

# Effect of Humic Acid, Biofertilizers and Mineral Phosphate on Soil Microbial Activity and Productivity of Pea Plants under Toshka Conditions

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## ABSTRACT

Two field experiments were conducted during the winter season of 2015/2016 & 2016/2017, at Toshka Research Station - Desert Research Center. The aim of the study was to investigate the effect of humic acid (HA) at rates of 0, 4 and 8 kg / fed. combined with phosphate dissolving bacteria(PDB) and mineral phosphate fertilizer at rates of 100, 90, 80 and 70% P<sub>2</sub>O<sub>5</sub> of the recommended dose in commercial production on pea plants. Treatment with humic acid at 8 kg / fed. combined with inoculation of PDB and 100% of mineral phosphate fertilizer significantly increased the total microbial count, *Bacillus megaterium* count, CO<sub>2</sub> evolution, organic carbon and phosphatase and dehydrogenase enzymes activities. The bio-fertilizer treatments (PDB) showed a clear superiority when added in combination with the phosphate fertilizer and humic acid compared to the results of using a mixture of humic acid and superphosphate only. Adding of humic acid and superphosphate with the presence of phosphate dissolving bacteria improved most of the vegetative growth characteristics of plants. Yield components had the highest positive response to humic acid combined with mineral fertilizer percentage with phosphate-dissolving bacteria. The highest concentration of N, P, and K were with the use of humic acid at (8 kg), phosphate-dissolving bacteria and 100% phosphate fertilization P<sub>2</sub>O<sub>5</sub>.

The study revealed that using 8 kg HA/fed in combination with PDB and high level of P<sub>2</sub>O<sub>5</sub> as a fertilizer application to improve soil properties, vegetative growth, mineral content and yield of pea plants in new soils was recommended.

**Key words:** Microbial activity, Pea, Growth, yield components, Humic acid, Bio-fertilizers, Super phosphate (P<sub>2</sub>O<sub>5</sub>), Toshka.

## INTRODUCTION

The over increased world population required both horizontal and vertical agriculture extension to meet the increased food demands. Vasil (1998) and Leisinger (1999) reported that increasing food productivity by about 50% in the next twenty years is needed to meet the population pressure. Horizontal and vertical agriculture extension in desert areas faced with the problem of low soil fertility. Vikram and Hamzehzarghani (2008) stated that phosphorus is the second major macronutrients for plants because it has an important role in plant metabolism. Yagodin (1990)

added that phosphorus has a great role in biosynthesis and translocation of carbohydrates, yield and fruits quality.

Most of soils contain large amounts of total phosphorus but only less than 10-15 % of that P content enter the plant – animal cycle and the rest amount remained inert–due to its fixation (Kucey *et al.*, 1989). Such inert phosphorus could become soluble and available to plants by the soil microorganisms (Pals, 1998; Hilda and Fraga, 1999). With this respect, Rodriguez and Fraga (1999) stated that strains from *Pseudomonas*, *Bacillus* and *Rhizobium* genera were among the most powerful phosphate solubilizers which, in turn, resulted in increases of P uptake and crop yield.

Using PDB inoculation was recommended to overcome the ever increasing cost of phosphorus mineral fertilizer and soil health maintenance (Babulkar *et al.*, 2000) and avoiding its harmful effect on environment (Bogatyre, 2000). Rhizobacteria was also used to increase bioavailability of P and K in soils which resulted in increasing their uptake and plant growth (Lin *et al.* 2002; Sahin *et al.* 2004; Girgis, 2006 and Eweda *et al.* 2007). Han and Lee (2005) added that Phosphate solubilizing bacteria has used to convert insoluble phosphate compounds into a available soluble form for plant uptake. As a result, El-Gizawy *et al.* (2009) found that adding 30 kg P<sub>2</sub>O<sub>5</sub> mineral fertilizer in combination with PDB markedly increased growth of bean plants as well as its yield, protein content and mineral uptake. Abdel-Kader and Selah (2017) found that growth of Roselle plants and its yield was significantly increased due to co-inoculation of PDB (*Bacillus megaterium* var. phosphaticum) and KSB (*Bacillus mucilaginosus*) combined with rock phosphate and feldspar.

Humic acid (HA) application is a wide spread compound used in agriculture development. It improves physical, chemical, fertility and biological properties of soils (Keeling *et al.*, 2003; Nardi *et al.*, 2004; Mikkelsen, 2005; Sarir *et al.*, 2005 and Mart, 2007). Such positive effects of humic acid on soil properties reflected on positive effects on plants (Ashraf *et al.*, 2005 and Susilawati *et al.*, 2009) through improving mineral availability (Mauromicale *et al.*, 2011) and

DOI: 10.21608/ASEJAIQJSAE.2020.129374

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Received November 05, 2020, Accepted, December 12, 2020

enhancing nutrients uptake (Mackowiak *et al.*, 2001 and Mauromicale *et al.*, 2011). Likewise, humic acid application increased yield of vegetables such as tomatoes, potatoes, onions, pepper, Peas and other leafy vegetables (Erik *et al.*, 2000; Albayrak, 2005; Vetayasuporn, 2006; Mohamed *et al.*, 2009 and Khan *et al.*, 2013).

Sarwar *et al.*(2014) found that rhizobacteria (PGPR) inoculation combined with humic acid (HA) and P<sub>2</sub>O<sub>5</sub> recorded the highest grain yield of mung bean and gave the highest concentration of P and N in mung bean shoot as well as improved P use efficiency (PUE) and enhanced P availability through chelating and reduce soil P fixation.

Keeping the declining soil fertility, ever increasing mineral fertilizer costs and continuous increasing demand for more food, the current study aimed to investigate the effect of humic acid, biofertilizers and phosphorus application as well as their interactions on microbial activity, mineral content and pea growth and yield grown at Toshka region.

## MATERIALS AND METHODS

Two experiments were carried out in 2016 / 2017 and 2017 /2018 at Toshka Experimental Station, South Egypt. The aim of the study was to investigate the effect of humic acid, biofertilizers and phosphorus application on microbial activity, mineral contents and growth and yield of pea plants.

The composite soil samples were collected before planting at depth of 0-30 cm; air dried and sieved (2 mm).

Some physical and chemical properties of the experimental farm soil and irrigation water were determined according to Klute (1986), Jackson (1973) and shown in Tables (A and B.).

### Treatments:

#### 1)Humic acid treatments:

Humic acid was applied as soil addition at rates of zero (control), 4 and 8 kg/fed. Twice after germination and at flowering. The source of humic acid is potassium humate, which contains 60% humic acids and 8% K<sub>2</sub>O.

#### 2)Biofertilizer treatments:

*Bacillus megaterium* as bacterial suspensions (10<sup>8</sup>cfu/ml) with Carboxy methyl cellulose 0.5% as an adhesive agent was applied to grains at planting time and the inoculation was repeated after 30 days of germination. Control treatment without bacterial inoculation was also designed. Isolates has been produced in soil microbiology laboratory, Desert Research Center (DRC).

#### 3)Phosphorus treatments:

Superphosphate (15.5%) was incorporated into the soil two weeks before planting at the following rates:

- 1)100 % of the recommended dose (200 kg superphosphate / fed)
- 2)90 % of the recommended dose (180 kg superphosphate / fed)
- 3)80 % of the recommended dose (160 kg superphosphate / fed)
- 4)70 % of the recommended dose (140 kg superphosphate / fed)

**Table A. Some physical and chemical properties of soil in studied area**

Particle size distribution				Organic	Chemical properties								
Sand (%)	Silt (%)	Clay (%)	Texture	matter (%)	pH	E.C. dS.m <sup>-1</sup>	Soluble anions (mg/L.)			Soluble cations (mg/L.)			
							HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>=</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>
50.88	21.84	27.28	SCL	0.67	8.62	3.98	7.61	11.71	20.48	8.21	3.49	26.99	1.11

SCL= Sandy clay loam.

**Table B. Analysis of the irrigation water:**

well No.	pH	EC (µS/cm)	TDS mg/l	Soluble cations				Soluble anions									
				Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	Cl <sup>-</sup>						
85	6.9	768	447.25	ppm	81.32	11.29	50.00	2.00	3.00	111.15	151.25	87.98					
				ppm	4.06	0.93	2.18	0.05	0.10	1.82	3.15	2.48					
				%	56.26	12.87	30.16	0.71	1.32	24.12	41.70	32.85					
<b>Trace elements (mg/L)</b>																	
	Ag	Al	B	Ba	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Si	Sr	V	Zn
	n.d.	n.d.	n.d.	0.05	n.d.	n.d.	0.15	0.02	0.25	0.002	n.d.	0.014	0.003	4	0.35	n.d.	0.008

Organic manure (EL-Nile Compost) was provided from ECARU (Egyptian Company for Agriculture Residues Utilization) Dokki, Giza, Egypt, and mixed into the soil surface two weeks before planting; its analysis was: pH 6.81, EC 2.91 dSm<sup>-1</sup>, total N 1.21%, total P 0.25 %, total K 0.62% and C/N 17.31. In addition, *Rhizobium leguminosarum* was added two times (during planting and after germination), which were isolated by microbiology laboratory at the Desert Research Center (DRC). 200kg ammonia sulfate/fed. and 100 kg potassium sulphate/fed were divided into two doses and added after germination and flowering.

