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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(1): 529-544. **OPEN ACCESS**

Physiological Effect of Bio-stimulant (Phosphate solubilizing bacteria) on growth and yield of two flax cultivars grown in sandy soil amended with rock phosphate

Talaat N. El Sebai[1], Bakry Ahmed Bakry[2], Maha Mohamed-Shater Abd Allah[3] and Hala Mohamed Safwat El-Bassiouny[3]

[1]Agriculural and microbiology, Agriculture and Biology Division, National Research Centre, Dokki, Giza, **Egypt**
[2]Agronomy Department, Agriculture and Biology Division, National Research Centre, Dokki, Giza, **Egypt**
[3]Botany Department, Agriculture and Biology Division, National Research Centre, Dokki, Giza, **Egypt**
33 El Bohouth st P.O. 12622
.

***Correspondence:** maha_eg1908@yahoo.fr **Accepted: 0**2 Nov 2018**Published online:** 27 Feb 2018

The availability of phosphorus for plants is severely limited, which reduces growth and yield in most terrestrial ecosystems. A field experiment was conducted at the experimental farm of National Research Centre, Nubaria, El-Behaira Governorate, Egypt, during two successive seasons. The aim of this work was to investigate the effect of mixed culture inoculums of Phosphate solubilizing bacteria (PSB) on growth, some biochemical aspects and yield of two flax cultivars (Letwania-1 and Sakha) grown in sandy soil amended with rock phosphate (RP). So, the effect of RP (120 and 240 Kg/ha) was compared with that of the half recommended and recommended doses of single supper phosphate (SSP 120 and 240 Kg/ha) in absence and presence of PSB inoculation (in soil inoculated and un-inoculated by PSB) to study the possibility of substituting synthetic phosphate fertilizer by natural one (RP). All treatments in the present study (phosphorus sources and their doses with and without PSB inoculation) improved morphological criteria of two flax varieties as well as yield and yield components when compared with the untreated controls. Data also showed that, all treatments increased oil% and oil yield (kg/fed), protein, phenol and flavonoids as compared with controls. A significant increasing in photosynthetic pigments, total soluble sugars, phenol contents and free amino acids were recorded for all treatments compared to untreated control. The highest values of all parameters were observed with the R dose of SSP in the presence or the absence of PSB followed by the high dose of rock phosphate but only in the presence of PSB. Our findings also indicated that mixed PSB soil inoculation led to a marked enhancement in all studied parameters that used during this study especially with high dose of RP. Sakha cultivar surpassed Letwania-1 cultivar in growth, yield quantity and quality. In conclusion, cultivation of flax using rock phosphate as phosphate source in the presence of PSB in sandy soil was effective and could be recommended.

Keywords: Flax, Growth, yield, Phosphate solubilizing bacteria, Chemical fertilizers, Rock phosphate.

INTRODUCTION

Phosphorus (P) is one of the major essential macronutrients for plants next to nitrogen. It plays an important role in plant growth, yield and all its major metabolic processes including cell division, photosynthesis and development of good root system, energy transfer, signal transduction, macromolecular biosynthesis and respiration (Khan et al., 2010). It considered as an essential component of cell structures, mainly as nucleic acids and phospholipids. It is principally in establishment the enzymatic machinery in energy storage and transport which in many cases participate membrane processes (Sinclair and Vadez 2002). Phosphorus is abundant in soils in both inorganic and organic forms; it is a major limiting factor for plant growth as it is in an unavailable form for root uptake. Inorganic P exists in soil, generally in insoluble mineral complexes, several of them appearing after repeated application of chemical fertilizers. These insoluble, precipitated forms cannot be mobilized rapidly and absorbed by plants (Rengel and Marschner 2005).

Chemical fertilizers have been used widely to improved food crop production. Yet the high cost of chemical fertilizers is now focusing interest on rock phosphate (RP) fertilizer like the natural phosphorus (P) source in addition, its relatively low cost (Lukiwati 2002). In order to of their extremely changeable and complex chemical composition, RP is a source of numerous nutrients other than P. Moreover, plants can use only a small amount of this P since 75–90% of added P is precipitated by metal–cation complexes, and rapidly becomes fixed in soils. Rock phosphate is the resource of chemical phosphorus fertilizers however it is weakly soluble in soil solution and one of the solutions for increasing its efficiency is the application of phosphate-solubilizing microorganisms (PSMs). In this regards (PSMs) has become importance as biofertilizers and seem to be as best eco-friendly means for P nutrition of crop.

