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Designing a Sustainable Agroecosystem for Wheat (*Triticum aestivum* L.) Production

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ABSTRACT

A two year study was conducted in Alborz province, Iran, to evaluate the effect of chemical, biological and organic sources of nutrients on wheat. The experimental design was factorial in the form of a randomized complete block design. Treatments of the first experiment were phosphorus, *Mycorrhiza* (*Glomus* sp.) and *Streptomyces* (*Streptomyces* sp.), in the second experiment were nitrogen, *Azospirillum* (*Azospirillum brasilense*) and *Streptomyces*, and in the third experiment were *Azospirillum*, *Mycorrhiza*, *Streptomyces* and animal manure. Results indicated that P and N fertilizers significantly affected most of the measured traits. Among the biofertilizers, *Azospirillum* and *Mycorrhiza* significantly affected most of the measured traits, but *Streptomyces* had no effect. Finally, results represented that application of animal manure synergistically affected activity of the microorganisms and improved wheat yield component; increasing grain yield by 9.1%.
Key words: *Azospirillum*, manure, *Mycorrhiza*, nitrogen, phosphorus, *Streptomyces*.

INTRODUCTION

As wheat production is vital to the human life on the earth, it is necessary to sustain and improve its production by proper agronomic practices in addition to breeding programs. Among the agronomic practices which are carried out today by the farmers, nutrient management is of a high importance. Wheat yield could increase through the application of chemical fertilizers, especially NPK containing ones.

Although the chemical sources of nutrients are effective on plant growth and yield, and are responsible for the boost of agricultural production, they are chemically synthetic products which their application may lead to undesirable consequences, such as soil, water and products pollution. It is clear that nutrients must be added to soils to keep up the current production records, so environmentally friendly sources of nutrients may be used to provide the nutrients to crops, and replace the chemical fertilizers. Some soil microorganisms are capable of improving soil nutrient and plant growth via different mechanisms.

Azospirillum is a free-living plant growth promoting rhizobacterium (PGPR), associated with the roots of numerous plant species, many of them of agronomic and ecological significance. This bacterium biologically fixes air nitrogen into soil, produces plant hormones and other plant growth promoting substances, improves plant root system development and nutrient uptake, solubilizes the insoluble phosphate in soil, alleviates environmental stresses and controls biotic stresses [1-3]. Ribaudo *et al*. (2006) observed that inoculation with *A. brasilense* FT 326 increased shoot and root fresh weight, main root hair length, and root surface of tomato plants [4]. In their experiment, the levels of indole-3-acetic acid (IAA) and ethylene, phytohormones related to plant growth, were also higher in the inoculated plants. Yadav *et al*. (2011) concluded that the combined application of *Azospirillum* and 60 kg N/ha produced comparable results (plant height and yield) to those obtained due to the application of the recommended dose of fertilizer (120 kg N/ha) [5].

Mycorrhizal symbiosis is a very common plant strategy to enhance nutrient absorption. The fungus hyphae provide an extra absorptive surface area for the plant roots which penetrates into soil; increasing the total soil volume available to the plant. This enables plant to absorb higher amount of water and nutrients; coping with the environmental stresses; improving growth and yield. *Mycorrhiza* fungi effectively provide phosphorus for their host plant. Phosphorus may be fixed in soil and become unavailable to plant roots. The fungal hyphae can release P from soil and make it available to plants [6-7]. In an experiment, mycorrhizal inoculated barley showed higher ear dry matter than non-inoculated plants. The inoculation improved growth (dry matter) by up to 43% compared with the non-inoculated control [8].

Streptomyces is a beneficial actinomycet and a plant growth promoting rhizobacterium (PGPR) with many effects on plant growth. *Streptomyces* produces compounds such as indole acetic acid, phosphate solubilizing substances, chitinase and siderophores which induce seed germination and growth. The bacterium has the potential to control plant pathogens because of producing antimicrobial compounds, and because *Streptomyces* colonize the root surface of host plant, protecting the root against pathogens invasion [9-10]. Sardi *et al*. (1992) found that *Streptomyces* protected wheat against fusarium and increased yield [11].

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In addition to the microorganisms, animal manure is another factor studied in this experiment. Application of animal manure, which contains high organic matter, improves soil physico-chemical properties, provides many nutrients to soil, affects soil pH, promotes the activity of soil microorganisms and finally, increases crop growth and yield [12]. Ojeniyi *et al*. (2007) reported that application of animal manure significantly increased leaf N and K content, plant height, the number of branches, leaf area, the number and weight of fruits of tomato [13]. This experiment was conducted to (1) find the best application rate of N and P fertilizer, with the highest effect on wheat yield and the lowest inhibitory effect on the tested microorganisms' activity, (2) determine the effect of *Azospirillum*, *Mycorrhiza* and *Streptomyces* on wheat and (3) evaluate the effect of animal manure on the activity of the tested microorganisms and wheat growth and yield.

MATERIALS AND METHODS

This series of three experiments were conducted during two years in Alborz province, Iran (51° 6' E, 35° 59' N and 1300 m above sea level), in an area characterized as a semiarid climate with dry warm summers and humid cold winters. The average annual precipitation 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, 42%; silt, 26%; clay, 32%). 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
290	8.5	0.85	0.80	7.9	1.3	38

All three experiments were conducted in a factorial experiment in the form of randomized complete block design (RCBD), but with different replications and factors:

The first experiment (1st year). This experiment was carried out with three replications and studied three factors:

Phosphorus fertilizer (P): phosphorous was applied in four levels of 0, 30, 60 and 90 kg/ha (P_0, P_1, P_2 and P_3, respectively) as triple super phosphate. Phosphorous fertilizer was all applied before sowing.

Mycorrhiza: in two levels of with 1 kg/ha (M_1) and without (M_0). Required amount of *Mycorrhiza* was weighted for each treatment and mixed with seeds right before sowing. The strain was from *Glomus* sp. with clay carrier which contained 10^5 spores/g.

Streptomyces: in two levels of with 0.5 kg/ha (S_1) and without (S_0). The strain was from *Streptomyces* sp. 557 in a clay carrier. It contained 10^8 cfu/g streptomyces and was applied as above for *Mycorrhiza*.

After preparing the field and before sowing wheat, 50% of 350 kg/ha urea fertilizer was broadcasted on soil surface and incorporated into soil by harrowing. Other 50% of this fertilizer was applied at stem elongation stage. The plots sites were then set in field and 0, 30, 60 and 90 kg P/ha were applied. Finally, 200 kg/ha wheat seed (*Triticum aestivum* L. var: Mahdavi) was weighted equally for all plots and inoculated with biofertilizers on Nov 12th. Seeds were broadcasted on soil surface and incorporated into soil by the means of a furrower. 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 treatments interference and movement of the microorganisms. On Jul. 1st, when plants were fully maturated, 4 m of the two middle rows were harvested and grains were detached from straw to weight the harvested grains. Before this final harvest, the number of panicles (only fertile panicles) was counted. Moreover, 20 fertile stems were selected randomly to study the yield components on them. To measure the 1000 kernels weight, samples were dried at 75°C in an oven for 24 hours.

The second experiment (1st year). This experiment was carried out in the same location and mostly the same method as the first experiment, but studied three different factors:

Nitrogen fertilizer (N): nitrogen was applied in four levels of 0, 50, 100 and 150 kg/ha (N_0, N_1, N_2 and N_3, respectively) as urea. Nitrogen fertilizer was split into two parts, one part applied before sowing and the other part at stem elongation stage.

Azospirillum: in two levels of 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* in a peat carrier and contained 10^8 cfu/g azospirillums.

Streptomyces: like the first experiment, here *Streptomyces* was in two levels of with 0.5 kg/ha (S_1) and without (S_0). *Streptomyces* properties were the same as *Streptomyces* applied in the first experiment.

