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Investigation Integrated Effect of Fertilizer and Biofertilizer (Nitrogen and Phosphorus) on Wheat (*Triticum aestivum* L.) Crop Production

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ABSTRACT

BACKGROUND: Fertilizer management plays an important role to achieve sustainable agriculture.
OBJECTIVES: Current study was conducted to assess response of agrophysiological traits of wheat to apply different level of fertilizer and biologic fertilizer (Phosphorus and Nitrogen).
METHODS: This research was carried out via factorial experiment based on randomized complete blocks design with three replications along 2016 year. The treatments included phosphorus fertilizer (P_1: 100% Triple superphosphate fertilizer, P_2: 70% Triple superphosphate fertilizer + Barvar 2, P_3: 40% Triple superphosphate fertilizer + Barvar 2, P_4: 100% Barvar 2) and nitrogen fertilizer (N_1: 100% urea fertilizer, N_2: 70% urea fertilizer + Azotobacter, N_3: 40% urea fertilizer + Azotobacter).
RESULT: According result of analysis of variance effect of different level of phosphorus, nitrogen fertilizer and interaction effect of treatments on all studied traits (instead harvest index) were significant. Evaluation mean comparison result of interaction effect of treatments on all measured traits revealed the highest amount of seed yield (6740.13 $kg.ha^{-1}$), 1000-Seed weight (41.33 gr), biologic yield (16061.37 $kg.ha^{-1}$), number of seed per spike (42.50) and number of spike per square meter (383.72) were noted for N_2P_2 (70% Triple superphosphate fertilizer + Barvar 2 and 70% urea fertilizer + Azotobacter) and lowest amount of mentioned traits belonged to N_1P_1 (100% urea fertilizer round and 100% Triple superphosphate fertilizer treatment).
CONCLUSION: Generally the results of this experiment showed that different levels of nitrogen and phosphorus were effective on seed yield and its components. Finally based on result of this research use 70% Triple superphosphate fertilizer + Barvar 2 and 70% urea fertilizer + Azotobacter improve crop production of bread wheat and can be advised to farmers.
KEYWORDS: *Azotobacter, Nutrition, Seed yield, Triple superphosphate, Urea.*

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1. BACKGROUND

Fertilizer management plays an important role for obtaining the satisfactory yields and to increase crop productivity. Nutrient management may be achieved by the involvement of organic sources, bio fertilizers, and micronutrients (Singh *et al*., 2002). Studies have shown that long-term use of fertilizers reduces crop yields. This decrease is due to the acidification of the soil, the reduction of biological activity of the soil and the inappropriate physical properties of soil (Alexandratos, 2003). Chemical fertilizers have several negative impacts on environment and sustainable agriculture. Therefore, bio fertilizers are recommended in these conditions and growth prompting bacteria uses as a replacement of chemical fertilizers (Wu *et al*., 2005). The growth and yield of a crop can be adversely affected by deficient or excessive supply of any one of the essential nutrients. However, in intensive agriculture nitrogen is the major nutrient which determining crop yield. Nitrogen as essential constituent of cell components having direct effect on growth, yield and quality of crop. Plant growth is affected more due to deficiency of nitrogen than that of any other nutrient. Nitrogen fertilization influences dry matter yield by influencing leaf area index, leaf area duration and photosynthetic efficiency (Mohan *et al*., 2015). Nitrogen (N) is essential for all biological process that occurs in the plant. A sub-optimal supply of N limits the expression of yield potentials of green bean varieties (Dauda *et al*., 2015). Nitrogen deficiency is frequently a major limiting factor for high yielding crops all over the world (Salvagiotti *et al*., 2008). The most important role of N in the plant is its presence in the structure of protein and nucleic acids which are the most important building and information substances of every cell. In addition, N is also found in chlorophyll that enables the plant to transfer energy from sunlight by photosynthesis. Thus, the supply of N to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Consequently, it influences cell size, leaf area and photosynthetic activity (Walley *et al*., 2005). Therefore, adequate supply of N is necessary to achieve high yield potential in crops. In general, N deficiency causes a reduction in growth rate, general chlorosis, often accompanied by early senescence of older leaves, and reduced yield (Erman *et al*., 2011). Mckenzie and Hill (1995) studied the effects of two levels of N applications (0 and 50 kg N ha^{-1}) on chickpea and reported that the increase of N rate from 0 to 50 kg N ha^{-1} significantly enhanced seed and dry matter yield, harvest index, number of pods per plant and 1000 seed weight. Nitrogen deficiency generally results in stunted growth and chlorotic leaves caused by poor assimilate formation that leads to premature flowering and shortening of the growth cycle (Lincoln and Edvardo, 2006). Beyranvand *et al*. (2013) suggested that effect of nitrogen and phosphate bio-fertilizers were evaluated the positively, there were an increase in plant height, ear weight, and number of grain per cob, grain yield and biomass yield. Increasing yield was attributed to the plant growth promoting substances by root colonizing bacteria more than the biological nitrogen fixation, stated that yield increased due to promoting root growth which in turn enhancing nutrients and water uptake from the soil (Lin *et al*., 1983). Combined application of organic fertilizer and urea fertilizer or combination urea fertilizer and polyamines significantly increased the yield, vegetative growth and the chlorophyll index (Zeid, 2008). Fertilizer management is one of the most important factors in successful cultivation of crops affecting yield quality and quantity (Tahmasbi *et al.,* 2011). A great attention has recently directed towards the application of bio-organic farming to avoid the heavy use of agrochemicals that result in the enormous environmental troubles (Abd El-Ghany, 2007). Inoculation with Azospirillum and Azotobacter increases the absorption of K, NO_3^-, H_2PO_4, so the root to stem ratio seems to increase. This makes the plant better deployed in the soil and access to limited resources of water and essential nutrients. Increasing the absorption of ions by inoculation can play an important role in increasing leaf growth. Also, the release of various phytohormones, such auxin, cytokinin, gibberellin, and unknown compounds by strains of these bacteria, increase the cell proliferation and cell division, thus increasing the leaf area index can be justified (Yazdani *et al.,* 2009). Hokm Alipour and Hamele Darbandi (2011) reported application of nitrogen fertilizer has positive effects on yield and physiological growth indices of maize cultivars, it can be suggested that use korduna cultivar with 180 kg N ha levels. Nitroxin biological fertilizer contains the most effective nitrogen fixation bacteria of Azotobacter and Azospirillium, which stabilizes the nitrogen, balance absorption of the micronutrient and macronutrient rate needed by plant, as it causes growth and development of root and shoots of plant by synthesis and excretion of stimulants of plant growth such as types of regulating hormones such as Oxine, and also production of different amino acids and types of antibiotics, Cyanide hydrogen, Siderophore, etc, and causes increase of quality and quantity of product by protecting root such as terrestrial pathogenic agents (Cardoso and Kuyper, 2006).

