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Effect of Zinc Fertilizer Rates on Grain and Straw Zn Content, and Grain Yield of Chickpea Varieties in Southern Ethiopia

Legesse Hidoto[1] Walelign Worku[2], BunyaminTar'an[3], and Hussein Mohammed[2]
[1]*Southern Agricultural Research Institute, P.O. Box 6, Hawassa, Ethiopia;* [2] *Hawassa University, P. O. Box 5, Hawassa, Ethiopia;* [3] *Crop Development Centre/ Department of Plant Sciences, University of Saskatchewan, 51Campus Drive, Saskatoon, SKS7N5A8, Canada*

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Abstract

Application of Zn had a significantly positive effect on grain Zn concentrations and also on grain yield especially under Zn deficient conditions. The amount of Zn required to alleviate Zn deficiency varied with severity of deficiency, soil types, nature of crops and cultivars. The response of chickpea varieties to Zn nutrition was studied in pots and on fields using zinc deficient soils during 2012 and 2013 cropping seasons to determine zinc fertilizer rate which improve zinc content and productivity of the crop. A factorial combination of three chickpea varieties and seven zinc fertilizer rates were laid in Randomized Complete Block design with three replications for both pot and field experiments. The result of pot experiment revealed that, variety Mastewal produced the highest grain yield (5.9 g pot^{-1}) and Habru produced highest (35.99mg kg^{-1}) straw zinc content. Conversely, local chickpea provided the highest (36.1mg kg^{-1}) grain Zn. Chickpea varieties and zinc fertilizer rates interaction on grain yield was *significant where 25kg ha^{-1} produced highest regardless of the varieties. Similarly, location had significant (p<0.01) effect of grain zinc content where Choroko produced 46.6 % more grain zinc content than both Taba and Jolle. Highest straw zinc (24.96 mg kg^{-1}) obtained from variety Habru, while highest grain zinc obtained from the application of 25 kg $ZnSO_4 .7H_2O$ ha^{-1} with either of the varieties which was at par with the highest Zinc rate (30kg ha^{-1}). Significant interaction effect of variety by location on grain yield and straw zinc content was observed. The variety Mastewal was superior in grain yield at Jolle and Choroko, while landrace performed better at Taba. The landrace and Habru were higher in straw zinc content across locations. Moreover, 25 kg $ZnSO_4 .7H_2O$ ha^{-1} resulted in 7 and 8% more grain and straw zinc content over the control, in that order. Thus, the current research inveterate a possibility of agronomic intervention for zinc fortification of chickpea through zinc fertilizer management.*

Keywords: biofortification, concentration, deficiency, enrichment, micronutrients, varieties.

Introduction

Chickpea (*Cicer arietinum L.*) is one of the major highland pulse crops widely grown in the highland and semi-highland regions of Ethiopia mainly on clay soils and is mainly cultivated in the country using residual moisture as a rain fed crop (MoANR, 2016). According to FAOSTAT (2018) report, Chickpeas covered an area of 241,212 ha with the production of 515,642 tons and productivity of 2.14 ton per hector. Raw chickpea seed (100 g) on an average provides about 5.45 mg of iron, 4.1 mg of zinc, 138 mg of magnesium and 160 mg of calcium. About 100g of chickpea seed can meet daily dietary requirements of iron (1.05 mg day^{-1} in males and 1.46 mg day^{-1} in females) and zinc (4.2 mg day^{-1} and 3.0 mg day^{-1}) and 200g can meet that of magnesium (260 mg day^{-1} and 220 mg day^{-1}) (FAO, 2002).

Unbalanced use of mineral fertilizers, low soil micronutrient status, and a decrease in the use of organic manures are the main causes of micronutrients deficiency in crop plants, which in turn produce deficient foods. The major reason for the widespread prevalence of Fe and Zn deficiencies in human populations is low dietary intake of Fe and Zn. In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet (Cakmak, 2008).

Application of Zn had a significantly positive effect on grain Zn concentrations and also on grain yield especially under Zn deficient conditions. The amount of Zn required to alleviate Zn deficiency varied with severity of deficiency, soil types, nature of crops and cultivars. According to Singh et al. (1983), Zn requirement of different crops varies significantly and plant species differ in their retort to Zn nutrition. Chickpea is relatively more sensitive to Zn deficiency than cereals (Tiwari and Dwivedi, 1990; Tiwari and Pathak, 1982); it is more likely to suffer from Zn deficiency when planted on Zn-deficient soils. According to Ahlawat *et* *al.* (2007), the main micronutrient that limits chickpea productivity is zinc and its deficiency is common among chickpea-growing regions of the world.

