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Effect of Biofertilizers on Yield and Yield Components of Wheat (*Triticum aestivum*. L) Under Iraqi Conditions

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Abstract: The objective of the study was to evaluate the efficiency of biofertilizers in improving wheat yield, yield components, and nutrients balance in soil. Local and imported biofertilizers were applied in a field experiment at Erbil, Iraq for the season 2016-2017. The experimental design was Randomized Complete Block Design (RCBD) with four replicates. The experiment consisted of five treatments: T1 = Natrusoil (Commercial Biofertilizer) only, T2 = Natrusoil + 25% Chemical Fertilizers (CF), T3 = Local Biofertilizer B1 + 25%CF, T4 = Local Biofertilizers B2 + 25%CF, and T5 = CF as a Control (Recommended: Urea = 260 kg/ha, DAP = 180 kg/ha). Management practices in soil preparation and crop management followed the common and general procedures. Most test biofertilizers significantly increased yield of wheat grain or yield components. Maximum significant grain yield was obtained from biofertilizers treatments Natrusoil+25%CF (4.659) and B1+25%CF (4.691) as compared with the Control (3.987 kg ha^{-1}). The increase in yield was in the range 17-18%. The application of biofertilizer resulted in a positive effect on nutrients balance in the soil at the end of season regardless of type of biofertilizrs as indicated by the increase in levels of NH_4, NO_3, P, and K. Among the benefits of using bioferilizers is the reduction in cost. The reduction was 47% in using the imported Natrusoil + 25%CF increased to 72% when using the Natrusoil alone.

Keywords: Biofertilizers, Bacteria, Fungi, Wheat, Yield, Mineral Fertilizers and Financial Cost

1. Introduction

Wheat is considered as one of the most important cereals crop. It has a special importance in Iraq because the local production is not sufficient to supply the annual demand thus, the country used to import wheat during the last sixty years according to almost all reports of central statistical belong to ministry of planning during the last decades. The best regions for wheat cultivation in terms of soil are areas with high amount of humus, enough aeration, and sufficient nutrients [1]. Researchers have estimated that 60% of land farms in the world lack nutrients availability for crops [2-3].

Agriculture practices in Iraq are heavily dependent on agrochemicals e.g, mineral fertilizers and pesticides. Irrational use of such agrochemicals would lead to the frequent soil pollution [4-5]. The use of chemical fertilizers and pesticides for promoting production level and protecting plants disturb ecological balance of the soil (Sharma 2002) [2]. Considerable quantities of heavy metals Cd, Pb, Ni and Cr have been reported in Iraqi soil with the use of DAP, NPK, rock phosphate and others [4-7]. In addition, organic matter in Iraqi soil is very low (nearly 1%). Therefore, much attention has focused on biofertilizers to increase agricultural production and minimize pollution of environment [8-9].

The biological formulation obtained from commercial production of Trichoderma sp. for instance as biopesticide and promoter were developed at biological factories and successfully demonstrated at several wheat fields in many countries [10]. Use of biofertilizers is helpful in improving restoration of environment leveraging agriculture [11]. It was reported that Rhizobium sp. used as plant growth promoters for wheat, whereas Azospirilum sp. as nitrogen fixer [10]. On other hand, Bacillus megaterium act as phosphate solubilizer, while Trichoderma harizianum is considered as plant growth promoters as well as biopesticides. Biofertilizers are products containing living cells of different types of microorganisms when applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by covering nutritionally important elements (nitrogen and phosphorus) from unavailable to available forms [11]. This is occurred through biological process such as nitrogen fixation and solubilization of rocks and phosphate with the combination of fungi and bacteria [8-9, 12].

The Plant Growth Promoting Rhizobacteria (PGPR) promoted plant growth directly through the process of fixation of atmospheric nitrogen, solubilization of phosphorus, production of siderophorus that soloubilize and sequester iron, and production of hormones regulators. Some bacteria support plant growth indirectly by improving and/or eliminating the growth restricting conditions either via production of antagonistic substances or by inducing resistance against plant pathogens [12].

