

## Effect of Different Fertilizing Treatments on Nutrient Uptake in Annual Medic (*Medicago scutellata* cv. Robinson) under Irrigated and Dry Farming Systems

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### ABSTRACT

To study the effect of different fertilizing systems on macro and micro nutrients uptake by annual medic (*Medicago scutellata* cv. Robinson) an experiment was conducted under dry farming and irrigated conditions at two research stations (Sararood Dryland Agricultural Research Institute and Soil Fertility Research Station in Mahidasht) during 2009 growing season. The experimental treatments consisted of the two experimental sites with different climatic conditions and cultural systems (dry farming and irrigated systems), while the fertilizer treatments consisted of the control (no fertilizer), chemical fertilizer, biological fertilizer, and different combinations of chemical and biological fertilizing systems. The results showed that, in both irrigated and dry farming conditions, all fertilizing treatments increased macro- and micro-nutrients uptake over the control. The highest concentration of nutrient elements such as Nitrogen (3.82%), Potassium, (4.16 mg kg<sup>-1</sup>), Iron (495 mg kg<sup>-1</sup>) and Cu (60.8 mg kg<sup>-1</sup>) were observed in integrated fertilizing treatments i.e. Nitrogen-fixing bacteria+Triple superphosphate. Application of integrated fertilizing treatments not only decreased the chemical fertilizer application (consequently, reducing the environmental pollutions), but it also enhanced forage quality in terms of higher macro- and micro-nutrients concentrations. According to the results of this study, it could be concluded that integrated fertilizing systems may be more efficient in dry farming agro-ecosystems than in irrigated systems.

**Keywords:** Annual Medic, Biological Fertilizer, Cultivation Systems, Fertilizing systems, Macro and Micro Nutrients.

### INTRODUCTION

In recent years, interest in application of bio-fertilizers has been increasing to attain sustainable agriculture, which has been set as the main objective for agricultural experts. For this purpose, optimizing the application of chemical fertilizers, enhancing the soil organic matter content, and guaranteeing the environmental protection seem to be crucial. Additionally, soil quality is not only

subordinated to the physical and chemical properties, but also it depends on its biological characteristics (Ebhin Masto *et al.*, 2006). Application the fertilizers such as mutualistic plant-fungus symbiosis, phosphate solubilizing microorganisms, and vermicompost has long been recognized as beneficial for plant growth and yield and the maintenance of soil fertility in agriculture (Arancon *et al.*, 2004; Gupta *et al.*, 2002; Gyaneshwar *et al.*, 2002). Chalk *et al.* (2006) reported a positive effect of

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Mycorrhiza on the performance of legumes and nitrogen uptake. It has been also stated that the environmental and different biological factors as well as soil are involved in symbiotic association of fungi with the roots of plants. Maleki Farahani *et al.* (2011) showed that phosphorous concentration in barley grains produced by nitrogen biofertilizers was significantly higher than those in other treatments. Grain Fe and Zn concentrations increased by vermicompost application. However, Mn concentration was higher in grains fertilized with chemical fertilizer. In their research, it was concluded that in barley production under water deficit conditions, grain mineral quality could be improved through integrated fertilizer application.

Application of integrated biological fertilizers (*Brady rhizobium japonicum* bacteria and phosphorus-solubilizing bacteria) not only improved nutrients absorption of minerals such as nitrogen through their collaboration, but also amended the biogeochemical cycle of nutrients in the soil and, consequently, led to increased efficiency of their uptake by plants by facilitating the solubility of mineral elements (Rosas *et al.*, 2002). Neeru *et al.* (2000) examined the inoculation effect of *Azotobacter crococom* strains and phosphorus-solubilizing bacteria on the uptake of N, P, and K under greenhouse conditions and observed that the uptake of these elements in all fertilizing treatments substantially increased in comparison with non-fertilizer treatments (control). It is indicated that the interactive effects of inoculation with *Rhizobium* bacteria, Mycorrhiza fungi and phosphorus-solubilizing bacteria in the Rhizosphere of medic increased the P and N uptake in this plant (Toro *et al.*, 1998). Dual inoculation of medic by *Glomus intraradices* fungi and *Sinorhizobium meliloti* bacteria significantly increased the dry weight, total P and N content in the plant tissues (Stancheva *et al.*, 2008). Generally, dual inoculation of medic under the Phosphorus deficiency conditions leads to an enhancement of weight, fixation of N<sub>2</sub> and Phosphorus content in plants compared to separately

inoculated treatments (Stancheva *et al.*, 2008).

Studies have shown that seed inoculation with a mixture of micro-organisms amended the uptake of nutritional elements and subsequently increased the yield compared to separate inoculation by each one of them (Perveen *et al.*, 2002; Zaidi *et al.*, 2003). The results of research on integrated biological fertilizers application shows that it may increase the nitrogen availability for the plant and likewise contribute to promote growth conditions and it may also strengthen the backbone of qualitative yield growth as the consequence.

Application of integrated fertilizer (a proper combination of biological fertilizer and urea) along with a proper irrigation system will lead to a better forage quality. The effects of biological fertilizers on the N and P content has been investigated extensively whilst the alluded effects on reservation of the micronutrient elements has not been examined considerably as yet. In general, the results of executed researches have illustrated that the application of biological fertilizers have a positive effect in terms of quantity and quality on different plant species. Our hypothesis was that integrated application of chemical and biological fertilizers can better contribute to mineral nutrients availability for medic plants.

