

# PRODUCTION OF PEA WITHOUT CHEMICAL FERTILIZERS VIA INTEGRATING BIOFERTILIZERS WITH VERMIWASH

## Saad Abou-El-Hassan<sup>1</sup> and Heba S. Elbatran<sup>2</sup>

<sup>1</sup>Central Lab of Organic Agriculture, Agricultural Research Center, Egypt. <sup>2</sup>Agricultural and Biological Division, National Research Center, Egypt.

## Abstract

Nowadays, the need for safe agricultural production is increasing, depending on the use of chemical fertilizer alternatives for achieving sustainable agricultural development. This study evaluates the production of green peas (cv. Master B) without using chemical fertilizers by integrating the biofertilizers with vermiwash (the liquid that is leached after passing the water through a column of vermiculture units). A field experiment was designed at Giza Agriculture Research Station, Agricultural Research Center, Egypt, during 2018/2019 and 2019/2020 seasons. Treatments of vermiwash only, vermiwash with inoculation of *Rhizobium* (Rh) and plant growth promoting rhizobacteria (PGPR) individual or in combination were compared to recommended chemical fertilizers. Applying vermiwash + Rh + PGPR gave the highest values of root nodulations, plant growth, nutritional content (N, P and K), yield, pod characteristics and seed compositions (protein, carbohydrates and dry matter) of green pea. The plants treated with vermiwash + Rh showed statistically similar results to the chemical fertilizers treatment in all growth, yield and quality traits. Application of vermiwash only gave the lowest values of the same traits. This study concluded the possibility of applying vermiwash with biofertilizers of *Rhizobium* and plant growth promoting rhizobacteria as a substitute of chemical fertilizers to produce a good yield with high quality of green peas.

Key words: Pea, Vermiwash, Vermicompost leachate, Rhizobium, PGPR.

#### Introduction

Pea (*Pisum sativum* L.) is one of the most important vegetable crops belong to Leguminaceae family. It is one of the most sustainable vegetable crops grown in the world, being nitrogen fixing legume. Cultivation of pea maintains soil fertility via atmospheric nitrogen fixation in its root nodules in symbiotic association with *Rhizobium*, thus plays a vital role in enhancing sustainable agriculture (Negi *et al.*, 2006). In Egypt the cultivated area of green pea is about 47000 feddans (feddan = 0.42 hectare), produced about 200000 tons (Economic Affairs Sector, Ministry of Agriculture and Land Reclamation, 2018).

Sustainable agricultural development needs to find alternatives to chemical fertilizers or limit their use to produce safe food for humans, maintain of soil fertility and environment without pollution. The continuous use of chemical fertilizers only in agriculture causes raising the pollutants in agricultural and groundwater, decreasing soil fertility, deterioration of soil health and reducing the production for area unit (Hernandez *et al.*, 2010).

Vermicompost is a product of organic matter

decomposing through integration between earthworms and microorganisms. In this process earthworms feed on the organic waste and convert it into a decomposing form with the help of microbial activity that accelerates degradation rates, as in composting without high temperature (Abduli *et al.*, 2013).

Vermiwash is the liquid leached after saturating the water for a vermicompost unit containing active earthworms and vermicompost at last maturing stages. It is a mixture of earthworm mucous secretions, nutrients, microorganisms and plant growth promoting substances (Gopal *et al.*, 2010), that are washed from the vermicompost into vermiwash leachate. This liquid is rich in amino acids, vitamins, nutrients like nitrogen, potassium, calcium, magnesium, iron, zinc and copper in addition to some growth hormones like auxins and cytokines (Suthar, 2010). In sum, vermiwash is a liquid fertilizer that is easier to add to crops compared to solid organic fertilizers, reduces the amount of solids required for crop production, facilitates fertilizing by farmers and reduces production costs (Pant *et al.*, 2011). Application of vermiwash led

ſ	Clay%	Silt%	Sand%	Texture	pН	ECdS/m		Cations	meq/l		An	ions me	q/l
	Clay 70	5111 70					Ca++	Mg++	Na <sup>+</sup>	<b>K</b> <sup>+</sup>	HCO <sub>3</sub> -	Cŀ	$SO_4^{=}$
ſ	41.5	35.2	23.3	Clay loam	7.66	1.73	3.12	1.15	2.83	1.74	2.27	3.75	3.62

Table 1: Physical and chemical properties of the experimental soil.

to improve plant growth, crop yield and nutritive quality (Gamaley *et al.*, 2001; Pant *et al.*, 2011). It is believed that soluble mineral nutrients, amino acids, vitamins and soluble plant growth regulators extracted in the vermicompost leachate have positive effects on initial root development and plant growth with both foliar and soil application (Edwards *et al.*, 2006; Arancon *et al.*, 2007).

