Absorption efficiency of N, P, K through triple inoculation of wheat (*Triticum aestivum* L.) by *Azospirillum brasilense*, *Streptomyces* sp., *Glomus intraradices* and manure application

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Abstract This study examined the effects of different biofertilizers and manure on the absorption and absorption efficiency of macronutrients by wheat in Alborz Province, Iran. The experimental design was factorial in the form of a randomized complete block design with three replications. The four factors of the experiment were *Azospirillum* (with 600 g/ha and without), mycorrhiza (with 1 kg/ha and without), *Streptomyces* (with 0.5 kg/ha and without) and manure (with 30 t/ha and without). To measure N, P and K, plants were harvested one time when grains were at dough stage and another time at plant maturity, oven dried, grinded and analyzed. *Azospirillum*, mycorrhiza and manure had a significant effect ($p \leq 0.01$) on most of the measured traits, but the effect of *Streptomyces* was not significant. The two-fold and three-fold interactions significantly affected the absorption and absorption efficiency of the three macronutrients. In most cases, four-fold inter-

actions yielded the highest values. Overall, the experimental results indicate that biofertilizers and manure can naturally meet most of the plant's nutritional requirements, improve the efficiency of nutrient absorption and replace part of chemical fertilizers in sustainable agricultural production systems.

Keywords *Azospirillum* · Manure · Mycorrhiza · *Streptomyces* · Macroelements

Introduction

A key factor with a major key role in sustainable agriculture is biofertilizers. Biofertilizers consist of beneficial microorganisms that provide nutrients to plants through symbiosis, association, and other mechanisms (Kuchaki et al. 1997; Siddiqui et al. 2008). Although these microorganisms naturally exist in most soils, their quantity and quality is usually insufficient, requiring inoculation. Biofertilizers are of different types and are categorized according to the component microorganisms: nitrogen-fixing bacteria (diazotrophs), mycorrhiza fungi, phosphate-solubilizing microorganisms, plant growth-promoting rhizobacteria (PGPR), etc. (Siddiqui et al. 2008; Sprent and Sprent 1990; Stacey et al. 1992).

Azospirillum is a nitrogen-fixing bacterium that associates with cereals. In this association, the bacterium fixes air nitrogen and produces plant growth promoters (Bashan and Levanony 1990; Dobereinea 1989; Elmerich 1986; Patriquin et al. 1983). Although the genus *Azospirillum* has five species, but most strains are from *A. brasilense*, which mostly associate with C3 cereals, and from *A. lipoferum*, which mostly associate with C4 cereals

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(Bashan and Levanony 1990; Bhattarai and Hess 1993; Elmerich et al. 1992).

Inoculating plants with *Azospirillum* significantly affects their growth, but the effect of the association on grain yield can be either increasing or non significant (Crossman and Hill 1987; Patriquin et al. 1983; Fallik and Okon 1996). This association can also increase total dry weight, total N in plant shoots, total number of tillers and fertile spikes, grain weight, leaf size, germination rate, root system development, nutrient uptake and phytohormone production. Mertens and Hess (1984) observed that the root/shoot ratio increased in wheat when inoculated with *Azospirillum* because of phytohormone production by the bacterium. Plants shoot growth may also increase by *Azospirillum* association because of more absorption of NO_3^- , NH_4^+ , PO_4^{3-} , K^+ and Fe^{2+} (Jain and Patriquin 1985; Lin et al. 1983; Marty and Ladha 1987). The association of *A. brasilense* with wheat and sorghum enhances total shoot and root weight, plant height, leaf size and total N (Kapulnik et al. 1981).

Mycorrhiza, which is a symbiotic fungus, has been under study for more than a century. In mycorrhizal symbiosis with plants, the fungus gains energy from carbon sources from the plant and in return, supplies many nutrients such as phosphorus, molybdenum, copper and iron to the plant (Allen 1991; Lambert and Weidensaul 1991; Miller et al. 1994). Mycorrhiza mycelium is connected to the plant root. Here, it serves as an additional and supplementary absorption organ for the root system, helping plants to use a greater volume of soil as a source of nutrients and water (George et al. 1994; Lin et al. 1983; Powell and Baggar 1986).

Although plants need high amounts of phosphate, its availability in soil for plants is low because mineral phosphate ions strongly bond with soil colloids and are also present in ferro-phosphate or aluminum phosphate form, making them immobile (Mostajeran and Zuie 1999). Mycorrhiza has a great effect on phosphorus absorption in such soils. Tarafdar and Marschner (1994) determined that mycorrhizal symbiosis with wheat increases the phosphorus absorption by the plant; the amount of absorption depends on the soil type, mycelium density and length, phosphatase activity and soil phosphorus content. Moreover, the formation and density of mycorrhiza colonization on plant roots are negatively correlated with the amount of phosphorus in the soil and plant (George et al. 1994; Plenchette et al. 1983).

The content of nitrogen and other elements in plants is reportedly enhanced in symbiosis with mycorrhiza (Mostajeran and Zuie 1999). Moreover, mycorrhiza helps plants to better tolerate drought and other stresses and reduces yield loss to some extent. Mycorrhiza mycelium can penetrate into pores and cracks that are too small for plant roots, helping plants to absorb more water. Moreover,

mycorrhiza in symbiosis with plants produces growth-promoting hormones such as auxin and cytokinin. Although mycorrhiza fungi exist in most soils, application of inoculants of highly active strains can maximize the efficacy of the symbiosis system (Daei et al. 2009; Marschner and Dell 1994; Siddiqui et al. 2008).

Streptomyces is an important beneficial soil-borne actinomycete that is categorized as a plant growth-promoting rhizobacteria (PGPR) and has many effects on plant growth. Colonies of this microorganism act like a seed coat and protect seeds against diseases. Treating corn seeds with S57 increased the germination rate from 80 % to 92 % (Sarachi et al. 1991; Sardi et al. 1992). Sardi et al. (1992) also observed that *Streptomyces* can protect wheat against fusarium and increases the crop yield. It also enhances the yield and sugar content in sugar beet. Different mechanisms are responsible for the effect of PGPRs on plant growth: a) hormone production by the microorganisms, b) improved absorption and transition of some nutrients and c) control of plant pathogens.

Clearly, the application of biofertilizers is the most natural and suitable way to keep soil biosystems alive and active. This calls for adding these microorganisms especially into soils with low organic matter and low microbial activity. In such soils they provide nutrients to plants, maintain soil biodiversity and bioactivity, and improve the quality of the soil and the environment. Many experiments have been conducted on soil organic matter and its role in soil fertility and sustainable agriculture systems. As such organic matter is source of energy for soil microorganisms, its presence will increase soil microbial activity. Organic matter improves the quality of clay soils by increasing their aeration and infiltrability; in sandy soils it enhances the water preservation capacity (Miller and Gardiner 1998; Violante et al. 2002). The present experiment was conducted to evaluate the effects of different biofertilizers and manure on the absorption and absorption efficiency of macronutrients in a sustainable wheat production system.

