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Full Length Research Paper

Investigating Fe and Zn foliar application on yield and its components of soybean (*Glycine max* (L) Merr.) at different growth stages

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In order to investigate Zn, Fe and Zn+Fe foliar application effects on soybean (*Glycine max* L.) yield and its components (number of pods per plant, number of seeds per pod and 1000 grain weight), a field experiment was conducted at Minodasht, Iran during 2009 and 2010 growth season. The experiment was arranged in split plot of a randomized complete block design and replicated three times. Parameters measured were grain yield, harvest index, first pod height, number of pods per plant, number of seeds per pod and 1000 grain weight. Results showed significant effect of Zn+Fe treatment on grain yield, number of pods per plant ($p < 0.05$) and 1000 grain weight ($p < 0.01$). The time of foliar application on number of pods per plant ($p < 0.05$) and 1000 grain weight ($p < 0.01$) was also significant. In general, the highest yield was produced by Zn+Fe combination treatment. There was a significant and positive correlation between grain yield and its components. Leaf area index in 407 growth degree days at the beginning of pod set (6/6), crop growth rate in 272 growth degree day (GDD) at the beginning of flowering (39/52) and net assimilation rate in 272 GDD at 10-leaf stage (3 g plant^{-1} GDD^{-1}) reached the highest point, respectively.

Key words: *Glycine max* L., nutrients, seed production, growth indexes.

INTRODUCTION

Soybean (*Glycine max* (L) Merr.) belongs to Papilionaceae, leguminous family and it is a herbaceous annual legume, normally erect, bushy and leafy that was cultivated in China for the first time which was ranked as an oilseed crop and it provides approximately 50% edible oil of the world (Akparobi, 2009). It has been recognized as a former crop plant since the origin of agriculture (Jandong et al. 2011). Soybean as a meal consists of important minerals, such as Ca, P and Fe. Due to the large amount of macro and micro nutrients, it has been considered as a nutritious food for human needs, livestock, industrial and medicinal purposes (Akparobi, 2009; Berglund, 2002). In Iran, soybean is cultivated after wheat and canola in rotation and soybean seed consists of 18 to 25% oil and 30 to 50% protein. Vahedi et al., (2010) and Salwa et al. (2011) stated that soybean is a crop that compensates shortage of oil and protein of other crops. Furthermore, it is a good source for height energy, protein and essential nutrients to human and animals. Soybean production has been increased to 36% since 2000 in the world. In 1961 to 1965, 28.6 million ton of soybean was produced and it was increased to 217.6 million ton per hectare in 2005 to 2007 (Masuda and Goldsmith, 2009).

Foliar spraying is a new method for crop feeding, which micronutrients in form of liquid are used into leaves (Nasiri et al., 2010). Foliar application of microelements is more beneficial than soil application. Since application rates are lesser as compared to soil application, same application could be obtained easily and crop reacts to nutrient application immediately (Zayed et al., 2011). Undoubtedly, soybean higher yield and quality as well as

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its oil will be obtained by microelements foliar spraying (Vahedi, 2011). Foliar spraying of microelements is very helpful when the roots can not provide necessary nutrients (Kinaci and Gulmezoglu, 2007; Babaeian et al., 2011). Moreover, soil pollution would be a major problem by micronutrients soil application. As people are concerned about the environment and plant leaves uptake nutrients better than soil application, foliar spraying was created (Bozorgi et al., 2011). Crop roots are unable to absorb some important nutrients such as zinc, because of soil properties, such as high pH, lime or heavy texture, and in this situation, foliar spraying is better as compared to soil application (Kinaci and Gulmezoglu, 2007). Narimani et al. (2010) reported that microelements foliar application improve the effectiveness of macronutrients. It has been found that microelements foliar application is in the same level and even more influential as compared to soil application. It was suggested that micronutrients could be applied successfully to compensate shortage of those elements (Arif et al., 2006). These authors found that based on soil properties, foliar spraying could be effective 6 to 20 times as compared to soil application. Resistance to different stresses will be increased by foliar application of micronutrients (Ghasemian et al., 2010). Since in field situation, soil features and environmental factors which affect nutrients absorption are extremely changeable, foliar application could be an advantage for crop growth (SeifiNadergholi et al., 2011). Also, effectiveness of foliar spraying is higher and the cost of foliar application is lower as compared to soil application (Yassen et al., 2010).