The magnitude of yield losses due to nutrient deficiency also varies among the nutrients (Ali *et al.*, 2008). In countries with high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet. According to UNICEF (2014) and Cakmak, (2008) reports sited by Legesse et al. 2017, micronutrient deficiency remains a significant public health burden in the country with deficiencies in iron, vitamin A, folic acid, iodine and zinc as the common deficiencies. The problem is more acute in southern Ethiopia where the livelihoods and diets are heavily dependent on cereals and root crops, which are relatively low in micronutrients and high in carbohydrates. Based on these facts, the experiments presented in this paper were conducted to examine the grain yield, grain and straw Zn content response of chickpea varieties to soil zinc application rates under pot and field conditions.

Materials and Methods

Study areas

Pot experiment was conducted using soils collected from farmers' field where the soil is zinc deficient. While field experiment was conducted at three locations in zinc deficient soils of Southern Ethiopia during the growing seasons from August to December of 2012 and 2013. These locations were, Jolle Andegna Kebele at Gurage zone with silty clay loam textured soil, Taba Kebele at Wolayita zone with silty loam textured soil and Huletegna Choroko Kebele at Halaba zone with clay loam. The locations coordinates for the three sites are 08^{o} 12' 25.9'' N and 038^{o} 28' 33.2'' E for Jolle Andegna, 07^{o} 01' 01.9'' N and 037^{o} 53' 57'' E for Taba and 07^{o} 20' 34'' N and 038^{o} 06' 30'' E for Huletegna Choroko. The elevations of the sites are 1923, 1915 and 1807, meters above sea level for Jolle Andegna, Taba and Huletegna Choroko, respectively. The sites receive an average annual rainfall of 922,989 and 774, mm in that order.

Experimental set-up and procedures

Pot experiment

Pot experiment was carried out using three chickpea varieties; (Mastewal: Desi type, Habru: Kabuli type and one landrace), and seven zinc fertilizer rates (0, 5, 10, 15, 20, 25, and 30 kg $ZnSO_4$ .7H O ha^{-1}) in plastic pots (210 mm diameter × 300 mm deep) filled with 4 kg of air dried zinc deficient soil collected from farmers' field in southern Ethiopia. The experiment was laid out in factorial combination using Randomized Complete Block Design with three replications. Zinc fertilizer as indicated for each treatment was placed in pot and mixed with soils using stick. Three chickpea grains placed per shallow hill of about 5 cm depth each and covered manually with fine soil. Fifteen days after planting, two plants were maintained as number of plants per pot. All necessary agronomic practices like weeding, watering, protecting were undertaken as required.

Field experiment

Similar to pot experiment, three chickpea varieties (Mastewal, Habru and one landrace with seven zinc fertilizer rates were laid out in factorial combination using Randomized Complete Block Design (RCBD) and replicated three times. A 3.2 m by 3.5 m long (11.2 m^2) gross plot size and 2.4 m by 3.5 m long (8.4 m^2) net plot size having 40 cm and 10 cm inter and intra raw spacing, respectively, was used. Zinc fertilizer ($ZnSO_4 .7H_2O$) was drilled in rows of experimental plots and mixed with soil using sticks before sowing the crop to manage seeds and fertilizer contact. Chickpea grains were tested for their viability for germination and were viable with germination of about 90%. Two chickpea grains placed per shallow hill of about 5 cm depth at 10 cm apart and covered manually with fine soil. Fifteen days after emergence, the extra plants thinned to maintain optimum population of 35 plants per row.

Data collection

Soil sample

Soil samples from a depth of 0-20 cm were collected using auger throughout the experimental field before filling pots and before sowing the crop, and mixed together as a composite sample to assess some physical and chemical properties including soil zinc content. The samples were air-dried, cleaned off any stones and plant residues, grounded in stainless steel soil grinder and allowed to pass a 2 mm sieve for analysis.

