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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of Plant growth Promoting Bacteria (*Azospirillum, Azotobacter, Pseudomonas*), Humic acid and Nitrogen Fertilizer on Growth and Yield of Wheat

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ABSTRACT
Application of bio fertilizers, especially nitrogen fertilizer as bacteria combined with the consumption of chemical fertilizers, is one of some charachteristics the important strategy for sustainable management of farming structures and increases their production in a sustainable farming system with enough inputs. To evaluate the effect of plant growth promoting bacteria (Azospirillum, Azotobacter, Pseudomonas), Humic acid and nitrogen fertilizer on growth and yield of Wheat. A factorial experiment in completely randomized design with four replications in 2010 at the Agricultural Research Station farm located in Karaj Mahdasht was performed. The factors examined included four levels of urea nitrogen fertilizer based on soil test values (0,100,200,300 kg/ ha) and two levels of bacteria and bacterial growth, including use as control and also, The third factor included two levels as well as the use and non application of an humic acid was is order. The survey results showed that the use of nitrogen fertilizers, growth stimulants and Humic acid bacteria on grain weight, grain yield, plant height, spike weight, biological yield. There was a significant difference compared to control (p<0.01). Interaction of two factors which also showed that there was a significant increase in most of characters. So that, Comparison of interaction between nitrogen(300 kg/h) with plant growth promoting rhizobacteria 5151 kg/h highest Grain yield and controls treatments lowest yield was 2943 kg/h. Results showed that Application of bio fertilizers activity increased to effect yield of Wheat.
Key-words: *Plant growth promoting bacteria, Nitrogen, Humic acid, yield of Wheat.*

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INTRODUCTION
In recent years, a major factor in increasing the yield of crops, consumed by a variety of inputs, especially chemical fertilizers. In recent years application of chemical fertilizers has created a revolution in the production of crops. So that, in 1998, about 365 million tons of fertilizer in the world, at a level equivalent to 1.4 billion hectares is being used. In recent decades, with the increasing use of chemical fertilizers serious environmental and economic problems in society imposed. In this regard, extensive efforts to find appropriate ways to improve the quality of soil, crop, and remove pollutants started. One of the ways to optimize crop production and maintaining a healthy environment, provide the necessary conditions and the need for greater use of Bio-fertilizers and organic. The use of humic acid and soil micro-organisms, particularly bacteria that perform a variety of biological processes in plant growth and nutrient cycles are involved increasingly enhanced.
Plant growth promoting rhizosphere bacteria (Plant Growth Promothing Rhizobactria) than free-living bacteria in the soil or contributor. PGPR bacteria on biological nitrogen fixation, the production of significant amounts of growth-stimulating hormones, especially the types of auxin, gibberellin and cytokinin plant growth and crop yield are affected. Of these, *Azospirillium brasil*ense, *Pseudomonas*

