Vol. 14(20), pp. 867-876, 16 May, 2019
DOI: 10.5897/AJAR2018.13593
Article Number: F09517F61023
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Foliar application of zinc sulphate to improve yield and grain zinc content in wheat (*Triticum aestivum* L.)

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Received 2 October, 2018; Accepted 6 December, 2018

Foliar application is a promising agronomic strategy as it involves direct adsorption and loading of nutrients from leaf surface to phloem in comparatively far less quantity than soil applications. Present investigation entails the evaluation of most suitable treatment of zinc sulphate to improve growth and yield components of wheat. Significant increase in leaf length, leaf area, plant height, number of tillers, spike length, number of grains per spike, plant fresh weight, yield per plant, total soluble proteins and grain zinc content at 4 and 6 mM foliar treatments of zinc sulphate advocates 4 mM treatment more appropriate from economic perspective.

Key words: Wheat, zinc deficiency, foliar application.

INTRODUCTION

Zinc deficiency in soils, is an important constraint after nitrogen, phosphorus and potassium (Quijano-Guerta et al., 2002; Khan et al., 2008). Soil parent material, its weathering process, and frequent cultivation are the factors that reduce soil zinc availability (Almendros, 2008; Das, 2014). Zinc becomes unavailable due to its adsorption to oxides and hydroxides of Fe and Al and because of antagonistic effects of other divalent cations such as P (Lohry, 2007). Soil organic matter and temperature increases zinc availability as certain chelating agents are released on decomposition; while leaching and soil leveling erase top soil decreasing availability of zinc (Broadley et al., 2007; Kabir et al., 2014). Crops grown on zinc deficient soils exhibit chlorotic or necrotic spots on leaves, short stature of plants, uneven crop stand, delayed maturity, improperly developed fruits, decresed yield and low nutritional quality (Broadley et al., 2007; Alloway, 2008). This is because zinc is an important cofactors of more than 300 enzymes involved in different physiological pathways; maintains integrity of plasma membrane preventing plants from pests and insects and controls auxin levels in shoots and buds of plants. (Auld, 2001; Alloway, 2008; Nishizawa, 2015). Consumption of such zinc deficient crops as major staple food augments its inadequacy in humans and one-third of the world's population suffers from zinc deficiency (Hotz and Brown, 2004; WHO, 2009; Stein, 2010) evident in the form of impaired growth, slow healing of wounds, dermatitis, impaired appetite and anemia (Kiekens, 1995; Hambridge, 2000). Children under the age of five suffer from impaired immunity leading to diarrhea, pneumonia and malaria due to zinc deficiency (Hotz and Brown, 2004; Wessel and Brown, 2012). Zinc is also important in nucleic acids and protein

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synthesis and acts as neurotransmitter for being involved in cell signaling mechanism (Tapeiro and Tew, 2003; Hershfinkel et al., 2007). Zinc deficiency in humans is prominent in the areas where people mainly depend upon cereals such as wheat as their major staple food (Gibson, 2011; Cakmak, 2008). Wheat is an important staple food all over the world and it is cultivated over 240 million hectares worldwide (Wajid, 2002). Cereal grains should ideally contain 50 to 60 mg kg^{-1} zinc to fulfill the recommended daily dietary intake of 15 mg for young adults (WHO, 2009). Since, most of the existing wheat varieties are reported to contain zinc up to 29 mg kg^{-1} (Losak et al., 2011). Therefore, it is essential to develop some sustainable agronomic strategy to combat zinc deficiency (Mayer et al., 2008). In this context, foliar application is reported to be an uncomplicated, nominal and sustainable solution to address micronutrient malnutrition. (Graham, 2008; Voogt et al., 2013; Cakmak et al., 2010b). This method requires careful monitoring of crop genotype; suitable treatment and phonological stage; and soil and environmental conditions in order to get effective results (Shehu and Jamala, 2010; Fageria et al., 2011; Yuan et al., 2013). This method has the advantage of direct absorption of zinc through leaf surface and its prompt loading to phloem resulting in zinc traslocation along with photosynthetic assimilates towards developing grains (Boonchuay, et al., 2013; Shivay et al., 2015). Foliar applications are also reported to be effective where low soil temperature and moisture interferes with the micronutrient absorption (Rehman et al., 2014). Antagonistic effects of P on soil applied Zn can also be mitigated by zinc foliar applications, so that P can be applied at desired level to achieve better yield (Zhang et al., 2012). This method ascertains the economic effectiveness as it usually requires minimal amount of zinc carrier (Rengel et al., 1999; Voogt et al., 2013). This is because zinc is not wasted due to soil fixation and because of leaching or removal of top soil (Rehman et al., 2014). Zinc sulphate is preferably used as a zinc source for foliar applications as it is sparingly soluble, comparatively cheap and is immediately absorbed through the leaf surface; and its little amount (2 to 2.5 kg ha^{-1}) can give desirable results through foliar application (Das et al., 2014; Sarwar et al., 2015). Zinc is reported to be adsorbed and translocated quickly in first 6 to 12 h after foliar application (Doolette et al., 2018).