Soil samples were collected from the soil at depth of 0-30 cm at 90 days from pea sowing to estimate density of total microbial and PDB which were quantified on yeast extract agar medium (Allen, 1959) and modified by Bunt and Rovira medium (Abd El-Hafez, 1966) using the dilution frequency method. CO<sub>2</sub> evolution (µg/g dry soil/ hr.), dehydrogenase activity (µg TPF g<sup>-1</sup>. dry soil 24h.) and phosphatase enzyme (PNP g/soil/h) in the rhizosphere were determined according to Pramer and Schmidt (1994), Thalmann (1967) and Tabatabai and Brimmer, (1969), respectively. Organic carbon content was determined by Walkley and Black's wet oxidation method (1934) and CO<sub>2</sub> evolution (µg/g dry soil/ hr.) in the rhizosphere were determined according to Pramer and Schmidt (1994). Total nitrogen percentage was determined by using the modified microkjeldahl method as described by Peach and Tracey (1956). Available phosphorous was extracted using 0.5 M NaHCO<sub>3</sub> at pH 8.5 according to Olsen *et al.* (1982) and measured colorimetrically using the chlorostannous phosphomolybdic-sulfuric acid method as described by Jackson (1973). Electrical conductivity (EC) and soil pH was determined in a 1: 2.5 soil to water extract using conductivity meter and Beckman pH meter, respectively according to Jackson (1973) and McLean (1982).

Plant height (cm), number of branches /plant, fresh and dry weights (gm) /plant of shoots and number of leaves /plant were recorded before harvest (after 95 days from sowing). Whereas, total chlorophyll (SPAD unit) was determined according to A.O.A.C. (1990). Nitrogen content of pea seeds (%) were determined using Micro-Kjeldahl method according to Peach and Tracey (1956). Phosphorus content of pea seeds (%) were estimated using Spectrocolorimeter and potassium content of pea seeds by using Flame photometer (Jackson, 1973).

At the harvest, plants of one row from each experimental plot were harvested to estimate yield parameters such as number of dry pods /plant, length of pods (cm), diameter of pods (mm), average seed number /dry pod, average weight of seeds (g) /pod and weight of seed yield.

### Experimental design and statistical analysis:

Split plot design was used with three replicates. Main plots were assigned for humic acid and sub plots were used for bio-fertilization; where phosphorus treatments were distributed in the sub sub plots. Obtained data were subjected to statistical analysis according to (Snedecor and Cochran, 1989).

## RESULTS AND DISCUSSION

### Microbial activity and Soil estimates:

Data concerned with the effect of humic acid, biofertilizers and phosphorus application on microbial activity expressed as total microbial counts, PDB density, CO<sub>2</sub> evolution, organic carbon, dehydrogenase activity (DHA) and phosphatase enzyme. Obtained data were presented in Tables (1, 2 and 3). As for soil estimates, Obtained data concerned with total nitrogen, available phosphorus, C/N ratio and C/P ratio in the soil cultivated with pea plants at Toshka region were presented in Tables (4- 5). Results indicated significant positive effect for either humic acid, biofertilizers and phosphorus application on the investigated characters, the highest values were obtained with 8 kg humic acid, PDB inoculation or 100% of phosphorus recommended dose (200 kg superphosphate / fed.). These results are in accordance with those reported by Pandya and Saraf (2010), Amal M. Omer (2010) who mentioned that bio-fertilizers application can increase the availability of nutrients by their biological activity, which in turn, improve soil fertility by increasing the number of such microorganisms and accelerate certain microbial processes. In addition, Yosefi *et al.* (2011) reported that biofertilizers improved soil fertility. It solubilized insoluble soil phosphates and increased plant growth substances in the soil.

With this respect, it is of interest to mention that multiple regression of *Bacillus megaterium* count (count×10<sup>4</sup> CFU) on total microbial count and total microbial count without *Bacillus megaterium* was presented in equation (1 and 2). Regression coefficients indicated that *Bacillus megaterium* count was increased in the first season an average of 0.00062 unit for each unit of total microbial count but only 0.00010 for each unit of total microbial count without *Bacillus megaterium*. The corresponding values in the second were 0.01102 and 0.0002. This indicated that total microbial was more effective than total microbial count without *Bacillus megaterium*; in the same time indicated that total microbial count other than *Bacillus megaterium* increased *Bacillus megaterium* count which could lead to conclude that there were mutual cooperation effect for some other bacteria on *Bacillus megaterium*. Such conclusion was true in both investigated seasons.

$Y^{\wedge} = 17.4 + 0.00062 * X_1 + 0.00010 * X_2$  Equation (1)  
for the first season

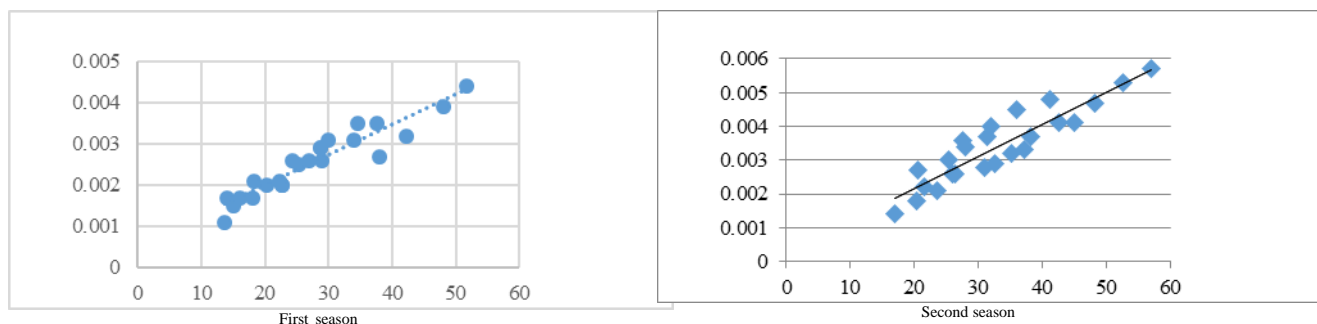
$Y^{\wedge} = 45.9 + 0.01102 * X_1 + 0.00023 * X_2$  Equation (1)  
for the second season

Where Y stand for the dependent variable *Bacillus megaterium* count (count×10<sup>4</sup> CFU), the independent variables X<sub>1</sub> stand for total microbial count (count×10<sup>4</sup> CFU) and x<sub>2</sub> stand for total microbial count without *Bacillus megaterium* (count×10<sup>4</sup>CFU)

It is, also, of great interest to know the relation between *Bacillus megaterium* density (count×10<sup>4</sup> CFU) and the available phosphorus in the soil (%). Linear correlation indicated that there was highly significant positive correlation between the available phosphorus in the soil and *Bacillus megaterium* density. Correlation coefficients (r) were 0.945 and 0.946 in the first and second seasons, respectively. Linear regression of the available phosphorus in the soil on the independent variable showed that regression coefficients were 7.31 and 9.9 in the first and second seasons, respectively. This means that soil available phosphorus would increase by 7.31 and 9.9 % in the first and second seasons, respectively, for each unit increase of *Bacillus megaterium*.

As for the interactions, the highest values were obtained generally with application of either 8 kg humic

acid / fed combined with PDB inoculation or 8 kg humic acid / fed combined with 100 % of phosphorus recommended dose. The beneficial effect of humic acid on microbial activity may be due to its activation through its positive effects on soil and plant characteristics (Zhang and Ervin, 2004), its various functional groups which, in turn, stimulate enzyme activity, membrane permeability, photosynthesis and respiration (Muscolo *et al.*, 2007 and Nardi *et al.*, 2002), its useful effects in minimizing the amount of mineral fertilization (Eman Abdel-Monem *et al.*, 2008). In addition, biofertilizer inoculation plays an important role in exchanges of CO<sub>2</sub> between land biosphere and atmosphere through soil microbial activity and CO<sub>2</sub> production (Luo and Zhou, 2006) as well as biofertilizer inoculation led to higher dehydrogenase activity than those in un-inoculated treatments (Amal *et al.* 2014). In this respect, Al-Haddad *et al.* (2014) showed that the highest significant increase in percentages of enzyme activity (dehydrogenase) was recorded in the Eucalyptus camaldulensis inoculated with a mixed microbial treatment of (*Azotobacter chroococcum*, *Bacillus circulans* and *Arbuscular mycorrhizal* fungi AMF) rather than those of individual and dual treatments in the two investigated seasons.



**Fig. 1. Regression of available P (Y, %) on *Bacillus megaterium* (X, count×10<sup>4</sup> CFU)**

$Y^{\wedge} = 0.000542 + 7.31E-05 X$ , r = 0.945 in the 1<sub>st</sub> season.

$Y^{\wedge} = 0.000693 + 9.9 X$ , r = 0.946 in the 2<sub>nd</sub> season.

