Effects of zinc application strategy on zinc content and productivity of chickpea grown under zinc deficient soils

Legesse Hidoto^{1,2}, Walelign Worku^{2*}, Hussein Mohammed² and Bunyamin Taran³

¹*Southern Agricultural Research Institute, P. O. Box 6, Hawassa, Ethiopia.* ²*Hawassa University, School of Plant and Horticultural Sciences, P. O. Box 5, Hawassa, Ethiopia.* ³*Crop Development Centre, Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada.*

*Corresponding author: walelignworku@yahoo.co.uk

Abstract

Field experiments were conducted at three locations with Zn deficient soils in southern Ethiopia during 2012 and 2013 cropping seasons to evaluate the effects of Zn fertilization strategies and varietal differences on Zn content and plant performance of chickpea (*Cicer arietinum* L.). Factorial combinations of three Zn fertilization strategies and five varieties were laid out in a randomized complete block design with three replications in each location and year. A combined analysis of variance was made using a mixed effects model. Zinc foliar application increased grain Zn content by 21 and 22% over Zn soil application and seed priming, respectively. The improvements were around four folds for straw Zn content for the same comparisons. Effects of Zn application strategies on grain and straw Zn contents were consistent across locations. The grain Zn concentration varied among the varieties ranging from 34 mg kg⁻¹ for Mastewal to 42 mg kg⁻¹ for the Landrace and variety Arerti. Zn application strategies did not affect the growth and yield parameters, except for pod bearing branches. Foliar Zn application and appropriate variety selection are potential approaches for Zn biofortification in chickpea. Further study aimed at identifying most effective spray timing for maximum grain quality response would be worthwhile to cut fertilizer and application costs.

Keywords: Zinc fertilizer, application strategy, varieties, biofortification, chickpea

1. Introduction

Chickpea is an important pulse crop widely used for food and fodder throughout the world. Among all pulse crops, the world's chickpea consumption is second only to dry beans and has shown an increase of 33.5% in area and 65.5% in volume of production since 2008 (FAOSTAT, 2015). Ethiopia is the largest producer of chickpea in Africa accounting for about 46% of the continent's production between 1994 and 2006 (MoA, 2014). The country is also the fifth largest producer of chickpea worldwide (FAO-STAT, 2015). During the period of 1996–2005, total acreage, production and productivity of chickpea in Ethiopia had increased by 41%, 217% and 122%, respectively (CSA, 2015).

Chickpea is high in protein, low in fat and sodium, cholesterol free and is an excellent source of both soluble and insoluble fiber, complex carbohydrates, vitamins, folate, and minerals especially calcium, phosphorus, iron, zinc and magnesium (Nwokolo and Smartt, 1996). These facts have made chickpea a potential staple food to help reduce micronutrient deficiencies in humans globally. Micronutrient deficiency also known as hidden hunger is a worldwide problem prevalent on more than half of the world population, particularly in developing countries (Mayer *et al.*, 2008) affecting mostly women, infants, and children (Kaya *et al.*, 2009). Hotz and Brown (2004) reported that Zn deficiency affects, on average, one-third of the world population, ranging from 4 to 73% in different countries. Low dietary intake of Fe and Zn appears to be the major reason for the widespread prevalence of Fe and Zn deficiencies in human populations (Cakmak, 2010).

Ethiopia has one of the highest rates of malnutrition in the world, which comprises about 52% of the country's rural population particularly children and women who fail to meet minimum requirements for calories

(CIFSRF, 2012). 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 (UNICEF, 2014). 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. In countries with high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet (Cakmak, 2008).