The goals of these experiments were to investigate the effects on nutrient uptake in the annual medic (*Medicago scutellata* cv. Robinson) of biological and chemical fertilizers in sole and integrated application systems in dry and irrigated farming conditions.

## MATERIALS AND METHODS

This experiment was conducted during 2009 growing season in two locations: (1) Sararood Dryland Agricultural Research Station with the geographic coordinates of 47°20' E and 34°20' N and elevation of 1,351 meters above the sea level, and (2) Mahidasht Soil Fertility Research Station with the geographic coordinates of 46°50'

**Table 1.** Selected physical and chemical characteristics of soil (0-30 cm depth) at two experimental sites.

Characteristic	Experimental stations	
	Sararood	Mahidasht
pH	7.7	7.9
Dissolved solids (EC.103)	30.0	55.0
Organic carbon (%)	0.31	0.62
CaCO <sub>3</sub> (%)	30	28
Olsen phosphorous (mg kg <sup>-1</sup> )	8.00	9.40
Available potassium (mg kg <sup>-1</sup> )	530	430
DTPA extractable Zn (mg kg <sup>-1</sup> )	0.4	1.6
DTPA extractable Cu (mg kg <sup>-1</sup> )	0.7	1.4
DTPA extractable Fe (mg kg <sup>-1</sup> )	2.00	4.8
DTPA extractable Mn (mg kg <sup>-1</sup> )	2.4	3.8
Soil texture	Loamy silt	Loamy clay

**Table 2.** The Average precipitation and temperature during growing season of annual medic at two experimental sites.

Month	Average precipitation (mm)		Average temperature (°C)	
	Sararood	Mahidasht	Sararood	Mahidasht
Feb.	18.3	21.2	7.3	6.4
Mar.	36.1	71.8	9.4	8.0
Apr.	15.2	12.4	16.2	14
May	0.2	0.9	22.7	19.7
Jun.	0	0	26.5	24.0

E and 34°16' N with elevation of 1,380 meters above the sea level. Some physical and chemical characteristics of soil and climatic information of the two experimental sites are shown in Tables 1 and 2.

The experimental sites in both locations were kept as fallow in the preceding year. The experimental treatments were arranged as split-split plots based on complete randomized block design with three replications. The locations (Sararoud Institute and Mahidash Research Stations) were considered as the main plots, the experimental conditions of irrigated system (D1) and dry farming system (D2) were assigned to the sub plots, and the fertilizing treatments were assigned to the sub-sub plots. The fertilizing treatments consisted of the control (without fertilizer), chemical, and biological and integrated fertilizing systems as follows:

T0: Control (No fertilizer application)

T1: Chemical fertilizer (135 kg ha<sup>-1</sup> urea fertilizer + 185 kg ha<sup>-1</sup> Triple superphosphate fertilizer)\*

T2: Urea chemical fertilizer (135 kg ha<sup>-1</sup>) + Phosphorous solubilizing bacteria

T3: Urea chemical fertilizer (135 kg ha<sup>-1</sup>) + Mycorrhiza

T4: Urea chemical fertilizer (135 kg ha<sup>-1</sup>) + Phosphorous solubilizing bacteria+Mycorrhiza

T5: Nitrogen fixing bacteria+Triple superphosphate fertilizer (185 kg ha<sup>-1</sup>)

T6: Nitrogen fixing bacteria+Phosphorous solubilizing bacteria

T7: Nitrogen fixing bacteria+Mycorrhiza

T8: Nitrogen fixing bacteria+Phosphorous solubilizing bacteria+Mycorrhiza

\* Chemical fertilizers consisting of triple superphosphate and Urea were applied according to soil test to fulfill the requirements of the crop in each site.

Soil samples were collected before the commencement of the experiment. Land preparation was carried out before annual medic planting in early March. Phosphate solubilizing bacteria (*Bacillus coagulans*),



Nitrogen fixing bacteria (*Sinorhizobium meliloti*), and Mycorrhiza (*Glomus intraradices*) inoculants were provided in the form of liquid solution by Soil and Water Research Institute of Iran. After calculating the number of seeds per treatment, the seeds were placed into an insulator polyethylene bag along with 4% of Arabic gum solution and 30 ml of distilled water for 100 g of seeds. The seeds and the adhesive substance were then gently shaken for 30 seconds and then one ml of each inoculum [at the rate of  $10^8$  CFU (Colony forming units)] was added to the adhesive seeds and again shaken for 45 seconds to ensure the inoculation substance was uniformly distributed among the seeds. The inoculated seeds were dispersed on an aluminum sheet to dry up in shade.

All experimental plots consisted of 6 planting rows of 25 cm apart with 5 meters in length. A buffer space of 2 m was considered between plots to prevent fertilizer exchange. The annual medic var. Robinson was planted at a rate of 20 kg of seed per hectare. Seed was sown on lines at the depth of one centimeter. Half of urea fertilizer and all phosphorous fertilizer (in treatments containing phosphorous chemical fertilizer) were applied to the soil, based on soil analysis and according to fertilizer recommendations for annual medic before sowing the seeds. N and P chemical fertilizers were applied to the soil in bands 5 cm from the seeding line. The rest of the N fertilizer was broadcasted on the specified plots when plants reached four-leaf stage.