Keeping in mind economic and staple importance of wheat, the present research work focused to evaluate the zinc foliar treatments growth and yield components of wheat. Signifcant results on different parameters at 4 and 6 mM zinc sulphate treatments help evaluating the economic effectiveness of 4 mM treatment in order to get healthy produce of wheat along with enhanced zinc accumulation in its grains. Consequently, people belonging to poor resource settings who mainly depend on wheat as their daily dietary staple food may benefit with its improved nutritional quality along with fulfilling their daily requirement of zinc.

MATERIALS AND METHODS

Experimental material

Wheat seeds of seven cultivars (Punjab-2011, Faisalabad-2008, Aass-2011, Galaxy-2005, Sehar-2008, Chakwal-50 and Lasani-2006) were collected from Punjab Seed Corporation, Lahore, Pakistan and zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) in 0, 4, 6 and 8 mM (milimolar) concentrations.

Treatments

Three foliar applications of 0, 4, 6 and 8 mM zinc sulphate were given at vegetative phase at an interval of 15 days. Two foliar treatments were then given during grain filling, that is, at milk and dough stage, respectively.

Wheat sowing

This experiment was laid out in a randomized complete block design with four zinc sulphate treatments in two blocks at Seed Centre, University of the Punjab, Lahore in *rabi* season of 2015-2016. The soil of the experimental area was loamy with pH 8.5, EC 0.8, SOM 0.79%, P 1.2 mg kg^{-1} and K 55 mg kg^{-1}. High pH and low organic matter of the soil suggested that soil type used in this investigation could possibly be classified as zinc deficient. Experimental area for wheat sowing was well-prepared with seed beds in rows 15 cm apart. Seeds were sown by hand drill at a seed rate of 60 kg ha^{-1}.

Data collection at vegetative and at reproductive phase

Leaf length, leaf width and leaf area were recorded after one week of each zinc foliar application. Data on chlorophyll content of three randomly selected plants of each stand in the respective subplot were also recorded twice during vegetative phase of crop growth at an interval of 4 weeks; while days to flowering, days to anthesis and days to grain maturity were recorded when 50% of each plant stand exhibited the attribute. Plant height, spike length and number of grains per spike were recorded by randomly selecting plants from each stand.

Crop harvesting and data collection of yield components

The crop was harvested from each sub-plot separately at complete physiological maturity. Three of harvested plants were then randomly selected from each stand of zinc sulphate treatment to record respective plant fresh weight and dry weight. Spikes of the same plants were then cut and threshed manually to record grain yield per plant. Harvest index was obtained by calculating the ratio between the grain yield per plant and the dry weight of respective plants. 1000-grains weight was also recorded for each treatment.

Total soluble protein and grain zinc analysis

Total soluble protein content of grains from each of the four treatments (0, 4, 6 and 8mM) was analyzed by using Biuret method

Table 1. Type III sum of square, mean square and probability of F value of vegetative growth parameters and yield components of wheat at 0, 4, 6 and 8 mM foliar treatments of zinc sulphate.

Trait	Type III sum of square	Mean square	Probability of F
Leaf length (cm)	519.44	173.14	**0.00**
Leaf width (cm)	0.314	0.104	0.32
Leaf area $(cm)^2$	1325.10	441.70	**0.00**
Days to flowering	5.33	1.77	0.31
Days to anthesis	3.05	1.01	0.34
Days to maturity	4.05	1.35	0.24
Number of tillers	66.40	22.13	**0.00**
Spike length (cm)	32.14	10.71	**0.00**
Grains per spike	611.97	203.99	**0.00**
Plant height (cm)	730.44	243.50	**0.00**
1000-grain weight (g)	29.72	9.90	0.72
Plant fresh weight (g)	1172.73	390.91	**0.01**
Plant dry weight (g)	55.84	18.61	0.72
Yield per plant (g)	355.70	118.56	**0.00**
Harvest index	1.58	0.53	0.16
Chlorophyll content index	157.95	52.65	0.13
Total soluble proteins (mg g^{-1})	8.87	2.96	**0.00**
Grain zinc content (mg g^{-1})	0.02	0.01	**0.00**

of Roenson and Johnson (1961). Grain zinc content of samples from each stand was also analyzed by using the method of zinc analysis reviewed by Shar and Bhanger (2001).

Statistical analysis

The data were analyzed by PROC MIXED and PROC GLM in SAS statistical software package 9.3.1 (SAS Institute Inc., Cary, NC, 2001). Least square means of zinc sulphate treatments were calculated through two-way analysis of variance (ANOVA). Type III sum of squares were computed by PROC GLM and means were compared using Duncan's multiple range tests to rank the different treatments.

