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ORIGINAL ARTICLE

Assessment of the Effect of Zinc Sulfate Biofortification on the Quantity and Quality Characteristics of Spring Wheat Cultivars

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ABSTRACT
Given the extent of zinc deficiency in the Iranian soils and human society, biofortification of wheat and other highly consumed foods with zinc content is one of the major challenges to lower zinc deficiency in plant communities and consequently in human societies. Accordingly, in this study, a factorial experiment was performed with 3 replications using a randomized complete block design to assess zinc biofortification impact on wheat. The study factors included the 2 cultivars of Shiroodi and Arta spring wheat and 7 zinc sulfate fertilizer treatments consisting of control (no spraying), spraying of 0.5% zinc sulfate in the booting stage, spraying of 0.5% zinc sulfate plus urea in the booting stage, spraying of 0.5% zinc sulfate in the milky stage, spraying of 0.5% zinc sulfate plus urea in the milky stage, spraying of 0.5% zinc sulfate in the dough stage, and spraying of 0.5% zinc sulfate plus urea in the dough stage of the grain conducted in the research field of College of Agriculture, University of Maragheh. The results obtained showed that spraying of zinc sulfate increased thousand kernel weight, economic yield, harvest index in the milky, and grain zinc concentration in the booting stage, while Arta cultivar was further affected by the spraying compared to Shiroodi cultivar. Thus, from this experiment, it is concluded that spraying wheat with zinc sulfate can lead to improved yield and enhanced zinc concentration of the grain.
Key Word: *Biofortification, Zinc Sulfate, Harvest Index, Zinc Content, Wheat*

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INTRODUCTION

Wheat is the first cereal in terms of nutritional value and the most important crop in the world. Because of a broad compatibility with a cultivar of weather conditions, the mentioned plant owns a higher scattering range than any other plants [13]. The cultivation of crops, especially wheat, comprises the main agricultural activity in semi-arid areas of the world, especially Iran [12].
Humans need at least 22 elements for health [35, 36, 14 and 15], which must be provided with proper nutrition. However, more than two-thirds of the world's population is facing the lack of one or more of an element of micronutrients in their diets. From among the elements, zinc and iodine account for the highest deficiencies in nutrition after iron [10]. This is more related to the lack of food diversity or small amounts of these elements in the meals. The deficiencies could be overcome by creating diversity in the diet, using supplements, enriching foods, enhancing concentrations, and providing bioavailability of micronutrients in the products. Due to the fact that in many cases, there is no possibility of creating diversity in the diet because of poverty or lack of access to food cultivar, the best solution to the problem would be addition of micronutrients such as zinc that provides a major contribution to human nutrition to the foods [17].
Like in humans, nutrients play a very important role in feeding and increasing yield of plants [7]. Lack of nutrients causes a limited plant growth and eventual reduction of plant yield. Based on plant quantitative needs, the essential elements are divided into the two groups of micronutrients and macronutrients. Micronutrients improve plant general conditions and act as a catalyst in the biochemical reactions in plants [24]. Iranian agricultural soils own a very small amount of micronutrients, the solubility of which is

