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Relationships Between Oil, Protein and Dry Matter in Soybean Seed with Some of Micronutrients Fertilization

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ABSTRACT

In order to investigate the relationships between oil percent, protein percent, pod, seed and total dry weight in soybean with zinc, iron and manganese applications, two field experiments were performed at the research field of the Islamic Azad University of Kermanshah, Iran in 2010-2011. The experimental design was a factorial experiment based on randomized complete block with three replicates. Treatments includes: Three level of Zn (0, 20 and 40 kg ha^{-1}), Fe (0, 25 and 50 kg ha^{-1}) and Mn (0, 20 and 40 kg ha^{-1}). At the harvesting time, ten plants were randomly selected from each plot and grains were separated from pods. Oil and protein percent in soybean seed were measured. For the measurement of pod, seed and total dry weight the samples were dried and finally were weighed. The results indicated that zinc and iron had significant effects on all of evaluated traits ($p<0.01$). Manganese had significant effects on oil, pod, seed and total dry weight ($p<0.01$) and on protein ($p<0.05$). Application of 20 kg Zn ha^{-1} had the most impact on pod dry weight and plant dry weight, while maximum oil and protein contents and seed dry weight were recorded with 40 kg Zn ha^{-1}. In excess amounts of iron application the oil and protein content was reduced, although was not statistically significant. The effect of zinc and manganese applications on oil percent in soybean seed was more than protein content.

Key words: Dry matter, micronutrient, oil, protein, soybean

INTRODUCTION

One of the most important agricultural productions in the world is oils that extracted from oilseeds. The oils not only are used for human nutrition (Dyer *et al*., 2008) and industry (Nikolau *et al*., 2008) but also even as replacement for fossil fuel (Durrett *et al*., 2008) is considered. Statistics show that the production of vegetable oils in the world increased more than 60% from 1997 to 2007 and more than 83% of the oil produced belonged to soybean, palm, rapeseed and sunflower (FAOSTAT., 2008). Soybean oil production in the world increased from 25,572,045 t in 2000 to 39,761,852 t in 2010 (+49.55%). At the same time in Iran, soybean oil production from 104600 t reached to 199600 t (+90.82%) (FAO., 2012). In the other side, soybean protein as a cheap substitute for animal protein in poor communities has been considered (Elsheikh *et al*., 2009). Plant nutrition and fertilization can be effective on the amount of oil and protein in soybean (Seguin and Zheng, 2006), corn (Sharafi *et al*., 2002) and canola (Morshedi and Naghibbi, 2004). Zinc plays an important role in nitrogen metabolism and amino acid synthesis; therefore, zinc deficiency can lead to protein reduction in plant (Broadley *et al*., 2007; Fageria, 2009). In addition, Popelkova *et al*. (2003) reported that the stability of plant proteins structure in the presence of manganese increases. Also, Hemantarajan and Trivedi (1997) stated that application of iron can increase the protein content in soybean seed. In previous studies, many researchers have emphasized the influence of zinc (Hinnawy *et al*., 1982), iron (Rahman, 1992) and manganese (Hinnawy *et al*., 1982; Salem, 1989) on oil content in soybean seeds. Thus, the main objective of this study was to determine the effects of zinc, iron and manganese fertilization on oil and protein of soybean in western parts of Iran.

