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ISOLATION AND DIAGNOSIS OF PHOSPHATE SOLVENT BACTERIA AND THEIR EFFECT AS BIOFERTILIZER ON GROWTH AND YIELD OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

The phosphate solvent bacteria were isolated from 10 agricultural soil samples using the solid culture media Pikovskaya and the isolates were identified using cultural and biological tests. Two solvent phosphate isolates were selected through the solubility index from two bacterial species: *Bacillus megaterium* and *Pseudomonas fluorescens*. The two isolates were grown in the fluid culture media for the preparation of the biological pollen and it is density was calculated. The gum arabic (10%) was prepared and the peat moss was used as a biological pollen holder as well as the fertilized seeds were planted in pots with a capacity of 10 kg of soil. The results showed that the treatment of mixed biological pollen and the third level of phosphate rock (500 kg.ha^{-1}) exceeded all treatments and achieved the highest plant height of 83.444 cm.plant^{-1}, dry vegetative weight of 12.581 g.plant^{-1}, yield weight 5.851 g.plant^{-1}, and protein content 8.744 % compared with other treatments that achieved low estimates for all studied criterions.

Keywords**:** Phosphate solvent bacteria, biological pollen, soil, wheat

Introduction

Phosphorus is one of the main nutrients that directly or indirectly affect biological processes and it is the main component of energy metabolism and bio-building of nucleic acids and membranes and the lack of phosphorus is one of the major determinants of crop production. Phosphate solvents bacteria have a high ability to dissolve phosphate in soil and used to increase the ability of the plant to absorb phosphorus and increase the yield. The phosphate solvents bacteria included *Pseudomonas*, *Bacillus* and *Rhizobium* (Patel, 2016). These bacteria can dissolve phosphate in several ways, Bacillus can produce organic acids such as Lactic, Gluconic, Citric and Succinic acids. Jastrzebska *et al*., 2015 noted the effect of bio-fertilizer produced from animal bones and ashes of the residues containing *Bacillus megaterium* in a field experiment using quaternary wheat. The objective of the study is to isolate and diagnose the phosphate solvent bacteria of the soil, and use it as biological fertilizer as well as study some of the growth criteria of wheat plant.

Materials and Methods

The phosphate solvent bacteria were isolated from the soil samples by making means of the decimals and cultivated on the solid culture media (Pikovskaya). The dishes were incubated at a temperature of 28 ° C. The colonies in the dish were identified by agricultural, microscopic and biochemical tests (Table 1). The ability of the bacterial isolates to dissolve the insoluble phosphate was then tested by growing them on the phosphate-containing medium according to the solubility index

Pot experiment

The efficient isolates in dissolving phosphorus compounds used a biological fertilizer, which was developed on the liquid nutrient medium (NB), and pollen density was calculated by planting it on the solid medium and counting the visible colonies. The peat moss was prepared and placed in polyethylene bags that were resistant to high heat and wet with sterile water at 20% of the peat moss weight ethyl alcohol and then washed with distilled water then after the wheat seeds were fertilized with the biological pollen, mixing 100 g of seeds with 10 ml of liquid bacterial pollen and 10 g of the gum was added and the bacterial pollen was treated with the holder in sterile bags. The experiment was carried out in a plastic house and loamy soil was used for planting. The soil was analyzed to investigate the physical, chemical and biological characteristics before planting (Table 2). After that, the soil placed in a 10 kg pot and planted with sterile seeds (each pot 10 seeds and after germination the number of seeds was reduced to 5 seed per pot). As recommended for the wheat crop, the chemical fertilizers (N. K) were added, while the phosphate fertilizer was added with half of the recommendation as well as the field capacity and water ready for soil for irrigation were calculated. The experiment included two factors: Biochemical pollen and phosphate rock.

