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Effect of Foliar Fertilization of Fe, B and Zn on nutrient concentration and seed protein of Cowpea "Vigna Unguiculata"

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Abstract : *Pot experiment was carried out under greenhouse conditions at Khabat, Iraqi Kurdistan during 2011 and 2012 growth season to investigate Fe, B and Zn foliar application effects on nutrient concentration and seed protein of cowpea (Vigna Unguiculata). The experimental design was a randomized complete block design with three replicates. In addition, three concentrations (0, 1 and 2 ppm) of micronutrient solutions were applied. Fe, B and Zn were sprayed every 15 days. Parameters measured were values of each nutrients and protein%, also, P, K, Ca, Mg, Na and Cl. The results of the analysis of variance showed that the effect of different treatments at 1% level on nutrient concentration and seed protein were significant. Iron treatment has a greater effect on the nutrient uptake and protein percentage of seed than other treatments. The study results explain that foliar fertilization with micronutrient may have a possibility role for increasing cowpea yield.*
Keywords : *Cowpea, Foliar Fertilization, Fe, B and Zn, Iraqi Kurdistan*

I. INTRODUCTION

Legumes are considered the most important source of food after cereals in the world, as they are main sources of protein and energy for human. Currently, it can be a good alternative for animal protein. Due to nutrition values and economic importance, it is necessary to develop new methods for increasing the crop production. Using foliar fertilization including micronutrients is one of these new methods that are considered more effective on yield, protein content and nitrogen fixation in pulses. In addition, cowpea is the most important crop of legume family that can be planted in semiarid region. In this area, the plant produces seeds containing18-32 % protein; as well as, nitrogen fixation ability. Therefore, it plays important role in human nutrition and sustainable farming systems (A. Hemmati, 2005). Cultivation importance of beans in Iraqi Kurdistan is clear, as *MOAKR, 2008* reported that individual needs for legume crops in 2008 was 7 kg/year, and it will rise to 12 kg/year by 2013. So, target production would be about 60 million tons for Kurdistan region.

However macronutrients such as NPK affect plant growth and development, they are depending on micronutrients availability. (Salwa et al., 2011) stated that microelements are crucial substances for crop's growth; however, they are used in lower amounts compared to macronutrients, such as N, P and K. They have a major role in cell division and development of meristematic tissues, photosynthesis, respiration and acceleration of plant maturity (Zeidan et al., 2010). In addition, iron (Fe), boron (B), zinc (Zn), copper (Cu), and manganese (Mn) are considered essential micronutrients for plants and humans (Kaya et al., 1999; Asad and Rafique, 2000; Hao *et al*., 2007); and micronutrients can maintain crop-physiology balance. Furthermore, these elements play vital roles in CO_2 flowing out, vitamin A improvement and resistant system activities (Narimani et al., 2010). So, deficiency of these nutrients can markedly reduce crop's yield, and even can cause ceasing plant growth. Mostly, amounts of iron, boron and zinc in soil are more than the plant needs but cannot readily be absorbed by plants. Thus, it is better to be used foliar application, as it is more effective than adding fertilizer to soil.

However, root is common to be first part of plant that adsorbes nutrients from soils, but nutrients availability might be restricted; then affects fertilizer efficiency. So, it is better to recommend foliar application that provides nutrients for plant (Altındişl et al., 1998). In fact, foliar fertilization is applied in small droplets on the leaves and stems of the plant; then nutrients are absorbed through these parts of plants (Kuepper, G. 2003). Also, foliar application can supply the nutrients for plants rapidly to obtain high performance guarantee. From an ecological perspective, foliar fertilization is more acceptable, because the small amounts of nutrients is used for rapid use by plants (Stampar et al., 1998).

On the other hand, legume seeds provide an exceptionally varied nutrient profile, including proteins, fibers, vitamins and minerals, and proteins are considered major components of legume seeds (Mitchell et al. 2009). Nitrogen that is used by seedling during germination is stored in seeds in the form of proteins. Seeds contain from 16% to 50% protein and provide one third of all dietary protein nitrogen (Graham and Vance, 2003). Of course, improving the protein content of grain legume seeds depend on genetic variability, environmental variability and heritability.

II. MATERIALS AND METHODS

The study was conducted under greenhouse conditions at experimental field of Khabat Agriculture Technical Institute, where is 30 km western Erbil (Figure1). The experiment was organized in randomized complete block design by three replications. *Black-eyed Varieties* of cowpea was sown in plastic pots containing 8 Kg soil. Fertilizers were applied at the following rates: 100 kg P/ha, 50 kg K/ha and 30 ton peatmoss/ha. Soil was sampled and analyzed as shown in (Table 1).

Foliar application treatments were applied by three levels which are 0, 1 and 2 ppm of micronutrients solution containing of Fe, B and Zn. For this purpose, Fe EDTA 6.8%, boric acid 5.17% and zinc sulfate 20% were used, respectively. Foliar spray was done according to the three levels of treatments. Then, 15 days after sowing micronutrients were applied, and the spraying was done every 15 days. Soil moisture was maintained at adequate levels to prevent water deficit and wilting.