The field was also prepared in the same way as the first experiment, but 90 kg P/ha (as triple super phosphate) was applied instead of the nitrogen fertilizer. The following traits were studied in this experiment: plant height, panicle length, the number of panicle/m^2, the number of kernels in panicle, 1000 kernels weight, grain yield, biomass, harvest index and biological nitrogen fixation (acetylene reducing activity; ARA). All these traits were measure in the same method as the first experiment but to study the biological nitrogen fixation, samples were taken at tillering, stem elongation and panicle stages. Sample roots were detached from root crown and placed in Erlen glass (300 ml) and blocked. Then, 10% of erlen volume was sucked out using a syringe and the same volume of acetylene gas was injected to the erlen. Erlens were then located in 25°C oven for 72 hours and after that, acetylene reduction (ethylene production) was measured by gas chromatography (perkin-elemer sigma 300). As the results of panicle stage seemed more logical, and were in agreement with those of Pedersen *et al*. (1978) [14], only these data were taken into consideration to obtain the acetylene reduction activity.

The third experiment (2nd year). The third experiment, the most comprehensive one which was planned based on the results of the first year experiments, was conducted in the same place and mostly the same method as the first year experiments, but with four replications and four factors:

Azospirillum: in two levels of with 600 g/ha (A_1) and without (A_0). *Azospirillum* was applied the same as the second experiment (first year).

Mycorrhiza: in two levels of with 1 kg/ha (M_1) and without (M_0). *Mycorrhiza* was applied the same as the first experiment (first year).

Streptomyces: in two levels of with 0.5 kg/ha (S_1) and without (S_0). *Streptomyces* was also applied the same as the first and the second experiments (first year).

Manure: in two levels of with 30 ton/ha (O_1) and without (O_0). The manure was fully decomposed cow dung stored for one year; its properties are listed in Table 2:

Table 2. Properties of the 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

The field preparation was conducted mostly the same as the first year experiments, but according to the results of the first year, 100 kg nitrogen and 60 kg phosphorus per hectare (that were shown to have the highest effect on wheat growth and yield and the lowest adverse effect on microorganisms activity) were applied. Then, the plot sites were set in field, and the manure was weighted and applied in the required plots based on 30 ton/ha and incorporated into the soil. Finally, wheat seeds were inoculated with the biofertilizers and planted on Nov 11th.

RESULTS

The first experiment. In this experiment, the effects of phosphorus, *Mycorrhiza* and *Streptomyces* were studied on wheat growth and yield. Analysis of variances (ANOVA) revealed the significant effect of phosphorus on the number of panicles/m^2, number of kernels/panicle, grain yield and the harvest index; the effect was not significant on rest of the measured traits (Table 3). The number of panicles/m^2 and kernels/panicle are directly related to wheat grain yield and are affected by the environmental factors. Determining the correlation of the measured traits (Table 4) also shows that wheat grain yield is significantly correlated to the number of panicles/m^2 and kernels/panicle. Among the four P application rates, P_3 (90 kg P/ha) was the most effective one which increased the number panicles/m^2, kernels/panicle and grain yield by about 23%, 39% and 18%, respectively, compared with P_0 (0 kg P/ha) (Table 5). Although P_3 was the best treatment in most cases, its effect was significantly the same as P_2 (60 kg P/ha) on grain yield. Harvest index is an important physiological index in crop plants which represents the distribution efficiency of assimilates between different organs. Results of this experiment also indicate that high P application rate (90 kg/ha) ended in the highest HI which means an increased P content in soil improves grain production rather than biomass production.

Table 3. Analysis of variances of the measured traits in the first experiment.

SOV	**df**	**Mean Squares (MS)**							
		Plant height	**Panicle length**	**1000 kernels weight**	**Panicles / m^2**	**Kernels in panicle**	**Grain yield**	**Biomass**	**HI**
Rep	2	**	ns	ns	*	**	ns	ns	ns
Phosphorus (P)	3	ns	ns	ns	**	**	**	ns	**
Mycorrhiza (M)	1	*	ns	*	**	**	**	ns	**
PM	3	ns	ns	**	**	**	ns	ns	**
Streptomyces (S)	1	ns	ns	ns	ns	ns	ns	ns	ns
PS	3	ns	ns	*	*	ns	ns	ns	**
MS	1	ns	ns	ns	**	**	ns	**	**
PMS	3	*	ns	**	**	**	ns	**	**
Error	30	15.81	0.816	1.64	1518.36	1.19	12.18	2.35	8.34
CV (%)	-	4.39	10.46	2.41	6.66	4.28	8.15	11.44	8.79

ns, nonsignificant; **, significant at P≤0.01; *, significant at P≤0.05.

Table 4. Correlation of the measured traits in the first experiment.

	Plant height	Panicle length	1000 kernels weight	Panicles / m^2	Kernels in panicle	Grain yield	Biomass	HI
Plant height	1.000 **							
Spike length	0.171 ns	1.000 **						
1000 kernels weight	0.143 ns	-0.022 ns	1.000 **					
Spike / m^2	0.299 *	0.223 ns	0.334 *	1.000 **				
Kernels in spike	0.249 ns	0.181 ns	0.413 **	0.857 **	1.000 **			
Grain yield	0.005 ns	0.319 *	0.272 ns	0.359 *	0.505 **	1.000 **		
Biomass	0.194 ns	0.009 ns	0.011 ns	-0.360 *	-0.387 **	0.259 ns	1.000 **	1.000 **
HI	0.157 ns	0.192 ns	0.131 ns	0.483 **	0.634 **	0.348 *	-0.878 **	

ns, nonsignificant; **, significant at P≤0.01; *, significant at P≤0.05.

Table 5. Effects of phosphorus (0, P_0; 30, P_1; 60, P_2; 90; P_3; kg/ha), *Mycorrhiza* (without, M_0; with, M_1) and *Streptomyces* (without, S_0; with, S_1) on the measured traits in the first experiment.