In each experimental site, the snail medic Robinson variety (*Medicago scutellata* cv. Robinson) was sown both in dry farming (D1) and irrigated conditions (D2) and fertilizing treatments were applied accordingly.

In the irrigated treatments, the conventional irrigation system was followed as practiced by farmers in the region (full irrigation to soil saturation at four growing stages): irrigation immediately after planting, at four-leaf stage, in the beginning of flowering stage, and at pod filling stage.

The concentration of N content in plants at the early stage of flowering of annual medic was determined by Kjeldahl method, P content by Olson method and with the aid of spectrophotometer (Olsen and Sommers, 1990). Potassium content in the dry ash was measured by Photometers film apparatus (Knudsen et al., 1982).

Furthermore, Iron, Zinc, Copper and Magnesium contents were quantified by extraction method with DTPA and then by the atomic absorption apparatus the data were cleared.

### Statistical Analysis

Data were analyzed using SAS and Graphs depicted by Microsoft Excel. Least significant difference (LSD) was used for mean separation test of different experimental factors with the alpha level of 0.05.

## RESULTS AND DISCUSSION

### Macro-elements

The results of analysis of variance for macro-nutrients concentrations in forage of annual medic are shown in Table 3. In both sites, the highest percentage of N and K contents of 3.82 percent and  $4.16 \text{ mg kg}^{-1}$ , respectively, were observed in the fertilizing treatment (T5) while the highest P content of  $0.335 \text{ mg kg}^{-1}$  was recorded in the fertilizing treatment (T8) (Table 4).

Results illustrated an interactive effect between production system and the experimental location (Table 5). The highest concentration of N and P were observed under irrigated conditions and the highest K content was measured at dry farming conditions, both in Mahidasht research site.

It seems that application of the N-fixing bacteria+triple superphosphate fertilizers

**Table 3.** ANOVA for forage nutrients.

SOV	df	N (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Fe (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	Cu (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
Location (L)	1	**	**	**	**	ns	**	**
Condition (C)	1	**	**	**	**	**	**	**
L×C	1	*	ns	**	**	**	**	**
Treatment (T)	8	**	**	**	**	**	**	**
L×T	8	**	**	**	**	**	**	**
C×T	8	**	**	**	**	*	**	**
L×C×T	8	**	**	**	**	**	**	**

ns: Non-significant; \* Significant at  $P \leq 0.05$ , \*\* Significant at  $P \leq 0.01$ .

**Table 4.** Macronutrients concentrations in forage was affected by different fertilizing systems at two experimental research stations.

Treatment <sup>a</sup>	N (%)			P (mg kg <sup>-1</sup> )			K (mg kg <sup>-1</sup> )		
	Location <sup>c</sup>		Mean	Location		Mean	Location		Mean
	L1 <sup>b</sup>	L2 <sup>c</sup>		L1	L2		L1	L2	
T0	2.09	2.38	2.23e	0.14	0.21	0.17g	2.56	2.46	2.51e
T1	3.18	2.92	3.04cd	0.23	0.29	0.26de	2.75	2.55	2.65de
T2	2.94	3.37	3.15bc	0.21	0.25	0.23f	2.71	2.98	2.85cd
T3	3.15	3.23	3.18bc	0.23	0.32	0.27cd	2.86	3.03	2.95c
T4	2.75	3.44	3.09bcd	0.22	0.28	0.25ef	3.31	2.93	3.12c
T5	3.76	3.90	3.82a	0.25	0.33	0.29bc	4.41	3.91	4.16a
T6	2.67	3.16	2.91d	0.32	0.28	0.30b	4.03	3.10	3.56b
T7	3.05	2.94	2.99cd	0.27	0.28	0.28cd	3.08	2.76	2.92cd
T8	3.28	3.27	3.27b	0.28	0.38	0.33a	4.40	3.38	3.89a
Mean	2.98b <sup>d</sup>	3.17a		0.24b	0.29a		3.35a	3.01b	
LSD Local.×Treat. (5%)	0.31			0.03			0.41		

<sup>a</sup> T0 to T8: Different fertilizing systems, <sup>b</sup> Sararoud Experimental Site, <sup>c</sup> Mahidasht Experimental Site.

<sup>d</sup> Means with the same letter in each column are not significantly different at 5 percent probability level.

**Table 5.** Interaction between two experimental conditions and Locations on each forage macro nutrient concentration with LSD values.

Experimental condition	N (%)		P (mg kg <sup>-1</sup> )		K (mg kg <sup>-1</sup> )	
	Location <sup>c</sup>		Location		Location	
	L1 <sup>a</sup>	L2 <sup>b</sup>	L1	L2	L1	L2
D1 <sup>c</sup>	2.45c <sup>e</sup>	3.51a	0.19c	0.28b	2.75c	3.94a
D2 <sup>d</sup>	2.76b	3.58a	0.24bc	0.35a	2.73c	3.29b
LSD						
Location×Experimental condition (5%)		0.16		0.05		0.10

<sup>a</sup> Sararoud Experimental Site, <sup>b</sup> Mahidasht Experimental Site, <sup>c</sup> Dry farming condition, <sup>d</sup> Irrigated system

<sup>e</sup> Means with the same letter in each column are not significantly different at 5 percent probability level.



improved plant growth and, as a result, the enhancement of N concentration was ascertained. Integrated application of P-solubilizing bacteria, Mycorrhiza fungi, and N-fixing bacteria had a synergetic effect on the plant P concentration. This result could be explained by PGPR positive contribution in the efficiency of nutrient absorption by plants, which improved uptake of minerals such as P. An increase in concentration of P was also recorded in (T8), which may be also explained by PGPR activities (ameliorated development in fungus hyphae) in rhizosphere to improve uptake of P. Solubilizing bacteria are able to provide adequate P required for annual medic plants in different growth stages by increasing the solubility of phosphate compounds in rhizosphere, thereby resulting in enhancement of P concentration in the forage.

