Middle-East Journal of Scientific Research 7 (4): 444-451, 2011
ISSN 1990-9233

Uptake of Micronutrients by Wheat (*Triticum aestivum* L.) in a Sustainable Agroecosystem

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Abstract: This experiment has been conducted in order to study the effects of different biofertilizers and manure on absorption of some micronutrients by wheat in Alborz Province, Iran. Experimental design was factorial in the form of randomized complete block design with three replications. Four factors of the experiment were Azospirillum (600 g/ha and without), Mycorrhiza (with 1 kg/ha and without), Streptomyces (0.5 kg/ha and without) and manure (30 ton/ha and without). Results showed significant effect of Azospirillum, Mycorrhiza and manure at $p \leq 0.01$ on measured traits but the effect of Streptomyces was not significant. All twofold and threefold interactions significantly affected absorption of four micronutrients, mostly at $p \leq 0.01$. Although because of adverse effect of Streptomyces on Mycorrhiza, fourfold interactions were not much better than threefolds, but the highest absorptions of Fe, Mn, Zn and Cu happened in fourfold interactions. In interaction of Azospirillum × Mycorrhiza × manure ($a_1m_1s_0o_1$), plants shoot contained 246.77 mg/kg Iron, 56.91 mg/kg Manganese, 13.03 mg/kg Zinc and 4.52 mg/kg Cupper that were much higher than any other treatments. Totally, results indicated that biofertilizers and manure can naturally meat large amount of plants nutritional requirements and replace chemical fertilizers in sustainable agricultural production systems.

Key words: Azospirillum · Cupper · Iron · Manganese · Mycorrhiza · Streptomyces · Zinc

INTRODUCTION

In most regions of the world, uncontrolled application of chemical inputs to gain high yield production in agriculture has raised costs of production and damaged soil, water and biological sources. Thus, the idea of sustainable low input agriculture was emerged in 1980s. One of the main factors that has key role in sustainable agriculture are biofertilizers. Biofertilizers are consisted of beneficial microorganisms that provide nutrients to plants through symbiosis, association, etc [1, 2]. Although these microorganisms naturally exist in most soils but their quantity and quality is not usually enough, so inoculation is necessary. Biofertilizers have different types and are categorized according to microorganisms used: nitrogen fixing bacteria (diazotrophs), Mycorrhiza fungi, phosphate solublizing microorganisms, plant growth promoting rhizobacteria (PGPR), etc [2-4].

Azospirillum is a nitrogen fixing bacteria that associate with cereals. In this association, the bacteria fix air nitrogen and produce plant growth promoters [5-8]. Although Azospirillum genus has five species but most strains are from *A. brasilense*, which mostly associate with C_3 cereals and *A. lipoferum*, which mostly associate with C_4 cereals [5, 9, 10].

Chemical agricultural inputs affect Azospirillum activity. A laboratory experiment showed that herbicides inhibit growth of Azospirillum. Nitrogen containing compounds also prevent Azospirillum to connect with plant root [5].

Inoculating plants with Azospirillum significantly affects their growth but effect of the association on grain yield can be either increasing or non significant [8]. This association can also increase total dry weight, total N in plant shoot, total number of tillers and fertile spikes, grain weight, leaf size, germination rate, root system development, nutrients uptake and phytohormones production. Mertens and Hess (1984) observed that ratio of root/shoot increased in wheat when inoculated with Azospirillum because of phytohormones production by the bacterium [11]. Plants shoot growth may also increase by Azospirillum association because of more absorption

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of NO_3^-, NH_4^+, PO_4^{2-}, K^+ and Fe [12, 13, 14]. Researchers found that association of *Azospirillum brasilense* with wheat and sorghum will enhance total shoot and root weight, plant height, leaf size and total N [15].