Materials and methods

The experiment was conducted in Alborz Province, Iran (51° 6' E, 35° 59' N and 1,300 m above sea level), in an area characterized by a semiarid climate with dry warm summers and humid cold winters. The average annual precipitation here is 242 mm, most of which falls from late autumn to early spring. The mean annual maximum air temperature was 26.1 °C (in July) and the minimum was 1 °C (in January). The soil type at the test site was clay loam (sand: 36 %, silt: 34, clay: 30 %). Other soil properties are listed in Table 1.

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 Percent
280	9.2	80	0.65	7.6	1.25	39

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

Azospirillum: with 600 g/ha (a_1) and without (a_0). The required amount of *Azospirillum* was weighed for each treatment and inoculated with seeds immediately before sowing. The strain was from *Azospirillum brasilense* in a peat carrier and contained 10⁸ cfu/g *Azospirillum*.

Mycorrhiza: with 1 kg/ha (m_1) and without (m_0), weighed for each treatment and inoculated with seeds immediately before sowing. The strain was from *Glomus intraradices*. The carrier was clay and contained 10⁵ spores per gram.

Streptomyces: with 0.5 kg/ha (s_1) and without (s_0). The strain was from *Streptomyces* sp. S57 in a clay carrier. It contained 10⁸ cfu/g *Streptomyces* and was applied as above for *Azospirillum* and mycorrhiza. *Streptomyces* sp. S57 refers to the strain which already selected among other different strains of *Streptomyces* with more efficiency.

Manure: with 30 t/ha (o_1) and without (o_0). The manure was fully decomposed cow dung stored for 1 year; its properties are listed in Table 2.

All of measured micro and macro elements were evaluated by the method of Mehlich 1978.

After preparing the field and before sowing wheat, N and P fertilizers were broadcasted on the soil surface and incorporated into soil by harrowing. According to the

results of an experiment conducted by the authors in the previous year, 100 kg nitrogen and 60 kg phosphorus per hectare (that were shown to have the least adverse effect on microorganism activity) were applied. The plot sites were then set in field, and the manure was weighed and applied in the required plots based on 30 t/ha and incorporated into the soil. Finally, on 11 Nov. 2008, 200 kg/ha wheat seed (*Triticum aestivum* L. var: Mahdavi) was weighed equally for all plots and inoculated with biofertilizers in separate containers. Seeds were broadcasted on the soil surface and, after that, a furrower was used. Each plot contained four rows, each 7 m long and 50 cm wide. An interspace of 1.5 m between plots and 4 m between replications were left uncultivated to prevent interference and movement of microorganisms.

To measure macronutrient absorption (N, P, K) by the plants, five plants were harvested from each plot when grains were at dough stage and dried at 70 °C in an oven for 72 h. Samples were then grinded and analyzed. On 22 June, when plants were fully matured, 4 m of the two middle rows of each plot was harvested and grains were detached from straw. Then, 50 g of seeds of each plot was dried at 70 °C in an oven for 48 h, grinded and analyzed to determine the percent of N, P and K in grains.

Finally, the macronutrient absorption efficiency of the plants and grains was determined based on the results of samples analysis. The following equations were used:

$$\text{Absorption efficiency of a nutrient in plant/grain} = \frac{\text{The nutrient content in plant/grain}}{\text{The nutrient content in soil before cultivation} + \text{Amount of the nutrient added to soil}}$$

$$\text{The nutrient content in plant} = \text{plant total dry weight before leaves dry} \times \% \text{ of the nutrient in whole plant before leaves dry}$$

Results and discussion

Absorption and absorption efficiency of nitrogen (N) in plants Analysis of variances showed significant effect of *Azospirillum* on plant N absorption ($p \leq 0.01$; Table 3) and

comparison of the means showed that applying *Azospirillum* increased plant N absorption by 17 % (Table 4). Increased absorption of NO_3^- and NH_4^+ is a result of *Azospirillum*. All strains of *Azospirillum* can biologically fix nitrogen and enhance the N content in plants and grains

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

Table 3 Analysis of variances of absorption and efficiency of absorption of N, P and K in plants

SOV	df	Mean squares (MS)					
		Absorption			Absorption efficiency		
		N	P	K	N	P	K
Rep	2	**	**	**	*	**	ns
<i>Azospirillum</i> (a)	1	**	**	**	**	**	**
Mycorrhiza (m)	1	**	**	**	**	**	**
<i>Azospirillum</i> × mycorrhiza (am)	1	**	**	**	**	**	**
<i>Streptomyces</i> (s)	1	ns	ns	ns	ns	ns	ns
<i>Azospirillum</i> × <i>Streptomyces</i> (as)	1	**	**	**	ns	ns	ns
Mycorrhiza × <i>Streptomyces</i> (ms)	1	**	**	**	**	**	**
<i>Azospirillum</i> × mycorrhiza × <i>Streptomyces</i> (ams)	1	**	**	**	**	**	**
Manure (o)	1	**	**	**	**	**	**
<i>Azospirillum</i> × manure (ao)	1	**	**	**	**	**	**
Mycorrhiza × manure (mo)	1	**	**	**	**	**	**
<i>Azospirillum</i> × mycorrhiza × manure (amo)	1	**	**	**	**	**	**
<i>Streptomyces</i> × manure (so)	1	**	**	**	ns	ns	ns
<i>Azospirillum</i> × <i>Streptomyces</i> × manure (aso)	1	**	**	**	**	**	*
Mycorrhiza × <i>Streptomyces</i> × manure (mso)	1	**	**	**	**	**	**
<i>Azospirillum</i> × mycorrhiza × <i>Streptomyces</i> × manure (amso)	1	**	**	**	**	**	**
Error	30	0.023	0.45	0.619	20.636	1708.51	23475.58
CV (%)	—	0.96	1.76	2.28	9.1	9.15	8.95

ns nonsignificant

*Significant at $p \leq 0.05$ **Significant at $p \leq 0.01$

(Bashan and Levanony 1990; Jain and Patriquin 1985; Lin et al. 1983; Marty and Ladha 1987). Applying mycorrhiza also had a significant ($p \leq 0.01$) effect on this trait, increasing it by 11 %. Other studies have shown that mycorrhizal symbiosis improves plant N absorption and

Table 4 The mean comparisons for effects of *Azospirillum* (a₀, without; a₁, with), mycorrhiza (m₀, without; m₁, with), *Streptomyces* (s₀, without; s₁, with) and manure (o₀, without; o₁, with) on absorption and absorption efficiency of N, P and K in plants

