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IMPACT OF LIVESTOCK MANURE, NITROGEN AND BIOFERTILIZER (AZOTOBACTER) ON YIELD AND YIELD COMPONENTS OF WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT. Integrated nutrient management strategies involving chemical and biologic fertilizer is a real challenge to stop using the high rates of agrochemicals and to enhance sustainability of crop production. In order to study the effects of livestock manure, chemical nitrogen, and biologic (*Azotobacter*) fertilizers on yield and yield components of wheat, an agricultural experiment in the form of split factorial design with three replications was conducted in Elam region, Iran. The aim of this research was assessment of the effects of these fertilizers separately and in integrated forms; and setting out the best fertilizer mixture. The results showed that treatment with livestock manure, *Azotobacter* and chemical nitrogen increased plant height, biological and grain yield. Using livestock manure and *Azotobacter* increased biologic yield through increase in plant height which cause to increase in grain yield without any significant changes in harvest index and other yield components, but the use of chemical nitrogen caused an increase in plant height, No. of spikelete/spike, No. of grain/spike, one thousand grain weight and harvest index, biologic and grain yield. In the light of the results achieved, we may conclude that using livestock manure and chemical nitrogen fertilizer together with the *Azotobacter* had the maximum impact on yield; and that we can decrease use of chemical fertilizers through using livestock manure and biologic fertilizers and to reach to the same yield when we use only chemical fertilizers.

Key words: Wheat; Livestock manure; *Azotobacter*; Nitrogen fertilizer.

INTRODUCTION

More recently, a real challenge for the workers in agricultural research field to stop using the high rates of agrochemicals which negatively affect human health and environment. Large quantities of chemical fertilizers are used to replenish soil N, resulting in high costs and severe environmental contamination (Dai *et al.,* 2004).

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Many attempt have been tried to replace a part of those harmful fertilizers by biofertilizer (El Kholy *et al.,* 2005). Integrated nutrient management strategies involving chemical fertilizer and biofertilizer have suggested to enhance the sustainability of crop production (Manske *et al.,* 1998). Biofertilizers are able to fix atmospheric nitrogen in the available form for plant (Chen, 2006) and have beneficial upon plant growth by production of antibiotic (Zahir *et al.,* 2004). *Azotobacter* is used as biofertilizer in the cultivation of most crops (Yasari *et al.,* 2007; Jarak *et al.,* 2005; Pati *et al.,* 1995). It can successfully grow in the rhizospheric zone of wheat, corn, rice and many other crops (Jadhav *et al.,* 1987). The goal of this paper was to study the effects of livestock manure, chemical nitrogen, and biologic fertilizers (*Azotobacter*) on yield and yield components of wheat.

MATERIALS AND METHODS

In order to study the impact of livestock manure, nitrogen, and biologic (mixing with stimulant bacteria) fertilizer on quantitative traits of wheat, an agricultural experiment was conducted in Elam region, Iran in the longitude of 46°,25’ and the latitude of 33°,38’ in the form of split factorial design in three replications. Experimental factors include livestock manure on two levels (used and unused), were allocated to the main plots, chemical nitrogen fertilizer on two level (used and unused), and *Azotobacter* biofertilizer on two level (inoculated and uninoculated) in the form of factorial were allocated to the sub plots. Livestock manure by the rate of 1 ton/ha was added in accordance with experimental design and nitrogen fertilizer was added in accordance with experimental design and soil test. Inoculation with *Azotobacter* was carried out as follows: after pouring wheat grains into a polyethylene sack, 30 ml of 20% sugar solution added to it, then the sack containing grains and adhesive material shake for a period of 30 seconds in such an extent that all surfaces of grains being adhesive homogenously. Then the inoculated liquid was added to adhesive grains sufficiently in such an extent that all surfaces to be covered by liquid. After 45 seconds of shaking and making sure of homogenous adhesion of inoculated liquid to grains, grains inoculated by liquid spread out on aluminum sheets under shade to dry. Then grains planted. Cultivation performed on rows with the depths of 3 to 5 cm. the cultivar we used was “Bahar M-79-7” which is among early cultivars certified by Agricultural Research Center of this region.