Mycorrhiza, which is a symbiotic fungus, has been under researches for more than a century. In Mycorrhiza symbiosis with plants, the fungus gets energy through carbon sources from plant and instead supplies many nutrients such as phosphorus, molybdenum, cupper and iron to plant [16, 17]. Mycorrhiza myceliums that are connected to plants root, act like an additional and supplementary absorption organ for plants root system that helps plants to use more volume of soil as source of nutrient and water [13, 18, 19].

Although plants need high amount of phosphate but its availability in soil for plants is low because mineral phosphate ions have strong bond with soil colloids and also present in ferro phosphate or aluminum phosphate form that make them immobile [20]. Mycorrhiza has great effect on absorption of phosphorus in such soils. Tarafdar and Marschner (1994) represented that Mycorrhizal symbiosis with wheat will increase phosphorus absorption by the plant and amount of absorption depends on soil type, density and length of mycelium, phophatase activity and soil phosphorus content [21]. Other researchers also concluded that formation and density of Mycorrhiza colonization on plants roots have negative correlation with the amount of phosphorus in the soil and plant [22].

Nitrogen content of some other elements in plants is reported to enhance in symbiosis with Mycorrhiza [20]. On the other hand, Mycorrhiza helps plants to tolerate drought stress better and reduces yield loss to some extent. Mycorrhiza mycelium can penetrate into pores and cracks that are too small for plant roots so they help plants to absorb more water. Moreover, Mycorrhiza in symbiosis with plants produces growth promoting hormones such as auxin and cytokinin that improve growth. This symbiosis is even effective on plants resistance to heat, salinity, heavy metals contamination and root diseases [23, 24, 2]. Although Mycorrhiza fungi exist in most soils but application of inoculants of highly active strains can maximize the efficacy of symbiosis system.

Streptomyces is an important beneficial soil born actinomycets that is categorized as plant growth promoting rhizobacteria (PGPR) and has many effects on plant growth. Colony of this microorganism acts like a seed coat and protect seeds against diseases. In laboratory condition, Streptomyces (S57 strain) could prevent growth and activity of some plant pathogens such as Fusarium, Rhizoctonia, Thielaviopsis, Cylindrocarpon, Trichoderma and Geotrichum. Moreover, treating corn grains with S57 increased germination rate to 92% from 80% [25, 26]. Sardi *et al*. (1992) also observed that Streptomyces can protect wheat against Fusarium and increase the crop yield and enhance yield and sugar content in sugar beet [26]. Different mechanisms are responsible for the effect of PGPRs on plants growth: a) hormones production by the microorganisms, b) improvement of absorption and transition of some nutrients and c) control of plant pathogens.

Totally, it's clear that application of biofertilizers is the most natural and suitable way to keep soil biosystems alive and active. So it's necessary to add these microorganisms specially into soils with low organic matters and microbial activity because they provide nutrients to plants, maintain soil biodiversity and bioactivity, improve quality of soil and environment, etc. Many experiments have studied soil organic matters and their role in soil fertility and sustainable agriculture systems. As these organic matters are sources of energy for soil microorganisms, their presence will increase soil microbial activity. Organic matters improve the quality of clay soils by increasing their aeration and infiltrability and in sandy soils; they enhance water preservation capacity [27, 28]. Finally, this experiment was conducted to evaluate the effects of different biofertilizers and manure on absorption of micronutrients in a sustainable wheat production system.

MATERIALS AND METHODS

This experiment was conducted in Alborz Province, Iran (51° 6' E, 35° 59' N and 1300 m above sea level), in the area where was located in semiarid climate with dry warm summers and humid cold winters. Average annual precipitation in this area is 242 mm that mostly happened during late fall to early spring. Mean annual maximum air temperature was 26.1°C (in July) and minimum was 1°C (in January). Soil type of the test site was clay loam (sand: 36%, silt: 34, clay: 30%). Other properties of the soil are mentioned in Table 1.

The study was conducted in a factorial experiment in the form of randomized complete block design (RCBD) with three replications and four factors:

Azospirillum: With 600 g/ha (a_1) and without (a_0). Required amount of Azospirillum was weighted for each treatment and inoculated with seeds right before sowing. The strain was from *Azospirillum brasilense* species in peat carrier and contained 10^8 cfu/g Azospirillums.