Phosphate solubilizing microorganisms are very attractive approach for using PRs as fertilizer because they are able to solubilize insoluble P by different mechanisms similar to secret organic acids and/or chelating procedure and/or ion exchange and/or lowering pH during proton production (Reyes et al., 2001).

The positive effect of PSB has been reported on food, fodder and medicinal plants (Gupta et al., 2012). The *Pseudomonas spp*. has been used for plant growth promotion and disease control in rice crop (Saikia et al., 2005). *Pseudomonas aeruginosa* is known to enhance the plant growth and suppress many fungal diseases by induced systemic resistance (Audenaert et al., 2002). Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* increased P uptake enhancing root and shoot elongation in canola, tomato and increased crop yields of potato, radishes, rice, sugar beet, tomato, beans, and wheat (Glick, 1997). The application of these inoculants by different methods can be positive to improve the effectiveness of naturally and unnaturally produced P resources and thus, optimize the chemical fertilizer application for the crop production (Salimpour et al., 2010).

Flax (or linseed) is one of the major industrial oils seed crops grown in temperate climates. The seed oil of this oilseed crop is enriched in α-linolenic acid (ALA). As a result of this, it is a good source for the human diet. Also, flax oil easily polymerizes on exposure to oxygen, making it useful for a variety of industrial products, including varnish and linoleum. Moreover, to industrial applications; meal from compressed, seed is useful as animal feed.

The aim of this study was to evaluate the performance of PSB consortium (mixed culture inoculums of *Acinetobacter sp.* and *Burkhoderia sp*.) for the growth, yield, some biochemical aspects, and nutrient uptake of flax plant in sandy soil under field condition and to investigate the possibility to substitute synthetic phosphate fertilizers by rock phosphate as a nature source of phosphorus.

MATERIALS AND METHODS

A field experiment was conducted in a sandy soil at the experimental Station of National Research Centre, Nubria district El-Behrea Governorate, Egypt, (latitude 30˚30'1.4"N, and longitude 30˚19'10.9"E, and mean altitude 21 m above sea level), during two successive winter seasons of 2015/2016 and 2016/2017. The experimental design was split plot design with four replication, with factors included two levels of bacteria treatments (with and without inoculation) occupy the main plots and super phosphate and rock phosphate concentrations were allocated at random in sub-plots. Flax seeds of two flax cultivars (Letwania-1 and Sakha) were sown on the 25th November in winter season in rows 3.5 meters long, and the distance between rows was 20 cm apart, plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The seeding rate was 1750 seeds/m^2. Nitrogen was applied after emergence in the form of ammonium nitrate 33.5% at rate of 179 Kg/ha in five equal doses. Potassium sulfate (48 % K_2O) was added at two equal doses of 120 kg/ha.

Table 1: Mechanical and chemical analysis of the experimental soil sites.

Mechanical analysis				
Sand		Silt 20-0µ%	Clay < 2µ%	Soil texture
Course 2000-	Fine 200-20µ %			
47.46	36.19	12.86	4.28	Sandy

Chemical analysis											
pH	EC dSm^{-1}	$CaCO_3$	OM %	Soluble Cations (meq/l)				Soluble anions (meq/l)			
				Na^+	K^+	Mg^+	Ca^{++}	CO_3^{--}	HCO	Cl^-	SO_4^{--}
7.60	0.13	5.3	0.06	0.57	0.13	0.92	1.0	0.0	1.25	0.48	0.89

Available nutrients						
Macro-element (ppm)			Micro-element (ppm)			
+N	P	K	Zn	Fe	Mn	Cu
52	12.0	75	0.14	1.4	0.3	0.00

Table (2): Different used treatments during this investigation

Treatments	With Inoculums	Without Inoculums
Without adding phosphate source	+	-
Half Recommended Dose of Single Super Phosphate (SSP) (1/2 R) (120 Kg/ha of SSP 15.5% ≡ 18.6 kg P_2O_5)	+	-
Recommended Dose of Single Super Phosphate (SSP) (240 Kg/ha of SSP 15.5% ≡ 37.2 kg P_2O_5) (R)	+	-
120 Kg/ha of rock phosphate (RP) 30.8% P_2O_5≡36.96 kg P_2O_5	+	-
240 Kg/ha of rock phosphate (RP) 30.8% P_2O_5≡73.92 kg P_2O_5	+	-

The soil of our experiment site is sandy soil where mechanical and physico- chemical analysis is reported in Table 1 according to (Chapman and Pratt, 1978) method. The experiment has ten treatments as summarized in Table 2.

