Effect of micronutrient application by different methods on yield, morphological traits and grain protein percentage of barley (*Hordeum vulgare* L.) in greenhouse conditions

Efecto de la aplicación de micronutrimentos mediante diferentes métodos sobre el rendimiento, caracteres morfológicos y porcentaje de proteína del grano de cebada (*Hordeum vulgare* L.) en condiciones de invernadero

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ABSTRACT

To study effects of different types of fertilization with elements of Iron, Zinc, Manganese and Cupper on quality and quantity performance of barley (*Hordeum Vulgare* L.) a pot style factorial experiment based on randomized block design with three replications was conducted in research greenhouse of Pardis-Saveh agricultural training center in 2010. Experimental factors were included in three levels of soil fertilization with Fe, Zn, Mn and Cu (Copper sulfate and Manganese sulfate 0, 2.5 and 5 mg/kg soil, and Iron sulfate and Zinc sulfate 0, 5 and 10 mg/kg soil) and two levels of solution spraying with these four elements (solutions of 0 and 0.002 Fe, Cu, Mn and Zn Chelate EDTA). None of the treatments used, showed any significant effect on grain protein percentage and number of tiller. Soil fertilization with Fe, Mn and Cu increased yield components significantly while soil fertilizing with Zn not only increased yield components significantly but also increased number of fertile tillers at level of 1%. Result analysis showed that application of Fe in form of solution spray increased grain yield and 1000 grain weight compared to the control while solution spraying of Zn increased grain yield significantly. Results derived from this experiment suggests that in order to increase yield components, number of tillers and number of fertile tillers in barley plant it is better to use soil fertilizing with Fe, Zn, Cu and Mn while for raising of grain protein percentage and improvement of grain yield qualitatively both method of soil fertilizing and solution spraying are recommended.

Key words: Barley, iron, zinc, manganese, copper, soil application, solution spraying

RESUMEN

Para estudiar los efectos de diferentes tipos de fertilización con elementos de hierro, zinc, manganeso y cobre sobre el comportamiento de la calidad y cantidad de cebada (*Hordeum vulgare* L.) se condujo un experimento factorial en macetas basado en el diseño de bloques al azar con tres repeticiones en el invernadero de investigación del centro de entrenamiento agrícola de Pardis-Saveh en 2010. Los factores experimentales fueron incluidos en tres niveles de fertilización del suelo con Fe, Zn, Mn y Cu (sulfato de cobre y sulfato de manganeso 0; 2,5 y 5 mg/kg de suelo y sulfato de hierro y sulfato de zinc 0, 5 y 10 mg/kg de suelo) y dos niveles de solución de pulverización con estos cuatro elementos (soluciones de 0 y 0,002 Fe, Cu, Mn y Zn Quelato EDTA). Ninguno de los tratamientos utilizados, mostró un efecto significativo sobre el porcentaje de proteína del grano y número de macollas. La fertilización de los suelos con Fe, Mn y Cu aumentó significativamente los componentes del rendimiento mientras la fertilización del suelo con Zn no sólo incrementó significativamente los componentes del rendimiento, sino que también aumentó el número de macollas fértiles al nivel de 1%. El análisis de los resultados mostró que la aplicación de Fe en la forma de solución de pulverización incrementó significativamente el rendimiento de granos y el peso de 1000 granos en comparación con el control mientras que la solución de pulverización de Zn aumentó significativamente el rendimiento de granos. Los resultados derivados de este experimento sugieren que para incrementar los componentes del rendimiento, número de macollas y número de macollas fértiles en la planta de cebada, es mejor el uso de la fertilización del suelo con Fe, Zn, Cu y Mn, mientras que para elevar el porcentaje de proteína del grano y el mejoramiento cualitativo del rendimiento de granos se recomienda tanto el método de la fertilización del suelo como la solución de pulverización.

