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Effect of Foliar Treatment of Micronutrients (Iron, Zinc and Manganese) on Nitrogen Yield and Biological Fixation of Bami Alfalfa (Medicago Sativa L.) With Inoculation of Bacteria in Kerman

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Abstract

This study was carried out aimed to evaluate the effect of foliar treatment of micronutrients including iron, zinc and manganese on nitrogen yield and biological fixation with inoculation of bacteria in the Bami alfalfa in order to obtain a cost- effective product with in Kerman in 2013 and was implemented for two years in Shahid Zendeh Rood agricultural education center in Kerman. The project was studied in an area of 2500 square meters using a split plot design, with four replications and with foliar treatment of micronutrients in 5 levels (a level as a control), and inoculation of bacteria in 3 levels (non-inoculated as control group). In all treatments, the distance between the lines was 30 cm, and all effects of the main factors and sub-factors on agronomic traits were analyzed statistically. Qualitative traits included plant height, yield, growth and nitrogen fixation, and quantitative traits included the amount of protein, chlorophyll and pigments, digestibility and the percentage of iron, zinc, manganese, nitrogen, phosphorus and potassium. Overall, the survey results indicate that, foliar treatment and inoculation had a significant impact on chlorophyll content and yield, and increased the amount of chlorophyll, protein, and the yield of Bami alfalfa.

Keywords: Bami alfalfa, Bacteria inoculation, Foliar Treatment, Iron, Nitrogen fixation, Zinc.

Introduction

Population growth and preparing food gradually over time will be more important. According to the terms of cultivated area, harvest takes place from 3 to 12 times which feeding the plant with micro elements in the time required, and in a short time according to the harvests seems necessary. Foliar treatment can play an important role in providing immediate nutritional elements (Barmaki & Eyvazi, 2009). Iron is immobile in the plant and is not transferred from the old tissue to younger tissue. Given that iron deficiency is considered as one of the yield limiting factors in crop production in the world, and also is of great importance in human health, anemia due to iron deficiency is important around the world. Iron deficiency is a complex disorder, and generated in response to multiple factors of soil, environmental and genetic (Barber, 1995).

According to (Goos & Johnson, 2000), one of the best management practices to overcome the problem of iron deficiency in plants is foliar treatment. (Fageria et al., 2002) reported that 30% reduction in wheat rice, maize yield and other crops is common in the case of zinc deficiency. According to the researchers, the lack of zinc has relationship with some environmental conditions to absorb and how to use it. Zinc deficiency in crop plants is due to low abortion of this element in the mother plant. (Shaver & Ronaghi, 2007) reported that zinc fertilizer with low solubility not only is not available over time, and is not available even two years during cultivation, and even when the plant shows the zinc deficiency, (1922), the necessary amount can't be provided, so foliar treatment is very important. Plants with manganese deficiency will be sensitive to Hypothermia and illnesses (1979), therefore, manganese plays an important role in plant nitrogen Fixation (Mengel et al., 2001). Hale et al stated that the increase in water-soluble manganese and the ability to exchange is one of the best cases for absorption which with regard to the short growing period and interval between harvest for *foliar treatment* the element, it is of special importance. *Bami alfalfa,* with the air nitrogen fixation with help of Rhizobium melilloti bacteria has increased production in its roots, and with plant roots rot in the soil, humus is created, which ultimately all these features improve the soil under cultivation, and improve and following the correction of soil physics (Sarmad Nia & Koochaki, 1989). This research was carried out aimed to evaluate the effect of foliar treatment of micronutrients including iron, zinc, manganese on nitrogen biological fixation and yield in Bami alfalfa in Kerman province and answered to the main question, "What is the effect of foliar treatment of iron, zinc, manganese on nitrogen biological fixation and yield in Bami alfalfa?

Materials and Methods

The experiment was conducted in two growing seasons in the teaching and research field of agricultural research center in Kerman province in a land of 50 * 50 meters. After soil sampling, and studying the test results, the slots at distance of 30 cm distance in line with land' slope were created by a opener. The statistical design considered for this study was a split-plot design with four replications, so that, in plots, the fertilizer factor was considered at 5 levels as the main factor, and bacteria in three levels as sub-factor. Each replication contains 15 treatments, and for each treatment, six 5-m lines was considered, and the distance between planting line was 30cm, when the land was prepared for planting, a 1-meter distance was considered for any repetitions, and according to plan, the profile of each plot was installed at the beginning of the plot. Inoculation with seeding was done by hand. The first irrigation was done immediately after planting. After mixing the bacteria and soaked in diluted sugar water, seed entered the bacteria and planting operations were performed immediately after mixing. After planting the seeds, the first irrigation immediately was performed using furrow irrigation method and after germination, on a regular basis once every seven days, irrigation was performed, when the plant height was 10 - 15 cm, the foliar treatment operations of iron, zinc and manganese as a four thousand were carried out individually for each harvest. When, plants height were 30 -40 cm, and they started flowering, harvest by shrubs was performed by separating the plants close to the ground, for this purpose, the beginning and end of lines (up to 0.5cm) as margins were removed, and other plants were harvested. Other samples were taken the same as before.