low due to the calcareous property, high pH, and low organic matter of the soil, bicarbonated irrigation water, drought and salinity stress, and persistence of unbalanced use of fertilizers [1]. Zinc is an essential element for plant growth and its constant and continuous use is necessary for plant optimal growth and maximum yield achievement [8]. This element is either used as a part of enzymatic structure or acts as the regulating cofactors in a large number of enzymes. Zinc is used to make DNA (2, 22, 5 and 37). Aside from this, it plays a role on the evolution of membrane structure and its stability against various injuries [28]. Zinc deficiency in calcareous soils is considered as a major problem for plant and human nutrition. Typically, its absorbable amount by plant is less than 0.7 mg/kg in the Iranian soils, while this amount should be about 1 mg/kg at optimum conditions. Plants that grow in such soil suffer from zinc deficiency. In addition, the unbalanced use of fertilizers, especially, excessive and abundant application of phosphate and nitrogen-containing fertilizers, unprevalence of zinc-containing fertilizers, and the adverse effect of phosphorus on zinc reduce the absorption of this element in agricultural products [28]. Zinc deficiency in humans can be solved by increasing its concentration in cereals (22 and 10). An important strategy for enhancing the concentration of micronutrients in grains is plant fertilization with soil or spraying [7 and 4]. Spraying is one of the primary methods in zinc application. For example, in a study in Egypt, zinc spraying significantly increased all the plant growth parameters in broad beans [32]. This technique also enhanced millet seed yield due to zinc appropriate concentration [40]. Furthermore, zinc spraying doubled its concentration in corn grains [26 and 38]. Velu *et al* [34]by using different methods of zinc sulfate application in different cultivars of wheat, Volvo et al. observed that it not only increases yield significantly, but also the concentration of this element in wheat grain, thus leading to enriched grains. Through the examination of the effects of iron, manganese, zinc, and copper on the yield and quality of wheat grain in the calcareous lands of Dorudzan area in Shiraz, Ziaeian and Malakooti [39] reported that seed yield enhances by the consumption of micronutrients. But given that zinc spraying brings about various results in different developmental stages and no consistent results are available from multiple studies, the current research investigated the effects of zinc sulfate at different spraying times on wheat seed yield, yield components, and zinc content.
Given the extent of zinc deficiency in the Iranian soils and human society, biofortification of wheat and other highly consumed foods with zinc content is one of the major challenges to lower zinc deficiency in plant communities and consequently in human societies.

MATERIALS AND METHODS

The investigation was conducted in the research field of Maragheh University of Agriculture located in the south of East Azerbaijan province in the city of Maragheh in 2012. Geographically, the area is located within 37/37 North Latitude and 46/27 East Longitude and at a height of 1475 m above sea level. The study was implemented with 3 replications by using a factorial experiment with a randomized complete block design. The study factors included 2 cultivars of Spring, Shiroodi, and Arta wheat associated with 7 zinc sulfate fertilizer treatments consisting of control (no spraying), spraying of 0.5% zinc sulfate in the booting stage, spraying of 0.5% zinc sulfate plus urea in the booting stage, spraying of 0.5% zinc sulfate in the milky stage, spraying of 0.5% zinc sulfate plus urea in the milky stage, spraying of 0.5% zinc sulfate in the dough stage, and spraying of 0.5% zinc sulfate plus urea in the dough stage of the grain, which comprised a total of 42 treatment combination. Each testing plot size was determined to be 6 m^2 and the seeds were cultivated in 6 rows per plot by regarding the distances of 20 cm and 1 cm between and on the rows in each plot, respectively. During the cultivation, topdressing fertilizer was released by hand-spraying in the 3-4 leaf stage only once. Farm irrigation was performed by flooding and the first irrigation began with the end of planting operation. The next irrigations were applied every 7 days, taking into account the seasonal rains. Weed combat operation was conducted as hand weeding in the field during the project implementation.
To study the characteristics of the field experiment, two-row side plantations of 25 cm from the beginning and end of each plot were considered as the marginal effect, but were excluded at the time of sampling. The characteristics and their method of measurement were as follows:
The number of spikes per unit area: Each test plot was obtained by counting the number of fertile spikes in 1 m^2.
Seed number per spike: Their number was counted in 15 spikes and then their average was estimated for 1 spike.
Thousand Kernel Weight (TKW): For its precise measurement, 3 samples of 1,000 kernels of seeds per plot (in each iteration) were counted separately after harvesting and weighed by sensitive scales. The average of the resulting numbers was considered as the TKW of the desired treatment in each iteration.

The number of fertile tillers: The fertile spikes of each of the 15 plants harvested were counted and their average was regarded as the number of fertile tillers per plant for the treatments.
Biomass: After elimination of the marginal effect, an area of 1 m^2 of the competing plants was harvested and dried in the sun for 5 days to determine the biomass. Then, the total weight of each plot in the mentioned area was measured by an exact scale and used as a biomass in the estimates after converting it to unit area (kg/ha), biological function.
Seed yield: The plants harvested from 1 m^2 were threshed and their grain weights were recorded as the seed yield per unit area (kg/ha) after weighing.
Harvest Index (HI): HI percentage was calculated based on constant moisture and dry sun from the following relation:

$$HI = (\text{economic yield} / \text{biomass}) \times 100$$

Grain protein content: The protein content of the grasins harvested seeds from the experimental plots was measured in triplicate with the use of Zeltex (ZX50) seed portable analyzer device. In each measurement, the device shows 2 numbers for grain protein content and the average of these two numbers is considered as one repetition.
Seed zinc concentration: Measurement of the zinc concentration of the seeds harvested from the experimental plots was performed by using wet ashing [16] in the laboratory of the Department of Chemistry, University of Maragheh and with the help of the atomic absorption spectrophotometer (Shimadzu) and the zinc concentrations of the seeds were subsequently determined.
After testing the normality of data distribution and homogeneity of variances, analysis of variance was conducted as factorial for the field characteristics based on randomized complete blocks. All the statistical analyses and mean comparisons were performed using SPSS software. For the comparison of means, Duncan's multi-range method was used at a probability level of 5%. Drawing of the shapes was carried out by utilizing Excel software.

RESULTS AND DISCUSSION

Based on the results of the current study, the number of tillers per plant and the number of spike per unit area were not affected by the spraying and cultivar (Table 1). Among the available cultivars, Arta cultivar represented a greater number of spikes compared to Shiroodi cultivar though the difference was not significant. It should be noted that there is no possibility of producing fertile spikes in the potential number of the plant tillers since the emergence of seedlings until producing 6-8 visible leaves on the main stems [33] and after the 4-6 leaf stage [19]. Thus, it is obvious that spraying zinc sulfate in the reproductive and seeding stages in the present study could have had no impacts on the mentioned traits. Khan *et al* [19] reported that soil zinc sulfate application leads to increasing wheat yield with the increase of spikes per unit area. To, the results of Shekari *et al* [29] confirmed the above finding by demonstrating that only the application of soil zinc sulfate was able to significantly enhance the number of spikes per unit area compared to the control and spraying treatments.

Table 1. Analysis of variance squares of the traits affected by the use of zinc sulfate treatments and drought stress.

Sources of Variation	Df	number of Spikes	number of tillers	TKW	number of grains per spike	Yield	Biological Yield	Harvest Index
Zinc Sulfate	6	3558.278	156.690	1.625*	55.690	233.174*	7190.898	272.37**
Cultivars	1	814.881	354.381	0.024*	146.720*	526.469	28600.380*	90.497*
Zinc Sulfate*Cultivars	6	4809.659	164.770	8.038	58.817	1374.220*	24423.061	5.092
Error	28	1264.676	145.423	18.529	36.652	514.709	4967.334	11.73

**Significant at 1 % * Significant at 5% ns Not-Significant

Based on the results of the analysis of variance (Table 1), seed number per spike was not affected by zinc sulfate treatment, but the figures revealed significant differences in the view of this trait. Also, zinc sulfate interactions in the cultivars were not significant based on this characteristic (Table 1). From among the cultivars, The cultivar Arta had a greater number of grains per spike compared to Shiroodi (Figure 1). The results of this research were expected since zinc sulfate was applied after the formation of grains per spike from a phenological viewpoint. In other words, achievement of such a result is normal with regard to zinc spraying stages in the current test, including the booting and subsequent stages. In fact, after the arrival of plant at the reproductive stage (after the formation of double ridges in APX) coinciding with the onset of the internodes, initiation of the end spikes would occur (11).