Palabras clave: cebada, hierro, cinc, manganeso, cobre, aplicación al suelo, solución de pulverización.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the modest grain which has a broad range of compatibility and transmittance among other crops. Following wheat, rice and maize barley is the fourth main grain in the globe (Khodabandeh, 2003). Since grains especially barley make up 60 to 70% of human calorie intake around the world to reach self-sufficiency in agricultural products surface unit yield must be increased, so micronutrients role in quality improvement of agricultural products are of great importance (Malakouti *et al*., 2000; Imam, 2007). Nutrient deficiency is widespread in majority of farms throughout the world. Level of deficiency varies from one region to another and from one plant to another plant (Al-Turki and Helal, 2004). Addition of nutrients to a plant not only improves its health and vitality but also strengthens it against pests and diseases. Moreover, human nutritional status and requirement to nutrients especially micronutrients meets (McCauley *et al*., 2009).

Necessity of iron for plant development was discovered by Knop and Vansachs in 1860s (Glass, 1989). Iron plays crucial role in plant metabolism particularly in chlorophyll synthesis which is vital for plant photosynthesis. In many enzymes such as catalases and peroxidases as well as flavoproteins iron is a key structural component (Smith, 1984; Ghazanshahi, 1999). Malakooti and Tehrani (2006) suggested that iron deficiency may adversely affect grain growth and eventually community's health.

Zinc usually diffuses in soil and plant absorbs it in form of ion (Malakooti and Tehrani, 2006). In many plant enzymatic systems zinc act as an activator catalyst or structural component and it helps in protein synthesis and degradation process (Brown *et al*., 1993). Decrease in growth regulators which is due to zinc deficiency causes dwarf reduction in distance between nodes of stem, twist in leaf margins and finally plant growth will cease. Signs of zinc deficiency usually present in young tissues (Malakooti and Keshavarz, 2006). Barley is one of the most resistant grains against zinc deficiency (Tandon, 1995)

Nutrition therapy with manganese in grain has been studied for many years. Barley is classified moderately sensitive to manganese deficiency (Malakooti and Tehrani, 2006). Seyedin (2006) suggested that in wheat, soil fertilizing and solution spraying of manganese sulfate may enhance growth and improve yield compared to the control and also increases level of manganese in seed and straw.

Copper also plays role in protein and carbohydrate metabolism as well as enzymatic systems. Its deficiency in crops first appears in leaf tip in tillering phase, however, in severe conditions it may appear earlier. Copper deficiency makes leaf narrow with white tip. Also growth between nodes diminishes. This element is immobile in crops; therefore, its deficiency appears in young plants. Barley is very sensitive to copper deficiency (Al-Turki and Helal, 2004; Balali and Malakooti, 2002; Zou *et al*., 2001; Mengel and Kirkby, 2001).

Several scientists following numerous studies on different plants especially grains concluded that application of micronutrients such as zinc, copper, iron and manganese either in forms of soil fertilization or foliar application lead to increase in grain yield components and protein percentage in seed; for instance wheat, maize, rice, barley and sorghum showed increase in yield components by application of micronutrients (Alloway, 2008; Zou *et al*., 2001).

This experiment was carried out to study the effect of two methods of solution spraying and soil fertilization with iron, copper, manganese and zinc in barley plants. Besides, we focused on whether different levels of such elements could influence qualitative or quantitative performance (by impact on one or more yield components) of barley plants.

MATERIALS AND METHODS

To study effect of Fe, Cu, Zn and Mn on qualitative and quantitative characteristics of barley plant, a factorial experiment based on randomized block design with three replications in research greenhouse of Pardis-Saveh agricultural training center located in 25 km south of city Saveh (35°1ˈN, 50°21ˈE, A1108 m) was conducted. First, a combined soil sample was prepared, air dried, screened through a 2 mm sieve and amount of Fe, Mn, Cu, Zn in soil were extracted by DTPA method then measured by atomic absorption device (Lindsay and Norvel, 1978). Soil nitrogen was determined by Kjeldahl method (Bremner and Mulvaney, 1982) and organic compounds by wet oxidation method (Nelson and Sommers, 1982). Extractable soil phosphorus was read by Olsen method (sodium carbonate, pH 8.2) and colorimetric method. Extractable soil potassium was determined by ammonium acetate solution (1.0 N) and flame photometry (Mohammad *et al*., 1990). It should be noted that soil pH was 7.25. Soil analysis results are given in Table 1. Treatments used in this experiment were as follow: Iron (0, 5, 10 mg/kg soil in form of iron sulfate in fertilizer and solution spraying with solutions of 0 and 0.002 iron chelate EDTA), zinc (0, 5, 10 mg/kg soil in form of zinc sulfate in fertilizer and solution spraying with solutions of 0 and 0.002 zinc chelate EDTA), copper (0, 2.5, 5 mg/kg soil in form of copper sulfate in fertilizer and solution spraying with solutions of 0 and 0.002 copper chelate EDTA), manganese (0, 2.5, 5 mg/kg soil in form of manganese sulfate in fertilizer and solution spraying with solutions of 0 and 0.002 manganese chelate EDTA). Solution spraying was carried out in tillering phase, stem elongation and spike development. In order to compensate lack of nutrition160 mg nitrogen per kg soil in form of urea was added to each pot in three occasions (80 mg before planting, 40 mg in tillering and 40 mg in stem elongation. With regard to soil test, phosphorus and potassium from sources of triple superphosphate and potassium sulfate were added to each pot according to recommended dosage before planting. In this experiment seeds of *H. sativum* were used and for vernalization of them, germinated seeds were incubated in 1°C for 31 days (Mahfoozi and Sasani, 2008).