The pH (H_2O) of the soils was measured potentiometrically in the supernatant suspension of a 1:2.5 soil: water mixture by using a pH meter. Electoro-conductivity (EC) was determined using 1:5 soil: water ratio. Soil texture was analyzed by Bouyoucos hydrometer method. Soil total N was analyzed by wet-oxidation procedure of the Kjeldahl method (Bremner and Mulvaney, 1982). Available P was determined using Olsen strategy by extracting the soil sample with 0.5M sodium bicarbonate at pH 8.5 (Olsen and Sommers, 1982). Soil organic carbon was determined following the Walkley (1947) procedure. The soil Zn content was extracted with Diethylene Triamine Penta acetic Acid (DTPA) and determined by Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978).

Plant samples

Subsamples of grains and straws for determination of micronutrients content were taken randomly from the entire harvested lots of each of three replicated randomized pots and that of field plots. Each replicated grain and straw samples were prepared by a standard HNO_2 O_2 digestion method (Thavarajah *et al.,* 2009), using wet digestion with nitric acid followed by atomic absorption spectrometry. Zinc concentrations measured by this method were validated using NIST standard reference material 1573a. Red berry lentil grains and organic wheat (*Triticum aestivum L.*) were used as laboratory reference materials and measured periodically to ensure consistency in the method. The analysis was conducted at the University of Saskatchewan, SK, Canada.

Statistical analysis

Grain yield, zinc content in grain and straw data were subjected to analysis of variance using the GLM procedure of SAS computer package (SAS, 2012). Effects were considered significant in all statistical calculations if the P-values were less than or equals to 0.05. Means were separated using Fisher's Least Significant Difference (LSD) test.

Results and Discussions

Soil analysis

The results of present study on soil test for both pot and field soils showed pH values ranging from 6.38 at Taba to 6.83 at Choroko. Similarly, the DTPA extracted zinc content ranged from 0.15 mg kg^{-1} at Taba to 0.98 mg kg^{-1} at Choroko (Table 1). The critical Zn concentrations in soils vary from 0.48 mg kg^{-1} to 2.5 mg kg^{-1} depending on soil type (Ahlawat *et al.* (2007). Similar figures with the range reported by Ahlawat et al., 2007, soils have low Zn availability when there is less than to 1.1 mg Zn kg^{-1} soil (DTPA extraction) (Ankerman and Large, 1974). Thus, the study soils were zinc deficient.

Table 1: Main physical and chemical properties of soils used in the pot and before field planting

Characteristics	Method of analysis	Unit	Pot soil	Soils before planting (field experiments)		
				Jole	Taba	Choroko
Texture	Bouyoucos hydrometer		SCL	SCL	SL	CL
OC	Walkley-Black	g kg^{-1}	1.70	1.71	1.05	1.76
pH	1:2.5, water		6.77	6.80	6.38	6.83
EC	1:5, water	dS m^{-1}	0.21	0.20	0.06	0.09
P	Olsen	mg kg^{-1}	27.1	28.65	36.05	37.8
Total N	Kjeldahl	%	0.57	0.61	0.77	0.45
Zn	DTPA	mg kg^{-1}	0.17	0.18	0.15	0.96

SCL= Silty clay loam SL= silty loam, CL= clay loam, OC=organic carbon, pH=the negative logarithm of the hydrogen ion concentration, EC= electro conductivity, ZN=zinc.

Pot experiment

The effect of varieties, zinc rates and their interaction on grain yield, and straw zinc content was significant (Table 2). The variety Mastewal produced the highest grain yield (5.9 g pot^{-1}) and Habru produced highest straw zinc content (35.99 mg kg^{-1}). While, the landrace provided the highest (36.1mg kg^{-1}) grain Zn (Table 3).

Several authors reported that there are significant variations in seed zinc concentration among chickpea varieties (Akay, 2011; Shaban et al. 2012; Diapari *et al.,* 2014). Similarly, Legesse *et al.* 2017, reported in their study conducted to evaluate the response of 15 Chickpea cultivars to zinc application showed marked variation in grain zinc concentration, agronomic efficiency, zinc efficiency, growth, and yield among the cultivars.