Improving soil fertility is one of the important strategies in increasing agricultural products through compensation application of chemical fertilizers by bio and organic fertilizers [13-14]. Combination of mineral and organic fertilizers can directly increase yield and quality parameters in comparison to mineral fertilizers applied alone and can cause environmentally improvements [8, 11, 15-16]. It is suggested to use a combination of organic and mineral fertilizers to improve grain quality that will lead to environment conservation achieve without a negative effect [17].

Several local contributions were achieved, out of them some were practically related to response of wheat to biofertilizers [18-19] who reported that biofertilizers alone or in combination significantly increased yield of wheat and improve soil characteristics.

The study was conducted to investigate the effect of both local and commercial biofertilizers in combination with mineral fertilizers on yield and yield components of wheat under climate conditions of northern Iraq. Also, reference has been made to local financial cost.

2. Materials and Methods

The experimental site is located in Erbil nearly 323.7 km North West Baghdad, Iraq with coordinates of 36.2 N and 44.0 E and elevation of 390 m. During the wheat growing season (2016 – 2017), the mean min temperature ranged from 3 to 17°C and mean max from 28 to 30°C. The average precipitation was 540 mm.

For characterization of soil, sampling was made at 0-30 cm depth. The soil samples were air-dried and grounded to pass 2 mm sieve. Physical and chemical characteristics of soil were determined using the general procedures described by Reference [20]. Particle size analysis was carried out using the hydrometer method. Chemical soil analysis was carried out as follows: pH and EC were determined with pH-meter and electrical conductivity meter respectively using 1:1 soil: water suspension. Nitrogen as NH_4-N was determined using micro-Kjeldahl and NO_3-N using Bremner and Keeney (1965) method, organic matter content using Walkley-Black acid digestion method. Soluble and extractable K was determined using flame photometer. Colorimetric method was applied to determine P using spectrophotometer method.

Table 1. *Chemical and physical characteristics of the soil before cultivation.*

Character	Unit	Value
Electrical conductivity (EC)	dS m^{-1}	1.2
pH	---	7.3
Organic Mater (OM)	%	1.1
N-NH_4		30
N-NO_3		35
P	mg kg^{-1}	12.6
K (Soluble)		79
K (Extractable)		203
Clay		30.5
Silt	%	17.5
Sand		52.5
Textural Class	Sand Clay Loam	

Wheat Barcelona cultivar was sowed on Dec. 12, 2016 at a rate of 120 kg seeds ha^{-1}. Five treatments were applied (Details of the treatments are given in Table 2):

* Crop managements were conducted as recommended practices for wheat crop.

T1: Natrusoil (Commercial Biofertilizer) only,

T2: Natrusoil + 25% of the recommended dose of chemical fertilizers (CF),

T3: Local Biofertilizer B1 + 25% of CF,

T4: Local Biofertilizer B2 + 25% of CF, and

T5: Control (CF) (Urea = 260 kg ha^{-1}, DAP = 180 kg ha^{-1}).

The size of block was 2.5 ha replicated four times. Each block containing the five treatments with 0.5 ha for each treatment (experimental unit).

Table 2. *Formulas of biofertilizer and chemical fertilizers.*

Treatments	Description
T1	Natrusoil only,* (Natrusoil is compatible mixture of humic acid 1.5% and some Bacillus species recorded up to 10 x10^{20} c.f.u. /mL- it is vincula product. Bacteria 02%, Bacillus subtillis, Bacillus magterium Bacillus polymyax, Bacillus licheniformis, Bacillus uniflagellatus Bacillus laterosporus, Bacillus chitinoporus).
T2	Natrusoil + 25% Chemical Fertilizer
T3	Local Formula B1+ 25% Chemical Fertilizer. (Formula B1 contains Rhizobium cicceri + Asospirillium baselines + Trichodermaharizium)
T4	Local Formula B2 + 25% Chemical Fertilizer. (contains Rhizobium cicceri + Azospirillium basilenes + Trichoderma harzianum + Bacillus megaterium)
T5	Control (The Recommended Chemical Fertilizer: Urea = 260 kg/ha and DAP = 180 kg/ha).

