

ZINC SEED PRIMING IMPROVES STAND ESTABLISHMENT, TISSUE ZINC CONCENTRATION AND EARLY SEEDLING GROWTH OF CHICKPEA

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ABSTRACT

Zinc (Zn) deficiency is one of the major factors causing substantial loss to chickpea production. Zinc can be applied by several means including seed priming. Before field application, optimization of Zn seed priming is required as both toxicity and deficiency of Zn badly impacts chickpea growth and productivity. Therefore, this study was conducted to optimize the Zn concentration for seed priming in both *desi* and *kabuli* chickpea. For this, four independent experiments were performed both in petri plates and sand filled small pots. In petri plates, seeds were sown between the layers of moist filter papers, while in sand filled pots, seeds were sown in small sand filled (500 g) pots. In the first two experiments, Zn was applied using different seed priming concentrations *viz.*, 0.01, 0.05, 0.1, 0.5, 1.0 M Zn and no Zn was taken as control. At all these Zn seed priming concentrations, there was no germination/emergence owing to Zn toxicity (data not given). In third and fourth experiments, based on above results, the Zn concentrations were reduced to 0.01, 0.001 and 0.0001 M Zn solutions, taking non-primed seeds as control. The mean germination/emergence time and seedling growth of both chickpea types were improved with Zn seed priming in 0.001 and 0.0001 M Zn solution except 0.01 M Zn that caused toxicity. In both experiments, seed priming with 0.001 M Zn decreased mean germination/emergence time, improved the seedling root (47.8 and 30.4%) and shoot length (27.1 and 10.9%), number of secondary roots (80.0 and 28.6%), seedling dry weight (25.5 and 42.5%) and seedling Zn concentration (30.3 and 47.2%), respectively in petri plates and sand filled pots in both chickpea types. In conclusion, seed priming ≥ 0.01 M Zn proved toxic. Chickpea seed may be primed with 0.001 M Zn to improve the seedling germination/emergence and early seedling growth.

Key words: Deficiency, toxicity, germination rate, root length, seedling dry weight.

INTRODUCTION

Zinc (Zn) is an essential micronutrient for plant growth; its deficiency decreases the yield of many crops. It is major micronutrient disorder which limits the chickpea (*Cicerarietinum* L.) productivity (Ahlawat *et al.* 2007), as it is required for pollen functionality and fertilization (Pandey *et al.* 2006). In chickpea, the deficiency of Zn reduces the rate of photosynthesis (Khan *et al.* 2004), delays the crop maturity and reduces the yield and the water use efficiency at whole plant level (Khan *et al.* 2003).

Zinc deficiency in Pakistani soils has been reported since decades and about 70% of Pakistan's agricultural soils are Zn deficient (Hamid and Ahmad 2001). Chickpea is mostly grown in southern Punjab and deficiency of Zn is more severe in southern Punjab due to sandy nature of soil (Ullah *et al.* 2019) with extent of Zn deficiency of above 75% (Maqsood *et al.* 2015). Deficiency of Zn affects the chickpea more severely compared with cereals (Khan, 1998). Chickpea varieties

also differ for response to the deficiency of Zn (Khan, 1998; Ahlawat *et al.* 2007).

Chickpea growth and development is badly affected owing to Zn toxicity as Zn above the needed level behaves as heavy metals (like lead and cadmium) and hinders the development and growth of plants (Ali *et al.* 2000). For instance, high dose of Zn (300 mg) decreased the root, shoot growth and dry weight of wheat (*Triticum aestivum* L.) (Glinska *et al.* 2016). In another study, the application of Zn (above 0.1 ZnCl₂ and 0.5 M ZnSO₄) was found toxic and affected the germination and seedling growth of wheat (Rehman *et al.* 2015). Zinc application at 10 mM decreased the germination up to 75% and cause reduction in gibberellic acid and zeatin contents in chickpea (Atici *et al.* 2005).