azotobacter and the ability to communicate with agronomically important crops, such as maize, sorghum and Wheat have attracted more attention.
According to the research conducted, the purpose of this study, to investigate the effect of humic acid bacteria contributor, and Wheat, and nitrogen fertilizer, to reduce the use of nitrogen fertilizer on Wheat yield in Karaj Mahdasht came into force. Wheat inoculated with various strains of Azotobacter positive effect on biological function, protein content, seed weight, leaf area, Zinc, Iron, Phosphorus and nitrogen are. Selected strains of *Azotobacter* on growth and yield considerably BM and seed quality under greenhouse conditions have influenced [1].
Highest plant height, spike, spike and grains per spike of Azotobacter and nitrogen composition are obtained. Least in the case of manure and Azotobacter without a predicate. The highest yield was obtained from a combination of nitrogen and Azotobacter [2]. Warembourg et al [3] The Effect of Azospirillum inoculation on the growth of Wheat, observed that the number of fertile tillers, root dry weight, shoot dry weight and root to shoot ratio in plants inoculated inoculation was lower than the control plants.
Research results Fallik & Okon [4] showed that application of *Azospirillum* inoculum concentrations resulted in a significant increase in root dry weight, root dry weight of foliage and corn to control. Ardekani et al [5], in examining the role of *Azospirillum* on Wheat, found that the use of this bacterium significant impact on increasing the number of spike, number of grains per spike and grain yield (compared to not using it) has. Cotton plants after inoculation of bacteria during the survey *P. fluorescens* 40-8 percent increase in plant growth was reported [6]. Zahir et al [8] increased by 19.8% in grain yield of maize after inoculation with Azotobacter, Pseudomonas reported [7] showed that the application of humic acid can absorb nutrients from including nitrate transporter genes in maize and activities of nitrate in plants.
Mackowiak et al [9] improving the availability of iron and zinc in Wheat'm using humic acid have been reported. Nordi et al [10] also declared that the process of seed germination of corn, humic acid can be absorbed nitrate and total mRNA production in plants. According to the research conducted, the purpose of this study, to investigate the impact of cooperative bacteria in the family cereals including Wheat and fertilizer nitrogen (to reduce the use of nitrogen fertilizer on Wheat yield in Mahdasht in Tehran was carried out.

MATERIALS AND METHODS

This experiment was carried out the 2010-2011 cropping season at the Agricultural Research Station, Islamic Azad University, and Tehran, located in Karaj Mahdasht. According to the results of soil tests, some of soil characteristics were: light clay loam, with electrical conductivity 0.58 (mmos/cm), organic carbon content of 0.6%, and the total nitrogen content 0.60. In this study, we treated 16 factorial experiments in a randomized complete block design, with four replications was used.
1: The use of urea fertilizer on soil test results (Table 3-1), with four levels: (A1) non-urea fertilizer (control), (A2) 100 kg/hectare (A3) 200 kg/hectare and the (A4) 300 kg/ hectare, it should be noted that nitrogen from urea in two stages, one at planting and the other before the flowering stage (heading) as roads were applied.
2: Using humic acid at two levels: a (B1) =not applicable Humic acid level (B2) = application rate of 15 liters/hectare is Humic acid. Humic acid, along with the first irrigation and at the beginning of stem elongation and flowering under different treatments were applied.
3 The use of microorganisms or bacteria growth on two levels: (C1) = no inoculation and (C2) =inoculation with bacteria. As a seed treatment and spraying the seed money and the second phase started on the road at the beginning of stem elongation and flowering in the treatments were applied. Bacteria that were used in this experiment, as a source of inoculum were formulated in liquid form. Source of hybrid Bacteria, including 12 strains of Azotobacter, Azospirillum strains of *Pseudomonas Florence* strains 169were.
Land preparation operations, in accordance with the custom of the region by conducting a deep tillage to disrupt the normal drive for aggregate crushing was done before planting. For each experimental unit (plot) was implanted in 6 lines (lines three meters in length). 20cm between rows was considered. After land preparation, planting row was done manually. Each line sowing, 30 g of seed was considered. 180 grams of seed for planting 6 lines in each plot at a depth of 4-3 cm was considered. It should be noted that on 08/22/89 the first irrigation began planting in late November.
It should be noted that, first, the control seeds without bacterial inoculum and the inoculated seeds (which about half an hour before planting the seeds were mixed in the pan) using disposable gloves, and was cultivated in a planned experiment. For review and determination of yield and yield components,

analyze data using statistical software SAS performed and graphs were plotted using Excel software. Means comparison by Duncan test at 5% and 1% as performed.

RESULTS AND DISCUSSION

Seed yield

Analysis of variance (Table 1) suggests that different levels of nitrogen from urea (factor A) has a significant effect on yield is 1% statistical level. Duncan's mean comparison test shows (Table 2) with increasing levels of nitrogen, grain yield increases, so treatment A4 (300 kg fertilizer) produced 4443.8 kg ha grain yield which is superior to control (without nitrogen fertilizer). Ozturk et al (2003) observed that with increasing nitrogen fertilizer, Wheat yield increased significantly. Thus, it appears that nitrogen plays an important role in increasing vegetative growth is due, ultimately increasing the performance of the plant.