Treatments	Absorption (%)			Absorption efficiency (g/kg)		
	N	P	K	N	P	K
a ₀	1.43	0.35	3.07	45.08	414.44	309.51
a ₁	1.74	0.41	3.84	54.73	489.20	384.23
m ₀	1.49	0.36	3.28	47.79	429.88	333.93
m ₁	1.68	0.41	3.64	52.03	473.76	359.80
s ₀	1.58	0.38	3.44	50.75	459.02	351.66
s ₁	1.59	0.38	3.48	49.06	444.62	342.07
o ₀	1.36	0.34	3.05	42.56	398.59	304.04
o ₁	1.81	0.42	3.87	57.25	505.05	389.70

external hyphae of mycorrhiza can provide 25 % of plant's N requirement (George et al. 1994; Powell and Baggara 1986). The increased amount of N in mycorrhizal plants is a result of higher phosphorus absorption. A higher P content in mycorrhizal plants stimulates the synthesis of nitrate reductase. Mycorrhiza also probably has a direct role in reducing nitrate (Mostajeran and Zuie 1999). The interaction of *Azospirillum* × mycorrhiza significantly affected plants N absorption ($p \leq 0.01$; Table 3). Comparing the means (Table 5) shows the highest N content in a₁m₁, with 1.77 % N in plants. The synergistic relation between *Azospirillum* and mycorrhiza improves plant growth and enables us to use these biofertilizers instead of phosphorus and nitrogen fertilizers, without reduction in yield (Triplett 1996).

Streptomyces had no effect on plant N absorption (Tables 3 and 4), but interactions of *Streptomyces* × *Azospirillum* and *Streptomyces* × mycorrhiza significantly affected this trait ($p \leq 0.01$; Table 5). Among interactions of mycorrhiza × *Streptomyces*, m₁s₀ had the highest N content (1.76 %). In m₁s₁, mycorrhiza did not grow well because of an adverse effect of *Streptomyces*, lowering absorption.

Table 5 The mean comparisons for effects of two-fold 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 absorption and absorption efficiency of N, P and K in plants

Treatments	Absorption (%)			Absorption efficiency (g/kg)		
	N	P	K	N	P	K
a_0m_0	1.27d	0.31d	2.75d	39.98c	365.04b	275.44c
a_0m_1	1.58c	0.39c	3.70c	50.18b	463.86a	343.57b
a_1m_0	1.71b	0.41b	3.80b	55.59a	494.71a	392.42a
a_1m_1	1.77a	0.42a	3.88a	53.87ab	483.69a	376.03a
a_0s_0	1.41c	0.34c	3.02c	45.61b	414.57b	312.17b
a_0s_1	1.45b	0.36b	3.13b	44.55b	414.31b	306.84b
a_1s_0	1.75a	0.42a	3.85a	55.89a	503.47a	391.15a
a_1s_1	1.73a	0.44a	3.82a	53.57a	474.93a	377.31a
m_0s_0	1.40c	0.33c	3.09c	45.48c	405.10c	319.12c
m_0s_1	1.59b	0.38b	3.47b	50.09b	454.65b	348.73b
m_1s_0	1.76a	0.43a	3.79a	56.02a	512.94a	384.20a
m_1s_1	1.59b	0.38b	3.48b	48.03bc	434.59bc	335.41bc
a_0o_0	1.11d	0.28d	2.39c	34.08c	326.52c	234.69b
a_0o_1	1.75b	0.42b	3.76b	56.08a	502.36ab	384.32a
a_1o_0	1.61c	0.39c	3.70b	51.05b	470.66b	373.39a
a_1o_1	1.87a	0.43a	3.98a	58.41a	507.74a	395.07a
m_0o_0	1.21d	0.30d	2.73d	37.91c	351.90c	273.03c
m_0o_1	1.78b	0.42b	3.83b	57.67a	507.85a	394.83a
m_1o_0	1.51c	0.38c	3.37c	47.22b	445.29b	335.05b
m_1o_1	1.84a	0.43a	3.91a	56.83a	502.24a	384.56a
s_0o_0	1.34d	0.33d	2.99c	42.94b	398.67b	305.75b
s_0o_1	1.83a	0.43a	3.89a	58.55a	519.37a	397.57a
s_1o_0	1.38c	0.35c	3.11b	42.18b	398.51b	302.32b
s_1o_1	1.79b	0.42b	3.85a	55.94a	490.72a	381.82a

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

Applying manure had a significant effect on N absorption (Table 3), increasing it by 24 %. Manure improves the physico-chemical conditions of the rhizosphere and helps plants to absorb more water and nutrients and grow better. Two-fold interactions of manure with all other factors significantly affected N absorption by plants, and in each group, a_1o_1 , m_1o_1 and s_0o_1 were the best treatments (Table 5).

Analysis of variances showed a significant effect of all three-fold and four-fold interactions on plant N absorption at $p \leq 0.01$ (Table 3). Comparison of means showed the highest N content in the three-fold interaction in $a_1m_1s_0$, $a_1m_1o_1$, $a_1s_0o_1$ and $m_1s_0o_1$ (Table 6) and in the four-fold interaction in $a_1m_1s_0o_1$ with 1.93 % (Table 7). Manure application in all treatments considerably increased plant N absorption.

The nitrogen absorption efficiency was significantly affected by applying *Azospirillum*, mycorrhiza and manure ($p \leq 0.01$; Table 3). Mean comparisons showed that *azospirillum*, mycorrhiza and manure increased this trait by 17 %, 8 % and 25 %, respectively (Table 4). *Azospirillum* improves plant root development and provides an additional absorptive surface for the absorption of water and nutrients; it also enhances plant photosynthesis, growth and total dry weight. As total nitrogen in plants is related to total dry

weight, we conclude that the total nitrogen content in the plant, and consequently the efficiency of N absorption, has increased. Mycorrhiza also increases the absorptive surface of plant root through myceliums and helps plants to take up more water and nutrients. Accordingly, more nutrient absorption means a higher nutrient content in plants and a better absorption efficiency. Another important factor is manure, which generally improves the physico-chemical conditions of the rhizosphere and provides many nutrients to plants through decomposition.

Application of *Streptomyces*, and its two-fold interactions with *Azospirillum* and manure, did not significantly affect N absorption efficiency (Table 3). Other two-fold, three-fold and four-fold interactions significantly affected this efficiency ($p \leq 0.01$; Table 3). The highest efficiency was recorded in $a_1m_0s_1o_1$ (61.02 g/kg), with no significant difference from $a_1m_1s_1o_1$ (Tables 6 and 7).