Zinc can be applied to crop plants via foliar and soil application and with seed treatments (priming and coating) (Farooq *et al.* 2012; Haider *et al.* 2018). In soil application, the uniform delivery of micronutrients is difficult and most of the nutrients may get fixed (Ryan *et al.* 2013). To counter the micronutrients deficiency, foliar application is an option, but it is inefficient if not applied

timely. It is expensive, require repetitive sprays and expertise as well (Takkar *et al.* 1986). However, Zn seed treatment is an economical and easy option (Farooq *et al.* 2018). Seed priming is a controlled pre-sowing hydration technique in which seeds are soaked in water or in any solution and then dried back to their original weight (Farooq *et al.* 2006). Seed priming with nutrients at higher concentrations inhibits the germination of crops e.g., barley (*Hordeumvulgare* L.) (Zelonka *et al.* 2005) and wheat (Rehman *et al.* 2015; Rehman and Farooq 2016). Therefore, for wide-scale application of nutrients through seed priming, optimizing the levels of nutrients is needed.

To the best of our knowledge, no information is available regarding the optimization of Zn seed priming in *desi* and *kabuli* chickpea. Therefore, this study was conducted to optimize different concentrations of Zn seed priming to improve stand establishment, tissue Zn concentration and seedling growth of both *desi* and *kabuli* chickpea.

MATERIALS AND METHODS

Plant material: Seeds of *kabuli* chickpea cv. Noor-2013 were collected from Pulses Research Institute, AARI, Faisalabad, Pakistan whereas seeds of *desi* chickpea cv. NIAB-CH-2016 were obtained from the Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

Experimental design and treatment details: This study was consisted of four independent experiments conducted in the Allelopathy Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan during 2016. The experimental treatments were executed in completely randomized design in factorial arrangement and repeated four times. The experiments were carried out in petri plates (90 mm × 1.5 mm) and in sand filled pots (150 mm × 90 mm). The growth conditions were maintained at 25 ± 2°C throughout the experimentation. The first two experiments were pre-optimization experiments and the Zn was applied as seed priming at 0.01, 0.05, 0.1, 0.5 and 1.0 M Zn for both petri plates and sand filled pots using zinc sulphate as source. In experiment, conducted in petri plates, both chickpea types' seeds were uniformly placed between the layers of moist filter papers. In the second experiment, seeds of both chickpea types were sown in plastic pots filled with river sand (500 g pot⁻¹). There was no germination in both chickpea types at ≥ 0.01 M Zn in both medium (Figure 1) (data not given).

Based on the result of pre-optimization experiments, Zn concentrations were reduced to (0.01, 0.001 and 0.0001 M Zn) for third and fourth experiments (Figures 2 and 3). For both chickpea types, seeds at 12% moisture content were soaked in aerated solution using commercial ZnSO₄·7H₂O (with 33% Zn) as source, for

eight hours. An aquarium pump was used to provide aeration. In all experiments, dry seed were taken as control. In sand filled pots and petri plates, eight seeds were sown.

In petri plates, seeds exhibited 2 mm radicles (length) were counted as germinated. While in pots, coleoptile appearance above the sand was scored as seedling emergence. After the constant germination the seedlings were thinned to five. After fifteen days of sowing the seedlings were harvested for growth traits (shoot length, root length, numbers of secondary roots per plant and seedling dry weight) and seedling Zn concentration determination.

Stand establishment, plant growth and seedling Zn concentration: The experiments were visited daily to record germination/emergence count till constant scores. Mean germination/emergence time (MGT/MET) was calculated using the formula of Ellis and Roberts (1981).

Fifteen days after sowing, seedlings were harvested to record seedling shoot and root length, number of secondary roots and seedling dry weight. The seedling shoot and root length was measured with measuring tap while the numbers of secondary roots were counted from the same plants to record the numbers of secondary roots per plant. Seedling dry weight was determined by drying in oven at 70°C till constant weight.

Zinc was estimated by grinding and digesting the roots and shoots samples in di acid (HClO₄:HNO₃ 1:2 v/v) on a digestion plate and Zn was determined using the atomic absorption spectrophotometer (Perkin Elmer, CA, USA) (Prasad *et al.* 2006).