The use of growth promoting bacteria (Azotobacter, Azospirillum, Pseudomonas) resulted to produce 4259.2 kg per hectare yield with an 23.1 percent increase compared with control treatment (Table 2). perera et al (1997) to enhance the performance of plants inoculated with Azospirillum mainly related to production growth, increasing nutrient uptake by plants inoculated with the bacterium known. Janow et al (1997); reported that Azotobacter and Azospirillum inoculation increased seed yield in Wheat and barley is 40 percent. Thus, the ability of the nitrogen-fixing Azospirillum and Pseudomonas ability to control plant pathogens, as well as the dissolution of insoluble phosphate was significantly increased grain yield

According to the analysis of simultaneous application of different levels of nitrogen and bacteria growth in a statistically significant effect on grain yield was 1%. (Table 1). Also Comparisons of mean (Table 3) the highest grain yield in treatments A_4C_2 (concurrent use of fertilizer rate of 300 kg with the use of bacteria) to produce 5151 kg of grain per hectare% 42.86 increased compared to control in the first place, and treatments 2C × 3A (concurrent use of fertilizer rate of 200 kg with the use of bacteria) were the next highest and the lowest grain yield by treatment of 2943.75 kilograms per hectare of A_1C_1 (increment non-use of fertilizer, bacteria stimulus) was. As can be seen, the use of different levels of nitrogen has a positive effect on bacteria growth and yield increases (Swedrzynsk&Sawicka, 2000)

The researchers reported that the number of bacteria, including Azospirillium brasilense, along with nitrogen increases, the yield is increased due to the positive effects of the bacteria. Tions between the two levels of nitrogen and humic acid interactions, the impact on grain yield were significant differences in the level of 5% was observed (Table 1). The mean (Table 3), indicating that the treatments A_3B_2 and A_4B_2 highest production values respectively 4557.2 and 4483.3 grain yield kg ha respectively when treated A_1B_1 yield 2561.6 kg ha in group was statistically significant. The growth of bacteria and humic acid interactions are significant at 1% level (Table 1). Comparisons based on average data (Table 3) was observed, B_2C_2 and B_1C_2 treatments compared to the control, respectively, 4.36 and 8/35 percent were considered in the first place.

1000 seed weight

Effect of nitrogen on seed weight was significant at the 1% level (Table 1). Comparison of means showed (Table 2), the maximum grain weight of the fertilizer 4A (300 kg ha) fertilizer recommendations based on soil test levels and has a weight equal to 49.16 mg is the lowest seed treatment Cody has a high level of evidence 1A (no use of nitrogen fertilizer) was equal to the 45.96 gr.

The use of growth promoting bacteria in their effects on grain weight by consuming bacteria, a significant difference was observed in 1% (Table 1). Thus, comparison of the data showed (Table 2), the maximum grain weight is related to the use of bacteria treated with 49.14 mg and 46.67 was the lowest heat treatment of non-application of the bacteria that cause the bacteria increased by 5% 1000 seed weight is. Zaied et al (2003) as well as the positive effects of these bacteria to produce more auxin in the presence of different levels of nitrogen fertilizer on grain weight were positive and statistically significant

Between use and non-use of humic acid affect seed weight, there was a significant difference at 1% level (Table 1). Therefore, consumption of grain weight humic acid could increase to 6% compared to control (Table 2). This increase, which may be due humic acid properties, including better rooting and open the macro and micro elements (Daei and Sardari, 2010). Interactions between bacterial growth and nitrogen, there was a significant difference at the 5% level (Table 1).