2. OBJECTIVES

Current study was conducted to assess response of agrophysiological traits of wheat to apply different level of fertilizer and biologic fertilizer (Phosphorus and Nitrogen).

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out via factorial experiment based on randomized complete blocks design with three replications along 2016 year. Place of research was located in Ahvaz city at longitude 48°40'E and latitude 31°20'N in the Khuzestan province (Southwest of Iran). The treatments included phosphorus fertilizer (P_1: 100% Triple superphosphate fertilizer, P_2: 70% Triple superphosphate fertilizer + Barvar 2, P_3: 40% Triple superphosphate fertilizer + Barvar 2, P_4: 100% Barvar 2) and nitrogen fertilizer (N_1:

100% urea fertilizer, N_2: 70% urea fertilizer + Azotobacter, N_3: 40% urea fertilizer + Azotobacter).

3.2. Farm Management

To apply phosphorus biofertilizer, the biofertilizer (100 $g.ha^{-1}$) was dissolved in a 10 liter container filled with water. Then the seeds were placed in these containers for 10 minutes before planting and impregnated with fertilizer solution and then they were planted. Phosphorus biofertilizer (brand Barvar 2) contains 10^7 to 10^8 phosphate-solubilizing bacteria (*Pantoea* agglomerans strain P5 and Pseudomonas strain P13) per gram of product, which are released phosphates ions by producing organic acids and phosphatase enzymes around the roots. Potassium fertilizer was applied from potassium sulfate source at 120 gr per plot. To combat broadleaf and narrow leaf weeds, Duplosan Super (2.5 $L.ha^{-1}$) and topic (1 $L.ha^{-1}$) herbicides was used at the end of tillage and before application of top-dressing fertilizer, respectively. The physical and chemical properties of studied soil mentioned in table 1.