Statistical analysis: The experimental data were subjected to analysis using statistical package Statistix 8.1 (Analytical Software, USA). The difference among treatments means was compared using Least Significant Difference (LSD) test at 5% probability level (Steel *et al.* 1997). Microsoft-Excel Program was used to calculate the Pearson correlation and graphical presentation of data.

RESULTS

Petri plates

Stand establishment, plant growth and Seedling Zinc Concentration: Seed priming with Zn significantly affected the mean germination time (MGT), shoot length (SL), number of secondary roots (NSRs), and seedling dry weight (SDW) of both (*kabuli* and *desi*) chickpea (Table 1 and 2). Moreover, the interaction of Zn seed priming × chickpea types was significant only for root length (RL) (Table 1). However, chickpea types only differed significantly for SDW (Table 2).

Seed priming with 0.001 M Zn solution reduced the MGT of both chickpea types but was statistically similar with 0.01 and 0.0001 M Zn solution. Maximum SL was recorded with 0.001 M Zn solution while minimum with 0.01 M Zn solution in both chickpea types (Table 1).

Maximum RL was recorded with 0.001 M Zn solution in *desi* chickpea, while minimum was with 0.01 M Zn solution in *kabuli* chickpea (Table 1). The maximum NSRs were recorded with 0.001 and 0.0001 M Zn and minimum with 0.01 M Zn solution in both chickpea types (Table 2). The maximum SDW was recorded with 0.001 M Zn, while minimum with 0.01 M Zn solution in both chickpea types. Among the chickpea types; maximum SDW was produced with *desi* chickpea compared to *kabuli* (Table 2).

In case of seedling Zn concentration, maximum seedling Zn concentration was measured with 0.01 M Zn which was toxic for both chickpea types (Figure 4a and 1). While, Zn seed priming concentration at which chickpea types performed best was 0.001 M Zn solution for both chickpea types (Figure 2a, 3a and 4a). However, minimum seedling Zn concentration was recorded where no Zn was applied in both chickpea types (Figure 4a).

Sand filled pots

Stand establishment, plant growth and Seedling Zinc Concentration: Seed priming with Zn significantly affected the mean emergence time (MET), shoot length (SL), root length (RL), numbers of secondary roots (NSRS) and seedling dry weight (SDW) of both (*kabuli* and *desi*) chickpea types (Table 3 and 4). Moreover, the

interaction of Zn seed priming \times chickpea types was significant for SL, NSRs, and SDW whereas non-significant for MET and RL. However, chickpea types didn't significantly differed for the Zn seed priming (Table 3 and 4).

Seed priming with 0.001 and 0.0001 M Zn solution reduced the MET in both chickpea types which is statistically similar to control. The maximum SL was recorded with 0.001 M Zn while, minimum with 0.01 M Zn solution in *kabuli* chickpea (Table 3).

Maximum RL was recorded with 0.001 M Zn solution in both chickpea types (Table 3). Maximum NSRs were recorded with 0.001 M Zn while, minimum with 0.01 M Zn solution in *kabuli* chickpea. Moreover, higher SDW was recorded with 0.001 M Zn solution in *kabuli* chickpea while, minimum with 0.01 M Zn in both chickpea (Table 4).

Correlation analysis showed that shoot and root length, and numbers of secondary roots per plant had strong positive correlation with seedling dry weight of both chickpea types grown in petri plates or sand filled pots under different concentrations of Zn seed priming. However, seedling emergence had strong negative correlation with seedling dry weight of both chickpea types grown in sand filled pots under different concentrations of Zn seed priming (Table 5). Maximum seedling Zn concentration was measured with 0.01 M Zn solution in both chickpea which was statistically similar with 0.001 M Zn solution in *desi* chickpea (Figure 4b). However, minimum seedling Zn concentration was recorded where no Zn was applied in *kabuli* chickpea (Figure 4b).

