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EFFECT OF THE BIOFERTILIZER (*AZOTOBACTER CHROOCOCCUM & TRICHODERMA HARZIANUM*) AND LEVELS OF PHOSPHATE ROCK ON GROWTH AND YIELD OF WHEAT (*TRITICUM AESTIVUM L.*)

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Abstract

Azotobacter chroococcum was isolated from 10 agricultural soil samples using the culture, Ashby. The bacterial isolates were diagnosed depending on morphological, microscopic, and biochemical properties. Diagnosis results showed there were 6 isolates belong to *A. chroococcum* given the symbol (A), then one isolate (more effective to fix nitrogen) was selected to evaluate the nitrogen quantity fixed into the culture (nitrogen free) pollinated with the fungi *T. harzianum*, and were purified and stored to be used as biofertilizer in subsequent experiments. *T. harzianum* was isolated from the same soil samples using the culture Potato Dextrose Agar (PDA) and the isolates were diagnosed according to the classification keys which showed that there was one isolate belong to *T. harzianum* given the symbol (T) and stored at 4 CR" to be use later as a biofertilizer. The two biofertilizers (bacterial and fungal) were used in pots experiment with phosphate rock levels of 0, 50, and 100 kg P. ha^{-1} and the half wheat fertilizer recommendation was used in all treatments. The results showed that the treatment of bacterial plus fungal inoculation (A+T) had superiority over all treatments in which all wheat growth and yield parameters were studied, and achieved a higher N and P concentration average of 3.00% N. $plant^{-1}$ and 0.39% P. $plant^{-1}$. The treatment (A+T) had superiority in the grain and biological yields of 18.01 and 43.06 g. pot^{-1}, respectively.

Key words : Azotobacter, Trichoderma, Biofertilizer, Wheat, Soil.

Introduction

Biofertilizers have an important role in maintaining soil fertility, can be used with organic and non-organic fertilizer, are environmentally friendly, are less expensive, and contribute to the development of an integrated nutrient management system in the soil, as well as a averages of preserving the environment (Mishra *et al.,* 2013). Biofertilizers have a role in dissolving non-dissolved phosphorus compounds, simplifying other nutrients, fixing nitrogen and increasing resistance to stress. They play a role in the formation of soil aggregates and improving the soil environment and fertility (Mitter *et al.,*2013). Zarrin *et al.,* 2009) mentioned that *A. chroococcum* forms compounds that promoting plant growth such as Indole Acetic Acid (IAA), enzymes, and hormones in different concentrations which have a positive impact on wheat seeds germination (achieved 100% in different plants). *A. chroococcum* has a high ability in non-symbiotic nitrogen fixation, and the biofertilization treatments proved the positive role of these bacteria in increasing the studied growth and yield parameters and grains content of nitrogen and protein compared to the control (Mikhailouskaya & Bogdevitch) (11). The study results of (Yousefi & Barzegar., 2009) mentioned that the treatment of wheat seeds with Azotobacter and Pseudomonas bacteria has increased the yield of the grains and the biological yield compared with the control, the inoculation of these bacteria can compensate for 25 – 50% of the added chemical fertilizers. Reyes *et al.,* 2006) mentioned that Trichoderma and Penicillium fungi had an ability to dissolve the phosphate rock through producing some organic acids(Kapari & Tewari 2010) found that the released phosphorus from tri-super phosphate gradually increased by increasing acidity of the culture at the inoculation with *T. harzianum*. (Windham *et al.,* 1986) noted the mechanism of producing

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plant hormones by *T. harzianum* which explained the plant growth promotion and called it as Plant Growth Promoter (PGP) (Harman *et al.,* 2001) found that *T. harzianum* enhances forming a dense and deep root system to achieve physiological benefits, especially when plant grows up in dry conditions. Wheat is one of the most important grain crops grown in the world, as it is an essential source of energy for human. This study aimed to i) isolate and diagnose *A. chroococcum* bacteria and *T. harzianum* fungi from the soil to produce a biofertilizer, and ii) Study of the effect of single inoculation and mixing of bacterial and fungal fertilizers on the growth and yield of wheat.