The results of the interaction between systems of production and fertilizing treatments are shown in Table 6. The highest concentration of N and K was observed in (T5) and the highest amount of Phosphorus

in (T6) and (T8) treatments, respectively.

Based on the results obtained in this experiment, concentrations of nutritional elements in the forage in annual medic was affected by interactions of fertilizing system, climatic conditions, and soil properties in different locations as well as the production system (dry farming and irrigated system). However, the roles of different fertilizing treatments were more prominent.

In both irrigated and dry farming conditions, all fertilizing treatments increased the concentrations of nutritional elements over the control. Also, the results revealed that P uptake through integrated fertilizing treatments were much more efficient compared to chemically provided P. Chabot *et al.* (1996) stated that in the soils with restricted P contents, inoculating the plants by P-solubilizing bacteria did not increase the P uptake. In contrast, in this experiment, the magnitude of P content in the plants in the fertilizing treatments with sole application of P-solubilizing bacteria was higher than the other fertilizing

**Table 6.** Interaction between different fertilizing systems and two experimental conditions on macronutrients concentration with LSD values.

Treatment <sup>a</sup>	N (%)			P (mg kg <sup>-1</sup> )			K (mg kg <sup>-1</sup> )		
	Experimental condition		Mean	Experimental condition		Mean	Experimental condition		Mean
	D1 <sup>b</sup>	D2 <sup>c</sup>		D1	D2		D1	D2	
T0	1.60	2.87	2.23	0.13	0.21	0.17	2.43	2.60	2.51
T1	2.54	3.54	3.04	0.23	0.30	0.26	2.45	2.85	2.65
T2	2.52	3.78	3.15	0.16	0.30	0.23	2.78	2.91	2.85
T3	2.64	3.73	3.18	0.21	0.33	0.27	2.63	3.26	2.95
T4	2.96	3.23	3.09	0.23	0.26	0.25	2.83	3.41	3.12
T5	3.52	4.13	3.82	0.20	0.39	0.29	2.70	5.63	4.16
T6	2.45	3.38	2.91	0.23	0.38	0.30	3.01	4.11	3.56
T7	2.51	3.47	2.99	0.25	0.30	0.28	2.81	3.03	2.92
T8	2.74	3.80	3.27	0.28	0.38	0.33	3.06	4.71	3.89
Mean	3.55	2.61		0.13	0.21		2.74	3.61	
LSD									
Experimental site	0.31			0.03			0.41		
×Condition×Treat.									
(5%)									

<sup>a</sup> T0 to T8: Different fertilizing systems, <sup>b</sup> Dry farming condition, <sup>c</sup> Irrigated system. Means with the same letter in each column are not significantly different at 5 percent probability level.

treatments. However, our results are supported by Singh and Kapoor (1998) who reported that inoculation with P-solubilizing microorganisms, with and without phosphate rock application, increased the yield of beans and P uptake by plants compared to the non-inoculated control.

T5 fertilizing treatment provided a better nutritional status and ultimately increased the percentage of N compared to the other fertilizing treatments. Behl *et al.* (2003) reported that simultaneous application of Mycorrhiza fungi and Azotobacter bacteria had a positive and synergistic effect on wheat plant. They explained their results by the positive effects of Azotobacter on increasing the growth of capillary roots and longitudinal growth of the fungus mycelium and their penetration into the lower layers of the soil which enhanced the plant access to more nutritional elements.

Phosphorus and nitrogen absorption in plants is enhanced by bio fertilizer application. The results obtained by Hamel and Smith (1991) implied that the high P content of the plants was due to the symbiotic relationship with Mycorrhiza fungi and the corn dependence on the colonization. This important issue demonstrates the high capacity of microorganisms in the enhancement of P uptake by the host plants. The increase in the P uptake happens in the symbiotic plants by developing the absorption contact surface of the root system, hence, it increases the phosphorous discharging area (Zaidi *et al.*, 2004). Kim *et al.* (1998) stated that the increase of P uptake in the inoculated plants was because of an increase in the number of uptake regions per unit area of the root and maximizing the ability of these roots to absorb P. In an investigation, it was specified that the integrated inoculation of the Rhizobial Mycorrhizal fungi and P-solubilizing bacteria in the Rhizosphere of medic increased the P and N uptake in this plant (Toro *et al.*, 1998). Pandey *et al.* (2000) reported that with a reduction in frequency and volume of irrigation, N

uptake in corn grain was reduced and this reduction occurred more often when fertilizer was not used.