Biofertilizers play an important role in maintaining soil productivity and sustainability, thus maximizing crop productivity. They serve as eco-friendly and beneficial to farmers in terms of low cost and ease of use for most crops (Khanna et al., 2019). Rhizobium is symbiotic bacteria, associate with the root systems of the legumes and form root nodules for fixing atmospheric nitrogen. The inoculation of legume crops with Rhizobium led to improve plant growth and yield (Tagore et al., 2013). Plant growth promoting rhizobacteria (PGPR) are a group of beneficial bacteria that fixing nitrogen, solubilizing phosphate, releasing potassium and promoting plant growth (Joseph et al., 2007; Souza et al., 2015). Application of PGPR lead to colonize the rhizosphere, promote growth, increase nutrient uptake and yield by a combination of mechanisms. They help in promoting free fixing nitrogen, increase supply of other nutrients, such as phosphorus, potassium, sulphur and micronutrients, produce plant hormones like cytokinins, Indole Acetic Acid, Gibberillins (Vessey, 2003; Hafeez et al., 2006; Egamberdieva, 2008; Gholami et al., 2009; Saharan and Nehra, 2011). Many studies showed that the combination of PGPR with *Rhizobium* further enhanced nodulation, root length, the growth, yield and quality of legume plants such as Remans et al., (2007) and Gharib et al., (2015) on bean; Ismail, (2002); Tilak et al., (2006); Gabr et al., (2007); Mishra et al., (2010); Rather et al., (2010) and Pramanik and Bera, (2012) on peas. Many studies showed that the combination of biofertilizers with organic fertilizers further enhanced the growth, yield and quality of plants such as Solaiman and Rabbani, (2006); Zaghloul et al., (2015) and Abo-Basha, (2016) on pea.

Therefore, this work investigates the possibility of integration of biofertilizers with vermiwash to produce green peas without chemical fertilizers.

## Material and Methods

A field experiment on pea was implemented at Giza Agriculture Research Station, Agricultural Research Center, Egypt, during two winter seasons of 2018/2019 and 2019/2020. This experiment was performed to produce green peas without using chemical fertilizers by integrating the biofertilizers with vermiwash. The experimental area was plowed and leveled before being divided into plots at area 14 m<sup>2</sup> (5 m length and 2.8 m width), each plot contained four ridges of 0.7 m width and 5 m length. Physical and chemical properties of the experimental soil were presented in table 1.

## **Plant Material**

Seeds of pea (cv. Master B) were sown in the field on the first week of November in both seasons. The seeds were sown twosome at a distance of 10 cm on one side of ridge; furrow system was applied for plant irrigation. The experiment was designed in complete randomized blocks with three replicates.

The Experimental Treatments.

- 1. Mineral fertilizer as N, P and K (control).
- 2. Vermiwash (V).
- 3. Vermiwash + Rhizobium leguminosarum (Rh)

4. Vermiwash + PGPR as *Azotobacter chroococcum*, *Bacillus megaterium* and *Bacillus circulans*.

5. Vermiwash + Rh + PGPR.

#### **Preparation of Vermiwash**

Vermiwash was produced by modifying the method described by Gopal et al., (2010), where a vermiculture unit consisting of 6 plastic cages ( $60 \times 40 \times 20$  cm) was installed on top of each other. The down cage was lined with black plastic to collect leachate, while the rest of the cages were lined with a plastic net intended for vermiculture. A layer (5 cm high) of rice straw (soaked in water for a day) was placed at the bottom of each cage, followed by a layer (12-15 kg) of mixture of rabbit manure and squash crop residues (1:1) after composting for two weeks. Steps build a vermiculture unit are demonstrated in fig. 1. Each cage contained 200 g of earthworms (Eisenia foetida and Lumbricus rubellas). The contents of each cage were watered by 2-3 liters/ week for two months with the leachate was reused in the watering. After two months, each cage was watered by 5 liters every week for month. During this period, the concentrated vermiwash is collected in the down cage and transported to a plastic barrel (100 liters capacity) at once a week for storage until use. The main chemical and microbiological properties of concentrated vermiwash



Fig. 1: Steps build a vermiculture unit, respectively.

are illustrated in table 2.