Inoculums preparation of PSB

Two phosphate solubilizing bacteria (PSB) were grown separately on Pikovskaya`s broth medium (PVKB) (Pikovskaya, 1948). It consists of these elements in gml^{-1}: Yeast Extract (0.50), Dextrose (10.0), Ammonium Sulfate (0.50), Magnesium Sulfate (0.10), Potassium Chloride (0.20), Sodium Chloride (0.20), Manganese Sulfate (0.002), Ferrous sulfate (0.002) and supplemented with 0.5% of TCP as a unique source of P. pH was adjusted to 7±0.3 using NaOH 2N. This medium was distributed into 250 ml Erlenmeyer flasks then inoculated separately with a portion of purified *Acintobacter sp* or *Burkholedria sp* that has been previously isolated from Egyptian soil. The inoculated flaks were incubated at laboratory conditions for 5 days in an orbital shaker (150 rpm). An equal volume of each PSB liquid culture was mixed together to make a PSB consortium inoculums. This mixture was used as consortium inoculums. This consortium inoculums were mixed with irrigation water then only the concerning plots was watered with them.

Rock phosphate (RP)

Rock phosphate (RP) was purchased from Abotartour Company, New Valley, Egypt. According to the company, it contained total P, 13.4% (30.8%P_2O_5), water soluble P, 0.037ppm.
Plant samples were taken after 60 days from sowing for measurements of growth characters and some biochemical parameters. Growth parameters in terms of, shoot length (cm), shoot fresh and dry weight (g), roots length (cm), root fresh and dry weight (g). Chemical analysis measured were photosynthetic pigments and total phenol contents. Plant samples were dried in an electric oven with drift fan at 70°C for 48 hr. till constant dry weight for determination of total soluble sugars (TSS), and free amino acids contents. Flax plants were pulled when signs of full maturity were appeared, then left on ground to suitable complete drying. Capsules were removed carefully. At harvest, plant height (cm), fruiting zone length (cm), number of fruiting

branches/plant, number of capsules/plant, seed yield/plant (g), biological yield/plant (g) and 1000 seeds wt (g), were recorded on random samples of ten guarded plants in each plot. Also, seed yield/fed (Kg/Fed), straw yield Kg/fed, and biological yield Kg/fed were studied. Some biochemical contents of the yielded seeds such as oil %, protein %, flavonoids, phenolics and nitrogen, phosphorus and potassium percentage were determined.

Chemical analysis:

Photosynthetic pigments: Total chlorophyll a and b and carotenoids contents in fresh leaves were determined using the method of (Lichtenthaler and Buschmann 2001). Total soluble sugars (TSS), were extracted and analyzed according to Homme et al (1992) and Yemm and Willis (1954). Free amino acid was determined with the ninhydrin reagent method Yemm and Cocking (1955). Phenolic compounds were determined by using Folin Ciocalteu reagent as described by Danil and George (1972). Total N was determined by using micro-Kjeldahl method as described in AOAC (1970). Seed oil content was determined using Soxhlet apparatus and petroleum ether (40-60ºC) according to AOAC (1990). Macroelement contents of the yielded grains were determined according to Chapman and Pratt, (1978). Phosphorus was determined using a Spekol spectrocolorimeter (VEB Carl Zeiss; Jena, Germany, while, estimation of K+ contents were done using a flame photometer. The resultant defatted meal is used for determination of protein, phenolic compound and flavonoids. The protein contents were determined by micro-kjeldahl method according to Badford (1976). Total flavonoid contents were measured by the aluminum chloride colorimetric assay as described by Chang et al., (2002).

Statistical analysis:

The data obtained were statistically analyzed according to (Snedecor and Cochran 1980). The combined analysis of the two growing seasons was carried out according to (Steel, and Torrie, 1960). Means were compared by using Least Significant Difference (LSD) at 5% levels of probability.