Awareness of responses of annual medics to different fertilizing treatments in well irrigated and dry conditions should be recognized as an important factor to reach success in crop production. It is expected that the plant N content (in protein and other components) in treatments with integrated application of bio and chemical N fertilizers will be higher than other fertilizing treatments. In fact, bio-fertilizers not only provide favorable conditions for plant growth through biological-fixation of N, but they also act to enhance plant ability in the process of N assimilation (by growth promoting enzymes, etc.). Researchers have shown that bio-fertilizers improved the growth, increased the nutrient uptake, and enhanced the drought resistance in the plants (Lucy *et al.*, 2004; Bano, 2006).

According to the mentioned benefits associated with bio-fertilizers, it can be assumed that bio-fertilizers increase the amount of accessible N in the plant and contribute to a suitable growth and, therefore, increase the qualitative yield in terms of N associated components such as protein. Therefore, integrated application of bio and chemical fertilizers in favorable irrigation conditions will lead to a substantial improvement in quantity and quality of yield.

### Micro-elements

In Table 3, the interactive effects of sites, production systems, and different fertilizing treatments on the concentration of micro-element nutrients in the annual medics are presented. The effects of different fertilizing treatments on the concentrations of Iron, Magnesium, Zinc and Copper are significant. The concentration of micro-element nutrients in the well irrigated conditions was significantly higher than dry farming conditions (Table 7). Comparison of



the mean interaction effects of the different fertilizing treatments in different sites (Table 8) shows that the maximum concentration of Iron, Magnesium, Copper and Zinc was reached in (T5), (T8), and (T6) fertilizing treatments, respectively. It could be possible that the bacterium and fungus components in these treatments acidified the Rhizosphere, which accelerated the availability of micronutrients such as Fe and Mg, leading to their higher concentration in medic seed.

Overall, the simultaneous application of the different microorganisms increased the concentration of all assessed elements in the seed (Table 8).

The interaction between experimental sites and the production systems (Table 9) on the concentration of micro-elements in annual medics were significant ( $P < 0.05$ ). The concentration of Fe, Mg and Cu in Mahidasht in dry farming system significantly increased compared to both irrigated and dry farming conditions in Sararoud. However, the concentration of Zn in L2D2 situation (Mahydsht under irrigated conditions) was significantly higher than the other conditions.

The interactions between fertilizing treatments and production systems on the concentration of micro-element nutrients were significant (Table 10). The highest concentrations of Fe, Cu, Mg, and Zn were observed in (T5), (T7) and (T6) treatments, respectively, while the lowest concentration of the micro-elements were recorded in the control treatment.

Based on the results shown in Table 8, in

both experimental sites at different fertilizing treatments, the concentration of micro-elements in plants were higher compared to the control. Concentrations of Fe, Mg and Cu in (T0), (T1) and (T2) fertilizing treatments at Sararoud Station were higher than the Mahidasht Station (Table 8). It seems that the concentrations of nutrients were affected by climatic conditions and location of the experimental sites. Azcon and Kapoor (1998) have reported that P-solubilizing bacteria can cause plant growth enhancement by synthesis of various hormones. In this process, roots in early stages of plant growth occupy a greater volume of the soil and, as a consequence, the level of nutrients absorption will increase.

High Fe concentration in shoots under (T5) fertilizing treatments can be explained by improved chemical, physical, and biological conditions of soil under this treatment. The concentration and uptake of Fe in integrated fertilizing treatments was significantly ( $P < 0.01$ ) higher compared to the chemical fertilizing treatment, thus, under the conditions of this experiment, the triple application of biological fertilizers (T8) improved the efficiency of Fe uptake by the plants.

It was noted that under irrigated conditions, Fe content of T5 treatment ( $603.66 \text{ mg kg}^{-1}$ ), and in the dry conditions, T8 treatment ( $400.66 \text{ mg kg}^{-1}$ ), were the highest concentrations in plants, respectively (Table 10). This result reveals that under deficit moisture conditions like dry farming system, the (T8) fertilizing treatments provided a more

**Table 7.** Comparison between two experimental conditions in each forage trait with LSD values.<sup>a</sup>

Experimental condition	N (%)	P ( $\text{mg kg}^{-1}$ )	K ( $\text{mg kg}^{-1}$ )	Fe ( $\text{mg kg}^{-1}$ )	Mg ( $\text{mg kg}^{-1}$ )	Cu ( $\text{mg kg}^{-1}$ )	Zn ( $\text{mg kg}^{-1}$ )
Dryland (D1) <sup>a</sup>	2.61b <sup>c</sup>	0.21b	2.74b	254b	57.0 b	30.7b	166 b
Irrigated (D2) <sup>b</sup>	3.55a	0.32a	3.61a	380a	62.2a	35.8a	333 a
LSD (5%)	0.11	0.01	0.07	14.10	1.30	0.78	2.61

<sup>a</sup> Dry farming condition, <sup>b</sup> Irrigated system, <sup>c</sup> Means with the same letter in each column are not significantly different at 5 percent probability level.