**Table 1. Influence of humic acid, biofertilizers and phosphorus applications on total microbial counts (Counts x 10<sup>6</sup> CUF g dry soil) and *Bacillus megaterium* count (count×10<sup>4</sup> CUF) during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	Total microbial count (count×10 <sup>6</sup> CUF)								<i>Bacillus megaterium</i> count (count×10 <sup>4</sup> CUF)									
		First Season				Second Season				First season			Second Season						
		Humic Acid								Humic Acid									
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean		
Without	100	31.33	35.33	39.33	35.33	37.33	41.00	48.67	42.33	22.33	28.67	34.67	28.56	26.33	37.33	42.67	35.44		
	90	28.67	32.67	35.67	32.33	35.00	38.33	46.00	39.78	18.00	25.33	30.00	24.44	23.67	32.67	38.33	31.56		
	80	27.00	31.33	34.67	31.00	33.33	36.67	42.67	37.56	15.00	20.33	27.00	20.78	20.33	26.00	35.33	27.22		
	70	21.00	30.67	33.00	28.22	31.33	34.00	39.00	34.78	13.67	16.00	22.67	17.44	17.00	21.67	31.00	23.22		
Mean		27.00	32.50	35.67	31.72	34.25	37.50	44.08	38.61	17.25	22.58	28.58	22.81	21.83	29.42	36.83	29.36		
PDB	100	38.33	47.00	84.00	56.44	42.67	51.33	91.00	61.67	28.67	37.67	51.67	39.33	31.33	41.33	57.00	43.22		
	90	32.67	42.67	78.33	51.22	38.33	47.67	86.33	57.44	24.33	34.00	48.00	35.44	28.00	36.00	52.67	38.89		
	80	29.67	38.00	68.67	45.44	36.67	43.33	77.00	52.33	18.33	29.00	42.33	29.89	25.33	32.00	48.33	35.22		
	70	25.33	33.00	62.67	40.33	34.00	37.00	66.00	45.67	14.00	22.67	38.00	24.89	20.67	27.67	45.00	31.11		
Mean		31.50	40.17	73.42	48.36	37.92	44.83	80.08	54.28	21.33	30.83	45.00	32.39	26.33	34.25	50.75	37.11		
P × HU	100	34.83	41.17	61.67	45.89	40.00	46.17	69.83	52.00	25.50	33.17	43.17	33.94	28.83	39.33	49.83	39.33		
	90	30.67	37.67	57.00	41.78	36.67	43.00	66.17	48.61	21.17	29.67	39.00	29.94	25.83	34.33	45.50	35.22		
	80	28.33	34.67	51.67	38.22	35.00	40.00	59.83	44.94	16.67	24.67	34.67	25.33	22.83	29.00	41.83	31.22		
	70	23.17	31.83	47.83	34.28	32.67	35.50	52.50	40.22	13.83	19.33	30.33	21.17	18.83	24.67	38.00	27.17		
Mean		29.25	36.33	54.54		36.08	41.17	62.08		19.29	26.71	36.79		24.08	31.83	43.79			
<b>LSD 5%</b>																			
Humic acid				3.037				2.883				0.914				0.921			
Biofertilizer				3.595				1.997				1.391				0.821			
Phosphrus				1.164				2.918				0.734				0.849			
Humic*Bio				6.226				3.459				2.410				1.423			
Humic*Phosphorus				2.016				5.054				1.272				1.470			
Bio*Phosphorus				1.366				3.423				0.861				NS			
Humic*Bio*Phosphorus				2.366				NS				1.492				NS			

\*- Initial total bacterial count was  $50 \times 10^3$  (CFU/g dry soil).

\*- Initial total *Bacillus* count was  $45 \times 10^2$  (CFU/g dry soil).

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Table 2. Influence of Humic acid, biofertilizers and Phosphorus applications on CO<sub>2</sub> evolution (mg CO<sub>2</sub>/100 g dry soil /24 hr.) and Organic carbon % during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	CO <sub>2</sub> evolution (mg CO <sub>2</sub> /100 g dry soil /24 hr.)									Organic carbon %										
		First Season			Second Season			First season			Second Season										
		Humic Acid									Humic Acid										
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean				
Without	100	10.33	14.41	16.96	13.90	15.10	19.10	24.67	19.62	1.34	1.64	1.95	1.64	1.50	1.73	2.13	1.79				
	90	9.30	12.65	14.74	12.23	11.83	16.23	21.70	16.59	1.15	1.42	1.67	1.41	1.31	1.54	1.92	1.59				
	80	8.43	10.61	13.18	10.74	9.67	13.63	18.33	13.88	1.05	1.22	1.42	1.23	1.17	1.32	1.70	1.40				
	70	7.30	8.93	12.34	9.52	8.50	11.93	15.80	12.08	0.88	1.03	1.22	1.04	1.00	1.15	1.47	1.21				
Mean		8.84	11.65	14.30	11.60	11.28	15.23	20.13	15.54	1.11	1.33	1.56	1.33	1.25	1.44	1.80	1.50				
PDB	100	11.40	17.73	21.43	16.86	18.37	27.60	37.80	27.92	1.43	1.95	2.40	1.93	1.68	2.07	2.57	2.11				
	90	10.53	16.38	19.61	15.50	15.73	22.93	34.60	24.42	1.25	1.64	2.15	1.68	1.50	1.90	2.40	1.93				
	80	9.43	14.68	17.88	14.00	13.37	20.63	30.27	21.42	1.05	1.37	2.00	1.47	1.25	1.67	2.13	1.68				
	70	8.62	12.78	16.24	12.55	10.30	17.70	27.03	18.34	0.92	1.17	1.75	1.28	1.08	1.38	1.87	1.44				
Mean		10.00	15.39	18.79	14.73	14.44	22.22	32.43	23.03	1.16	1.54	2.08	1.59	1.38	1.75	2.24	1.79				
P × HU	100	10.87	16.07	19.20	15.38	16.73	23.35	31.23	23.77	1.39	1.80	2.18	1.79	1.59	1.90	2.35	1.95				
	90	9.92	14.51	17.17	13.87	13.78	19.58	28.15	20.51	1.20	1.53	1.91	1.55	1.40	1.72	2.16	1.76				
	80	8.93	12.64	15.53	12.37	11.52	17.13	24.30	17.65	1.05	1.30	1.71	1.35	1.21	1.49	1.92	1.54				
	70	7.96	10.85	14.29	11.03	9.40	14.82	21.42	15.21	0.90	1.10	1.48	1.16	1.04	1.27	1.67	1.33				
Mean		9.42	13.52	16.55		12.86	18.72	26.28		1.13	1.43	1.82		1.31	1.60	2.02					
<b>LSD 5%</b>																					
Humic acid				0.981					2.479					0.051				0.105			
Biofertilizer				0.561					1.228					0.033				0.069			
Phosphrus				0.414					0.636					0.038				0.033			
Humic*Bio				0.972					2.127					0.057				0.120			
Humic*Phosphorus				0.717					1.102					0.065				0.058			
Bio*Phosphorus				NS					0.746					NS				0.039			
Humic*Bio*Phosphorus				NS					NS					0.077				NS			

\*- Initial total bacterial count was 50 × 10<sup>3</sup> (CFU/g dry soil).  
 \*- Initial total *Bacillus* count was 45 × 10<sup>2</sup> (CFU/g dry soil).  
 \*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Table 3. Influence of Humic acid, biofertilizers and Phosphorus applications on phosphatase enzyme (PNP g/soil/h) and dehydrogenase activity (DHA) ( $\mu\text{g TPF g}^{-1}$ . dry soil 24hr.) during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	phosphatase enzyme (PNP g/soil/h)								(DHA) ( $\mu\text{g TPF g}^{-1}$ . dry soil 24hr.)									
		First Season				Second Season				First season				Second Season					
		Humic Acid								Humic Acid									
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean		
Without	100	1.050	1.467	1.767	1.428	1.143	1.637	1.840	1.540	2.38	4.08	5.12	3.86	3.38	4.73	6.39	4.83		
	90	0.933	1.240	1.433	1.202	1.040	1.333	1.667	1.347	2.10	3.57	4.60	3.42	2.71	4.05	5.38	4.05		
	80	0.813	1.037	1.260	1.037	0.983	1.197	1.533	1.238	1.39	3.31	4.17	2.96	2.38	3.73	4.71	3.61		
	70	0.700	0.943	1.050	0.898	0.923	1.083	1.373	1.127	0.72	2.68	3.62	2.34	1.71	3.36	4.36	3.14		
	Mean	0.874	1.172	1.378	1.141	1.023	1.313	1.603	1.313	1.65	3.41	4.37	3.14	2.55	3.97	5.21	3.91		
PDB	100	1.933	2.600	2.867	2.467	2.133	2.733	3.167	2.678	3.24	5.67	8.81	5.91	4.74	6.73	9.37	6.95		
	90	1.500	2.203	2.567	2.090	1.833	2.433	2.833	2.367	2.73	5.34	8.11	5.39	3.73	6.05	8.37	6.05		
	80	1.270	2.077	2.400	1.916	1.467	2.237	2.533	2.079	2.56	4.65	7.73	4.98	3.05	5.37	7.73	5.38		
	70	1.043	1.950	2.090	1.694	1.333	2.117	2.333	1.928	1.90	3.64	6.25	3.93	2.39	3.73	6.05	4.06		
	Mean	1.437	2.208	2.481	2.042	1.692	2.380	2.717	2.263	2.61	4.83	7.72	5.05	3.48	5.47	7.88	5.61		
P × HU	100	1.492	2.033	2.317	1.947	1.638	2.185	2.503	2.109	2.81	4.88	6.96	4.88	4.06	5.73	7.88	5.89		
	90	1.217	1.722	2.000	1.646	1.437	1.883	2.250	1.857	2.42	4.46	6.35	4.41	3.22	5.05	6.88	5.05		
	80	1.042	1.557	1.830	1.476	1.225	1.717	2.033	1.658	1.98	3.98	5.95	3.97	2.72	4.55	6.22	4.50		
	70	0.872	1.447	1.570	1.296	1.128	1.600	1.853	1.527	1.31	3.16	4.93	3.14	2.05	3.55	5.20	3.60		
	Mean	1.155	1.690	1.929	1.357	1.846	2.160	2.13	4.12	6.05	3.01	4.72	6.55						
LSD 5%																			
Humic acid				0.087				0.124				0.250				0.418			
Biofertilizer				0.054				0.088				0.212				0.181			
Phosphrus				0.074				0.053				0.189				0.388			
Humic*Bio				0.094				0.152				0.367				0.313			
Humic*Phosphorus				NS				NS				NS				NS			
Bio*Phosphorus				0.087				0.062				0.222				0.456			
Humic*Bio*Phosphorus				0.150				0.107				0.384				NS			

\*- Initial phosphatase enzyme 0.65 (PNP g/soil/h)

\*- Initial Dehydrogenase activity 2.01 ( $\mu\text{g TPF g}^{-1}$  dry soil 24h.)