MATERIALS AND METHODS

Two field experiments were conducted at the research field of the Islamic Azad University of Kermanshah province, Iran (34023'N, 4708'E; 1351 m elevation) in 2010 and 2011. The experimental design was a 3×3×3 factorial experiment based on randomized complete block with three replicates. Every year, Williams [*Glycine max* (maturity group III)], supplied by the oilseed company of the Kermanshah Agricultural Administration, Iran], was selected as the experimental material. Soil samples were collected from experimental area at 0-30 cm depth for soil analyses. The texture of the soil based on silty clay with pH 7.6, total organic matter 2.3%, electrical conductivity (ECe) 0.61 dS m^{-1}, total nitrogen 0.18%, available phosphorus 9.9 mg kg^{-1}, available potassium 563 mg kg^{-1}, zinc 0.71, iron 6.2 and manganese 4.3 mg kg^{-1} soil for year of 2010 and pH 7.4, total organic matter 2.1%, electrical conductivity (ECe) 0.52 dS m^{-1}, total nitrogen 0.14%, available phosphorus 10.1 mg kg^{-1}, available potassium 389 mg kg^{-1}, zinc 0.83, iron 3.6 and manganese 4.0 mg kg^{-1} soil for year of 2011. Before planting of soybean, fertilizers were used as follows: 200 kg P_2O_5 ha^{-1} and 50 kg N ha^{-1} and mixed with soil and land was ploughed once and harrowed twice. Soybean seed was inoculated with *Bradyrhizobium japonicum*. This experiment included 27 treatments that were placed in 81 plots. The plots consisted of six rows, 5 m in length spacing 60 cm apart. The distance between plants within a row was 5 cm and plant density was 333000 plants/ha. The plant density was achieved by over planting and thinning at V3 stage. Usage amounts of fertilizers zinc (0, 20 and 40 kg ha^{-1} from $ZnSO_4$ source), iron (0, 25 and 50 kg ha^{-1} from $FeSO_4$ source) and manganese (0, 25 and 40 kg ha^{-1} from $MnSO_4$ source) were calculated based on plots area surface; next, fertilizers were mixed with soft soil at the ratio of 1: 5 and placed on furrows made manually next to the stacks. At the end of growth season and harvesting time, ten plants were randomly selected from each plot, were cut from the soil surface with shears and grains were separated from pods. The soybean plants were harvested at maturity when 95% of total pods turned brown. Oil and protein percent in soybean seed were measured according to Emami (1996) and Jung *et al*. (2003). For the measurement of pod, seed and total dry weight the samples were dried at 70°C for 48 h and finally were weighed. All of data obtained from the measurements were evaluated statistically by MSTATC software. Combined variance analysis was performed after Bartlet test for checking uniformity of data variance (p = 0.05). Least Significant Difference test (LSD) was used to compare means and finally, Excel software (version of 2007) was applied to construct diagrams.

RESULTS AND DISCUSSION

The results of analysis of variance combined of oil, protein and dry matter production in soybean affected by zinc, iron and manganese fertilization at two years shown in Table 1. Based on results obtained zinc and iron had significant effects on all of evaluated traits at 1% levels

Table 1: Analysis of variance of oil, protein and dry matter in pod, seed and plant of soybean affected by zinc, iron and manganese fertilization at 2010 and 2011 years

		MS				
				Dry matter production		
Source of variation	df	Oil (%)	Protein (%)	Pod	Seed	Plant
Year (Y)	1	0.71^{ns}	2.78^{ns}	0.11^{ns}	0.49^{ns}	0.08^{ns}
Zn	2	481.44**	143.01**	7.59**	31.40**	24.76**
Y×Zn	2	1.68^{ns}	0.33^{ns}	0.15^{ns}	0.29^{ns}	1.83^{ns}
Fe	2	50.35**	30.99**	0.80**	21.23**	457.22**
Y×Fe	2	0.06^{ns}	2.85^{ns}	0.001^{ns}	0.33^{ns}	0.06^{ns}
Zn×Fe	4	14.01**	1.98^{ns}	0.02^{ns}	4.22**	32.57**
Y×Zn×Fe	4	0.95^{ns}	0.17^{ns}	0.22^{ns}	1.84**	1.48^{ns}
Mn	2	29.00**	5.19*	3.84**	110.96**	132.88**
Y×Mn	2	0.08^{ns}	0.46^{ns}	0.07^{ns}	0.38^{ns}	2.17^{ns}
Zn×Mn	4	1.54^{ns}	1.81^{ns}	1.41**	1.43*	6.05^{ns}
Y×Zn×Mn	4	0.54^{ns}	0.34^{ns}	0.11^{ns}	0.21^{ns}	0.97^{ns}
Fe×Mn	4	1.06^{ns}	0.49^{ns}	0.38**	0.71^{ns}	0.91^{ns}
Y×Fe×Mn	4	0.19^{ns}	0.41^{ns}	0.10^{ns}	0.83^{ns}	0.54^{ns}
Zn×Fe×Mn	8	2.22*	1.84^{ns}	0.47**	2.37**	5.87^{ns}
Y×Zn×Fe×Mn	8	1.03^{ns}	0.40^{ns}	0.09^{ns}	0.40^{ns}	1.52^{ns}
Error	104	1.02	1.39	0.09	0.51	3.17
Coefficient of variation (%)	-	8.76	7.49	8.97	7.39	7.76

ns: Non significant, *,**significant at 5 and 1% levels of probability, respectively