Table 1: Soil properties of the experimental field

K (ava.) ppm	P (ava.) ppm	Total N %	O.C %	pH	EC × 10^3 dS/m	Saturation %
260	8.2	0.80	0.65	7.4	1.25	37

Table 2: Properties of manure

Cu mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg	EC ds/m	pH	O.C %	K %	P %	N %
32	72	286	0.41	14.26	8.77	26.1	4.45	0.74	2.30

Mycorrhiza: With 1 kg/ha (m_1) and without (m_0) that was weighted for each treatment and inoculated with seeds right before sowing. The strain was from *Glomus intraradices*. The carrier was clay and contained 10^5 spores per gram.

Streptomyces: With 0.5 kg/ha (s_1) and without (s_0). The strain was from *Streptomyces sp*. in clay carrier. It contained 10^8 cfu/g Streptomyces and was applied similar to Azospirillum and Mycorrhiza.

Manure: With 30 ton/ha (o_1) and without (o_0). The manure was one year stored, fully decomposed cow dung and its properties are mentioned in Table 2:

After preparing the field and before sowing wheat, N and P fertilizers were broadcasted on soil surface and incorporated into soil by harrowing. According to the results of previous year experiment conducted by the authors, 100 kg nitrogen and 60 kg phosphorus per hectare, that showed to have the lowest adverse effect on activity of microorganisms, were applied. Then, place of plots were set on field, manure was weighted and applied in required plots on the basis of 30 ton/ha and incorporated into soil. Finally, on Nov. 11th, 200 kg/ha wheat seed (*Triticum aestivum* L. var: Mahdavi) was weighted equally for all plots and inoculated with biofertilizers in separate containers. Grains were broadcasted on soil surface and after that, furrower was used. Each plot contained four rows with 7 m length and 50 cm width. 1.5 m between plots and 4 m between replications were left uncultivated to prevent interference and movement of microorganisms.

To measure the absorption of micronutrients (Fe, Mn, Zn and Cu) by plants, five plants were harvested from each plot when grains were at dough stage and dried at 70°C Oven for 72 hours. Then samples were grinded and analyzed. Finally, data were analyzed using MSTAT-C and SPSS.

RESULTS AND DISCUSSION

Iron (Fe): According to analysis of variances (ANOVA), application of Azospirillum significantly affected Fe absorption (Table 3) at $p \leq 0.01$ and increased up to

Table 3: Analysis of variances of measured traits

		Mean Squares (MS)			
SOV	df	Fe	Mn	Zn	Cu
Rep	2	61.064**	24.008**	2.236**	10.656**
Azospirillum (a)	1	23303.778**	1334.364**	66.741**	1002.841**
Mycorrhiza (m)	1	2249.456**	347.949**	17.28**	255.763**
Azospirillum × mycorrhiza (am)	1	4532.047**	168.75**	6.601**	167.253**
Streptomyces (s)	1	0.001^{ns}	0.114^{ns}	0.013^{ns}	0.403^{ns}
Azospirillum × streptomyces (as)	1	44.602*	17.497*	0.907**	6.163**
Mycorrhiza × streptomyces (ms)	1	5390.949**	331.696**	14.963**	238.52**
Azospirillum × mycorrhiza × streptomyces (ams)	1	4112.365**	149.743**	5.467**	160.601**
Manure (o)	1	28836.015**	1842.145**	94.641**	1337.741**
Azospirillum × manure (ao)	1	17622.984**	321.368**	10.453**	575.468**
Mycorrhiza × manure (mo)	1	4699.532**	130.680**	5.201**	185.653**
Azospirillum × mycorrhiza × manure (amo)	1	4109.775**	160.747**	4.813**	155.520**
Streptomyces × manure (so)	1	29.657*	26.731**	1.268**	2.253**
Azospirillum × streptomyces × manure (aso)	1	30.544*	20.202*	1.08**	7.363**
Mycorrhiza × streptomyces × manure (mso)	1	4298.435**	127.727**	4.688**	169.501**
Azospirillum × mycorrhiza × streptomyces × manure (amso)	1	4219.312**	120.777**	4.563**	142.141**
Error	30	5.924	3.422	0.046	0.227
CV (%)	-	1.12	4.06	2.08	6.822

ns; nonsignificant; **, significant at p = 1%; *, significant at p = 5%.

