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Effect of Foliar Application of Micronutrients on Quantitative and Qualitative Characteristics of Soybean (*Glycine max* L.)

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ABSTRACT

To investigate the impact of important micronutrients including iron, manganese and Biomax (complete fertilizer) on growth characteristics, performance and proteins of soybean (Williams cultivar), a study randomized complete block design with three replications and five treatments (control, iron, manganese, iron/Mn and Biomax) were conducted at the Agricultural Research Station, College of Agriculture and Natural Resources, Razi University. In this research, the amount of fertilizers was based on soil tests and was applied before and after flowering. The results of this research showed that in soybean plants, for the use of micronutrient elements, there is a significant difference in traits, plant height, the distance between the crown and the first sub-stem, stem diameter, the number of pods in the sub-branches, the number of pods per stem, the number of pods per plant, protein mg/pr and yield. There was no significant difference in pod length and number of seeds in pod traits. The comparison table showed that for most traits of all micronutrients, Biomax fertilizer had the greatest effect.

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1. Introduction

Among agricultural plants, oilseed plants are of special importance, despite the annual production of more than 271 thousand tons of oilseeds in Iran most of the used oil is supplied from foreign sources (Alijani *et al.*, 2017). More than 80% of vegetable oil consumed in Iran is imported, therefore, there is a big demand to introduce a new source of vegetable oil to cover and compensate for the shortage of this product in the daily diet of the people (Piravi-Vanak *et al*., 2021).

Soybean (*Glycine max* L.) is one of the most cultivated legume crops in the world. In 2019, its global production was about 334 million metric tons from a harvested area of 121 million hectares In the U.S., soybean is the second largest crop after corn (*Zea mays* L.) and is primarily grown in the Midwest region, where about 75% of the total agricultural area (38.5 million hectares) is used for corn and soybean productions, The usage of soybeans ranges from human consumption to animal feed to non-food Products (Thapa *et al.*, 2021).

Soybeans contain 30%-52% protein, 17%-27% oil and 20% carbonated water. The high prevalence of soybean in the world is related to the quality of grain and protein. The amount and proportion of protein, oil and other important organic and mineral substances in the grain allow it to be used in various industries. Soybean grain is used to make butter, margarine, cheese, milk, flour, confectionery and canned food. Soybean oil amounts to 40% of the world's vegetable oil production (Nazarovna *et al.*, 2020).

Micronutrients have many advantages for the production of crops in different climatic conditions and different living and non-living stress conditions. These benefits include a positive effect on nitrogen absorption. Increase plant biomass production above

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NPK levels and improving tolerance to abiotic (such as drought and salinity) and biotic (pests and diseases) stresses through increasing water use efficiency, plant health and systemic response. In addition, micronutrients enhance the nutritional quality of food crops by providing nutrients such as zinc and iron that are critical to human health but often lacking in diets worldwide (Dimkpa *et al.*, 2019).

Micronutrients or in other words essential mineral elements are very important in the development of plants, Low-consumption and micronutrient elements are often not present in the soil and are used as fertilizers to increase the productivity of plants, especially when conventional fertilizers (NPK) are not effective (Dimkpa *et al.*, 2016).

Growth hormones, soluble elements, increased absorption and transport of nutrients, detoxification, increased transfer of sugar and amino acids in plant roots, and enhanced induction resistance to environmental stresses and can increase the growth and development of plants (Fazeli-Nasab *et al*., 2022).

The use of nutrients increases the resistance of plants to drought stress (Dimkpa *et al*., 2017). Soil consumption of micronutrient elements, in addition to the low efficiency of their absorption by the plant, is also very expensive from an economic point of view, and therefore alternative methods such as spraying foliar can be used (Siavashi *et al*., 2004).

Management scenarios in agricultural systems. Nano-based substances are easily absorbed by target plants based on their specific. Application; the NP amendment could help plant populations experience sustainable growth (Rasouli *et al.*, 2020).

Foliar application of micronutrient fertilizers can guarantee plants' access to nutrients to achieve a high yield. From an ecological point of view, foliar fertilization is more acceptable because less amounts of nutrients for fast consumption is provided by the plant (Alijani *et al*., 2017). Iron increases the thousand-seed weight by increasing the photosynthetic activity and the production of protein and carbohydrates in the plant. Iron element is also used in the structure of cytochromes (Yilmaz *et al.*, 2008). Iron deficiency causes a decrease in protein production and prevents the formation of chlorophyll. Considering that soybeans grow in alkaline soils, iron deficiency is one of the problems for the growth and development of soybeans (Yilmaz *et al*., 2008).