Effective biofortification strategies need to be identified and implemented in order to enrich the micronutrient content in the staple crops especially in developing countries. Fertilizer application is one of the agronomic approaches that can help to enhance the nutrition security through improving the quality of grains in addition to its role in increasing productivity (Pathak *et al.*, 2012; Márquez-Quiroz *et al.*, 2015). Soil zinc deficiency is common in most of the chickpea growing areas of the world (Khan *et al.*, 2000). Zinc deficiency is not only the cause of low productivity of the crops, but it also results in low Zn concentration in seeds, which leads to poor dietary Zn intake when consumed (Pathak *et al.*, 2012). Concentration of micronutrients in grains has been improved with varying applications of micronutrients in various pulses (Brennan *et al.*, 2001; Mohammad *et al.*, 2011; Pathak *et al.*, 2012). However, fertilizer delivery methods may vary in convenience, effectiveness and cost. Among many others, foliar spray, soil application and seed treatment are found to be effective application practices for some micronutrients (Melash *et al.*, 2016). Each method has the potential to influence the extent of micronutrient concentration both in the treated plant directly and indirectly through enrichment of the seeds by micronutrient treatment of the

parent (Johnson *et al.*, 2005). Much of the research on crop responses to foliar fertilization was done on soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.) (Fageria *et al.*, 2009) and rice (*Oryza sativa* L.) (Guo *et al.*, 2016) with only limited studies on chickpea. Foliar Zn application increased grain and straw Zn concentration in chickpea (Pathak *et al.*, 2012). Soil Zn application also had positive effect on grain Zn concentration (Kaya *et al.*, 2009), and grain and shoot Zn contents in chickpea (Shivay *et al.*, 2014). On the other hand, soil fertilization with Zn has no effect on Zn concentration in chickpea and lentil (*Lens culinaris* Medik.) seeds (Johnson *et al.*, 2005). Seed hydro-priming with Zn increased chickpea Zn concentration in seeds (Arif *et al.*, 2007; Harris *et al.*, 2008). In contrast, Johnson *et al.* (2005) reported that priming seeds with Zn solution had no effect on micronutrient content of the progeny seeds in chickpea and lentil, and even prevented germination of chickpea in one year. In addition to the inconsistency in the reported results, no study has been done to compare the response of chickpea or other pulses to different Zn application strategies.

Crop responses to micronutrient fertilization in terms of productivity has been mixed. In chickpea, grain yield increased with the application of Zn through foliar spraying (Pathak *et al.*, 2012), soil application (Kaya *et al.*, 2009) and by seed priming (Harris *et al.*, 2008). On the other hand, Johnson *et al.* (2005) did not find grain yield improvement neither from Zn seed priming nor from soil Zn fertilization in chickpea and lentil. In summer maize (*Zea mays* L.) Zn applied as both basal and foliar spray increased the grain yield with application rates varying between locations (Liu *et al.*, 2016).

In general, the micronutrient content of crops has received less attention and crop selection took place with little or no regard for the mineral and other micronutrient components of the seeds that may cover a

very large part of the human diet (Ray *et al.*, 2014). Variations in Zn concentration and discrepancies in the extent of response to Zn application have been observed among chickpea genotypes (Khan *et al.*, 2000; Bueckert *et al.*, 2011; Diapari *et al.*, 2014; Ray *et al.*, 2014). For instance, Bueckert *et al.* (2011) observed that in addition to differences in grain Zn concentration among individual genotypes, the kabulis have superior Zn concentration than the desi types. Potential variations in seed Zn concentration and crop performance in response to Zn application methods among the desi and kabuli chickpea genotypes currently under production in Ethiopia have not been examined. Thus, the objectives of the study were: 1) to compare Zn fertilizer application strategies on chickpea grain and straw Zn concentrations and the overall crop productivity and 2) to examine variability among the desi and kabuli chickpea varieties for grain and straw Zn concentration and their possible variation in response to Zn application strategies.

2. Materials and Methods

2.1. Description of the study areas

Field experiments were conducted during the 2012 and 2013 cropping seasons at three locations with Zn deficient soils in the selected administrative districts of Southern Ethiopia. These locations were Huleteegna Choroko Kebele at Halaba district, Taba Kebele at Damot Galle district and Jolle Andegna Kebele at Meskan district. The location coordinates for the three sites are 07° 20' 34" N and 038° 06' 30" E for Huleteegna Choroko, 07° 01' 01.9" N and 037° 53' 57" E for Taba and 08° 12' 25.9" N and 038° 28' 33.2" E for Jolle Andegna. The elevations of the sites are 1807, 1915 and 1923 metres above sea level for Huleteegna Choroko, Taba and Jolle Andegna, respectively. The sites receive an average annual rainfall of 774, 989 and 922 mm in that order.