Statistical Analysis

SAS-Excel -Minitab software was used for data analysis and variance analysis in the comparison of means using Duncan at confidence level 5% and Excel was used to draw the charts and Word was used to insert the tables.

Results and Discussion

The results of the data analysis about the main effect of micronutrients on iron in the first and second years were significant at P < 0.05. The most effect was related to the first year with (type-2) inoculation and the use of iron (Table 1). In general, it seems that, independent fertilizer treatments couldn't have a significant effect on traits. Each independent treatment is affected by genetic factors and the lack of other elements. No significant differences in some traits are well justified (Figure 1). Because of the effectiveness of iron in some traits, as well as lack of its impact on other traits can be justified in such a way that there is remobilization of iron between the zinc and manganese.

Table 1. Variance analysis of amount of iron (Fe) at first and second year (inoculation of bacteria and micronutrients)

Sources of changes	Degrees of freedom	Mean Square		F calculated	
		The second year	The first year	The second year	The first year
Fertilizer A	4	74419.700	83273.213	633.3292**	1111.6439*
Error	12	117.506	74.910		
Bacteria B	2	27793.908	32122.806	159.8678**	368.7446*
Fertilizer* Bacteria AB	8	3439.231	2623.111	19.7821**	30.1113**
Error	30	173.856	87.114		
Year C	1	1147.008	99.190	4.4398**	1.4037 ns
Year* Fertilizer AC	4	419.008	78.386	1.6219 ns	1.1093 ns
Year* Bacteria BC	2	466.158	6.696	1.8044 ns	0.0948 ns
Bacteria *Year* Fertilizer ABC	8	935.940	25.051	2.4616 ns	0.3545 ns
Error	45	258.347	70.664		
Compound error	119				
CV%	11.39 6.01				

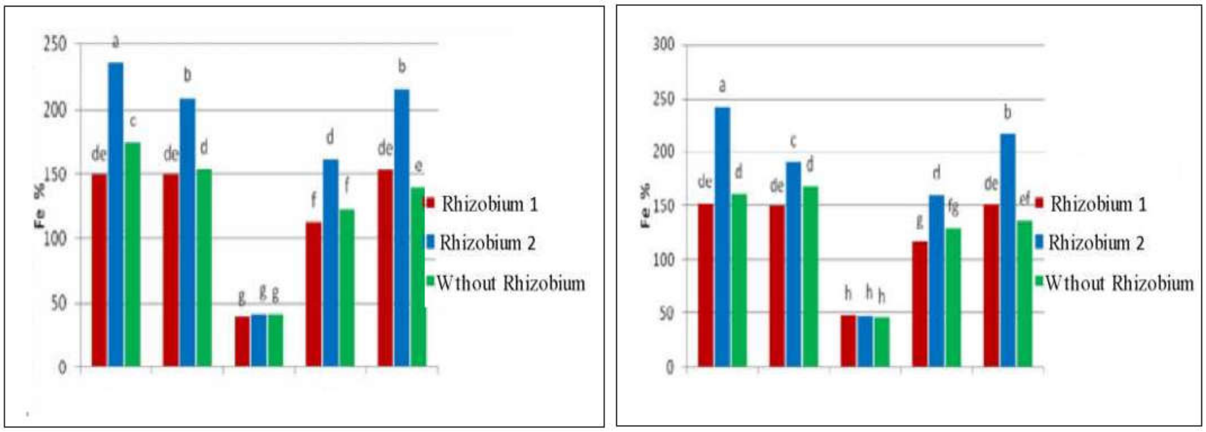

Figure 1. Comparison of mean interaction of nutrient and bacteria on the iron content in the first year (left) and in the second year (right)

Iron, Zinc, Manganese, Iron, control, micronutrients

The results of the data analysis about the amount under inoculation of bacteria and micronutrients in first and second year with the probability P <0.005 were significant. It can be concluded that, in general, the first year has been more effective than the second year. Zinc is effective on plant growth, and is involved in photosynthesis and chlorophyll formation, and its deficiency causes an imbalance of Nutrients in a plant, and reduces water use efficiency, and reduces the quality of the product. (Danso & Maky, 1990), reported that the plants facing a shortage of manganese gather the excess amounts of manganese. Van Borstel, reported that with manganese consumption in rice, transport and deposition of zinc increased [9] (Table 2) & (Figure 2).