* Vincula Industries, Inc. P.O. Box 2549, Haker Heights, TX 76548 USA, phone. 760-900-9927. Email: subhi@vincula.com.

The local bacterial isolates were isolated and purified in the organic laboratories and grown on 1000 mL nutrient broth flasks and incubated at 28 oC in cooled shaking incubator for 2 days till attain uniform density 107- 108 cfu/mL (Majed et.al 2017). The culture then carried on the solid sterilized carrier containing charcoal + peatmos and incubated at 28oC for 24 – 48 h. Seed coating was the method used with the local bacterial isolates whereas spraying the field by diluted sample was used for Natrusoil. For the later imported biofertilizer, 1.0 L of Natrusoil was diluted in 400 L of clean water enough for 1.0 ha area and sprayed evenly on land just before sowing and plowing.

Natrusoil is a compatible commercial liquid mixture of humic acid 1.5 % and of some Bacillus spp recorded up to (10*1020 c.f.u/mL). The microorganisms of Natrusoil are several species of bacteria (Table 1), considered as source of B. subtilis as biopesticides, B. megaterium as phosphate solubilizes, Bacillus polymyxa as N2 fixer, and B. licheniformis secreted many enzymes and antibiotics. Similarly, B. uniflagellates are considered as source of biopesticides, B. laterosporus helps in balancing of normal flora and as source of pesticides while B. chitinoporus works on hydrolysis of compound. Information and the product "Natrusoil" are from Vincula Industries, Inc., USA.

Feasibility study was performed to compare the financial cost of mineral fertilizers and biofertilizers alone or in combination with the mineral fertilizer.

3. Results

3.1. Effect of Biofertilizer on Wheat Yield, Yield Components, and Soil Nutrients Status

Most test biofertilizers significantly increased yield of wheat grain or yield components (Table 3). Natrusoil+25%CF and B1+25%CF increased yield by 17-18% over the control. Similarly, number of spikes/m^2 for Natrusoil, B1+25%CF, and B2+25%CF increased by 6 to 27% over the control. Weight of 1000 kernel for Natrusoil+25%CF, B1+25%CF, and B2+25%CF increased by 18-25% over the control. The number of spikes and weight of 1000 kernel are the main factors responsible for the increase in yield. On the other hand, no significant differences were found for the weight of spikes.

Table 3. *Biofertilizers effect on wheat yield and its components during the season 2016-2017 in Erbil province, Iraq.*

Treatment	No. of spikes m^{-2}	Grain $spike^{-1}$ (g)	Weight 1000 grain gm)	Yield (ton ha^{-1})
T1 (Natrusoil)	413.3*	34.33	36.67	4.427
T2 (Natrusoil+25%CF^{+})	384.1	40.53	42.20*	4.659*
T3 (B1+25%CF)	426.7*	34.00	41.13*	4.691*
T4 (B2+25%CF)	436.7*	34.17	39.80*	4.578
T5 (Control, 100%CF)	343.7	39.60	33.70	3.987
$LSD_{0.05}$	55.23	3.697	4.347	0.615

$^{+}$ CF=Chemical Fertilizer.
* significant at the 0.05 level of probability.

It is clearly evident that the levels of nutrients in the soil after harvest have been increased with the application of chemical fertilizer or biofertilizers regardless of type of biofertilizrs (Table 4). The increase in nutrients of biofertilizer treatments was in the ranged of 33-77%, 20-80%, 56-74%, 11-39%, and 3-25% for NH_4, NO_3, P, K_{sol}, and K_{ext}, respectively.

Table 4. *Effect of application of biofertilizers on nutrients status in the soil after harvest of wheat crop. (Data for nutrients in the soil before cultivation are included for comparison).*

Soil Sample		NH_4	NO_3	P	K (Sol)	K (Ext)
		mg kg^{-1}				
Before cultivation		30	35	12.6	79	203
	T1 (Natrosoil)	53	42	19.9	88	221
	T2 (Natrsoil+25%CF^{+})	40	43	21.5	72	254
After Harvest	T3 (B1+25%CF)	51	63	19.6	110	243
	T4 (B2+25%CF)	45	59	5.0	98	210
	T5 (Control, CF)	63	60	22.0	136	213

$^{+}$ CF = Chemical Fertilizer.