Table2. Mean square values of Analysis of variance for the effect of zinc rate on grain yield, grain and straw zinc content of Chickpea genotypes

Source	df	Grain Yield	Grain Zinc	Straw Zinc
Rep	2	0.11**	2.48	26.12*
Var	2	3.92**	261.09**	743.25**
Znr	6	2.77**	11.20	52.75**
Var*Znr	12	2.68**	35.30**	60.19**
Error	40	0.81	6.44	7.45
Total	62			
CV%		16.64	7.74	8.62

df=degree of freedom, *=significant at P<0.05, ***=significant at P<0.01
Rep= replication, Var= variety, Znr=Zinc rate

Table 3. Effect of zinc rate on grain yield (g pot^{-1}), grain and straw Zn content (mg kg^{-1}) of chickpea varieties

Variety	Grain yield	Grain Zinc	Straw Zinc
Habru	5.10b	33.32b	35.99a
Mastewal	5.90a	29.05c	24.87c
Local	5.24b	36.05a	34.09b
$LSD_{5\%}$	0.56	1.58	1.70

Means in a column followed by the same letter are not significantly different (P>0.05) according to LSD test; LSD = Least significant difference

The effect of zinc fertilizer was irregular for the parameters tested (Table 4). However, 25 kg $ZnSO_4.7H_2O$ ha^{-1} produced the highest grain yield, grain and straw zinc content and is at par with the highest zinc rate in the experiment. Similarly, Valenciano et al (2010) conducted an experiment on "Response of chickpea (Cicer arietinum L.) yield to zinc, boron and molybdenum application under pot conditions" and reported that growth and yield characteristics were positively affected by the Zn application. Moreover, other authors also reported that the soil Zn application increased plant growth and at maturity plants that were fertilized with Zn had a greater total production of DM (Khan et al. 2000; Brennan et al. 2001).

Table 4. Effect of zinc rate (kg ha^{-1}) on grain yield (g pot^{-1}), grain and straw Zn (mg kg^{-1}) content of chickpea genotypes

Zn rate	Grain Yield	Grain Zinc	Straw Zinc
0	4.78c	34.02ab	28.53d
5	5.22bc	31.57c	29.11d
10	5.00bc	31.98bc	32.81abc
15	5.44bc	32.43abc	30.51cd
20	5.22bc	32.69abc	31.74bc
25	6.44a	34.64a	33.67ab
30	5.78ab	32.32abc	35.16a
$LSD_{5\%}$	0.86	2.42	2.60

Means in a column followed by the same letter are not significantly different (P>0.05) according to LSD test; LSD = Least significant difference

The interaction effect of varieties and zinc rates on grain yield, grain and straw zinc content was significant where 25kg ha^{-1} produced highest grain yield regardless of the varieties. However, Mastewal with 15 kg ZnSO4 gave 7g pot^{-1} was the exceptional (Fig.1). Whereas, the landrace produced highest grain zinc at all zinc fertilizer rates and the variety Habru exhibited highest grain zinc under no zinc fertilization (Fig.2). The variation in seed zinc concentration of the current chickpea varieties could be due to variation in seed physiology, morphology, and tissue zinc distribution which all are under genetic control (Moraghan *et al*,. 2005; Ariza- Nieto *et al.,* 2007).

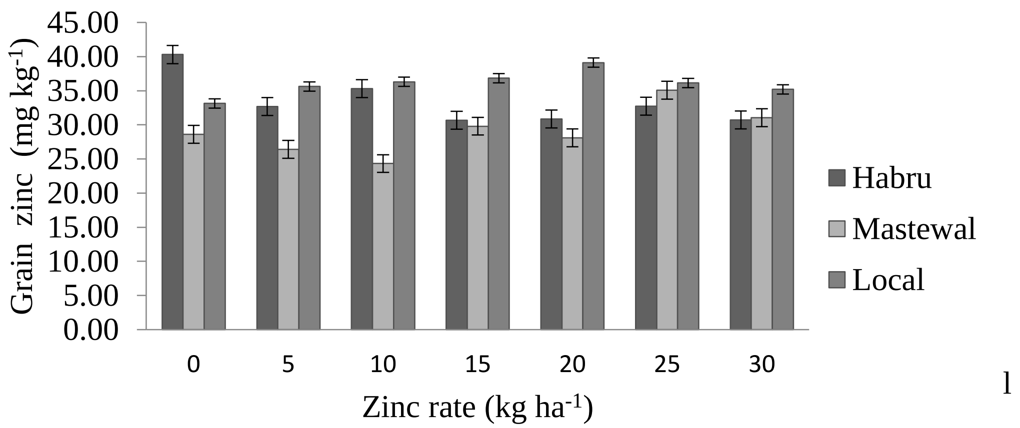

Figure 1. Effect of zinc fertilizer rate and chickpea variety interaction on grain yield (g pot^{-1})