Manganese plays a role in the synthesis of photosynthetic and respiratory enzymes and plays an active role in plant metabolism. In plants with manganese deficiency, absorption of phosphorus and calcium, amount of chlorophyll, photosynthesis and the amount of oil in the seed decrease (Rezaei Chiyaneh *et al.*, 2015).

Remroudi et al. (2011) reported the effect of foliar application of iron and manganese on the biological performance and grain and other morphological characteristics of Plantago ovata seeds. Brighteni et al. (2008) stated in their study that the consumption of boron element increases the grain yield and oil percentage and stated that by increasing the pollen grain fertility and as a result of increasing the number of full grains, it has increased the grain yield of sunflower (Brighteni *et al*., 2008).

Zinc is one of the low-consumption elements that is necessary for the growth and reproduction of agricultural plants and is used in the synthesis of proteins and plant hormones such as auxin. In some reports, soy is mentioned as a plant sensitive to zinc deficiency (Banks, 2004).

Molybdenum is an essential micronutrient for plants. Many agricultural soils are deficient in molybdenum (Liu, 2002). Hasanpour et al. (2015) in a study on wheat, showed that molybdenum foliar application helps wheat yield during stress. Molybdenum is more involved in the nitrogen mechanism, one of the enzymes that play a key role in the series of processes related to nitrate consumption is nitrate reductase. There are many evidences of the complexity of regulating this process and the role of molybdenum in its activity level (Sigel, 2002). Yildirim, effects of three different concentrations of maxi crop foliar fertilizer (including 1.2% nitrogen, 939 ppm P2O5, 16.2% K2O, 4.5% manganese, 3% sodium, 520 ppm iron, 2050 ppm investigated magnesium, 15 ppm zinc, 2.8 ppm copper, 43 ppm boron and 13 ppm molybdenum) in different stages of growth on yield and yield components of soybean and reported that the use of foliar nutrition increased grain yield, but no significant difference was observed between different amounts of fertilizer for the weight of 1000 seeds (Yildirim *et al*., 2008).

The purpose of this research was to study the effect of important micronutrient elements iron, and manganese biomax (complex of six micronutrient elements) on some morphological and physiological characteristics of the soybean plant.

2. Materials and methods

This experiment was conducted in the form of a randomized complete block design (RCBD) with 3 replications and 5 treatments in the research farm of Kermanshah's Agriculture and Natural Resources Campus of Razi Universit, Kermanshah, Iran at 2018.

The treatments used in this research were: 1-Control, 2- Iron, 3-Manganese (Mn EDTA), 4- Biomax (complex of six micronutrients iron, zinc, manganese, boron, molybdenum and copper) of Manvert Company of Spain, 5- Iron/Mn.

Fertilizer amounts were used based on the soil test measured from the field, according to which, iron in the amount of 0.7 grams per 10 liters of water (7 kg per hectare), manganese in the amount of 10 ml per 10 liters of water (4 liters per hectare) and Biomax was also used in the amount of 10 grams per 10 liters of water (4 kg per hectare) and iron/manganese in an amount equal to their single state.

Fertilizers were sprayed in two stages for all experimental units with Manual spray solution, the first stage was at the beginning of flowering and the second stage was at the pod filling stage. For each treatment, 2 stacks were allocated at a distance of 60 cm and a length of 1 meter, and two rows of seeds were planted at a distance of 30 cm in each stack. To prevent spraying solutions on other rows, plastic buffers were used during spraying between rows.

Traits such as number of pods per plant, number of seeds per pod, hundred seed weight, pod diameter, pod length, length of the flowering period, number of days to maturity, plant height and harvest index were measured. The seeds were sent to the laboratory of the Medical Biology Research Center (MBRC), Kermanshah University of Medical Science, Kermanshah, Iran, to evaluate the percentage of protein. After pod production and seed ripening, traits were measured and data was recorded. Software such as Path2, MSTAT-C, SPSS, MINITAB, etc. was used for statistical analysis.