The mean maximum and minimum temperatures for Huletegna Choroko, Taba and Jolle Andegna were 28.6 °C and 13.6 °C, 24.9 °C and 13.6 °C, and 27.1 °C and

10.7 °C, respectively. Soil properties of the experiment sites and weather information during the crop growth period are presented in Tables 1 and 2, respectively.

Table 1. Soil physico-chemical properties of the experimental sites at Jolle Andegna, Taba and Huletegna Choroko in 2012 and 2013.

Location	Year	pH	EC (mmho cm ⁻¹)	Zn (mg kg ⁻¹)*	Soil Zn status*	OC (%)	Total N (%)	Available P (mg kg ⁻¹)	Texture class
Jolle Andegna	2012	6.77	0.20	0.17	Very low	1.71	0.57	27.1	SCL
	2013	6.82	0.22	0.19	Very low	1.70	0.65	30.2	SCL
Taba	2012	6.36	0.05	0.13	Very low	0.99	0.71	36.5	SL
	2013	6.40	0.07	0.16	Very low	1.10	0.83	35.6	SL
Huletegna Choroko	2012	6.73	0.08	0.98	Low	1.78	0.44	37.6	CL
	2013	6.93	0.09	0.94	Low	1.73	0.46	38.0	CL

*, Soils have low Zn availability when there is less than to 1.1 mg Zn kg⁻¹ soil (DTPA extraction) (Ankerman and Large, 1974); SCL, silt clay loam; SL, silt loam; CL, clay loam.

Table 2. Monthly total rainfall (mm) during crop growth period (August to December) of 2012 and 2013 and long term average between 2000-2011.

Location	Year	Rainfall (mm)					
		August	September	October	November	December	Total
Jolle Andegna	2012	143.9	88	15.8	8.8	3.5	260
	2013	164.7	48.1	50.1	33.5	2.7	299.1
	2000-2011	207.5	113.7	48.4	24	8.3	401.9
Taba	2012	169.7	135.3	13	32.6	16.8	367.4
	2013	178.4	210.2	149.7	97.3	2.3	637.9
	2000-2011	152.6	134.3	69.5	48.8	41	446.2
Huletegna Choroko	2012	89.5	100.7	10	9	2.6	211.8
	2013	178.1	138.8	118.9	60.3	0.2	496.3
	2000-2011	121.81	114.1	53.7	42.1	21	352.71

Source: National Meteorology Agency, Addis Ababa, Ethiopia (2013).

2.2. Experimental treatments, design and management

The experiment was based on factorial combinations of three Zn application strategies and five chickpea varieties. Zinc fertilizer application strategies consisted of foliar application, soil application and seed priming. The five chickpea varieties were composed of four improved varieties that were released between 1999 and 2007 and one local landrace. The improved varieties consisted of two desi types (Mastewal and Naatolii) and two Kabuli types (Arerti and Habru) while the land race was a desi type. The treatment combinations were arranged in a randomized complete block design with three replications. The improved varieties were previously tested for performance and were selected to expand chickpea production in southern Ethiopia. The foliar application was carried out by spraying 0.5% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solution three times: at early vegetative stage, at early flowering and at pod filling. The amount of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ fertilizer used for each spray was 8.93 kg ha^{-1} . For soil Zn application, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at the rate of 25 kg ha^{-1} was drilled in rows and incorporated into soil before the sowing of chickpea. Seed priming involved soaking of chickpea seeds in 0.05% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.45 kg ha^{-1}) solution for 12 hours and drying the seeds under shade for ease of handling before sowing.

The chickpea seeds were sown at 40 cm between rows and 10 cm between plants spacing. Sowing took place at the end of the main cropping season: at Huletegna Choroko on 8 September 2012 and 23 August 2013, at Jolle Andegna on 20 September 2012 and 4 September 2013 and at Taba on 14 September 2012 and 17 September 2013. A week after emergence the seedlings were thinned to maintain a density of 25 plants m^{-2} . Gross and net plot sizes for the experiments were 11.2 and 8.4 m^2 , respectively. Weeds were controlled by hand weeding and

cultivation as frequently as required. All agronomic practices other than the treatment factors were kept uniform across all treatments.