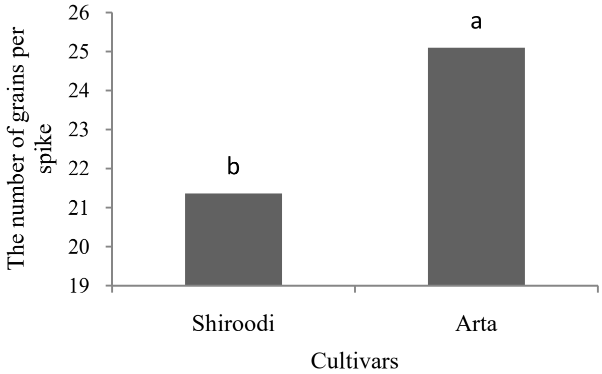

Figure 1. Changes in the number of grains per spike in wheat

According to the results of the data analysis of variance (Table 1), it was found that the effect of zinc sulfate spraying at the level of 5% is significant on TKW components. Accordingly, from among the spraying of zinc sulfate treatments, the highest TKW related to the use of zinc sulfate with urea in the milky stage of the grains (Figure 2). Within the entire spraying process, the greatest effect on TKW was obtained when zinc sulfate was used with urea, the values of which were significantly different from those of pure zinc sulfate. The mean comparison of the main effects of zinc sulfate spraying on TKW components in the study cultivars indicated that seeds Shiroodi grains had a higher TKW compared to Arta grains (Figure 3). Due to the fact that the spraying was carried out during the grain filling and taking into account that the element deficiency is considered as one of the most important factors in reducing yields in the mentioned scope (14), spraying of zinc sulfate with urea with a direct effect on grain filling on the one hand and retardation of aging of the photosynthesizing organs resulting in increasing leaf area duration (LAD) on the other hand can lead to improved TKW. It should be noted that due to the larger number of grains per spike in Arta cultivar, the assimilates produced were distributed among a greater number of seeds and, therefore, the share of each grain was reduced compared to when less number of grains existed and the assimilates received by each grain were greater. Similar results were reported by Soleimani [31] and Pol Shekane Pahlevan *et al* [27]. In another study, the effect of zinc spraying was examined on bread wheat at the grain filling stage and it was observed that zinc spraying increased TKW in some of the study cultivars [25].

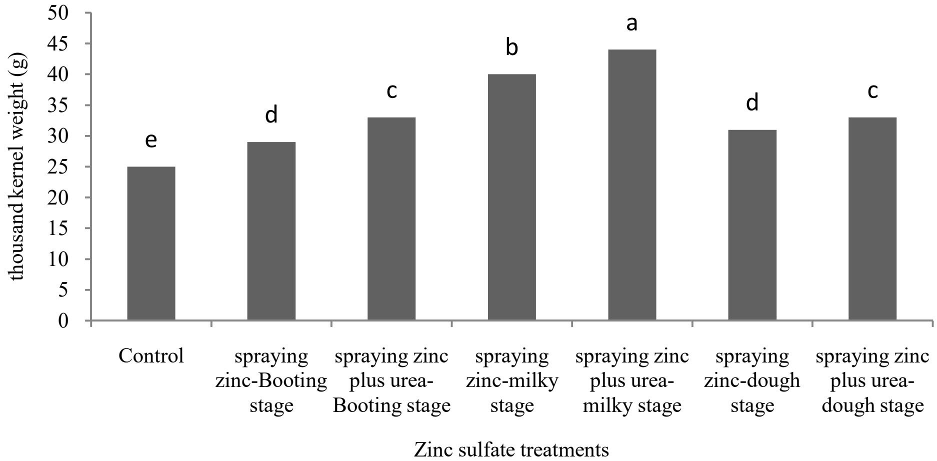

Figure 2. Changes in thousand kernel weight by spraying the compounds of zinc sulfate

Zinc sulfate treatments did not show significant differences in terms of biomass. However, there were significant differences among the cultivars based on this trait (Table 1). In this regard, Arta cultivar had a higher biomass compared to Shiroodi cultivar (Figure 4). As mentioned in the case of number of seeds per plant, plant biomass was completed before the spraying treatment and hence the treatments had no effects on this feature. In a similar research, Khan *et al* [18] confirmed the same result.