Tions between the two levels of nitrogen and humic acid interactions in their effects on grain yield were significant differences in the level of 5% was observed (Table 1). The mean (Table 3), indicating that treatment of A_4B_2 and A_3B_2 maximum production yield and the treatment group and A_1B_1 and A_2B_1 in groups were statistically identical. The growth of bacteria and humic acid interactions are significant at

1% level (Table 1). Comparisons based on average data (Table 3), the highest seed weight of B_2C_2 treated with 49.38 mg and the lowest B_1C_1 treated with 44.20 gr.

Biological yield

Effect of different levels of nitrogen on biological function, shows a significant difference at 1% level (Table 1). Comparison of data obtained from nitrogen fertilizer levels (Table 2) show that the level of fertilizer A4 (300 kg per ha) and biological yield of 14.401 kg per hectare in group I and group A1 (without fertilizer) and biological yield of 10.474 kg per hectare was in the last group. Affect the growth of bacteria (Azotobacter, Azospirillum, Pseudomonas) biological yield showed a 1% statistical level (Table 1). Comparison of the data also indicated (Table 2), the most biologically relevant application performance by 14093.7 kg ha-mentioned bacteria that 19.6 % represents an increase in total biomass than the control plants was biologically (weight whole plant) and proper nutrition without any environmental stress, nutrition etc. It is also effective in increasing. Nitrogen-fixing bacteria may increase the shoot dry weight of leaves and stems are also increasing mineral (Suba Rao, 1988). Effect of humic acid on the biological function also exhibits the 1% statistical level (Table 1). The mean (Table 2), indicated that the biological function of the 13934.3 kg ha treatments applied to the treatment of non-application of humic acid and acid humic biological function 11488.8 kg per hectare in second place. Interactions between bacterial growth and nitrogen was significant at the 1% level (Table 1). By comparison average data (Table 3), were treated by A_4C_2 and A_3C_2 respectively 15572.2 and 15781.2 kg per hectare, the highest level of biological yield is allocated to the first group of clusters. A_1C_1 and A_2C_1 treatments with biological yield, respectively, 10635.4 and 10052 kg ha were in the last group. Spike of between 30 to 89 percent. Baldany et al (1983) conducted a series of field experiments in Brazil, said that Wheat seed inoculated with bacteria such as Azospirillum, shoot dry weight increased from 16 to 96 percent. Also, the total nitrogen of shoot spike in production from 30 to 89 percent. Tions between the two levels of nitrogen and humic acid interactions in their effects on grain yield were significant differences in the level of 5% was observed (Table 1). The mean (Table 3) showed that the treatment group A_3B_2 and A_4B_2 Most of the biological function values, respectively, 15395.8 and 15197.9 kg per ha were in the top group. A_1B_1, whereas treatment with 8937.5 kg ha biological yield statistically accounted for the lowest place in the final group. The growth of bacteria and humic acid interactions at the level of 1%, there were no significant differences (Table 1). Comparisons based on average data (Table 3) was observed compared to the control treatment B_2C_2 36.8 percent, in the first place.

Weight of Spike

As in the analysis of variance (Table 1) are observed between the different levels of nitrogen fertilizer impact on spike weight difference is significant at 1% statistical level there is a comparison of levels of factor A (Table 2) show that the highest spike weight of treatment A4 (with application of 300 kg nitrogen) to yield 6953.1 kg per ha and the lowest control (no nitrogen fertilizer) to spike 5271.5 kg per hectare yield in the latter group, the there.

Also, the bacterial growth (Azotobacter, Azospirillum, Pseudomonas) according to analysis of variance (Table 1) at the 1% statistical significant difference existed when comparing average data (Table 2) showed that of those who consume bacteria could ear weight compared to control 24.1 percent increase. Due to the ability to stimulate growth in nitrogen-fixing bacteria in the heading and spikelet formation can be realized due to it. Between consumption and non-consumption of acid humic impact on spike weight were significant differences in the level of 5% was observed (Table 1). As compared to average data (Table 2) showed that the humic acid produces the characteristic spike weight at a rate equal to 6408.7 to 5972.1 kg per hectare compared to non-consumers kg per hectare, respectively.