RESULTS

Improvement in vegetative growth of wheat plants was exhibited by statistically significant increase in leaf length, leaf area and plant height. Reproductive growth also exhibited significant increase in number of tillers, spike length, number of grains per spike, plant fresh weight, yield per plant, increase in total soluble proteins and grain zinc accumulation (Table 1).

Leaf length (cm), leaf width (cm) and leaf area (cm^2)

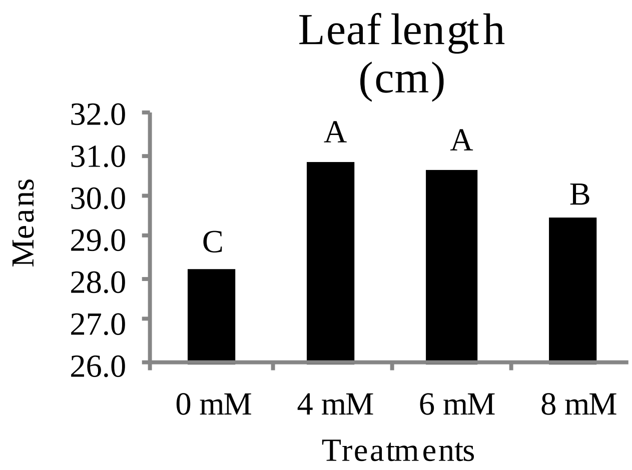
Least square means of observations exhibited maximum increase in leaf length (30.79 cm) at 4 mM. Leaf length increase was in the same range at 4 and 6 mM treatments (Graph 1). Leaf width did not exhibit significant increase and it was recorded to be in the same range at all the four treatments (Graph 2). A significant increase in leaf area was recorded at 4 mM treatment (33.50 cm^2) (Graph 3).

Days to flowering, days to anthesis, and days to maturity

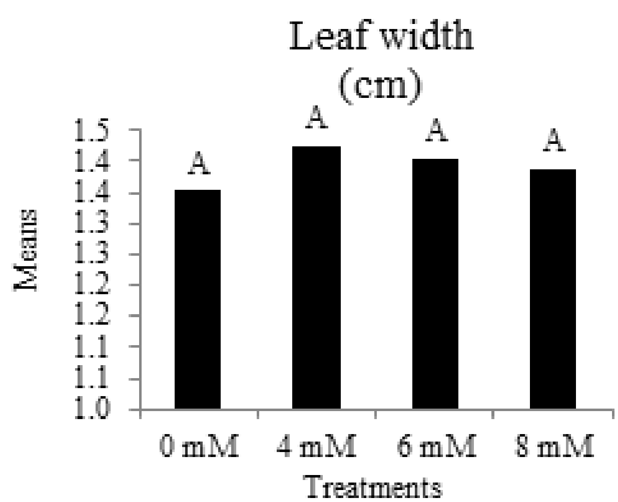
A non-significant change in days to flowering, days to anthesis and days to maturity was recorded at all the treatments. Maximum days to flowering (91.60) were observed at 8 mM; maximum days to anthesis (95.57) were exhibited at 6 mM; and maximum days to maturity (135.57) were exhibited at 6 mM (Graph 4, 5 and 6).

Number of tillers, spike length (cm) and number of grains per spike

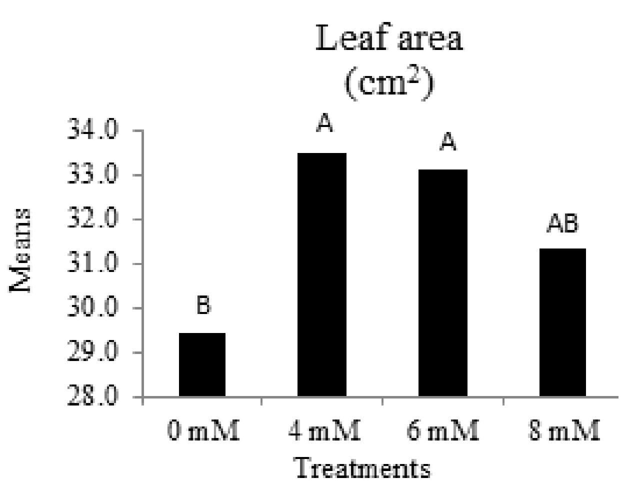
Maximum increase in number of tillers (5.61) was recorded at 4 mM; spike length exhibited significant increase at 6 mM (12.95 cm); and maximum number of grains (73.20) was observed at 4 mM (Graph 7). Spike length was in the same range at 4, 6 and 8 mM with a significant increase over 0 mM (Graph 8). Means and relative grouping of number of grains per spike based on DMRT also displayed that all the three treatments of zinc sulphate were in the same range with significant increase over 0 mM (Graph 9).