## Quantities and Methods of Application

The control treatment was a chemical fertilizers of N, P and K at rates 60 kg N, 30 kg  $P_2O_5$  and 48 kg  $K_2O/$ fed expressed as 293 kg of ammonium sulphate (20.5% N), 194 kg of calcium super phosphate  $(15.5\% P_2O_5)$ and 100 kg of potassium sulphate (48% K<sub>2</sub>O), respectively. Calcium super phosphate was added as one dose during soil preparation, whereas ammonium sulphate and potassium sulphate were added at three equal portions, after 2, 4 and 6 weeks from sowing. Seeds of pea were inoculated with Rhizobium leguminosarum by using seed coating method at rate 40 g/kg of seeds just before sowing. Plant growth promoting rhizobacteria were applied as a liquid mixture of Azotobacter chroococcum (N fixing bacteria), Bacillus megaterium (P solubilizing bacteria) and Bacillus circulans (K releasing bacteria). Each kind was added at a rate of 5 L/fed (1ml contains 106-108 cell) according to Mashhoor et al., (2002). PGPR were supplemented to the soils after ten days from sowing seeds. Vermiwash was diluted with water at a ratio of 1:10 approximately at EC 2.5 dS/m (Hashem and Abd-

 Table 2: The main chemical and microbiological properties of concentrated vermiwash.

Item	Vermiwash
pH	8.43
Ec (ds/m)	6.63
N (mg/l)	82.08
P (mg/l)	31.23
K (mg/l)	29.16
Fe (mg/l)	2.49
Mn (mg/l)	0.94
Zn (mg/l)	0.59
Total count (cfu ml <sup>-1</sup> )	
Bacteria	$2.6 \times 10^{7}$
Fungi	$1.5 \times 10^{2}$
Actinomycetes	$3.2 \times 10^{5}$

Elrahman, 2016) and applied as a soil application every ten days at a rate of  $1L/m^2$  (Emam *et al.*, 2020). Addition of vermiwash started after ten days from sowing seeds and continued for two months.

## **Data Recorded**

After 60 days from sowing pea seeds, six plants were randomly taken from each replicate for measuring plant growth characteristics expressed as plant length, leaf number/plant and shoot fresh weight. As well as nutritional content (N, P and K) in pea plants were determined in dry matter of the most recent fully developed trifoliate leaves according to Cottenie *et al.*, (1982). Total nitrogen, phosphorus and potassium were determined by Micro Kjeldahl, Spectrophotometer and Flame photometer respectively according to FAO, (1980). Nodules were collected from roots of the same plants manually, counted and weighted, then dried at 70°C for 48 hr to determine the number of root nodules/plant, fresh and dry weight of the nodules.

After 85 days from pea seeds sowing, the pods were harvested weekly for three times. Yield per plant and plot of pea pods were recorded after each harvesting accumulatively. Ten of pea pods from each plot were taken randomly at harvest to estimate pod characteristics as weight, length and diameter. After harvest directly, 150 g of pea seeds were sampled from each experimental unit to determine seed compositions of total protein, total carbohydrates and dry matter. Protein content was determined by N content by 6.25 (AOAC, 2000). Total carbohydrates were determined colorimetrically according to the methods described by Dubois *et al.*, (1956). Dry matter was calculated by the following equation:

% Dry matter = 
$$\frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

#### Statistical analysis

Data of the two seasons were arranged and

statistically analyzed by the analysis of variances according to Snedecor and Cochran, (1980) with SAS software, version 2004. Treatment means were compared using Tukey test at significance level 0.05.

## **Results and Discussion**

Data in table 3 show the effect of biofertilizers and vermiwash on nodules characters in root of pea plants during the two winter seasons. In this respect, data mentioned that all nodules parameters (nodule number/ plant, nodule fresh and dry weight) were the lowest in plants fertilized with chemical fertilizers compared to the plants inoculated with the different biofertilizers either in the presence or absence of vermiwash application. This result is agreement with Abo-Basha, (2016) who stated that chemical fertilizers reduced root nodulation in pea plants compared to biofertilizers. The highest values of all nodule characters were monitored in the plants inoculated with Rh + PGPR in the presence of vermiwash. These results are consistent with Remans et al., (2007) and Gharib et al., (2015) on bean; Ismail, (2002); Tilak et al., (2006) and Mishra et al., (2010) on peas; they found

that co-inoculation of plants with Rhizobium and other biofertilizers resulted in significant increasing nodule number/plant, nodule fresh and dry weight. As well as, the presence of vermiwash as a liquid organic fertilizer that contains the many of growth promoters may have promoted root growth and increased its branching, which led to an increase in the number of nodules on plant roots, especially in the presence of *Rhizobium*. This finding is consistent with Solaiman and Rabbani, (2006) and Abo-Basha, (2016), they indicated that all nodule characters on roots of pea plants enhanced by application of biofertilizers in combination with organic nitrogen fertilizer.