At ripening stage samples were taken for plant growth measurements and determine of nutrient contents. Leaves and seeds were collected after harvest to determine seed protein percentage and total N by using Kejldahl method; as well as, iron, boron and zinc contents in leaves by using atomic absorption. Other elements such as Na, Cl, Ca, Mg, P, and K also were determined after harvesting for each treatment (Table 2). Data were analyzed using SPSS 18 statistical package. The least significant difference (TUKEY`S HSD) at 1% level was used to compare the means of treatments.

Figure1. The experience location map

Table 1: Analysis of soil sample before sowing

Sand	Silt	Clay	OM	pH	EC	N	K^+	Ca^{2+}	Mg^{2+}	Na^+	Cl^-	P	Zn	Fe	B
%	%	%	%		$dS.m^{-1}$	%	mmolic.L^{-1}					$mg.g^{-1}$	ppm		
33	26	41	0.8	7.9	1.6	0.16	0.14	7.28	2.96	3.58	1.28	4.15	20	30	5

III. RESULTS

Nutrient concentration: Analysis of variance shows that application of iron, boron and zinc significantly increased nutrient concentration of cowpea compared to control treatment (Table 2). The results indicate that the highest concentration of iron absorbed at (Fe, 2pmm) treatment which was 210 $mg.kg^{-1}$, but both levels of B and Zn had no noticeable effect on the iron absorption in cowpea. Also, the results show that the highest concentration of boron and zinc absorbed at (Fe, 2pmm) treatment which were 220 and 80 $mg.kg^{-1}$, respectively. Furthermore, there was a similar effect in concentrations of elements P, K, Ca, Mg, Na and Cl (in $mg.g^{-1}$) for treatments, and the results are presented in Table 3. All treatments had significant effects ($P < 0.01$) to increase macro and micro nutrients concentration in cowpea, and Na concentration was the highest in (Zn, 2pmm) treatment. Nutrient concentration was also significantly affected by treatments.

It might be due to participation of iron in the formation of chlorophyll (Reddy, K.B. et al, 1993), Zinc plays a role in plant enzymes (Cakmak, I. et al, 1996), and boron plays an important role in improving the overall quality and quantity of products (Umesh, 1993). It has been found that iron is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions (Brittenham, 1994). Hence, iron has many essential roles in plant growth and development, including chlorophyll synthesis, thylakoid synthesis and chloroplast development (Miller et al., 1995). Also, iron is required at several steps in the biosynthetic pathways. Zinc is an essential element for plants that act as a metal component of various enzymes or as a functional structural or regulatory cofactor and for protein synthesis, photosynthesis, the synthesis of auxin, cell division, and sexual fertilization (Marschner, 1995). Also, zinc plays a special role in synthesizing proteins, RNA and DNA (Kobraee et al., 2011). It can be noted that the main functions of boron are related to cell wall strength and development, cell division, fruit and seed development, sugar transport, and hormone development. Some functions of boron interrelate with those of nitrogen, phosphorus, potassium and calcium in plants. However, balancing nutrition is essential for optimum crop growth.

Another important point is interactions between micronutrients which affect their uptake, distribution, and utilization in plants (Imtiaz *et al.*, 2003). Many studies have examined these interactive effects, especially between Fe and Zn. (Sliman, 1990) explained also, antagonism between Fe and Zn in soybean. There is a complex interplay between elements, and how they affect each other in the soil plant. Some elements can enhance or suppress each other.

Table 2: Effect of Fe, B and Zn on nutrient concentration in cowpea

Treatments		Fe	B	Zn
			$mg.kg^{-1}$	
Control, 0 pmm		40.00	16.00	8.00
Fe, 1 ppm		90.00	31.00	25.00
Fe, 2 ppm		154.00	47.00	42.00
B, 1 ppm		51.00	31.00	18.00
B, 2 ppm		58.00	40.00	24.00
Zn, 1 ppm		47.00	26.00	13.00
Zn, 2 ppm		50.00	37.00	17.00
Tukey's HSD	Treat. & concentration	1.28	1.35	1.35
	Interaction	2.61	2.94	2.94

Table 3: Effect of Fe, B and Zn on macro and micro nutrients in leaves in cowpea

Treatments		P	K^+	Ca^{2+}	Mg^{2+}	Na^+	Cl^-
					$mg.g^{-1}$		
Control, 0 pmm		2.4	25	2.90	2.30	2.10	1.50
Fe, 1 ppm		5.5	80	3.50	2.70	2.30	2.70
Fe, 2 ppm		8	85	3.55	2.44	3.10	2.88
B, 1 ppm		4	70	3.13	2.60	2.45	2.75
B, 2 ppm		9	85	3.55	2.44	3.40	2.89
Zn, 1 ppm		3.5	60	3.02	2.32	2.44	2.70
Zn, 2 ppm		7	65	3.95	2.94	3.14	2.88
Tukey's HSD	Treat. & concentration	0.927	4.786	0.246	0.275	0.166	0.227
	Interaction	2.008	10.367	0.534	0.595	0.360	0.492