3.2. Economic Evaluation

Financial evaluation of using the imported biofertilizer (Natrosoil) alone or in combination with 25% of chemical fertilizer (T1 and T2) as compared with using of chemical fertilizer T5 (Control) is given in Table 5. The evaluation is based on the subsided prices of the chemical fertilizer, but not on prices in local market. The latter is much higher than the subsided price. The reduction in cost per one hectare area when using Natrusoil + 25%CF was 126,000 ID (ID, Iraqi Dinar = 1,200 US Dollar). Further, the reduction in cost reached 193,000 ID when using the Natrusoil alone. In percent, the reduction in cost was 47% and 72% for Natrasoil + 25%CF and Natrusoil, respectively. Application of biofertilizers resulted in decreasing agricultural cost worldwide which supported the existing contribution (Abd El-Gawad and El-Sayed 2009, Sharma et. al 2012; Yasin et. al 2012).

Table 5. *Financial cost of fertilizers under study per hectare on wheat fields in Iraq.*

Fertilizer	Dose/ha	Cost /kg or L (ID[+])	Total Cost/ha (ID)
Urea	260 kg	500	130,000
DAP	180 kg	770	138,600
Both Urea + DAP (CF[++])	180 kg + 260 kg		268,600
Natrusoil	1 liter	75,000	75,000
25%Urea	65 kg	500	32,500
25%DAP	45 kg	770	34,650
Natrusoil + 25%CF	1 L + 65 kg + 45 kg		142,150
Reduction in cost when using Natrusoil alone (T1)	268,600 - 75,000 = 193,600		
Reduction in cost when using Natrusoil + 0.25%CF (T2)	268,600 - 142,000 = 126,600		

[+] ID = Iraqi Dinar (One US. Dollar = 1200 ID).
[++] CF = Chemical Fertilizer

4. Discussion

Pollution in Iraqi soils has been indicated in several reports due to the application of DAP, NPK, and rock phosphate. Such application resulted in buildup of Cd, Pb, Ni, Cr in soil and also in contamination of wheat by Cd and Pb [7]. The levels of N in the forms of NO_3 and NH_4, P, and K increased in the soil after harvesting due to the application of biofertilizer as well as the chemical fertilizer. The lowest value of P was noticed in the biofertilizer treatments (T1 - T4) demonstrated that Plant Growth Promoting Rhizobacteria (PGPR) dissolved the mineral phosphorus and increased its available for crop uptake. This result is supported by Ref. [11]. Results presented in Table 1 indicated that biofertilizers have positive effect on plant growth and play a great role in making the nutrients N, P and K available for plant uptake. In the same line Ref. Similar results on enhancing the availability of nutrients in soil with application of biofertilizer are achieved [21].

Plant growth and yield parameters indicated that inoculation with biofertilizers B1 and B2 as solid or Natrusoil as liquid fertilizers individually or in combination gave an enhancement effect on both plant growth and yield (Table 3). These results are agreed and supported by several researchers [17, 22-24]. They indicated that in some species and strains of Azotobacter, Azospirillium, Bacillus, Pseudomonas and some fungal species, Trichoderma and others have significant effect on number of spikes, seed weight, and number of seeds as well as yield.

The superiority of biofertilizers application when accompanied with reasonable amounts of mineral fertilizer can be due to N fixation, P solubility, supplying humic acid, growth promoting, and diseases resistance. On the other hand, chemical fertilizer alone can be imbalanced and insufficient.

5. Conclusions

Response of Wheat to biofertilizers alone or in combination with 25% of recommended dose of mineral fertilizers was positive. The biofertilizer increased yield and yield components of wheat, improve soil nutrients balance, and minimize environmental pollution. Among the benefits of using bioferilizers was the reduction in cost as compared with the use of full dose of mineral fertilizer.

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