Materials and Methods

Isolation and diagnosis

Ten soil samples were: collected from the fields of the college of Agriculture – University of Baghdad, taken from the area of the rhizosphere (soil and plant roots), placed in bags of polyethylene, and brought to the laboratory and series of dilutions were prepared. 0.5 ml (10^{-5} and 10^{-6} dilutions) was spread on the hard Ashby culture with three replicates to isolate Azotobacter; the culture contents were: 20 g. L^{-1} mannitol, 0.2 g. L^{-1} calcium phosphate, 0.1 g. L^{-1}, 0.1 g. L^{-1} sodium chloride, 0.2 g. L^{-1} hydrated magnesium sulfate, 0.1 g. L^{-1} potassium sulfate, 5 g. L^{-1} calcium carbonate, 20 g. L^{-1} Agar, and 1 L distilled water (Islam *et al.,*2008). The dishes were incubated at 28 ° C for 48 hours. After the appearance of the bacterial colonies, the following morphological, microscopic, and biochemical tests were carried out: colony shape, colony color, cells shape, chromium chromatography, oxidase, catalase, gelatinase, indole, starch decomposition, motion, and growth at 37 C. bacterial isolates were diagnosed (Holt *et al.,*1994). Four *A. chroococcum* isolates were diagnosed. In order to select more effective isolate to fix nitrogen, accumulative nitrogen quantity was evaluated for 7 days using 25ml of semi – solid (L G) nitrogen – free (Girish *et al.,*2010), the contents of this culture were: 0.1 g.L^{-1} $FeSO_4.7H_2O$, 0.2 g. L^{-1} $MgSO_4.7H_2O$, 1.0 g. L^{-1} K_2HPO_4, 1.0 g. L^{-1} $CaCO_3$, 5 g. L^{-1} sucrose, 5 g. L^{-1} Bromophenol blue. The nitrogen fixed into the semi – solid culture was evaluated using Keldahl device.

Trichoderma fungi

T. harzianum fungi was isolated, from the same soil samples that *A. chroococcum* bacteria was isolated before, using PDA. One *T. harzianum* isolate was diagnosed among four fungi isolates after studying their properties according to(Barnett & Hunter, 1972), and (Domsch *et al.,* 1980) and stored on a slant.

Preparation of bacterial inoculum

The most efficient nitrogen fixation *A. chroococcum* was used to prepare the bacterial inoculum. 100 ml of liquid nutrient culture (N.B) was prepared in a 250 ml flask and, after sterilization, inoculated with *A. chroococcum* and incubated at 28 ° C for 72 h. The density of the inoculum was calculated based on counting dishes method.

Preparation of fungal inoculum

250 g of millet seeds were placed in a 500 ml flask, 50 ml distilled water were added, sterilized by autoclave for 20 minutes, incubated for 2 days at 28 ° C. A part of the fungal culture was added to the flask, millet seeds were mixed with the fungi, incubated for a week with moving the contents of the flask daily for the purpose of distributing the fungus on the seeds equally, and the inoculum density was estimated using dilution and dishes method.

Treatments

The treatment of the bacterial inoculum was symbolized as (A), fungal inoculum as (T), and mixed inoculum as (A+T). Phosphate rock was added at three levels (0, 50, and 100) kg P ha^{-1}. Mineral fertilizers (N. P. K) were added as a half of the fertilizer recommendation for the wheat crop. The experimental units were 36.

Greenhouse experiment

Soil samples were taken from one of the agricultural fields in the College of Agriculture for the purpose of growing wheat seeds in 36 pots. The soil was analyzed: pH and EC in extract 1: 1 (Thomas,1982), organic matter was estimated using the Walkley-Black method (Nelsonand Sommers,1982), calcium carbonate was evaluated using calcimeter method (Thomas,1982), available phosphorus (Olsen *et al.,*1954), available nitrogen (Thomas,1082), CEC (Polemioand Rhoades,1977), and soil texture using hydrometer (Gee and Bauder,1986). (Table 1) showed the physical and chemical and physical soil properties:

In this experiment, Plastic pots (10 kg capacity) were used. The seeds were sterilized with 1% sodium hypo-chloride and then washed with distilled water. 250 sterile seeds were placed in sterile beaker and the 100 ml of bacterial inoculum was added with 10% of acacia gum left for an hour. 10 seeds were planted into each pot. 1 g of fungal inoculums taken from the culture and put with the seeds. After seed germination, the number of plants reduced to 5 plants per pot. This experiment was carried out in the greenhouse of Department of Soil Science and water resources, College of Agriculture – University of Baghdad. The growth parameters (such as the percentage of nitrogen and phosphorus, and the grain and biological

Table 1: Some soil chemical and physical properties.