Treatments	Plant height (cm)	Spike length (cm)	1000 kernels weight (g)	Spike / m^2	Kernels in spike	Grain yield (ton/ha)	Biomass (ton/ha)	HI (%)
P_0	89.50a	8.28a	52.26b	511.30c	21.08d	3.91c	13.16a	0.30b
P_1	90.56a	8.52a	53.25ab	571.74b	24.44c	4.23b	13.38a	0.31b
P_2	90.70a	8.79a	53.57a	625.30a	27.03b	4.35ab	13.43a	0.32b
P_3	91.93a	8.93a	53.59a	632.97a	29.41a	4.63a	13.60a	0.34a
M_0	89.46	8.46	52.77	555.63	23.77	4.05	13.04	0.31
M_1	91.87	8.80	53.56	615.03	27.21	4.51	13.73	0.32
S_0	90.65	8.58	52.81	580.52	25.21	4.18	13.01	0.32
S_1	90.70	8.68	53.51	590.13	25.77	4.38	13.76	0.32
P_0M_0	86.58b	7.85a	50.43b	441.25e	17.45f	3.46c	12.30b	0.28d
P_0M_1	88.32ab	8.71a	54.08a	581.35c	24.70d	4.10b	12.96ab	0.31bc
P_1M_0	89.96ab	8.43a	53.11a	520.57d	22.50e	4.06b	12.38b	0.32b
P_1M_1	91.47ab	8.76a	54.01a	622.90abc	26.37c	4.36ab	13.60ab	0.32b
P_2M_0	91.44ab	8.62a	53.43a	601.41bc	25.96cd	4.36ab	13.88ab	0.31bc
P_2M_1	92.36a	8.81a	54.10a	649.20ab	28.09b	4.63a	14.44a	0.32b
P_3M_0	92.41a	8.80a	53.06a	656.65bc	29.16ab	4.56a	13.60ab	0.33a
P_3M_1	92.80	9.06a	53.08a	659.28a	29.67a	4.70a	13.93ab	0.33a
P_0S_0	88.74a	8.08a	51.10b	498.16e	20.89d	3.70c	12.09b	0.30b
P_0S_1	89.85a	8.48a	53.41a	524.44de	21.26d	4.13b	13.16a	0.31b
P_1S_0	90.24a	8.36a	52.93a	562.83cd	23.91c	4.06bc	12.86ab	0.31b
P_1S_1	90.80a	8.68a	54.20a	580.65bc	24.97c	4.40ab	13.66ab	0.32b
P_2S_0	90.60a	8.70a	52.60ab	589.54b	26.72b	4.30ab	13.53ab	0.31b
P_2S_1	91.62a	8.73a	53.85a	627.45ab	27.34b	4.40ab	13.98ab	0.31b
P_3S_0	91.27a	8.85a	53.33a	638.48a	29.31a	4.60a	13.58ab	0.35a
P_3S_1	92.16a	9.16a	53.90a	661.06a	29.51a	4.66a	14.22a	0.32b
M_0S_0	88.73b	8.42a	52.62b	494.17d	21.70d	4.00c	11.72b	0.34a
M_0S_1	90.19ab	8.50a	52.91b	617.09b	24.57c	4.10bc	14.31a	0.28c
M_1S_0	92.67a	8.85a	54.11a	686.10a	29.84a	4.66a	14.36a	0.32bc
M_1S_1	91.11ab	8.75a	53.00a	543.95c	25.84b	4.36b	13.15a	0.33a
$P_0M_0S_0$	85.38c	7.76a	49.33e	337.60h	13.68h	3.46d	9.32d	0.37b
$P_0M_0S_1$	86.44bc	7.93a	51.53de	544.91ef	21.22g	3.46d	9.83d	0.35b
$P_0M_1S_0$	91.72abc	8.80a	55.30ab	658.72bc	28.84bc	4.73a	11.73cd	0.33bc
$P_0M_1S_1$	88.44bc	8.40a	52.86cd	503.97f	20.55g	3.93bcd	11.55cd	0.41a
$P_1M_0S_0$	90.77abc	8.66a	51.90cd	436.36g	20.75g	4.16bcd	13.86bc	0.28cd
$P_1M_0S_1$	91.27abc	8.86a	54.33abc	604.78cde	24.25ef	4.20abc	13.73bc	0.31bc
$P_1M_1S_0$	92.61abc	8.80a	52.36cd	689.30ab	29.19bc	4.53abc	13.78bc	0.30c
$P_1M_1S_1$	92.11abc	8.83a	51.53de	556.51ef	23.56f	3.86cd	13.46bc	0.33bc
$P_2M_0S_0$	91.23abc	8.30a	52.83cd	571.51def	24.63ef	4.26abc	14.20bc	0.27d
$P_2M_0S_1$	94.05ab	8.43a	54.03bc	631.30bcd	27.30cd	4.80a	14.77ab	0.28cd
$P_2M_1S_0$	97.16a	8.56a	56.50a	750.60a	30.05ab	4.53abc	17.33a	0.27d
$P_2M_1S_1$	86.71bc	8.96a	53.76bcd	547.78ef	26.12de	4.73a	13.33bc	0.33bc
$P_3M_0S_0$	91.11abc	8.36a	52.80cd	631.21bcd	27.72cd	4.40abc	13.00bc	0.36b
$P_3M_0S_1$	91.77abc	8.63a	55.40ab	687.36ab	30.59ab	4.80a	14.00bc	0.31bc
$P_3M_1S_0$	89.82abc	8.53a	53.86bcd	645.76bc	31.30a	4.60ab	14.86ab	0.32bc
$P_3M_1S_1$	90.11abc	8.76a	52.30cd	567.54def	28.04cd	4.70a	15.44ab	0.29c

Means in a column followed by the same letter are not significantly different at P≤0.01.

Inoculating wheat seeds with *Mycorrhiza* significantly affected all the measured traits except for panicle length and biomass (Table 3). Panicle length which is effective on the number of grains was not affected by any treatment of the experiment. This is mainly because this feature is controlled genetically, not by environmental factors, so panicle length was significantly correlated only to grain yield (Table 4). Mycorrhizal symbiosis increased the number of panicles/m^2 by about 10.16% compared with the control. As planting density was the same in the both treatments, improved number of panicles/m^2 may be attributed to the enhancement of the number of tillers/m^2 which is caused by *Mycorrhiza*. Plant height, the number of kernels/panicle and 1000 kernels weight were also favored by *Mycorrhiza* application (by 2.69%, 14.47% and 15%, respectively). Grain yield is significantly correlated to panicle length, the number of panicles/m^2 and kernels/panicle (Table 4), so grain yield increased as the mentioned traits increased (Table 5). Harvest index was also slightly enhanced (by 3.22%) in mycorrhizal symbiosis.

Streptomyces, which is a plant growth promoting rhizobacterium (PGPR), had no significant effect on any of the measured traits (Table 3).

The three-fold interaction of phosphorus × *Mycorrhiza* × *Streptomyces* significantly affected all the measured traits except for panicle length and grain yield (Table 3). Plant height, the number of panicles/m^2, 1000 kernels weight and biomass were the highest in $P_2M_1S_0$ and increased by 13.80%, 122.33%, 14.53% and 85.94%, respectively, compared with the control ($P_0M_0S_0$; Table 5).

The second experiment. This experiment studied the effects of nitrogen, *Azospirillum* and *Streptomyces* on wheat growth, yield and biological nitrogen fixation. Analysis of variances indicated that application of nitrogen significantly affected all the measured traits (Table 6). Nitrogen is vital for plant growth and directly affects plant height; applying 150 kg N/ha (N_3) increased plant height by 18.2% (Table 7) compared with the control (0 kg N/ha). The number of panicles/m^2 is depended on the number of main stems and fertile tillers, and tillering in wheat is affected by the environmental factors especially nutrition. N_3 was the best treatment here which increased the number of panicles/m^2 by 24.3% compared with the control. Presence of sufficient N in soil at grain filling stage severely affects kernels weight and that is why N_3 gave 8.6% heavier kernels compared with N_0 when 1000 kernels weight was measured. Grain yield is controlled by genotype and environmental factors. Increased grain yield is the results of improvement in other yield components such as the number of panicles/m^2, number of kernels in panicle and 1000 kernels weight. Results of this experiment represented that grain yield was significantly correlated to all other measured traits except for nitrogen fixation (Table 8). Grain yield was also increased by 63% in N_3 compared with N_0. Finally, biological nitrogen fixation which was measured by the method of acetylene reducing activity was decreased when nitrogen fertilizer was applied. The highest biological nitrogen fixation occurred in N_0 which was about 6.7% higher than N_3 (data not shown).

Table 6. Analysis of variances of the measured traits in the second experiment.

SOV	df	Mean Squares (MS)							
		Plant height	Spike length	1000 kernels weight	Spike / m^2	Kernels in Spike	Grain yield	Biomass	HI
Rep	2	ns	ns	ns	ns	ns	ns	ns	ns
Nitrogen (N)	3	**	**	**	**	*	**	**	**
Azospirillum (A)	1	**	*	ns	*	*	**	**	**
NA	3	**	ns	ns	ns	ns	ns	ns	*
Streptomyces (S)	1	ns	ns	ns	ns	ns	ns	ns	ns
NS	3	ns	ns	ns	ns	ns	ns	ns	ns
AS	1	ns	ns	ns	ns	ns	ns	ns	ns
NAS	3	**	ns	ns	ns	ns	ns	ns	ns
Error	30	22.53	0.25	1.16	1725.43	2.95	0.128	0.331	6.51
CV (%)	-	5.65	6.47	2.09	7.52	10.31	10.18	6.00	6.80

ns, nonsignificant; **, significant at P≤0.01; *, significant at P≤0.05.

Application of *Azospirillum* significantly affected all the measured traits except for 1000 kernels weight (Table 6). *Azospirillum* is a nitrogen fixing bacterium which affects plant vegetative growth. Applying *Azospirillum* (A_1) increased plant height by 4.6%, the number of panicles/m^2 by 6% and biomass by 9.95% compared with the control (A_0; Table 7). Grain yield was also increased by 15.29% as the result of *Azospirillum* association. Neither *Streptomyces* nor the two and the three-fold interactions significantly affected the measured traits (Table 6). The only exception was plant height which was significantly affected by the two-fold interaction of nitrogen × *Azospirillum* and the three-fold interaction.