3. Results

The results of the analysis of variance and mean comparing of the traits showed that foliar spraying had a significant difference for the traits of plant height, crown distance to the first sub-stem, stem diameter, the number of pods per sub-stem of the pod, the number of pods per the main stem, the number of pods per plant had the amount of protein in mg/pr and the yield in grams, and no significant difference was observed for the characteristics of pod length and the number of seeds in the pod. Also, the average comparison table showed that for most traits Biomax fertilizer, which was a complex of all micronutrient elements, had the greatest effect.

3.1. Plant height

The results of variance analysis of the data (Table 1) showed that foliar spraying has a significant effect at the 1% level on plant height. According to the average comparison table (Table 2) Biomax with a height of 146.05 cm had the greatest effect. In this study, a positive correlation with a coefficient of r=0.836 was obtained between the height of the stem and the performance of dry fodder, which indicates that any factor that increases the height of the stem can also increase the performance of the whole plant (Table 3).

3.2. The distance from the crown to the first sub-stem

The results of variance analysis of the data (Table 1) showed that foliar spraying has a significant effect at the 1% level on the distance between the crown and the first sub-stem, which according to the average comparison table (Table 2) has the greatest effect on Biomax with a height of 13.92 cm. In this study, the positive and high correlation coefficient between the two attributes of plant height and the distance between the crown and the first sub-stem r=0.885 shows that with the increase in the distance between the crown and the first sub-stem, the height of the plant increases significantly (Table 3).

3.3. Stem diameter

The results of variance analysis of the data (Table 1) showed that foliar spraying has a significant effect at the level of 1% on the diameter of the stem, which according to the average comparison table (Table 2) had the most effect on Biomax with a height of 2.08 cm. Studies have shown that increasing the stem diameter in plants increases the amount of dry matter in plants.

3.4. Number of pods per sub-stem

The results of variance analysis of the data (Table 1) showed that foliar application has a significant effect at the 1% level on the number of pods per secondary stem, which according to the mean comparison table (Table 2), Biomax had the most effect with 54 numbers. In this study, the high correlation coefficient between this trait and grain yield, r=0.881, showed that foliar spraying can increase the yield by increasing the number of pods in the sub-stem (Table 3).

Table 1. Variance analysis of studied traits in foliar application of micronutrients on soybean.

S.O.V	df	Plant Height	Distance from the crown to the first sub-stem	Stem diameter	Number of pods per sub-stem	Number of pods per main stem	Number of pods per plant	Pod length	Number of seeds per pod	Amount of protein mg/pr	Yield
R	2	21.36	0.59	0.012	18.2	7.26	26.46	0.084	0.46	1.13	55/06
T	4	644.01**	21.33**	0.82**	195.66**	129.93**	642.43**	0.492ns	0.56ns	224.41**	3408.96**
E	8	242.77	3.12	0.01	62.93	21.46	5.38	0.21	0.21	1.65	237.81
CV%	-	14.33	15.99	16.31	16.52	15.36	13.15	11.12	17.02	15.51	15.41

*: significant at the 5% probability level, **: significant at the 1% probability level, ns: non-significant.

Table 2. Mean comparison of studied traits in the foliar application of micronutrients on soybean.

Treatment	Plant Height	Distance from the crown to the first sub-stem	Stem diameter	Number of pods per sub-stem	Number of pods per main stem	Number of pods per plant	Pod length	Number of seeds per pod	Amount of protein mg/pr	Yield
Test	106.21d	7.15e	0.76d	34.00d	23.66d	57.66e	4.30ab	2.33b	14.60d	246.33d
Manganese	121.65c	8.69d	1.54c	37.33bc	25.00d	62.33d	4.33ab	2.33b	15.38d	262.36cd
Iron	129.13bc	10.31c	1.82b	41.66c	30.33c	72.00c	4.76a	3.33a	23.15c	288.00bc
Manganese/Iron	132.49b	12.00b	1.95ab	48.00b	34.00b	82.00b	3.73b	3.00ab	28.84b	292.83b
Biomax	146.05a	13.92a	2.08a	54.00a	39.66a	93.66a	3.90ab	2.66ab	34.70a	334.63a

*: significant at the 5% probability level, **: significant at the 1% probability level, ns: non-significant.

Table 3. Correlation table of studied traits in foliar application of micronutrients on soybean.