Absorption and absorption efficiency of phosphorus (P) in plants Analysis of variance shows that applying *Azospirillum* significantly affected plant P absorption (Table 3) and mean comparison showed a 14 % increase in plant P content as a result of *Azospirillum* association (Table 4). *Azospirillum* enhances the root's absorptive surface, helping

Table 6 The mean comparisons for effects of three-fold interactions of *Azospirillum* (a₀, without; a₁, with), mycorrhiza (m₀, without; m₁, with), *Streptomyces* (s₀, without; s₁, with) and manure (o₀, without; o₁, with) on absorption and absorption efficiency of N, P and K in plants

Treatments	Absorption (%)			Absorption efficiency (g/kg)		
	N	P	K	N	P	K
a ₀ m ₀ s ₀	1.11e	0.26e	2.38e	35.55c	315.04d	244.62c
a ₀ m ₀ s ₁	1.44d	0.36d	3.12d	44.41b	415.04c	306.26b
a ₀ m ₁ s ₀	1.71bc	0.42b	3.66c	55.67a	514.09a	379.73a
a ₀ m ₁ s ₁	1.45d	0.36d	3.14d	44.70b	413.59c	307.42b
a ₁ m ₀ s ₀	1.70c	0.41c	3.79b	55.41a	495.17ab	393.62a
a ₁ m ₀ s ₁	1.73b	0.41c	3.81b	55.77a	494.26ab	391.21a
a ₁ m ₁ s ₀	1.81a	0.44a	3.92a	56.36a	511.78a	388.67a
a ₁ m ₁ s ₁	1.73b	0.41c	3.38ab	51.37a	455.59bc	363.40a
a ₀ m ₀ o ₀	0.83h	0.21f	1.77f	24.45e	231.88c	166.23c
a ₀ m ₀ o ₁	1.71d	0.41c	3.73cd	55.51abc	498.20a	384.64a
a ₀ m ₁ o ₀	1.38g	0.35e	3.00e	43.71d	421.17b	303.14b
a ₀ m ₁ o ₁	1.79c	0.42b	3.80c	56.66ab	506.51a	384.00a
a ₁ m ₀ o ₀	1.58f	0.39d	3.68d	51.36bc	471.92ab	379.82a
a ₁ m ₀ o ₁	1.85b	0.42b	3.93b	59.83a	517.51a	405.01a
a ₁ m ₁ o ₀	1.64e	0.40c	3.73cd	50.73c	469.41ab	366.95a
a ₁ m ₁ o ₁	1.90a	0.44a	4.03a	57.00ab	497.97a	385.12a
a ₀ s ₀ o ₀	1.04h	0.26f	2.26d	32.23c	300.34d	222.85c
a ₀ s ₀ o ₁	1.77c	0.42b	3.79b	58.99a	528.80a	401.50a
a ₀ s ₁ o ₀	1.17g	0.31e	2.52c	35.93c	352.71c	246.53c
a ₀ s ₁ o ₁	1.72d	0.41c	3.74b	53.17ab	475.92ab	367.15ab
a ₁ s ₀ o ₀	1.63e	0.40c	3.71b	53.66ab	497.00a	388.65ab
a ₁ s ₀ o ₁	1.88a	0.44a	4.00a	58.11a	509.95a	393.64ab
a ₁ s ₁ o ₀	1.59f	0.39d	3.69b	48.43b	444.32b	358.12b
a ₁ s ₁ o ₁	1.86b	0.43b	3.59a	58.71a	505.53a	396.49ab
m ₀ s ₀ o ₀	1.04f	0.25e	2.35e	31.91c	289.65c	232.22c
m ₀ s ₀ o ₁	1.77c	0.41bc	3.82b	59.06a	520.56a	406.02a
m ₀ s ₁ o ₀	1.38e	0.35d	3.10d	43.90b	414.15b	313.83b
m ₀ s ₁ o ₁	1.79b	0.42b	3.84b	56.27a	495.15a	383.64a
m ₁ s ₀ o ₀	1.64d	0.41c	3.62c	53.98a	507.69a	379.27a
m ₁ s ₀ o ₁	1.89a	0.45a	3.97a	58.08a	518.18a	389.13a
m ₁ s ₁ o ₀	1.38e	0.35d	3.11d	40.46b	382.88b	290.82b
m ₁ s ₁ o ₁	1.80b	0.42bc	3.85b	55.60a	486.30a	380.00a

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

plants to take up more PO_4^{-2} (Bashan and Levany 1990; Jain and Patriquin 1985; Lin et al. 1983). The effect of mycorrhiza on P absorption was significant ($p \leq 0.01$) and increased it by 21 %. Mycorrhiza makes more distant soil phosphorus available to plant roots through mycelia. After complete growth of plant roots, mycorrhiza mycelia meet large amount of plant's P requirement from soil volumes that are further away from the roots. The phosphorus content in plants was significantly affected by the interaction of *Azospirillum* $\times$ mycorrhiza ($p \leq 0.01$; Table 3), and the highest P content (0.42 %) was recorded in a₁m₁. The synergistic relation of *Azospirillum* and mycorrhiza is responsible of high P absorption here.

Streptomyces had no significant effect on the amount of P in plant shoots (Table 3), but the interaction of *Streptomyces* $\times$

mycorrhiza significantly affected this trait and a₁s₀ and a₁s₁ were the best treatments (Table 5). The interaction of mycorrhiza $\times$ *Streptomyces* was also significant at $p \leq 0.01$. The phosphorus uptake in m₁s₁ was not high because of an adverse effect on *Streptomyces* on mycorrhiza activity.

Manure and its two-fold interactions with *Azospirillum*, mycorrhiza and *Streptomyces* significantly affected P content ($p \leq 0.01$; Table 3). Manure releases many nutrients into the soil through decomposition and helps plants to grow more and take up more nutrients. In two-fold interactions of manure with other factors, a₁o₁ (0.43 %), m₁o₁ (0.43 %) and s₀o₁ (0.43 %) were the best treatments (Table 5).

Analysis of variance showed that plant P content was significantly affected by all three-fold and four-fold interactions. Comparisons of the means showed the highest

Table 7 The mean comparisons for effects of four-fold 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 absorption and absorption efficiency of N, P and K in plants

Treatments	Absorption (%)			Absorption efficiency (g/kg)		
	N	P	K	N	P	K
$a_0m_0s_0o_0$	0.51h	0.11i	1.04g	11.62f	97.01e	75.87d
$a_0m_0s_0o_1$	1.70d	0.41e	3.72cd	59.48ab	533.07a	413.36a
$a_0m_0s_1o_0$	1.16g	0.30h	2.50f	37.28e	366.74cd	256.59c
$a_0m_0s_1o_1$	1.72d	0.41de	3.74cd	51.53bcd	463.33ab	355.93ab
$a_0m_1s_0o_0$	1.58ef	0.40ef	3.47e	52.83abcd	503.66ab	369.82ab
$a_0m_1s_0o_1$	1.85bc	0.44b	3.86bc	58.51abc	524.52a	389.64ab
$a_0m_1s_1o_0$	1.18g	0.31h	2.53f	34.59e	338.67d	236.47c
$a_0m_1s_1o_1$	1.72d	0.41de	3.74cd	54.81abcd	488.51ab	378.37ab
$a_1m_0s_0o_0$	1.57f	0.39g	3.66d	52.20abcd	482.28ab	388.57ab
$a_1m_0s_0o_1$	1.84c	0.42c	3.92b	58.63abc	508.05a	398.67ab
$a_1m_0s_1o_0$	1.60e	0.39fg	3.69d	50.53cd	461.55ab	371.07ab
$a_1m_0s_1o_1$	1.86bc	0.43c	3.94b	61.02a	526.97a	411.35a
$a_1m_1s_0o_0$	1.70d	0.42cd	3.76cd	55.13abc	511.72a	388.72ab
$a_1m_1s_0o_1$	1.93a	0.46a	4.08a	57.60abc	511.85a	388.61ab
$a_1m_1s_1o_0$	1.58ef	0.39g	3.69d	46.34d	427.10bc	345.17b
$a_1m_1s_1o_1$	1.87b	0.43c	3.97ab	56.40abc	484.09ab	381.63ab