Salwa et al. (2011) stated that microelements are defined substances that are crucial for crop growth; however, they are used in lower amounts as 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). One of the most important roles of micronutrients is keeping balanced crop physiology. Furthermore, these elements play vital roles in CO_2 flowing out, improvement in vitamin A and immune system activities (Narimani et al., 2010). Zinc plays a special role in synthesizing proteins, RNA and DNA (Kobraee et al., 2011). Zinc and iron take over different roles in crop, such as formation, partitioning and utilization of photosynthesis assimilates (Sawan et al., 2008). The major role of zinc element in crops is not clear Nasri et al. (2011). These authors found that although zinc is an important element which should be provided for crop growth, but it could be poisonous in large amount. As a matter of fact, the importance of zinc foliar application is due to being given to crop immediately (Alloway, 2003). Gul et al. (2011) claimed that profitability of micronutrients will be obtained in combination with macro elements, such as nitrogen and potassium. Ghasemian et al. (2010) declared that zinc element is essential in chlorophyll production and pollen function. Iron (Fe) contributes in very enzymatic activities, such as cytochromes, ferredoxine, superoxide dismutase (SOD), catalase (CAT), peroxidase and nitrate reductase. It was reported by Kobraee et al. (2011) that growth limitation, symbiosis, nodulation, photosynthesis, dry matter production and plant nutrient disorder were caused by the lack of zinc and iron. Furthermore, reactions of electron transportation are needed for zinc and iron.

Cakmack (2002) found that an important reason of zinc and iron deficiencies around the world is the consumption of cereals which have low amounts of Zn and Fe micronutrients and high levels of bicarbonate in irrigation water increases Fe shortage of soils. The main factors which affect the amount of zinc in soil are pH, carbonate content, organic matter, soil texture and interaction between zinc and other microelements, such as iron (Bukvić et al., 2003). Zinc and iron deficiency in soils could be a restricted factor of yield and extremely decrease crop yield quality (Salwa et al., 2011). Bozoglu et al. (2007) found that in 25 countries, zinc deficiency is a soil common problem. Babaeian et al. (2011) announced that zinc and iron deficiencies are common in 30 and 50% of soils, respectively in the world. These authors claimed that microelements deficiency is mainly due to their low amounts and soil features which hinder crop roots to obtain them. Vahedi (2011) stated that lack of zinc is a major problem in the world and shortage of zinc will reduce crop yield. Generally, dry matter production and its division into different parts of plant will be weakened by lack of micronutrients (Sawan et al., 2008). Lack of zinc in crop plants is due to the fact that this element cannot be solved in soils, and reduction of cell growth and development is one of the symptoms of Zn deficiency (Ghasemian et al., 2010). Kobraee et al. (2011) and Ghasemian et al. (2010) claimed that young leaves chlorosis and plant metabolism disorder will be caused by lack of iron, and in condition of iron stress, the absorption of Fe would be enhanced. Since iron is not soluble in soil, foliar application of this nutrient is necessary. Ai-Qing et al. (2011) and Sliman and Motto (1990) reported that there is an antagonistic interaction between zinc and iron in soybean which influences their absorption, partitioning and utilization. These authors announced that zinc affects absorption and translocation of iron and vice versa. The aim of this study was to investigate zinc and iron foliar application effects on soybean yield and its components (grain yield, number of pods per plant, number of seeds per pod and 1000 grain weight).

MATERIALS AND METHODS

This study was conducted at Minodasht, Iran during 2009 and 2010 growth seasons. The experiment was arranged in split plot of a randomized complete block design and replicated three times. Main factor were three different stages (10 leaf stage, beginning of flowering and beginning of pod set), sub plots were (control, Zn 116

Table 1. Analysis of soil sample taken from 0 to 30 cm depth.