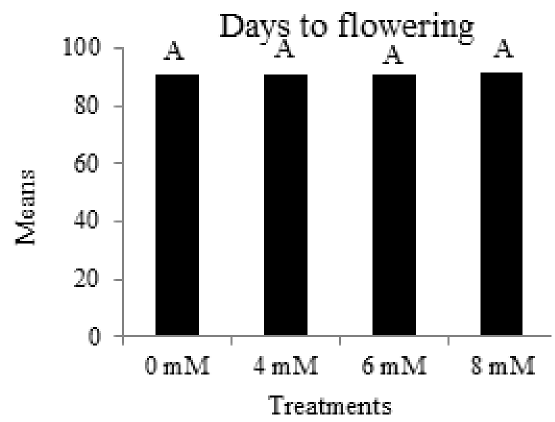
Graph 1. DMRT grouping of leaf length.



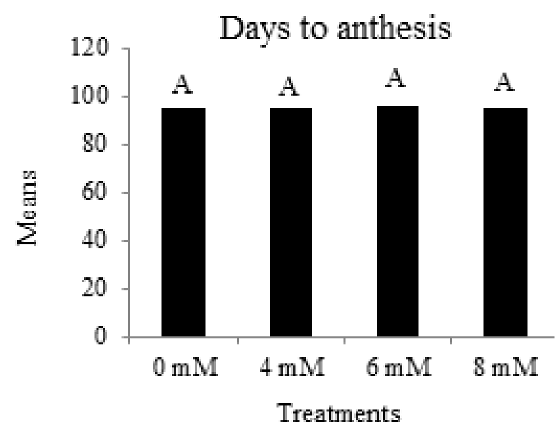
Graph 2. DMRT grouping of leaf width.



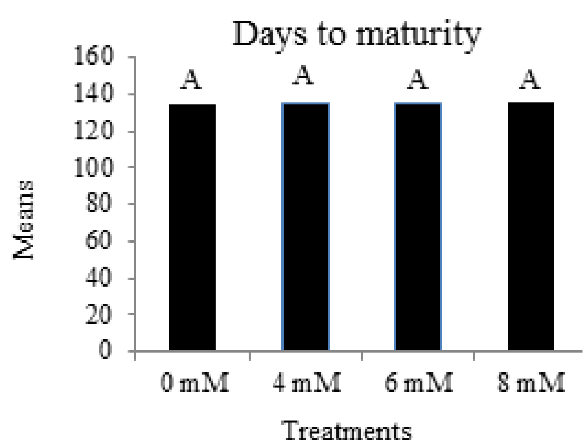
Graph 3. DMRT grouping of leaf area



Graph 4. DMRT grouping of days to flowering



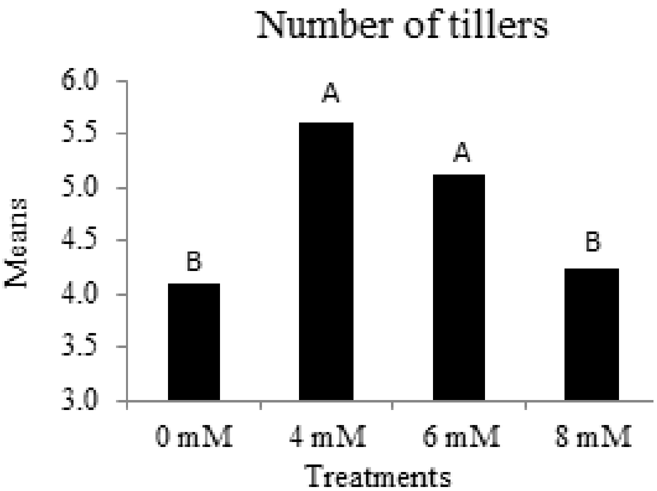
Graph 5. DMRT grouping of days to anthesis



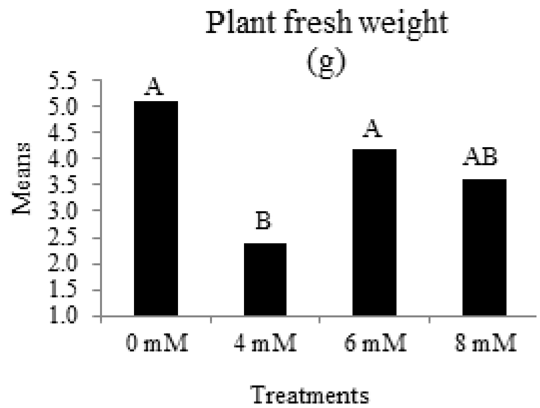
Graph 6. DMRT grouping of days to maturity

Plant fresh weight (g), plant height (cm) and 1000-grain weight (g)