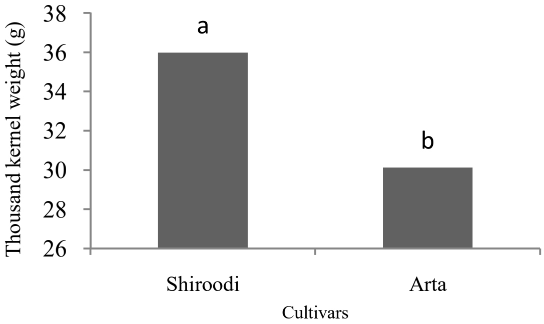

Figure 3. Changes in Thousand kernel weight in wheat

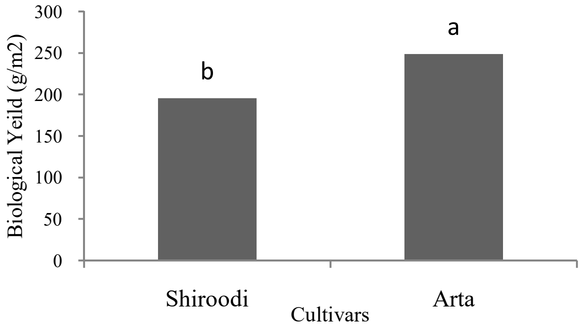

Figure 4. biological yield changes in wheat

From among the main effects, only the use of zinc sulfate had a significant effect on economic yield and the cultivars did not show a significant difference in terms of this trait (Table 1). From among the zinc sulfate spraying treatments, the best economic yield was obtained when the fertilizer was sprayed with urea. Also, concerning the time of spraying, the best time was at the milky stage. It seems that regarding TKW significance in the evaluation of yield components, the significance of the mentioned traits was related to this part of practice. As mentioned above, by affecting the process of photosynthesis and increasing LAD, zinc sulfate led to the enhancement of the grain filling duration and eventually TKW. With respect to the aforementioned issues, Shiroodi seeds were larger than Arta seeds, while seed number per spike was reverse in the former cultivar. Thus, the higher economic yields in Shiroodi and Arta cultivars could be affected by TKW and seed number per spike, respectively. In this way, by having a strong point, each cultivar represented a similar economic yield. The interaction between zinc sulfate application and the cultivars was significant based on economic yield (Table 1). According to Figure 5, it is observed that the use of pure zinc sulfate is better than its application with urea for Arta cultivar based on economic yield, while the application of zinc sulfate associated with urea has a higher effect on the improvement of this trait in the case of Shiroodi cultivar (Table 3). Bybordi and Mamedov [6] showed that the use of zinc increased Rapeseed yield. In general, it appears that depending on the cultivar, the type of zinc fertilizer used, time of application, amount of fertilizer, and the interaction with environmental factors, the consumption of this element can enhance the number of spikes, Number of spikes per square meter, grain weight, starch, protein, indole acetic acid (IAA), delay aging, and thus increase the yield. Pol Shekane Pahlevan *et al* [27] and Soleimani [31] observed that zinc sulfate increased wheat seed yield. A number of researchers consider zinc fertilizer application beneficial for the improvement of yield and yield components [9, 30 and 17].