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

P content in $a_1m_1s_0$, $a_1m_1o_1$, $a_1s_0o_1$ and $m_1s_0o_1$ (Table 6). In four-fold interactions, $a_1m_1s_0o_1$ had the highest P content (0.46 %) and was the best treatment (Table 7). The effective impact of manure is clear in those treatments in which manure was applied.

Among the four factors of the experiment, only *Streptomyces* did not improve the phosphorus absorption efficiency in plants. In contrast, the effect of *azospirillum*, mycorrhiza and manure was significant at $p \leq 0.01$ (Table 3). Mean comparison indicated a 15 %, 9 % and 21 % increase in plant P absorption efficiency as a result of applying *Azospirillum*, mycorrhiza and manure, respectively (Table 4). *Azospirillum* and mycorrhiza increase the absorptive surface of plant roots, enabling plants to absorb more nutrients and water. This enhances plant growth and total dry weight. Clearly, the total P content in plants is related to total dry weight, and a higher amount of P in plants therefore means a more efficient P absorption. Manure also plays an important role in the availability of soil nutrients to plants and helps the roots to absorb more nutrients.

Although the application of *Streptomyces* and its two-fold interaction with *Azospirillum* and manure had no effect on this trait, the interaction of *Streptomyces* $\times$ mycorrhiza did significantly affect the efficiency of P absorption ($p \leq 0.01$); the best treatment was m_1s_0 with 512.94 g/kg phosphorus (Table 5).

The effect of all three-fold and four-fold interactions was significant on P absorption efficiency ($p \leq 0.01$; Table 3). Comparing the means showed the best efficiency in the four-fold interaction in $a_1m_1s_0o_1$, $a_1m_1s_0o_0$,

$a_1m_0s_1o_1$, $a_0m_0s_0o_1$ and $a_0m_1s_0o_1$ (Table 7). Manure had a considerable positive impact in all treatments in which it was applied.

Absorption and absorption efficiency of potassium (K) in plants Analysis of variance showed that applying *Azospirillum* significantly affected plant K absorption ($p \leq 0.01$), increasing it by 20 %. *Azospirillum* increases the absorptive surface of roots, and plants absorb more K^+ (Bashan and Levanony 1990; Jain and Patriquin 1985; Lin et al. 1983). Applying mycorrhiza significantly increased K absorption (about 10 %; Table 4). K absorption is a debated issue: some researchers state that mycorrhiza helps plants to absorb more K, while others deny such an effect (Mostajeran and Zuie 1999; Tarafdar and Marschner 1994). In our experiments, the interaction of *Azospirillum* $\times$ mycorrhiza had a significant effect on the K content in plants, and mean comparison showed that a_1m_1 had the highest value (3.88 %). Comparing a_0m_1 and a_1m_0 reveals that *Azospirillum* is more effective for K absorption than mycorrhiza.

Based on analysis of variance, *Streptomyces* had no effect on plant K content (Table 3), but the interaction of *Azospirillum* $\times$ *Streptomyces* and mycorrhiza $\times$ *streptomyces* significantly affected the trait. In the interaction *Azospirillum* $\times$ *Streptomyces*, a_1s_1 and a_1s_0 were the best treatments with 3.82 % and 3.85 %, respectively (Table 5). m_1s_0 was the best treatment in the interaction mycorrhiza $\times$ *Streptomyces*, with 3.79 % K in the plant shoot. The reduced plant K content in m_1s_1 (3.48 %) reflects the inhibitory effect of *Streptomyces* on mycorrhiza (Table 5).

Table 8 Analysis of variances of absorption and efficiency of absorption of N, P and K in grains

SOV	df	Mean Squares (MS)					
		Absorption			Absorption efficiency		
		N	P	K	N	P	K
Rep	2	ns	**	**	ns	ns	ns
<i>Azospirillum</i> (a)	1	*	*	**	**	*	**
Mycorrhiza (m)	1	ns	*	*	**	**	*
<i>Azospirillum</i> × mycorrhiza (am)	1	ns	**	ns	**	**	ns
<i>Streptomyces</i> (s)	1	ns	ns	ns	ns	ns	ns
<i>Azospirillum</i> × <i>Streptomyces</i> (as)	1	ns	ns	ns	**	**	**
Mycorrhiza × <i>Streptomyces</i> (ms)	1	ns	ns	**	*	**	**
<i>Azospirillum</i> × mycorrhiza × <i>Streptomyces</i> (ams)	1	ns	ns	**	ns	ns	ns
Manure (o)	1	ns	ns	ns	**	*	**
<i>Azospirillum</i> × manure (ao)	1	ns	ns	**	**	ns	**
Mycorrhiza × manure (mo)	1	ns	*	**	ns	**	**
<i>Azospirillum</i> × mycorrhiza × manure (amo)	1	ns	*	ns	*	**	ns
<i>Streptomyces</i> × manure (so)	1	**	ns	ns	**	ns	*
<i>Azospirillum</i> × <i>Streptomyces</i> × manure (aso)	1	**	ns	**	**	**	**
Mycorrhiza × <i>Streptomyces</i> × manure (mso)	1	ns	ns	*	**	**	**
<i>Azospirillum</i> × mycorrhiza × <i>Streptomyces</i> × manure (amso)	1	ns	*	ns	**	**	**
Error	30	1.004	16.117	1.288	7.286	7.854	2.089
CV (%)	—	3.89	6.00	2.49	8.57	9.08	8.90

ns nonsignificant

*Significant at $p \leq 0.05$ **Significant at $p \leq 0.01$

Applying manure increased plant K content by 21 % (Table 4). Manure affects the physico-chemical conditions of the rhizosphere and improves plant growth and nutrient uptake. The two-fold interaction of manure with *azospirillum*, mycorrhiza and *Streptomyces* had a significant effect