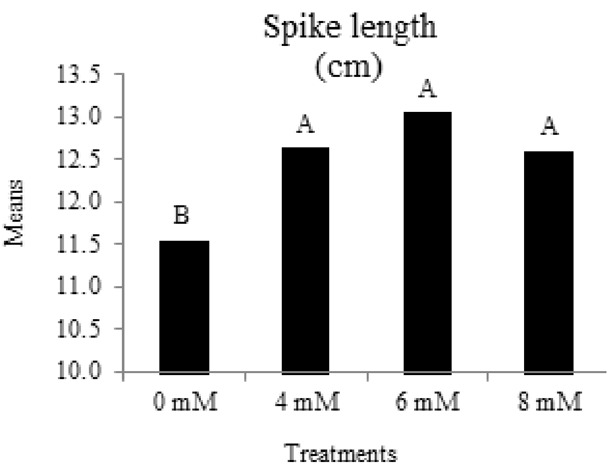
Significant increase in plant fresh weight (34.22 g) was



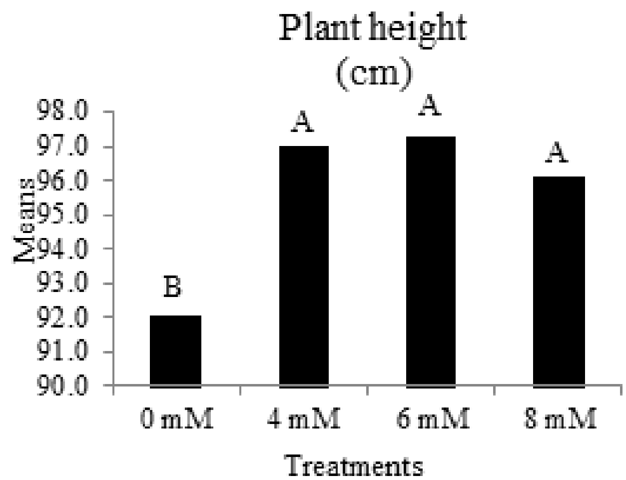
Graph 7. DMRT grouping of number of tillers.



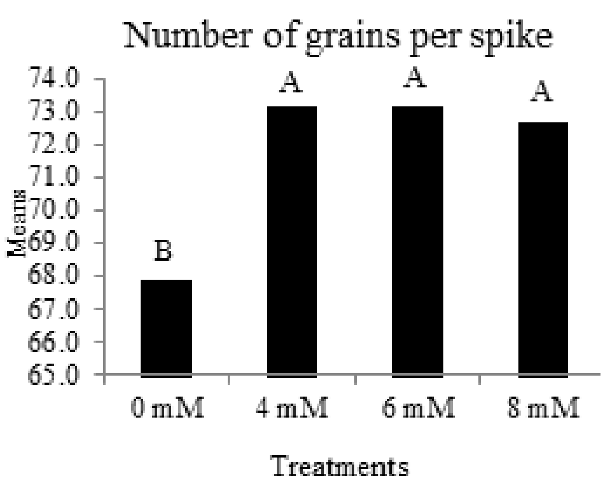
Graph 10. DMRT grouping of plant fresh weight



Graph 8. DMRT grouping of spike length.



Graph 11. DMRT grouping of plant height.

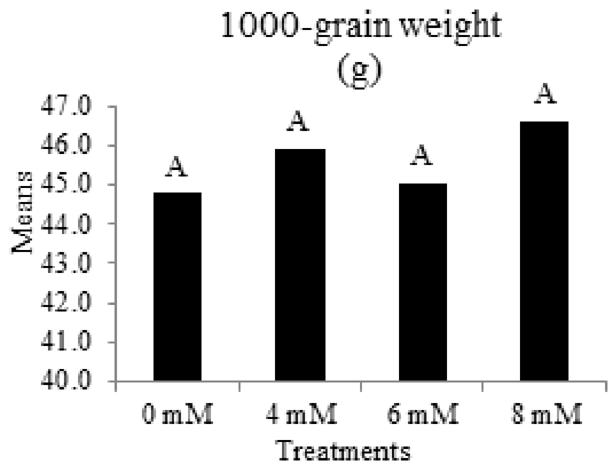


Graph 9. DMRT grouping of grains per spike.

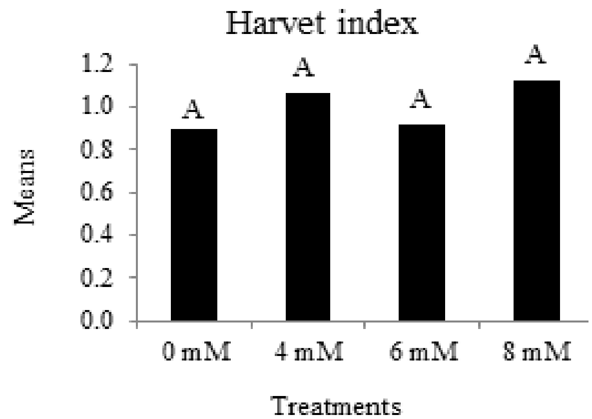
recorded at 4 mM zinc sulphate. Maximum increase (97.27 cm) in plant height was observed at 6 mM zinc sulphate treatment. Non-significant increase in 1000-grain weight was recorded at all the zinc sulphate treatments. Means and relative grouping of plant fresh weight also exhibited significantly higher range at 4 mM (Graph 10). Plant height also displayed significant increase at all foliar treatments of zinc sulphate over control (Graph 11). Non-significant increase in 1000-grain weight over control was also verified by means and their relative grouping based on DMRT in Graph 12.