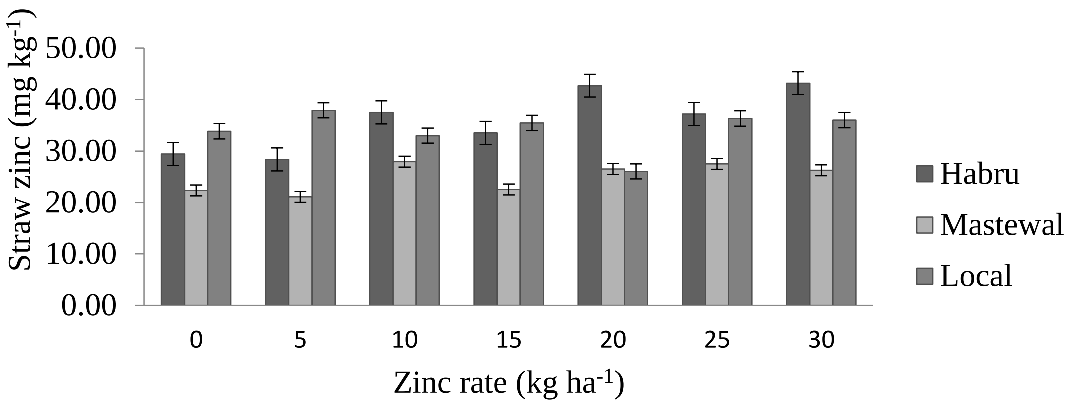

Figure 2. Effect of zinc fertilizer rate and chickpea variety interaction on grain zinc (mg kg^{-1})

As presented in Fig 3, varieties Habru and landrace responded better to all zinc rates while at 20 kg ha^{-1} the desi types yielded analogous to each other. Similarly, chickpea is generally considered sensitive to Zn deficiency, although there are differences in sensitivity to Zn deficiency between varieties (Khan, 1998; Ahlawat *et al.,* 2007).

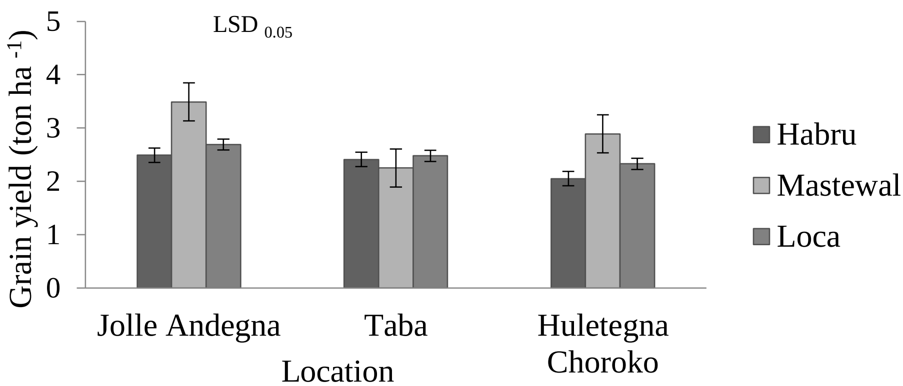

Figure 3. Effect of zinc fertilizer rate and chickpea variety interaction on straw zinc (mg kg^{-1})

Field Experiments

Despite chickpea grain yield responded significantly to different rates of Zn fertilizer under pot conditions, it was not confirmed under field condition (Table 5). Based on DTPA-extractable Zn, grain yield response to Zn application would not be expected at Choroko site because the average baseline level of DTPA-extractable Zn at this site was 0.96 mg kg^{-1} soil, which is above the minimum critical threshold of soil Zn concentration. Nevertheless, the baseline of DTPA-extractable Zn measured at Taba (0.15 mg kg^{-1}) and Jolle (0.18 mg kg^{-1}) were below the critical threshold; yet, application of $ZnSO_4.7H_2O$ failed to produce an improvement in chickpea yield. Similarly, Khan *et al.* (1998) conducted an experiment on 13 chickpea varieties, and reported that, the Zn application created different effects on the varieties regarding the dry matter production of the aerial parts. Although dry matter production maintained an increase in some varieties, it did not cause a significant change on grain yield in certain others at the end of the growing period. In the same way, Hatice *et al.* (2007) demonstrated chickpea grain yield increase with increasing rate of Zn application, but not statistically significant. Akay (2011) also reported that application of Zn did not give a significant increase in yield of chickpea varieties. Soil application of Cu, Zn, and Mo is more efficient than Mn and Fe fertilization, on most soils, but all transition metal nutrients are not readily translocated within plants on deficient soil (Yilmaz *et al.* 1998). Moreover, Ullah, *et al.* (2018), reported that application of P and Zn and their dose adjustment require special techniques and knowledge of the behavior of each nutrient in soil in presence of the other. For instance, better results with regards to number of pods $plant^{-1}$ at medium levels of P and Zn in the study points out their better adjustment to subside their mutual antagonism at these levels, which at the highest levels of one nutrient and being antagonistic would, otherwise, have reduced the availability of the other and decreased the growth and yield of the crop, resulted to the dilution effect of soil Phosphorus content (Wijebandara , 2007).