3.3. Measured Traits

In order to determine the seed yield and its components, the two side rows and half a meter of the beginning and end of each plot were eliminated as the marginal effects and finally the ultimate samples were taken from an area of 1 m^2. In order to determine the number of spikes per area unit, the spikes were taken from an area of 1 m^2 of then three middle lines of each plot after considering half a meter of beginning and end of each line as the margin and after counting the spikes their mean was considered as the number of spikes per area unit. As many as 10 spikes were randomly selected from the middle lines of each plot and the number of seeds was counted carefully and their mean was recorded. Two 500-seed samples were randomly selected from the produced seeds by each plot and if the weight difference of the two samples was less than 5%, the total weight of the two samples was considered as weight of 1000-seed. After full maturity of the seeds, the spikes were taken from the 3 middle lines of each plot in an area of 1 m^2 and the seed yield of each plot with moisture of 14% was calculated per area unit and then was recorded. Harvest index (HI) was calculated according to formula of Gardener *et al.* (1985) as follows: **Equ.1.** Harvest Index= (Seed yield/Biologic yield) ×100.

Table 1. Physical and chemical properties of the experiment field

Acidity (pH)	7.51
Electrical conductivity ($ds.m^{-1}$)	5.96
Organic carbon (%)	0.45
Phosphorus (ppm)	6.8
Potassium (ppm)	123
Clay (%)	27
Silt (%)	38
Sand (%)	35
Soil texture	Clay loam

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS (Ver.8) software and Duncan multiple range test at 5% probability level. Curve was drawn by Excel Software (Ver.2014).

4. RESULT AND DISCUSSION

4.1. Number of spike per square meter

According result of analysis of variance effect of phosphorus, nitrogen and interaction effect of treatments on number of spike per square meter was significant at 5% probability level (Table 2). Evaluation mean comparison result of interaction effect of treatments indicated maximum number of pods per m^2 (383.72) was noted for 70% urea fertilizer + Azotobacter and 70% Triple superphosphate fertilizer + Barvar 2 and lowest one (353.28) belonged to 100% urea fertilizer with 100% Triple superphosphate fertilizer treatment (Table 3). The correct and proportional nitrogen application rate of fertilizers increases wheat grain yield by increasing the number of spikes per unit area, and increasing the number of seeds per spike has a lower role in raising the yield (Fowler and Brydon, 2001). Researchers reported that the increase in nitrogen consumption increases the number of spikes per unit area, which can increase vegetative growth and, consequently, increase the amount of tillering due to nitrogen consumption. In such a situation, the number of fertilized tillers per unit area increases and the number of spikes per unit area also increases (Mosanaei *et al*., 2017).

Table 2. Result analysis of variance of studied traits

S.O.V	df	No. spike per m^{-2}	No. seed per spike	1000-Seed weight	Seed yield	Biologic yield	Harvest index
Replication	2	896.61^{*}	31.707^{*}	25.593^{*}	19047.14^{*}	42886.49^{*}	15.03^{ns}
Phosphorus (P)	3	1370.52^{*}	66.285^{*}	34.137^{*}	20359.71^{*}	54858.55^{*}	9.259^{ns}
Nitrogen (N)	2	988.41^{*}	54.72^{*}	26.823^{*}	18542.60^{*}	67559.42^{*}	43.581^{**}
N × P	6	582.61^{*}	27.936^{*}	15.428^{*}	16975.22^{*}	49766.57^{*}	7.926^{ns}
Error	22	175.24	8.93	5.02	2715.48	8349.51	5.22
CV (%)	-	3.57	8.20	6.30	10.9	7.50	5.70

$^{ns,\ *\ and\ **}$: no significant, significant at 5% and 1% of probability level, respectively.

4.2. Number of seed per spike

Result of analysis of variance revealed effect of phosphorus, nitrogen and interaction effect of treatments on number of seed per spike was significant at 5% probability level (Table 2). Assessment mean comparison result of interaction effect of treatments showed maximum number of seed per spike (42.50) was noted for 70% urea fertilizer + Azotobacter and 70% Triple superphosphate fertilizer + Barvar 2 and lowest one (32.03) belonged to 100% urea fertilizer with 100% Triple superphosphate fertilizer treatment (Table 3). According to the research of Mosanaei *et al*. (2017), the effect of nitrogen fertilizer on the number of wheat spikes was significant, which was consistent with the results of the present study. Nitrogen increases the biomass production and increases the possibility of retransmission of photosynthetic materials, producing more seeds per spike and better filling them after flowering, which will increase seed yield (Shanggan *et al*., 2000).