Table 4: Mean comparison of main effects of Azospirillum (a_0, without; a_1, with), Mycorrhiza (m_0, without; m_1, with), Streptomyces (s_0, without; s_1, with) and manure (o_0, without; o_1, with) on measured traits

Treatments	Fe	Mn	Zn	Cu
	mg/kg			
a_0	199.05	40.29	9.17	3.36
a_1	239.12	50.83	11.53	4.27
m_0	206.63	40.87	9.75	3.59
m_1	227.54	48.25	10.95	4.05
s_0	217.09	45.51	10.33	3.81
s_1	217.09	45.61	10.37	3.83
o_0	192.58	39.37	8.95	3.29
o_1	241.60	51.76	11.75	4.35

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$

Table 5: Mean comparison of twofold interactions of Azospirillum (a_0, without; a_1, with), Mycorrhiza (m_0, without; m_1, with), Streptomyces (s_0, without; s_1, with) and manure (o_0, without; o_1, with) on measured traits

Treatments	Fe	Mn	Zn	Cu
	mg/kg			
a_0m_0	174.88c	35.72d	8.2d	2.94d
a_0m_1	215.23b	44.86c	10.14c	3.78c
a_1m_0	238.38a	50.02b	11.30b	4.23b
a_1m_1	239.86a	51.65a	11.76a	4.32a
a_0s_0	194.09b	39.64b	9.02d	3.31d
a_0s_1	196.02b	40.94b	9.33c	3.41c
a_1s_0	238.16a	50.28a	11.41b	4.25b
a_1s_1	240.08a	51.39a	11.65a	4.30a
m_0s_0	196.03c	40.19c	9.17c	3.35c
m_0s_1	217.23b	45.55b	10.33b	3.82b
m_1s_0	238.14a	50.83a	11.49a	4.26a
m_1s_1	216.95b	45.67b	10.41b	3.83b
a_0o_0	151.35d	31.51d	7.30d	2.49d
a_0o_1	238.72b	49.07b	11.04b	4.23b
a_1o_0	233.77c	47.23c	10.59c	4.09c
a_1o_1	244.47a	54.44a	12.47a	4.45a
m_0o_0	172.22c	35.03d	8.02d	2.86d
m_0o_1	241.03a	50.71b	11.48b	4.31b
m_1o_0	212.93b	43.71c	9.87c	3.72c
m_1o_1	242.16a	52.80a	12.03a	4.38a
s_0o_0	191.79b	38.57c	8.77d	3.26c
s_0o_1	240.81a	51.06a	11.61b	4.33a
s_1o_0	193.36b	40.16b	9.13c	3.32b
s_1o_1	242.38a	52.45a	11.90a	4.36a

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$

40 mg/kg (Table 4). Azospirillum has important role in root development thus; plants absorb more nutrients[12-14]. Mycorrhiza also affected Fe absorption at $p \leq 0.01$ and plants absorbed 9% more Fe in symbiosis with Mycorrhiza. Mycorrhiza myceliums provide a supplementary absorption organ to plants roots and help plants to absorb more nutrients from soil. Interaction of

Table 6: Mean comparison of threefold interactions of Azospirillum (a_0, without; a_1, with), Mycorrhiza (m_0, without; m_1, with), Streptomyces (s_0, without; s_1, with) and manure (o_0, without; o_1, with) on measured traits