Effect of biofertilizers and vermiwash on growth characteristics of pea plants in both seasons is presented in Table 4. The results cleared that the fertilized pea plants with chemical fertilizers gave the highest values in plant lengths with insignificant differences compared to the plants treated with V + Rh + PGPR. While the treatment of V + Rh + PGPR was superior to other treatments in the number of leaves per plant and shoot fresh weight. Treatment of V + Rh recorded the second greatest leaf number and shoot fresh weight per plant without significant differences with the treatment of chemical fertilizers. Treatment of V + PGPR came in the third order. The lowest values in all growth characteristics were resulted from application of vemiwash only. Increment in the growth of pea plants with using V + Rh + PGPR, might be due to the positive synergistic effect between Rhizobium and PGPR for fixing atmospheric nitrogen, increase supply of phosphorus, potassium and micronutrients in the rhizosphere zone that improved the nutrition of the plants, which was reflected on increasing plant growth (Joseph et al., 2007; Tagore et al., 2013; Souza et al., 2015). As well as, this might be due to positive effects of the integration of vermiwash and biofertilizers in providing soluble mineral nutrients, amino acids, vitamins and plant growth regulators like cytokinins, Indole Acetic Acid and Gibberillins that promoted root development and plant growth (Gamaley et al., 2001; Edwards et al., 2006;

 Table 3: Effect of biofertilizers and vermiwash on root nodule of characters of pea plants during 2018/2019 and 2019/2020 seasons.

Turturati	Nod N		Nodul weigl		Nodule dry weight (g)				
Treatments	1 <sup>st</sup> 2 <sup>nd</sup>		1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>			
	season	season	season	season	season	season			
ChF	2.733 e	2.733 e 2.960 e		0.161 d	0.015 c	0.015 c			
Vermiwash (V)	4.767 d	5.843 d	0.170 d	0.174 d	0.016 c	0.016 c			
V+Rh	V+Rh 12.933 b 14	14.063 b	0.388 b	0.390 b	0.033 a	0.034 a			
V+PGPR	8.833 c	9.943 c	0.215 c	0.219 c	0.020b	0.020 b			
V+Rh+PGPR	18.333 a	19.626 a	0.413 a	0.411 a	0.035 a	0.036 a			
Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test; ChF = Chemical fertilizers of N, P and K;									
Rh = h	Rhizobium le	0	,		ter chrooc	occum,			
	Bacillus n	negaterium	and <i>Bacillu</i>	s circulans					

 Table 4: Effect of biofertilizers and vermiwash on vegetative growth characteristics of pea plants 2018/2019 and 2019/2020 seasons.

	Plant l	neight	Le	eaf	Shoot fresh weight					
Transformer	(cr	n)	nun	ıber						
Treatments	1 <sup>st</sup> 2 <sup>nd</sup>		1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>				
	season	season	season	season	season	season				
ChF	55.97 a 57.20		23.38b	26.00 b	46.64 b	48.473 bc				
Vermiwash (V)	43.53 d	45.00 d	18.05 d	20.60 d	36.04 d	38.183 d				
V+Rh	51.50 bc	53.33 b	23.51 b	26.67 b	47.31 b	49.120 b				
V+PGPR	49.47 c	50.20 c	21.96 c	23.97 c	41.98 c	44.930 c				
V+Rh+PGPR 53.60 ab 56.60 a 25.86 a 28.33 a 53.46 a 55.5										
Means followed in same column by similar letters are not statistically different										
	at 0.05 level according to Tukey test; ChF = Chemical fertilizers of N, P and K; Rh = Rhizobium leguminosarum; PGPR = Azotobacter chroococcum,									
Rh = I		0				coccum,				
	Bacillus n	negaterium	and <i>Bacillu</i>	s circulans	1					