Protein percentage of seed: Analysis of variance showed statically significant differences between fertilizer application levels; as well as, significant effects for interaction of Fe, B and Zn fertilizer usage on the protein percentage of seed (Figure 2). The highest protein percentage was obtained when cowpea treated with Fe, 2ppm an amount of 28.9%. This is related to the role of iron in chlorophyll formation and respiration, photo-synthesis and symbiotic nitrogen fixation in plant. On the other hand, the lowest protein percentage in seed (25.3%) was from (B, 1 ppm) and (Zn, 1ppm) treatments. However, foliar application and interaction between Fe and other micronutrients had significant effect on protein yield (Table 4).

According to results of (Hemmati A., 2005) studies, application of micronutrient iron, zinc and manganese on average two percentage has increased seed protein in bean. In this study, an average increase of 3.5% protein occurred for treatments. Similarly, the highest percentage (5.5) was obtained from Fe, 2 ppm treatment.

Table 4: Effect of Fe, B and Zn on protein percentage in cowpea seed

Treatments		Protein percentage of seed
Control, 0 pmm		23.4
Fe, 1 ppm		26.7
Fe, 2 ppm		28.9
B, 1 ppm		25.3
B, 2 ppm		26.8
Zn, 1 ppm		25.3
Zn, 2 ppm		28.4
Tukey's HSD	Treat. & concentration	0.002
	Inter action	0.008

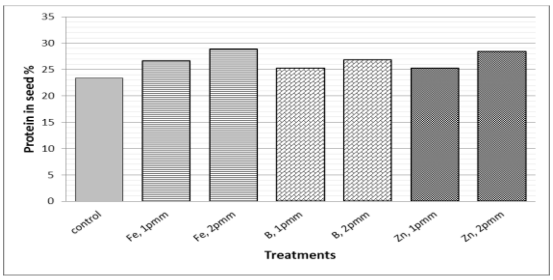

Figure 2: Effect of Fe, B and Zn on protein percentage in seed in cowpea

Effect on soil chemical properties: As shown in Table 5 after experiment, soil organic matter percentage increased but pH and electric conductivity reduced compared to data in Table 3. Thus there has been a change in soil elements: potassium content was constant and magnesium content was almost constant, while nitrogen and chlorine content increased but sodium and calcium reduced. (Hemmati A., 2005) noted that soil nitrogen content increases with direct application of micronutrients on soil or foliar application.

Table 5: Analysis of soil sample after experiment

OM	pH	EC	N	K^+	Ca^{2+}	Mg^{2+}	Na^+	Cl^-	P
%		$dS.m^{-1}$	%			$mmolic.L^{-1}$			$mg.g^{-1}$
1.4	7.7	0.9	2.8	0.14	3.05	2.65	1.50	1.75	3.30

IV. DISCUSSION

In common fertilization, elements are often added to soil as cation form and mostly are fixed by the soil. The availability of micronutrients such as Fe, B and Zn is greatly affected by pH and $CaCO_3$ content as well as soil texture. Usually, micronutrient-deficiency problems are found in calcareous soil of arid and semi-arid regions (Fouly M.M., 1983). Thus, it is better to be sprayed cations on the leaves, as foliar application supplies nutrients for plants faster compared to fertilizer application to soil. In addition, the experiment results show that foliar application of these elements was effective on the legume family (Hemmati A., 2004).

However, a number of studies have reported that the application of micronutrient fertilizers to soil or crop foliage increased micronutrient concentrations in seeds. In contrast, other researchers found that foliar sprays results nutritional disorders and imbalances (Ghasemi-Fassaei et al., 2008; Pahlavan- Rad and Pessarakli, 2009).

During the past decade, numerous researches were conducted to explain effects of micronutrient on protein content of seeds. Results showed that adding of each micronutrient (Fe, Zn, Cu, and B) or a combination of Fe + Zn + Cu + B to NPK fertilizer increase grain quality. (Sharma et al., 2008) observed an increased protein content and yield in their experiments. Similar results were obtained by (Peterson et al., 1986) and (Han and Shepherd, 2009). They reported that higher copper and zinc content result in higher protein content. A site study showed that NPK+ micronutrients increased significantly protein content of wheat kernel (M.J. Malakouti, et al., 2008) this result shows that due to the use of micronutrients grain yield increased by 17% in the greenhouse experiment. The field experiment also showed a mean yield increase (6.6% increases), due to balanced fertilization.

V. CONCLUSION

Foliar application treatment with Fe exceeds on the other treatments followed by B and Zn application. Such effects of foliar application with micronutrients (Fe, B and Zn) might be due to their critical role in crop growth, involving in photosynthesis processes, respiration and other biochemical and physiological activates and thus their importance in achieving higher yields. Similar results were reported by researchers.

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