Each experimental plot comprises 10 rows with the row distances of 25 cm and along 4 m each. Phosphorous and potassium fertilizers were used in accordance with soil test and requirements of plants. According to the result of soil test, 280 kg/ha urea (0.46 nitrogen) and 60 kg/ha P_2O_5 in the form of triple superphosphate, and 10 Kg/ha K_2O in the form of potassium sulfate were added to the soil.

A half quantity of urea and all quantities of other fertilizers were mixed by soil before cultivation. The rest of urea fertilizer was used at the stages of tillering and stem elongation. Weeds were also controlled manually during the growth season. subject matter of our measurement were: plants height, number of spike per square meter, number of grain per each spike, one thousand grain weight, grain yield, biologic yield, and harvest index. The data were analyzed by SAS software and the means were compared by Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Livestock manure

The result of variance analysis indicated that the treatment of livestock manure had significant effect (P=0.01) on plant height, grain yield and biologic yield (*Table 1*). This research showed that biologic yield was at its maximum rate i.e. 6822 kg/ha with the treatment of using livestock manure and was at its minimum rate, standing at 5800 kg/ha while no livestock manure was used (treatment of unused livestock manure) (*Table 2*). Livestock manure had increased biologic yield by the rate of 15%. We may conclude that livestock manure with a proper decay rate will prepare good nutritional condition for plant through gradual freeing of nutritional elements and amending soil texture and this lead to increase in plant height and increase in biomass so that although the harvest index was not affected by the treatment, grain yield was increased through increase in biomass. Demotes *et al.* (2004) also reported that using organic fertilizers led to increase in biologic and grain yield.

Biofertilizer

The result of analysis of variance showed that biofertilizer had significant effect on height, grain yield and biologic yield (*Table 1*).

Infecting wheat grains by *Azotobacter* led to a height increase equal to 14% (*Table 3*). This is because of increase in absorption of nutrients by plant, improvement of soil characteristics such as contents of organic materials and increase in accessible nitrogen. In general, accessibility of water and essential nutrients would affect the height of wheat crops via increasing nodes and internodes. Shaalan (2005) showed that infecting grains of fennel (*Nigella sativa*) by biologic fertilizers such as *Azospirillum* and *Azotobacter* resulted in improvement of growing traits of plant, such as height. Zahir *et al.* (1998) also reported an 8.5% increase in height of corn which were infected by *Azotobacter* and *Pseudomonas.*

According to *Table 3,* the treatment of using *Azotobacter* leading to grain yield of 3360 kg/ha has a significant effect of 15% more than treatment without inoculation by *Azotobacter* with the grain yield of 2839 kg/ha. It is may be due to positive relation of wheat and this bacterium. Wani *et al.* (2007) studied synergetic impacts of inoculation of pea by *Azotobacter* and found that combined inoculation by *Azotobacter* and *Pseudomonas* would considerably improve node creation and absorption of nutrients and would help better absorption of nitrogen, phosphate and root expansion. Using these bacteria increased growth and production of pea. Narula *et al.* (2000) presented the impact of *Azotobacter* on solving inorganic phosphate and on an increase of growth of wheat.

Table 1 - Analysis of variance of the effect of manure, *Azotobacter* and nitrogen on yield and yield component of wheat (mean square)