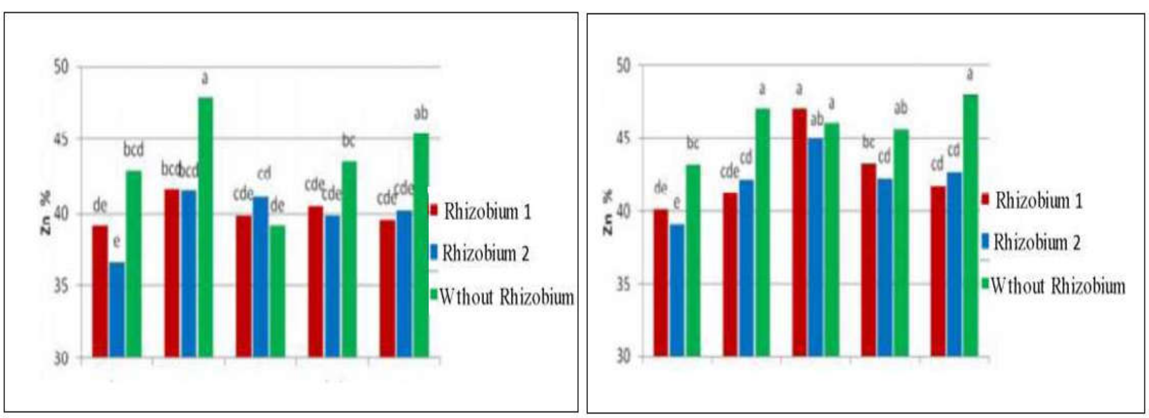

Figure 2. Comparison of mean interaction of nutrient and bacteria on the zinc content in the first year (left) and in the second year (right)

Table 2. Variance analysis of amount of zinc (zinc) at first and second year (inoculation of bacteria and micronutrients)

Sources of changes	Degrees of freedom	Mean Square		F calculated	
		The second year	The first year	The second year	The first year
Fertilizer A	4	89.875	62.821	5.8385**	4.461*
Error	12	15.394	15.526		
Bacteria B	2	159.003	187.008	52.2616**	27.6708**
Fertilizer* Bacteria AB	8	18.088	29.071	5.9454**	4.3015**
Error	30	3.042	6.758		
Year C	1	75.843	114.075	19.1539**	29.4810**
Year* Fertilizer AC	4	74.901	40.221	18.9161**	10.3945**
Year* Bacteria BC	2	5.833	0.175	1.4731 ns	0.0452 ns
Bacteria *Year* Fertilizer ABC	8	89.760	96.008	22.6686**	24.8119**
Error	45	3.960	70.664		
Compound error	119				
CV%	4.56 4.77				

The results of analysis of variance for the main effect of micronutrients on manganese in both years were significant at $P < 0.01$. It can be concluded that the first year and the use of zinc and Rhizobium (type-1) was better. (Von Borstel & lesins, 1977) observed that the use of zinc and iron and manganese increases the seed weight and seed number per panicle wheat significantly, in this experiment, manganese and iron compete with each other in the process of root, and the competition is possible for this reason, because in some traits, manganese concentration was reduced after foliar treatment of iron. The effectiveness of manganese in some traits can be justified in such a way that, remobilization of zinc is possible from the leaves to the grain, and vice versa, while the remobilization of manganese is low (Jones, 1965) (Table 3) & (Figure 3).

Table 3. Variance analysis of amount of manganese at first and second year (bacteria Inoculation and micronutrients)

Sources of changes	Degrees of freedom	Mean Square		F calculated	
		The second year	The first year	The second year	The first year
Fertilizer A	4	207.655	398.825	174.0365*	92.4514**
Error	12	1.193	4.314		
Bacteria B	2	421.018	661.056	98.0967**	172.2310**
Fertilizer* Bacteria AB	8	112.781	117.478	26.2779**	30.6077**
Error	30	4.292	3.838		
Year C	1	429.408	178.852	67.2860**	19.0170**
Year* Fertilizer AC	4	80.533	14.488	12.6192**	1.5404ns
Year* Bacteria BC	2	19.536	2.190	3.0612 ns	0.2328 ns
Bacteria *Year* Fertilizer ABC	8	6.839	7.778	1.0717ns	0.9334ns
Error	45	6.382	9.405		
Compound error	119				
CV%	5.31 6.94				