Germinated seeds were planted in early October and in each pot 10 seed were planted, after 10 days number of plants was decreased to 4. Pots were kept inside the greenhouse under controlled temperature (25±3°C during daytime and 17±3°C during nighttime) and shrubs grew under normal daytime duration (about 12 h). During experiment we tried to keep soil humidity up to farm capacity by weighing pots and adequate irrigation (relative humidity about 30%). Experiment period was 64 days. After they matured, plants were cut in place of crown. Samples of spikes from each pot were washed by distilled water and dried in oven at 65°C. To measure protein proportion of barley corn, samples were milled and nitrogen amount of grain was calculated through Kjeldahl method with coefficient of 5.83 in nitrogen content (Bremner and Mulvaney, 1982). Data were analyzed by SAS software and multiple comparisons was done through Duncan's multiple range test at the $p<0.5$ level.

RESULTS

Grain yield

Results showed that effect of soil fertilizing with Fe, Zn, Cu and Mn on grain yield was significant at level of 1%, however, solution spraying of Fe and Zn became significant at level of 5% (Table 2). As it is shown in Table 3, the maximum grain yield took place by soil application with 10 mg/kg Fe and Zn as soil fertilizer that increased grain yield to 28.71% and 35.11%, respectively compared to the control. As for Mn and Cu, maximum grain yield occurred in treatment with 5 mg/kg Mn and Cu as soil fertilizer that increased grain yield to 23.64% and 20.74%, respectively compared to the controls. Results of table 4 shows that solution spraying of Zn and Fe may increase grain yield to 22.01% and 23.32%, respectively compared to the control.

1000 grain weight

As it is shown in Table 2, effect of soil application with Fe, Zn, Cu and Mn on 1000 grain weight was significant at level of 1%. However, solution spraying of elements showed no significant effect on 1000 grain weight. In table 3 it is shown that highest amount of 1000 grain weight resulted from application of 10 mg/kg Zn and Fe to the level of 28.32 g and 29.95 g that increased 1000 grain weight to 10.49% and 15.36% compared to the control.

Table 1. Results of chemical analysis of soil test at Research Greenhouse of Pardis-Saveh Agricultural Training Center, Saveh, Iran.

Mn	Zn	Cu	Fe	P	K	CEC $mmol^{+}$/kg	OC %	Clay %	Lime %	pH	EC dS/m
mg kg^{-1}											
0.58	0.64	0.41	52.0	11	68	2.18	48.0	29	11.3	7.25	5.1

CEC: Cation exchange capacity; OC: Organic carbon; EC: Electrical conductivity

Number of grains per spike

Analysis of variance table showed that effects of soil application of Zn, Fe, Cu and Mn and solution spraying of Fe affected significantly number of grains per spike at level of 1%. As it is shown in table 3, maximum number of grains in spike was obtained by application of 10 mg/kg Fe and Zn as soil fertilizer and 5 mg/kg Cu and Mn. According to Table 3, treatment with 10 mg/kg Zn and Fe increased number of grains per spike to 16.41% and 20.50%, respectively. However, treatment with 5 mg/kg Mn and Cu increased number of grains per spike to 9.37% and 8.94%, respectively. Solution spraying of Fe improved number of grains per spike to 13.45%.