2.3. Data collection

Grain and straw Zn concentration analyses were conducted at the University of Saskatchewan, Saskatoon, Canada. Subsamples of grains and straws (25 g) for determination of Zn concentration were taken randomly from the entire harvested plot of each of the three replications at each location. The samples were oven dried at 70°C for 24 hours and a ground sample of 0.5 g was weighed on a balance sensitive to the nearest 0.00001 g . The grain and straw samples from each replication were prepared by a standard HNO_3 – H_2O_2 digestion method (Thavarajah *et al.*, 2009) using wet digestion with nitric acid. Zinc concentration was measured by flame Atomic Absorption Spectrometry (AJ ANOVA 300, Lab Synergy). Zinc concentrations measured by this method were validated using NIST standard reference material 1573a. Lentil (*Lens culinaris*) seeds (cv, CDC Redberry) and organic wheat (*Triticum aestivum*) seeds were used as laboratory reference materials and were measured periodically to ensure consistency in the method.

Plant height, number of pod bearing branches and number of pods plant⁻¹ were recorded from 10 randomly selected plants of the middle six rows in each plot. Above ground biomass and grain yield were measured from a harvest made on the middle six rows ($2.4 \text{ m} \times 3.5 \text{ m} = 8.4 \text{ m}^2$) of each plot at ripening. Grain yield was adjusted to storage moisture content of 10% based on the value of actual grain moisture read by digital grain moisture tester (Hoh-Express HE-50, Pfeuffer, Germany). Grain weight was determined by counting 250 grains randomly from each plot and converting it to 1000 grain weight after adjusting to storage moisture content. Grain Zn yield

ha⁻¹ was calculated based on grain Zn concentration (mg kg⁻¹) and grain yield (t ha⁻¹).

Fifteen soil samples from a depth of 0-30 cm were randomly collected before sowing across each experimental site using an auger to assess the physical and chemical properties including soil Zn concentration. The collected soil samples were mixed together on location basis, air dried, cleaned off any stones and plant residues and were grinded in stainless steel soil grinder and allowed to pass a 2 mm sieve. The soil Zn concentration was extracted with DTPA and determined by Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978). Available P was determined 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. Details of the soil properties for the experimental fields are shown in Table 1.

2.4. Data analysis

Prior to combined analysis of variance, separate analysis of each location during 2012 and 2013 was carried out using general linear model of SAS software (SAS, 2008). The data were tested for homogeneity of error variance. The result of this test revealed that grain yield, above ground biomass and straw Zn content were heterogeneous. The regression approach has indicated that Log transformation was the appropriate method. Grain yield and above ground biomass were transformed using log (data+1), while log (data) was applied for straw Zn concentration (Gomez and Gomez, 1984). All data were subjected to combined analysis of variance using the mixed effects model (SAS, 2008) where year and replication were considered random effects while location, variety and Zn application strategy were assigned as fixed effects. Differences among treatment means were compared using least significant difference (LSD) test at 5%

whenever effects were found significant at $P < 0.05$ from the analysis of variance.

3. Results

3.1. Weather

Total precipitation during the growing season (August–December) of 2013 was much more than either 2012 at all locations or the long term average at the two locations (Table 2). For instance, the total rainfall for the growing season at Huletegn Choroko was greater by 285 mm and 244 mm compared to 2013 and the long-term amounts, respectively. Particularly, rainfall in October and November at Jolle Andegna in 2012 was meagre with amounts about one third of the long-term averages and 31% to 26% of 2013. The amount of rainfall during the growing season in 2013 was greater by 15, 74 and 134% at Jolle Andegna, Taba and Huletegn Choroko as compared to the year 2012, respectively. The long term rainfall also exceeded that of 2012 at Taba (43%) and Huletegn Choroko (14%) but was lower at Jolle Andegna by 34%. Moreover, the distribution of rainfall during the 2012 growing period was uneven with most of the days without rain and some days with less than 5 mm precipitation. Relatively high and evenly distributed rainfall in 2013 meant longer crop growth duration leading to larger biomass accumulation and higher grain yield compared to 2012. Location wise, Taba received the highest amount of rainfall (367 mm) in 2012 followed by Jolle Andegna (260 mm) and Huletegn Choroko (212 mm).