Table 7. Effects of nitrogen (0, N_0; 50, N_1; 100, N_2; 150; N_3; kg/ha), *Azospirillum* (without, A_0; with, A_1) and *Streptomyces* (without, S_0; with, S_1) on the measured traits in the second experiment.

Treatments	Plant height (cm)	Spike length (cm)	1000 kernels weight (g)	Spike / m^2	Kernels in spike	Grain yield (ton/ha)	Biomass (ton/ha)	HI (%)
N_0	75.34c	6.99b	48.84c	500.3c	15.44b	2.78d	7.76d	0.36b
N_1	82.83b	7.86a	51.65b	523.1c	16.70ab	3.35c	8.34c	0.40a
N_2	88.72a	8.19a	52.29ab	564.5b	16.81ab	3.81b	10.52b	0.36b
N_3	89.05a	8.20a	53.03a	621.9a	17.69a	4.53a	11.77a	0.38a
A_0	82.10	7.66	51.29	536.38	16.45	3.27	9.14	0.36
A_1	85.87	7.96	51.61	568.52	16.87	3.77	10.05	0.38
S_0	83.82	7.74	51.44	543.97	16.52	3.43	9.46	0.37
S_1	84.16	7.87	51.45	560.93	16.80	3.61	9.73	0.38
N_0A_0	70.66d	6.75c	48.83c	480.10d	14.97b	2.57f	7.17e	0.36b
N_0A_1	80.03c	7.23bc	48.85c	520.50cd	15.91b	2.98ef	8.36d	0.35b
N_1A_0	82.53bc	7.62ab	51.53b	501.40cd	16.33b	3.03de	8.13d	0.37b
N_1A_1	83.14bc	8.10a	51.77b	544.80bc	16.77b	3.67bc	8.56d	0.43a
N_2A_0	86.97ab	8.13a	52.27b	543.00bc	16.64b	3.45cd	9.97c	0.35b
N_2A_1	90.47a	8.25a	52.32b	586.10ab	16.92b	4.17a	11.07b	0.37b
N_3A_0	87.64ab	7.14a	52.28b	621.10a	16.71b	4.02ab	11.32b	0.35b
N_3A_1	90.47a	8.26a	53.77a	622.70a	19.06a	4.28a	12.23a	0.34b
N_0S_0	73.33e	6.88b	46.65c	481.50d	14.82c	2.75d	7.63d	0.36bc
N_0S_1	77.36de	7.10b	49.03c	519.00cd	16.05bc	2.80d	7.90cd	0.35c
N_1S_0	82.06cd	7.83a	51.47b	522.70cd	16.36abc	3.33c	8.27cd	0.40a
N_1S_1	83.61bc	7.88a	51.83b	523.50cd	16.49ab	3.37c	8.42c	0.40a
N_2S_0	87.50abc	8.13a	52.28ab	562.10bc	16.75abc	3.67bc	10.38b	0.35c
N_2S_1	88.17a	8.25a	52.30ab	567.00bc	16.92abc	3.95b	10.66b	0.37abc
N_3S_0	89.94a	8.14a	53.02a	603.9ab	17.26abc	4.40a	11.59a	0.38abc
N_3S_1	89.94a	8.26a	53.03a	639.8a	18.64a	4.65a	11.59a	0.39ab
A_0S_0	81.11a	7.55a	51.18a	516.06b	16.44a	3.21b	8.96b	0.35b
A_0S_1	83.10a	7.76a	51.40a	556.70ab	16.45a	3.33b	9.32b	0.35b
A_1S_0	85.23a	7.94a	51.49a	565.15a	16.60a	3.87a	9.96a	0.38a
A_1S_1	86.53a	7.97a	51.73a	571.89a	17.15a	4.06a	10.14a	0.39a
$N_0A_0S_0$	66.66e	6.57e	49.23e	448.50f	15.81bc	2.53h	6.97h	0.36cd
$N_0A_0S_1$	74.66d	6.92de	48.47e	511.70def	15.99bc	2.60h	7.36gh	0.35d
$N_0A_1S_0$	80.00cd	7.20cde	48.07e	514.50def	16.11bc	2.97gh	8.29fg	0.35d
$N_0A_1S_1$	80.05cd	7.27bcde	49.60de	526.40cdef	13.82c	3.00fgh	8.44f	0.35d
$N_1A_0S_0$	82.23cd	7.43abcde	52.20abc	502.40ef	16.92bc	3.00fgh	7.93fgh	0.37bcd
$N_1A_0S_1$	83.94abc	7.80abcd	51.33cd	500.30ef	16.92bc	3.07fgh	8.32fg	0.36cd
$N_1A_1S_0$	81.78cd	8.22ab	51.47bcd	544.50bcde	15.82bc	3.67def	8.60f	0.42a
$N_1A_1S_1$	83.28bcd	7.97abc	51.60bc	545.20bcde	16.78bc	3.67def	8.52f	0.43a
$N_2A_0S_0$	87.28abc	8.07abc	52.17abc	527.90cde	17.09bc	3.40efg	9.86e	0.34d
$N_2A_0S_1$	86.67abc	8.20ab	52.37abc	558.20bcde	16.19bc	3.50defg	10.07de	0.34d
$N_2A_1S_0$	92.61a	8.20ab	52.40abc	606.10abc	16.76bc	3.93cde	10.89cd	0.36d
$N_2A_1S_1$	88.33abc	8.30a	52.23abc	566.10bcde	17.60ab	4.40bc	11.25bc	0.39abcd
$N_3A_0S_0$	88.16abc	8.15abc	52.00abc	585.50abcd	15.78bc	3.90cde	11.10bc	0.35d
$N_3A_0S_1$	87.11abc	8.13abc	52.57abc	656.70a	16.87bc	4.13cd	11.53abc	0.35d
$N_3A_1S_0$	91.72ab	8.13abc	54.03a	622.40ab	17.73ab	4.90ab	12.08ab	0.40abc
$N_3A_1S_1$	89.22abc	8.38a	53.50ab	623.00ab	20.40a	5.17a	12.37a	0.41ab

Means in a column followed by the same letter are not significantly different at P≤0.01.

Table 8. Correlation of the measured traits in the second experiment.

	Panicle length	Spike / m^2	1000 kernels weight	Kernels in spike	HI	Biomass	Grain yield	Plant height
Spike length	1.000**							
Spike / m^2	0.762**	1.000**						
1000 kernels weight	0.843**	0.707**	1.000**					
Kernels in spike	0.481ns	0.444ns	0.617*	1.000**				
HI	0.378ns	0.231ns	0.392ns	0.531*	1.000**			
Biomass	0.812**	0.911**	0.795**	0.534*	0.181ns	1.000**		
Grain yield	0.817**	0.863**	0.817**	0.672**	0.525*	0.930**	1.000**	1.000**
Plant height	0.900**	0.803**	0.813**	0.446ns	0.176ns	0.856**	0.790**	

ns, nonsignificant; **, significant at P≤0.01; *, significant at P≤0.05.

The third experiment. The effects of *Azospirillum*, *Mycorrhiza*, *Streptomyces* and manure on wheat growth and yield were studied in this experiment. Analysis of variances showed the significant effect of *Azospirillum* on plant height, the number of tillers in plant, root weight, the number of panicles/m^2 and kernels in panicle and grain yield; the effect was not significant on rest of the measured traits (Table 9). *Azospirillum* is a nitrogen fixing bacterium and fixes air nitrogen into the rhizosphere. Nitrogen is directly related to plant growth so application of *Azospirillum* increased plant height by 4.8%, the number of tillers in plant by 5.7%, root weight by 9.56% and grain yield by 4.6% (Table 10). As was earlier pointed out, *Azospirillum* fixes air nitrogen into the rhizosphere and the results of this experiment indicated that wheat root weigh, which represents root system development was increased.

Mycorrhizal symbiosis significantly affected all the measured traits except for plant height, panicle length, stem diameter, biomass and biological N fixation (Table 9). *Mycorrhiza* is a phosphate solubilizing fungus and phosphorous is an essential element for plant reproduction and that is why in this experiment *Mycorrhiza* affected reproductive yield components better than the vegetative ones. For example, mycorrhizal symbiosis increased the number of grains in panicle by 3.72%, 1000 kernels weight by 3.84% and grain yield by 3.86% (Table 10), but had no significant effect on plant height and biomass.