	N	%	Р	%	K%					
Treatments	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>				
	season	season	season	season	season	season				
ChF	4.787 b	4.790 b	0.355 ab	0.364 b	2.788 ab	2.768 b				
Vermiwash (V)	3.771 d	3.867 d	0.262 d	0.287 d	2.081 d	2.146 d				
V+Rh	4.730 b	4.773 b	0.325 c	0.334 c	2.520 c	2.524 c				
V+PGPR	4.382 c	4.527 c	0.346 b	0.355 b	2.726 b	2.746 b				
V+Rh+PGPR	4.868 a	4.993 a	0.370 a	0.385 a	2.846 a	2.860 ab				
Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test; ChF = Chemical fertilizers of N, P and K;										
	Rh = Rhizobium leguminosarum; PGPR = Azotobacter chroococcum,									
	Bacillus n	negaterium	and Bacillu	is circulans	7					

 
 Table 5: Effect of biofertilizers and vermiwash on nutritional content of pea plants during 2018/2019 and 2019/2020 seasons.

Arancon et al., 2007; Suthar, 2010).

Data in table 5 are mentioned that the highest concentrations of N, P and K in pea leaves were found in plants that treated by V + Rh + PGPR. Treatment of chemical fertilizers recorded the second highest concentrations of N, P and K in leaves. The treatment of V + Rh statistically gave the same N content that resulted from chemical fertilizers, while the treatment of V + PGPR statistically gave P and K content similar to the chemical fertilizers. On the other hand, the lowest

26b2.746b46a2.860 ab46a2.860 ab46a2.860 ab2.860 ab(Abo-Basha, 2016). Where, vermiwash<br/>is rich in dissolved nutrients, amino acids,<br/>vitamins and some growth hormones like<br/>auxins and cytokines that are easily<br/>available for plants (Suthar, 2010; Pant<br/>et al., 2011). Besides, the role of *Rhizobium* in supplying<br/>plants with N by fixing atmospheric nitrogen in the root<br/>nodules (Tagore et al., 2013). As well as, the role of<br/>PGPR in free fixing nitrogen solubilizing phosphate

concentrations of N, P and K in plants

resulted from application of vermiwash only. Similar findings were noticed in the second season. The cause of the superiority of nutritional content in plants treated with V + Rh + PGPR may be due to the integration of these factors (organic and biofertilizers) in improving

PGPR in free fixing nitrogen, solubilizing phosphate, releasing potassium and producing plant growth promoters in the rhizosphere zone (Vessey, 2003; Hafeez *et al.*, 2006; Egamberdieva, 2008; Gholami *et al.*, 2009; Saharan and Nehra, 2011). All these beneficial substances led to improve of growth and nutritional status of plants.

Effects of biofertilizers and vermiwash on yield and

pod characteristics of pea in both seasons are presented in tables 6 and 7. The results suggested that the plants fertilized with V + Rh + PGPR produced the highest yield per plant and plot, as well as gave the best pod characteristics compared to other treatments. The treatment of V + Rh came in the second order without statistical differences with the chemical fertilizers treatment, whether in relation to the yield or pod characteristics. The treatment of V + PGPR came in fourth order, whereas the treatment of vermiwash only produced the lowest values of yield and pod characteristics. This preference in yield quantity and pod characteristics of V + Rh + PGPR treatment over other treatments, might be explained to its superiority in root nodule number and weight (Table 3), nutrient content of N, P and K in plants (Table 5) which helped in stimulating plant growth (Table 4), resulting increased in photosynthesis and better carbohydrate construction that translocate towards the pods, thus enhanced of yield quantity and pod

 Table 6: Effect of biofertilizers and vermiwash on yield component of pea plants during 2018/2019 and 2019/2020 seasons.

	Yield/	plant g	Yield/plot	t (14 m <sup>2</sup> )kg	Yield t/fed.						
Treatments	1 <sup>st</sup> 2 <sup>nd</sup>		1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>					
	season	season	season	season	season	season					
ChF	66.597 b	66.697 b	18.727 b	19.027 b	5.873 b	5.883 b					
Vermiwash (V)	50.067 d	52.790 d	14.117 d	14.640 d	4.413 d	4.657 d					
V+Rh	66.310b	67.013 b	18.760 b	19.107 b	5.847 b	5.910b					
V+PGPR	59.470 c	60.880 c	17.013 c	17.383 c	5.247 c	5.370 c					
V + Rh + PGPR 72.577 a 74.243 a 20.627 a 21.073 a 6.400 a 6.5											
at 0.05 level acc	ording to T	Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test; ChF = Chemical fertilizers of N, P and K; Rh = Rhizobium leguminosarum; PGPR = Azotobacter chroococcum,									

Bacillus megaterium and Bacillus circulans

 
 Table 7: Effect of biofertilizers and vermiwash on the characteristics of pea pods during 2018/2019 and 2019/2020 seasons.