Table 1. Effect of different concentrations of zinc seed priming on the mean germination time, shoot and root length of chickpea types (Petri plate experiment)

Zn application	Chickpea type			Chickpea type			Chickpea type		
	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)
	Mean germination time (days)			Shoot length (cm)			Root length (cm)		
Control	2.51	2.26	2.39 A	3.14	2.61	2.88 AB	1.58 bcd	2.68 b	2.13
SP (0.01 M Zn)	1.83	1.89	1.86 B	0.48	1.38	0.93 C	0.69 d	1.14 cd	0.92
SP (0.001 M Zn)	1.40	1.55	1.47 B	3.23	4.08	3.66 A	1.62 bcd	4.68 a	3.15
SP (0.0001 M Zn)	1.56	1.66	1.61 B	2.16	2.28	2.22 B	1.48 bcd	2.14 bc	1.81
Mean (C)	1.82	1.84		2.25	2.59		1.34	2.66	
LSD (p \leq 0.01)		0.41			0.81			1.33	

SP=Seed priming; Mean (T)=Mean of treatment; Mean (C)=Mean of chickpea types; C=Chickpea types; T=Treatment

Table 2. Effect of different concentrations of zinc seed priming on the numbers of secondary roots and seedling dry weight of chickpea types (Petri plate experiment)

Zn application	Chickpea type			Chickpea type		
	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)
	Number of secondary roots			Seedling dry weight (mg)		
Control	06	05	05 AB	381	475	428 B
SP (0.01 M Zn)	01	03	02 B	213	276	244 C
SP (0.001 M Zn)	08	10	09 A	475	599	537 A
SP (0.0001 M Zn)	04	08	06 A	327	525	426 B
Mean (C)	05	06		349 B	468 A	
LSD ($p \leq 0.01$)	3.81			C=57; T=81		

SP=Seed priming; Mean (T) = Mean of treatment; Mean (C)=Mean of chickpea types; C=Chickpea types; T=Treatment

Table 3. Effect of different concentrations of zinc seed priming on the mean emergence time, shoot and root length of chickpea types (Sand filled pot experiment)

Zn application	Chickpea type			Chickpea type			Chickpea type		
	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)
	Mean emergence time (days)			Shoot length (cm)			Root length (cm)		
Control	3.52	3.41	3.46 B	27.6 ab	21.9 bcd	24.7	11.6	15.4	13.5 ^{AB}
SP (0.01 M Zn)	4.13	4.33	4.23 A	02.4 e	16.9 ab	09.7	01.7	11.6	06.7 ^B
SP (0.001 M Zn)	2.63	2.98	2.80 B	29.3 a	25.5 abc	27.4	16.9	18.3	17.6 ^A
SP (0.0001 M Zn)	3.05	3.31	3.18 B	19.4 cd	23.9 abc	21.6	12.8	13.6	13.2 ^{AB}
Mean (C)	3.33	3.51		19.7	22.1		10.7	14.7	
LSD ($p \leq 0.01$)	0.67			6.3			6.8		

SP=Seed priming; Mean (T) = Mean of treatment; Mean (C)=Mean of chickpea types; C=Chickpea types; T=Treatment

Table 4. Effect of different concentrations of zinc seed priming on the numbers of secondary roots and seedling dry weight of chickpea types (Sand filled pot experiment)

Zn application	Chickpea type			Chickpea type		
	<i>Kabuli</i>	<i>Desi</i>	Mean (T)	<i>Kabuli</i>	<i>Desi</i>	Mean (T)
	Number of secondary roots			Seedling dry weight (mg)		
Control	15 ab	12 b	14	425 ab	400 b	412
SP (0.01 M Zn)	02 c	12 b	07	325 b	375 b	350
SP (0.001 M Zn)	20 a	17 ab	18	650 a	525 ab	587
SP (0.0001 M Zn)	13 b	14 ab	13	457 ab	500 ab	478
Mean (C)	12	14		433	481	
LSD ($p \leq 0.01$)	7.0			240		

SP=Seed priming; Mean (T) = Mean of treatment; Mean (C)=Mean of chickpea types; C=Chickpea types; T=Treatment