($p<0.01$). Also, manganese had significantly effects on oil percent, pod, seed and total dry weight at 1% levels ($p<0.01$) and on protein at 5% level ($p<0.05$). Oil, seed dry weight and total dry weight of plant were affected at 1% levels by interaction effect of zinc with iron (Zn×Fe). In addition, interaction effects of zinc with manganese (Zn×Mn) had significantly effects on pod and seed dry weight at 1 and 5% levels, respectively. Except for pod dry weight, interaction effects of iron with manganese (Fe×Mn) had no effects on other evaluated traits. Triple interaction effects of Zn×Fe×Mn had significantly effects on oil ($p<0.05$), pod and seed dry weight ($p<0.01$) but protein percent and total dry weight unaffected by this treatment (Table 1). The results of interaction effects of Zn×Fe and Zn×Mn on oil and protein percentage and also pod, seed and total dry weight of soybean plants at 2010 and 2011 years was shown in Table 2. Application of 20 kg Zn ha^{-1} had the most impact on pod dry weight (3.7 g) and plant dry weight (30.1 g), while maximum oil (22.9%) and protein (34.5%) content and seed dry weight (10.1 g) were recorded with 40 kg Zn ha^{-1}. Reduction in dry weight of plant organs due to zinc deficiency, also previously been reported by researchers (Cakmak *et al*., 1996; Brennan, 2001; Thalooth *et al*., 2006). Wang and Jin (2007) reported that zinc application had the significantly effect on dry matter accumulation and growth of corn. It is important to note that, application of 20 kg Zn ha^{-1} with 25 and 50 kg Fe ha^{-1} have the same effect on pod and seed dry weight and the obtained difference was not statistically significant. The most total dry weight was obtained by application of 50 kg Fe ha^{-1} and without the use of zinc. Indeed, iron had the more impact on plant dry weight compared zinc. A similar result was obtained by Caliskan *et al*. (2008). Application of 40 kg Zn with 25 kg Fe ha^{-1} led to increased oil and protein concentration in soybean seed by 24.3 and 35.4%, respectively (Table 2). In excess amounts of iron application the oil and protein content was

Table 2: Interaction effects of Zn×Fe and Zn×Mn on oil and protein percentage and also pod, seed and total dry weight of soybean plants at 2010 and 2011 years

	Iron (kg ha^{-1})			Manganese (kg ha^{-1})		
Zinc (kg ha^{-1})	0	25	50	0	20	40
Pod dry weight (g)						
0	2.9^{d}	3.1^{cd}	3.2^{c}	3.1^{d}	3.3^{cd}	2.9^{f}
20	3.7^{a}	3.8^{a}	3.8^{a}	3.4^{c}	3.9^{ab}	4.0^{a}
40	3.5^{b}	3.7^{ab}	3.8^{a}	3.2^{de}	3.8^{b}	3.9^{ab}
Seed dry weight (g)						
0	7.7^{c}	9.5^{b}	9.3^{b}	7.6^{h}	9.2^{e}	9.7^{d}
20	9.1^{b}	10.6^{a}	10.1^{a}	8.1^{g}	10.5^{c}	11.2^{ab}
40	10.1^{a}	10.2^{a}	10.5^{a}	8.6^{f}	10.8^{bc}	11.5^{a}
Plant dry weight (g)						
0	20.1^{d}	23.4^{c}	27.7^{a}	22.6^{c}	23.9^{ab}	24.1^{ab}
20	20.1^{d}	24.3^{bc}	23.6^{c}	20.3^{d}	23.5^{abc}	24.5^{a}
40	19.2^{d}	23.2^{c}	25.1^{b}	20.6^{d}	23.5^{abc}	23.4^{bc}
Oil (%)						
0	17.4^{e}	17.9^{de}	18.3^{d}	17.3^{e}	18.1^{d}	18.3^{d}
20	20.1^{c}	22.7^{b}	23.8^{a}	21.1^{c}	22.5^{b}	23.0^{b}
40	22.9^{b}	24.3^{a}	23.7^{a}	22.8^{b}	24.2^{a}	23.8^{a}
Protein (%)						
0	31.2^{e}	32.4^{d}	32.4^{d}	32.0^{d}	32.0^{d}	32.0^{d}
20	33.3^{c}	34.7^{ab}	35.3^{a}	33.9^{c}	34.9^{ab}	34.5^{bc}
40	34.5^{b}	35.4^{a}	35.3^{a}	34.5^{bc}	35.4^{a}	35.3^{a}