Table 9 The mean comparisons for effects of *Azospirillum* (a₀, without; a₁, with), mycorrhiza (m₀, without; m₁, with), *Streptomyces* (s₀, without; s₁, with) and manure (o₀, without; o₁, with) on absorption and absorption efficiency of N, P and K in grains

Treatments	Absorption (%)			Absorption efficiency (g/kg)		
	N	P	K	N	P	K
a ₀	2.53	0.66	0.45	30.42	29.75	17.17
a ₁	2.61	0.68	0.46	32.60	31.96	18.36
m ₀	2.57	0.65	0.45	30.97	29.66	17.25
m ₁	2.57	0.68	0.46	32.06	32.05	18.28
s ₀	2.56	0.66	0.45	32.03	31.14	17.80
s ₁	2.60	0.67	0.46	31.00	30.57	17.72
o ₀	2.54	0.66	0.45	29.97	29.80	17.14
o ₁	2.60	0.67	0.46	33.06	32.06	18.39

on K absorption ($p \leq 0.01$; Table 3), and a₁o₁ (3.98 %), m₁o₁ (3.91 %) and s₀o₁ (3.89 %) were the best treatments (Table 5).

All three-fold and four-fold interactions of *Azospirillum*, mycorrhiza, *Streptomyces* and manure significantly improved plant K content ($p \leq 0.01$; Table 3). The highest value was recorded in a₁m₁s₀o₁ (4.08 %), although had no significant difference from a₁m₁s₁o₁ (3.97 %) (Tables 6 and 7).

Azospirillum, mycorrhiza and manure significantly increased the efficiency of K absorption ($p \leq 0.01$; Table 3) by 19 %, 7 % and 21 %, respectively (Table 4). Mycorrhiza mycelia and hyphae help plants to use a greater volume of soil as source of water and nutrients, enabling plants to absorb more nutrients. This supports previous studies (Tarafdar and Marschner 1994). Manure makes the soil condition suitable for roots to grow and absorb nutrients; it also releases many nutrients into the soil. Accordingly, plants grow faster, take up more nutrients, and the efficiency of nutrient absorption is increased.

Streptomyces and its two-fold interaction with *Azospirillum* and manure did not affect this trait. In contrast, *Streptomyces* × mycorrhiza had a significant effect ($p \leq 0.01$), and m₁s₀ was the best treatment (384.20 g/kg potassium in shoots; Table 5).

Table 10 The mean comparisons for effects of two-fold interactions of *Azospirillum* (a₀, without; a₁, with), mycorrhiza (m₀, without; m₁, with), *Streptomyces* (s₀, without; s₁, with) and manure (o₀, without; o₁, with) on absorption and absorption efficiency of N, P and K in grains

Treatments	Absorption (%)			Absorption efficiency (g/kg)		
	N	P	K	N	P	K
a ₀ m ₀	2.52b	0.62b	0.44b	28.69b	26.51b	16.24b
a ₀ m ₁	2.55ab	0.69a	0.45b	32.15a	33.00a	18.10a
a ₁ m ₀	2.60ab	0.67a	0.45b	33.24a	32.81a	18.26a
a ₁ m ₁	2.63a	0.70a	0.47a	31.97a	31.11a	18.46a
a ₀ s ₀	2.50b	0.64b	0.44c	29.33b	27.91c	16.15c
a ₀ s ₁	2.57ab	0.66ab	0.45bc	31.51b	31.61b	18.19b
a ₁ s ₀	2.59a	0.67ab	0.46a	34.72a	34.38a	19.46a
a ₁ s ₁	2.63a	0.69a	0.46a	30.50a	29.54bc	17.26bc
m ₀ s ₀	2.54a	0.65b	0.44c	30.60b	28.74b	16.46c
m ₀ s ₁	2.60a	0.66ab	0.45b	31.33ab	30.58b	18.04ab
m ₁ s ₀	2.60a	0.69a	0.47a	33.45a	33.55a	19.15a
m ₁ s ₁	2.55a	0.67a	0.46ab	30.67b	30.57b	17.41bc
a ₀ o ₀	2.48b	0.65b	0.44c	27.73b	28.33b	15.77b
a ₀ o ₁	2.59a	0.66ab	0.45b	33.11a	31.18a	18.57a
a ₁ o ₀	2.60a	0.67ab	0.45b	32.20a	31.28a	18.51a
a ₁ o ₁	2.61a	0.69a	0.47a	33.01a	32.64a	18.21a
m ₀ o ₀	2.53a	0.64b	0.44c	28.79b	27.44b	15.89b
m ₀ o ₁	2.60a	0.66ab	0.45b	33.14a	31.88a	18.61a
m ₁ o ₀	2.55a	0.67ab	0.45b	31.14a	32.17a	18.39a
m ₁ o ₁	2.61a	0.69a	0.46a	32.98a	31.94a	18.17a
s ₀ o ₀	2.50c	0.66a	0.45a	28.77c	29.42b	16.65b
s ₀ o ₁	2.60b	0.67a	0.45a	35.28a	32.86a	18.96a
s ₁ o ₀	2.50c	0.67a	0.45a	31.16b	30.18b	17.63b
s ₁ o ₁	2.70a	0.67a	0.46a	30.84bc	30.96ab	17.83ab

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

All three-fold interactions of *Azospirillum*, mycorrhiza, *Streptomyces* and manure significantly improved K absorption efficiency ($p \leq 0.01$; Table 3). Comparison of the means showed that the best treatments in each group of three-fold interactions were a₁m₀s₀, a₁m₀o₁, a₀s₀o₁ and m₀s₀o₁, although they were not significantly different from many other treatments (Table 6). In four-fold interactions, which significantly affected this trait, a₀m₀s₀o₁ (413.36 g/kg) and a₁m₀s₁o₁ (411.35 g/kg) were the best treatments.

Absorption and absorption efficiency of nitrogen (N) in grains Among the four factors of the experiment, only *Azospirillum* significantly improved the N content in grains ($p \leq 0.05$); the effect of other three factors was not significant (Table 8). Other experiments indicated that applying *Azospirillum* increases the absorption of NO₃⁻ and NH₄⁺ (Jain and Patriquin 1985; Lin et al. 1983; Marty and Ladha 1987). Moreover, as *Azospirillum* can fix air nitrogen, plants absorb more N and some of this N will be stored in reproductive organs such as grains to form protein (Bashan and Levanony 1990).

In two-fold interactions, *Streptomyces* × manure significantly ($p \leq 0.01$) affected N content in grains, and s₁o₁ was

the best treatment (Table 10); other interactions, however, had no effect on the trait (Table 8). *Azospirillum* × *Streptomyces* × manure was the only three-fold interaction to significantly affect the grain N content, and the best treatment was a₁s₁o₁ (Table 11). The effect of the four-fold interaction of *Azospirillum* × mycorrhiza × *Streptomyces* × manure was not significant, and mean comparison showed that a₀m₀s₀o₀ (2.36 %) was the worst and a₀m₀s₁o₁ (2.7 %) the best treatments (Table 12).