Streptomyces had no significant effect on any of the measured traits (Table 9). Application of manure significantly affected all measure traits except for the harvest index (Table 9). Results indicated that application of manure increased plant height by 2.44%, the number of tillers in plant by 7.3% and root weight by 8.2% (Table 10). Stem diameter, which represents plant tolerance to lodging, was significantly enhanced (by 8.4%) as the result of manure application. Manure is considerably important for soil improvement and affects soil chemical, physical and biological properties. In this experiment, manure application increased wheat grain yield by 9.1% compared with the no manure treatment (Table 10).

The mean comparison of the two-fold and three-fold interactions is listed in Table 11 and Table 12, respectively. Results indicated that the four-fold interaction of *Azospirillum* × *Mycorrhiza* × *Streptomyces* × manure significantly affected all the measured traits except for panicle length, the number of kernels in panicle and the harvest index (Table 9). In most cases, $A_1M_1S_0O_1$ was the best four-fold interaction which increased plant height by 23.58%, stem diameter by 50.74%, the number of tillers in plant by 36.93% and grain yield by 102.76% compared with the control (Table 13).

Table 9. Analysis of variances of the measured traits in the third experiment.

SOV	df	Mean Squares (MS)										
		Plant height	Spike length	Stem diameter	Tillers / plant	Root dry weight	Spike / m^2	Kernels / spike	1000 kernels weight	Grain yield	Biomass	HI
Rep	3	ns	*	**	ns	ns	ns	ns	**	ns	ns	ns
A	1	**	ns	ns	**	*	**	**	ns	*	ns	ns
M	1	ns	ns	ns	**	*	**	*	*	*	ns	*
AM	1	ns	**	ns	**	*	**	**	ns	**	**	ns
S	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
AS	1	**	ns	**	ns	ns	**	*	ns	**	ns	**
MS	1	ns	ns	ns	**	ns	**	**	ns	**	ns	ns
AMS	1	ns	*	*	ns	ns	**	**	ns	**	ns	ns
O	1	**	**	**	**	*	**	**	**	**	*	ns
AO	1	*	ns	ns	ns	ns	**	**	ns	*	*	ns
MO	1	*	ns	*	ns	*	**	**	ns	**	*	ns
AMO	1	**	ns	**	*	ns	**	**	ns	*	ns	ns
SO	1	**	ns	**	ns	ns	**	**	**	**	ns	ns
ASO	1	*	**	**	*	*	ns	**	*	**	*	ns
MSO	1	**	**	ns	ns	ns	**	ns	ns	**	**	ns
AMSO	1	**	ns	**	**	**	**	ns	**	**	*	ns
Error	45	8.571	0.138	4.330	0.087	0.044	816.057	5.823	11.018	0.143	1.064	0.207
CV (%)	-	3.13	4.63	6.11	7.13	13.70	6.08	6.3	7.27	7.15	8.25	10.74

A, *Azospirillum*; M, *Mycorrhiza*; S, *Streptomyces*; O, manure
ns, nonsignificant; **, significant at P≤0.01; *, significant at P≤0.05.

Table 10. Effects of *Azospirillum* (without, A_0; with, A_1), *Mycorrhiza* (without, M_0; with, M_1), *Streptomyces* (without, S_0; with, S_1) and manure (without, O_0; with, O_1) on the measured traits in the third experiment.

Treatments	Plant height (cm)	Spike length (cm)	Stem diameter (mm)	Tillers / plant	Root dry weight (g)	Spike / m^2	Kernels / spike	1000 kernels weight (g)	Grain yield (ton/ha)	Biomass (ton/ha)	HI (%)
A_0	91.22	7.98	3.36	4.03	1.45	446.13	36.78	45.50	5.16	12.31	0.41
A_1	95.60	8.06	3.44	4.26	1.59	494.03	39.89	45.78	5.40	12.70	0.42
M_0	93.06	8.01	3.40	3.92	1.46	458.53	37.63	44.78	5.18	12.40	0.41
M_1	93.75	8.02	3.41	4.37	1.58	481.63	39.03	46.50	5.38	12.61	0.42
S_0	93.00	7.99	3.39	4.10	1.52	470.03	38.14	45.03	5.26	12.36	0.42
S_1	93.81	8.04	3.41	4.11	1.53	470.13	38.53	46.22	5.30	12.65	0.41
O_0	92.28	7.88	3.32	4.00	1.46	441.00	35.77	44.40	5.05	12.20	0.41
O_1	94.53	8.15	3.60	4.30	1.58	499.16	40.90	46.87	5.51	12.81	0.43

Table 11. Effects of two-fold interactions of *Azospirillum* (without, A_0; with, A_1), *Mycorrhiza* (without, M_0; with, M_1), *Streptomyces* (without, S_0; with, S_1) and manure (without, O_0; with, O_1) on the measured traits in the third experiment.

Treatments	Plant height (cm)	Spike length (cm)	Stem diameter (mm)	Tillers / plant	Root dry weight (g)	Spike / m^2	Kernels / spike	1000 kernels weight (g)	Grain yield (ton/ha)	Biomass (ton/ha)	HI (%)
A_0M_0	90.56b	7.83b	3.34a	3.63c	1.33b	422.19c	34.21c	44.62a	4.91b	12.00b	0.40a
A_0M_1	91.87b	8.00ab	3.39a	4.38ab	1.57a	470.06b	39.35ab	46.94a	5.41a	12.20b	0.44a
A_1M_0	95.56a	8.13a	3.42a	4.20b	1.59a	493.19a	38.71b	46.06a	5.35a	12.61ab	0.42a
A_1M_1	95.62a	8.19a	3.46a	4.40a	1.60a	494.87a	41.06a	46.94a	5.45a	13.22a	0.41a
A_0S_0	89.44c	7.88a	3.30a	4.03b	1.45b	428.75c	36.35b	45.00a	4.88c	12.23a	0.39b
A_0S_1	93.00b	8.08a	3.36a	4.13ab	1.46b	463.50b	37.21b	45.12a	5.07c	12.32a	0.41ab
A_1S_0	95.60ab	8.10a	3.42a	4.30a	1.54ab	476.56b	39.07a	45.87a	5.45b	12.43a	0.43a
A_1S_1	96.00a	8.10a	3.52a	4.32a	1.60a	511.50a	40.70a	46.56a	5.72a	12.98a	0.44a
M_0S_0	92.12a	7.95a	3.39a	3.80c	1.47c	432.06d	35.06c	43.62b	5.04c	12.07a	0.41b
M_0S_1	93.62a	8.06a	3.41a	4.03b	1.47c	485.00b	40.21a	46.50a	5.33bc	12.65a	0.42a
M_1S_0	93.87a	8.09a	3.42a	4.62a	1.63a	508.19a	41.23a	46.50a	5.56a	12.73a	0.43a
M_1S_1	93.62a	8.07a	3.40a	4.12b	1.54b	455.06c	36.84b	45.93ab	5.20bc	12.57a	0.41b
A_0O_0	89.18c	7.86b	3.17b	3.83b	1.40c	403.44c	33.20c	43.56b	4.81b	11.73b	0.41a
A_0O_1	93.25b	8.07ab	3.55a	4.23a	1.51b	488.81b	40.36a	46.31a	5.51a	12.74a	0.43a
A_1O_0	95.37a	7.88b	3.25b	4.17a	1.52b	478.56b	38.33b	45.25ab	5.29a	12.67a	0.41a
A_1O_1	95.81a	8.23a	3.63a	4.35a	1.66a	509.50a	41.44a	47.44a	5.52a	12.88a	0.42a
M_0O_0	91.18b	7.87a	3.15b	3.83c	1.33c	406.00c	33.11c	43.75b	4.82b	11.97b	0.40b
M_0O_1	94.73a	8.10a	3.53a	4.00bc	1.58b	487.25b	39.64b	45.81ab	5.48a	12.43b	0.44a
M_1O_0	93.37a	7.90a	3.27b	4.17b	1.57b	476.00b	38.42b	45.06b	5.28a	12.37b	0.42ab
M_1O_1	94.77a	8.15a	3.65a	4.57a	1.60a	511.06a	42.16a	47.94a	5.54a	13.25a	0.41ab
S_0O_0	90.37b	7.81b	3.18d	4.02b	1.44b	432.34b	33.63c	42.62b	4.89c	12.12b	0.40b
S_0O_1	94.18a	8.17a	3.47b	4.30a	1.53ab	490.44a	39.15b	46.25a	5.31b	12.35ab	0.42ab
S_1O_0	93.44a	7.96ab	3.31c	4.08b	1.47b	449.63b	37.90b	46.18a	5.21b	12.20b	0.42ab
S_1O_1	95.62a	8.23a	3.71a	4.32a	1.64a	507.87a	42.65a	47.50a	5.70a	13.09a	0.43a

Means in a column followed by the same letter are not significantly different at P≤0.01.

