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Effect of foliar fertilization with zinc and manganese sulfate on yield, dry matter accumulation, and zinc and manganese contents in leaf and seed of chickpea (*Cicer arietinum*)

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ABSTRACT

To investigate the effects of zinc (Zn) and manganese (Mn) foliar fertilization on yield, dry matter accumulation, Zn and Mn concentrations in leaf and seed of chickpea cultivars, a field experiment was conducted in 2014. The experimental design was a split plot in randomized complete block with three replications. The phenological stages recorded were as follows: Emergence, flowering, 50% flowering, and harvest maturity. At harvest, economic yield and its components, biological yield, harvest index, stem, leaf, pod, seed, and total dry weight were measured. The Zn and Mn contents were determined by atomic absorption spectrometry. The results indicated that the spraying of Zn and Mn elements did not have a significant effect on the time from emergence to 50% flowering and the time from emergence to maturation. Spraying treatments had a significant effect on dry weight of stems, leaves, pods, seeds, and total plant. In all three cultivars, Zn spraying had the greatest effect on the plant height, number of pods per plant, number of seeds per plant, 100-seed weight, grain yield, biological yield, and dry weight of leaves, seeds, and total plant, while Mn spraying had the greatest effect on the increase in stem and pod dry weight and protein content.

1. INTRODUCTION

Chickpea (*Cicer arietinum*) is one of the most important food legume crops which is grown as a source of cheap protein for human. Chickpea contains 13–33% protein, 40–55% carbohydrate, 4–10% oil (50% oleic and 40% linoleic acid) [1], B-group vitamins (thiamin, riboflavin, and niacin) [2], folate, β-carotene, and mineral elements such as Zn, Se, Fe, Ca, Mg, K, Cu, and P [3]. In Iran, chickpea is cultivated on an area of 433,356 hectares with an annual seed production of 177,493 tones and average seed yield of 409.6 kg/ha [4]. In our country, productivity of chickpea is still low and unstable due to its cultivation on marginal soils, especially under rainfed conditions. Nutrient management is one of the important factors for increasing its productivity. Recently, more attention has been paid to the application of microelement fertilizers. In calcareous soils, zinc (Zn) and manganese (Mn) deficiency are common [5,6], and root availability to these elements is reduced [7]. Soils in many arid and semi-arid regions of Iran due to high pH and low organic matter are faced with Zn and Mn deficiencies. Soil application is a more common method to supply macronutrients to plants, but this approach will create problems for micronutrients [8], thus, in these soils, foliar application is more effective [9]. On the other hand, when root activity is reduced due to water deficit, foliar fertilization is more advantageous in absorption compared to soil application. According to Heidarian *et al*., and Narimani *et al.* [10,11] studies under drought stress, plant roots cannot absorb micronutrients, and foliar spraying of micronutrients will be useful. In brief, the main objectives of this study were to investigate changes in Zn and Mn concentrations in leaves and seeds, dry matter production, and yield and yield components of chickpea, after Zn and Mn foliar fertilization.

2. MATERIALS AND METHODS

2.1. Site Description and Soil Analysis

A field experiment was conducted on a research field of the Islamic Azad University of Kermanshah Province, Iran (34°23’ N, 47°8’ E; 1351 m elevation) in 2014. The soil type of the experimental areas is silty clay with cold and rainy winters and hot and dry summers, and annual average precipitation is 478 mm before planting, soil samples were collected from an experimental area at a depth of 0 cm–30 cm for soil analysis. The texture of the soil corresponded to silty clay. The soil had the following properties: pH 7.1, total organic matter 1.8%, electrical conductivity 0.48 ds/m, total nitrogen 0.15%, available phosphorus 9.5 mg/kg, available potassium 346 mg/kg, and Zn 0.42 and Mn 1.6 mg/kg soil.

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2.2. Treatment and Experimental Design

The experimental design was a split plot in randomized complete block with three replications. The main plot includes three cultivars: C_1=Flip 90–96C, C_2=Flip 93–93C, and C_3=Flip 84–48C; and three foliar fertilization which consisted of (F_0) spray with distilled water (control treatment), (F_{Zn}) Zn spray from $ZnSO_4$ source, and (F_{Mn}) Mn spray from $MnSO_4$ source which are replaced in subplot. A common dose of 50 kg P_2O_5/ha and 30 kg N/ha was applied to all plots as basal dose at the time of sowing. Before planting, seeds were presoaked in 10% sugar solution and then inoculated with *Bradyrhizobium leguminosarum* (2 g/kg of seeds). The crop was sown on March 14, 2014. The experiment included nine treatments placed on 27 plots. The plots consisted of eight rows; 4 m in length and sand spaced at 25 cm. The distance between plants in a row was 5 cm and the plant density was 800,000 plants/ha. Plots were hand weeded 2 times when needed. At the late of vegetative growth stage and/or before flowering, the plants were sprayed twice (with 1-week interval) with 0.5% (*w/v*) or distilled water until the leaves were wet based on [12] the study.