	Pod w	eight g	Pod ler	ngth cm	Pod diameter cm			
Treatments	1 <sup>st</sup> 2 <sup>nd</sup>		1 <sup>st</sup>	2 <sup>nd</sup>	1 st	2 <sup>nd</sup>		
	season	season	season	season	season	season		
ChF	6.390 b	6.410b	9.484 b	9.489 bc	1.020 bc	1.035 bc		
Vermiwash (V)	5.093 d	5.163 d	8.043 d	8.188 d	0.884 d	0.903 d		
V+Rh	6.420 b	6.497 b	9.445 b	9.558 b	1.042 b	1.055 b		
V+PGPR	5.877 c	6.037 c	9.019 c	9.204 c	0.970 c	1.012 c		
V+Rh+PGPR	7.083 a	7.180 a	10.421 a	10.563 a	1.143 a	1.159 a		
Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test; ChF = Chemical fertilizers of N, P and K; Rh = Rhizobium leguminosarum; PGPR = Azotobacter chroococcum,								
	Bacillus	megateriu	<i>m</i> and <i>Bacill</i>	'us circulans				

Table 8:	Effect	of biofertilizers	and	vermiwash	on tl	he com	position	of pea	seeds
	during	2018/2019 and 2	2019	/2020 seasor	1S.				

Turotanonta	Pro (g/10		Carboh (g/10	•	% Dry matter				
Treatments	1st2ndseasonseason		1 <sup>st</sup> season	2 <sup>nd</sup> season	1 <sup>st</sup> season	2 <sup>nd</sup> season			
ChF	5.548b	5.545 b	14.387 b	14.447 b	23.443 b	23.410b			
Vermiwash (V)	4.490 d	4.629 d	13.920 c	14.020 c	22.653 c	22.670 c			
V+Rh	5.492 b	5.559b	14.406 b	14.507 b	23.553 ab	23.653 ab			
V+PGPR	4.915 c	5.140 c	14.198 bc	14.297 b	23.257 b	23.323 b			
V+Rh+PGPR	5.793 a	5.964 a	14.909 a	15.067 a	23.953 a	24.013 a			
V + Rh + PGPR 5.793 a 5.964 a 14.909 a 15.067 a 23.953 a 24.013 a Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test; ChF = Chemical fertilizers of N, P and K; Rh = Rhizobium leguminosarum; PGPR = Azotobacter chroococcum, Bacillus megaterium and Bacillus circulans									

characteristics of green pea. These results are consistent with those obtained by Tilak *et al.*, (2006); Gabr *et al.*, (2007); Rather *et al.*, (2010) and Pramanik and Bera, (2012). They mentioned that application of *Rhizobium* and PGPR for pea plants led to improve root nodulation, plant growth and yield quality. These results also are confirmed by Zaghloul *et al.*, (2015) and Abo-Basha, (2016). They explained that integrating biofertilizers with organic fertilizers led to the production of plants with strong growth, high nutritional content, good yield and quality of peas.

Data in table 8 are showed the effects of biofertilizers and vermiwash on seed compositions of green pea in the two seasons. The results illustrated that application of V + Rh + PGPR produced the highest contents of protein, carbohydrates and dry matter in seeds compared to other treatments. The treatment of V + Rh ranked second order without significant differences with the chemical fertilizers treatment in all these contents, as well as without significant differences with V + PGPR treatment in the contents of carbohydrates and dry matter. Increment of seed combinations with V + Rh + PGPR treatment may be attributed to the promoting effect for integration of biofertilizers (*Rhizobium* and PGPR) with vermiwash, which helped to enhance vegetative growth (Table 4) and nutritional status (Table 5) of pea plants, resulting in increased photosynthesis products of protein, carbohydrates and dry matter that subsequently transferred to storage places in pea pods. These results are harmony with those demonstrated by Zaghloul et al., (2015) and Abo-Basha, (2016), they indicated that integrating biofertilizers with organic fertilizers led to increase the compositions of protein and carbohydrates in pea seeds.

# Conclusion

It could be concluded possibility of applying vermiwash with biofertilizers of *Rhizobium* and plant growth promoting rhizobacteria as a substitute of chemical fertilizers to produce a good yield with high quality of green peas.