Treatments	Fe	Mn	Zn	Cu
	mg/kg			
$a_0m_0s_0$	154.06e	30.67d	7.15e	2.49e
$a_0m_0s_1$	195.70d	40.77e	9.25d	3.39d
$a_0m_1s_0$	234.11c	48.60b	10.88c	4.14c
$a_0m_1s_1$	196.34d	41.11c	9.40d	3.42d
$a_1m_0s_0$	238.00b	49.71b	11.20b	4.22b
$a_1m_0s_1$	238.76b	50.33b	11.40b	4.24b
$a_1m_1s_0$	242.17a	53.07a	12.10a	4.39a
$a_1m_1s_1$	237.56b	50.23b	11.42b	4.25b
$a_0m_0o_0$	112.06f	23.46e	5.68g	1.69f
$a_0m_0o_1$	237.70bc	47.99bc	10.72d	4.19c
$a_0m_1o_0$	190.70e	39.55d	8.92f	3.28e
$a_0m_1o_1$	239.75b	50.16b	11.37c	4.27b
$a_1m_0o_0$	232.39d	46.59c	10.35e	4.03d
$a_1m_0o_1$	244.37a	53.45a	12.25b	4.43a
$a_1m_1o_0$	235.15cd	47.87c	10.83d	4.15c
$a_1m_1o_1$	244.57a	55.44a	12.68a	4.48a
$a_0s_0o_0$	148.83e	29.46e	6.83g	2.38f
$a_0s_0o_1$	239.34b	49.81b	10.88d	4.25b
$a_0s_1o_0$	153.93d	33.55d	7.77f	2.59e
$a_0s_1o_1$	238.11b	48.33bc	11.20c	4.22b
$a_1s_0o_0$	234.75c	47.69bc	10.70de	4.14c
$a_1s_0o_1$	243.52a	53.79a	12.33b	4.47a
$a_1s_1o_0$	232.80c	46.77c	10.48e	4.05d
$a_1s_1o_1$	245.42a	55.09a	12.60a	4.45a
$m_0s_0o_0$	151.37e	29.97e	6.97e	2.42e
$m_0s_0o_1$	240.68b	50.41b	11.38b	4.29b
$m_0s_1o_0$	193.07d	40.08d	9.07d	3.30d
$m_0s_1o_1$	241.38ab	51.01b	11.58b	4.33b
$m_1s_0o_0$	232.20c	47.17c	10.57c	4.10c
$m_1s_0o_1$	244.08a	54.49a	12.42a	4.43a
$m_1s_1o_0$	193.65d	40.25d	9.18d	3.34d
$m_1s_1o_1$	240.24b	51.11b	11.63b	4.33a

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$

Azospirillum × Mycorrhiza significantly affected Fe absorption at $p \leq 0.01$ (Table 3) and mean comparison (Table 5) showed the highest Fe absorption in a_1m_0 and a_1m_1 so it indicated that effect of Azospirillum is greater than Mycorrhiza.

Streptomyces had no effect on Fe absorption (Table 3). Interaction of Azospirillum × Streptomyces was significant only at $p \leq 0.05$ and mean comparison showed the highest Fe absorption in a_1s_0 and a_1s_1. Interaction of Mycorrhiza × Streptomyces was significant at $p \leq 0.01$ and the highest Fe absorption happened in m_1s_0. Fe absorption declined in m_1s_1 because of the effect of Streptomyces on activity of Mycorrhiza. Threefold interaction of Azospirillum × Mycorrhiza × Streptomyces was significant at $p \leq 0.01$ (Table 3) and mean comparison showed the highest Fe absorption in $a_1m_1s_0$ (Table 6).

Comparing $a_1m_1s_0$ with $a_1m_0s_1$ indicates that effect of co application of Azospirillum and Mycorrhiza on Fe absorption is greater than the effect of co application of Azospirillum and Streptomyces.