**Table 8.** Micronutrients concentrations in forage was affected by different fertilizing systems at two experimental research stations.<sup>a</sup>

Treatment <sup>a</sup>	Fe (mg kg <sup>-1</sup> )		Mg (mg kg <sup>-1</sup> )		Cu (mg kg <sup>-1</sup> )		Zn (mg kg <sup>-1</sup> )	
	Location		Location		Location		Location	
	L1 <sup>b</sup>	L2 <sup>c</sup>	Mean	Mean	L1	L2	L1	L2
T0	162	117	139g	33.8	44.0	33.8	22.6	19.6
T1	317	243	279e	53.1	34.6	43.8f	27.2	23.1
T2	428	196	311d	52.5	46.4	49.4e	26.1	24.3
T3	321	165	242f	69.5	47.1	58.3d	31.7	34.2
T4	250	252	251f	55.5	50.0	52.7e	27.4	24.7
T5	543	447	495a	82.8	74.8	78.7b	74.0	47.8
T6	385	234	309d	56.0	66.3	61.1d	27.0	27.3
T7	308	488	397c	59.4	73.8	66.5c	31.6	26.5
T8	393	467	429b	72.6	101.7	87.1a	43.8	60.6
Mean	354a <sup>d</sup>	289b		60.5 a	58.7a		34.5a	31.9b
LSD local*treat (5%)	28.8			6.13			2.87	15.8

<sup>a</sup> T0 to T8: Different fertilizing systems, <sup>b</sup> Sararoud Experimental Site, <sup>c</sup> Mahidasht Experimental Site, <sup>d</sup> Means with the same letter in each column are not significantly different at 5 percent probability level.

**Table 9.** Interaction between two experimental conditions and location in each forage micronutrients concentration with LSD values.

Experimental condition	Fe (mg kg <sup>-1</sup> )		Mg (mg kg <sup>-1</sup> )		Cu (mg kg <sup>-1</sup> )		Zn (mg kg <sup>-1</sup> )	
	Location		Location		Location		Location	
	L1 <sup>c</sup>	L2 <sup>d</sup>	L1	L2	L1	L2	L1	L2
D1 <sup>d</sup>	261.25 <sup>c</sup>	429.00a	51.42d	69.74a	33.21b	35.97a	162.62d	312.77b
D2 <sup>b</sup>	246.81c	332.88b	62.59b	54.82c	28.33c	35.64a	170.22c	353.37a
LSD Location×Experimental condition (5%)	2.05		1.84		1.106		3.699	

<sup>a</sup> Dry farming condition, <sup>b</sup> Irrigated system, <sup>c</sup> Sararoud Experimental Site, <sup>d</sup> Mahidasht Experimental Site, <sup>e</sup> Means with the same letter in each column are not significantly different at 5 percent probability level.



**Table 10.** Interaction between two experimental conditions and fertilizing treatments on micronutrients concentrations in annual medic forage with LSD values.

Treatment <sup>a</sup>	Fe (mg kg <sup>-1</sup> )		Mg (mg kg <sup>-1</sup> )		Cu (mg kg <sup>-1</sup> )		Zn (mg kg <sup>-1</sup> )	
	Experimental condition <sup>c</sup>		Experimental condition		Experimental condition		Experimental condition	
	D1 <sup>b</sup>	D2 <sup>c</sup>	D1	D2	D1	D2	D1	D2
T0	115.00	163.66	35.75	48.08	18.75	24.09	49.66	74.50
T1	182.83	377.00	39.63	52.66	26.20	30.60	94.50	180.66
T2	228.50	395.33	46.25	61.62	19.76	35.85	98.50	206.33
T3	172.83	312.83	55.00	50.66	30.08	24.63	102.66	297.50
T4	172.83	329.16	54.79	79.00	27.38	61.83	135.00	411.16
T5	386.83	603.66	78.58	63.25	59.91	30.37	162.00	363.66
T6	291.50	327.33	58.96	71.83	23.83	35.06	354.16	533.16
T7	335.33	460.33	61.29	91.41	23.05	56.33	223.83	452.83
T8	400.66	459.16	82.83	48.08	48.00	24.09	277.50	477.83
Mean	254.03	380.94	57.01	62.95	30.77	35.87	166.42	333.07
LSD Experimental site× Condition×Treat. (5%)	28.84		6.139		2.87		15.86	

<sup>a</sup> T0 to T8: Different fertilizing systems, <sup>b</sup> Dry farming condition, <sup>c</sup> Irrigated system, Means with the same letter in each column are not significantly different at 5 percent probability level.

favorable status than the other treatments to enhance efficiency of micro nutrient elements absorption. Accordingly, it could be concluded that the soil moisture had a significant effect on the plant micro-nutrient uptake.

By a general law, as the availability of the moisture in the soil increases from the permanent wilting point, the cation and anion uptake by plants follow an increasing trend. The increase in soil moisture status facilitates higher absorption of nutrients. Water deficits could adversely affect the uptake and accumulation of mineral elements in the plant tissues through restricting of root growth and development (Kramer and Boyer, 1995).

Application of different fertilizing treatments as in chemical, biological, and integrated treatments, increased the plant nutrients concentration compared to the control. It seems that the appropriate combination of biological and chemical fertilizers alongside with reducing the use of chemical fertilizer may alleviate the environmental pollution, and eventually lead towards a more desirable quality of the forage by more efficient macro and micro nutrients absorption. Findings of this research indicated the superiority of integrated application of chemical and biological fertilizers in nutrients uptake and increased yield in annual medic are supported by other researchers (Patidar and Mali, 2001; Rong Xiang *et al.*, 2001). It seems that improving the chemical, biological, and especially physical properties of soils, with the aid of a proper combination of the biological and chemical fertilizers, causes the appropriate nutritional conditions and increases the nutrients uptake and leads to growth improvement.