# Acknowledgement

This work has been supported by Central Laboratory for Organic Agriculture, Agricultural Research Center; Agricultural and Biological Division, National Research Center.

# References

- Abduli, M.A., L. Amiri, E. Madadian, S. Gitipour and S. Sedighian (2013). Efficiency of vermicompost on quantitative and qualitative growth of tomato plants. *Int. J. Environ. Res.*, 7(2): 467-472.
- Abo-Basha, D.M.R. (2016). Impact of interaction between organic nitrogen and bio fertilizers on quality and productivity of Pea (*Pisum sativum* L.) plants. *Int. J. Pharm. Tech. Res.*, 9(10): 543-550.
- AOAC (2000). Association of Official Analytical Chemists. Official Methods of Analysis. 17<sup>th</sup> ed. International Maryland, USA, 1250.
- Arancon, N.Q., C.A. Edwards, R. Dick and L. Dick (2007). Vermicompost tea production and plant growth impacts. *BioCycle*, 48: 51-52.
- Cottenie, A., M. Verloo, L. Kiekers, G. Velghe and R. Camrbynek (1982). Chemical Analysis of Plants and Soils. Hand Book, 1-63, Ghent, Belgium.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Robers and F. Smith (1956). Colorimetric method for determination of sugar and related substances. *Anal. Chem.*, **28(3)**: 350-356.
- Economic Affairs Sector (EAS), Ministry of Agriculture and Land Reclamation (2018). The Indicators Agriculture Statistics.
- Edwards, C.A., N.Q. Arancon and S. Greytak (2006). Effects of vermicompost teas on plant growth and disease. *BioCycle*, **47:** 28-31.
- Egamberdieva, D. (2008). Plant Growth Promoting properties of rhizobacteria isolated from Wheat and Pea grown in loamy sand soil. *Turkish J. Biol.*, **32:** 9-15.
- Emam, M.S.A., L.M.M. Hamed and T.R. Elsayed (2020). Sweet corn performance and rhizosphere microbial densities in response to mineral and organic amendments. *Egypt. J. Soil. Sci.*, 60(1): 43-52.

FAO (1980). Soil and Plant Analysis. Soils Bulletin 38/2,250.

Gamaley, A.V., M.A. Nadporozhskaya, A.I. Popov, O.G. Chertov, N.V. Kovsh and O.A. Gromova (2001). Non-root nutrition with vermicompost extracts as the way of ecological optimization. Plant nutrition: food security and sustainability of agro-ecosystems through basic and applied research. 14<sup>th</sup> Int. Plant Nut. Colloq. Springer Netherlands, Hannover, Germany, 862-863.

- Gabr, S.M., H.A. Elkhatib and A.M. El-keriawy (2007). Effect of different biofertilizer types and nitrogen fertilizer levels on growth, yield and chemical contents of pea plants (*Pisum* sativum L.). J. Agric. & Env. Sci. Alex. Univ., Egypt, 6(2): 192-218.
- Gharib, A.A., M.M. Shahen and A.A. Ragab (2015). Influence of Rhizobium inoculation combined with *Azotobacter chroococcum* and *Bacillus megaterium* var phosphaticum on growth, nodulation, yield and quality of tow snap bean (*Phaseolus vulgaris* L.) cultivars. *Annals Agric. Sci.*, *Moshtohor*, 53(2): 249-261.
- Gholami, A., S. Shahsavani and S. Nezarat (2009). The effect of Plant Growth Promoting Rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *Int. J. Biol. Life Sci.*, 1(1): 35-40.
- Gopal, M., A. Gupta, C. Palaniswami, R. Dhanapal and G.V. Thomas (2010). Coconut leaf vermiwash: a bio-liquid from coconut leaf vermicompost for improving the crop production capacities of soil. *Curr. Sci. India*, **98(9):** 1202-1210.
- Hafeez, F.Y., S. Yasmin, D. Ariani, Y. Mehboob-ur-Rahman Zafar and K.A. Malik (2006). Plant growth promoting bacteria as biofertilizer, *Agronomy for Sustainable Development*, 26: 143-150.
- Hashem, F.A. and S.H. Abd-Elrahman (2016). Soil chemical characteristics and growth of broccoli and cauliflower plants as affected by liquid organic fertilizers and irrigation water levels. *Global J. Adv. Res.*, **3(10):** 881-895.
- Hernandez, A., H. Castillo, D. Ojeda, A. Arras, J. Lopez and E. Sanchez (2010). Effect of vermicompost and compost on lettuce production. *Chilean J. Agric. Res.*, **70(4)**: 583-589.
- Ismail, R.H. (2002). Physiological studies on biofertilization in pea plants (*Pisum sativum* L.) under calcareous soil conditions. Ph. D. Thesis, Fac. Agric., Ain Shams. Univ., Egypt.
- Joseph, B., R.R. Patra and R. Lawrence (2007). Characterization of plant growth promoting Rhizobacteria associated with chickpea (*Cicer arietinum* L). *Int. J. Plant Prod.*, **1(2)**: 141-152.
- Khanna, R., J. Pawar, S. Gupta, H. Verma, H. Trivedi, P. Kumar and R. Kumar (2019). Efficiency of biofertilizers in increasing theproduction potential of cereals and pulses: A review. J. Phar. Phytochemistry, 8(2): 183-188.
- Mashhoor, W.A., M.A. El-Borollosy, H.H.A. Abdel-Azeem, S.A. Nasr and S.M. Selim (2002). Biofertilization of wheat plants exposed to environmental conditions. J. Agric. Sci. Ain. Shams. univ., 10(2): 543-565.