Manure had significant effect on Fe absorption at $p \leq 0.01$ and enhanced it 20%. By decomposition of manure in soil, different nutrients become available to plants so plants absorb more nutrients. Interaction of Azospirillum × manure and Mycorrhiza × manure and also Azospirillum × Mycorrhiza × manure significantly affected Fe absorption at $p \leq 0.01$ but interaction of Streptomyces × manure was significant only at $p \leq 0.05$ (Table 3). In threefold interaction of Azospirillum × Streptomyces × manure, that was significant at $p \leq 0.05$, $a_1s_0o_1$ and $a_1s_1o_1$ were the best treatments with the highest Fe content in plants. Interaction of Mycorrhiza × Streptomyces × manure significantly affected Fe absorption at $p \leq 0.01$ and $m_1s_0o_1$ was the best treatment (Table 6). Finally, interaction of Azospirillum × Mycorrhiza × Streptomyces × manure had significant effect on Fe absorption at $p \leq 0.01$ (Table 3) and mean comparison showed the highest Fe absorption in $a_1m_1s_0o_1$. Increasing effect of manure in all treatments is obvious compared with treatments without manure.

Table 7: Mean comparison of fourfold interactions of Azospirillum (a_0, without; a_1, with), Mycorrhiza (m_0, without; m_1, with), Streptomyces (s_0, without; s_1, with) and manure (o_0, without; o_1, with) on measured traits

Treatments	Fe	Mn	Zn	Cu
	mg/kg			
$a_0m_0s_0o_0$	70.82h	13.80g	3.70j	0.82i
$a_0m_0s_0o_1$	237.30d	47.55de	10.60efg	4.16e
$a_0m_0s_1o_0$	153.30g	33.12f	7.67i	2.56h
$a_0m_0s_1o_1$	238.09cd	48.42de	10.83def	4.22de
$a_0m_1s_0o_0$	226.85f	45.12e	9.97g	3.94g
$a_0m_1s_0o_1$	241.38cd	52.08bc	11.80c	4.34c
$a_0m_1s_1o_0$	154.56g	33.99f	7.87i	2.62h
$a_0m_1s_1o_1$	238.12cd	48.24de	10.93de	4.21de
$a_1m_0s_0o_0$	231.93e	46.14de	10.23gh	4.02f
$a_1m_0s_0o_1$	244.07ab	53.97b	12.17b	4.41bc
$a_1m_0s_1o_0$	232.84e	47.04d	10.47fg	4.04f
$a_1m_0s_1o_1$	244.67ab	53.61b	12.33b	4.44ab
$a_1m_1s_0o_0$	237.56d	49.23cd	11.17d	4.25d
$a_1m_1s_0o_1$	246.77a	56.91a	13.03a	4.52a
$a_1m_1s_1o_0$	232.75e	46.50de	10.50fg	4.05f
$a_1m_1s_1o_1$	242.36bc	53.28ab	12.33b	4.45ab

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$

Manganese (Mn): Analysis of variances showed significant effect of Azospirillum on Mn absorption at $p \leq 0.01$ (Table 3) and mean comparison indicated 20% increase in Zn absorption as a result of Azospirillum association (Table 4). Azospirillum helps plants to develop their root system and absorb more nutrients so it enhances nutrients content in plants shoot [29, 12-14]. Mycorrhiza also had significant effect on Mn absorption at $p \leq 0.01$ and increased it by 15%. Marschner and Dell (1994) in their experiments observed that Mycorrhizal symbiosis had no effect on plants Mn absorption [24]. Interaction of Azospirillum × Mycorrhiza (Table 3) was significant on Mn absorption and best treatment was a_1m_1. Comparing a_1m_0 with a_0m_1 indicates that Azospirillum has more increasing effect on Mn absorption than Mycorrhiza (Table 5). Streptomyces had no effect on this trait.