4.3. 1000-Seed weight

According result of analysis of variance effect of phosphorus, nitrogen and interaction effect of treatments on 1000-Seed weight was significant at 5% probability level (Table 2). Evaluation mean comparison result of interaction effect of treatments indicated maximum 1000-Seed weight (41.33 gr) was noted for 70% urea fertilizer + Azotobacter and 70% Triple superphosphate fertilizer + Barvar 2 and lowest one (30.55 gr) belonged to 100% urea fertilizer with 100% Triple superphosphate fertilizer treatment (Table 3). Bio-fertilizers by increasing nitrogen the efficiency and uptake cause most shoot growth and consequently increasing the biological yield. Other reports have indicated that seed inoculation of corn with plant promoting bacteria in addition to 30 to 35% reduction of nitrogen fertilizer improved plant growth. Increased microbial biomass is directly related to soil health; it enhances the balance of nutrient elements and nutrient availability in root rhizosphers that promotes growth and ultimately affects a higher yield (Biari *et al*. 2008). Nouraki *et al*. (2016) reported mixing of biological fertilizers with chemical fertilizers could reduce the needs of chemical fertilizers up to 25% and these results are comparable to the application of 100% chemical fertilizers. Therefore, the best hybrid maize is the single cross 704 that has good yield potential when the chemical fertilizer is used at either 25% or 50% of the current application when mixed with the biofertilizer. Other studies determined that plant growth was improved even when the nitrogen fertilizer applied was reduced by 30-35% as long as the seeds had been inoculated with growth promoting bacteria.

Table 3. Mean comparison interaction effect of treatments on studied traits

Treatment		No. spike per m^{-2}	No. seed per spike	1000-Seed weight (gr)	Seed yield ($kg.ha^{-1}$)	Biologic yield ($kg.ha^{-1}$)
N_1	P_1	353.28^{d}	32.03^{d}	30.55^{de}	3481.72^{dg}	9166.11^{fh}
	P_2	371.46^{bc}	36.14^{bc}	36.73^{b}	4930.84^{bd}	12928.43^{cd}
	P_3	367.02^{c}	34.39^{c}	35.80^{bc}	4518.61^{c}	11878.03^{de}
	P_4	356.81^{d}	32.07^{cd}	32.11^{d}	3674.31^{df}	9488.06^{fg}
N_2	P_1	362.35^{cd}	35.33^{bd}	34.92^{c}	4470.39^{cd}	11293.57^{e}
	P_2	383.72^{a}	42.50^{a}	41.33^{a}	6740.13^{a}	16061.37^{a}
	P_3	379.17^{ab}	40.29^{ab}	39.47^{bc}	6029.73^{ab}	14677.29^{b}
	P_4	368.23^{c}	37.02^{b}	36.07^{c}	4917.01^{cd}	11838.37^{de}
N_3	P_1	360.42^{ce}	32.11^{cd}	33.14^{cd}	3835.32^{de}	9125.35^{fh}
	P_2	378.39^{ab}	39.25^{ac}	38.28^{ac}	5685.27^{b}	13823.17^{bc}
	P_3	374.08^{b}	37.30^{b}	36.63^{b}	511.05^{bc}	12483.40^{cd}
	P_4	363.78^{cd}	34.01^{c}	32.76^{cd}	4053.11^{d}	9769.52^{f}

*Means with similar letters in each column are not significantly differentt by Duncan test at 5% probability level.
N_1: 100% urea fertilizer, **N_2:** 70% urea fertilizer + Azetobacter, **N_3:** 40% urea fertilizer + Azetobacter
P_1: 100% Triple superphosphate fertilizer, **P_2**: 70% Triple superphosphate fertilizer + Barvar 2, **P_3**: 40% Triple superphosphate fertilizer + Barvar 2, **P_4**: 100% Barvar 2

An increase in the biomass of the microbial community was related to the soil health as this had an effect on the balance and availability of nutrients in the rhizosphere of the roots that lead to a higher yield (Biari *et al*., 2008).

4.4. Seed yield

Result of analysis of variance revealed effect of phosphorus, nitrogen and interaction effect of treatments on seed yield was significant at 5% probability level (Table 2). Assessment mean comparison result of interaction effect of treatments showed maximum seed yield (6740.13 $kg.ha^{-1}$) was noted for 70% urea fertilizer + Azotobacter and 70% Triple superphosphate fertilizer + Barvar 2 and lowest one (3481.72 $kg.ha^{-1}$) belonged to 100% urea fertilizer with 100% Triple superphosphate fertilizer treatment (Table 3). Jafari Haghighi and Yarmahmodi (2011) in conclusion for reach to high yield in corn stated biological fertilizer cannot sufficient but integrated application of fertilizers (Biological and chemical fertilizers) became causes significant increase in yield. Use of the biofertilizers offers agronomic and environmental benefits to intensive farming systems in Egypt country, and the data showed that using *Azospirillum brasilense* or commercial bio fertilizers in cereals crops with a half amount of nitrogen rate (144 $kgN.ha^{-1}$) caused a significant increase in amount of crop yield. Further, seed inoculation with Rhizobium, phosphorus solubilizing bacteria, and organic amendment increased the seed production of the crop (Panwar *et al*., 2006).