RESULTS

Plant Growth:

Effect of flax cultivars:

Different growth parameters (plant height, fresh, dry weight of shoot and root) are presented in Table (3). Data show marked differences between cultivars under study. Sakha cultivar significantly surpassed Letwania-1 in most of the studied growth parameters.

Effect of super phosphate and rock phosphate:

Table (4) represents the effect of different concentrations of single super phosphate (SSP 1/2 R and R dose) and rock phosphate (RP120 or 240 Kg/ha) on morphological criteria of flax cultivars (Letwania-1 and Sakha). Treating flax cultivars with different treatments increased all growth criteria (plant height, fresh and dry weight of shoot and root). The maximum increases in all the growth criteria were obtained by using SSP (R dose) followed by RP (R dose) and SSP (1/2 R).

Effect of interaction between flax cultivars, SSP, RP and phosphate solubilizing bacteria:

Table (5) represents the effect of SSP (1/2 R and R dose) and RP (120 or 240 Kg/ha) on morphological criteria of flax cultivars (Letwania-1 and Sakha) in absence or presence of phosphate solubilizing bacteria (PSB).Treating flax cultivars with different treatments increased all growth criteria in the presence or absence of PSB when compared with corresponding control. The maximum increases in all the growth criteria were obtained by using SSP (R dose) in absence or present of PSB followed by RP (R dose) in the presence of PSB. The soil inoculated with PSB leads to a significant increase in all studied morphological parameter when compared to the corresponding treatments in absence of PSB in both cultivars.

Photosynthetic pigments contents:

Effect of flax cultivars:

Table (6) shows the effect of flax cultivars on photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) . Data clearly show the non significant differences between cultivar (Letwania-1 and Sakha) on photosynthetic pigments.

Effect of super phosphate and rock phosphate:

Table (7) represents the effect of different concentrations of single super phosphate (SSP) (1/2 R and R dose) and rock phosphate (RP) (120 or 240 Kg/ha) on photosynthetic pigments of flax cultivars (Letwania-1 and Sakha). Treating flax cultivars with different treatments increased photosynthetic pigments.

Table (3): Effect of cultivars on morphological criteria of flax cultivars (Letwania-1 and Sakha) (at 60 days from sowing) (combined analysis of two seasons).

Cultivar	Plant height (Cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
Letwania-1	71.18	13.501	2.91	1.67	0.33
Sakha	80.66	14.519	3.17	2.12	0.38
LSD at 5%	0.30	0.95	0.82	0.82	0.05

Table (4): Effect of single super phosphate (SSP) (½ R & R) or rock phosphate (RP 120 & 240 Kg/ha) on morphological criteria of flax cultivars (at 60 days from sowing) (combined analysis of two seasons).

Treatment	Plant height (Cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
Control	66.02	6.99	1.62	1.08	0.21
RP (120 Kg ha^{-1})	71.15	13.78	2.38	0.93	0.25
RP (240 Kg ha^{-1})	77.73	15.51	3.50	1.83	0.34
SSP (½ R)	78.61	15.03	3.40	1.75	0.26
SSP (R)	86.10	18.75	4.30	3.88	0.46
LSD at 5%	1.08	0.80	0.20	0.20	0.02

Table (5): Effect of single super phosphate (SSP) (½ R & R) dose or rock phosphate (RP 120 & 240 Kg/ha) on morphological criteria of flax cultivars (Letwania-1 and Sakha) (at 60 days from sowing) in absence (-) or presence (+) of phosphate solubilizing bacteria (combined analysis of two seasons).

Cultivar	Treatment	plant height (Cm)		Fresh weight of shoot (g)		Dry weight of shoot (g)		Fresh weight of root (g)		Dry weight of root (g)	
		-	.+	-	.+	-	.+	-	.+	-	.+
Letwania-1	Control	61.51	63.51	5.79	7.39	1.43	1.62	0.92	0.86	0.24	0.33
	RP (120 Kg ha^{-1})	62.90	71.90	11.93	14.16	1.84	2.67	0.64	1.21	0.22	0.36
	RP (240 Kg ha^{-1})	65.56	77.90	12.59	17.63	2.99	3.77	0.86	2.49	0.23	0.41
	SSP (½ R)	75.90	76.43	14.03	15.16	3.22	3.25	1.39	1.43	0.29	0.34
	SSP (R)	77.93	78.26	17.66	18.67	4.08	4.19	3.25	3.59	0.38	0.46
Sakha	Control	69.18	69.90	6.77	7.99	1.66	1.74	1.04	1.48	0.10	0.14
	RP (120 Kg ha^{-1})	71.90	77.90	13.39	15.64	2.07	2.93	0.97	1.10	0.18	0.24
	RP (240 Kg ha^{-1})	75.56	91.90	13.73	18.09	3.24	4.01	1.18	2.78	0.24	0.47
	SSP (½ R)	80.56	81.55	15.69	15.69	3.43	3.71	2.02	2.14	0.12	0.28
	SSP (R)	90.93	97.26	19.34	19.32	4.2	4.68	3.79	4.91	0.44	0.56
LSD at 5%		5.1		0.77		0.11		0.22		0.02	