2.3. Plant Sampling

The phenological development of the crop was monitored at 2–3-day intervals for all seasons from the onset of flowering. The phenological stages recorded were as follows: Emergence (E), flowering (F), 50% flowering (F_{50}), and harvest maturity (HM). When 50% of the plants in any plot had emerged or had at least one open flower, or 50% of the plants in any plot had flowered, then this was designated as emergence, flowering, and 50% flowering stage, respectively. HM was reached when >95% of the plants in the plot had completely senesced and turned brown. At harvest, plant height, number of subbranch, pod and seed per plant, and 100-seed weight were measured on 10 randomly selected plants in each subplot. Furthermore, for measuring of stem, leaf, pod, seed, and total dry weight, the samples were dried at 70°C for 48 h. 1 m^2 from each sub-plot was harvested and blended and seed and biological yields (g m^{-2}) were weighed. Harvest index (%) was obtained by dividing economic yield by biological yield multiplied by 100. To measure leaf and seed concentrations of micronutrients, 10 g of each leaf and seed sample were collected from the plots of each treatment. Samples (leaves and seeds, separately) were washed with distilled water, dried in an oven at 70°C for 48 h, weighed, and incinerated at 550°C. Dry ash samples were solved in concentrated HNO_3 and $HClO_4$. The Zn and Mn contents were determined by atomic absorption spectrometry according to Kacar [13]. Nitrogen measurements for total N were based on the Kjeldahl method. The Kjeldahl method was performed according to AOAC international [14].

2.4. Statistical Analyses

All the data obtained from the measurements were evaluated statistically with MSTATC software. Analysis of variance was performed after the Bartlett test for checking uniformity of data variance (P = 0.05). Least significant difference test was used to compare the means. Excel software was used to plot diagrams.

3. RESULTS AND DISCUSSION

3.1. Effect of Cultivar and Foliar Treatments on TF_{50} and THM and Dry Weight Accumulation in Different Parts of Chickpea Plants

The results of analysis of variance performed to test the effect of foliar fertilization with Zn and Mn sulfate on dry matter accumulation in different parts of chickpea plant shown in Table 1, indicated that there were significant differences between cultivars in terms of total dry weight (P < 0.05) and days from emerging (E) to HM (THM) (P < 0.01). The days from emerging to HM ranged from 96 to 112 days, and Flip 93–93C was the earliest in reaching HM (112.61 days) compared to Flip 84–48C (99.13 days) and Flip 90–96C (96.12 days) cultivars. However, Flip 84–48C cultivar had shorter period of duration from emerging to 50% flowering (46 days). These results were consistent with the results obtained by Sabaghnia and Janmohammadi [15]. Based on Pearson correlation [Table 2], there was no significant correlation between chickpea growth period and spraying of Zn and Mn. In fact, the spraying of Zn and Mn elements did not have a significant effect on the time from emergence to 50% flowering (TF50) and the time from emergence to maturation (THM). These results were somewhat different from those obtained by Janmohammadi *et al*. [16]. In their research entitled, "the impacts of nanostructured nutrients on chickpea performance under supplemental irrigation," the application of Zn and Mn on the number of days from emergence to 50% flowering had a very significant effect, such that Zn consumption in comparison with other treatments has reduced the duration of up to 50% flowering in chickpea. Spraying treatments had a significant effect on dry weight of stems, leaves, pods, seeds, and total plant (P < 0.01). In contrast, dry weight of stems, leaves, pods, seeds, and total plant was not affected by interaction effects between cultivars and foliar × cultivar treatments [Table 1]. The

Table 1: Analysis of variance for the impact of Zn and Mn sulfate on dry matter accumulation in different parts of chickpea plant and times for emergence to 50% flowering and harvest maturity

SOV	df	Ms						
		Dry weight of					Days after sowing	
		Stem	Leaf	Pod	Seed	Total plant	TF50	THM
Replication	2	0.052	0.016	0.061	0.155	4.670	8.775	1.886
Cultivar (C)	2	0.427^{ns}	1.002^{ns}	0.128^{ns}	0.120^{ns}	13.388*	50.500^{ns}	690.145**
Error a	4	0.308	0.227	0.921	0.096	1.445	10.983	35.909
Foliar treatment (F)	2	5.176**	5.675**	8.019**	26.095**	106.019**	46.359^{ns}	3.322$_{ns}$
Cultivar×Foliar treatment (C×F)	4	0.147^{ns}	0.400^{ns}	0.369ns	0.020$_{ns}$	1.003^{ns}	6.421$_{ns}$	12.553$_{ns}$
Error b	12	0.074	0.320	0.611	0.251	3.209	9.026	16.256
Coefficient of variation (%)	-	6.69	9.08	18.78	8.66	10.28	6.21	5.99

Ns,* and **: Non-significant, significant at 5% and 1% levels of probability, respectively. TF50: Time to 50% flowering, THM: Time to harvest maturity, SOV: Source of variation, Zn: Zinc, Mn: Manganese