Harvest index	Biologic yield (kg)	Grain yield (kg)	Spike/m^2	1000 grain weight	Grain/ spike	Spikelet /spike	Stem diameter (cm)	Height (cm)	DF	s.v
26.74 ^{ns}	8717737 *	3066714 ^{ns}	2906.5 ^{ns}	143.3 ^{ns}	14.72 ^{ns}	128.1 ^{ns}	0.79 ^{ns}	668.64 ^{ns}	2	Block
32.55 ^{ns}	6267926 **	2703459 **	25480.1 ^{ns}	191.53 ^{ns}	149 ^{ns}	79.9 ^{ns}	3.41 ^{ns}	1626.9 **	1	Manure (M)
20.56	60430	115724.6	1577.7	4.74	12.14	12.56	0.12	28.98	2	E_a
101.72 ^{ns}	1823259 *	1628125 *	5520.6 ^{ns}	55.81 ^{ns}	38.00 ^{ns}	22.8 ^{ns}	0.77 ^{ns}	596 **	1	Azotobacter (A)
132.96 **	10006542 **	5145782 **	45762.6 **	340.50 **	197.22 **	365.04 **	1.70 ^{ns}	1109.7 **	1	Nitrogen (N)
74.65 ^{ns}	375750 ^{ns}	80157 ^{ns}	352.6 ^{ns}	1.50 ^{ns}	0.20 ^{ns}	0.20 ^{ns}	0.40 ^{ns}	226.93 ^{ns}	1	A*M
3.51 ^{ns}	285798 ^{ns}	89426 ^{ns}	2.6 ^{ns}	1.21 ^{ns}	3.84 ^{ns}	3.22 ^{ns}	0.21 ^{ns}	77.04 ^{ns}	1	N*M
9.23 ^{ns}	76275 ^{ns}	1080 ^{ns}	204.1 ^{ns}	0.73 ^{ns}	2.4 ^{ns}	38.50 *	0.007 ^{ns}	8.88 ^{ns}	1	N*A
5.73 ^{ns}	421085 ^{ns}	32047 ^{ns}	2521.5 ^{ns}	3.22 ^{ns}	1.70 ^{ns}	4.86 ^{ns}	0.042 ^{ns}	64.02 ^{ns}	1	N*A*M
13.36	184120	79223	3041.38	13.20	13.28	2.65	0.34	60.79	12	E_{bc}
7.52	6.7	9.07	18.9	10.9	13.2	6.9	21.02	12.17	-	Cv%

ns=Non significant, *=$p<0.05$,**=$p<0.01$

Table 2 - Means comparison of livestock manure levels on yield and yield component of wheat

Harvest index	Biologic yield (kg)	Grain yield (kg)	Spike/m^2	1000 grain weight	Grain/ spike	Spikelet /spike	Stem diameter (cm)	Height (cm)	Treatment
49.75 a	58000 b	2764 b	258.5 a	30.35 a	25.06 a	21.46a	2.40 a	55.82 b	With manure
47.42 a	6822 a	3435 a	323.0 a	36.00 a	30.05 a	25.11 a	3.16 a	72.29 a	Without manure

The averages in each column with the same letters have no significant difference with each other on 5% level of Duncan test.

Table 3 - Means comparison of *Azotobacter* levels on yield and yield component of wheat

Harvest index	Biologic yield (kg)	Grain yield (kg)	Spike / m^2	1000 grain weight	Grain /spike	Spikelet /spike	Stem diameter (cm)	Height (cm)	Treatment
46.52 a	6035 b	2839 b	275 a	31.65 a	26.3 a	22.31 a	2.96 a	59.07 b	With Azotobacter
50.64 a	6586 a	3360 a	306 a	34.7 a	28.81 a	24.26 a	2.60 a	69.04 a	Without Azotobacter

The averages in each column with the same letters have no significant difference with each other on 5% level of Duncan test.

Table 4 - Means comparison of nitrogen levels on yield and yield component of wheat

Harvest index	Biologic yield (kg)	Grain yield (kg)	Spike/m^2	1000 grain weight	Grain/ spike	Spikelet /spike	Stem diameter (cm)	Height (cm)	Treatment
46.23 b	5665 b	2637 b	247 b	29.41 b	24.69 b	19.39 b	2.51 a	57.25 b	Without nitrogen
50.94 a	6956 a	3563 a	334 a	36.95 a	30.42 a	27.19 a	3.05 a	70.85 a	With nitrogen

The averages in each column with the same letters have no significant difference with each other on 5% level of Duncan test.