Table (6): Effect of cultivars on photosynthetic pigments (mg/g fresh weight), total soluble sugars (TSS) free amino acids (FAA) and phenol contents, as (mg/ 100g dry weight) of flax cultivars (Letwania-1 and Sakha) (at 60 days from sowing) (combined analysis of two seasons).

Cultivar	Chlo a	Chlo b	Carotenoids	Total pigments	TSS	FAA	Phenols
Letwania-1	1.53	0.64	1.96	4.12	441.50	339.20	64.04
Sakha	1.60	0.65	1.96	4.20	622.40	374.63	64.90
LSD at 5%	0.25	0.18	0.78	0.41	5.28	2.00	9.90

The maximum increases in photosynthetic pigments were obtained by using SSP (R dose) followed by SSP (1/2 R) and RP (R dose).

Effect of interaction between flax cultivars, SSP, RP and phosphate solubilizing bacteria:

Data in Table (8) reveal that, significant increase in chlorophyll a, chlorophyll b, carotenoids, and consequently total pigments contents in response to treatment with either rock phosphate or single supper phosphate in both cultivars, in the presence or absence of PSB as compared with control plants. The results showed that there was no significant increase in photosynthetic pigments of both flax cultivars plants between soil inoculated and uninoculated by PSB in all treatments. Plants treated with recommended dose (R) of SSP and amended with PSB had the highest total chlorophyll content by 73% and 80% as compared with the corresponding controls of flax cultivars (Letwania-1 and Sakha) respectively. The soil inoculated with (PSB) leads to a significant increase in photosynthetic pigments when compared to the corresponding treatments in absence of PSB in both cultivars.

Phenol contents, total soluble sugars and free amino acids.

Effect of flax cultivars:

Table (6) shows the effect of flax cultivars on total soluble sugars, free amino acids and phenol contents. Sakha cultivar significantly surpassed Letwania-1 in TSS and FAA contents by 40 and 10% respectively and non significant different in phenol contents.

Effect of super phosphate and rock phosphate:

Table (7) represents the effect of SSP (1/2 R and R dose) and RP (120 or 240 Kg/ha) on total soluble sugars, free amino acids and phenol contents of flax cultivars. Treating flax cultivars with different treatments increased total soluble sugars, free amino acids and phenol contents. The maximum increases in these parameters were obtained by using SSP (R dose) followed by SSP (1/2 R) and RP (R dose).

Effect of interaction between flax cultivars, SSP, RP and phosphate solubilizing bacteria:

Data recorded in the present study (Table 8) demonstrated that, application of either rock phosphate or single supper phosphate in both flax cultivars (Letwania-1 and Sakha) induced an additive stimulatory effect on the accumulation of total soluble sugars, free amino acids and phenol contents in presence and absence of PSB as compared with those of the corresponding controls. Application of single super phosphate at (R) in the soil inoculation with PSB is the most effective treatment in presence of PSB as compared of the corresponding controls of flax cultivars (Letwania-1 and Sakha).

Yield and yield components*:*

Effect of flax cultivars:

Different yield and yield components (plant height, number of branches, flowering zone length, seeds weight /plant, 1000 seed wt (g), straw yield (ton/fed) and seed yield (kg/fed). are presented in Table (9). Data show marked differences between cultivars under study. Sakha cultivar significantly surpassed Letwania-1 in most of the studied yield and yield components.