Property	Value
2.9	EC (Dsm^{-1})
pH	7.2
OM (%)	0.6
$CaCO_3$ (%)	24.25
CEC ($Cmol. Kg^{-1}$)	18.4
N ($mgkg^{-1}$)	13.41
P ($mgkg^{-1}$)	5.12
Total *Bacteria Count cfu* g^{-1} soil × 10^5	1.2
Sand (%)	53.52
Silt (%)	36.14
Clay (%)	10.34
Soil Texture	Sandy loam

yield) were evaluated. A factorial experiment, Complete Randomized Design (CRD) with three replicates, was used and significant differences among the averages, by choosing Least Significant Difference (LSD), were compared using SAS (2004) program.

Results and Discussion

Azotobacter isolation and diagnosis

The isolation and diagnosis results showed that there were four bacterial isolates belonged to *A. chroococcum* based on morphological, microscopic and biochemical tests. One bacterial isolate was selected for its ability to fix nitrogen in the nitrogen-free culture, which was able to fix a higher nitrogen quantity of 5.65 mg N. ml^{-1} compared with the lower fixed nitrogen quantity of 1.75 mg $N.ml^{-1}$ by other isolates.

Concentrations of nitrogen and phosphorus (%) in the plant

The results, in tables 2 and 3, showed that there were significant differences, for the treatments of single and mixed bacterial and fungal biofertilizers, in % of N and P per plant. The mixed biofertilizer treatment (A+T) had a superiority on other treatments (at 100 $kg.ha^{-1}$ of phosphate rock) achieving 3.33% $N.plant^{-1}$ and 0.38% $P.plant^{-1}$ compared with the control which achieved 2.22 and 0.22%, respective-ely. A single biofertilizer (A) achieved an average of 3.15% $N.plant^{-1}$ and 0.32% $P.plant^{-1}$ compared with the control, while the treatment (T) achieved a significant superiority of 2.23% $N.plant^{-1}$ and 0.24% $P.plant^{-1}$ compared to the control.(Al-Erwy, 2016) pointed to the positive effect of the bacterial inoculum in supplying plant with nutrients such as nitrogen through the nitrogen fixation as well as the important role of growth regulators in improving plant growth. (Pu Guixin *et al.,* 2008) mentioned that biofertilization is important for atmospheric nitrogen fixation, the production of growth regulators, and nutrient absorption. When *T. harzianum* used as a fertilizer, works to form a dense and deep root system which has physiological benefits for the plant (Harman, 2001).

Grain and biological yield ($g.pot^{-1}$)

The results in (Tables 4, 5) showed that there were significant differences among the averages of single and the mixed biofertilizer in the grain and the biological yield ($g.pot^{-1}$). (A+T) treatment was significantly superior over all treatments at 100 $kg.ha^{-1}$, which achieved a grain yield of 19.80 g-1 and a biological yield of 49.13 g-1 compared with the averages of the two control treatments of 12.31 and 21.82 g. pot^{-1}, respectively. While the treatment of the single inoculum (A) and the fungal inoculum (T) showed significant differences among the averages. The inoculum (A) treatment achieved a grain yield of 18.17 $g.pot^{-1}$ and biological yield of 43.23 $g.pot^{-1}$ compared with the two control treatments of 12.31 and 21.82 g.pot-1at

Table 2: Concentration of N (%$N.plant^{-1}$).