In 2013, all sites had adequate rainfall except Jolle Andegna (299 mm). In spite of better rainfall amount at Taba, chickpea yields were relatively low because of poor rainfall distribution in 2012 and excessive initial moisture followed by extended rainfall in 2013.

3.2. Grain and straw Zn concentrations and grain Zn yield

Zinc application strategy significantly influenced both grain and straw Zn concentrations (Table 3). Zinc foliar application resulted in the highest grain and straw Zn content as compared to both soil application and seed priming and this effect was consistent across locations. Accordingly, Zinc foliar application

had 21 and 22% grain Zn content advantage over soil application and seed priming, respectively (Table 4). Moreover, foliar application resulted in 383 and 437% increase in straw Zn content over soil application and seed priming methods, respectively. Grain Zn yield of chickpea also varied significantly among application strategies (Table 3) with foliar application having the highest grain Zn yield of 85 g ha⁻¹.

Table 3. Mean square values for combined analysis of variance over locations and years for grain and straw Zn concentration, grain Zn yield, grain yield, thousand grain weight, and pods per plant.

Source	DF	Grain Zn	Straw Zn ^a	Grain Zn yield ^a	Grain yield ^a	TGW	Pods per plant
Strategy (S)	2	1905*	246764**	0.32*	0.003	54	524
Variety (V)	4	563 *	802	0.20	0.066	230114*	7359
Location (L)	2	1485	4566	2.39	0.536	67096	25145
S*V	8	27	1000	0.02	0.005	165	354
S*L	4	17	1230	0.01	0.002	2	310
V*L	8	83	1514	0.09	0.029*	21821	3023
S*V*L	16	18	1103	0.01	0.004	234	346
CV%		21.5	(19.2)	(20.2)	(25.8)	7.5	23.3

DF, degree of freedom; *, Significant at 5% level; **, significant at 1% level; TGW, thousand grain weight; a, Log transformed data.

Grain Zn content also varied significantly among the varieties (Table 3) ranging between 34 and 42 mg kg⁻¹ (Table 4). The Local landrace and variety Arerti had 19% more grain Zn than variety Mastewal, which had the lowest Zn content (34 mg kg⁻¹) of all tested varieties (Table 4). Unlike grain Zn content, the variation in straw Zn content and grain Zn yield among varieties

were not significant (Table 3). Location effects and their interactions were not significant for grain and straw Zn concentrations and grain Zn yield in spite of a wide variation in the later (Table 3). This was due to a highly significant location by year interaction effects against which the trait was tested for significance.

Table 4. The effect of Zn application strategies and variety on grain and straw Zn concentrations, grain Zn yield, grain yield, grain weight and pods per plant of chickpea.

Treatments	Grain Zn (mg kg ⁻¹)	Straw Zn (mg kg ⁻¹) ^a	Grain Zn yield (g ha ⁻¹) ^a	Grain yield (ton ha ⁻¹) ^a	1000 grain wt (g)	Pod no. plant ⁻¹
Strategy						
Soil appl.	36.58b	23.32 (1.28b)	71 (1.68b)	1.88 (0.41)	250	63
Seed priming	36.24b	21.02 (1.26c)	68 (1.68b)	1.84 (0.41)	251	68
Foliar appl.	44.37a	112.80 (2.02a)	85 (1.78a)	1.94 (0.42)	251	64
SE±	0.91	1.45 (0.01)	9.08 (0.01)	0.02 (0.001)	1.58	2.79
LSD _{5%}	2.47	(0.01)	(0.05)	NS	NS	NS
Variety						
Arerti	41.55a	55.86 (1.57)	80 (1.74)	1.95 (0.41)	251d	65
Habru	37.64b	50.83 (1.52)	65 (1.65)	1.69 (0.38)	303a	58
Mastewal	34.11c	51.89 (1.47)	75(1.71)	2.08 (0.45)	273c	67
Naatolii	40.19ab	47.02 (1.49)	83(1.80)	2.06 (0.45)	286b	52
Local landrace	41.87a	56.38 (1.55)	70(1.67)	1.66 (0.37)	139e	82
SE±	1.14	4.98(0.04)	6.42(0.04)	0.14 (0.02)	24.00	5.38
LSD _{5%}	3.19	NS	NS	NS	7.17	NS
Location						
JolleAndegna	34.42	43.22 (1.33)	75 (1.61)	2.12 (0.41)	245	78
Taba	40.76	55.52 (1.62)	49 (1.64)	1.30 (0.34)	280	46
Huletegn Ch.	42.00	55.62 (1.62)	100 (1.91)	2.25 (0.50)	226	71
SE±	6.22	7.95 (0.10)	33.56 (0.26)	0.82 (0.12)	47.44	27.30
LSD _{5%}	NS	NS	NS	NS	NS	NS