Number of tiller

Effect of two methods of solution spraying and soil fertilizing with Fe, Zn, Cu and Mn on trait of number of tiller was not statistically significant (table2). According to Table 3, application of Zn at level of 10mg/kg as soil fertilizer increased number of tiller up to 3.27% compared to the control. Treatment of 10 mg/kg Fe as soil fertilizer increased number of tiller to 3.99% compared to the control. As for copper treatment with 5 mg/kg as soil fertilizer increased number of tiller about 2.28% compared to the control, but soil fertilizing of manganese not only did not increase number of tillers but also had opposite effect on number of tillers compared to the control. As it is

Table 2. Results of analysis of variance of barley traits at Research Greenhouse of Pardis-Saveh Agricultural Training Center, Saveh, Iran.

Source of variation	df	Grain yield	1000-grain weight	Grain number in spike	Numbers of tiller	Fertile tillers	Grain protein (%)
Soil application with Zn	2	321.21 **	458.72 **	438.24 **	1.14 ns	65.48 **	1.68 ns
Solution spraying with Zn	1	7.21 *	1.04 ns	48.4 ns	0.12 ns	0.24 ns	1.00 ns
Soil application with Mn	2	25.35 **	24.32 **	64.25 **	0.09 ns	0.008 ns	2.01 ns
Solution spraying with Mn	1	0.19 ns	009.0 ns	1.33 ns	1.57 ns	0 ns	0.002 ns
Soil application with Fe	3	84.53 **	387.45 **	658.35 **	0.21 ns	2.35 ns	0.68 ns
Solution spraying with Fe	1	5.48 *	0.31 ns	68.34 **	0.02 ns	0.54 ns	0.22 ns
Soil application with Cu	2	65.32 **	27.34 **	38.47 **	0.12 ns	0.004 ns	1.01 ns
Solution spraying with Cu	1	0.36 ns	1.00 ns	0.001 ns	0.54 ns	0.01 ns	0.02 ns
Error	16	00.1	12.1	89.3	52.0	37.0	50.1
Coefficient of variation (%)	-	90.9	66.2	38.4	5.14	28.20	54.9

df: Degree of freedom. :**Significant at 1% :* ، Significant at 5% ، ns :Non-significant.

Table 3. Trait average of barley at different quantity of soil application with Zn, Mn, Fe and Cu at Research Greenhouse of Pardis-Saveh Agricultural Training Center, Saveh, Iran.

Treatment	Level	Grain yield (g/pot)	1000-grain weight (g)	Grain number in spike	Numbers of tiller	Fertile tillers	Grain protein (%)
Zn	0	7.30 b	25.35 b	19.35 b	3.85 a	3.75 b	10.23 a
	5 mg kg^{-1}	7.56 b	25.66 b	19.85 b	3.87 a	3.80 b	10.27 a
	10 mg kg^{-1}	10.24 a	28.32 a	23.15 a	3.98 a	4.23 a	10.35 a
Mn	0	7.30 b	25.35 b	19.35 b	3.85 a	3.75 a	10.23 a
	2.5 mg kg^{-1}	7.54 b	25.98 b	19.42 b	3.83 a	3.70 a	10.38 a
	5 mg kg^{-1}	9.56 a	27.39 a	21.35 a	3.88 a	3.80 a	10.25 a
Fe	0	7.30 b	25.35 b	19.35 b	3.85 a	3.75 a	10.23 a
	5 mg kg^{-1}	8.01 b	25.85 b	20.12 b	3.93 a	3.75 a	10.32 a
	10 mg kg^{-1}	11.25 a	29.95 a	24.34 a	4.01 a	3.94 a	10.36 a
Cu	0	7.30 b	25.35 b	19.35 b	3.85 a	3.75 a	10.23 a
	2.5 mg kg^{-1}	7.45 b	25.54 b	19.50 b	3.85 a	3.80 a	10.42 a
	5 mg kg^{-1}	9.21 a	27.33 a	21.25 a	3.94 a	3.80 a	10.54 a

Significant differences ($P < 0.05$) are indicated by different letters according to Duncan's multiple range test shown in Table 4, solution spraying of Zn, Fe, Cu and Mn increased number of tillers to 0.77%, 2.28%, 1.28% and 0.77%, respectively compared to the controls but this boost was not significant.