Table 2: The effect of Zn and Mn sulfate on dry matter accumulation in different parts of chickpea plant and times for emergence to 50% flowering and harvest maturity

	Means						
	Dry weight of (g plant^{-1})					Days after sowing	
	Stem	Leaf	Pod	seed	Total plant	TF50	THM
Cultivar (C)							
C1	4.26±0.79	6.21±1.09	4.09±0.99	5.92±1.49	17.49±3.37	47.12±3.70	96.12±3.12
C2	3.83±0.55	5.90±0.53	4.30±1.31	5.72±1.61	16.17±3.02	51.09±2.41	112.61±4.42
C3	4.08±0.83	6.57±0.85	4.10±0.92	5.71±1.47	18.61±3.63	46.86±3.06	99.13±3.28
LSD value	0.726	0.62	1.26	0.41	1.57	4.34	7.84
Foliar treatment (F)							
F0	3.18±0.35	5.31±0.63	3.08±0.35	3.82±0.34	13.57±1.42	49.16±3.27	102.01±4.44
FZn	4.46±0.41	6.74±0.57	4.59±0.61	6.86±0.45	20.15±2.36	45.80±4.24	102.63±4.89
FMn	4.53±0.41	6.62±0.56	4.81±1.05	6.67±0.42	18.55±1.74	50.12±3.89	103.26±5.13
LSD value	0.28	0.58	0.80	0.51	1.84	3.09	4.14
Cultivar×Foliar treatment (C×F)							
C1F0	3.25±0.8	4.85±0.73	2.96±0.31	3.97±0.24	13.71±1.95	47.52±2.11	95.16±2.45
C1FZn	4.89±0.8	6.91±0.11	4.86±0.83	6.95±0.36	20.34±2.20	45.50±4.32	97.71±4.01
C1FMn	4.65±0.43	6.86±0.27	4.44±0.43	6.83±0.45	18.41±1.35	48.35±3.94	95.48±2.49
C2F0	3.19±0.31	5.37±0.52	3.07±0.46	3.67±0.42	12.87±1.65	51.62±4.01	112.11±3.96
C2FZn	4.03±0.32	6.19±0.37	4.80±0.17	6.83±0.71	18.75±2.48	49.51±4.96	110.23±5.24
C2FMn	4.27±0.30	6.14±0.35	5.03±1.80	6.67±0.44	16.89±0.79	52.14±2.25	115.39±3.32
C3F0	3.11±0.61	5.71±0.46	3.21±0.36	3.82±0.41	14.12±0.57	48.35±2.87	98.65±5.17
C3FZn	4.47±0.12	7.12±0.69	4.12±0.53	6.79±0.41	21.35±2.41	42.38±1.99	100.03±2.39
C3FMn	4.67±0.50	6.87±0.74	4.97±0.84	6.52±0.51	20.35±0.84	49.86±2.63	98.70±2.97
LSD value	0.48	1.06	1.39	0.82	3.19	5.34	7.17

Values are means of three replicates and standard deviations. C_1: Flip 90–96C, C_2: Flip 93–93C, and C3: Flip 84–48C; F_0: Spray with distilled water (control treatment), F_{Zn}: Zn spray, and (F_{Mn}) Mn spray; TF50: Time to 50% flowering, THM: Time to harvest maturity, Zn: Zinc, Mn: Manganese

results of the comparison of mean dry weight of stems, leaves, pods, seeds, and total plant length and from the time of emergence up to 50% flowering (TF50 and THM) are shown in Table 3. According to the results, although there was no significant difference between different cultivars in terms of dry weight of stems, leaves, pods, and seeds, the highest dry weight of stem (4.26 g) and seed (5.92 g) belonged to Flip 90–90C. In addition, the highest leaf dry weight (6.57 g) and dry weight of the whole plant (18.61 g) were recorded in Flip 84–48C cultivars. The lowest stem dry weight (3.83 g), leaf dry weight (5.90 g), and dry weight of the whole plant (16.17 g) belonged to Flip 93–93C. Spraying of both Zn and Mn in comparison with the control treatments increased the dry weight of stems, leaves, pods, seeds, and total plant, but the effect of spray application on leaf dry weight increase (6.74 g), dry weight of seed (6.86 g), and dry weight of total plant (20.15 g) was effective, and the effect of Mn spraying increased the stem dry weight (4.53 g) and pod dry weight (PDW) (4.81 g). Previously, also Shaver *et al.,* and Wang and Jin [17,18] reported a high correlation between the application of Zn fertilizers and their effect on plant growth. On the other hand, the reaction of different cultivars of chickpea was different from that of Zn and Mn foliar treatments [Table 3]. The highest dry weight of stem (4.89 g) and seed (6.95 g) belonged to Flip 90–96C, and the highest leaf dry weight (7.12 g) and total plant (21.35 g) belonged to Flip 84–48C and Zn spray application. In fact, in all three cultivars, Zn spraying had the greatest effect on the dry weight of stems, leaves, seeds, and total plant. According to Yang *et al.* [19], various crop varieties differ greatly in terms of the utilization of microelements. In this regard, researchers in the past also stated that there are many differences between different genotypes of a crop in terms of the response to a deficiency or excess amount of an element [20-22]. Based on the results obtained in this experiment, Mn sprays increased the PDW in Flip 93–93C more than other treatments (5.03 g). Izaguirre-Mayoral and Sinclair [23] reported that with increase in Mn concentration, leaf dry weight, and shoot dry weight increased, and this increase is dependent on the genetic characteristics of the plant. The results of correlation analysis between traits [Table 2] revealed that there was a positive and significant correlation between dry weight of grain with stem dry weight ($r = 0.84$**), dry weight of pod ($r = 0.76$**), and dry weight of whole plant ($r = 0.80$**).