Table 5 - Mean comparison of interaction between livestock manure* *Azotobacter* on measured traits

Harvest index	Biologic yield (kg)	Grain yield (kg)	Spike/m^2	1000 grain weight	Grain / spike	Spikelet /spike	Stem diameter (cm)	Height (cm)	Treatment
47.13 b	5399 b	2561 c	247 a	28.58 b	23.71 b	20.58 c	2.35a	53.9 b	A_1B_1
47.72 ab	6200 a	2967 bc	269 a	32.13 ab	26.41 ab	22.35 bc	2.45 a	57.7 b	A_1B_2
45.93 ab	6671 a	3117 b	304 a	34.73 ab	28.88 ab	24.05 ab	2.85 a	64.2 b	A_2B_1
53.58 a	6972 a	3754 a	342 a	37.29 a	31.21 a	26.18 a	3.47 a	80.3 a	A_2B_2

The averages in each column with the same letters have no significant difference with each other on 5% level of Duncan test.
A_1= without manure; A_2 = with manure; B_1 = without ***Azotobacter***; B_2 = with ***Azotobacter***

Biofertilizer had positive impact on the biologic yield (*Table 3*). The results show that the maximum biologic yield belongs to the treatment by biologic fertilizer, standing at 6586 kg/ha, and the minimum one belongs to treatment without biologic fertilizer, standing at 6035 kg/ha. Using *Azotobacter* would result in significant increase of biologic yield by the rate of 9%. Stancheva *et al.* (1992) showed that dry weight of corn would increase by inoculation by *Azotobacter*. Bath. *et al.* (2005) stated that inoculation of vetch by *Azotobacter* resulted in significant increase in biologic yield of this plant. They concluded that it was because of better accessibility and absorption of nutrients leading to an increase in dried biomass in vetch. Tilak *et al.* (1982) stated that the effect of inoculation of corn and sorghum by *Azotobacter* and *Azospirillum* on aerial organs of these plants is considerable. All these reports attest the results of our experiment.

Nitrogen fertilizer

The result of analysis of variance in *Table 4* shows significant effect of treatment by nitrogen fertilizers on the traits of wheat subject matter of our research except for the trait of stem diameter. The comparison of means of different levels of nitrogen fertilizer shows that using such fertilizer would increase plant height so that the maximum height was 70.87 cm and the minimum height was 57.25 cm. This result shows the positive effect of nitrogen fertilizer on increase of height (*Table 4*).

Number of spikelet per spike was 27.19 and 19.39 in treatment of use and non-use of chemical nitrogen fertilizer, respectively.

Number of spikelet in each spike shall be numerated one of the major elements of yield. Nitrogen fertilizer may be increased primordial cells in spikelet, therefore they increased in treatment by nitrogen fertilizer finally ending on increase in number of grains. Therefore, grain yield increased at this treatment.

Means comparison of different levels of nitrogen fertilizer shows that maximum number of grain per spike was 30.42 at the treatment of nitrogen fertilizer and the minimum one was 24.69 at the treatment in which such fertilizer was not used. Our result was in consents with other research. Rasmussen *et al.* (1997). Demotes *et al.* (2004) showed that nitrogen deficiency during the growth period of spike or after pollination would decrease dry weight of spike as well as number of grains in spike.

Use of nitrogen fertilizer would increase weight of one thousand grains by the rate of 13% (36.95 g in comparison with 29.41 in the treatment of use and without use of nitrogen, respectively *Table 4*). Results shows that although number of grains in spike increased, as the result of increase in nitrogen fertilizer, photosynthesis and leaf area duration was increased resulted in source strength and finally increase in grain weight. El-kholy *et al.* (2005) reported that optimal use of nitrogen will significantly increase grain weight and grain yield.