Fungus (T)	Bacteria (A)	Levels of Phosphate Rock (Kg . ha^{-1})			Average	
		0	50	100	T+A	T
no adding	without inoculation	2.10	2.20	2.35	2.22	2.68
	inoculation	3.05	3.17	3.25	3.15	
adding	without inoculation	2.14	2.26	2.30	2.23	2.78
	inoculation	3.25	3.36	3.40	3.22	
0.15 T+A				LSD	LSD T+A 0.6	LSD T 0.03
T+PR	no adding	2.12	2.13	2.16	LSD T+ PR 1.22	
	adding	2.15	2.19	2.21	Average A	
A+PR	no adding	2.10	2.16	2.23	2.12	
	adding	2.30	2.39	2.45	2.38	
LSD A+PR 0.41					0.02 LSD A	
PR		2.16	2.21	2.51	LSD RP 0.04	

A= bacterial inoculum T= fungal inoculum A+T= mixed PR= phosphate rock levels.

Table 3: Concentration of P (%P.plant^{-1}).

Fungus (T)	Bacteria (A)	Levels of Phosphate Rock (Kg . ha $^{-1}$)			Average	
		0	50	100	T+A	T
no adding	without inoculation	0.20	0.21	0.22	0.22	0.26
	inoculation	0.29	0.31	0.36	0.32	
adding	without inoculation	0.21	0.24	0.28	0.24	0.28
	inoculation	0.24	0.33	0.39	0.38	
LSD A+T+PR 0.09					LSD T+A0.05	LSD T0.03
T+PR	no adding	0.20	0.22	0.23	LSD T+PR 0.01	
	inoculation	0.26	0.32	0.39	Average A	
A+PR	adding	0.23	0.24	0.26	0.24	
	inoculation	0.27	0.33	0.38	0.32	
LSD A+PR 0.12					LSD A 0.03	
PR		0.24	0.27	0.31	LSD PR 0.04	

Table 4: Grain yield (g.pot^{-1}).

Fungus (T)	Bacteria (A)	Levels of Phosphate Rock (Kg . ha $^{-1}$)			Average	
		0	50	100	T+A	T
no adding	without inoculation	10.27	12.24	14.43	12.31	15.24
	inoculation	15.18	19.25	20.10	18.17	
adding	without inoculation	11-75	13.17	16.00	13.64	16.72
	inoculation	16.80	20.11	22.50	19.80	
A +T 1.07		LSD PR			LSD T+A 0.43	LSD T 0.38
T+PR	no adding	10.30	12.31	14.00	LSD T + PR 1.62	
	adding	13.11	14.80	15.55	Average A	
A+PR	without inoculation	12.10	13.00	14.75	13.28	
	inoculation	14.32	14.88	15.85	15.01	
LSD A + PR 0.52					LSD A 0.31	
PR		12.45	13.74	15.03	LSD PR 0.34	

Table 5: The biological yield (g.pot-1).

Fungus (T)	Bacteria (A)	Levels of Phosphate Rock (Kg . ha $^{-1}$)			Average	
		0	50	100	T+A	T
no adding	without inoculation	20.61	21.65	23.22	21.82	29.19
	inoculation	30.79	33.81	35.11	36.57	
adding	without inoculation	23.75	24.50	25.85	24.70	29.94
	inoculation	31.22	35.15	39.13	35.17	
LSD A + T + PR 1.20					LSD T+A 1.75	LSD T 0.97
T+RP	no adding	20.68	21.81	24.11	LSD T + PR 0.01	
	adding	35.60	39.41	42.33	Average A	
A+RP	without inoculation	21.00	22.13	24.05	22.39	
	inoculation	30.35	34.20	35.88	33.47	
LSD A + PR 0.74					LSD A 0.37	
PR		26.90	29.38	31.59	LSD PR 0.52	

100 kg.ha^{-1}, respectively. All treatment had superiority in the grain and the biological yields at the level of 100 kg.ha^{-1} of phosphate rock compared with the level of 50 kg.ha^{-1}. The role of bacterial and fungal fertilizers was to increase nitrogen in the soil and release of growth-promoting substances, especially making some unavailable nutrients available to be absorbed (Kumar,1998)(Patil,2010)(Reyes *et al.*,2006).

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