Means with the same letter are not significantly different at 5% level; NS, not significant; a, transformed values are given in parenthesis.

3.3. Grain yield, seed weight and pods per plant

Zinc fertilizer application strategy did not influence grain yield, seed weight and pods per plant (Table 3). Similarly, there was no significant difference among varieties for these traits except seed weight. The variety Habru had the largest seed weight compared to all other varieties; it has more than twofold larger seeds than the Local landrace (Table 4). The improved varieties of both kabuli and desi types had greater seed size than the Local landrace.

The location effect was not significant on any of the traits (Table 3). However, there was a significant variety by location interaction for grain yield. The interaction indicated that the two improved desi types, Mastewal and Naatolii, were particularly superior at Taba (Figure 1, A). These varieties also performed well at Jolle Andegna, which were at par with variety Arerti, a kabuli type. At Huletegn Choroko, the kabuli type Arerti was superior while the rest had similar productivity.

Figure 1. The location by variety interaction effects on (A) grain yield and (B) total biomass of chickpea.

3.4. Plant height, pod bearing branches, above ground biomass and harvest index

None of the traits were significantly influenced by Zn application strategies or varietal differences except number of pod bearing branches which varied significantly among varieties (Table 5). The varieties Arerti (kabuli), Mastewal and Local landrace (desi) produced greater number of pod bearing branches (Table 6). However, this did not translate into higher number of pods except the Local landrace, which had the highest number of pods plant⁻¹. Performance for these traits was also similar except for a location by variety interaction for above ground biomass (Figure 1, B). The interaction showed that the improved desi type, Mastewal and Naatolii, had better performance at Taba, while the differences were not significant for the kabulis in the other two sites (Figure 1, B).

The trends expressed for total biomass were more or less similar with that of grain yield due non significant variation for harvest index among the varieties.

3.5. Correlation and group comparison

Grain yield did not show significant correlation either with grain Zn content ($r=0.06$) or straw Zn content ($r=-0.07$). Also, grain size did not significantly associate either with grain Zn ($r=0.00$) or straw Zn ($r=0.07$) concentrations. On the other hand, grain Zn concentration had a positive and significant correlation with straw Zn content ($r=0.41$; $P<0.01$). The Local landrace had greater grain and straw Zn concentrations than the mean of improved genotypes though the difference was significant only for the former (Table 7). On the other hand, the kabulis and the desis had similar grain and straw Zn content as a group.

Table 5. Mean square values for combined analysis of variance over locations and years for plant height, number of pod bearing branches, above ground biomass and harvest index.

Source	DF	Plant height	Pod bearing branches	Above ground biomass ^a	Harvest index
Strategy (S)	2	26	8	0.001	0.007
Variety (V)	4	418	211**	0.09	0.088
Location (L)	2	2787	4516	1.287	0.287
S*V	8	16	8	0.026	0.010
S*L	4	13	16	0.000	0.003
V*L	8	42	42	0.024*	0.015
S*V*L	16	9	8	0.003	0.008
CV%		9.3	25.5	25.3	14.3

DF, degree of freedom; *, significant at 5% level; **, significant at 1% level; a, Log transformed data.