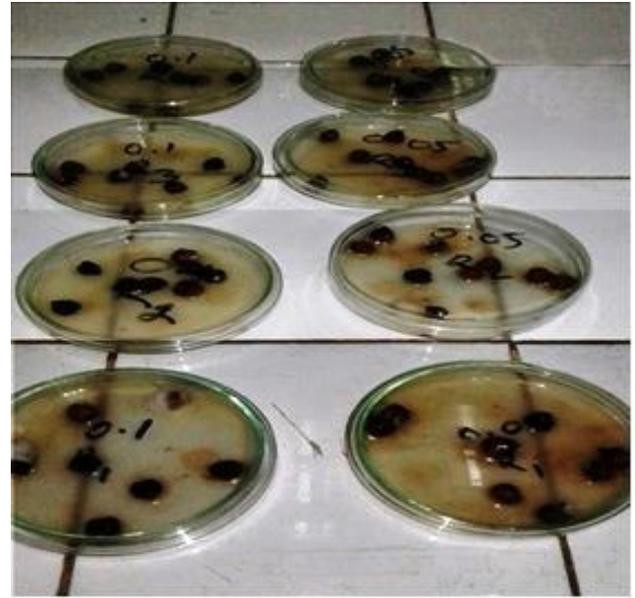
Table 5. Correlation of some stand establishment and growth traits with seedling dry weight of chickpea types in petri plate and sand filled pot experiments (n=4)

Variables	Petri plate experiment		Sand filled pot experiment	
	<i>Kabuli</i> chickpea	<i>Desi</i> chickpea	<i>Kabuli</i> chickpea	<i>Desi</i> chickpea
Mean emergence time (days)	-0.16	-0.43	-0.94**	-0.83**
Shoot length (cm)	0.94**	0.88**	0.77*	0.91**
Root length (cm)	0.89**	0.85**	0.90**	0.66*
Numbers of secondary roots	0.99**	0.94**	0.88**	0.91**

** = Significant at $p \leq 0.01$; * = Significant at $p \leq 0.05$



Kabuli chickpea

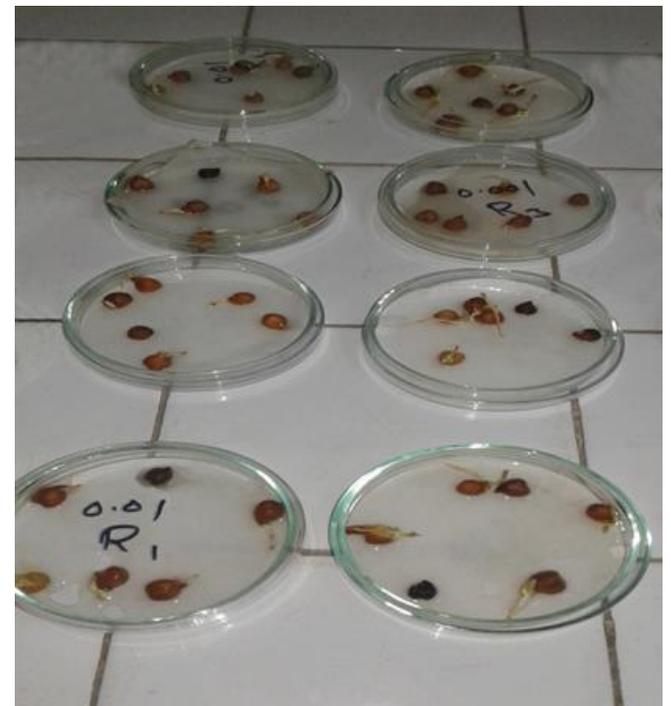


Desi chickpea

Figure 1. Effect of different concentrations of Zn seed priming(0.01; 0.05; 0.1; 0.5 and 1.0 M Zn) on the germination of *kabuli* and *desi* chickpea in petri plates



Kabuli chickpea



Desi chickpea

Figure 2. Effect of different concentrations of Zn seed priming(0.01; 0.001 and 0.0001 M Zn) on the germination of *kabuli* and *desi* chickpea in petri plates