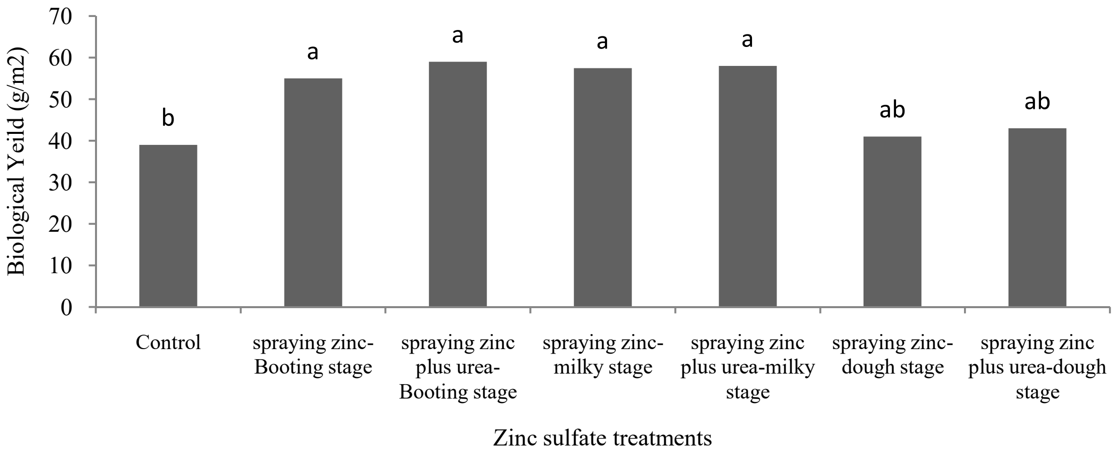

Figure 5. Changes in economic yield by spraying the compounds of zinc sulfate

Harvest Index (HI) represents the amounts of stored and sent materials in and from the grain and generally the entire material produced within the vegetative and reproductive periods, which is always emphasized by researchers as a valuable trait in evaluating the production of crops (11). Zinc sulfate treatments at the level of 5% showed significant differences based on harvest index (Table 1). In this regard and in most cases, the use of zinc sulfate increased HI. In particular, in the milky stage, it was remarkable compared to control and other spraying treatments (Figure 7). It has been reported that any factors that alleviates the existing limitations to plants will lead to the improvement of HI at the reproductive and mature stages of wheat. One of these cases is the bioavailability and optimization of plant nutrition (28). It should be noted that at the time of wheat seeding, plant root organ is actually disabled. Therefore, spraying of micronutrients can improve HI (41). Also, wheat cultivars had significant differences based on HI. From among the cultivars, Shiroodi cultivar represented a greater harvest index than Arta cultivar (Figure 8). In a study, Bakht *et al* [3] reported that zinc sulfate enhances harvest index in wheat.

Table 3. compare means of economic yield in Homa cultivars under the influence of Zinc Sulfate and drought stress

Economic Yield							Treatment
Spraying Zinc Plus Urea-dough Stage	Spraying Zinc-Dough Stage	Spraying Zinc Plus Urea-Milky Stage	Spraying Zinc-Milky Stage	Spraying Zinc Plus Urea-Booting Stage	Spraying Zinc-Booting Stage	Control	
48.11 ab	44.5 ab	55.9 ab	62.32 a	53.12 ab	64.4a	40.16 bc	Arta
46.11 ab	42 ab	60.01 a	53.13 ab	65.21 a	44.13 ab	36.12 bc	Shiroodi

According to Figure 9, all the levels of zinc sulfate spraying increased grain zinc concentrations compared to the control seeds. Also, among the spraying treatments, spraying at the booting stage had a greater effect on the grain zinc reserves. From among the cultivars, the seed zinc amount of Arta cultivar was significantly higher than that of Shiroodi cultivar. Assessment of the mean comparisons of the treatments revealed that based on the biofortification of seed zinc, the use of zinc sulfate in the treatments of the booting and dough stages had the highest accumulation of this element in the Arta cultivar. Accordingly, the lowest grain zinc concentration was related to the control treatment of Shiroodi cultivar. Furthermore, Ozturk *et al* [23] showed the highest zinc levels of the grain are obtained when spraying it at the milky stage. Cakmak *et al* [9] and Kutman *et al* [21] noted that zinc spraying of wheat leaves is much more effective in increasing the grain zinc concentration than its soil application and its use in the early stages of seed development can be further beneficial. They also stated that the repetition of this practice can significantly enhance zinc concentration of the grain. In addition to the above study, Zhang *et al* [38] concluded that the use of Zn biofortification in wheat causes an increase of the grain zinc concentration by 50% and that of the flour up to 76%.

Table 4. compare means of Zinc Content in Homa cultivars under the influence of Zinc Sulfate and drought stress

Zinc Content							**Treatment**
Spraying Zinc Plus Urea-dough Stage	Spraying Zinc-Dough Stage	Spraying Zinc Plus Urea-Milky Stage	Spraying Zinc-Milky Stage	Spraying Zinc Plus Urea-Booting Stage	Spraying Zinc-Booting Stage	Control	
0.38 c	0.28 g	0.3 e	0.3 e	0.29 f	0.5 a	0.25 h	Arta
0.28 g	0.32 d	0.32 d	0.39 b	0.38 c	0.29 f	0.21 i	Shiroodi

The results of the present research revealed that the types of cultivar and zinc sulfate application had no significant effects on the components of grain protein content. Shekari *et al* (2015) stated that the use of zinc sulfate had no significant impact on grain protein level. Unlike in this study, Sadri and Malakooti reported that Zinc sulfate consumption increases protein content besides yield enhancement. Omidian et al. showed that zinc sulfate spraying has a significant effect on canola seed protein content.

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