Sample	Value
Clay (%)	16
Silt (%)	78
Sand (%)	6
Fe (ppm)	7.68
Zn (ppm)	0.9
Mn (ppm)	7.0
Cu (ppm)	2.8
Texture silty loam	-
pH	7.7

Table 2. Analysis of variance of seed yield, harvest index and first pod height.

Source of variation	df	Mean of square		
		Seed yield	Harvest index	First pod height
Time (T)	2	188235.111^{ns}	0.001^{ns}	4.405^{ns}
Fertilizer (F)	3	2676456.4*	0.003^{ns}	3.019^{ns}
T × F	6	581802.519^{ns}	0.003^{ns}	3.739^{ns}
Error	18	580269.370	0.003	2.798

**, *, ns: Significant at 1 and 5% probability levels and non significant, respectively.

ppm treatment, Fe 116 ppm treatment and Zn+Fe combination treatment). Minodasht (latitude 160 29 ' N, longitude 260 55 ' E) is situated at 200 m above sea level. Annual rainfall average is 600 mm and the climate is mild-semi humid. Annual temperature average is 11/9°C. Soybean cultivar used in this experiment was Sahar (Persian) which was sown on the 14th July, 2009. Analysis of soil sample taken from 0-30 cm depth explained in Table 1. Seeds were pretreated with *Rhizobium japonicum* and weed control was done manually. The trial was harvested after physiologic maturity. Each plot consisted of six rows with 6 m length and the row spacing was 50 cm, ten selected plants were used to take the data from each plot of each replication. Data were recorded for grain yield, first pod height, harvest index, number of pods per plant, number of seeds per pod and 1000 grain weight. Leaf area index (LAI), crop growth rate (CGR) and net assimilation rate (NAR) was calculated based on growth degree day. Data collected were analyzed statistically using SAS and correlation coefficient by statistical processor system support (SPSS). The means differences among the treatments were compared by least significance difference test (LSD) at 0.05 level of possibility.

LAI = LA/SA

CGR = w2-w1/GDD

$$\text{GDD} = \sum \left(\frac{T\max + T\min}{2} - 10 \right)$$

NAR = CGR/LAI

where LA is the leaf area, SA is the ground area that occupied a plant, W is the dry matter and GDD is the growth degree day.

RESULTS AND DISCUSSION

Seed yield

Analysis of variance showed that the effect of micronutrients foliar application on grain yield was significant ($p < 0.05$), but the effect of foliar spraying at different growth stages was not significant (Table 2). Mean comparison showed that Zn+Fe combination treatment produced maximum yield which was 15.75 ton/ha. Zn treatment and Fe treatment yields were 25 and 11.41% higher than control treatment, respectively (Table 3). Our results were in line with the findings of Banks (2004) in soybean and Arif et al. (2006) in wheat. In contrast, Mallarino et al. (2001) and Gerwing et al. (2003) showed that zinc foliar spraying did not increase soybean yield. SeifiNadergholi et al. (2011) indicated that ZnSo4 foliar application at flowering and seed set noticeably raised soybean yield components. Berglund (2002) noted that Zn foliar application particularly at vegetative growth stage increased soybean seed yield. Bozoglu et al. (2007) stated that foliar application of micronutrients could be implemented for higher yield and quality. These authors reported that shortage of some micronutrients decreased yield in chickpea. Zinc foliar application enhanced soybean yield by influencing the number of seeds per plant and seed weight (Kobraee et al., 2011). Zeidan et al. (2006) reported that yield components in

Table 3. Mean Comparison of seed yield and yield components.

Treatment	Seed yield (ton/ha)	Pod/plant number	Seed/pod number	1000 grain weight (g)
Control	6.66^{c}	24.23^{b}	1.68^{b}	144.4^{c}
Zn	7.42^{b}	35.97^{a}	2.16^{a}	167.3^{b}
Fe	8.33^{ab}	31.31^{a}	2.23^{a}	178.2^{ab}
Zn + Fe	15.75^{a}	36.36^{a}	2.17^{a}	200^{a}

Means, in each column, following similar letter(s) are not significantly different at the 5% level of probability- using Duncan's multiple range test.

Table 4. Mean comparison of yield components at different growth stages.