Table 1 : Results of agricultural, microscopic and biochemical tests of phosphate solvent bacteria isolates

Isolates	Cell form	Gram bacteria	Production of oxides	Production of catalase	Production of gelatin	Decomposition of starch	Production of indole	Production of urease	Consumption of citrates	The movement
1	bacilli	-	+	+	-	-	-	+	+	+
2	bacilli	-	+	+	+	-	-	-	+	+
3	bacilli	+	+	+	-	+	-	-	+	+
4	bacilli	-	+	+	+	-	-	-	+	+
5	bacilli	+	+	+	+	-	-	-	+	+
6	bacilli	+	+	+	+	-	-	+	+	+
7	bacilli	-	+	-	-	-	-	-	+	+
8	bacilli	-	+	+	+	-	-	-	+	+
9	bacilli	-	+	-	-	+	-	+	+	-
10	bacilli	+	+	+	+	-	-	-	+	-

Table 2 : Some chemical, physical and biological characteristics of pre-planting soil

Measured quality		Value	Measuring unit
Electrophoresis Ec 1: 1		2.4	ds.m^{-1}
pH 1: 1		7.3	
Nitrogen (N)		23.0	Mg.kg^{-1} soil
Phosphorus (P)		5.16	
Potassium (K)		97.81	
Organic matter (OM)		0.65	%
Dissolved calcium Ca^{+2}		14.25	Mme.l^{-1}
Dissolved sodium		1.88	
Bicarbonate dissolved (Hco_3)		1.23	
Dissolved chlorine		22.53	
Magnesium soluble (Mg)		7.62	
Soil separators	Sand	4.6	g.kg soil^{-1}
	Silt	2.4	
	Clay	7.3	
Preparation of total bacteria		23.0	Cfu.g^{-1} dry soil
Preparation of *Bacillus* bacteria		5.16	
Preparation of *Pseudomons* bacteria		97.81	

Treatments and Experimental Design

The experiment was of Complete Random Design (CRD) with two factors, the factor is the biological agent that included the single pollen *Bacillus megaterium* or *Pseudomonas fluorescens*, the bacterium mixed pollen and the non-addition of the biological pollen. The second factor is the phosphate rock which included three levels (0, 250 and 500 kg ha^{-1}) of phosphate rock. The growth parameters were measured and included plant height, dry vegetative weight and the calibrations of the crop (Weight of plant^{-1} and protein content in grains).

Results and Discussion

The results of the solubility index showed that there were two efficient isolates in the phosphate solubility, namely, isolates 2 and 5, which achieved more solubility of phosphatic rock on the surface of the plant than the rest of the isolates. The isolates of numbers 2 and 5 were then tested for biochemical preparation, where isolation (2) belong to *Bacillus megaterium* bacteria and isolation (5) belong to *Pseudomonas fluorescens* bacteria.

Plant height (cm): The results of the statistical analysis showed that there was a significant difference between the experimental treatments (Table 3). The combined treatment with the third level of phosphate rock (500 kg ha^{-1}) exceeded on all the treatments, which achieved a height of 83.444 cm compared to the average of the comparison treatment which achieved 70.444 cm while single pollen treatment for *Bacillus* bacteria achieved height of 81,000 cm and *Pseudomonas* recorded value of 77.667 cm. These results showed that the biological pollen has a clear effect on the height of the plant and especially the mixed pollen which may be attributed to the positive role of bio-fertilization using the mixture of bacterial isolates that stimulate plant growth by facilitating phosphorus and increasing the concentrations of nutrients and soil nutrients that affect the properties of total vegetation, including plant height. There were significant differences between the averages of phosphate rock with the biological pollen where the level of 500 kg.he^{-1} gave an average of 81.667 cm compared to the level of 250 kg.he^{-1} and level (0) kg ha-1 phosphate rock which recorded means of 78.000 and 74.750 cm respectively. This combination (*Bacillus megaterium* and *Pseudomonas fluorescens*) plays a role in stimulating growth by producing growth hormones such as acetic acid and oxinate which have a significant role in stimulating cellular division and plant elongation (Verma et al. 2010).