Use of chemical nitrogen fertilizer caused an increase in number of spike per square meter. Generally, nitrogen fertilizer would improve tillering and this caused an increase in the number of fertile spike per square meter. Ayoub *et al.* (1994) showed that proper use of nitrogen would increase grain yield in wheat through increasing numbers of spike; and that number of grain per spike would have lesser role in increasing of grain yield.

Treatment with chemical nitrogen fertilizer with a grain production rate of 3563 kg/ha had the maximum rate of this trait and treatment without chemical fertilizer had the minimum rate of grain yield with a quantity of 2637 kg/ha (*Table 4*). This shows that in treatment with chemical fertilizer grain yield will increase as the result of nutrients accessibility for the plant because of more absorption of them by plant and increase in growth by increase of leaf area index.

Chemical fertilizer would increase biologic yield. Increase in biologic yield (biomass) is the result of efficient benefit from ambient conditions and nutrients by plant; when nutritional and growth condition is good, plant will grow well and with established with a proper density, and will reach to a proper leaf area index in a short period of time; reaching to an optimal leaf area index will impact both biologic and grain yield. Mc Donald (1992) in his study on the effects of different levels of nitrogen fertilizer on yield of different cultivars of wheat in 10 regions of Australia reported that dry biomass of plant would significantly increase during pollination while use of nitrogen increases.

Using nitrogen fertilizer would significantly increase harvest index by the rate of 8% (*Table 4*). In fact, harvest index represents efficiency of plant in transfer of photosynthetic materials from source to sink. Nitrogen fertilizer having impact on partitioning of dry matter and allocating much dry biomass to grain would increase grain yield and harvest index. Paknezhad (1995) having studied the effect of different levels of nitrogen fertilizer on the growth and qualitative and quantitative yield of Falat cultivar of wheat reported that higher levels of nitrogen had higher harvest index.

There was no significant interactions between use of livestock manure and *Azotobacter*, livestock manure and chemical fertilizer on any trait subject matter of our experiment. However, comparison of means shows that combined use of these fertilizers had increased all traits of plant in comparison with control treatment (*Tables 5 and 6*). The result of research on corn also shows that combined treatments of livestock manure and biologic fertilizers would increase such traits as number of grain per row, weight of one thousand grains, and grain yield, which is somehow complied with our research (Mentler *et al.,* 2002).

Table 6 - Mean comparison of interaction between livestock manure* chemical nitrogen fertilizer on measured traits

Harvest index	Biologic yield (kg)	Grain yield (kg)	Spike/m^2	1000 grain weight	Grain / spike	Spikelet /spike	Stem diameter (cm)	Height (cm)	Treatment
44.69b	5236c	2362c	214b	26.8c	22.6b	17.2d	2.04b	47.2b	A_1C_1
50.16ab	6336b	3166b	302ab	33.9ab	27.5ab	25.7b	2.76ab	64.4a	A_1C_2
47.78ab	6067b	2911b	280ab	32.1bc	26.7ab	21.5c	2.98ab	67.2a	A_2C_1
51.72a	7577a	3959a	366a	40.0a	33.3a	28.6a	3.33a	77.3a	A_2C_2

The averages in each column with the same letters have no significant difference with each other on 5% level of Duncan test.
A1= A_1= without manure; A_2 = with manure; C_1 = without chemical nitrogen; C_2 = with chemical nitrogen;

Table 7 - Mean comparison of interaction between *Azotobacter** chemical nitrogen fertilizer on measured traits

Harvest index	Biologic yield (kg)	Grain yield (kg)	Spike/m^2	1000 grain weight	Grain/spike	Spikelet /spike	Stem diameter (cm)	Height (cm)	Treatment
43.5ab	5446c	2383c	229b	27.71c	23.11b	19.68c	2.34a	52.8b	B_1C_1
49.5ab	6624ab	3296b	322ab	35.6ab	29.48ab	24.95b	2.86a	65.2ab	B_1C_2
48.91ab	5884bc	2890b	265ab	31.11bc	26.26ab	19.1c	2.69a	61.6b	B_2C_1
52.38a	7288a	3330a	346a	38.3a	31.36a	29.43a	3.23a	76.4a	B_2C_2