Treatment	Pod/plant	1000 grain weight
10-leaf	35.42^{a}	168.3^{a}
Flowering	33^{ab}	163.8^{c}
Pod set	31^{b}	166^{b}

Means in each column, following similar letter(s) are not significantly different at the 5% level of probability- using Duncan's multiple range test.

lentil are enhanced by foliar application of micronutrients. Due to the enzymatic activity enhancement, micro-elements effectively increased photosynthesis and translocation of assimilates to the seed. Zayed et al. (2011) announced that due to the synergistic effect, zinc + iron treatment as compared to Zn treatment and Fe treatment was more helpful in rice. Arif et al. (2006) reported that higher yield could be achievable by foliar spraying which ensures crop dry matter production. Kobraee et al. (2011) claimed that zinc and iron application at the same time could be lead to higher dry matter and seed yield as compared to using them separately. Kakiuchi and Kobata (2008) revealed that seed production in soybean is lower as compared to the other legumes, and for production, one gram of seed, two gram photosynthesis assimilates will be required. Shiraiwa et al. (2004) declared that pod number and seed number are two major factors which affect soybean seed yield. Variation in dry matter production in seed filling period is a prime factor for different seed yield in soybean genotypes. In soybean, as a result of increasing the ratio of source-sink during flowering stage, seed yield was increased by the seed number enhancement (Cumudini et al., 2011).

Number of pod per plant

Analysis of variance showed that the effect of micronutrients foliar application and foliar spraying at different growth stages were significant ($p < 0.05$) (Table 5). Mean comparison showed that the highest number of pod per plant was produced at 10 leaf stage (35.42) and the least number was at the beginning of pod set (31) (Table 4). In addition, there was no statistical difference among all the fertilizer treatments and Zn+Fe combination treatment had the maximum number of pod per plant (36.36) and minimum number was produced by control (24.23) (Table 3). Our results were in agreement with Bozorgi et al. (2011) in faba bean, Kobraee et al. (2011) in soybean and SeifiNadergholi et al. (2011) in common bean who showed that the number of pods per plant was enhanced by zinc foliar application. Elballa et al. (2004) revealed that microelements application enhanced the number of pods per plant. Zeidan et al. (2006) found that foliar spray of micronutrients considerably enhanced the number of pods per plant, 1000 seed weight and seed yield. Kakiuchi and Kobata (2006) claimed that one of the most important factors which are determiner in soybean seed yield is pod number per plant. These authors indicated that the rate of pod set in soybean raised with an enhancement in source vigor at the time at which sink/source ratio was altered by flower thinning and defoliation. Furthermore, the ratio of dry matter enhancement in soybean seeds to its shoot is a crucial factor which affects the rate of podding in soybean. SeifiNadergholi et al. (2011) reported that highest pods per plant was produced by foliar spraying at flowering and podding stage and increase of number of pods per plant due to foliar application could be attributed to significant effect of microelements on reproductive organs, such as stamens and pollens. These authors revealed that since soybean is a self-pollinated crop, stamen activity enhances the number of flowers that can

Table 5. Analysis of variance of yield components.

Source	df	Mean of Square		
		Pod/plant	Seed/pod	1000 grain weight
Time (T)	2	117.693*	0.001^{ns}	60.778**
Fertilizer (F)	3	294.940*	0.579^{ns}	2050.185**
T × F	6	35.253^{ns}	0.059*	34.407^{ns}
Error	18	71.422	0.021	60.333

**, *, ns: Significant at 1 and 5 % probability levels and non significant, respectively.

Table 6. The interaction of fertilizer and time of fertilizer application on seed/pod.

Treatments	10-leaf stage	Beginning of flowering	Beginning of pod set
Control	1/7de	1/5^{e}	1/86^{cd}
Zn	2/2^{ab}	2/13^{abc}	2/16^{ab}
Fe	2/2^{ab}	2/36^{a}	2/13^{abc}
Zn + Fe	2/2^{ab}	2/26^{ab}	2/06^{bc}

Means, in each column, following similar letter(s) are not significantly different at the 5% level of probability- using Duncan's multiple range test.

fertile well and as a result, larger number of pods per plant will be produced.