\*- para-nitrophenol (PNP)

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Table 4. Influence of Humic acid, biofertilizers and Phosphorus applications on total nitrogen in soil % and C/N ratio during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	Total nitrogen in soil %								C/N ratio								
		First Season				Second Season				First season				Second Season				
		Humic Acid								Humic Acid								
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	
Without	100	0.20	0.31	0.36	0.29	0.27	0.39	0.43	0.36	6.70	5.29	5.46	5.82	5.49	4.48	5.00	4.99	
	90	0.17	0.27	0.34	0.26	0.23	0.35	0.40	0.33	6.75	5.27	4.96	5.66	5.76	4.37	4.83	4.99	
	80	0.13	0.22	0.28	0.21	0.19	0.30	0.36	0.29	8.08	5.48	5.01	6.19	6.08	4.35	4.72	5.05	
	70	0.11	0.15	0.24	0.17	0.16	0.28	0.33	0.26	8.03	7.03	5.01	6.69	6.37	4.15	4.41	4.98	
	Mean	0.15	0.24	0.31	0.23	0.21	0.33	0.38	0.31	7.22	5.59	5.13	5.98	5.85	4.35	4.76	4.99	
PDB	100	0.29	0.40	0.67	0.45	0.34	0.47	0.78	0.53	4.99	4.84	3.57	4.47	4.95	4.43	3.28	4.22	
	90	0.25	0.34	0.60	0.40	0.31	0.41	0.71	0.48	5.00	4.88	3.57	4.48	4.89	4.63	3.38	4.30	
	80	0.20	0.28	0.55	0.34	0.27	0.38	0.66	0.43	5.25	4.90	3.66	4.60	4.68	4.39	3.25	4.11	
	70	0.17	0.25	0.54	0.32	0.23	0.33	0.58	0.38	5.49	4.69	3.26	4.48	4.71	4.15	3.22	4.03	
	Mean	0.23	0.32	0.59	0.38	0.29	0.40	0.68	0.46	5.15	4.83	3.52	4.50	4.82	4.41	3.28	4.17	
P × HU	100	0.24	0.36	0.52	0.37	0.31	0.43	0.61	0.45	5.71	5.03	4.22	4.99	5.19	4.45	3.88	4.51	
	90	0.21	0.30	0.47	0.33	0.27	0.38	0.55	0.40	5.70	5.06	4.07	4.94	5.25	4.51	3.90	4.55	
	80	0.17	0.25	0.42	0.28	0.23	0.34	0.51	0.36	6.36	5.15	4.12	5.21	5.27	4.36	3.77	4.47	
	70	0.14	0.20	0.39	0.24	0.19	0.31	0.46	0.32	6.52	5.57	3.80	5.30	5.40	4.15	3.65	4.40	
	Mean	0.19	0.28	0.45	0.25	0.25	0.36	0.53	0.32	6.00	5.15	4.06	5.27	5.27	4.38	3.81	4.40	
LSD 5%																		
Humic acid						0.017				0.015				0.849				0.395
Biofertilizer						0.013				0.008				0.571				0.157
Phosphrus						0.013				0.009				0.464				0.102
Humic*Bio						0.023				0.014				0.989				0.271
Humic*Phosphorus						0.023				0.016				0.804				0.176
Bio*Phosphorus						0.015				0.011				0.545				0.119
Humic*Bio*Phosphorus						NS				0.019				0.944				0.206

\*- Initial total nitrogen in soil 0.09%

\*- Initial C/N ratio in soil 9.33 %

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

\*- C/N ratio = Organic Carbon % / Total nitrogen %



**Table 5. Influence of Humic acid, biofertilizers and Phosphorus applications on available phosphorus % and C/P ratio during 2016/2017 seasons**

Bacterial Inoculations	Phosphorus %	Available phosphorus %								C/P ratio in soil									
		First Season				Second Season				First season				Second Season					
		Humic Acid								Humic Acid									
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean		
Without	100	0.0021	0.0029	0.0035	0.0028	0.0026	0.0033	0.0041	0.0033	638.1	565.5	557.1	586.9	576.9	525.2	520.2	540.8		
	90	0.0017	0.0025	0.0031	0.0025	0.0021	0.0029	0.0037	0.0029	674.7	569.2	539.7	594.5	622.4	532.1	518.1	557.5		
	80	0.0015	0.0020	0.0026	0.0020	0.0018	0.0026	0.0032	0.0025	700.0	611.5	545.0	618.8	651.7	506.5	531.3	563.2		
	70	0.0011	0.0017	0.0020	0.0016	0.0014	0.0022	0.0028	0.0021	802.7	607.6	608.5	673.0	714.3	522.7	523.9	587.0		
	Mean	0.0016	0.0023	0.0028	0.0022	0.0020	0.0028	0.0035	0.0027	690.6	578.3	558.6	609.2	622.5	512.9	515.4	550.3		
PDB	100	0.0029	0.0035	0.0044	0.0036	0.0037	0.0048	0.0057	0.0047	494.1	557.1	545.5	532.2	454.9	430.6	450.4	445.3		
	90	0.0026	0.0031	0.0039	0.0032	0.0034	0.0045	0.0053	0.0044	480.8	530.0	551.3	520.7	441.2	422.2	452.8	438.7		
	80	0.0021	0.0026	0.0032	0.0026	0.0030	0.0040	0.0047	0.0039	500.0	528.1	625.0	551.0	416.7	416.8	453.8	429.1		
	70	0.0017	0.0020	0.0027	0.0021	0.0027	0.0036	0.0041	0.0035	539.4	586.5	648.1	591.4	401.1	384.2	455.4	413.5		
	Mean	0.0023	0.0028	0.0036	0.0029	0.0032	0.0042	0.0050	0.0041	505.7	548.2	576.4	543.4	430.9	417.6	448.4	432.3		
P × HU	100	0.0025	0.0032	0.0040	0.0032	0.0031	0.0041	0.0049	0.0040	554.8	560.9	543.8	553.2	513.5	463.4	479.6	485.5		
	90	0.0022	0.0028	0.0035	0.0028	0.0028	0.0037	0.0045	0.0037	544.5	547.5	546.3	546.1	501.1	465.4	479.6	482.0		
	80	0.0018	0.0023	0.0029	0.0023	0.0024	0.0033	0.0040	0.0032	583.3	564.3	589.0	578.9	505.0	452.1	479.3	478.8		
	70	0.0014	0.0019	0.0024	0.0019	0.0020	0.0029	0.0035	0.0028	642.9	580.5	617.9	613.8	521.0	436.9	476.3	478.1		
	Mean	0.0020	0.0025	0.0032		0.0031	0.0041	0.0049		567.0	573.2	568.8		423.2	389.0	412.9			
<b>LSD 5%</b>																			
Humic acid				0.00015				0.00016				NS				NS			
Biofertilizer				0.00005				0.00007				77.4				58.5			
Phosphrus				0.00006				0.00008				40.9				NS			
Humic*Bio				0.00009				0.00011				134.0				NS			
Humic*Phosphorus				0.00011				0.00013				NS				30.6			
Bio*Phosphorus				0.00007				NS				NS				20.7			
Humic*Bio*Phosphorus				NS				NS				NS				35.9			

\*- Initial available phosphorus in soil 0.0001%

\*- Initial C/P ratio in soil 8400 %

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

\*- C/P ratio = Organic Carbon % / Available phosphorus %

**Pant growth:**

Data concerned with the effect of humic acid, biofertilizers and phosphate application on plant measurements expressed as plant height (cm), number of branches, fresh weight, dry weight, number of leaves, chlorophyll, number of dry pods, length of pods, diameter of pods, average seed number, average weight of seeds and weight of seed yield of both investigated seasons were presented in Tables (6 - 8). Obtained results indicated significant positive effect for either humic acid, biofertilizers and phosphorus application on the investigated characters; the highest values were obtained with 8 kg humic acid, PDB inoculation and 100% of phosphorus recommended dose (200 kg superphosphate / fed).

Multiple regression was, also, carried out between plant dry weight, the most expressive growth parameter, on total microbial count and total microbial count without *Bacillus megaterium* and presented in equations (3 and 4). Regression coefficients indicated that plant dry weight (gm /plant) was increased in the first season an average of 0.00533 (gm /plant) for each unit of total microbial count, but only by 0.00012 for each unit of total microbial count without *Bacillus megaterium*. The corresponding values in the second were 0.00927 and 0.000113. This means that total microbial was more effective than total microbial count without *Bacillus megaterium*; in the same time indicated that total microbial count without *Bacillus megaterium* increased plant dry weight which could lead to conclude that microbial inoculation is very important in new reclaimed lands. Such conclusion was true in both investigated seasons.