Number of fertile tillers

According to variance analysis table (Table 2), soil fertilizing with Zn affected significantly number of fertile tillers at level of 1% , however soil fertilizing with Fe, Mn and Cu and even solution spraying with Fe, Zn, Mn and Cu did not significantly affect number of fertile tillers. Analyzing of mean comparison table showed that soil application of zinc had positive effect on number of fertile tillers as maximum number of fertile tillers was obtained through soil fertilization of Zinc (10 mg/kg as soil fertilizer) which increased 11.36% compared to the control (Table 3). For soil application with Fe, maximum number of fertile tillers obtained in treatment with 10 mg/kg as soil fertilizer which showed 4.82% increase compared to the control. Results from table 3 show that soil application with Cu and Mn lead to increase in number of fertile tillers. For Mn, treatment with 5mg/kg as soil fertilizer and for Cu, treatments with 2.5 and 5 mg/kg as soil fertilizer showed maximum number of fertile tillers which is more than 1.32% compared to the control. Solution spraying with Zn, Fe and Cu increased number of fertile tillers to 0.10%, 2.11%, 0.79% compared to the control but spraying of Mn showed no effect on number of fertile tillers.

Grain protein

Different types of soil fertilizing and solution spraying with Fe, Zn, Mn and Cu on level of grain protein did not show any statistically significant effect (Table 2). Application of Zn to the amount of 10mg/kg soil increased grain protein to 1.16% compared to the control (Table 3). Also, treatment with 10 mg/kg Fe as soil fertilizer increased grain protein to 1.25% compared to the control. Treatment with 5 mg/kg Cu as soil fertilizer resulted in highest amount of grain protein which was 2.94% more compare to the control, however, soil fertilizing with 2.5 mg/kg Mn showed maximum amount of grain protein which was 1.45% more than the amount of the control. According to Table 4, solution spraying with Zn, Fe and Cu increased grain protein 0.77%, 2.28%, 3.22% and 3.03% respectively compared to the control.

DISCUSSION

In this experiment, it was found that increase in consumption level of Zn, Fe, Cu and Mn whether as soil fertilizer or solution spraying increases grain yield, 1000 grain weight and number of grains per spike in barley plant. Fathi and Enayat Gholizadeh (2010a), Abdel-Hady (2007), Malakooti and Tehrani (2006) reported similar results. Because Fe plays crucial role in plant respiration, photosynthetic reactions and chlorophyll synthesis and decrease in level of Fe reduces plant growth as a result diminishes food production, addition of Fe improves the grain yield components. Increase in Zn level may enhance chlorophyll content of plan also may increase level of indole acetic acid (IAA) which is one of the most vital hormones necessary for growth and development. Zinc also takes part in nitrate conversion to ammonia in plant (Alloway, 2008; McCauley *et al*., 2009; Hemantaranjan and Grag, 1988). Since Cu is one of the most essential elements

Table 4. Average traits that studied in barley at different quantity of solution spraying with Zn, Mn, Fe and Cu at Research Greenhouse of Pardis-Saveh Agricultural Training Center, Saveh, Iran.

Treatment	Level	Grain yield (g/pot)	1000-grain weight (g)	Grain number in spike	Numbers of tiller	Fertile tillers	Grain protein (%)
Zn	0	7.30 b	25.35 a	19.35 a	3.85 a	3.75 a	10.23 a
	2 mg/1000L	9.36 a	26.12 a	20.54 a	3.88 a	3.80 a	10.24 a
Mn	0	7.30 a	25.35 a	19.35 a	3.85 a	3.75 a	10.23 a
	2 mg/1000L	7.85 a	25.65 a	19.98 a	3.85 a	3.75 a	10.45 a
Fe	0	7.30 b	25.35 a	19.35 b	3.85 a	3.75 a	10.23 a
	2 mg/1000L	9.52 a	26. 32 a	22.46 a	3.94 a	3.94 a	10.57 a
Cu	0	7.30 a	25.35 a	19.35 a	3.85 a	3.75 a	10.23 a
	2 mg/1000L	7.93 a	25.48 a	20.02 a	3.90 a	3.78 a	10.55 a