Number of seeds per pod

Analysis of variance showed that the effect of foliar application and foliar spraying at different growth stages were not significant (Table 5). There was no statistical difference among all the fertilizer treatments (Table 3). Mean comparison revealed that Fe treatment had the maximum number of seed per pod (2.23) and the minimum was produced by control (1.68) (Table 3). The interaction of fertilizer and time of fertilizer application showed that Fe treatment at the beginning of flowering produced maximum number of seed per pod (2.36) and control treatment produced the least (1.5) at the beginning of flowering (Table 6). These results coincided with SeifiNadergholi et al. (2011) in common bean and Banks (2004) in soybean who reported that zinc foliar application increased the number of seeds per pod. This author showed that zinc foliar application in eight-leaf stage with increasing leaf area, dry weight and length of flowering period enhanced the number of seeds per pod. Nasri et al. (2011) reported that it was increased considerably by zinc foliar spraying in *Phaseolous vulgaris*. Ghasemian et al. (2010) declared that maximum number of seeds per plant was produced by zinc application in soybean.

1000 grain weight

Analysis of variance revealed that the effect of micronutrient foliar spraying and time of foliar application on 1000 grain weight were significant ($p < 0.01$) (Table 5). Mean comparison showed that maximum 1000 grain weight was found at 10-leaf stage (168.3 g) and the minimum was obtained by the beginning of flowering stage (163.8 g) (Table 4). Zn+Fe combination treatment produced maximum 1000 grain weight (200 g) and minimum 1000 grain weight was obtained by control treatment (144/4 g) (Table 3). The results are in agreement with Arif et al. (2006) who found considerable enhancement in 1000 seed weight by foliar spraying in wheat crop. Nasri et al. (2011) in *P. vulgaris* and Ghasemian et al., (2010) in soybean reported that 100 seed weight was increased considerably by zinc foliar spraying. When photosynthesis assimilates is translocated from vegetative organs to the other parts, seed weight will be enhanced considerably Kakiuchi and Kobata (2008). It was shown that in common bean, microelements affects leaf and as a result larger amounts of assimilate was produced. Since seeds are major source of assimilate, they moves to the seeds and in conclusion larger seeds were produced (SeifiNadergholi et al., 2011).

Correlation coefficient of yield and its components

Correlation coefficients (Table 7) indicated that soybean yield had positive and significant correlation with 1000 grain weight (r = 0.711), number of pod per plant (r = 0.577) and number of seed per pod (r = 0.483). Correlation of soybean yield with first pod height was negative and significant (r = -0.433). Number of pods per

Table 7. Correlation coefficients of various agronomic characteristics of soybean (*Glycine max* (L.) Merr).

	Number of pod per plant	First pod height	Number of seed per pod	1000 grain weight
Seed yield	0/577**	- 0/433**	0/483**	0/711**
Number of pod per plant	-	- 0/384*	0/498**	0/588**
Number of seed per pod	-	-	-	0/615**

**, *, ns: Significant at 1 and 5 % probability levels and non significant, respectively.

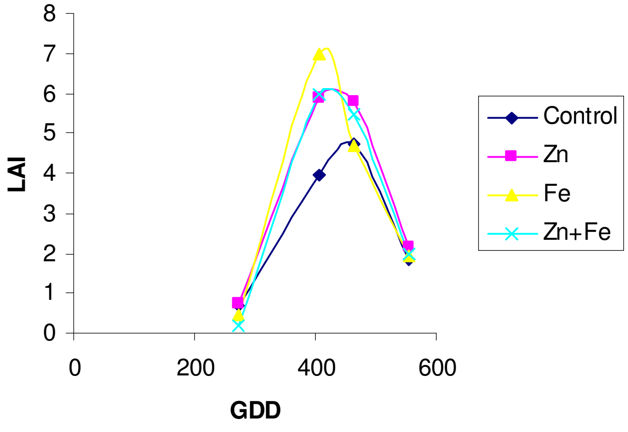

Figure 1. Leaf area index at different growth stages.