	Plant Height	Distance from the crown to the first sub-stem	Stem diameter	Number of pods per sub-stem	Number of pods per main stem	Number of pods per plant	Pod length	Number of seeds per pod	Amount of protein mg/pr	Yield
Plant Height	1									
Distance from the crown to the first sub-stem	0.885**	1								
Stem diameter	0.845**	0.873**	1							
Number of pods per sub-stem	0.898**	0.907**	0.787**	1						
Number of pods per main stem	0.883**	0.93**	0.793**	0.877**	1					
Number of pods per plant	0.92**	0.94**	0.814**	0.97**	0.961**	1				
Pod length	-0.363ns	-0.342ns	-0.171ns	-0.372ns	-0.381ns	-0.388ns	1			
Number of seeds per pod	0.199ns	0.302ns	0.461ns	0.303ns	0.155ns	0.245ns	0.28ns	1		
Amount of protein mg/pr	0.863**	0.968**	0.823**	0.907**	0.964**	0.961**	-0.359ns	0.321ns	1	
Yield	0.836**	0.884**	0.797**	0.881**	0.897**	0.908**	-0.146ns	0.381ns	0.906**	1

*:significant at the 5% probability level, **:significant at the 1% probability level, ns: non-significant

3.5. Number of pods per the main stem

The results of variance analysis (Table 1) showed that foliar spraying has a significant effect at the 1% level on the number of pods per the main stem, which according to the average comparison table (Table 2) has the greatest effect on Biomax with an average of 39.66 had a number In this study, the high correlation coefficient between this trait and seed yield, r=0.897, showed that foliar spraying can increase the yield by increasing the number of pods in the main stem (Table 3).

3.6. Number of pods per plant

The results of variance analysis of the data (Table 1) showed that foliar spraying has a significant effect at the 1% level on the number of pods per plant. According to the average comparison table (Table 2), Biomax had the most impact with an average of 93.66.

3.7. Pod length

The results of variance analysis of the data (Table 1) showed that foliar application has no significant effect on the pod length, but according to the mean comparison table (Table 2), iron had the greatest effect with an amount of 4.76 cm. In this research, this case can be well demonstrated by the negative correlation coefficient r=-0.146 of this trait with seed yield in this study (Table 3).

3.8. Number of seeds per pod

The results of variance analysis of the data (Table 1) showed that foliar application has no significant effect on the number of seeds in the pod, but according to the average comparison table (Table 2) iron had the greatest effect with an average of 3.33. It seems that the iron element by increasing the rate of photosynthesis and plant metabolism increases the pollination of the plant and as a result the number of seeds in the pod increases.

3.9. Amount of protein mg/pr

The results of variance analysis of the data (Table 1) showed that foliar spraying has a significant effect at the 1% level on the amount of protein, and based on the average comparison table (Table 2) Biomax had the most effect with a rate of 37.4 mg/pr.

3.10. Yield (gr)

The results of variance analysis of the data (Table 1) showed that foliar spraying has a significant effect at the 1% level on yield, and based on the average comparison table (Table 2), Biomax had the most effect with 334.63 gr. Iron plays a vital role in the production of chlorophyll and electron transfer in photosynthesis, and ferredoxin is an iron-carrier protein that is involved in electron transfer (Kroh and Pilon, 2020). Therefore, it is natural that with the increase of iron in the leaf, the amount of chlorophyll in the leaf also increases, the photosynthetic activity increases and finally, the yield increases. Goss and Johnson also reported in 2000 that the yield of soybeans increased by 303 kg/ha with iron foliar application. In general, according to the role of zinc and iron elements in photosynthesis, it can be said that low-consumption elements increase the yield of plants by increasing the photosynthetic power and the amount of photosynthesis of plants.

Table 4. Path analysis and determining the direct and indirect effects of the studied traits.