According to the analysis of simultaneous application of different levels of nitrogen and bacteria growth in Varanasi on the statistical spike weight at 1% significant difference (Table 1). The mean comparisons (Table 3) Maximum number of spikes per treatment A_4C_2 to produce 7869.7 kg per ha performance 35.6 % showed an increase compared to the control. Tions between the two levels of nitrogen and humic acid interactions in their effects on spike yield significant differences at the 5% level, respectively (Table 1). The mean (Table 3), indicating that the treatments A_4B_1 and A_3B_2 and A_2B_3 Most of the spike Rating 7192.7 and 6747.9 and 6713.5, respectively, and the values of kg acres were in the top group. A_1B_1, whereas treatment with 4752.8 kg per hectare yield statistically significant spike in last place. Interactions between bacteria and humic acid at 1% growth in the influence of spike weight, there were no significant differences (Table 1). Comparisons based on average data (Table 3), B_1C_2 compared to the control group showed a 41% increase.

Tabal 1- Results of variance analysis of measured characters								
S.O.V	df	Length Spike (cm)	Spike (m^2)	Spike weight (Kg/ha)	Kernel weight (g)	Grain yield (Kg/ha)	Biological yield (Kg/ha)	Harvest index
Rep	3	2.21 ns	177.44 ns	561900.8 ns	0.62 ns	43750.94 ns	2527534.9 ns	10.84 ns
nitrogen	3	2.57 ns	45.86 ns	7806529**	34.99**	5933822**	46648213**	12.58 ns
Humic	1	6.26 ns	54.2 ns	3050852*	117.93**	5400953**	95733178**	12.06 ns
Bacteria	1	7.08 ns	0.08 ns	46313656**	97.61**	13532282**	1222216701**	7.55 ns
Nitrogen* Humic	3	0.99 ns	33.79 ns	1716378*	2.11*	846234*	4457560.5*	5.04 ns
Nitrogen*Bacteria	3	0.07 ns	76.06 ns	3112700**	1.6*	1312801**	7530210.6**	29.14 ns
Humic*Bacteria	1	3.21 ns	173.91 ns	32494204**	79.92**	12290003**	86974299**	24.19 ns
Humic*Bacteria* nitrogen	3	0.28 ns	18.12 ns	243216.5 ns	0.77ns	157739.4 ns	1619172.8 ns	11.26 ns
Error	45	1.79	47.7	557636.2	0.53	211983.5	1119371.2	11.56
VC	-	13.53	14.42	12.06	1.53	12.17	8.32	11.39
Ns,*,**: not significant and significant at 5 and 1% level of probability respectively								

Table 2- Comparison of interaction mains effect nitrogen, plant growth promoting rhizobacteria, humic acid on traits test								
		Length Spike (cm)	Spike (m^2)	Spike weight (Kg/ha)	Kernel weight (g)	Grain yield (Kg/ha)	Biological yield (Kg/ha)	Harvest index
	A1	9.38 a	45.8 a	5271.5 c	45.96 c	3000.6 c	10474 d	28.67 a
Nitrogen	A2	9.86 a	47.8 a	6155.5 b	47.56 b	3722.4 b	12380.2 c	29.77 a
	A3	10.00 a	47.8 a	6381.8 b	48.95 a	4030.6 b	13592.2 b	30.07 a
	A4	10.30 a	49.9 a	6953.1 b	49.16 a	4444.8 a	14401.0 a	30.81 a
Humic Acid	B1	9.59 a	46.9 a	5972.1 b	46.55 b	3508.8 b	11488.8 b	29.40 a
	B2	10.20 a	48.8 a	6408.7 a	49.26 a	4089.8 a	13934.9 a	30.26 a
Bacteria	C1	9.57 a	47.8 a	5339.7 b	46.67 b	3339.5 b	11330 b	29.49 a
	C2	10.20 a	47.8 a	7041.1 a	49.14 a	4259.2 a	14093.7 a	30.17 a
Means followed by similar letters in the same column are not significantly different based Duncan multiple range at 5 percent level probability								