Effect of super phosphate and rock phosphate:

Table (10) represents the effect of different concentrations of single super phosphate (SSP 1/2 R and R dose) and rock phosphate (RP120 or 240 Kg/ha) on on yield and yield components of flax cultivars. Treating flax cultivars with different treatments increased all on yield and yield components. The maximum increases in on yield and yield components were obtained by using SSP (R dose) followed by RP (R dose) and SSP (1/2 R).

Table (7): Effect of single super phosphate (SSP) (½ R & R) or rock phosphate (RP 120 & 240 Kg/ha) on photosynthetic pigments (mg/g fresh weight), total soluble sugars (TSS) free amino acids (FAA) and phenol contents, as (mg/ 100g dry weight) of flax cultivars (Letwania-1 and Sakha) (at 60 days from sowing) (combined analysis of two seasons).

Treatments	Chlor a	Chlor b	Carotenoids	Total Pigments	TSS	FAA	Phenols
Control	1.01	0.53	1.61	3.15	325.00	207.25	35.86
RP (120 Kg ha^{-1})	1.43	0.57	1.86	3.88	426.50	275.50	53.46
RP (240 Kg ha^{-1})	1.55	0.61	1.90	4.05	598.75	394.75	73.84
SSP (½ R)	1.73	0.73	2.15	4.56	600.00	403.33	69.87
SSP (R)	2.11	0.79	2.29	5.17	709.50	503.75	89.30
LSD at 5%	0.05	0.13	0.27	0.28	10.30	9.89	8.67

Table (8): Effect of single super phosphate (SSP) (½ R & R) dose or rock phosphate (RP 120 & 240 Kg/ha) on photosynthetic pigments (mg/g fresh weight, total soluble sugars (TSS) free amino acids (FAA) and phenol contents, as (mg/ 100g dry weight) of flax cultivars (Letwania-1 and Sakha) in absence or presence (+) of phosphate solubilizing bacteria (at 60 days from sowing) (combined analysis of two seasons)

Cult	Treatment	Chlorophylla		Chlorophyllb		Carotenoids		Total Pigments		TSS		FAA		Phenols	
		-	+	-	+	-	+	-	+	-	+	-	+	-	+
Letwania-1	Control	0.97	1.07	0.50	0.57	1.58	1.66	3.04	3.29	289	299	187	219	31.15	37.72
	RP (120 $Kgha^{-1}$)	1.28	1.46	0.57	0.55	1.79	1.90	3.69	3.91	333	386	225	284	47.99	64.27
	RP (240 $Kgha^{-1}$)	1.39	1.61	0.60	0.58	1.81	2.02	3.80	4.22	405	548	301	433	65.17	84.47
	SSP (½ R)	1.67	1.72	0.71	0.74	2.07	2.19	4.40	4.60	423	492	320	414	65.07	70.47
	SSP (R)	2.02	2.14	0.78	0.81	2.25	2.37	5.00	5.27	600	636	501	504	84.97	89.12
Sakha	Control	0.89	1.10	0.52	0.52	1.55	1.67	2.94	3.34	333	377	189	232	34.21	40.38
	RP (120 $Kgha^{-1}$)	1.42	1.56	0.60	0.55	1.84	1.92	3.87	4.05	478	509	278	315	43.72	57.87
	RP (240 $Kgha^{-1}$)	1.49	1.70	0.64	0.61	1.77	2.01	3.90	4.30	675	765	403	440	66.38	79.37
	SSP (½ R)	1.74	1.81	0.72	0.77	2.14	2.20	4.53	4.72	713	770	434	443	70.80	73.17
	SSP (R)	2.08	2.19	0.76	0.83	2.26	2.30	5.10	5.30	789	811	498	510	87.94	95.17
LSD at 5%		0.15		0.07		0.23		0.28		32.39		32.61		10.75	

Table (9): Effect of cultivars on yield on yield and yield components of flax cultivars (Letwania-1 and Sakha) (combined analysis Of two seasons).

Cultivar	Plant height (Cm)	No of branches / plant	Flowering zone length (Cm)	Seed wt /plant (g)	1000 seed wt (g)	Straw yield (ton/fed)	Seed yield (kg/fed)
Letwania-1	79.55	2.90	25.88	4.09	3.17	2.22	552.60
Sakha	84.57	3.39	28.71	4.40	3.64	2.71	581.60
LSD at 5%	4.08	0.37	0.51	0.50	0.31	1.08	24.66

Table (10): Effect of single super phosphate (SSP) (½ R & R) or rock phosphate (RP 120 & 240 Kg/ha) on yield and yield compounds (combined analysis of two seasons).