Table 6. Effects of Zn fertilizer application strategy and variety on plant height, number of pod bearing branches, above ground biomass and harvest index

Treatments	Plant height (cm)	Pod bearing branch no.	Above ground biomass (ton ha ⁻¹) ^a	Harvest index
Strategy				
Soil appl.	48.2	17	4.86 (0.68)	0.42
Seed priming	47.8	17	4.76 (0.67)	0.43
Foliar appl.	48.9	16	4.87 (0.67)	0.44
SE±	0.15	0.79	0.07(0.01)	0.01
LSD _{5%}	NS	NS	NS	NS
Variety				
Arerti	48.4	18a	5.39 (0.69)	0.39
Habru	52.3	15b	4.86 (0.67)	0.39
Mastewal	45.2	17a	4.74 (0.70)	0.48
Naatolii	49.4	14c	4.49 (0.70)	0.45
Local landrace	46.2	18a	3.84 (0.60)	0.45
SE±	1.48	0.43	0.45(0.02)	0.02
LSD _{5%}	NS	1.60	NS	NS
Location				
Jolle Andegna	49.2	21	4.66 (0.61)	0.50
Taba	42.3	8	3.35 (0.61)	0.40
Huleteegna Choroko	53.4	20	6.48 (0.81)	0.40
SE±	4.37	3.47	1.84(0.15)	0.06
LSD _{5%}	NS	NS	NS	NS

Means with the same letter are not significantly different at 5% level; NS, not significant; a, transformed values are given in parenthesis.

Table 7. Contrast means for grain and straw Zn concentrations.

Component	Kabul vs	Desi	Improved vs	Local
Grain Zn content (mg kg ⁻¹)	38.72a	39.60a	38.37b	41.87a
Straw Zn content (mg kg ⁻¹)	53.34a	51.76a	51.4a	56.38a

Contrast means with same letter are not significantly different at 5% level

4. Discussion

Grain and straw Zn content of chickpea were significantly affected by Zn fertilizer application strategies. The results were consistent across locations. Foliar Zn fertilizer application resulted in significantly higher grain and straw Zn content as compared to soil application and seed priming. Similarly, Pathak *et al.* (2012) observed that there was an increase in Zn content in the grains and leaves of chickpea after foliar Zn application to Zn deficient as well as Zn sufficient plants. Also, Alloway (2004) observed that foliar application of Zn led to an increase in Zn content in both the grain and the vegetative parts of the plants. Application of micronutrients by foliar spray was more effective because of the small amounts required, more uniform application and efficiency of uptake by the plants compared to soil fertilization (Fageria *et al.*, 2009). Mousavi (2011) also indicated that foliar application of micronutrients was more suitable than soil application due to the rapid availability, ease of use and reduced toxicity that could be caused by accumulation and element stabilization in the soil. Moreover, nutrient availability to plant from soil application could be reduced due to binding in the soil and restricted uptake when moisture is limiting (Yadegari, 2016). On the other hand, the need for repeated foliar applications due to poor mobility of micronutrients led to increased expenditure and extended application time.

Soil Zn application and seed Zn priming did not influence either grain or straw Zn concentration. Other studies have also indicated that seed priming with Zn may not be effective in fulfilling Zn requirements of different crops. For example, soil fertilization with Zn has no effect on Zn content of chickpea and lentil seeds (Johnson *et al.*, 2005). Moreover, Johnson *et al.* (2005) reported that Zn primed seeds have no effect on micronutrient contents of the progeny seeds in chickpea and lentil and hampered germination of chickpea in one year. On the other hand, soil Zn application had a significant positive effect on grain Zn concentration (Kaya *et al.*, 2009) and grain and shoot Zn content (Shivay *et al.*, 2014) in chickpea. Also, seed hydro-priming with Zn increased chickpea Zn content (Harris *et al.*, 2008; Arif *et al.*, 2007).