Yield per plant (g), plant dry weight (g), and harvest index

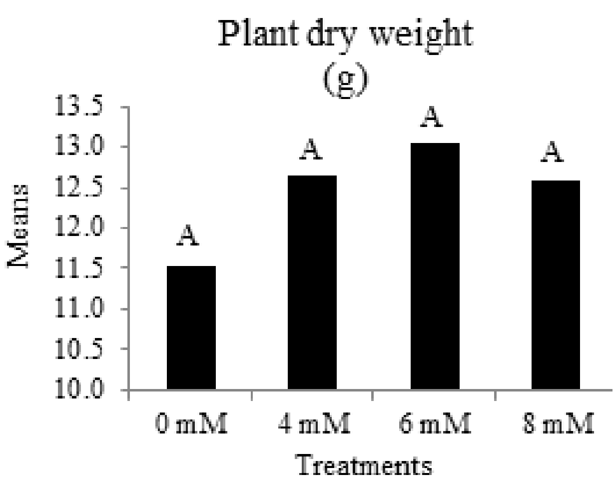
Maximum significant increase in yield per plant (15.60 g) was exhibited at 4 mM zinc sulphate treatment. Plant dry weight however, exhibited a non-significant increase at all



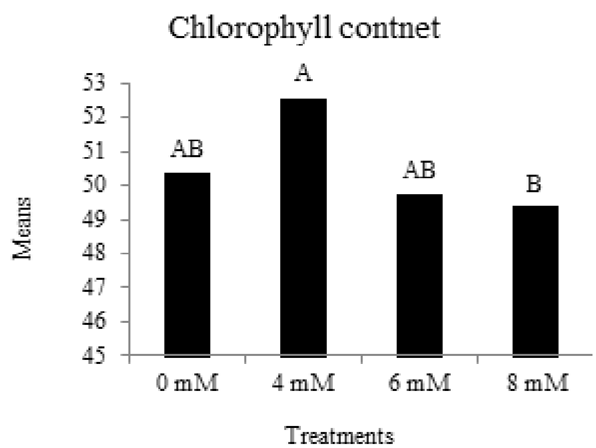
Graph 12. DMRT grouping of 1000-grain weight



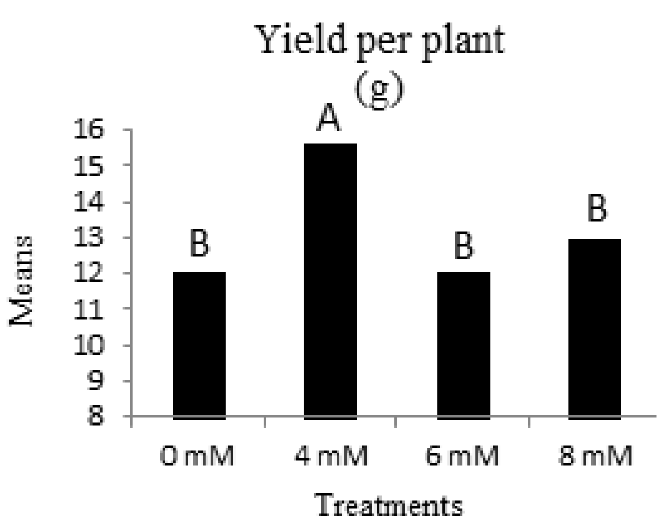
Graph 15. DMRT grouping of harvest index



Graph 13. DMRT grouping of plant dry weight



Graph 16. DMRT grouping of chlorophyll content.