3.2. Effect of Cultivar and Foliar Treatments on Yield and Its Components

The results of analysis of variance of yield and yield components of chickpea under the influence of variety and different treatments of Zn and Mn spraying are shown in Table 4. According to the results, there was a significant difference between cultivars in terms of plant height and 100-seed weight at 1% level ($P < 0.01$) and in terms of number of branches and harvest index at 5% level ($P < 0.05$). In contrast, there was no significant difference between number of pods per plant, number of seeds per plant, grain yield, and biological yield. On the contrary, spray application was not only effective on plant height but also other traits were affected by spray treatments, as such; the effect of spraying on the number of branches at 5% level and other traits at 1% level was significant. Apart from the weight of 100 seeds of chickpea which was affected by interaction effects of cultivars and spraying at 5% level, the interaction effect of these two factors did not have a significant effect on the other studied traits [Table 4]. Research by Thalooth *et al.,* and Hu *et al.* [24,25] showed that Zn fertilization has little or no effect on plant height, but Mn spraying can increase plant height in some plants. On the other hand, according to Babaeian *et al.* [26], although the effect of Zn and Mn fertilization was not statistically significant on sunflower seed yield, simultaneous consumption of these two elements led to a slight increase in yield of sunflower. However, Yousefi [27] stated that Zn and Mn application can significantly increase the crop yield. The results of comparison of mean of cultivars and different treatments of foliar application on yield and yield components of chickpea have been shown in Table 5. According to the results, the highest plant height (43.51 cm) and number of seeds per plant (21.01) were in Flip 90–90C and the highest number of branches (3.65), number of pods per plant (17.84), grain yield (110.58 g m^{-2}), and biological yield (212.03 g m^{-2}) were recorded in the Flip 84–48C cultivar. Furthermore, Flip 9–93C had the highest grain weight (36.60 g) and harvest index (49.13%). On the contrary, compared to other spray treatments, Zn spraying increased plant height (42.53 cm), number of pods per plant (19.29), number of seeds per plant (23.02), 100-seed weight (34.89 g), grain yield (11640 g m^{-2}), and biological yield (222.37 g m^{-2}) in chickpea. Mansur *et al.* [28] reported that Zn consumption will increase the grain yield by increasing the number of seeds per plant and seed weight. Numerous research studies have highlighted the role and importance of Zn in enhancing the yield and production of crops [29-31]. Mn spraying increased the number of branches (3.56) and harvest index (51.75%) in chickpea. The results

Table 3: The effect of Zn and Mn sulfate on [Zn]leaf and [Zn]seed and [Mn]leaf and [Mn]seed

	Means			
	Concentration (mg g^{-1})			
	[Zn]leaf	[Zn]seed	[Mn]leaf	[Mn]seed
Cultivar (C)				
C1	26.79±7.38	44.53±8.45	21.82±7.13	28.92±5.22
C2	29.86±8.47	45.66±9.79	22.56±9.86	30.90±7.46
C3	26.65±6.70	48.10±9.68	24.19±8.91	26.95±5.23
LSD value	3.09	5.76	5.79	1.04
Foliar treatment (F)				
F0	16.34±1.85	28.65±4.14	11.90±1.75	21.59±2.91
FZn	42.75±5.06	72.21±5.67	17.58±3.84	24.54±3.39
FMn	24.22±2.41	37.43±3.37	39.09±5.89	40.65±3.73
LSD value	2.89	3.76	3.62	3.73
Cultivar×Foliar treatment (C×F)				
C1F0	16.31±2.55	26.25±2.73	12.17±1.51	21.69±3.02
C1FZn	41.28±0.57	67.61±1.93	17.41±2.32	26.47±4.15
C1FMn	22.79±2.82	39.73±4.01	35.89±±4.74	38.61±4.00
C2F0	15.73±2.21	27.20±1.98	13.34±0.35	23.72±1.94
C2FZn	48.25±4.96	75.31±7.35	15.61±4.12	25.81±1.67
C2FMn	15.61±2.58	34.48±1.77	38.72±6.34	43.17±3.21
C3F0	16.97±1.05	32.49±4.84	10.18±1.55	19.35±2.62
C3FZn	38.73±2.22	73.72±4.71	19.73±4.90	21.33±1.75
C3FMn	24.26±2.61	38.08±2.14	42.65±5.31	40.16±3.65
LSD value	5.00	6.51	6.28	6.41