$Y^{\wedge} = 82.7 + 0.00533 * X_1 + 0.00012 * X_2$  Equation (3)  
for the first season

$Y^{\wedge} = 120 + 0.00927 * X_1 + 0.000113 * X_2$  Equation (4)  
for the second season

Where Y stand for the dependent variable plant dry weigh (gm / plant), the independent variables  $X_1$  stand for total microbial count (count $\times 10^4$  CFU) and  $X_2$  stand for total microbial count without *Bacillus megaterium* (count $\times 10^4$  CFU)

Data showed that humic acid (8 kg/fed.), PDB inoculation and phosphate application (concentration 100 %) gave the highest values of the investigated plant growth measurements in the first and second seasons. Ramana, V. *et al.* (2010) studied the effect of bio-fertilizers VAM (Vesicular Arbuscular Mycorrhizae) and PSB (Phosphorus Solubilizing Bacteria) along with their graded dose of fertilizers on growth of French bean. Their results revealed that the application of 75 per cent recommended Dose of Fertilizer + VAM + PSB significantly increased the plant height (cm), number of branches per plant, leaf area (cm<sup>2</sup>) and dry weight (g) of plant. In addition, Yosefi *et al.* (2011) reported that biofertilizers improved soil fertility by fixing atmospheric nitrogen both in association with plant roots as well as solubilized insoluble soil phosphates and increased plant growth substances in the soil. Furthermore, Hala Kandil (2014) reported that pea growth as well as of other legumes was affected by both phosphorous and humic acid application. In this respect, Agamy *et al.* (2012) showed that the application of Bio and/or FM in combination with NPK on wheat (*Triticum aestivum* L.) significantly increased all growth characters i.e., plant height, number of spikes/plant, leaf area and fresh and dry weights of both shoot and spikes / plant. Shehata *et al.* (2006) added that there was some microorganism which stimulates the *Azotobacter* population in soil thereby increasing the nitrogen fixation by *Azotobacter*. They showed that the maximum increments of vine length and leaf number as well as fresh and dry weight of shoots were recorded by the inoculation of squash seeds with *Azotobacter*. Sarhan *et al.* (2011) added that Biogein and Netropein produced the intermediate values.

**Table 6. Influence of Humic acid, biofertilizers and phosphorus applications on plant height (cm) and number of branches /plant of pea during 2016/2017 seasons**

Bacterial Inoculations	Phosphorus %	Plant height (cm) of pea								Number of branches /plant of pea							
		First Season				Second Season				First season				Second Season			
		Humic Acid								Humic Acid							
		With out	4 Kg /fed.	8 Kg /fed.	Mean	With out	4 Kg /fed.	8 Kg /fed.	Mean	With out	4 Kg /fed.	8 Kg /fed.	Mean	With out	4 Kg /fed.	8 Kg /fed.	Mean
Without	100	42.7	55.7	60.9	60.9	46.3	67.1	70.9	70.9	8.7	15.3	13.4	13.4	12.3	26.3	24.4	24.4
	90	41.3	54.3	59.8	59.8	44.9	65.3	67.8	67.8	8.3	15.0	12.8	12.8	10.7	23.7	21.9	21.9
	80	40.3	52.7	58.2	58.2	43.1	62.2	65.0	65.0	8.0	14.3	12.3	12.3	9.3	20.7	19.6	19.6
	70	38.7	50.7	56.6	56.6	42.2	58.6	62.5	62.5	7.3	12.7	11.2	11.2	8.0	18.7	17.0	17.0
Mean		40.8	53.3	62.5	58.9	44.1	63.3	69.2	66.5	8.1	14.3	14.9	12.4	10.1	22.3	29.8	20.7
PDB	100	47.7	93.3	87.2	87.2	55.6	102.2	96.7	96.7	13.3	20.7	20.7	20.7	18.3	32.3	29.4	29.4
	90	47.3	89.3	82.0	82.0	52.3	96.7	91.7	91.7	11.7	19.3	18.7	18.7	15.3	28.7	26.0	26.0
	80	45.7	87.3	77.7	77.7	49.7	92.1	86.3	86.3	10.3	17.7	16.3	16.3	13.7	24.7	23.0	23.0
	70	43.3	72.7	67.7	67.7	47.7	86.9	80.5	80.5	9.0	16.0	14.8	14.8	12.3	22.3	21.0	21.0
Mean		46.0	85.7	104.3	78.6	51.3	94.5	120.6	88.8	11.1	18.4	23.3	17.6	14.9	27.0	32.7	24.9
P × HU	100	45.2	74.5	74.1	74.1	51.0	84.7	83.8	83.8	11.0	18.0	17.1	17.1	15.3	29.3	26.9	26.9
	90	44.3	71.8	70.9	70.9	48.6	81.0	79.7	79.7	10.0	17.2	15.7	15.7	13.0	26.2	23.9	23.9
	80	43.0	70.0	67.9	67.9	46.4	77.1	75.7	75.7	9.2	16.0	14.3	14.3	11.5	22.7	21.3	21.3
	70	41.0	61.7	62.1	62.1	44.9	72.7	71.5	71.5	8.2	14.3	13.0	13.0	10.2	20.5	19.0	19.0
Mean		43.4	69.5	93.4		47.7	78.9	106.4		9.6	16.4	19.1		12.5	24.7	31.2	
LSD 5%																	
Humic acid		3.8				3.7				1.8				1.3			
Biofertilizer		2.4				2.5				2.5				0.5			
Phosphrus		3.4				1.4				1.4				0.5			
Humic*Bio		4.1				4.3				NS				0.8			
Humic*Phosphorus		5.9				2.5				NS				0.8			
Bio*Phosphorus		4.0				1.7				1.7				0.6			
Humic*Bio*Phosphorus		6.9				2.9				NS				1.0			

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Table 7. Influence of humic acid, biofertilizers and phosphorus applications on fresh weight (g)/plant and dry weight (g)/plant during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	Fresh weight (g)/plant								Dry weight (g)/plant									
		First Season				Second Season				First season			Second Season						
		Humic Acid								Humic Acid									
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean		
Without	100	245.3	1899.7	1849.7	1849.7	346.7	2330.0	2143.3	2143.3	72.2	132.4	227.8	144.1	102.3	175.9	309.8	196.0		
	90	215.7	1775.7	1401.7	1401.7	311.7	2238.3	1991.7	1991.7	54.5	123.0	215.2	130.9	84.2	165.8	291.9	180.6		
	80	210.3	1460.3	1225.0	1225.0	290.0	2040.0	1749.4	1749.4	36.9	108.2	171.7	105.6	58.8	148.0	240.0	149.0		
	70	144.7	410.7	763.7	763.7	246.7	1511.7	1464.4	1464.4	22.1	87.0	137.5	82.2	43.7	116.5	194.1	118.1		
	Mean	204.0	1386.6	2339.4	1310.0	298.8	2030.0	3182.9	1837.2	46.4	112.6	188.0	115.7	72.3	151.6	259.0	160.9		
PDB	100	365.3	3200.3	2856.7	2856.7	565.7	4041.7	3568.0	3568.0	102.4	192.1	365.2	219.9	143.1	258.7	513.4	305.0		
	90	330.7	3000.7	2612.3	2612.3	528.3	3537.0	3160.1	3160.1	93.1	169.1	329.3	197.2	137.3	234.0	461.7	277.7		
	80	275.3	2175.7	2018.2	2018.2	510.7	3170.7	2899.6	2899.6	75.2	147.5	282.5	168.4	107.3	201.1	388.8	232.4		
	70	254.7	2154.7	1971.3	1971.3	460.0	2883.3	2590.6	2590.6	61.6	113.8	223.3	132.9	87.4	158.9	333.2	193.2		
	Mean	306.5	2632.8	4154.6	2364.6	516.2	3408.2	5239.3	3054.6	83.1	155.6	300.1	179.6	118.8	213.2	424.3	252.1		
P × HU	100	305.3	2550.0	2353.2	2353.2	456.2	3185.8	2855.7	2855.7	87.3	162.2	296.5	182.0	122.7	217.3	411.6	250.5		
	90	273.2	2388.2	2007.0	2007.0	420.0	2887.7	2575.9	2575.9	73.8	146.1	272.3	164.0	110.8	199.9	376.8	229.2		
	80	242.8	1818.0	1621.6	1621.6	400.3	2605.3	2324.5	2324.5	56.0	127.8	227.1	137.0	83.1	174.6	314.4	190.7		
	70	199.7	1282.7	1367.5	1367.5	353.3	2197.5	2027.5	2027.5	41.8	100.4	180.4	107.6	65.6	137.7	263.6	155.6		
	Mean	255.3	2009.7	3247.0		407.5	2719.1	4211.1		64.7	134.1	244.1	147.6	95.5	182.4	341.6	206.5		
LSD 5%																			
Humic acid				9.8				202.6				9.0				25.1			
Biofertilizer				6.0				83.0				5.8				12.2			
Phosphrus				9.2				76.6				13.0				11.9			
Humic*Bio				10.4				143.7				10.1				21.1			
Humic*Phosphorus				16.0				132.6				22.5				20.6			
Bio*Phosphorus				10.8				89.8				NS				13.9			
Humic*Bio*Phosphorus				18.7				155.6				NS				NS			