Considering the concentration and the magnitude of nutrients uptake in the annual medics, it was highlighted that the highest concentration and nutrient uptake was achieved by integrated fertilizing treatments. Application of integrated fertilizing treatments not only optimized and lowered the rate of sole chemical fertilizer application (consequently, reducing the environmental pollutions), but it also enhanced the forage quality in terms of higher macro and micro nutrients concentration in plant tissues.

According to the results of this study, it could be concluded that integrated fertilizing

treatments may be considered more efficient in dry farming than in irrigated agro-ecosystems.

## REFERENCES

1. Arancon, N., Edwaeds, C.A., Bierman, P., Welch, C. and Metzger, J.D. 2004. Influences of Vermicomposts on Filed Strawberries. 1. Effects on Growth and Yields. *Biores., Technol.*, **93**: 145-153.
2. Azcon, S. and Kapoor, K. 1998. Effects of Inoculation of Phosphate Solubilizing Microorganisms and an Arbuscular Mycorrhizal Fungus on Mung Bean Grown under Natural Soil Condition. *Mycorrhiza*, **7**(5): 249-253
3. Bano, A. 2006. Altitudinal Variation in Azospirillum Species Collected from the Rhizosphere and Roots of *Zea mays* (L.). *Asian J. Plant Sci.*, **5**(6): 1051-1053
4. Behl, R. K., Sharma, H., Kumar, V. and Narula, N. 2003. Interactions among Mycorrhiza, *Azobacterchroococcum* and Root Characteristics of Wheat Varieties. *J. Agron. Crop Sci.*, **189**(3): 151-155
5. Brundrett, M. C. 1991. Mycorrhizas in Natural Ecosystems. *Adv. Ecol. Res.*, **171**: 313-321.
6. Chabot, R., Antoun, H. and Cescas, M. 1996. Growth Promotion of Maize and Lettuce by Phosphate-solubilizing *Rhizobium Legumin osarumbio* var Phaseoli. *Plant Soil*, **184**: 311-321
7. Chalk, P. M., Souza, RdF, Urquiaga, S., Alves, B. J. R. and Boddey, R. M. 2006. The Role of Arbuscular Mycorrhiza in Legume Symbiotic Performance. *Soil Biol. Biochem.*, **38**(9): 2944-2951
8. EbhinMasto, R., Chhonkar, P. K., Singh, D. and Patra, A.K. 2006. Changes in Soil Biological and Biochemical Characteristics in a Long-Term Field Trial on a Sub-tropical Inceptisol. *Soil Biol. Biochem.*, **38**: 1570-1582
9. Gupta, M. L., Prasad, A., Ram, M. and Kumar, S. 2002. Effect of the Versicular- Arbuscular Mycorrhizal (VAM) Fungus *Glomus fasciculatum* on Essential Oil Yield Related Characters and Nutrient Acquisition in the Crops of Different Cultivars of Menthol (*Menthaarvensis* L.) under Field Condition. *Biores. Technol.*, **81**: 77-79
10. Gyaneshwar, P., Naresh Kumar, G., Parekh, L. J. and Poole, P. S. 2002. Role of soil Microorganisms in Impairing P Nutrition of Plants. *Plant Soil*, **93**: 245-283.
11. Hamel, C. and Smith, D. L. 1991. Plant Development in a Mycorrhizal Field-grown Mixture. *Soil Biol. Biochem.*, **23**(7): 661-665.
12. Kim, K. Y., D. Jordan and G. A. McDonald. 1998. Effect of Phosphate-Solubilizing Bacteria and Vesicular-Arbuscular Mycorrhizae on Tomato Growth and Soil Microbial Activity. *Biol. Fert. Soils*, **26**: 79-87.
13. Kramer, J. K. and Boyer, J. S. 1995. *Water Relations of Plants and Soils*. Academic Press, California, PP. 1-495.
14. Knudsen, D., Peterson, G. A. and Pratt, P. F. 1982. Lithium, Sodium, and Potassium. 2. Chemical and Microbiological Properties. In: "Methods of Soil Analysis", (Eds.): Page, A. L., Miller, R. H. and Keeney, D. R. American Society of Agronomy, Madison, Wisconsin, USA, PP. 225-246
15. Lucy, M., Reed, E. and Bernard, R. Click. 2004. Applications of Freelifving Plant Growth Promoting Rhizobacteria. *Antonie van Leuwenhoek*, **86**: 1-25
16. MalekiFarahani, S., Chaichi, M. R., Mazaheri, D., TavakkoAfshari, R. and Savaghebi, Gh. 2011. Barley Grain Mineral Analysis as Affected by Different Fertilizing Systems and by Drought Stress. *J. Agr. Sci. Tech.*, **13**: 315-326.
17. Neeru, N., Vivek, K., Rishi, K. and Wolfguncy, M. 2000. Effect of P-solubilizing *Azotobacter chroococcum* on N, P, K Uptake in P-respponsive Genotypes Grown under Greenhouse Condition. *J. Plant Nutr. Soil Sci.*, **163**: 393-398.
18. Olsen S. R., Sommers L. E. 1990. Phosphorus, In: "Methods of Soil Analysis" (Eds.): Page ASL et al. Part 2.2. Agron. Monogor ed., Madison, WI. Pp. 403-431.
19. Pandey, R. K., Maranville, L. W. and Admou, A. 2000. Deficit Irrigation and Nitrogen Effects on Maize in Sahelian Environment. *Agric. Water Manage.*, **45**(1): 1-13
20. Patidar, M. and Mali, A. L. 2001. Integrated Nutrient Management in Sorghum (*Sorghum bicolor*) and Its Residual Effects on Wheat (*Triticumaestivum* L.). *Microbiol. Res.*, **168**(2): 341-347
21. Perveen, S., Khan, M. S. and Zaidi, A. 2002. Effect of Rhizospheric Microorganisms on Growth and Yield of Green Gram (*Phaseolus radidtus*). *Indian J. Agric. Sci.*, **72**: 421-423
22. Rong Xiang, M., Jian Rong, J., Zhu, H., Liu, Q. F. Z., Liu, J. and Yue, Z. H. 2001. Effects of Application of Inorganic Fertilizer in Combination with Organic Fertilizer to Red