Table 5. ANOVA for grain yield, grain and straw Zn content response of chickpea varieties to zinc fertilizer rates

Sources of variation	df	Grain yield	Grain zinc	Straw Zinc
Loc (Location)	2	10.26	9065**	3457
Var	2	10.23	48	1297*
Loc*Var	4	4.86*	8	232*
Znr (zinc rate)	6	0.29	320**	51
Loc*Znr	12	0.28	36	29
Var*Znr	12	0.18	9	30
Loc*Var*Znr	24	0.33	5	20
CV%		6.71	9.25	7.85

df=degree of freedom, *=significant at P<0.05, ***=significant at P<0.01

The interaction effect of varieties and location on grain yield was significant (Table 5). Mastewal produced significantly superior grain yield at Jolle and Choroko. This variety had 40 and 30%; 41 and 24 % grain yield advantage over the varieties Habru and landrace at the specified locations, respectively (Fig.4).

Several authors reported that crop response to Zn is positively depends on crop type. For example, improved yield have been observed in rice (Shivay et al. 2008), corn (Singh et al. 1979), and wheat (Cakmak et al. 1999) grown on soils ranging in pH 7.2-8.8 and initial DTPA-extractable soil levels of 0.01-0.78 mg Zn kg-1 when soil applied ZnSO4 had been broadcast and incorporated ranging from 5 to 23 kg Zn ha^{-1}. However, Singh et al. (1987) reported that no significant yield response in several dryland annual crops from Zn fertilizer application. On the other hand, grain Zn content showed significant response to Zn application (Table 5). The highest grain Zn content of 39.55 mg kg^{-1} was obtained from the application of 25 kg $ZnSO_4.7H_2O$ ha^{-1}. This is at par with 30 kg $ZnSO_4.7H_2O$ ha^{-1} from field experiments (Table 6).

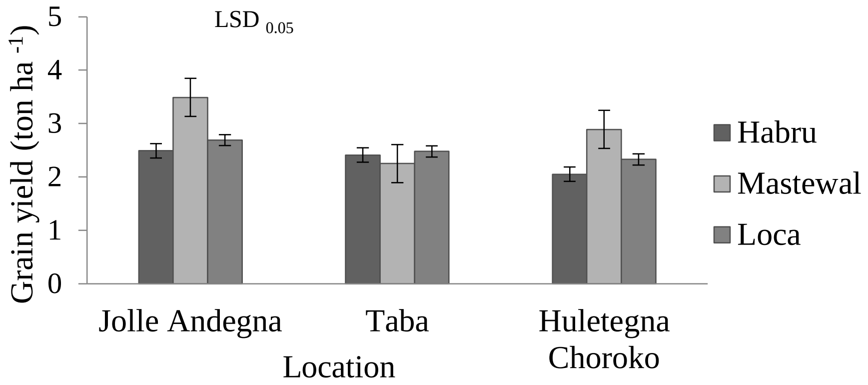

Figure 4. Effect of location and variety interaction on grain yield (ton ha^{-1}) of chickpeas

As presented in Table 5, there was highly significant ($P<0.01$) difference in grain zinc content observed among locations. Choroko produced 46.6% more grain zinc content than both Taba and Jolle (Table 6). Similarly, Valenciano *et al.* 2010 reported that, the environmental conditions during experiments affected the plant's response differently and there were significant differences between environments (soils). Application of 25 kg $ZnSO_4.7H_2O$ ha^{-1} significantly ($P<0.05$) improved grain Zn content to 39.55 mg kg^{-1} compared to 37.05 mg kg^{-1} under no zinc treatment. A higher rate (30 kg ha^{-1}) of $ZnSO_4.7H_2O$ did not increase grain Zn content any further.