Values are means of three replicates and standard deviations. C_1: Flip 90–96C, C_2: Flip 93–93C, and C_3: Flip 84–48C; F_0: Spray with distilled water (control treatment), FZn: Zn spray, and (FMn) Mn spray; [Zn]leaf: Leaf Zn concentration, [Zn]seed: Seed Zn concentration, [Mn]leaf: Leaf Mn concentration, [Mn]seed: Seed Mn concentration, Zn: Zinc, Mn: Manganese

Table 4: Analysis of variance for the impact of Zn and Mn sulfate on [Zn]leaf and seed, [Mn]leaf and seed, and seed protein content

SOV	df	Ms				
		Concentration				Seed protein content
		[Zn]leaf	[Zn]seed	[Mn]leaf	[Mn]seed	
Replication	2	0.906	18.576	9.467	1.664	0.603
Cultivar(C)	2	29.613^{ns}	29.874^{ns}	13.170ns	35.165**	14.011**
Error a	4	5.586	19.389	35.646	0.634	0.425
Foliar treatment (F)	2	1655.357**	4777.536**	1851.041**	947.277**	13.159**
Cultivar×Foliar treatment (C×F)	4	25.176^{ns}	37.61^{ns}	20.931ns	9.386^{ns}	0.950^{ns}
Error b	12	7.912	13.377	12.487	13.186	1.168
Coefficient of variation (%)	-	10.13	7.93	15.46	12.55	5.37

Ns and **: Non-significant and significant at 1% levels of probability, respectively, [Zn]leaf: Leaf Zn concentration, [Zn]seed: Seed Zn concentration, [Mn]leaf: Leaf Mn concentration, [Mn]seed: Seed Mn concentration, SOV: Source of variation, Zn: Zinc, Mn: Manganese of the comparison of the means on the interaction between cultivars and spraying treatments [Table 5] show that Zn spray application had the highest effect on number of pods per plant (20.61), grain yield (125.11 g m^{-2}), and biological yield (230.65 g m^{-2}) in Flip 84–48C, while Zn spray application had the highest effect on plant height (44.71 cm) and number of seeds per plant (24.07) in Flip 90–96C cultivar. The results of Heitholt *et al.* [32] indicated that with Zn consumption, the number of seeds per plant increased by about twice compared to control treatment. The highest harvest index belonged to Mn spraying in Flip 84–48C cultivar. De Varennes and Goss [33] stated that if the consumption of nutrients causes a balance between nutrients in soil and plant, it can have a positive effect on the growth and production of crops. On the other hand, researchers have linked the decline in growth and crops production with the competition of Zn with other elements in absorption [3], the existence of known and/or unknown interaction between Zn and other elements [34], the activity of certain enzymes that affect plant metabolism [30], decrease in plant photosynthesis [35,36], and a decrease in chlorophyll synthesis [34]. Research by Liagat *et al*. [37] demonstrated that Zn application could increase grain yield in cotton. They stated that the plants grown in calcareous soils are usually faced with Zn deficiency and supplying the plant nutrition requirements will not only increase the quality and quantity of crop products but also increase the level of health in the community. The effect of Zn on the increased biological yield of chickpea in Balai *et al.* [38] experiment has also been emphasized. The results of Pearson correlation [Table 2] showed that there was a positive and significant correlation between the number of seeds per plant with grain yield (r = 0.85**) and number of pods per plant (r = 0.70**). Furthermore, there was a positive and significant correlation between 100-seed weight and harvest index at 1% level (r = 0.64**).

3.3. Effect of Cultivar and Foliar Treatments on [Zn]Leaf and Seed, [Mn]Leaf and Seed, and Seed Protein Content

The results of analysis of variance of Zn and Mn concentrations in leaves and seeds as well as protein content in chickpea seed are shown in Table 6. According to the results, there was no significant difference between different cultivars in terms of Zn concentration in leaf and seed and Mn concentrations in chickpea leaves, while Mn concentration and protein content in chickpea grain were significantly different at 1% level (P < 0.01). In contrast, different spraying treatments caused a significant difference at 1% level in all traits studied in Table 6. The interaction effect of cultivar with Zn and Mn fertilizations (F × C) was not affected by Zn concentration in leaves and seeds, Mn concentration in leaves and seeds, and protein content of chickpea seed. The results of the comparison of means [Table 7] show that although the Zn concentration in the leaves and seed of chickpea cultivars had no significant difference, the highest Zn concentrations in leaves and seeds were observed in Flip 93–93C (129.86 mg/g) and Flip 84–48C (40.10 mg/g), respectively. In addition, the highest concentrations of Mn in leaves and seeds were recorded in Flip 84–48C (24.19 mg/g) and Flip 93–93C (30.90 mg/g), respectively. As expected, the use of Zn and Mn elements led to a sharp increase in the concentration of these elements in the leaves and seeds of the plant so that the highest Zn concentrations in the leaf (42.75 mg/g) and grain (72.21 mg/g) of chickpea were observed