The interaction between Azospirillum × Streptomyces significantly affected Mn absorptionat $p \leq 0.05$. Both a_1s_1 and a_1s_0 were in the same group and had the highest Mn contend in plants. Interaction of Mycorrhiza × Streptomyces was significant at $p \leq 0.01$ and the highest Mn absorption happened in m_1s_0 (50.83 mg/kg). Comparing m_0s_1 with m_1s_0 showed greater effect of Mycorrhiza than Streptomyces on Mn absorption and also indicated that m_1s_1 could not absorb high amount of Mn because of inhibitory effect of Streptomyces. Mn absorption was significantly affected by application of manure ($p \leq 0.01$) and manure increased it 23%. Interaction of Azospirillum × Manure was significant on Mn absorption at $p \leq 0.01$ (Table 3) and mean comparison showed the highest Mn absorption in a_1o_1 (Table 5). Interaction of Mycorrhiza × manure was significant on Mn absorption and m_1o_1 was the best treatment. Interaction of Streptomyces × manure significantly affected Mn absorption and s_1o_1 (52.45 mg/kg) and s_0o_1 (51.06 mg/kg) had the highest Mn content in plants shoot.

Among threefold interactions, only Azospirillum × Streptomyces × manure was significant at $p \leq 0.05$ and others were significant at $p \leq 0.01$ (Table 3). In four threefold interaction, $a_1m_1s_0$, $a_1m_1o_0$ along with $a_1m_0o_1$, $a_1s_1o_1$ along with $a_1s_0o_1$ and $m_1s_0o_1$ were the best treatments (Table 6). Fourfold interaction of Azospirillum × Mycorrhiza × Streptomyces × manure significantly affected Mn absorption at $p \leq 0.01$ (Table 3) and mean comparison showed the highest Mn absorption in $a_1m_1s_0o_1$ (Table 7). In treatments that Streptomyces reduced activity of Mycorrhiza, application of manure helped plants to absorb higher amount of Mn.

Zinc (Zn): Studying the effect of Azospirillum on Zn absorption showed a significant effect at $p \leq 0.01$ (Table 3) and mean comparison indicated that application of Azospirillum increased Zn absorption in plants 18% (Table 4). Other studies showed that inoculating plants with Azospirillum raise absorption of nutrients [12-14] and it is because of the effect of Azospirillum hormonal activity on root development [5, 30]. Application of Mycorrhiza significantly affected Zn absorption at $p \leq 0.01$ and increased Zn content by 10%. Marschner and Dell (1994) represented that hyphae of Mycorrhiza could absorb and transfer Zn, so in plants inoculated with Mycorrhiza Zn content was higher [24]. The interaction between Azospirillum × Mycorrhiza significantly affected Zn absorption at $p \leq 0.01$ (Table 3) and a_1m_1 was the best treatment (Table 5). Comparing a_0m_1 with a_1m_0 indicates that effect of Azospirillum on Zn absorption is greater than the effect of Mycorrhiza.

Streptomyces had no significant effect on Zn absorption but interaction of Azospirillum × Streptomyces significantly affected the trait at $p \leq 0.01$ (Table 3) and mean comparison showed the highest Zn content in a_1s_1 (Table 5). Interaction of Mycorrhiza × Streptomyces was also significant at $p = 0.01$ and m_1s_0 was the best treatment (11.49 mg/kg).

Application of manure significantly affected Zn absorption at $p \leq 0.01$ (Table 3) and increased it 23% (Table 4). Manure releases many nutrients in rhizosphere so plants absorb higher amount of nutrients. Interaction of Azospirillum × Manure significantly affected Zn absorption at $p \leq 0.01$ and mean comparison showed that co application of Azospirillum and manure had the greatest effect on plants Zn content (12.47 mg/kg). The interaction between Mycorrhiza × manure was also significant at $p \leq 0.01$ and m_1o_1 was the best treatment. Comparing m_0o_1 with m_1o_0 shows that manure is more effective on Zn absorption than Mycorrhiza.The interaction betweenStreptomyces × manure significantly affected Zn absorption at $p \leq 0.01$ (Table 3) and the highest Zn absorption happened in s_1o_1 (Table 5).