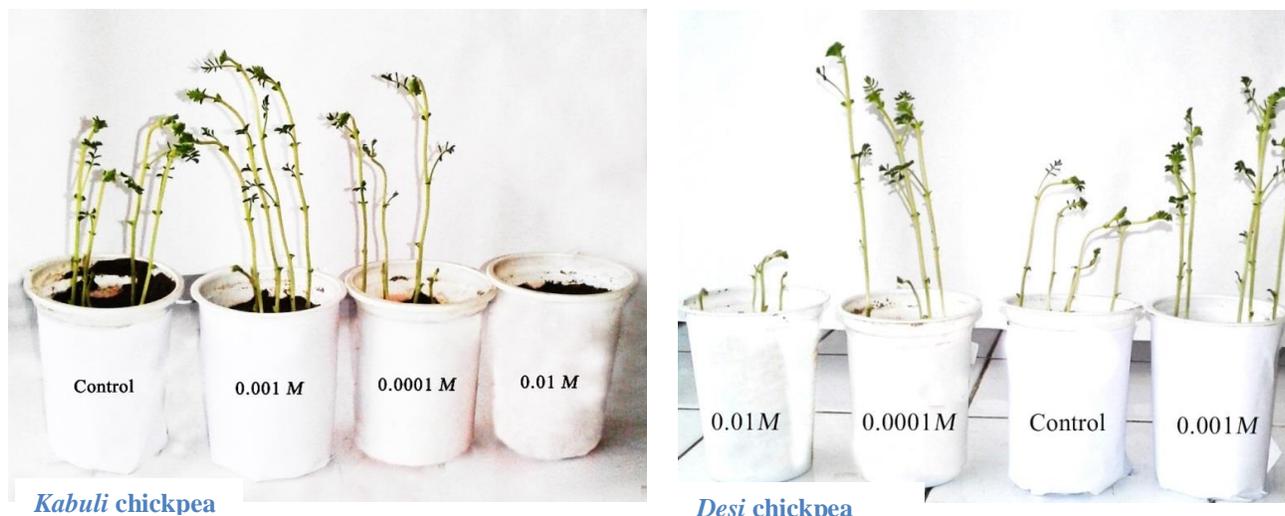


Figure 3. Effect of different concentrations of Zn seed priming (0.01; 0.001 and 0.0001 M Zn) on the emergence of *kabuli* and *desi* chickpea in sand filled pots