Table 5: The effect of Zn and Mn sulfate on yield and yield components of chickpea

	Means							
	H (cm)	NS	NPP	NSP	WS (g)	SY (g m^{-2})	BY (g m^{-2})	HI (%)
Cultivar (C)								
C_1	43.51±2.81	3.01±0.45	17.50±3.58	21.01±4.08	28.26±2.01	99.72±13.74	208.62±22.59	45.47±3.83
C2	40.22±3.69	3.44±0.61	17.50±3.47	19.27±3.67	36.60±2.81	106.31±13.27	196.78±26.76	49.13±4.88
C3	36.18±3.34	3.65±0.52	17.84±4.47	20.13±5.26	34.41±4.01	110.58±19.16	212.03±25.84	47.75±6.81
LSD value	2.05	0.43	3.11	3.47	3.37	14.45	26.53	1.88
Foliar treatment (F)								
F0	37.68±3.89	3.02±0.46	13.24±1.80	15.51±2.13	29.98±3.42	91.27±6.05	180.91±18.58	41.93±3.39
FZn	42.53±3.43	3.53±0.60	19.29±2.94	23.02±2.35	34.89±4.16	116.40±12.52	222.37±17.31	48.66±2.92
FMn	39.69±4.73	3.56±0.55	18.31±3.60	21.89±3.54	34.40±3.07	108.9±14.19	214.11±17.61	51.75±3.97
LSD value	4.13	0.47	3.25	3.34	1.16	13.01	19.05	2.99
Cultivar×Foliar treatment (C×F)								
C1F0	42.31±1.62	2.71±0.52	14.31±1.58	16.80±2.83	26.43±1.87	89.91±8.56	189.58±10.56	41.35±1.63
C1FZn	44.71±2.64	3.25±0.22	19.57±3.19	24.07±1.46	30.19±1.35	108.48±6.67	221.72±26.07	46.71±2.30
C1FMn	43.52±3.21	3.08±0.50	18.63±2.79	22.17±3.72	28.17±0.55	100.72±20.89	214.39±20.01	48.35±2.73
C2F0	37.21±3.56	3.09±0.38	12.23±2.59	14.68±0.77	33.35±0.71	93.15±5.54	167.32±12.55	44.37±1.61
C2FZn	43.28±2.96	3.50±0.85	17.68±2.96	21.91±2.27	38.65±1.22	115.67±14.94	214.72±17.96	50.30±3.41
C2FMn	40.16±3.55	3.72±0.61	16.59±2.83	21.23±1.50	37.81±2.27	110.06±7.88	208.29±20.13	52.71±5.10
C3F0	33.52±1.93	3.25±0.46	13.19±0.67	15.04±2.39	30.15±1.44	90.76±10.91	185.76±26.19	40.08±4.35
C3FZn	39.61±3.26	3.83±0.65	20.61±1.30	23.08±3.36	35.82±3.21	125.11±12.36	230.65±12.01	48.97±1.32
C3FMn	35.40±1.90	3.87±0.26	19.71±3.36	22.27±3.92	37.23±3.11	115.89±15.79	219.56±18.42	54.19±1.48
LSD value	7.16	0.82	5.63	5.78	2.02	22.54	33.01	5.18

Values are means of three replicates and standard deviations. C_1: Flip 90–96C, C_2: Flip 93–93C, and C_3: Flip 84–48C; F_0: Spray with distilled water (control treatment), F_{Zn}: Zn spray, and (F_{Mn}) Mn spray; H: Plant height, NS: Number of subbranch, NPP: Number of pod per plant, WS: 100-seed weight, SY: Seed yield, BY: Biological yield, and HI: Harvest index, Zn: Zinc, Mn: Manganese

Table 6: Correlation coefficients among evaluated traits in chickpea influenced Zn and Mn sulfate fertilizers