Table 12. Effects of three-fold interactions of *Azospirillum* (without, A_0; with, A_1), *Mycorrhiza* (without, M_0; with, M_1), *Streptomyces* (without, S_0; with, S_1) and manure (without, O_0; with, O_1) on the measured traits in the third experiment.

Treatments	Plant height (cm)	Spike length (cm)	Stem diameter (mm)	Tillers / plant	Root dry weight (g)	Spike / m^2	Kernels / spike	1000 kernels weight (g)	Grain yield (ton/ha)	Biomass (ton/ha)	HI (%)
$A_0M_0S_0$	88.50d	7.58c	3.38ab	3.56e	1.31c	343.63e	30.71d	42.50b	4.31d	11.60c	0.37c
$A_0M_0S_1$	92.62bc	8.07ab	3.38ab	3.71e	1.36bc	469.25c	37.70b	45.12ab	5.44ab	12.40abc	0.43ab
$A_0M_1S_0$	93.37bc	8.17ab	3.46ab	4.70a	1.58ab	513.87ab	38.75b	46.75a	5.51ab	13.03ab	0.42ab
$A_0M_1S_1$	90.37cd	8.08ab	3.22b	4.15cd	1.56ab	426.25d	35.00c	46.37a	5.39abc	12.19bc	0.44ab
$A_1M_0S_0$	95.37ab	8.06ab	3.40ab	4.03d	1.62a	500.75ab	39.40b	44.75ab	5.14bc	12.90ab	0.39c
$A_1M_0S_1$	95.75ab	8.32a	3.43ab	4.36bc	1.58ab	502.50ab	42.73a	46.62a	5.75a	13.54a	0.42ab
$A_1M_1S_0$	97.37a	8.07ab	3.61a	4.55ab	1.66a	520.50a	43.70a	47.50a	5.70a	12.42abc	0.45a
$A_1M_1S_1$	93.87b	7.96abc	3.32b	4.10cd	1.50abe	483.87bc	38.67b	45.50ab	5.10bc	11.96bc	0.42ab
$A_0M_0O_0$	86.75d	7.65b	3.03d	3.57c	1.21c	321.50f	27.04d	43.25c	4.31b	11.01c	0.39b
$A_0M_0O_1$	94.37abc	7.97ab	3.70a	3.70c	1.46b	466.63de	39.19bc	46.25abc	5.51a	12.78ab	0.43a
$A_0M_1O_0$	91.62c	8.10ab	3.31c	4.08b	1.06ab	454.75e	39.34bc	44.25abc	5.25a	12.44ab	0.42ab
$A_0M_1O_1$	92.12bc	8.16a	3.37bc	4.38b	1.58ab	519.75ab	41.37ab	47.62ab	5.44a	12.99ab	0.41ab
$A_1M_0O_0$	95.50a	8.06ab	3.27c	4.08b	1.46b	466.63de	37.47c	43.87bc	5.33a	11.96bc	0.44a
$A_1M_0O_1$	95.62a	8.32a	3.56ab	4.31b	1.59ab	499.25abc	39.95bc	46.62abc	5.52a	12.92ab	0.42ab
$A_1M_1O_0$	95.12ab	7.80b	3.23cd	4.26b	1.57ab	485.37cd	39.36bc	45.00abc	5.31a	12.42ab	0.42ab
$A_1M_1O_1$	96.12a	8.13a	3.73a	4.76a	1.73a	522.87a	42.94a	48.25a	5.57a	13.52a	0.41ab
$A_0S_0O_0$	85.00c	7.60c	2.93e	3.87b	1.29c	373.50d	30.69d	44.25c	4.13d	11.26b	0.36c
$A_0S_0O_1$	93.87b	8.16a	3.51bc	4.18a	1.60ab	484.00b	40.09b	47.25ab	5.49ab	12.66a	0.43ab
$A_0S_1O_0$	92.62b	7.74bc	3.41cd	3.88b	1.43bc	433.37c	35.71c	43.00c	5.21bc	12.19ab	0.42b
$A_0S_1O_1$	93.37b	7.97abc	3.43cd	4.27a	1.50abc	491.25b	41.31ab	47.62ab	5.62ab	12.82a	0.43ab
$A_1S_0O_0$	95.75ab	8.02ab	2.30cd	4.17a	1.60ab	465.87b	36.57c	44.12bc	5.41ab	12.20ab	0.44a
$A_1S_0O_1$	95.00ab	8.17a	3.67ab	4.41a	1.69a	493.63b	41.57ab	47.75ab	5.65a	13.14a	0.42b
$A_1S_1O_0$	94.25ab	8.18a	3.21d	4.17a	1.44bc	487.25b	36.99c	44.87abc	5.10ab	12.40a	0.41b
$A_1S_1O_1$	97.37a	8.29a	3.75a	4.29a	1.63ab	531.75a	43.73a	48.25a	5.79a	13.37a	0.43ab
$M_0S_0O_0$	87.25c	7.61c	3.00e	3.80c	1.31c	346.87e	28.37d	41.37c	4.27d	11.26d	0.37c
$M_0S_0O_1$	93.50b	8.04ab	3.47bc	3.87c	1.57ab	498.50ab	38.89b	45.87ab	5.38bc	12.67bc	0.42ab
$M_0S_1O_0$	92.87b	8.00abc	3.31cd	3.85c	1.36bc	434.13d	35.73c	43.87bc	5.28bc	12.31bcd	0.42ab
$M_0S_1O_1$	94.25ab	8.13ab	3.47bc	4.21bc	1.62a	517.25a	42.57a	46.25ab	5.61ab	13.14ab	0.42ab
$M_1S_0O_0$	93.25b	8.01abc	3.23d	4.45b	1.58ab	476.00bc	37.85bc	46.12ab	5.52ab	12.63bc	0.43a
$M_1S_0O_1$	97.00a	8.30a	3.82a	4.80a	1.67a	517.87a	43.56a	49.12a	5.80a	13.88a	0.41ab
$M_1S_1O_0$	94.00ab	7.79bc	3.31cd	3.90c	1.48abc	465.13c	37.95bc	45.75ab	5.05c	11.72cd	0.43a
$M_1S_1O_1$	95.12ab	8.26a	3.60b	4.35b	1.58ab	504.87ab	41.74a	46.75ab	5.35bc	12.43bc	0.43a

Means in a column followed by the same letter are not significantly different at P≤0.01.

Table 13. Effects of four-fold interactions of *Azospirillum* (without, A_0; with, A_1), *Mycorrhiza* (without, M_0; with, M_1), *Streptomyces* (without, S_0; with, S_1) and manure (without, O_0; with, O_1) on the measured traits in the third experiment.

