70	0.35	0.31	6.93	3.67
		P- value							
*V	<.0001	0.4673	0.4301	0.0278	0.1124	0.6125	0.2895	0.0046	0.0006
Zn	0.9613	0.8245	0.3312	0.0002	<.0001	0.2241	0.2703	0.7137	0.7412
V × Zn	0.9937	0.8144	0.2548	0.7379	0.8878	0.1312	0.0725	0.9971	0.6544
Location	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

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

*V= variety; H.Dume = Hawassa Dume

The application of increasing levels of zinc fertilizer significantly increased tissue Zn concentrations (Table 7). Sharma and Bapat (2000) and Shaheen et al. (2007) reported that both the grain and straw Zn concentrations of wheat significantly increased with the application of Zn. Ai-Qing et al. (2011) and Ai-qing et al. (2013) also reported that the foliar application of Zn fertilizer significantly increased leaf and seed Zn concentrations, respectively, in wheat. Another report by Fageria et al. (2014) also indicated that Zinc concentration of tropical legume cover crops was increased with the addition of Zn to the soil. The highest concentrations of both leaf Zn (24.06 mg kg^{-1}) and seed Zn (25.97 mg kg^{-1}) were observed at the application of the highest level of zinc fertilizer (1.5%), whereas the lowest values of both leaf and seed Zn, 20.64 and 21.95 mg kg^{-1}, respectively, were recorded with no zinc fertilizer. These highest values of Zn in leaves and seeds were 16.57 and 18.31% higher, respectively, than the control. The value of seed Zn obtained with the application of 0.5% $ZnSO_4{\cdot}7H_2O$ was also 11.98% greater than the value obtained from the control. Although the highest seed Zn was observed at the application of 1.5% $ZnSO_4{\cdot}7H_2O$, it was not significantly higher than the values obtained at applications of 0.5 and 1% $ZnSO_4{\cdot}7H_2O$.

Therefore, the application of 0.5% $ZnSO_4{\cdot}7H_2O$ is recommended for producing better quality haricot beans. The amount of seed Zn obtained from the 0.5% application was 24.58 mg kg^{-1}, which is enough for 1.64 days per person (Hafeez et al., 2013), i.e., 1 kg of haricot beans produced with the application of 0.5% $ZnSO_4{\cdot}7H_2O$ can supply a single person's Zn needs for 1.64 days. Fertilization with Zn did not significantly influence the yield of haricot beans (Table 7). Ai-qing et al. (2013) also reported that the foliar application of Zn fertilizer did not significantly increase the grain yield of wheat in China. Contrarily, Kobraee et al. (2011) reported that Zn fertilizer significantly increased the yield of soybeans. Goh and Karamanos (2003) likewise reported that foliar application of Zn significantly increased the yield of haricot beans compared to an unfertilized control. Application of Zn fertilizer did not significantly influence leaf and seed concentrations of Fe, Cu and Mn in haricot beans.

Nasir produced its highest yield and tissue Zn concentrations at Butajira. Ibado, Hawassa Dume, and Sari-1 produced their highest grain yield at Butajira, but they produced the highest tissue Zn concentrations at Halaba. Yield and seed Zn concentration were higher in the fields than pots with all varieties and Zn rates at all locations, which could be attributed to better root growth and distribution that could intern, enable the beans to absorb more Zn and other nutrients. This higher nutrient absorption resulted in more vigorous plant growth that could produce higher yield in the fields than pots. Leaf Zn concentration was lower in field experiments than the pots, which could be attributed to dilution effect caused by higher vegetative growth.

Table 7. Tissue micronutrients concentrations of haricot bean varieties as influenced by Fe fertilization

$ZnSO_4.7H_2O$ (%)	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	2741.3a	144.32a	98.84a	20.64c	21.95b	6.34a	6.45a	127.24a	34.28a
1%	2729.5	147.66a	87.21a	22.72ab	24.58a	6.53a	6.55a	128.18a	34.39a
2%	2810.0a	145.37a	88.66a	22.54b	25.64a	6.59a	6.71a	125.42a	32.57a
3%	2748.4a	148.77a	88.52a	24.06a	25.97a	6.27a	6.44a	124.49a	33.43a
CV (%)	40.92	24.91	54.40	23.45	24.39	19.04	16.71	19.02	38.35
LSD (5%)	320.54	10.37	14.03	1.50	1.7	0.35	0.31	6.93	3.67

Means followed by the same letter(s) within a row are not significantly different at $P \leq 0.05$.

Lohry (2007) indicated adequate concentrations and ranges of micronutrients in plants. According to this author, Cu ranges from 2-50 ppm and the adequate amount is 6 ppm; Zn ranges from 10-250 ppm, and 20 ppm is adequate; Iron ranges from 10-600 ppm, and 100 ppm is adequate; manganese ranges from 10-800 ppm, and 50 ppm is adequate. According to this data, the result of the current study indicated that the concentrations of Zn, Fe, Cu and Mn in the plants of the beans are adequate.

4. Conclusion

Both pot and field experiments revealed that haricot bean production varied significantly among soils (locations) and bean varieties in terms of yield and tissue micronutrients concentrations. The highest values for yields were observed in soils of Butajira. Nasir and Sari-1 had the highest seed Zn, 20.13 and 20.14 mg kg^{-1}, respectively, while Hawassa Dume gave the highest grain yield (3149.3 kg ha^{-1}) though not significantly different from Nasir did (2870.4 kg ha^{-1}). The application of increasing levels of zinc fertilizer significantly increased tissue Zn concentrations, but not significantly affected the concentrations of the other micronutrients considered in the study. The highest leaf and seed Zn concentrations, 50.71 and 20.53 mg kg^{-1}, respectively, were observed at applications of 1.5 and 1% zinc sulfate, respectively, indicating that the levels of Zn fertilizer yielding the highest concentrations of Zn in leaves and seeds may differ. Although the highest seed Zn was observed with the application of 1.5% $ZnSO_4{\cdot}7H_2O$, it was not significantly higher than the values obtained at applications of 0.5 and 1% $ZnSO_4{\cdot}7H_2O$. Thus, Nasir was found to be the best bean variety, and 0.5% $ZnSO_4{\cdot}7H_2O$ was the best rate for the quality bean production.

The supply of Zn from haricot beans can be improved through foliar application of zinc sulfate but the application of Zn fertilizer couldn't affect the concentrations of Fe, Cu and Mn. Selection of bean varieties with high capacity to accumulate Zn and Fe can supply the minerals and the consumption of such haricot beans can significantly improve the Zn and Fe status of people who consume the crop as a major component of their diet.

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