Table 3: Plant height rate (cm)

Pseudomonas	***Bacillus* bacteria**				
	0	**1**	**2**	**3**	**Average**
0	68.667	76.667	74.333	79.333	74.750
1	70.333	81.000	77.333	83.333	78.000
2	72.333	85.333	81.333	87.667	81.667
LSD5%	N.S				2.086**
Average	70.444	81.000	77.667	83.444	78.139
LSD5%	2.409**				

The total dry weight of g.plant^{-1}: The results showed that there were significant differences between the means of the treatments (Table 4). The treatment of the biological pollen with the level of 500 kg.ha^{-1} of phosphate rock gave an average of 12.581 g.plant^{-1} compared to the average of comparison treatment which gave value of 10.318 g.plant^{-1} while the treatment of single pollen of *Bacillus* and *Pseudomonas* achieved means of 12.210 and 11.487 g.plant^{-1} respectively. There was a significant difference between the average of the three levels of phosphate rock, which ranged between 12.745 and 10.655 g.plant^{-1}. These results indicated the ability and efficacy of the two bacterial isolates in facilitating and processing phosphorus and potassium, affecting the vegetative growth (Jastrzebska, 2015 and Za rjani, 2013).

Table 4: The weight of the total vegetation (g.plant $^{-1}$)

Pseudomonas	***Bacillus* bacteria**				
	0	**1**	**2**	**3**	**Average**
0	9.353	11.190	10.691	11.387	9.353
1	9.672	12.175	11.533	12.809	9.672
2	11.929	13.265	12.238	13.548	11.929
LSD5%	N.S				0.3648**
Average	10.318	12.210	11.487	12.581	11.649
LSD5%	0.4213**				

The weight of the yield (g. plant^{-1}): The results showed a significant difference between the averages of the biological pollen and the interaction with phosphate rock levels and their effect on the yield weight of the plant (Table 5). The combined biological pollen with 500 kg ha^{-1} of the phosphate rock was superior on all the other treatments and achieved 5.851 $g.plant^{-1}$ compared to both treatments of single pollen and control treatment that achieved 4,877, 4.199, and 2.180 respectively. This was also reflected in phosphate rock levels where the third level achieved the highest level of 5.243 $g.plant^{-1}$ compared to the levels of 0 and 250 Kg ha^{-1} that recorded values of 3,153 and 4,435 $g.plant^{-1}$ respectively. These results reflect the high microbial activity of bacterial isolates by providing the nutrients needed by the plant to contribute to increase the plant yield through the balanced absorption of nutrient by the plant (Yousef and Barzegar, 2014).

Table 5: The average of Plant yield weight ($g.plant^{-1}$)

Pseudomonas	***Bacillus* bacteria**				
	0	**1**	**2**	**3**	**Average**
0	1.843	3.307	2.933	4.527	3.153
1	2.180	5.043	4.363	6.153	4.435
2	2.517	6.280	5.300	6.873	5.243
LSD5%	0.6081**				0.3040**
Average	2.180	4.877	4.199	5.851	4.277
LSD5%	0.3511**				

Protein content in grains (%): The results showed that significant differences between the averages of biological pollen and phosphate rock levels in the protein content of the grains. The mixed biological pollen treatment was superior with mean of 8.744% compared with the comparison treatment that achieved 7.271%, while the two treatments of single pollen recorded percentage of 7.966 and 7.641% respectively. The treatment of the third level of phosphate rock (8.347%) has exceeded the level of 0 and 250 $kg.he^{-1}$ (7.366 and 8.00% respectively). The increased absorption of phosphorus available in the soil by the plant will lead to an increase in the size of the total root and thus increase the absorption of major nutrients, including the element of nitrogen, which is reflected on the increase in protein in the grains**.**

Table 6 : Protein percentage in grains (%)

Pseudomonas	***Bacillus* bacteria**				
	0	**1**	**2**	**3**	**Average**
0	7.154	7.570	7.393	7.345	7.366
1	7.238	8.003	7.580	9.194	8.004
2	7.421	8.324	7.951	9.693	8.347
LSD5%	N.S				0.511**
Average	7.271	7.966	7.641	8.744	7.905
LSD5%	0.590**				

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