Treatments	Plant height (cm)	Spike length (cm)	Stem diameter (mm)	Tillers / plant	Root dry weight (g)	Spike / m^2	Kernels / spike	1000 kernels weight (g)	Grain yield (ton/ha)	Biomass (ton/ha)	HI (%)
$A_0M_0S_0O_0$	79.50d	7.05d	2.70d	3.52e	0.896f	181.75g	20.87g	38.75e	2.90e	9.28d	0.31b
$A_0M_0S_0O_1$	94.50abc	8.12abc	3.92ab	3.62de	1.73abc	505.50bc	40.23bc	46.25abcd	5.72ab	12.75abc	0.44a
$A_0M_0S_1O_0$	91.25c	7.82bc	3.37bc	3.60e	1.20bcde	461.25de	31.77f	42.00de	5.30abcd	11.63c	0.45a
$A_0M_0S_1O_1$	94.00abc	8.32ab	3.40bc	3.80cde	1.52cde	534.50ab	40.55bc	43.75bcde	5.72ab	13.92a	0.41a
$A_0M_1S_0O_0$	90.50c	8.15ab	3.17c	4.62ab	1.48cde	462.50de	37.27cd	45.75abcd	5.36abcd	12.82abc	0.41a
$A_0M_1S_0O_1$	92.75bc	8.20ab	3.27bc	4.77a	1.69abc	540.20ab	42.20b	49.25ab	5.52abc	13.25abc	0.41a
$A_0M_1S_1O_0$	90.25c	8.05abc	3.45bc	3.55e	1.20ef	450.50f	33.21ef	42.50cde	5.27abcd	11.46c	0.45a
$A_0M_1S_1O_1$	94.00abc	8.12abc	3.47bc	4.75a	1.63abcd	447.00e	38.23cd	46.00abcd	5.51abc	11.80c	0.46a
$A_1M_0S_0O_0$	95.00abc	8.17ab	3.30bc	4.07cd	1.52bcde	512.00bc	35.87de	44.00bcd	5.63abc	12.65abc	0.44a
$A_1M_0S_0O_1$	96.50ab	8.47a	3.57b	4.10c	1.73abc	529.00ab	42.93b	45.50abcd	5.70ab	12.75abc	0.44a
$A_1M_0S_1O_0$	96.25ab	7.95abc	3.25bc	4.00cde	1.50bcde	469.00cde	42.50b	46.75abcd	5.03cd	13.25abc	0.37a
$A_1M_0S_1O_1$	97.50ab	8.17ab	3.55b	4.62ab	1.95a	469.50cde	42.95b	49.00ab	5.68ab	13.21abc	0.42a
$A_1M_1S_0O_0$	96.50ab	7.87abc	3.30bc	4.27bc	1.46cde	470.50cde	40.50bc	47.50abcd	5.26abc	12.00c	0.43a
$A_1M_1S_0O_1$	98.25a	8.40ab	4.07a	4.82a	1.86ab	565.25a	46.90a	49.75a	5.88a	13.83a	0.42a
$A_1M_1S_1O_0$	93.75bc	7.52cd	3.17c	3.95cde	1.31de	462.75de	37.67cd	42.00de	4.83d	11.80c	0.40a
$A_1M_1S_1O_1$	94.00abc	8.40ab	3.47bc	4.25bc	1.68abc	505.00bcd	39.67bcd	45.75abcd	5.17bcd	12.11bc	0.42a

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

DISCUSSION

Results of the first experiment indicated that phosphorus application significantly affected most of the measured traits, mainly the reproductive ones such as the number of kernels in panicle and grain yield. P application also increased harvest index (HI), representing that high P concentration in soil conducts assimilates to the reproductive organs and results in the improvement of grain production rather than vegetative growth (Tables 3 and 5). Phosphorus is a macronutrient highly required by plants. It plays role in energy transfer, cell membranes, nucleic acids and other key compounds [7, 15]. The effect of P on plant growth and yield is well reported by other researchers. The results of a study represented that the maximum number of tillers/plant and grain yield were achieved when 80 kg P/ha was applied and the minimum number of tillers/plant was obtained in the control [16]. In another experiment it was concluded that increased P application rate enhanced grain yield and protein content of two cowpea cultivars [17]. Concerning the effect of P fertilizer on HI, Malik *et al.* (2006) reported the significant effect of phosphorus application rates on HI of soybean [18].

Application of nitrogen in the second experiment significantly affected all the measured traits. Presence of sufficient N in soil is vital for both vegetative and reproductive growth stages; affecting all the measured traits from plant height to grain yield (Table 6). N is a component of amino acids, nucleic acids, enzymes and proteins, chlorophyll and cell wall structure and is involved in many physiological and biochemical processes in plants [7, 19]. Regarding the key roles N plays in plants, numerous experiments have studied the effect of N application in approximately all plants. In an experiment it was concluded that N application considerably affected wheat root length at different soil depths, root weight and root-shoot ratio [20]. Njuguna *et al.* (2010) found that N application increased the number of tillers, panicles and grain yield in wheat [21]. They observed that the highest N rate (46 kg/ha) resulted in the highest grain yield (1176.7 kg/ha) compared with the control (0 kg N/ha) which resulted in the lowest grain yield (1022.9 kg/ha). Shekoofa and Emam (2008) also reported that application of 200 kg N/ha increased wheat plant height by 4.1% and grain yield by 84.86%, compared with the control (0 kg N/ha) [22].

Azospirillum was the factor studied in both the second and the third experiments. In the second experiment, inoculating wheat seeds with *Azospirillum* significantly affected all the measured traits except for 1000 kernels weight; however, in the third experiment, application of *Azospirillum* only affected some of the measured traits. For example, panicle length, biomass and HI are the traits significantly affected by *Azospirillum* only in the second experiment not in the third (Tables 6 and 9). The reason, in addition to the effect of site and year, may be attributed to the functions of *Azospirillum* and the effects of chemical N on them. The most important roles of *Azospirillum* are biological nitrogen fixation (BNF) and phytohormones production. In our second experiment it was observed that grain yield was significantly the same in N_3A_0 (150 kg N/ha without *Azospirillum*) and N_1A_1 (50 kg N/ha + *Azospirillum*); indicating that *Azospirillum* can fix atmospheric N and compensate low chemical N application rate and give acceptable yield (Table 7). Yadav *et al.* (2011) also concluded that the combined application of *Azospirillum* and 60 kg N/ha produced comparable results (plant height and yield in maize) to those obtained due to the application of the recommended dose of fertilizers (120 kg N/ha) [5]. About the phytohormones production ability of *Azospirillum* spp., researchers found that the levels of indole-3-acetic acid (IAA) and ethylene, phytohormones related to plant growth, were higher in *A. brasilense* FT 326 inoculated tomato plants which resulted in the improvement of shoot and root system development [4].

In addition to BNF and phytohormones production, *Azospirillum* affects plant growth by improving plant root system development and nutrient uptake. In our third experiment it was detected that *Azospirillum* inoculation significantly increased root dry weight by about 9.65%, compared with the control (Table 10). Other researchers have reported the effect of *Azospirillum* on plant root system development and nutrient uptake. In an experiment on tomato it was observed that inoculation with *A. brasilense* FT 326 increased shoot and root fresh weight, main root hair length, and root surface of tomato plants [4]. Ardakani *et al*. (2011 b) reported that inoculating wheat seeds by *Azospirillum* increased plant NPK content by 21.68%, 17.14% and 25.08%, respectively [23].

Azospirillum inoculation is also effective on plant's tolerance against environmental stresses such as drought and salinity. In an experiment, lettuce seeds tolerated NaCl better when inoculated with *Azospirillum* and had superior germination and growth compared with the non-inoculated control [24].

Now, it can be discussed why the application of *Azospirillum* was more effective on wheat yield and yield components in the second experiment rather than the third experiment. It was mentioned that *Azospirillum* affects plant growth mainly through BNF. On the other hand, in the second experiment, nitrogen was one of the treatments so there were plots which received no N fertilizer before or after planting wheat but were inoculated with *Azospirillum*. Therefore, low soil N content made *Azospirillum* BNF system more efficient; the effect was more visible on the measured traits. However, in the third experiment, N fertilizer was not among the treatments and was added to soil in all plots in the same rate; the improved soil N content made *Azospirillum* BNF system less efficient.