Similar letters in each column shows non-significant difference according to LSD test in 5% level

reduced, although this reduction was not statistically significant. The results of means comparison of zinc and manganese interaction (Zn×Mn) (Table 2) showed that maximum pod dry weight of soybean (4 g plant^{-1}) was obtained in $Zn_{20}Mn_{40}$ treatment. While, application of 40 kg Mn ha^{-1} individually led to decreased pod dry weight severely (2.9 g plant^{-1}). The highest seed dry weight per plant was recorded in $Zn_{40}Mn_{40}$ treatment with 11.5 g plant^{-1}. In the other side, with application of 20 kg Zn ha^{-1} and 20 kg Mn ha^{-1}, simultaneously produced maximum total dry weight of plant which was equal to 24.5 g plant^{-1}. In our experiment the effect of zinc applied on oil and protein of soybean was more than the iron and manganese application. Manganese increased seed oil content but had little impact on grain protein percentage. These results are in agreement with Hebbern *et al.* (2005), Mirzapour and Khoshgoftar (2006), Sarkar *et al.* (2007) and Movahhedy-Dehnavy *et al.* (2009). Also, iron used up to 25 kg ha^{-1} increased oil and protein content in soybean seed but in excess amounts of iron these traits were decreased, however, this reduction was not statistically significant. The $Zn_{40}Mn_{20}$ treatment had the most impact on oil and protein contents in soybean seed, so that increased oil and protein contents by 24.2 and 35.4%, respectively. The $Zn_{40}Mn_{40}$ treatment could not be effective on soybean oil and protein contents. Wilson *et al.* (1982) and Horest (1991) emphasized that with manganese fertilization, oil content in soybean seed increased significantly. Also, oil and protein in safflower seed increased with zinc application (Babhulkar *et al.*, 2000). Interaction effect of iron and manganese interaction (Fe×Mn) on oil and protein percentage and also pod, seed and total dry weight of soybean plants shown in Table 3. The results indicated that maximum pod dry weight; total dry weight, oil and protein

Table 3: Interaction effect of Fe×Mn on oil and protein percentage and also pod, seed and total dry weight of soybean plants at 2010 and 2011 years

Fertilizers treatment (kg ha^{-1})		Means				
Iron	Manganese	Pod dry weight (g)	Seed dry weight (g)	Plant dry weight (g)	Oil (%)	Protein (%)
0	0	3.1^{e}	7.4^{f}	18.0^{e}	19.5^{c}	32.8^{d}
	20	3.4^{cd}	9.4^{d}	26.3^{d}	20.3^{b}	33.3^{cd}
	40	3.6^{bc}	10.1^{c}	21.0^{cd}	20.6^{b}	32.9^{cd}
25	0	3.2^{e}	8.3^{e}	21.8^{c}	20.9^{b}	33.6^{bc}
	20	3.8^{ab}	10.8^{ab}	24.6^{b}	22.1^{a}	34.4^{ab}
	40	3.7^{b}	11.3^{a}	24.6^{b}	22.0^{a}	34.4^{ab}
50	0	3.4^{d}	8.6^{e}	23.7^{b}	20.8^{b}	34.0^{ab}
	20	3.9^{a}	10.3^{bc}	26.5^{a}	22.6^{a}	34.6^{a}
	40	3.6^{bcd}	11.1^{a}	26.2^{a}	22.4^{a}	34.4^{ab}

Similar letters in each column shows non-significant difference according to LSD test in 5% level

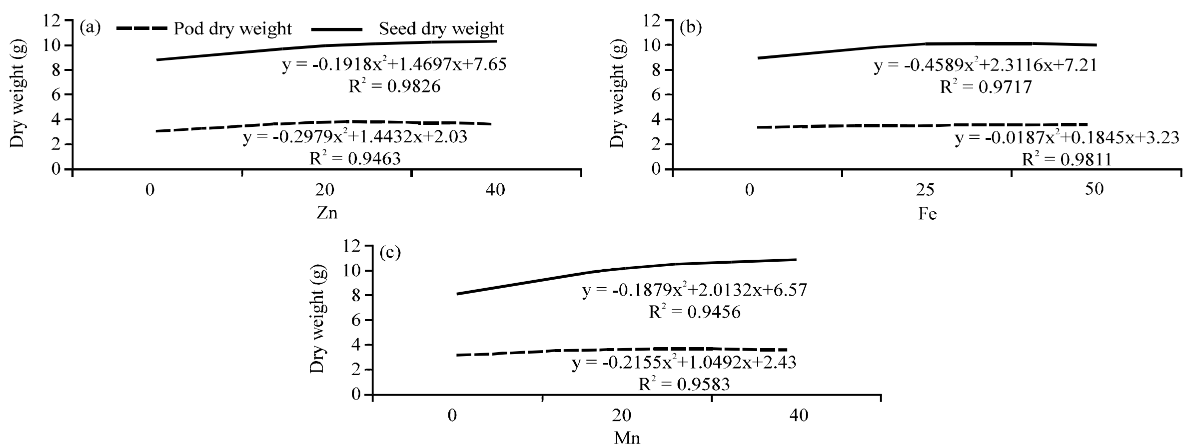

Fig. 1(a-c): Impact of (a) Zinc, (b) Iron and (c) Manganese (individually) on pod and seed dry weight (g plant^{-1}) of soybean