Independent traits	Direct effects	Indirect effects							Simple correlation with performance (sum of direct and indirect effects)
		Plant Height	distance from the crown to the first sub-stem	Number of pods per sub stem	Number of pods per main stem	Number of pods per plant	Pod length	Number of seeds per pod	
Plant Height	0.187	-------	0.331	0.902	0.959	-1.285	-0.068	0.497	0.861
Distance from the crown to the first sub-stem	-0.374	0.165	-------	0.911	1.01	-1.313	-0.064	0.557	0.892
Number of pods per sub stem	1.005	0.168	-0.399	-------	0.952	-1.335	-0.069	0.052	0.434
Number of pods per main stem	1.086	0.165	-0.348	0.881	-------	-1.342	-0.071	0.027	0.64
Number of pods per plant	-1.397	0.172	-0.351	0.974	1.043	-------	-0.072	0.042	0.412
Pod length	0.185	-0.069	0.127	-0.374	-.414	0.541	-------	0.048	0.044
Number of seeds per pod	0.174	0.037	-0.113	0.304	0.168	-0.343	0.051	-------	0.278

3.11. Path analysis

The results of the path analysis (Table 4) showed that the yield as a dependent variable and the traits number of seeds per pod, length of the pod, number of pods per plant, number of pods in the main stem, number of pods in the secondary stem, distance from the crown to the first secondary stem, Plant height is considered as the independent variable. Also, in this analysis, it was found that the trait of plant height has direct and small effects on the yield, but this trait can increase the yield through the indirect effects of increasing the number of pods per main and secondary stems. Also, the results of this table showed that the attributes of crown distance to the first secondary branch and pod length have negative direct effects and affect yield through indirect effects. The results of this table showed that these traits affect the yield through direct effects and their direct effects are more important.

4. Discussion

Khalili Mahalleh (2008) showed that foliar application of micronutrient elements has a significant difference in plant height.

Research shows that foliar application of micronutrients iron, zinc, and manganese increases the dry matter yield in plants by increasing the stem height (Whitty and Chambliss, 2005).

Improving nutritional conditions and the positive role of iron and zinc can be effective in photosynthesis and the performance of light photosystems in increasing growth indicators such as stem diameter (Malakoti and Tehrani,1999).

The number of pods in a plant can be considered as one of the most important components of seed yield because the pods contain seeds and participate in the growth and development of seeds in the early stages of seed filling through photosynthesis (Shirani-Rad *et al.*, 2010).

The results of Morshidi and Naghibi (2004) showed that zinc foliar application in rapeseed increased the number of pods per plant. Yang et al. (2009) reported the positive effect of zinc on the number of pods per plant, the number of seeds per pod, the amount of oil and the yield of rapeseed. Grewal et al. (2001) also reported similar results about the lack of significance of zinc foliar spraying along the length of the pod.

The pod length determines the number of seeds in each pod and thus affects the yield of the plant. Pod length in rapeseed is one of the variety-dependent traits that is less affected by agricultural factors. Cultivars with longer pods produce fewer pods per plant, and on the contrary, cultivars with shorter pods produce more pods per plant. According to researchers, although pod length does not have a significant effect on increasing yield, cultivars with longer pod length produce more yield (Chay and Thurling, 1989).

Rehem et al. (1998) showed that the use of micronutrient elements increased the number of seeds in the pod in canola compared to the control treatment (without foliar application). They stated that the reason for the low number of seeds in the pod in the control treatment was the limitation of the supply of growing material to the tip of the inflorescence, and the increase in the number of seeds in the pod due to the foliar application of micronutrient elements was due to the key role of these elements in transporting water and nutrients from the roots to the Aerial organs announced.

Zinc foliar spraying increased the number of seeds per pod in rapeseed. It seems that the consumption of zinc during the flowering stage allows the direct flow of nutrients to the seeds (Morshedi and Naghibi, 2004). Because micronutrient elements have a direct effect on increasing the amount of protein, oil and seed yield of plants, including oil plants (Riley *et al.*, 2000).

Therefore, in plants that lack these elements, the amount of seed protein is reduced and amino acids do not accumulate. It was shown in Morshedi and Naghibi's experiment (2004) that zinc foliar application increased rape seed protein.

Bron et al. (2003) reported that the amount of total protein in most plants decreases drastically under conditions of zinc deficiency, while the amount of protein compounds remains almost unchanged.

Cakmak et al. (1989) stated in their reports that the amount of bean seed protein decreased in the treatment of zinc deficiency. The obtained results are consistent with the findings of Banks in soy about increasing the amount of seed protein with the consumption of micronutrient elements (Banks, 2004).

Conflict of Interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed Consent

The authors declare not to use any patients in this research.

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