N absorption efficiency in grains was affected by *Azospirillum*, Mycorrhiza and manure ($p \leq 0.01$; Table 8). *Azospirillum* and mycorrhiza help plants to develop their root systems and absorb more nutrients. Manure also makes soil conditions suitable for root development and releases many nutrients into the soil. This boosts growth and nutrient uptake, promoting nutrient absorption efficiency. *Streptomyces* had no effect on this trait.

Except for Mycorrhiza × manure, all other two-fold interactions significantly affected N absorption efficiency in grains (Table 8). In three-fold interactions, only *Azospirillum* × mycorrhiza × *Streptomyces* had no effect on the trait; the other interactions significantly affected it. Comparison of the means showed the best efficiency in a₁m₀s₀ (35.20 g/kg),

Table 11 The mean comparisons for effects of three-fold 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 absorption and absorption efficiency of N, P and K in grains

Treatments	Absorption (%)			Absorption efficiency (g/kg)		
	N	P	K	N	P	K
$a_0m_0s_0$	2.48c	0.60b	0.43c	26.00d	23.06d	14.36d
$a_0m_0s_1$	2.51bc	0.63b	0.44bc	31.38bc	29.97bc	18.12bc
$a_0m_1s_0$	2.58abc	0.68a	0.47a	32.67abc	32.76ab	17.94bc
$a_0m_1s_1$	2.56abc	0.64b	0.45b	31.64bc	33.25ab	18.26bc
$a_1m_0s_0$	2.61ab	0.69a	0.45b	35.20a	34.42a	18.56b
$a_1m_0s_1$	2.61ab	0.69a	0.45b	31.28bc	31.19abc	17.96bc
$a_1m_1s_0$	2.65a	0.70a	0.48a	34.23ab	34.33a	20.36a
$a_1m_1s_1$	2.56abc	0.68a	0.46ab	29.71c	27.89c	16.56c
$a_0m_0o_0$	2.46c	0.60d	0.43b	24.47b	22.57c	13.75b
$a_0m_0o_1$	2.58ab	0.63cd	0.45b	32.91a	30.46ab	18.73a
$a_0m_1o_0$	2.50bc	0.65bc	0.45b	30.99a	31.91ab	17.79a
$a_0m_1o_1$	2.60ab	0.66bc	0.45b	33.31a	34.09a	18.41a
$a_1m_0o_0$	2.61ab	0.65bc	0.45b	33.12a	32.31ab	18.03a
$a_1m_0o_1$	2.61ab	0.70ab	0.46b	33.36a	33.31ab	18.49a
$a_1m_1o_0$	2.56ab	0.68b	0.45b	31.29a	30.25b	18.98a
$a_1m_1o_1$	2.65a	0.73a	0.48a	32.66a	31.97ab	17.94a
$a_0s_0o_0$	2.38c	0.64b	0.43c	22.91c	24.35c	13.29 d
$a_0s_0o_1$	2.61b	0.68ab	0.45b	35.76a	31.47ab	19.01ab
$a_0s_1o_0$	2.41c	0.64b	0.45b	32.54ab	32.31a	18.25abc
$a_0s_1o_1$	2.60b	0.65ab	0.46b	30.47b	30.90ab	18.13abc
$a_1s_0o_0$	2.58b	0.68ab	0.45b	34.63a	34.50a	20.01a
$a_1s_0o_1$	2.65b	0.69a	0.46b	34.80a	34.25a	18.91ab
$a_1s_1o_0$	2.61b	0.65ab	0.45b	29.78b	28.05b	17.00c
$a_1s_1o_1$	2.76a	0.69a	0.47a	31.22b	31.02ab	17.52bc
$m_0s_0o_0$	2.48d	0.63c	0.43c	25.61d	24.52c	13.73d
$m_0s_0o_1$	2.70ab	0.66bc	0.46b	35.59a	32.96ab	19.19ab
$m_0s_1o_0$	2.50cd	0.65bc	0.45b	30.69c	30.36b	18.06abc
$m_0s_1o_1$	2.60bc	0.67abc	0.46b	31.97bc	30.80ab	18.03abc
$m_1s_0o_0$	2.51cd	0.67abc	0.45b	31.93bc	32.76ab	19.57a
$m_1s_0o_1$	2.71a	0.71a	0.47a	34.97ab	34.33a	18.72abc
$m_1s_1o_0$	2.50cd	0.66bc	0.44bc	30.33c	30.01b	17.20c
$m_1s_1o_1$	2.58bc	0.67abc	0.46b	31.00c	31.12ab	17.62bc

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

$a_1m_0o_1$ (33.36 g/kg), $a_0s_0o_1$ (35.76 g/kg) and $m_0s_0o_1$ (35.59 g/kg), although they did not differ significantly from many other treatments (Table 11). The effect of four-fold interaction was significant, with the lowest efficiency in the control and in treatments that contained both mycorrhiza and *Streptomyces* (Table 12).

Absorption and absorption efficiency of phosphorus (P) in grains Analyses of variance showed that *Azospirillum* and mycorrhiza significantly affected grain P content ($p \leq 0.05$), but the effect of *Streptomyces* and manure was not significant (Table 8). *Azospirillum* and mycorrhiza increased P content in grains by about 3 % and 4.4 %, respectively (Table 9). Both *Azospirillum* and mycorrhiza enhance root development and absorptive surface, helping plants to take

up more nutrients (Bashan and Levanony 1990; Jain and Patriquin 1985; Lin et al. 1983).

Among two-fold interactions, only *Azospirillum* $\times$ mycorrhiza and mycorrhiza $\times$ manure had a significant effect, and in these interactions a_1m_1 (0.70 %) and m_1o_1 (0.69 %) were the best treatments, although the values did not differ significantly from other treatments (Table 10). In three-fold interactions, *Azospirillum* $\times$ mycorrhiza $\times$ manure significantly affected this trait ($p \leq 0.05$); the effect of other three-fold interactions was not significant. Comparison of the means showed that $a_1m_1o_1$ had the highest P content (0.73 %; Table 11). The four-fold interaction of *Azospirillum* $\times$ mycorrhiza $\times$ streptomyces $\times$ manure significantly affected this trait ($p \leq 0.05$).