4.5. Biologic yield

According result of analysis of variance effect of phosphorus, nitrogen and interaction effect of treatments on biologic yield was significant at 5% probability level (Table 2). Evaluation mean comparison result of interaction effect of treatments indicated maximum biologic yield (16061.37 $kg.ha^{-1}$) was noted for 70% urea fertilizer + Azotobacter and 70% Triple superphosphate fertilizer + Barvar 2 and lowest one (9166.11 $kg.ha^{-1}$) belonged to 100% urea fertilizer with 100% Triple superphosphate fertilizer treatment (Table 3). Eydizadeh *et al*. (2010) stated that biological fertilizers increase the root contact with soil and ultimately increase the absorption of nutrients. Mentioed researchers also stated that the production of various acids by bacteria could lead to more organic solubility of the soil. It seems that the effect of bio-fertilizers provides up to 50% of the plant's nutritional requirements, and the rest of the plant's needs must be provided through the use of chemical fertilizers. Hojattipor *et al.* (2014) reported that the maximum total dry weight was obtained in wheat with increasing nitrogen fertilizer up to 225 $kg.ha^{-1}$, along with biological nitrogen fertilizer of nitrokara. Nouraki *et al.* (2017) reported that the spraying of biological fertilizers containing amino acids along with nitrogen fertilizers increases the growth and production of dry matter. Application of fertilizer of triple super phosphate 50% with the bio-phosphate had a significant effect on increasing total dry weight of the corn crop.

4.6. Harvest index

Harvest index is also an important factor in increasing yield, in grains, the increase in biomass has reached its final limit, hence the increase in seed yield through the allocation of more photosynthetic materials to the sink (seeds) is possible, in which case the harvest index will significantly increase (Krishnan *et al.*, 2003). Result of analysis of variance showed effect of nitrogen on harvest index was significant at 1% probability level but effect of phosphorus and interaction effect of treatments was not significant (Table 2). The variability of the harvest index in the plants depends on the difference in the production of the assimilates during the seed filling and re-transplantation of the assimilates before the pollination of each genotype and the strength of the reservoir (Nour mohammadi *et al.*, 2001). Han and Lee (2006) attributed the increase in corn harvest index in biofertilizer treatment to better absorb nutrients. Because the plant with better absorption of nutrients and increasing leaf area index can use better solar radiation and send more photosynthetic materials to seed and thus increase dry matter. Compare different level of nitrogen showed that the maximum and the minimum amount of harvest index belonged to 40% urea fertilizer + Azotobacter (39%) and 100% urea fertilizer (35%) treatments (Fig.1). Increase of harvest index due to the increase of nitrogen fertilizer in maize can physiologically attribute to the increase of leaf area continuity and, nitrogen availability. In fact by creating balance between the nutrients bio-fertilizers increase both vegetative and reproductive growth and by creating adequate destination (seed), the assimilates will mobilize into seeds and ultimately the harvest index of plant seed increase (Araei *et al.*, 2014). Veisi Nasab *et al.* (2015) by evaluate the effect of different level of vermicompost on maize production reported the maximum harvest index (31.04%) was obtain from consume 12 $t.ha^{-1}$ vermicompost.

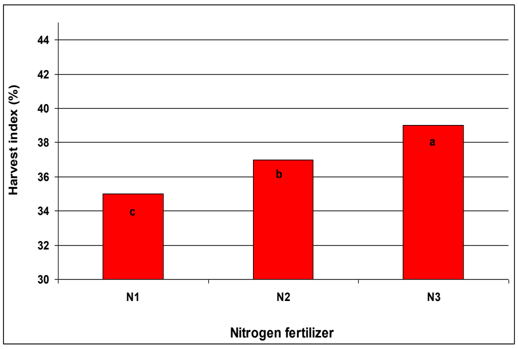

Fig.1. Effect of different level of nitrogen on harvest index by Duncan test at 5% probability level.
N_1: 100% urea fertilizer**, N_2:** 70% urea fertilizer+Azetobacter**, N_3:** 40% urea fertilizer+Azetobacter

5. CONCLUSION

Generally the results of this research showed that different levels of nitrogen and phosphorus were effective on seed yield and its components. Finally based on result of this research use 70% Triple superphosphate fertilizer + Barvar 2 and 70% urea fertilizer + Azotobacter improve crop production of bread wheat and can be advised to farmers.

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FOOTNOTES
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