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Table 8. Influence of humic acid, biofertilizers and phosphorus applications on number of leaves /plant and chlorophyll (SPAD unit) during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	Number of leaves /plant								Chlorophyll (SPAD unit)								
		First Season				Second Season				First season				Second Season				
		Humic Acid								Humic Acid								
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	
Without	100	14.3	28.0	26.6	26.6	21.7	33.0	33.2	33.2	19.3	23.0	39.0	27.1	22.3	26.5	43.1	30.6	
	90	12.7	24.3	22.8	22.8	19.0	28.7	29.4	29.4	17.7	18.3	35.6	23.9	19.6	23.7	40.1	27.8	
	80	11.3	22.3	20.1	20.1	16.7	24.7	26.4	26.4	14.9	14.4	29.3	19.5	16.8	19.0	35.4	23.7	
	70	9.7	18.0	17.7	17.7	13.3	21.7	23.2	23.2	12.9	10.9	23.0	15.6	14.6	14.7	28.0	19.1	
	Mean	12.0	23.2	30.2	21.8	17.7	27.0	39.6	28.1	16.2	16.7	31.7	21.5	18.3	21.0	36.7	25.3	
PDB	100	20.7	35.3	40.6	40.6	25.3	46.7	47.8	47.8	26.2	44.4	59.1	43.2	28.5	47.7	70.6	48.9	
	90	18.3	31.7	35.1	35.1	22.7	43.0	42.7	42.7	22.8	39.7	54.6	39.0	25.1	43.2	62.2	43.5	
	80	15.7	27.3	31.6	31.6	19.3	38.7	38.1	38.1	18.6	35.2	47.2	33.7	21.5	39.0	57.0	39.2	
	70	11.7	24.7	25.6	25.6	16.3	34.7	34.6	34.6	14.8	27.9	43.1	28.6	17.9	34.5	52.6	35.0	
	Mean	16.6	29.8	53.3	33.2	20.9	40.8	60.7	40.8	20.6	36.8	51.0	36.1	23.2	41.1	60.6	41.6	
P × HU	100	17.5	31.7	33.6	33.6	23.5	39.8	40.5	40.5	22.8	33.7	49.0	35.2	25.4	37.1	56.8	39.8	
	90	15.5	28.0	28.9	28.9	20.8	35.8	36.1	36.1	20.2	29.0	45.1	31.5	22.3	33.5	51.2	35.7	
	80	13.5	24.8	25.8	25.8	18.0	31.7	32.3	32.3	16.7	24.8	38.3	26.6	19.2	29.0	46.2	31.5	
	70	10.7	21.3	21.6	21.6	14.8	28.2	28.9	28.9	13.8	19.4	33.0	22.1	16.2	24.6	40.3	27.0	
	Mean	14.3	26.5	41.7		19.3	33.9	50.1		18.4	26.7	41.4		20.8	31.0	48.6		
<b>LSD 5%</b>																		
Humic acid				3.3					3.8					2.6				
Biofertilizer				2.2					3.2					2.1				
Phosphrus				1.3					2.1					1.7				
Humic*Bio				3.7					5.6					3.7				
Humic*Phosphorus				2.3					NS					3.0				
Bio*Phosphorus				1.6					NS					NS				
Humic*Bio*Phosphorus				2.7					NS					NS				

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Yield and its components:**

Data concerned with the effect of humic acid, biofertilizers and phosphate application on Yield measurements expressed as number of dry pods, length of pods, diameter of pods, average seed number, average weight of seeds and weight of seed yield were presented in Tables (9 - 11). Obtained results indicated significant positive effect of all of humic acid, biofertilizers and phosphorus application on the investigated characters; the highest values were obtained with 8 kg humic acid, PDB inoculation or 100% of phosphorus recommended dose (200 kg superphosphate / fed). Data showed that humic acid (8 kg/fed.), PDB inoculation and phosphate application (100 % of recommended dose) gave the highest values. Numbers of dry pods were 14.427 and 16.158, lengths of pods were 9.967 and 10.950 (cm), diameters of pea pods were 0.915 and 0.978 (mm), average seeds number per pod were 8.342 and 8.967, average seeds weight per pod were 3.488 and 3.850 (g) and weight of seeds yield / m<sup>2</sup> was 1356.7 and 1356.7 (g) in the first and second seasons respectively.

As for the relationship of seed yield and bacterial counts, multiple regression of seed yield (gm / m<sup>2</sup>) on total microbial count and total microbial count without *Bacillus megaterium* was estimated and presented in equations (5 and 6). Regression coefficients indicated that seed yield was increased in the first season an average of 0.31157 gm/m<sup>2</sup> for each unit of total microbial count but only by 0.00028 gm / m<sup>2</sup> for each unit of total microbial count without *Bacillus megaterium*. The corresponding values in the second

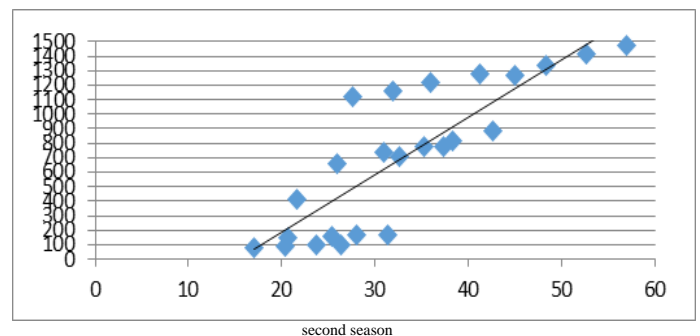
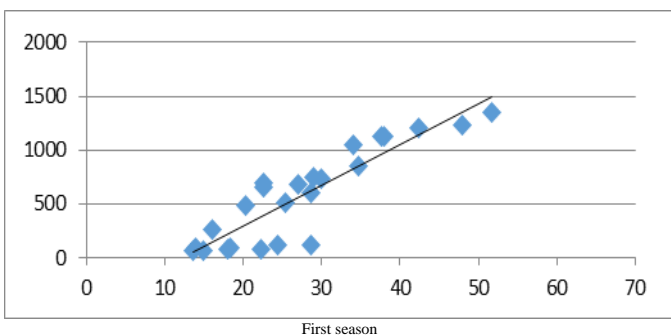
season were 0.393 and 0.00028. This means that total microbial was more effective than total microbial count without *Bacillus megaterium*; in the same time indicated that total microbial count without *Bacillus megaterium* increased seed yield which could lead to conclude, again, that microbial inoculation is very important in new reclaimed lands for increasing seed yield. Such conclusion was true in both investigated seasons.

$Y^{\wedge} = 1012 + 0.31157 * X_1 + 0.00028 * X_2$  Equation (5)  
for the first season

$Y^{\wedge} = 1442 + 0.393 * X_1 + 0.00028 * X_2$  Equation (6)  
for the second season

Where Y stand for the dependent variable seed yield (gm / m<sup>2</sup>), the independent variables X<sub>1</sub> stand for total microbial count (count×10<sup>4</sup> CFU) and X<sub>2</sub> stand for total microbial count without *Bacillus megaterium* (count×10<sup>4</sup> CFU).

It is, also, of great interest to know the relation between *Bacillus* density (count×10<sup>4</sup>) and seed yield of pea plants. Linear correlation indicated that there was highly significant positive correlation between seed yield (gm/m<sup>2</sup>) and *Bacillus megaterium* density (count×10<sup>4</sup> CFU). Correlation coefficients (r) were 0.9 and 0.84) in the first and second seasons, respectively. Linear regression of seed yield on the independent variable showed that regression coefficients were 37.66 and 39.43 in the first and second seasons, respectively. This means that seed yield would increase by 37.66 and 39.43 (gm/ m<sup>2</sup>) in the first and second seasons, respectively, for each unit increase of *Bacillus megaterium*



**Fig. 2. Regression of seed yield (Y, gm /m<sup>2</sup>) on Bacillus megaterium (X, count×10<sup>4</sup> CFU)**

$Y^{\wedge} = 455.10 + 37.66 X, r = 0.9.$  in the 1<sub>st</sub> season

$Y^{\wedge} = -600.091 + 39.43384 X, r = 0.84$  in the 2<sub>st</sub> season

On the other hand, uninoculated treatment gave the lowest values of the qualities listed earlier as following: Number of dry pods / plant were 7.768 and 8.392, lengths of pods were 6.817 and 8.021 (cm), diameters of pea pods were 0.645 and 0.698 (mm), average seeds number / pod were 6.800 and 7.929, average seeds weight / pod were 1.748 and 1.904 (g) and weights of seeds yield /m<sup>2</sup> were 730.7 and 803.3(g) in the first and second season, respectively. That consistent with Afifi *et al.* (2010) results who found that humic acid improved nutrient status and yield components of faba bean plants. In addition, Ramana, V. *et al.* (2010) studied the effect of bio-fertilizers VAM (*Vesicular Arbuscular Mycorrhizae*) and PSB (Phosphorus Solubilizing Bacteria) along with their graded dose of fertilizers on yield attributes and yield of french bean. Their results revealed that the application of 75 per cent recommended Dose of Fertilizer + VAM + PSB significantly increased number of pods per plant, number of pods per cluster, number of seeds per pod, 100 seed weight (g), pod length, pod yield per plant (g) and pod yield per hectare. As for phosphorus effect on plant growth, Sharma (2002) reported that one of the advantages of plant feeding with phosphorus is to create deeper and more abundant roots. Omar *et al.* (1990) and Tesfaye *et al.* (2007) added that phosphorus is one of the most important elements significantly affecting plant growth and metabolism. The crop

production on more than 30% of the world arable lands related to P availability. Tsvetkova and Georgiev, (2007) added that phosphorus may be a critical constraint of legumes under low nutrient environments because there is a substantial need for P in the N<sub>2</sub> fixation process.

#### **Seed analysis:**

Regarding chemical constituents of pea seeds, nitrogen, phosphorus and potassium were estimated and shown in Tables (12, 13). It was clearly that pea plant treatments with only chemical fertilizers gave lower values than plants treated with biofertilizers in all the measurements in both investigated seasons. That result was in harmony with those obtained by El-Sayed *et al.* (2018 and Pandya and Saraf (2010). Also, Suke *et al.* (2011) reported that treated maize (*Zea mays* L.) with recommended dose fertilizer + *Azotobacter* + PSB led to increase of nitrogen, phosphorus and potassium contents in leaves.

#### **RECOMMENDATION**

The study revealed that using 8kg HA/fed in combination with PDB and high level of P<sub>2</sub>O<sub>5</sub> as a fertilizer application to improve soil properties, vegetative growth, mineral content and yield of pea plants in new soils was recommended.