	H	^{Ns}	NP	^{Ns}P	WS	EY	BY	HI	SDW	LDW	PDW	GDW	TDW	[Zn]leaf	[Zn]seed	[Mn]leaf	[Mn]seed	P%	TF50	THM
H	1.0																			
^{Ns}	−0.16^{ns}	1.0																		
NP	0.11^{ns}	0.31^{ns}	1.0																	
^{Ns}P	0.37^{ns}	0.5^{ns}	0.70**	1.0																
WS	−0.18^{ns}	0.61**	0.30^{ns}	0.31^{ns}	1.0															
EY	0.10^{ns}	0.62**	0.63**	0.85**	0.57**	1.0														
BY	0.23^{ns}	0.31^{ns}	0.64**	0.60**	0.25^{ns}	0.50*	1.0													
HI	0.06^{ns}	0.54*	0.50*	0.55*	0.64**	0.53*	0.53*	1.0												
SDW	0.35^{ns}	0.32^{ns}	0.60**	0.76**	0.24^{ns}	0.55*	0.75**	0.64**	1.0											
LDW	0.12^{ns}	0.29^{ns}	0.66**	0.61**	0.24^{ns}	0.56*	0.48*	0.49*	0.73**	1.0										
PDW	0.31^{ns}	0.28^{ns}	0.51*	0.67**	0.37^{ns}	0.59**	0.68**	0.53*	0.77**	0.50*	1.0									
GDW	0.44^{ns}	0.39^{ns}	0.72**	0.80**	0.42^{ns}	0.64**	0.75**	0.66**	0.84**	0.68**	0.76**	1.0								
TDW	0.27^{ns}	0.65**	0.70**	0.71**	0.37^{ns}	0.69**	0.68**	0.59**	0.77**	0.71**	0.57**	0.80**	1.0							
[Zn]leaf	0.41^{ns}	0.25^{ns}	0.54*	0.59**	0.45^{ns}	0.55*	0.56*	0.38^{ns}	0.51*	0.49*	0.46^{ns}	0.72**	0.66**	1.0						
[Zn]seed	0.35^{ns}	0.30^{ns}	0.57**	0.55*	0.37^{ns}	0.57**	0.57**	0.27^{ns}	0.47^{ns}	0.52*	0.39^{ns}	0.66**	0.69**	0.96**	1.0					
[Mn]leaf	−0.03^{ns}	0.37^{ns}	0.41^{ns}	0.53*	0.35^{ns}	0.45^{ns}	0.37^{ns}	0.66**	0.62**	0.47^{ns}	0.59**	0.57**	0.40^{ns}	−0.06^{ns}	−0.14^{ns}	1.0				
[Mn]seed	0.08^{ns}	0.37^{ns}	0.30^{ns}	0.41^{ns}	0.28^{ns}	0.29^{ns}	0.26^{ns}	0.65**	0.50*	0.32^{ns}	0.54*	0.52*	0.37^{ns}	−0.05^{ns}	−0.18^{ns}	0.88**	1.0			
P%	−0.20^{ns}	0.42^{ns}	0.21^{ns}	0.31^{ns}	0.75**	0.46^{ns}	0.24^{ns}	0.55*	0.29^{ns}	0.25^{ns}	0.45^{ns}	0.46^{ns}	0.20^{ns}	0.20^{ns}	0.18^{ns}	0.64**	0.52*	1.0		
TF50	−0.18^{ns}	0.14^{ns}	−0.17^{ns}	−0.14^{ns}	0.22^{ns}	−0.03^{ns}	−0.38^{ns}	0.55*	−0.28^{ns}	−0.31^{ns}	0.05^{ns}	−0.17^{ns}	−0.21^{ns}	−0.26^{ns}	−0.40^{ns}	0.21^{ns}	0.45^{ns}	0.21^{ns}	1.0	
THM	−0.01^{ns}	0.13^{ns}	−0.15^{ns}	−0.15^{ns}	0.56*	0.02^{ns}	−0.20^{ns}	0.09^{ns}	−0.22^{ns}	−0.18^{ns}	0.09^{ns}	−0.01^{ns}	0.13^{ns}	0.15^{ns}	0.03^{ns}	−0.04^{ns}	0.23^{ns}	−0.43^{ns}	0.55*	1.0

Ns, * and **: Non-significant, significant at 5% and 1% levels of probability, respectively. H: Plant height, ^{Ns}: Number of subbranch, NPP: Number of pod per plant, WS: 100-seed weight, SY: Seed yield, BY: Biological yield, HI: Harvest index, SDW: Stem dry weight, LDW: Leaf dry weight, PDW: Pod dry weight, GDW: Grain dry weight, TDW: Total plant dry weight, [Zn]leaf: Leaf Zn concentration, [Zn]seed: Seed Zn concentration, [Mn]leaf: Leaf Mn concentration, [Mn]seed: Seed Mn concentration, TF50: Time to 50% flowering, and THM: Time to harvest maturity, Zn: Zinc, Mn: Manganese

Table 7: Analysis of variance for the impact of Zn and Mn sulfate fertilization on yield and yield components of chickpea