All threefold interactions had significant effect on Zn absorption at $p \leq 0.01$ (table 3) and mean comparison showed the highest Zn absorption in $a_1m_1s_0$, $a_1m_1o_1$, $a_1s_1o_1$ and $m_1s_0o_1$ (Table 6). Fourfold interaction of Azospirillum × Mycorrhiza × Streptomyces × manure had also significant effect on Zn absorption at $p \leq 0.01$ (Table 3) and $a_1m_1s_0o_1$ was the best treatment containing the highest amount of Zn in plants shoot (Table 7). Comparing $a_1m_1s_0o_1$ with $a_1m_0s_1o_1$ indicates that co application of Azospirillum and Mycorrhiza and manure is more effective than co application of Azospirillum and Streptomyces and manure, on Zn absorption. In all cases, treatments with manure had better Zn absorption than the same treatments without manure.

Cupper (Cu): According to analysis of variances (Table 3), application of Azospirillum had significant effect on Cu absorption at $p \leq 0.01$. As Azospirillum has important role in plants root development, it increased plants Cu content 21% (Table 4). Mycorrhiza had significant effect on this trait at $p \leq 0.01$ and increased it by11%. Lambert and Weidensaul (1991) observed an increase in Cu content in soybean plants inoculated with Mycorrhiza [31]. Marschner and Dell (1994) also reported that Mycorrhiza hyphae have role in higher Cu absorption in Mycorrhizal plants [24]. The interaction between Azospirillum × Mycorrhiza showed significant effect on Cu absorption at $p \leq 0.01$ (Table 5) and the highest Cu absorption happened in a_1m_1.

Streptomyces had no effect on Cu absorption but interaction of Azospirillum × Streptomyces significantly affected this trait at $p \leq 0.01$ and a_1s_1 was the best treatment with the highest Cu content in plants shoot. Comparing a_1s_0 with a_0s_1 indicated that application of Azospirillum is more effective on Cu absorption than application of Streptomyces. Interaction of Mycorrhiza × Streptomyces showed significant effect at $p \leq 0.01$ and mean comparison indicated the highest Cu absorption in m_1s_0. Low Cu absorption in m_1s_1 is because of adverse impact of Streptomyces on activity of Mycorrhiza.

Application of manure increased Cu absorption 24% (Table 4) so had significant effect on the trait at $p \leq 0.01$ (Table 3). Manure contains high amount of nutrients and by decomposition release them into soil so plants root absorb more nutrients. The interaction between Azospirillum × manure significantly affected Cu absorption at $p \leq 0.01$ and mean comparison showed the highest Cu content in a_1m_1. Interaction of Mycorrhiza × manure was also significant on this trait at $p \leq 0.01$ and the most Cu absorption happened in m_1o_1 (4.38 mg/kg). Comparing m_0o_1 with m_1o_0 indicates that effect of manure on Cu absorption is greater that the effect of Mycorrhiza. Co application of Streptomyces and manure had significant effect on Cu absorption at $p \leq 0.01$ and both s_1o_1 and s_0o_1 had the highest content of Cu in plants shoot.

All threefold and fourfold interactions showed significant effect on Cu absorption at $p \leq 0.01$ and the highest absorption happened in $a_1m_1s_0o_1$.

CONCLUSION

Totally, results of this experiment indicated that main effect of Azospirillum, Mycorrhiza and manure was significant on absorption of four measured micronutrients but the effect of Streptomyces was non-significant. In most cases, application of Streptomyces damaged Mycorrhiza fungi and decreased their activity so it is better to use Streptomyces only as biofungicide for seeds. Co application of Azospirillum and Mycorrhiza had synergistic effect on measured traits. Finally, effects of fourfold interaction of Azospirillum × Mycorrhiza × Streptomyces × manure was not much better than threefold interactions because of the adverse impact of Streptomyces on Mycorrhiza.

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