contents were observed in $Fe_{50}Mn_{20}$ treatment by 3.9 (g plant^{-1}), 26.5 (g plant^{-1}), 22.6 and 34.6%, respectively. Application of 25 kg Fe ha^{-1} with 40 kg Mn ha^{-1} had more effects on seed dry weight and it increased to 11.3 g plant^{-1}. Furthermore, the lowest values of evaluated traits were recorded in check treatment. With zinc application up to 40 kg ha^{-1}, seed dry weight was increased, while values greater than 20 kg Zn ha^{-1} was reduced pod dry weight. Iron use up to 25 kg ha^{-1} increased pod and seed dry weight and excess amount of iron had no significantly effects on these traits. Response of seed dry weight to manganese was more than pod dry weight. Manganese significantly increased seed dry weight (Fig. 1). The effects of different levels of zinc, iron and manganese on total dry weight of soybean plant shown in Fig. 2. Total dry weight was increased by Zn, Fe and Mn applied, while the effect of iron on this attribute is greater than the other micronutrients. Application of 50 kg Fe ha^{-1} enhanced total dry weight to 25.47 g plant^{-1} (Fig. 2). These results are agreed with Ghasemi-Fasaei *et al*. (2002). Impact of different levels of zinc, iron and manganese (individually) on oil and protein concentration in soybean seed shown in Fig. 3. With zinc application from 0-40 kg ha^{-1} oil and protein contents were increased. Similar

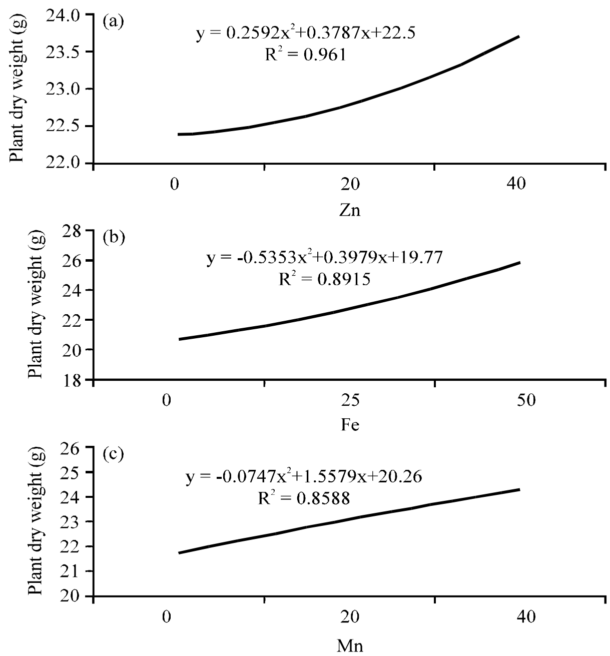

Fig. 2(a-c): Impact of (a) Zinc, (b) Iron and (c) Manganese (individually) on plant dry weight (g plant^{-1}) of soybean

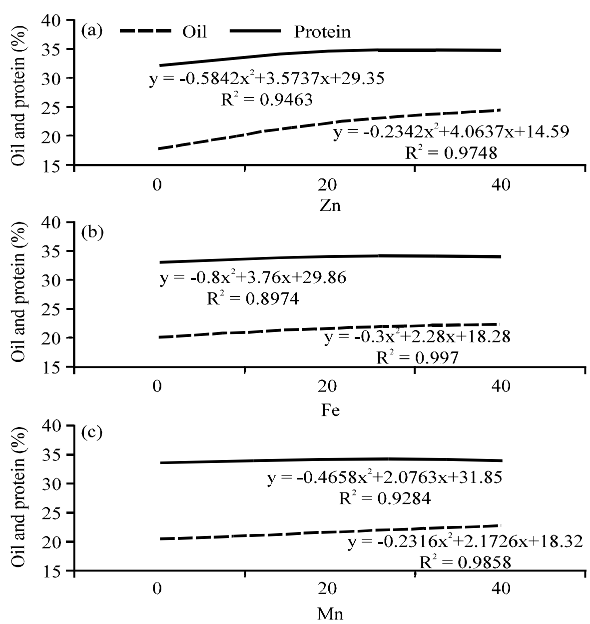

Fig. 3(a-c): Impact of (a) Zinc, (b) Iron and (c) Manganese (individually) on oil and protein (%) in soybean seed

trends (but milder) were observed when iron was used. As it can be seen in Fig. 1 the effect of zinc and manganese applications on oil percent in soybean seed was more than protein content. Moreover, responses of oil and protein in seed to iron fertilization are almost similar.

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