Table 3- Comparison of interaction between nitrogen, plant growth promoting rhizobacteria, humic acid on trait test								
Treatment		Length Spike (cm)	Spike (m^2)	Spike weight (Kg/ha)	Kernel weight (g)	Grain yield (Kg/ha)	Biological yield (Kg/ha)	Harvest index
	A1×B1	8.76 a	44.19 a	4752.9 g	48.82 f	2561.98 g	8937.5 g	28.67 a
	A1×B2	10.00 a	47.50 a	5790.1 def	47.10 cde	3439.58 def	12010.41 e	28.67 a
	A2×B1	9.75 a	47.69 a	5927.1 def	46.59 de	3565.62 de	11625 ef	30.70 a
Nitrogen*humic	A2×B2	10.31 a	48.10 a	6383.3 bc	48.54 b	3879.16 cd	13135.41 cd	29.45 a
	A3×B1	9.52 a	50.70 a	6015.6 bc	47.19 cd	3503.89 def	11788.58 ef	29.74 a
	A3×B2	10.21 a	49.27 a	6747.9 ab	50.71 a	4557.29 a	15395.83 a	29.81 a
	A4×B1	10.33 a	45.26 a	7192.7 a	47.60 bc	4404.16 abc	13604.16 c	31.95 a
	A4×B2	10.35 a	50.34 a	6713.5 abc	50.72 a	4483.33 ab	15197.91 ab	29.66 a
	A1×C1	9.09 a	43.62 a	5061.02 d	45.08 f	2943.75 fg	10052.08 f	29.22 bcd
	A1×C2	9.67 a	48.06 a	5482.06 cd	46.84 de	3057.52 fg	10895.83 ef	28.12 bcdef
	A2×C1	9.68 a	47.69 a	5065.62 d	46.40 e	3112.50 ef	10635.41 edf	29.43 bcd
Nitrogen*Bacteria	A2×C2	10.37 a	48.38 a	7244.79 ab	48.73 b	4332.29 bc	14125 b	30.72 ab
	A3×C1	9.44 a	47.41 a	5195.83 d	47.30 cd	3565.35 ed	11611.49 d	30.62 ab
	A3×C2	10.29 a	49.00 a	7567.70 ab	50.60 a	4495.83 b	15572.90 a	28.92 bcdef
	A4×C1	10.07 a	50.67 a	6036.45 c	47.92 c	3736.45 d	13020.83 c	28.69 bcdef
	A4×C2	10.61 a	44.93 a	7869.79 a	50.40 a	5151.04 a	15781.24 a	32.93 a
	B1×C1	9.03 a	48.65 a	6546.87 c	44.20 c	2610.80 c	8941.16 c	29.31 a
	B1×C2	10.15 a	45.27 a	7535.30 a	48.90 ab	4406.88 a	14036.45 ab	31.22 a
Humic*Bacteria	B2×C1	10.11 a	47.19 a	6270.61 b	49.15 ab	4068.22 b	13718.75 ab	29.67 a
Treatment	B2×C2	10.32 a	41.50 a	6546.87 b	49.38 a	4111.45 ab	14151.04 a	29.13 a
Means followed by similar letters in the same column are not significantly different based Duncan multiple range at 5 percent level probability								

CONCLUSIONS

According to the results of this research, it was found that the use of bio fertilizer can be compared to no inoculation; the plants provide the nitrogen needed help. And when combined with nitrogen fertilizer applied, can be effective in improving performance. Bio fertilizer used in this research study, likely through mechanisms enabling the production of plant growth hormones, such as auxin, gibberellic acid and cytokinins increased yield, disease control, soil and plant stimulate the immune system and increase the uptake the food. It is also common in cultivation due to low production per unit area of bio-organic fertilizers can be effective in increasing production per unit area. Considering the low price of these inputs, taking in all the area under Wheat cultivation can result in a substantial increase in the product.

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