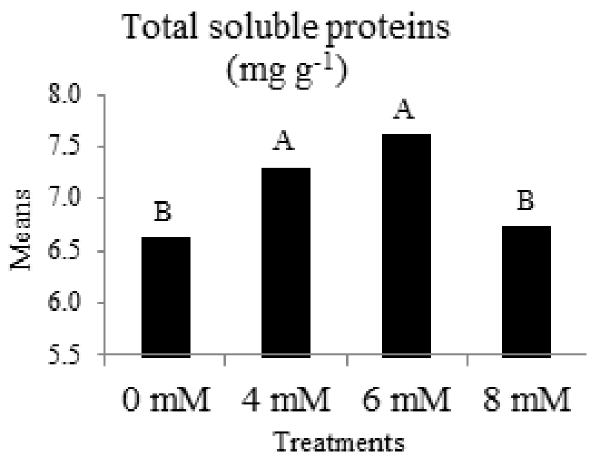


Graph 14. DMRT grouping of yield per plant

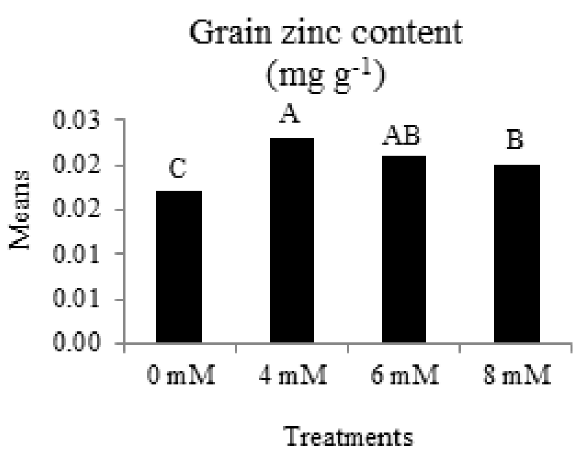
zinc sulphate foliar treatments with highest value (16.12 g) at 4 mM. The harvest index also exhibited non-significant increase at all zinc sulphate treatments and it was 1.12 at 8 mM. Increase in plant dry weight was in the same range at all zinc sulphate treatments (Graph 13). Graph 14 confirmed that yield per plant increased significantly at 4 mM. Similarly non-significant difference in harvest index by all treatments could be seen in Graph 15.

Chlorophyll content, total soluble proteins (mg g^{-1}) and grain zinc content (mg kg^{-1})

There was non-significant increase in chlorophyll content at all zinc sulphate treatments over control. A very slight increase (50.60) in chlorophyll content could be observed at 8 mM (Graph 16). Total soluble proteins exhibited significant increase over control with maximum value at 6 mM (7.61 mg g^{-1}). Grain zinc content was significantly increased up to 0.023 mg g^{-1} at 4 mM. Significant



Graph 17. DMRT grouping of total soluble proteins



Graph 18. DMRT grouping of grain zinc content.

increase in total soluble proteins was statistically in the same range at 4 and 6 mM treatments, whereas protein content at 0 and 8 mM treatment exhibited non-significant difference (Graph 17). Grain zinc content increase was in the same range at 4 and 6 mM treatment while, 0 and 8 mM treatments showed closely similar values of grain zinc content (Graph 18).

DISCUSSION

Wheat is reported to be a poor source of zinc having less than 20 mg kg^{-1} in most of the cultivars which should be more than 50 mg kg^{-1} dry weight of wheat grains (Zeidan et al., 2010). Zinc improves not only wheat growth and its yield components but also increases its water use efficiency (Singh, 2004). Dry matter accumulation and duration of reproductive growth is reported to be reduced at higher temperature during grain development and grain filling stage (Gibson and Paulsen, 1999). Zinc application may also combat with this yield limiting stress by increasing thermo-tolerance of the photosynthetic apparatus of wheat during high temperatures during ripening stage and maturation of wheat crop (Graham and McDonald, 2001). In the present experiment, many of the vegetative and yield components of wheat improved by foliar application of zinc. This could be related to the improved physiology of plants like photosynthesis, enhanced nutrient uptake, auxin activity, thermo-tolerance and water use efficiency.

The present observations on significant increase in leaf area index was in accordance with Khan et al. (2008) and Abdoli et al. (2014) who also reported an increase in leaf area index through zinc application. A minor reduction in flowering time at 4, 6 and 8 mM treatments of zinc sulphate supported a comparative lengthier grain filling duration. Findings of Abdoli et al. (2014) were in agreement with the present results as they also related increase in yield components and grain zinc components with reduced days to flowering. This led to lengthier grain filling duration which finally influenced the reproductive attributes of the crop.

The results on number of tillers, spike length and number of grains per spike were in great analogy with the work of Asad and Rafique (2000) and Hussain et al. (2005) who also reported increase in number of grains per spike and spike length by zinc application. Soleimani (2006) and Ali et al. (2009) reported a significant increase in number of grains per spike upon zinc application. Gomez-Beccera et al. (2010) explained that different cultivars behave differently in different locations, thus a combined effect of cultivar and treatment and particular agronomic managerial practices should be taken in to account while comparing the effect of different zinc treatments. The present results were analogous to the work of Arif et al. (2006), Jain and Dhama (2007) and Ranjbar and Bahmaniar (2007) who also noticed increase in grain yield by zinc application. Non-significant increase in plant dry weight of wheat cultivars was in great analogy with the work of Wang et al. (2012) who also did not notice any significant effects of zinc treatment in increasing biomass. The present results on non-significant increase in harvest index were found to be in agreement with the work of Hussain et al. (2005) and Abdoli et al. (2014) who also reported non-significant increase in harvest index by zinc treatments, while Imtiaz et al. (2003) and Ozkutlu et al. (2006) reported a reduction in harvest index owing to greater biomass. Jiang et al. (2013) and Aslam et al. (2014) reported a significant increase in chlorophyll with foliar application of 4 mM zinc sulphate. Potarzycki and Grzebisz (2009) also reported that zinc foliar application increased nitrogen uptake and protein quality which ultimately improved growth and yield components of the crop.