In this study, Zn concentration in the straw was highly enriched by foliar application when compared to the grain Zn owing to the mechanism of ion uptake. Zinc from foliar application penetrates the cuticle and the cellulose wall via limited or free diffusion (Franke, 1967) and ions are also absorbed through the stomata on the leaves (Eichert and Burkhardt, 2001). The altered subcellular compartmentation of Zn in the shoot enhances more efficient biochemical utilization of Zn in cells of the shoot while only part of the nutrient can be translocated from the shoot to the grain (Franke, 1967). Also, most of the micronutrients mobility in plant tissues is poor unlike macronutrients (Fageria

et al., 2009). Straw obtained from crops grown on nutrient deficient soils may also be deficient with the same nutrients (Nube and Voortman, 2006), which in turn causes nutrient deficiency in animals that fed on this straw (Rengel *et al.*, 1999). Chickpea plants foliar sprayed with Zn had greater straw Zn concentration compared to the grain, which could contribute in preventing human Zn deficiency through consuming the animals that fed on the straw (Cakmak, 2002).

The tested varieties differed for grain Zn content while contrast comparison between desi and kabuli was not significant for either grain or straw Zn content, indicating the importance of focusing on individual selection. Variation in grain Zn concentration among chickpea genotypes was previously reported (Khan *et al.*, 2000; Kaya *et al.*, 2009; Bueckert *et al.*, 2011; Diapari *et al.*, 2014; Ray *et al.*, 2014). Responses to Zn applications were variable depending on the genotypes (Khan *et al.*, 2000), which is not the case in our study. Moreover, Bueckert *et al.* (2011) reported that the kabulis had better grain Zn concentration than the desis in a study involving four kabuli and six desi genotypes. Grain and straw Zn contents were positively correlated, which indicated selection for high grain Zn content may ensure greater Zn concentration in the straw. On the other hand, neither grain nor straw Zn concentrations were correlated either with grain yield or grain weight. Diapari *et al.* (2014) also reported that association of Zn content with grain weight was mostly not significant though it was negatively associated with grain yield among 94 genotypes.

The Zn fertilizer application strategies did not influence most of the growth and productivity parameters in this study. Findings on the effect of Zn fertilization on plant growth and yield are mixed. Grain yield has increased with the application of Zn through foliar spraying (Pathak *et al.*, 2012), soil application (Kaya *et al.*, 2009) and seed priming (Harris *et al.*, 2008), in chickpea. On the other hand, Johnson *et al.* (2005)

did not find grain yield response neither from Zn seed priming nor from soil fertilization with Zn in chickpea and lentil. Source of variation among reported results could be differences in initial soil Zn content, prevalence of limiting factors other than Zn, genotype variation and differences in timing, frequency and rate of Zn application.

The tested chickpea varieties did not vary on growth and yield parameters except for number of pod bearing branches and grain weight. However, varietal performance has not been consistent across locations following a significant interaction with locations. Previous tests have also shown that the five tested varieties differed in productivity across locations (Beyene *et al.*, 2013) though variety rankings were not consistent. Average productivity of the varieties has been low compared to their potential especially at Taba. This was because of a very low performance in 2012 due to poor rainfall amount and distribution and further yield drop at Taba in 2013 because of excess rainfall during early establishment since the crop is sensitive to waterlogging (Hawando, 1987).

5. Conclusions

The study showed that foliar Zn spraying is an effective method for biofortification of chickpea compared to soil application and seed priming. Foliar application has even greater advantage for improving straw Zn concentration, thereby, improving animal feed quality, which could in turn improve human nutrition. On the other hand, none of the Zn application strategies influenced most of the growth and productivity parameters. Varietal differences were observed for grain Zn concentration showing the potential use of genotype selection for improving Zn availability in human diets. Based on the results, foliar application of 0.5% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at the specified times and the use of varieties such as Naatolii and Arerti can be recommended as a Zn

biofortification strategy for chickpea in Ethiopia. On the other hand, though fertilizer requirement for repeated spraying is nearly similar to that of soil fertilization it would be still too costly to farmers, especially in the absence of yield response. Thus, a study aimed at identifying the most effective spray timing for maximum response of grain quality would be worthwhile as a first step. On the other hand, fertilization with Zn alone may not enhance the productivity of chickpea in southern Ethiopia perhaps 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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