- Upland Soil. *J. Human Agril. Univ.*, **27**: 453-456
23. Rosas, S., Rovera, M., Andres, J., Correa, N., 2002. Effect of phosphat –solubilization bacteria on the rhizobial – legume symbiosis In: Proceedings of the 15th International Meeting on Microbial Phosphate Solubilization. Salamanca University, 16-19 July 2002 Salamanca, Spain.
24. Samarah, N. H., Mullen, R. and Cianzio, S. 2004. Size Distribution and Mineral Nutrients of Soybean Seeds in Response to Drought Stress. *J. Plant Nutr.*, **27**(5): 815-835
25. Singh, S. and Kapoor, K. 1998. Effects of Inoculation of Phosphate Solubilizing Microorganisms and an Arbuscular Mycorrhizal Fungus on Mungbean Grown under Natural Soil Condition. *Mycorrhiza*, **7**(5): 249-253
26. Stancheva, M., Geneva, E., Djonova, N., Kaloyanova, M., Sichanova, M. and Boychinova, G. 2008. Response of Alfalfa (*Medicago sativa* L.) Growth at Low Accessible Phosphorus Source to the Inoculation with Mycorrhizal Fungi and Nitrogen Fixing Bacteria. *GEN Appl. Plant Physiol.*, **34**(3-4): 319-326
27. Toro, M., Azcon, R. and Barea, J. M. 1998. Improvement of Arbuscular Mycorrhiza Development by Inoculation of Soil with Phosphate-solubilizing Rhizobacteria to Improve Rock Phosphate Bioavailability (32P) and Nutrient Cycling. *Appl. Environ. Microbiol.*, **63**(11): 4408-4412
28. Zaidi, A., Khan, M. S. and Aamil, M. 2003. Interactive Effect of Rhizotrophic Microorganisms on Yield and Nutrient Uptake of Chickpea (*Cicerarietinum* L.). *Eur. J. Agron.*, **19**: 15-21
29. Zaidi, A., Khan, M. S. and Aamil, M. 2004. Bioassociative Effect of Rhizospheric Microorganisms on Yield and Nutrient Uptake of Green Gram. *J. Plant Nutr.*, **27**: 599-610

### اثر تیمار های مختلف کود شیمیایی بر جذب عناصر غذایی در یونجه یکساله (*Medicago scutellata*) رقم رایینسون تحت شرایط آبی و دیم

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#### چکیده

به منظور مطالعه اثر تیمار های مختلف کود شیمیایی بر برخی شاخص های کیفی یونجه یکساله رقم رایینسون، آزمایشی در سال زراعی ۸۸-۱۳۸۷ در دو مکان (ایستگاه معاونت موسسه تحقیقات کشاورزی دیم (سرارود) و ایستگاه تحقیقات حاصلخیزی خاک ماهیدشت) در دو سامانه کشت آبی و دیم بطور مجزا اجرا شد. تیمارهای مورد بررسی در این تحقیق شامل شاهد؛ سامانه تغذیه ای شیمیایی؛ بیولوژیک و تلفیقی بودند. نتایج نشان داد هم در شرایط آبی و هم در شرایط دیم تمامی سامانه های تغذیه ای باعث افزایش جذب عناصر میکرو و ماکرو نسبت به تیمار شاهد می گردد. بیشترین میزان نیتروژن (۳/۸۲ درصد)؛ پتاسیم (۴/۱۶ درصد)؛ آهن (۴۹۵ میلیگرم بر کیلوگرم) و روی (۶۰/۸ میلی گرم در کیلوگرم) در سامانه تغذیه ای تلفیقی (باکتری تثبیت کننده نیتروژن+ سوپر فسفات تریپل) بدست آمد. نظام تغذیه تلفیقی علاوه بر صرفه جویی در مصرف کود شیمیایی و به تبع آن کاهش آلودگی محیط زیست، خصوصیات کیفی علوفه را نیز بهبود بخشید. با توجه به نتایج حاصل از این تحقیق می توان چنین استنباط کرد که کاربرد سامانه تغذیه تلفیقی در شرایط دیم از موفقیت بیشتری برخوردار است.