Bharti et al. (2013) observed an increase in methionine content with progressive application of zinc. Jiang et al.

(2013) also observed an increase in different enzymes activity in zinc treated plants. Liu et al. (2014), highlighted that increase in protein content and grain zinc content is mostly parallel to each other. Abdoli et al. (2014) reported that zinc foliar application increased grain zinc concentration from 9.4 to 19.7 mg kg^{-1}. Kutman et al. (2011) and Zhang et al. (2012) described that accumulation of zinc in vegetative tissue had a positive correlation with increase in grain zinc concentration up to 30 mg kg^{-1}. Abdoli et al. (2014) and Jiang et al. (2008) also noticed a three-fold increase in grain zinc content in comparison with control from 18.7 to 50 mg kg^{-1}. Up to 83.5% increase in grain zinc content was reported by Zou et al. (2012) who recorded almost consistent results over a wide range of 23 locations in seven different countries with their local cultivars and agronomic practices. Waters et al. (2009) and Liu et al. (2014) discussed the source and sink limitations in grain zinc accumulation. They emphasized that zinc translocation towards grains was not proved to be the limiting factor. Thus grains could accumulate quite high amounts of zinc by increasing its supply. Zhao (2011) also recommended that foliar application of zinc was preferable as it could increase yield attributes and grain zinc content up to 80%. Karim et al. (2012) reported a simultaneous increase in yield and grain zinc content in wheat. Cakmak et al. (2010b) reported that 10 mg kg^{-1} increase in grain zinc concentration was sufficient to combat zinc deficiency while foliar application increased grain zinc up to 20 mg kg^{-1}. This was helpful in achieving targeted levels of zinc in cereal grains. Zinc foliar application at early milk stage of grain filling is reported to significantly increase zinc concentration in wheat grain (Arif et al., 2006). Similarly Ozturk et al. (2006) also emphasized that frequent application of zinc at early milk stage (up to 10 or every third day) increased grain zinc content considerably along with progressive increase in in seed size and weight. Foliar application of zinc at milk and dough stage has also been reported to accumulate more zinc in grains than its application at earlier stages like stem elongation and booting stage by McCauley et al. (2009).

Figure 1. Wheat sowing.

Figure 2. Seedling emergence.

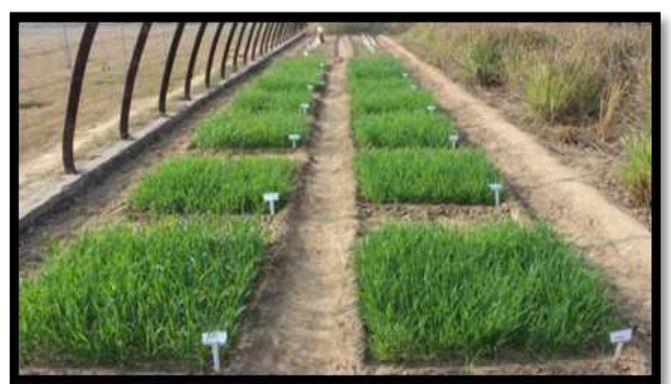


Figure 3. Tillering stage.

Conclusion

In current experiment on wheat most of the significant results were obtained at 4 mM (0.11%) and at 6 mM (0.17%) foliar treatments of zinc sulphate. Zinc sulphate was applied five times to wheat crop in 100 ml dose to each subplot of (2 × 2) ft. Grain zinc accumulation at 4mM (011%) zinc sulphate treatment was 0.023 mg g^{-1} in wheat experiment. This suggested that from its 100 g flour we may obtain 2.3 mg zinc. Use of an average of 300 g of this wheat will provide us with 6.9 mg zinc which may add well our daily need of zinc intake which is recommended to be in the range of 15 mg as per WHO (2009). These facts and figures may further help us to decide future perspectives of our research in terms of number and timely application of zinc foliar applications (Figures 1 to 6). Although, most of the significant results in this experiment were observed at 4 and 6 mM treatment of zinc sulphate however, for per hectare application 4 mM or 0.11% zinc sulphate may prove to be preferable from economic perspective.

Figure 4. Stem elongation stage.

Figure 5. Grain filling stage.

Figure 6. Mature crop of wheat.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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