Significant differences ($P<0.05$) are indicated by different letters according to Duncan's multiple range test in chlorophyll synthesis, enhance plant photosynthesis and carbohydrate metabolism, help to regulate water movement through plant and play vital role in seed production, addition of this element to barley would increase grain yield components (Mengel and Kirkby, 2001; McCauley *et al*., 2009). Manganese is important in chlorophyll synthesis as chloroplast is the most sensitive organism in plant cells to Mn deficiency. Mn also takes part in nitrogen and carbohydrate metabolism and along with other elements is necessary for plant growth. That is why addition of Mn to barley may increase yield and yield components (Mengel and Kirkby, 2001; McCauley *et al*., 2009).

As we found in this experiment, increase in amount of micronutrients (Fe, Cu, Zn and Mn) showed any significant effect regarding number of tiller and fertile tillers. Tandon (2003) suggested that number of tiller in crops especially in wheat and barley is determined by genetic factors and nutrition plays negligible role in this matter. However in our experiment we observed that increase in amount of micronutrients whether in form of soil fertilizer or solution spray increased number of tillers somehow and had positive effect, though very little, on number of tillers. The results of the experiment were in consistence with studies previously reported by Arora and Singh (2004), Fathi and Enayat Gholizadeh (2010 b).

Studies showed that with increase on Fe level, photosynthesis and plant dry matter production will also increase, therefore, plant can fertilize more tiller then more grain will form (Agrawal, 1992). Zinc increases enzymes containing Zn (e.g. anhydrase) which are important in metabolism of carbohydrates and morphological traits, so increases the number of tillers (Hemantaranjan and Grag, 1988; Vankhadeh, 2002). Manganese also plays positive effect on number of fertile tillers. Soluble carbohydrates especially in roots following an increase in level of Mn and eventually increase in photosynthesis will increase (Rennan *et al*., 2007). Barley is very sensitive to Cu deficiency. Addition of Cu to the plant whether in form of soil fertilizer or solution spray will increase photosynthesis and carbohydrate metabolism as well as enzymatic activity. Copper deficiency may reduce number of fertile tillers. Grains are very sensitive to Cu deficiency in tillering phase. If Cu requirements met in this period of time, plant will generate more tiller and fertile tillers (Mengel and Kirkby, 2001; McCauley *et al*., 2009; Al-Turki and Helal, 2004).

In the experiment results show that neither of soil fertilization nor solution spraying with Fe, Mn, Zn and Cu had any effect on grain protein proportion, however, grain protein percentage showed a rising trend. Smith (1984) believed that amount of wheat grain protein is influenced by environmental circumstances including available water and nitrogen content of soil. Since in our experiment, these two factors were applied at same level, lack of any significant change in grain protein could be justified. In general, Zn as a key structural component of RNA polymerase participates in protein synthesis, Cu is essential in photosynthesis and exists in chloroplast protein compounds which its deficiency reduces net photosynthesis and finally grain protein diminishes. Iron and Mn are important for activity of number of enzymes and protein synthesis so deficiency of them decreases the amount of grain protein (Ziaiian, 2003; Hemantaranjan and Grag, 1988; Sadri and Malakooti, 2001; Vankhadeh, 2002; Yilmaz *et al*., 1997; Romheld and Marschner, 1991; Seyedin, 2006; Sharma *et al*., 1994).

In our experiment results suggest that yield components, number of tiller and number of fertile tillers in barley plant mostly affected by soil application of elements rather than spraying. Maximum yield obtained in highest level of Fe and Zn (10 mg/kg as soil fertilizer) Cu and Mn (5 mg/kg soil) treatments. Highest percentage of grain protein obtained from soil fertilizing and solution spraying of elements in upmost level of application (soil fertilizing treatment with 5mg/kg Cu and Mn, and 10 mg/kg Fe and Zn, solution spraying with solution of 0.002 Fe, Zn, Cu and Mn chelate EDTA). Therefore, it can be concluded that in order to increase barley grain yield quantitatively, soil fertilizing with Fe, Zn, Mn and Cu is suggested but for increasing the percentage of grain protein and improvement of grain yield qualitatively both method of soil fertilizing and solution spraying are recommended.

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