The averages in each column with the same letters have no significant difference with each other on 5% level of Duncan test.
B_1 = without ***Azotobacter***; B_2 = with ***Azotobacter***; C_1 = without chemical nitrogen; C_2 = with chemical nitrogen

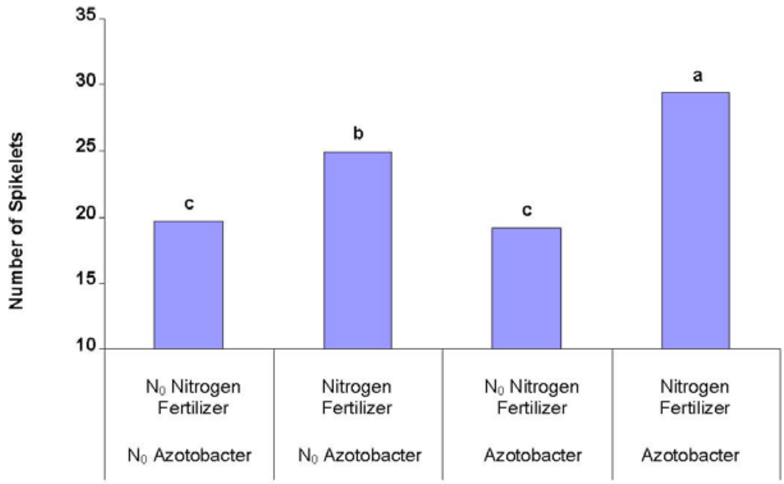

Figure 1 – Means comparison of correlative use of biologic and chemical (nitrogen) fertilizers on number of spikelet

Perhaps livestock manure has decreased its activity in the presence of nitrogen fertilizer in such an extent that has failed to have significant effect on the traits. Alexander and Zuberer (1988) reported that existent of ammonium with concentration of 4 mg/liter had decreased acetylene reduction activity in corn.

The results of variance analysis in *Table 1* show that there was a significant interaction between use of biologic and chemical (nitrogen) fertilizers only on the trait of "number of spikelet /spike" and no significant effect on other traits (*Table 7*). In reactions of treatment with chemical fertilizer and inoculation by *Azotobacter* it was revealed that use of nitrogen fertilizer together with *Azotobacter* will produce the highest mean of number of spikelet i.e. 29.4 (*Fig. 1*). Therefore, we can conclude that presence or absence of *Azotobacter* would have no effect on number of spikelet when nitrogen fertilizer is not used. But when nitrogen fertilizer is used, inoculation by *Azotobacter* would be effective on this trait; in another word, *Azotobacter* would play role in absorption of soil's nitrogen more than in biologic al nitrogen fixation. Considering the role of *Azotobacter* in production of Indole Acetic Acid (IAA) and in increasing IAA in rhizospher area, it would increase growth and development of roots; and whereas wheat had nitrogen fertilizer in access, it better absorbed nutrients with its developed root; and could increase number of spikelet per spike; this complies with the report of Vande Broek (1999).

The results show that combined use of livestock manure, biologic and chemical (nitrogen) fertilizers had no significant effect on the traits subject matter of our experiment. However, in means comparison of correlative use of these three fertilizers it was revealed that using livestock manure and nitrogen fertilizer in presence of *Azotobacter* would have maximum effect on the traits subject matter of our experiment.

CONCLUSIONS

Use of chemical nitrogen led to an increase in yield components through which yield increase, but livestock manure and biologic nitrogen fertilizer increase grain yield through increase in plant height which cause to an increase in biologic yield without any changes in harvest index. Although there was no significant difference between these forms of nitrogen fertilizers in yield increase, we may conclude that using livestock manure and biologic nitrogen fertilizer together could reduce N application without any significant reduction of grain yield.

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