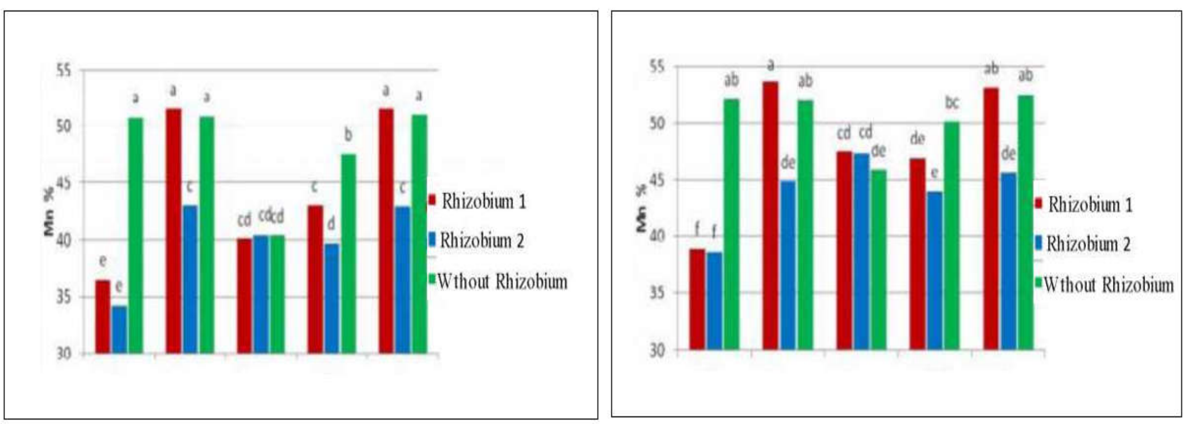

Figure 3. Comparison of mean interaction of nutrient and bacteria on the manganese content in the first year (left) and in the second year (right)

The nitrogen fixation was not significant in the first year, and this could be because, although the nitrogen will affect specific leaf weight, but their interactions on other traits was significant. It also appears that the difference between the result and the geographical location, soil texture causes lack of consistency and lack of its significance. Perhaps a certain kind of bacteria or feeding other elements improves the situation. Jong stated that some bacteria which lead to nitrogen fixation, although generates more nodes with more weigh, but they have less nitrogen fixation and this issue has relationship with the lower relative performance of the nodes. The amount of nitrogen fixed in this experiment is consistent with the amount of nitrogen fixed in the other tests. In this experiment, there is a direct relationship (1971) between the number of nodes and nitrogen fixation (Ayanaba & Dart, 1977). Harper, in one experiment which was done on Soybean, concluded that there is a direct relationship between the number of nodes generated and nitrogen fixation, which was consistent with the above. Nitrogen Fixation depends on the amount of carbohydrate which be provided the nodes through foliage. Usually per gram of nitrogen fixed, 3 to 4 grams of carbohydrate should be provided for nodes (Harper & Cooper, 1971) (Table 4) & (Figure 4).

Table 4. Analysis of variance on nitrogen fixation at first and second years (under inoculation of bacteria and micronutrients)

Sources of changes	Degrees of freedom	Mean Square		F calculated	
		The second year	The first year	The second year	The first year
Fertilizer A	4	1197.28	27.71	29.9**	7 17**
Error	12	0.614	0.064	0.051 ns	0.005 ns
Rhizobium B	2	57.53	1.28	28.76**	0.0642ns
Fertilizer* Rhizobium AB	8	16.04	0.188	2 ns	0.023 ns
Error	30	7.08	0.165	0.23 **	0.005 ns.
Compound error	59				
CV%	6.44 7.13				