Table 6. Effect of location, variety and Zn fertilizer rates on grain yield, grain and straw zinc contents

Treatments		Grain yield ($t\ ha^{-1}$)	Grain Zn ($mg\ kg^{-1}$)	Straw Zn ($mg\ kg^{-1}$)
Location	Jolle	2.89	31.75b	22.14
	Taba	2.38	31.64b	16.32
	Choroko	2.42	46.39a	26.77
	$LSD_{5\%}$	NS	0.84	NS
Variety	Habru	2.34	36.11	24.96a
	Mastewal	2.88	36.38	18.55c
	Local	2.50	37.29	21.72b
	$LSD_{5\%}$	NS	NS	0.42
Zn (kg $ZnSO_4.7H_2O$ ha^{-1})	0	2.52	37.05b	20.63
	5	2.67	37.54b	20.48
	10	2.60	34.20d	23.24
	15	2.63	33.11d	22.15
	20	2.46	35.52c	21.82
	25	2.54	39.55a	21.57
	30	2.53	39.18a	22.31
	$LSD_{5\%}$	NS	1.28	NS

Means in a column followed by the same letter are not significantly different (P>0.05) according to LSD test; LSD = Least significant difference

The effect of location on straw zinc content of chickpea was not significant. However, the effect of variety and location by variety interaction exhibited significant ($P<0.05$) influence on chickpea straw zinc content (Table 5) where, Habru had superior across location followed by land race except at Choroko where the variety Mastewal found to be better than Landrace (Fig. 5).

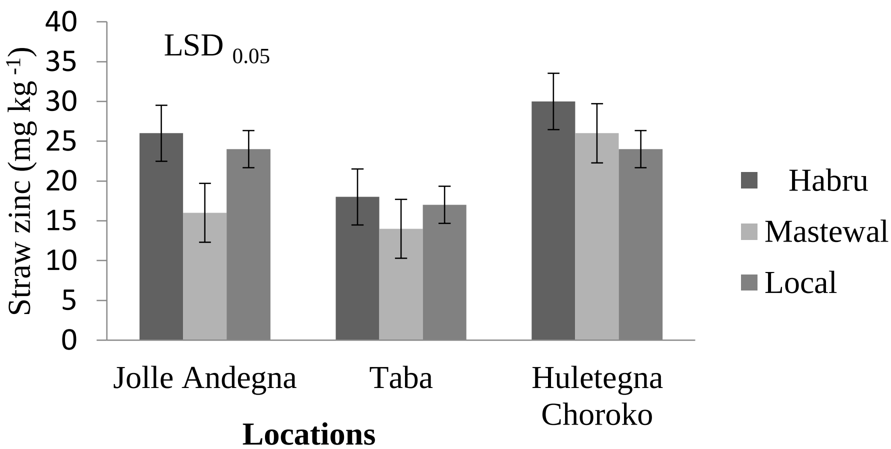

Figure 5. Effect of location and variety interaction on straw zinc content ($mg\ kg^{-1}$) of chickpeas

Conclusion and Recommendation

Pot experiment result indicated significant grain yield, grain and straw zinc content difference among varieties. The variety Mastewal produced the highest grain yield, while Habru produced highest straw zinc content and the landrace provided the highest grain Zn. Similarly, the effect of zinc rate exhibited significant influence on grain yield and straw zinc content. The highest chickpea grain yield and straw zinc content obtained from the application of 25 kg $ZnSO_4.7H_2O$. Likewise, the highest grain Zn content obtained from the application of 25 kg $ZnSO_4.7H_2O$ ha^{-1} with either of the varieties and this is at par with 30 kg $ZnSO_4.7H_2O$ ha^{-1} from field experiments. The results of present studies confirmed possibilities of chickpea bio-fortification through zinc fertilizer application. Moreover, improvement of chickpea grain zinc through zinc fertilizer application is an attractive option in solving zinc deficiency-related health problems for resource poor farmers who cannot afford fortified foods for their nutrition security. However, Zinc fertilization alone may not improve the productivity of chickpea in the study areas may be due to the prevalence of other limiting elements, which needs further research on the effects of Zn in conjunction with other micronutrients.

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