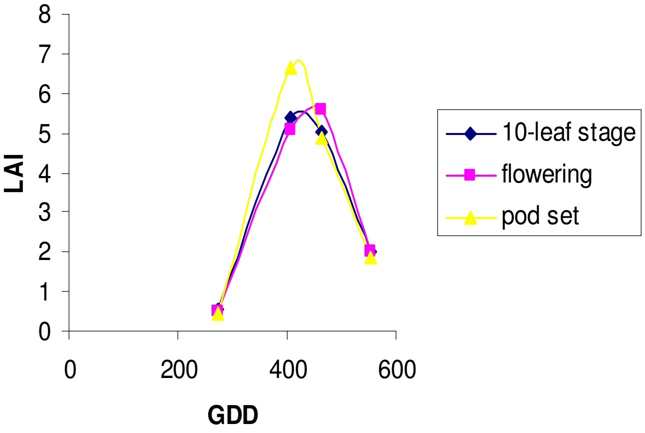

Figure 2. Effect of foliar application on LAI.

plant showed positive and significant correlation with number of seed per pod (r = 0.498) and 1000 grain weight (r = 0.588). Furthermore, number of seed per pod had positive significant correlation (r = 0.615) with 1000 grain yield. However, a negative and significant correlation (r = - 0.384) was found between number of pods per plant and first pod height. Our findings were in confirmation with the previous studies carried out by Showkat and Tyagi (2010) and Arshad et al. (2006) who indicated that there was positive and significant correlation between seed yield with number of pods per plant and 100 seed weight. Cumudini et al. (2001) found that there is a significant and positive correlation between seed yield and number of seeds per plant, number of seeds per pod and 1000 seed weight.

Leaf area index (LAI)

Results of this study indicated that Leaf area index during the experiment decreased at different growth stages so that in 407 GDD and at the beginning of pod set reached to the highest point (6.6) (Figure 1), but after that because of accelerating in leaves, ascending was declined. Leaves ascending decreased 23.73 in LAI. Furthermore, the highest LAI was found by foliar application with Fe (6.9) in 407 GDD and there was no difference among other treatments (Figure 2). Micronutrients foliar application increases enzymes activities which lead to higher crop production and leaf area (LA) expansion (Zayed et al., 2011). However, Mallarino et al. (2001) found that foliar fertilization could be harmful for leaf area expansion. Zinc foliar application increases tryptophan amino acid and indol acetic acid hormone which were the two main factors in leaf area expansion in *P. vulgaris* (SeifiNadergholi et al., 2011).

Crop growth rate (CGR)

Maximum CGR was obtained at the beginning of flowering (39.52) in 272 GDD (Figure 3). In addition, maximum CGR was found by zinc and iron combination treatment application (40.05) in 407 GDD and minimum CGR belong to control treatment (5.62) in 553 GDD (Figure 4). Pedersen and lauer (2004) stated that crop growth rate is the most important factor in growth analysis of soybean, because it shows the amounts of canopy assimilate and influences on the amount of dry matter and balances by changing leaf are index (LAI) and net assimilation rate (NAR). These authors showed that optimum CGR will be achievable by LAI 3 to 3.5. It was indicated by Cumudini et al. (2001) that throughout early reproductive stage, there is a significant and positive correlation between crop growth rate and seed number in maize and also, between dry matter production and seed

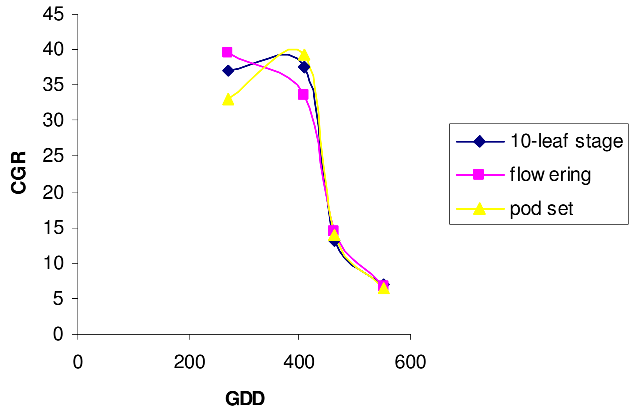

Figure 3. Crop growth rate at different growth stages.