**Table 9. Influence of humic acid, biofertilizers and phosphorus applications on number of dry pods /plant and length of pods (cm) of pea during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	Number of dry pods /plant of pea								Length of pods (cm) of pea									
		First Season				Second Season				First season				Second Season					
		Humic Acid								Humic Acid									
		Without t	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean		
Without	100	5.7	7.0	9.4	7.4	6.6	7.5	10.1	8.1	3.9	5.4	8.7	6.0	5.1	6.3	9.5	6.9		
	90	4.0	6.2	9.0	6.4	5.5	6.8	9.1	7.1	2.9	5.0	7.7	5.2	4.3	5.9	8.4	6.2		
	80	3.2	5.3	6.9	5.1	4.9	5.9	7.8	6.2	2.8	4.4	5.7	4.3	3.3	5.0	7.8	5.4		
	70	2.5	4.4	5.8	4.3	3.7	5.3	6.6	5.2	2.3	3.9	5.1	3.8	2.9	4.1	6.5	4.5		
	Mean	3.9	5.7	7.8	5.8	5.2	6.4	8.4	6.6	3.0	4.7	6.8	4.8	3.9	5.3	8.0	5.7		
PDB	100	6.7	14.5	18.0	13.0	7.8	15.2	18.7	13.9	6.0	8.4	11.2	8.5	6.7	9.6	12.4	9.5		
	90	6.1	12.5	15.8	11.5	7.2	13.5	17.2	12.6	5.1	7.5	10.5	7.7	6.1	9.0	11.4	8.8		
	80	4.5	9.5	13.0	9.0	6.5	11.5	15.4	11.1	4.1	6.2	9.5	6.6	5.7	8.0	10.6	8.1		
	70	3.1	7.7	11.0	7.3	5.8	10.5	13.5	9.9	3.7	4.2	8.7	5.5	4.6	6.4	9.4	6.8		
	Mean	5.1	11.0	14.4	10.2	6.8	12.7	16.2	11.9	4.7	6.6	10.0	7.1	5.7	8.2	11.0	8.3		
P × HU	100	6.2	10.7	13.7	10.2	7.2	11.4	14.4	11.0	4.9	6.9	10.0	7.3	5.9	7.9	10.9	8.2		
	90	5.1	9.3	12.4	8.9	6.3	10.1	13.1	9.9	4.0	6.2	9.1	6.4	5.2	7.4	9.9	7.5		
	80	3.9	7.4	9.9	7.1	5.7	8.7	11.6	8.7	3.5	5.3	7.6	5.4	4.5	6.5	9.2	6.7		
	70	2.8	6.0	8.4	5.8	4.8	7.9	10.0	7.5	3.0	4.1	6.9	4.6	3.7	5.2	8.0	5.6		
	Mean	4.5	8.4	11.1		6.0	9.5	12.3		3.8	5.6	8.4		4.8	6.8	9.5			
LSD 5%																			
Humic acid				2.9				1.4				0.8				0.3			
Biofertilizer				0.6				0.6				0.4				0.2			
Phosphrus				0.5				0.3				0.3				0.2			
Humic*Bio				1.1				1.0				0.8				0.4			
Humic*Phosphorus				0.9				0.5				0.5				0.4			
Bio*Phosphorus				0.6				0.3				0.4				NS			
Humic*Bio*Phosphorus				1.0				0.6				0.6				NS			

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.



**Table 10. Influence of humic acid, biofertilizers and phosphorus applications on diameter of pods (mm) of pea and average seed number /dry pod of pea during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	Diameter of pea pods (mm)								Average seed number /dry pea pod									
		First Season				Second Season				First season				Second Season					
		Humic Acid								Humic Acid									
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean		
Without	100	0.37	0.57	0.71	0.55	0.52	0.63	0.78	0.64	4.05	5.61	7.85	5.84	4.78	6.52	9.07	6.79		
	90	0.32	0.52	0.67	0.50	0.41	0.57	0.72	0.57	3.48	5.35	7.02	5.28	4.38	5.95	8.45	6.26		
	80	0.28	0.46	0.63	0.46	0.35	0.49	0.68	0.51	3.68	4.90	6.52	5.03	3.85	5.25	7.52	5.54		
	70	0.23	0.37	0.57	0.39	0.26	0.42	0.61	0.43	3.27	4.52	5.82	4.54	3.52	4.77	6.68	4.99		
	Mean	0.30	0.48	0.65	0.48	0.38	0.53	0.70	0.54	3.62	5.09	6.80	5.17	4.13	5.62	7.93	5.89		
PDB	100	0.48	0.87	1.02	0.79	0.61	0.96	1.16	0.91	4.71	7.25	9.18	7.05	5.98	8.65	9.88	8.17		
	90	0.44	0.82	0.94	0.73	0.52	0.88	0.99	0.80	4.63	6.62	8.92	6.72	5.35	7.67	9.43	7.48		
	80	0.37	0.75	0.88	0.67	0.46	0.84	0.91	0.74	4.31	6.28	8.08	6.23	4.78	6.95	8.70	6.81		
	70	0.34	0.67	0.83	0.61	0.38	0.75	0.86	0.66	3.78	5.90	7.18	5.62	3.88	6.02	7.85	5.92		
	Mean	0.41	0.78	0.92	0.70	0.49	0.86	0.98	0.78	4.36	6.51	8.34	6.40	5.00	7.32	8.97	7.10		
P × HU	100	0.42	0.72	0.86	0.67	0.56	0.80	0.97	0.78	4.38	6.43	8.52	6.44	5.38	7.58	9.48	7.48		
	90	0.38	0.67	0.81	0.62	0.47	0.72	0.85	0.68	4.06	5.99	7.97	6.00	4.87	6.81	8.94	6.87		
	80	0.32	0.61	0.75	0.56	0.41	0.67	0.80	0.62	4.00	5.59	7.30	5.63	4.32	6.10	8.11	6.18		
	70	0.28	0.52	0.70	0.50	0.32	0.59	0.74	0.55	3.53	5.21	6.50	5.08	3.70	5.39	7.27	5.45		
	Mean	0.35	0.63	0.78	0.56	0.44	0.69	0.84	0.64	3.99	5.80	7.57	5.79	4.57	6.47	8.45	6.44		
LSD 5%																			
Humic acid				0.03				0.01				0.45				0.16			
Biofertilizer				0.01				0.02				0.25				0.18			
Phosphrus				0.01				0.02				0.16				0.17			
Humic*Bio				0.02				0.04				0.44				0.32			
Humic*Phosphorus				0.02				NS				0.27				0.29			
Bio*Phosphorus				NS				NS				NS				0.20			
Humic*Bio*Phosphorus				NS				0.04				NS				0.34			

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Table 11. Influence of humic acid, biofertilizers and phosphorus applications on average weight of seeds (g) /pod of pea and weight of seed yield (g/m of pea during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus %	Average weight of seeds (g) /pod of pea								weight of seed yield (g)/m <sup>2</sup> of pea									
		First Season				Second Season				First season				Second Season					
		Humic Acid								Humic Acid									
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean		
Without	100	1.017	1.117	2.183	1.439	1.100	1.167	2.417	1.561	81.7	605.3	853.3	513.4	103.0	774.0	885.0	587.3		
	90	0.840	0.970	1.917	1.242	1.017	1.083	2.083	1.394	75.0	507.3	735.0	439.1	94.3	708.3	815.0	539.2		
	80	0.800	0.887	1.583	1.090	0.867	0.983	1.733	1.194	65.3	488.3	679.0	410.9	87.3	660.0	775.0	507.4		
	70	0.683	0.767	1.307	0.919	0.717	0.917	1.383	1.006	63.0	261.7	655.3	326.7	82.3	418.3	738.3	413.0		
	Mean	0.835	0.935	1.748	1.173	0.925	1.038	1.904	1.289	71.3	465.7	730.7	422.5	91.8	640.2	803.3	511.8		
PDB	100	1.150	2.483	4.050	2.561	1.200	2.667	4.317	2.728	124.3	1123.3	1356.7	868.1	171.3	1280.0	1473.3	974.9		
	90	0.993	2.317	3.817	2.376	1.117	2.483	3.967	2.522	114.7	1046.7	1233.3	798.2	163.7	1220.0	1416.7	933.4		
	80	0.887	2.107	3.267	2.087	1.033	2.167	3.667	2.289	95.0	745.3	1207.7	682.7	153.7	1161.7	1341.7	885.7		
	70	0.823	1.883	2.817	1.841	0.933	1.983	3.450	2.122	86.7	694.0	1121.7	634.1	144.3	1121.7	1263.7	843.2		
	Mean	0.963	2.198	3.488	2.216	1.071	2.325	3.850	2.415	124.3	1123.3	1356.7	745.8	158.3	1195.8	1356.7	909.3		
P × HU	100	1.083	1.800	3.117	2.000	1.150	1.917	3.367	2.144	103.0	864.3	1105.0	690.8	137.2	1027.0	1179.2	781.1		
	90	0.917	1.643	2.867	1.809	1.067	1.783	3.025	1.958	94.8	777.0	984.2	618.7	129.0	964.2	1115.8	736.3		
	80	0.843	1.497	2.425	1.588	0.950	1.575	2.700	1.742	80.2	616.8	943.3	546.8	120.5	910.8	1058.3	696.6		
	70	0.753	1.325	2.062	1.380	0.825	1.450	2.417	1.564	74.8	477.8	888.5	480.4	113.3	770.0	1001.0	628.1		
	Mean	0.899	1.566	2.618		0.998	1.681	2.877		88.2	684.0	980.3		125.0	918.0	1088.6			
<b>LSD 5%</b>																			
Humic acid				0.546				0.476				45.096				56.967			
Biofertilizer				0.149				0.061				13.711				7.070			
Phosphrus				0.070				0.046				12.427				11.260			
Humic*Bio				0.257				0.106				23.747				12.246			
Humic*Phosphorus				0.122				0.080				21.523				19.502			
Bio*Phosphorus				0.082				NS				14.579				13.210			
Humic*Bio*Phosphorus				NS				0.094				25.252				22.880			