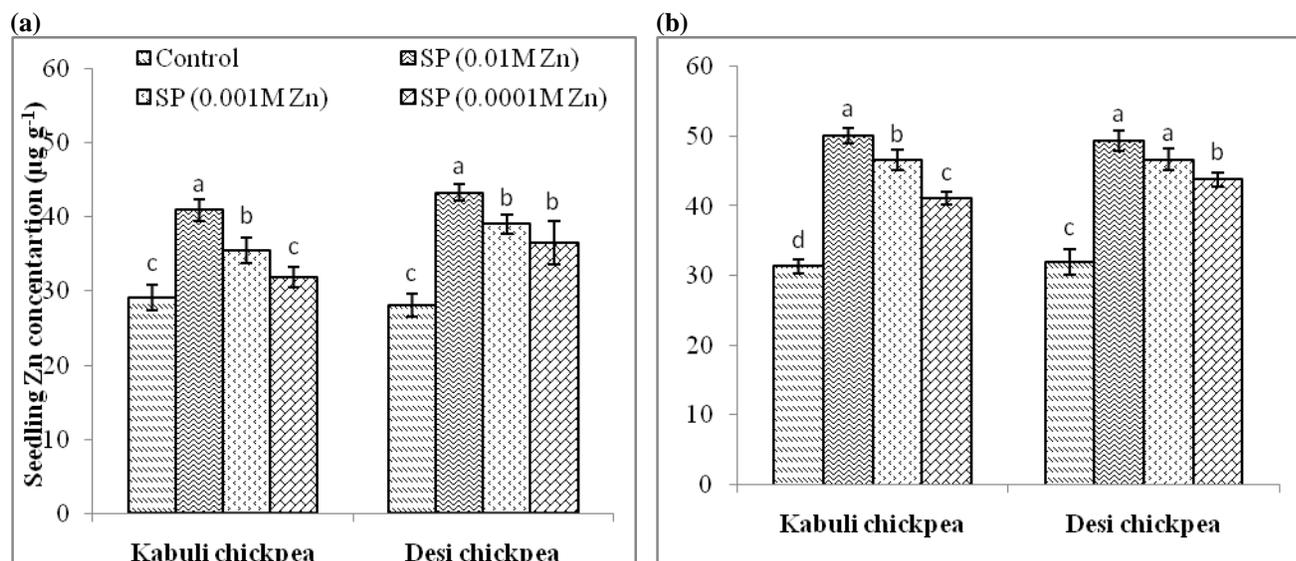


Figure 4. Effect of different concentrations of Zn seed priming on the seedling Zn concentration of *kabuli* and *desi* chickpea; (a) petri plate experiment, (b) sand filled pots

DISCUSSION

In this study, the optimization and potential of Zn seed priming was evaluated for the early stand establishment, tissue Zn concentration and improving seedling growth of both (*desi* and *kabuli*) chickpea. Seed priming with Zn improved the emergence/germination and seedling development of chickpea (Tables 1-4) as priming of seeds change the physiology of embryo and activates the hydrolytic enzymes by which emergence occur at a rapid pace than normal seedling emergence (Bam *et al.* 2006). Moreover, germination/emergence and seedling growth was improved by the application of Zn in priming solution (Tables 1-4) as Zn is involved in the

radicle development and in the early stages of coleoptile growth and in auxin synthesis (Ozturk *et al.* 2006). Seedling dry weight was increased with Zn seed priming due to early and uniform emergence/germination, which in result improved the seedling growth (Tables 1-4) as the deficiency of Zn decreases the root growth in chickpea (Khan 1998). Moreover, the improvements in seedling growth of both chickpea types with Zn seed priming might be due to significant improvement in shoot and root length and numbers of secondary roots per plants as seedling dry weight had strong positive correlation with growth traits (Table 5).

Zinc seed priming at 0.01 M Zn was proved toxic which suppressed the germination/emergence and

seedling growth of chickpea (Tables 1-4). Root growth is restricted at higher concentration of Zn as Zn toxicity suppresses the process of cell division (Prasad *et al.* 1999) (Tables 1-4). Moreover, the root and leaf development were reduced at higher concentration of Zn owing to substantial decrease in NADPH production in chloroplasts (Mousavi 2011). The different chickpea varieties behave different to the deficiency of Zn (Ahlawat *et al.* 2007). The chickpea types differed for response to Zn levels and the Zn above the required level was toxic for both chickpea types (Figures 1-4).

The higher application of Zn inhibit germination and reduced the growth and development of chickpea (Tables 1-4; Figure 1) as application of Zn at higher concentration (1.0 and 10 mM Zn) cause delay and impeded the germination in chickpea due to decrease in gibberellic acid and zeatin in germinating seeds (Atici *et al.* 2005). The over dose of Zn inhibits the growth and development of plants (Ali *et al.* 2000) owing to its involvement in metabolic processes linked with normal development (Gadallah and El-Enany 1999). Heavy metals including Zn decrease the germination of seeds (Ali *et al.* 2000) as heavy metals in high concentration disturb the physiological and biochemical processes, for example, injure cell membranes, affect the enzyme activity, damage photosynthetic apparatus and rate, and break the protein synthesis (Monni *et al.* 2001; Atici *et al.* 2003). Among the tested chickpea types, *desi* chickpea performed better in stand establishment and growth compared with *kabuli* chickpea (Tables 1-4). However, regarding germination/emergence *kabuli* chickpea was better than *desi* chickpea (Tables 1-4).

Application of micronutrients above the required level behaves as heavy metal and cause stress in plants. Chickpea is very sensitive to Zn deficiency and toxicity thus, there is need to optimize the Zn concentrations for seed priming for field application to improve the growth, productivity and nutritional value of grain.

In conclusion, Zn seed priming with 0.001 M Zn solution performed best in improving the early seedling germination/emergence, tissue Zn concentration and growth of both chickpea types compared with other Zn seed priming concentrations. However, at high concentration ≥ 0.01 M Zn solution seedlings germination and growth suppressed which indicated Zn toxicity for chickpea.

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