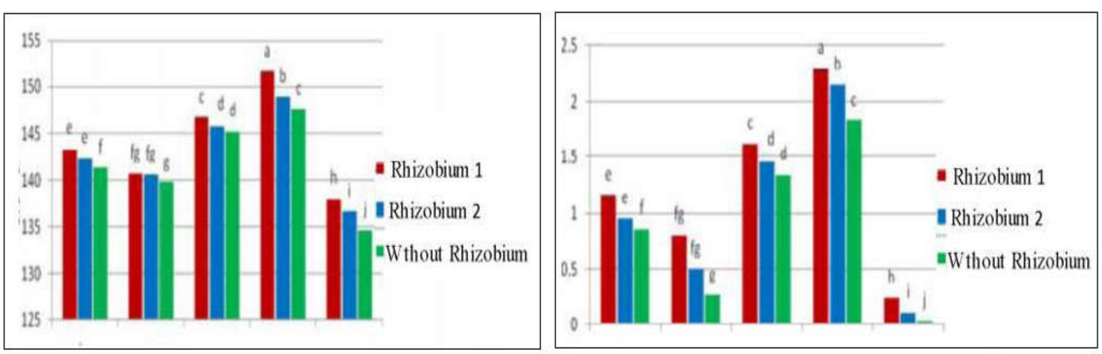

Figure 4. Comparison of the interaction of nutrient and bacteria on the number of nodes per plant (left) on nitrogen fixation (right)

The results of the data analysis on shoot dry weight under inoculation of bacteria and micronutrients showed that, among all the effects, the effect of year on the wet weight was significant in the first year with the probability P < 0.01. Other effects were not significant. In both years, the highest dry weight was related to the control (without use). Reducing the dry weight of the plant can be due to reducing the net production of the plant, which by a reduction in the phloem sap, contribution of different parts is reduced, therefore, the weight of the plant will be reduced. Reduced photosynthesis decreases the amount of chlorophyll, and by increasing the role of nitrogen metabolism in to make combinations such as proline which is used to adjust the osmotic, will be reduced, Heydari Sharif Abbad believes that it is due to an imbalance of nutrients which ultimately reduces the yield of the plant. Climate changes, the interaction of plant competition in nutrient uptake are some significant cases that have a role in determining the dry weight. It seems that the balance of nutrients and changes in pH, and freeing them from compounds stabilized in soil reduce the dry weight of a plant (Haideri Sharif Abad, 1983). The results of this study (1998) are consistent with the results of the effect of fertilizers in wheat by Kafi and on in Ruiz by (Roeiz, 1998). During the imbalance of nutrients, with by increasing osmotic potential, greatly nutrient uptake is reduced, and its yield will be less (Roeiz, 1998). According to the results, competitive power of a plant in the vicinity of the *foliar treatment* is reduced and increase in nutrient at the same time can also increase the concentration of salts, and lead to decreased absorption. This showed that the sensitivity of leaf and its effectiveness is affected by nutritional conditions. Also, changes in nutritional conditions affect carbon dioxide concentration. Reduction of carbon dioxide reduces significantly the leaf dry weight, with the reduction of carbon dioxide, dry leaf weight is reduced. Comparison of dry weight in different scenarios for foliar treatment, and inoculation, is considered as an indicator of the competitive power of the plant (Table 5).

Table 5. Analysis of variance on dry weight at the first and second year (under inoculation of bacteria and micronutrients)

Sources of changes	Degrees of freedom	Mean Square		F calculated	
		The second year	The first year	The second year	The first year
Fertilizer A	4	4090.429	37826.992		0.9972 ns
Error	12	10723.612	37931.464	0.3814 ns	
Bacteria B	2	2939.608	14400.633		0.8388 ns
Fertilizer* Bacteria AB	8	12286.098	19668.404	0.3709 ns	1.1456 ns
Error	30	7925.333	17169.150	1.5502 ns	
Year C	1	41218.133	3305060.208		241.9227**
Year* Fertilizer AC	4	1083	21998.542	13.1168**	1.6102 ns
Year* Bacteria BC	2	2544.008	2631.233	0.3449 ns	0.1926 ns
Bacteria *Year* Fertilizer ABC	8	2119.706	7031.879	0.8096 ns	0.5147 ns
Error	45	3142.374	13661.636		
Compound error	119				
CV%	16.36 30.32				

Conclusion

The results of this survey indicate that, foliar treatment and inoculation had significant effect on chlorophyll content and yield, and increase the amount of chlorophyll, protein and Bami alfalfa yield in a way that stresses created during year caused more losses than the testing levels.

Recommendations

a. Further investigation of legumes with higher production in the nitrogen fixation studies
b. The use of legume in which nitrogen-fixing period is longer.
c. Wider and more extensive experiments to determine the status of nitrogen fixation during the stabilization period
d. More studies on nitrogen fixation in agriculture lands that are limited by a lack of nitrogen-fixing microorganisms.
e. The use of Rhizobium that can quickly form a node use on the roots, so we will not have to use nitrogen.
f. Wider use of Legomitos plants in agriculture to prevent the import of chemical fertilizers with nitrogen
g. More research on foliar treatment of other micronutrients in other Microelements, especially forage plants

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