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Table 12. Influence of humic acid, biofertilizers and phosphorus applications on nitrogen and phosphorus concentration in seeds of pea during 2016/2017 seasons**

Bacterial Inoculations	Phosphorus %	Nitrogen content in seeds of pea (%)								Phosphorus concentration (ppm)									
		First Season				Second Season				First season				Second Season					
		Humic Acid								Humic Acid									
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean		
Without	100	1.387	1.877	2.320	1.861	1.91	2.41	3.01	2.44	249.40	364.30	457.65	357.12	268.61	398.63	487.19	384.81		
	90	1.200	1.650	1.850	1.567	1.72	2.27	2.66	2.22	209.70	345.53	425.08	326.77	247.52	367.72	461.13	358.79		
	80	0.967	1.400	1.573	1.313	1.51	2.14	2.45	2.03	188.40	295.92	396.91	293.74	210.95	349.90	427.65	329.50		
	70	0.760	1.050	1.397	1.069	1.11	1.89	2.28	1.76	165.93	259.19	363.12	262.75	189.82	318.68	375.47	294.66		
	Mean	1.078	1.494	1.785	1.453	1.56	2.18	2.60	2.11	203.36	316.24	410.69	310.10	229.23	358.73	437.86	341.94		
PDB	100	1.630	2.487	4.007	2.708	2.16	3.62	4.28	3.35	294.53	436.71	635.96	455.74	335.30	445.68	652.17	477.72		
	90	1.083	2.113	3.633	2.277	2.02	3.42	4.04	3.16	268.63	377.77	564.61	403.67	311.22	405.60	625.73	447.52		
	80	0.917	1.867	3.383	2.056	1.82	3.19	3.74	2.92	250.10	351.26	547.98	383.11	267.95	372.08	562.21	400.75		
	70	0.740	1.677	2.850	1.756	1.47	2.94	3.37	2.59	217.60	331.37	450.24	333.07	245.43	335.55	537.00	372.66		
	Mean	1.093	2.036	3.468	2.199	1.87	3.29	3.86	3.01	257.72	374.28	549.70	393.90	289.98	389.73	594.28	424.66		
P × HU	100	1.508	2.182	3.163	2.284	2.04	3.01	3.64	2.90	271.97	400.51	546.81	406.43	301.96	422.16	569.68	431.26		
	90	1.142	1.882	2.742	1.922	1.87	2.85	3.35	2.69	239.17	361.65	494.84	365.22	279.37	386.66	543.43	403.15		
	80	0.942	1.633	2.478	1.684	1.67	2.67	3.10	2.48	219.25	323.59	472.44	338.43	239.45	360.99	494.93	365.12		
	70	0.750	1.363	2.123	1.412	1.29	2.41	2.83	2.18	191.77	295.28	406.68	297.91	217.63	327.12	456.24	333.66		
	Mean	1.085	1.765	2.627		1.72	2.74	3.23		230.54	345.26	480.19		259.60	374.23	516.07			
LSD 5%																			
Humic acid				0.3865				0.2502				18.253				19.045			
Biofertilizer				0.3062				0.0880				15.460				17.722			
Phosphrus				0.0711				0.0690				14.847				17.112			
Humic*Bio				0.5303				0.1525				26.777				30.695			
Humic*Phosphorus				0.1232				0.1196				25.717				NS			
Bio*Phosphorus				0.0835				NS				NS				NS			
Humic*Bio*Phosphorus				0.1446				NS				30.171				NS			

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

**Table 13. Influence of humic acid, biofertilizers and phosphorus applications on potassium concentration in seeds of pea during 2016/ 2017 seasons**

Bacterial Inoculations	Phosphorus	Potassium concentration (%)								
		First Season				Second Season				
		Humic Acid								
		Without	4 Kg /fed.	8 Kg /fed.	Mean	Without	4 Kg /fed.	8 Kg /fed.	Mean	
Without	100	0.91	1.28	1.37	1.19	1.38	1.76	1.94	1.69	
	90	0.84	1.18	1.28	1.10	1.14	1.40	1.70	1.41	
	80	0.77	1.11	1.24	1.04	1.01	1.17	1.42	1.20	
	70	0.73	1.02	1.14	0.97	0.94	1.05	1.19	1.06	
	Mean	0.81	1.15	1.26	1.07	1.12	1.35	1.56	1.34	
PDB	100	1.13	1.66	2.02	1.60	1.73	2.12	2.56	2.13	
	90	1.08	1.54	1.95	1.52	1.47	1.85	2.28	1.87	
	80	1.03	1.45	1.86	1.45	1.17	1.49	1.98	1.55	
	70	0.92	1.31	1.74	1.33	1.05	1.34	1.78	1.39	
	Mean	1.04	1.49	1.89	1.47	1.36	1.70	2.15	1.74	
P × HU	100	1.02	1.47	1.69	1.40	1.55	1.94	2.25	1.91	
	90	0.96	1.36	1.61	1.31	1.31	1.63	1.99	1.64	
	80	0.90	1.28	1.55	1.24	1.09	1.33	1.70	1.37	
	70	0.83	1.17	1.44	1.15	1.00	1.19	1.49	1.22	
	Mean	0.93	1.32	1.58		1.24	1.52	1.86		
LSD 5%										
Humic acid					0.033					0.069
Biofertilizer					0.016					0.149
Phosphrus					0.023					0.083
Humic*Bio					0.028					NS
Humic*Phosphorus					0.040					NS
Bio*Phosphorus					0.027					NS
Humic*Bio*Phosphorus					NS					NS

\*- P × HU= Interaction of Phosphorus treatment with humic acid treatments.

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## الملخص العربي

### تأثير حامض الهيوميك والأسمدة الحيوية والفوسفات المعدني على النشاط الميكروبي بالتربة وإنتاجية نباتات البسلة تحت ظروف توشكا

محمود على محمد السيد، السيد محمد طه

مقارنة بنتائج إستخدام خليط من حامض الهيوميك والسوبر فوسفات فقط. كما أشارت الدراسة أيضا الى أن معدلات الإضافة من حامض الهيوميك والسوبر فوسفات مع وجود البكتريا المذيبة للفوسفات أدت الى تحسين معظم صفات النمو الخضري للنباتات والمتمثلة في (ارتفاع النبات، عدد الأفرع / نبات ، الوزن الأخضر والجاف/نبات، نسبة الكلوروفيل). كما أن النتائج المحصولية المبينة في (عدد القرون الجافة على النبات، طول القرن/سم، قطر القرون /مليميتر، متوسط عدد البذور في القرن، متوسط وزن البذور في القرن، وزن محصول البذور/ المتر المربع) قد استجابت للزيادة المستخدمة من تلك الإسمدة مع البكتريا المذيبة للفوسفات. وبتقدير نسب عناصر النيتروجين والفوسفور واليوتاسيوم في البذور تبين أن أعلى تركيز لتلك العناصر مع إستخدام حامض الهيوميك (٨ كجم/ فدان) مصحوبا بالتلقيح الحيوي بالبكتريا المذيبة للفوسفات وإضافة نسبة ١٠٠٪ من التسميد الفوسفاتي فو٢أ الموصى به في الإنتاج التجاري.

أوصت الدراسة بموجب هذا التطبيق باستخدام (HA + PDB + فو٢أ) كتطبيق سمادى لتحسين خواص التربة والنمو الخضري والمحتوى المعدني والنواتج المحصولي لنباتات البسلة في الأراضي الحديثة.

أجريت دراسة حقلية خلال الموسم الشتوي لعامي ٢٠١٦/٢٠١٧ & ٢٠١٦/٢٠١٧، بمحطة بحوث توشكا . مركز بحوث الصحراء، لدراسة تأثير الأحماض الدبالية HA (حامض الهيوميك ، بثلاث معاملات - بدون إضافة، ٤ ، ٨ كجم/فدان) مع معاملتين من التسميد الحيوي (بدون لقاح + PDB البكتريا المذيبة للفوسفات) مع أربع معاملات من التسميد الفوسفاتي (١٠٠ ، ٩٠ ، ٨٠ ، ٧٠ ٪ فو٢أ) من المعدل الموصى به في الإنتاج التجاري) على نباتات البسلة. أوضحت الدراسة إن زيادة معدل التسميد بحامض الهيوميك الى ٨ كجم/فدان، مع التلقيح بال PDB + ١٠٠٪ من التسميد الفوسفاتي كان له أثر بالغ في زيادة الأعداد الكلية للميكروبات والبكتريا المذيبة للفوسفات وإنبعاث ثاني أكسيد الكربون والمادة العضوية بالتربة. بالإضافة الى زيادة نشاط الإنزيمات المرتبطة بميكروبات التربة مثل إنزيم الفوسفاتيز والديهيدروجينيز. كما أن المحتوى المعدني في التربة مثل النيتروجين الكلي والفوسفور الميسر ونسبة الكربون الى النيتروجين (C/N ratio) ونسبة نسبة الكربون الى الفوسفور (C/P ratio) في التربة أظهرتا إستجابة معنوية مرتفعة لجميع عوامل الدراسة خصوصا التركيزات المرتفعة منها مع البكتريا المذيبة للفوسفات. ولقد أظهرت معاملات التسميد الحيوي (PDB) تفوق واضح عند إضافتها مع معاملات الأسمدة الفوسفاتية وحامض الهيوميك مجتمعة