- Mishra, A., K. Prasad and G. Rai (2010). Effect of bio-fertilizer inoculations on growth and yield of dwarf field pea (*Pisum sativum* L.) in conjunction with different doses of chemical fertilizers. J. Agron., **9(4):** 163-168.
- Negi, S., R.V. Sing and O.K. Dwivedi (2006). Effect of Biofertilizers, nutrient sources and lime on growth and yield of garden pea. *Legume research*, **29(4)**: 282-285.
- Pant, A., T.J.K. Radovich, N.V. Hue and N.Q. Arancon (2011). Effects of Vermicompost Tea (Aqueous Extract) on Pak Choi Yield, Quality and on Soil Biological Properties. *Compost Sci. and Util.*, **19(4):** 279-292.
- Pramanik, K. and A.K. Bera (2012). Response of biofertilizers and phytohormone on growth and yield of chick pea (*Cicer arietinum* L.). J. Crop and Weed, **8(2):** 45-49.
- Rather, S.A., M.H. Hussain and M.L. Sharma (2010). Effect of biofertilizers on growth, yield and economics of field pea (*Pisum sativum* L.), *Int. J. Agri. Sci.*, 6(1): 65-66.
- Remans, R., A. Croonenborghs, R.S. Gutierrez, J. Michiels and J. Vanderleyden (2007). Effects of plant growth-promoting rhizobacteria on nodulation of *Phaseolus vulgaris* L. are dependent on plant P nutrition. *Eur. J. Plant Path.*, **119**: 341-351.
- Saharan, B.S. and V. Nehra (2011). Plant Growth Promoting Rhizobacteria: A Critical Review. *Life Sci. Medic. Res.*, 21.
- Snedecor, G.W. and W.G. Cochran (1980). Statistical methods. Sixth Edition, Iowa state university press, Ames., Iowa, U.S.A.
- Solaiman, A.R.M. and Z. Rabbani (2006). Effects of *Rhizobium* inoculant, compost and nitrogen on nodulation, growth and yield of pea. *Korean J. Crop Sci.*, **51(6):** 534-538.
- Souza, R., A. Ambrosini and L.M.P. Passaglia (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.*, 38: 401-419.
- Suthar, S. (2010). Evidence of plant hormone like substances in vermiwash: an ecologically safe option of synthetic chemicals for sustainable farming. *Ecol. Eng.*, **36(8)**: 1089-1092.
- Tagore, G.S., S.L. Namdeo, S.K. Sharma and N. Kumar (2013). Effect of *Rhizobium* and phosphate solubilizing bacterial inoculants on symbiotic traits, nodule legheamoglobin and yield of chickpea genotypes. *Inter. J. Agro.*, 58: 16-27.
- Tilak, K.V.B.R., N. Ranganayaki and C. Manoharachari (2006). Synergistic effects of plant-growth promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by pigeon pea. *Eur. J. Soil Sci.*, **57(1):** 67-71.
- Vessey, J.M. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, **255**: 571-586.
- Zaghloul, R.A., H.E. Abou-Aly, R.M. El-Meihy and M.T. El-Saadony (2015). Improvement of growth and yield of pea plants using integrated fertilization management. *Universal J. Agri. Res.*, **3(4):** 135-143.