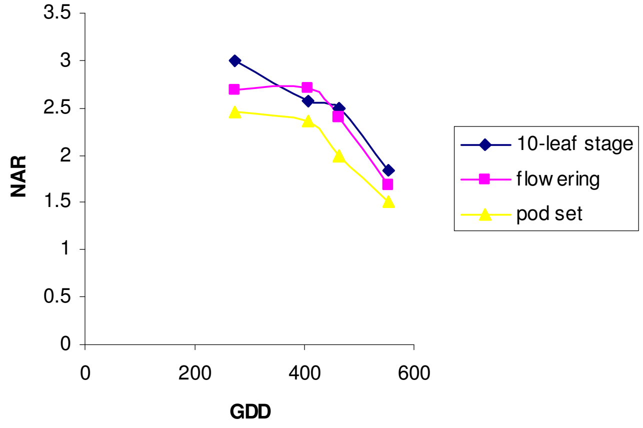

Figure 5. Net assimilation rate at different growth stages.

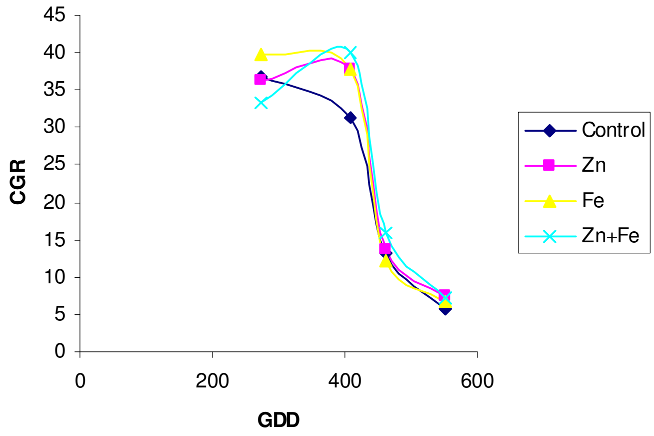

Figure 4. Effect of foliar spraying on CGR.

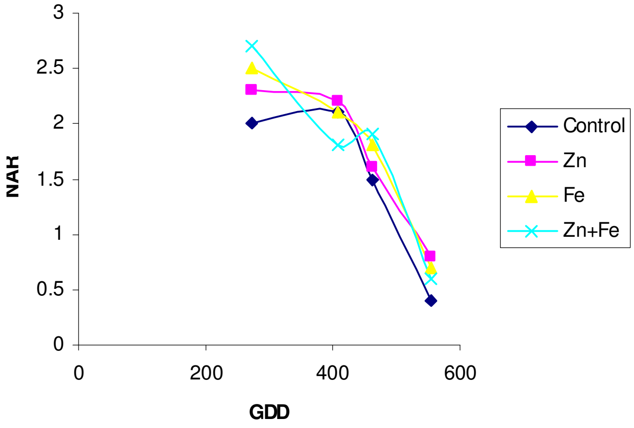

Figure 6. Effect of foliar application on NAR.

number in soybean. Ruhul et al. (2009) declared that the highest amount of crop growth rate was observed up to 60 days after sowing and it was decreased at 90 days after sowing. It was reported by Oya et al. (2004) that soybean seed yield was considerably correlated with crop growth rate during the reproductive growth stage. These authors believed that soybean has a special period in which reproductive and vegetative growth stages happen at the same time.

Net assimilation rate (NAR)

Results of this study revealed that NAR variations during the experiment were descending (Figure 5 and 6). Apparently, NAR is influenced by many factors which are complex and practically are not recognizable. For this reason, results of many researches are different with each other. Maximum NAR was observed at 10-leaf stage (3 g plant^{-1} GDD^{-1}) in 272 GDD and minimum NAR belong to pod set stage (1.5 g plant^{-1} GDD^{-1}) in 553 GDD (Figure 5). With regard to treatments, Zn+Fe combination treatment (35%) was more than control treatment (Figure 6). So, maximum NAR was found by foliar application of Zn+Fe (2.7 g plant^{-1} GDD^{-1}) in 272 GDD and minimum NAR was observed by control treatment (0.4 g plant-1 GDD^{-1}) in 553 GDD (Figure 6).

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