The P absorption efficiency of grains was significantly affected by all factors of the experiment except for

Table 12 The mean comparisons for effects of four-fold 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 absorption and absorption efficiency of N, P and K in grains

Treatments	Absorption (%)			Absorption efficiency (g/kg)		
	N	P	K	N	P	K
$a_0m_0s_0o_0$	2.36d	0.56d	0.42e	15.78f	14.03e	8.99d
$a_0m_0s_0o_1$	2.5bc	0.64bc	0.46abc	36.22a	32.08abc	19.73ab
$a_0m_0s_1o_0$	2.4d	0.62c	0.45cd	33.15abcde	31.10abcd	18.52bc
$a_0m_0s_1o_1$	2.7a	0.65bc	0.47a	29.61de	28.83cd	17.72bc
$a_0m_1s_0o_0$	2.4d	0.64bc	0.44de	30.04cde	34.66ab	17.59bc
$a_0m_1s_0o_1$	2.7a	0.73a	0.45cd	35.29abc	30.86abcd	18.28bc
$a_0m_1s_1o_0$	2.4d	0.68abc	0.45cd	31.94abcde	33.53abc	17.99bc
$a_0m_1s_1o_1$	2.6b	0.71a	0.45cd	31.33abcde	32.97abc	18.53bc
$a_1m_0s_0o_0$	2.6b	0.67abc	0.43de	35.45ab	35.00a	18.47bc
$a_1m_0s_0o_1$	2.6b	0.70a	0.44de	34.96abc	33.84abc	18.65bc
$a_1m_0s_1o_0$	2.6b	0.67abc	0.47a	30.80bcde	29.62abcd	17.60bc
$a_1m_0s_1o_1$	2.6b	0.72a	0.47a	31.77abcde	32.77abc	18.33bc
$a_1m_1s_0o_0$	2.5bc	0.68abc	0.46abc	33.81abcde	34.01abc	21.56a
$a_1m_1s_0o_1$	2.6b	0.72a	0.51a	34.65abcd	34.66ab	19.16abc
$a_1m_1s_1o_0$	2.5bc	0.63c	0.44de	28.76e	26.49d	16.41c
$a_1m_1s_1o_1$	2.5bc	0.65bc	0.46abc	30.66bcde	29.28bcd	16.71c

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

Streptomyces (Table 8). *Azospirillum* and mycorrhiza influence root development and help plants to take up more nutrients. Mycorrhiza produces phosphatase enzymes that affect the unavailable forms of phosphates in the soil and make them available to plants (Tarafdar and Marschner 1994). Manure releases many nutrients in the rhizosphere during decomposition and makes the soil condition more suitable for root growth. The overall result of all these factors is better root development, higher nutrient absorption and, finally, better plant growth.

In two-fold interactions, *Azospirillum* $\times$ manure and *Streptomyces* $\times$ manure did not affect the P absorption efficiency of grains, but the other interactions did (Table 8). Except for *Azospirillum* $\times$ mycorrhiza $\times$ *Streptomyces*, which had no effect on this trait, all other three-fold interactions had a significant effect ($p \leq 0.01$). Mean comparison (Table 11) indicated that in each group of the three-fold interactions, $a_1m_0s_0$ (34.42 g/kg), $a_0m_1o_1$ (34.09 g/kg), $a_1s_0o_0$ (34.50 g/kg) and $m_1s_0o_1$ (34.33 g/kg) were the best treatments. Note, however, that they did not differ significantly from many other treatments. The four-fold interaction of *Azospirillum* $\times$ mycorrhiza $\times$ *Streptomyces* $\times$ manure significantly improved the efficiency, with the best values occurring in $a_1m_0s_0o_0$ (35.00 g/kg) (Table 12).

Absorption and absorption efficiency of potassium (K) in grains Only *Azospirillum* and mycorrhiza significantly affected grain K content (analysis of variance); the effect of *Streptomyces* and manure was not significant (Table 8). Other researchers have also indicated increased K absorption in the presence of *Azospirillum* (Jain and Patriquin 1985; Lin et al. 1983). The opinions regarding mycorrhiza

and K absorption vary, with some believing that mycorrhizal symbiosis increases K absorption, others not (Mostajeran and Zuie 1999; Tarafdar and Marschner 1994).

Two-fold interactions of mycorrhiza $\times$ *Streptomyces*, *Azospirillum* $\times$ manure and mycorrhiza $\times$ manure significantly affected K absorption in grains, but other two-fold interactions did not. All three-fold interactions had a significant effect, except for *Azospirillum* $\times$ mycorrhiza $\times$ manure (Table 8). In each group of three-fold interactions, $a_1m_1s_0$ (0.48 %), $a_1s_1o_1$ (0.47 %) and $m_1s_0o_1$ (0.47 %) were the best treatments (Table 11). The Four-fold interaction of *Azospirillum* $\times$ mycorrhiza $\times$ *Streptomyces* $\times$ manure had no effect, but mean comparison showed the highest values in $a_1m_1s_0o_1$ (0.51 %), although with no significant difference from many other treatments (Table 12).

The efficiency of K absorption in grains was also significantly affected by *Azospirillum*, mycorrhiza and manure; the effect of *Streptomyces* was not significant (Table 8). *Azospirillum* and mycorrhiza improve the development of root systems, enhancing the roots absorptive surface. Manure improves the physical and chemical properties of soil in the rhizosphere and releases many nutrients during decomposition. Combination of all these factors improves nutrient absorption.

The effect of all two-fold interactions was also significant on K absorption efficiency in grains; an exception is *Azospirillum* $\times$ mycorrhiza. Among three-fold interactions, *Azospirillum* $\times$ *Streptomyces* $\times$ manure and mycorrhiza $\times$ *Streptomyces* $\times$ manure significantly affected the trait ($p \leq 0.01$), but *Azospirillum* $\times$ mycorrhiza $\times$ *Streptomyces* and *Azospirillum* $\times$ mycorrhiza $\times$ manure did not. In the two significant three-fold interactions,

$a_1s_0o_0$ (20.01 g/kg) and $m_1s_0o_0$ (19.57 g/kg) were the best treatments (Table 11). The four-fold interaction of *Azospirillum* $\times$ mycorrhiza $\times$ *Streptomyces* $\times$ manure significantly affected K absorption efficiency in grains ($p \leq 0.01$), but mean comparison put most treatments in the same group (Table 12). The best treatment was $a_1m_1s_0o_0$ (21.56 g/kg), although it did not differ significantly from $a_0m_0s_0o_1$ (19.73 g/kg) and $a_1m_1s_0o_1$ (19.16 g/kg).

Conclusion

Overall, the results of this experiment indicate that the main effect of applying the four factors was an improved absorption and absorption efficiency of N, P and K in plants rather than in grains. Manure significantly improved all measured traits in the plant and grain (except for absorption of the three nutrients in grain). *Streptomyces* had no significant effect on any of the measured traits. The co-application of *Azospirillum* and mycorrhiza typically had a synergistic effect on most of the measured traits. The effects of the four-fold interaction of *Azospirillum* $\times$ mycorrhiza $\times$ *Streptomyces* $\times$ manure was not much better than that of three-fold interactions because of the adverse impact of *Streptomyces* on mycorrhiza. Finally, the absorption efficiency of the three macronutrients was mostly improved, especially in the plant, by the factors tested in this experiment as these factors help root system development and facilitate nutrient uptake.

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