Mycorrhiza inoculation, in the first and the third experiments, significantly affected most of the measured traits. *Mycorrhiza* is a symbiotic fungus with several beneficial effects on plants. The fungus hyphae are capable of absorbing water and nutrients and when connected to plant roots, provide an extra absorptive organ to plants. These hyphae penetrate into soil pores and cracks; helping plant roots to take up water and nutrients more efficiently. This enables plants to use more volume of soil as water and nutrient pool, and grow better under low soil nutrient and water content [6, 25]. Neumann and George (2009) conducted an experiment to evaluate the effect of mycorrhizal symbiosis on cowpea (*Vigna unguiculata*) growth and nutrient uptake and concluded that the symbiosis increased water and nutrients uptake under drought stress condition [26]. In other experiments it was proved that mycorrhizal inoculation significantly affected lucerne root system development [27] and improved wheat Fe, Mn, Zn and Cu uptake by 10.12%, 18.06%, 12.31% and 12.81%, respectively [28].

Mycorrhiza is also a phosphate solubilizing fungus which effectively provides soil fixed P to plants because of some organic acids production. Result of our study (the first experiment) showed that P_3M_0 (90 kg P/ha without *Mycorrhiza*) and P_1M_1 (30 kg P/ha + *Mycorrhiza*) gave significantly the same grain yield (Table 5); this verifies that *Mycorrhiza* may be used instead of higher doses of chemical P fertilizer because of the ability to release the fixed P in soil. Other studies have also reported the effect of mycorrhizal inoculation on plant growth, nutrient uptake and yield. In an experiment on *Eucalyptus globulus*, application of *Mycorrhiza* significantly increased shoot dry weight [29]. Al-Karaki *et al*. (2004) studied the effect of mycorrhizal inoculation on wheat and attributed the enhanced productivity to enhanced uptake of nutrients such as P, Zn and Cu [30]. Farzaneh *et al*. (2009) concluded that inoculating barley with *Mycorrhiza* increased the total dry matter at maturity by up to 43% [8]. Pharudi (2010) also observed an improved growth and health in wheat and maize when inoculated with *Mycorrhiza* and reported that the improvement may be resulted by the enhanced water and nutrient uptake and moreover, the synergistic relation of *Mycorrhiza* with other beneficial soil microorganisms [31]. Our results also confirm the synergistic relation of *Mycorrhiza* and *Azospirillum* as A_1M_1 was more effective on all the measured traits than A_1M_0 or A_0M_1, although in some cases the differences were not significant (Table 11).

Streptomyces had no significant effect on any of the measured traits in any of the experiments. However in two-fold interactions, *Streptomyces* showed synergistic effect on P, N, *Azospirillum* and manure. For example in the third experiment, although *Streptomyces* had no effect on grain yield, in two-fold interaction with *Azospirillum* influenced grain yield in the way that A_1S_1 was significantly superior to A_1S_0 (Table 11). *Streptomyces* is a Plant Growth Promoting Rhizobacterium (PGPR) which affects plant growth and seed germination by producing compounds such as indole acetic acid, phosphate solubilizing substances, chitinase and siderophores. In addition, *Streptomyces* produces antimicrobial compounds and also colonize the root surface of host plant which protect plant root against pathogens [9-10]. Soe *et al*. (2010) represented that inoculating soybean plants with *Streptomyces* increased root nodules dry weight at V6 stage from 0.17 g/plant to 0.19 g/plant [32]. Sardi *et al*. (1992) also found that *Streptomyces* protected wheat against fusarium and increased yield [11]. Unlike the synergistic effect, *Streptomyces* may have negative impacts on some soil microorganisms because it produces many antimicrobial compounds and some isolates may also have direct negative impacts on plants [9]. In our experiment it was observed that *Streptomyces* had antagonistic relation and inhibited activity of *Mycorrhiza*. For example in both the first and the third experiments, M_1S_0 gave significantly higher grain yield than M_1S_1 (Tables 5 and 11). Venkatachalam *et al*. (2010) found that *S. gibosonni* and *S. grieseoletus* increased seed germination by producing polyamines while *S. viridochrogenes* and *S. clavifer* inhibited seeds germination by producing phosphinothricin [33]. Samaca *et al*. (2003) also studied the effect of

Streptomyces on alfalfa nodulation under in-vitro conditions and reported that colonization and activity of alfalfa nodules and the associated microorganisms were inhibited by *Streptomyces* [9].

The last factor evaluated in this study (only in the third experiment) was animal manure. Application of manure significantly affected all measured traits except for HI (Table 9). Application of animal manure has many beneficial effects on soil and plants. Animal manure may contain high or low amount of macro and micronutrients required for plant growth, depending on the animals type and their forage, manure storage, processing and application methods [12, 19]. Although animal manure may have low and variable nutrient contents, application of animal manure affects soil physical, chemical and biological properties. The most important impact of manure on soil is the improvement of soil organic matter which consequently balances soil pH, CEC and temperature, stabilizes soil aggregates and reduces erosion, increases aeration, enhances soil water-holding and buffering capacity, provides food and energy for soil microorganisms and creates a perfect soil environment for plant growth [12, 19, 34, 35, 36].

The previously mentioned effects of animal manure on soil and plants have been reported by various researchers. Manure may be a source of nutrients which releases macro and micronutrients during decomposition. In an experiment on the effect of manure and NPK on soil, it was indicated that application of manure increased the total N content to 0.36% from 0.22% in the control [35]. Zhang *et al*. (2006) also reported that application of liquid cattle manure significantly increased soil mineral N concentration and exchangeable K compared with the control [37].

Soil pH is an important factor which influences the availability of mineral nutrients to plant roots. Schoenau (2006) reported that animal manures with low organic matter and high ammonium nitrogen content (such as liquid hog manure) can significantly decrease soil pH when repeatedly applied at high rates but manures with high organic matter and carbonate content can raise the pH of acid soils [12]. Soil Cations Exchange Capacity (CEC) is the maximum cations holding capacity of a soil and represents the fertility of the soil. Mujiyati and Supriyadi (2009) concluded that application of manure increased soil CEC by 24.11% compared with the control [35].

Application of animal manure improves soil physical properties and may protect soil against erosion, depending on the manure type. Assefa (2002) concluded that applying hog manure on the Gray Luvisolic soils increased aggregation of the soil [38]. Moreover, cattle and hog manure application increased water infiltration rate compared with the control. Ramos *et al*. (2006) also detected that applying slurry on the soil surface had a protective effect on soil and reduced soil detachment by up to 70% [36].

Another function of animal manure is the improvement of soil microorganisms' population as it provides food and is source of energy for soil microorganisms. Niewiadomska *et al*. (2010) studied the effects of manure application on soil bacteria in maize cropping and concluded that incorporating manures into soil slightly increased the number of ammonification bacteria [39]. Mujiyati and Supriyadi (2009) found that application of manure increased *Azotobacter* population by 29% and *Azospirillum* population by 68%, compared with the control [35]. Roeslan (2004) also reported that the manure application can promote soil *Azospirillum* population [40]. Improving soil microorganisms' activity as the result of manure application was also observed in our experiment. Acetylene Reducing Activity (ARA) which represents the biological nitrogen fixation by *Azospirillum* was significantly affected by manure. In the third experiment, application of manure increased ARA by 9.6%, indicating that manure has encouraged the activity of *Azospirillum* (data not shown).

From the above discussed benefits of animal manure it can be finally concluded that manure creates a perfect soil environment for plant growth. In our experiment it was indicated that application of manure increased wheat root dry weight by 8.2%, plant height by 2.44% and grain yield by 9.1% compared with the control (Table 10). Mujiyati and Supriyadi (2009) tested the effect of manure on chili and observed that application of manure significantly increased yield from 0.850 kg/plant to 0.973 kg/plant [35]. Ojeniyi *et al*. (2007) found that application of animal manure significantly improved leaf N and K, plant height, the number of branches, leaf area, the number and weight of fruits of tomato [13]. Ardakani *et al*. (2011 b) also reported that application of manure increased wheat grain and plant P content by 1.15% and 23.53%, respectively [23].

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