SOV	df	Ms							
		H	NS	NPP	NSP	WS	SY	BY	HI
Replication	2	1.199	1.018	10.677	4.549	13.819	99.818	218.992	36.441
Cultivar	2	121.484**	0.946*	14.377ns	6.821ns	168.103**	269.883ns	577.271ns	30.705*
Error a	4	2.461	0.106	5.646	7.041	6.645	121.968	410.814	2.057
Foliar treatment	2	53.483ns	0.831*	94.720**	147.740**	65.906**	1501.200**	4331.940**	226.757**
Cultivar×Foliar treatment	4	3.807ns	0.033ns	1.512ns	0.772ns	5.596*	60.919ns	69.484ns	10.708ns
Error b	12	16.186	0.211	10.036	10.568	1.282	160.472	344.059	8.495
Coefficient of variation (%)	-	10.07	13.65	18.69	16.14	4.42	12.00	9.01	6.14

ns, * and **: Non-significant, significant at 5% and 1% levels of probability, respectively, H: Plant height, NS: Number of subbranch, NPP: Number of pod per plant, WS: 100-seed weight, SY: Seed yield, BY: Biological yield, HI: Harvest index, SOV: Source of variation, Zn: Zinc, Mn: Manganese

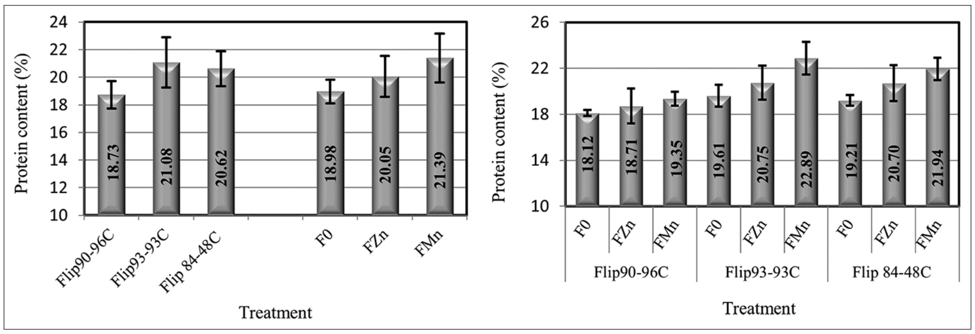

Figure 1: The effect of zinc and manganese sulfate on seed protein content of chickpea

in Zn spraying, and the highest concentration of Mn in the leaves (39.09 mg/g) and grain (40.65 mg/g) was recorded in Mn spraying. Balai and Hidoto [38,39] studies showed that with Zn application, Zn concentrations in seeds and shoots of chickpea were increased. Interaction effects of cultivars and Zn and Mn spraying on Zn and Mn concentration in chickpea leaves and stems were not statistically significant, but according to Table 7, the highest Zn concentrations in leaf (48.25 mg/g) and grain (75.31 mg/g) in Flip 93–93C cultivar and the highest Mn concentration in leaf (42.65 mg/g) and seed (40.16 mg/g) were observed in Flip 84–48C and Flip 93–93C cultivars, respectively. Existence of sufficient amounts of nutrients in crop seed is one of the important factors in increasing the quality of crop production, which depends on the process of absorption and remobilization from other parts of the plant to the seeds [40,41]. There is a lot of difference between elements in terms of remobilization process so that some elements have little ability to retransfer from other organs to the grain [20]. In our experiment with Zn fertilization, Mn concentration in leaf and seed in all of the cultivars was increased. Furthermore, similar results were obtained with Mn spraying. Zn concentrations increased in leaves and grains by Mn spraying [Table 7]. There was a positive and significant correlation at 1% level between Zn concentration in leaf with dry weight of grain (r = 0.72**) and dry weight of total plant (r = 0.66**). Furthermore, there was a positive and significant correlation between Zn concentration in grain with dry weight of grain (r = 0.66**) and dry weight of total plant (r = 0.69**) [Table 2]. The highest and lowest protein content in chickpea seed were measured in Flip 93–93C (21.08%) and Flip 90–96C (18.73%), respectively [Figure 1]. On the other hand, Mn spraying had the greatest effect on the increase in protein content of chickpea seed compared to other treatments so that Mn spraying increased the protein content of chickpea seeds by 12.69% compared to the control treatment (non-spraying). Research conducted by Balai *et al*., and Choudhary *et al.* [38,42] showed that Zn intake could increase protein in chickpea seeds. They stated that Zn consumption as compared to non-intake treatment could increase the protein content of chickpea seed. According to Seguin and Zheng [43], the balance nutrition of the plant and the optimal use of chemical fertilizers can affect the amount of oil and protein in the crops. As can be shown in Figure 1, Mn spray had a great influence on the protein content of the Flip 93–93C variety (22.89%). In all cultivars, the least amount of protein belonged to the check treatment. In this experiment, increase in the amount of protein resulting from the Zn spraying in all three varieties was very low.

4. CONCLUSION

The results of this experiment showed that Zn and Mn elements increased the dry weight of the pod, and there was no significant difference between these two elements. Among cultivars, the Flip 84–48C had more ability to transfer Zn from leaves to seeds. In this study, synergistic effects between Zn and Mn were observed so that the application of each of these elements increased the concentration of others in chickpea leaves and seeds. Mn application increased Zn concentration in leaves and chickpea seed. In fact, Mn improves the storage of Zn in the leaf and transfers it to chickpea seeds.

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