Treatments	Plant height (Cm)	No of branches	Flowering zone length (Cm)	Seed weight/ plant (g)	1000 seed weight (g)	Straw yield (ton /fed)	Seed yield (kg/fed)
Control	68.33	2.48	23.74	2.92	2.48	1.21	314.25
RP (120 Kg ha^{-1})	80.85	2.88	26.44	3.55	2.76	1.99	475.00
RP (240 Kg ha^{-1})	83.16	3.12	27.58	4.32	3.93	2.91	644.00
SSP (½ R)	84.61	3.48	28.31	4.54	3.83	2.89	656.00
SSP (R)	93.36	3.77	30.43	5.89	4.51	3.34	771.25
LSD at 5%	8.35	0.40	0.40	0.06	0.48	0.02	9.94

Table (11): Effect of single super phosphate (SSP) (½ R & R) or rock phosphate (RP 120 & 240 Kg/ha) on yield of flax cultivars (Letwania-1 and Sakha) in absence (-) or presence (+) of phosphate solubilizing bacteria.

Cult	Treatment	plant height (Cm)		No of Branches		flowering zone length (Cm)		Seed wt/plant (g)		1000 seed wt (g)		Straw yield (ton/fed)		Seed yield (kg/fed)	
		-	.+	-	.+	-	.+	-	.+	-	.+	-	.+	-	+
Letwania-1	Control	65.08	70.08	2.0	2.3	24.1	25.8	2.19	3.28	2.20	2.33	1.06	1.11	278	315
	RP (120 Kgha^{-1})	82.65	81.31	2.3	2. 8	21.3	27.9	2.82	3.89	2.59	2.76	1.81	1.90	466	496
	RP (240 Kgha^{-1})	80.31	83.00	2.7	3.0	25.1	27.1	3.65	4.27	3.52	4.13	2.21	2.80	592	676
	SSP (½ R)	82.41	83.09	3.0	3. 6	26.1	26.5	4.59	4.62	3.66	3.76	2.52	2.66	603	659
	SSP (R)	84.08	85.38	3.4	3. 9	27.4	27.6	5.72	5.84	4.30	4.45	3.06	3.10	769	775
Sakha	Control	66.08	72.08	2.6	3.0	20.8	24.4	2.53	3.70	2.60	2.81	1.24	1.43	320	344
	RP (120 Kg ha^{-1})	77.38	82.08	3.0	3.4	27.4	29.1	3.45	4.03	2.85	2.86	2.09	2.17	435	503
	RP (240 Kg ha^{-1})	79.08	92.08	3.3	3.5	28.1	30.1	4.45	4.90	3.75	4.33	2.94	3.71	615	695
	SSP (½ R)	86.85	86.09	3.5	3.8	28.4	32.3	4.23	4.71	3.88	4.03	3.09	3.29	667	697
	SSP (R)	101.02	103.0	3.7	4.0	30.6	36.2	5.90	6.08	4.34	4.98	3.24	3.97	752	790
LSD at 5%		8.75		0.23		2.17		0.12		0.13		0.11		9.91	

Table (12): Effect of cultivars on yield on on nutritional value on the yielded seeds of flax cultivars (Letwania-1 and Sakha) (combined analysis of two seasons).

Cultivar	Oil%	Protein %	Flavonoids	Phenol	N%	P%	K%
			mg /100g dry wt				
Letwania-1	22.10	18.56	21.94	211	2.08	0.18	0.58
Sakha	21.90	21.51	24.12	308	2.38	0.17	0.60
LSD at 5%	0.95	1.02	1.09	30.55	0.77	0.02	0.02

Table (13): Effect of single super phosphate (SSP) (½ R & R) or rock phosphate (RP 120 & 240 Kg/ha) on nutritional value on the yielded seeds (combined analysis of two seasons).

Treatments	Oil%	Protein %	Flavonoids	Phenol	N%	P%	K%
			mg /100g dry wt				
Control	19.11	17.45	18.34	220	1.31	0.12	0.49
RP (120 Kg ha^{-1})	21.85	20.11	21.78	244	1.85	0.14	0.62
RP (240 Kg ha^{-1})	22.40	20.09	24.69	269	2.67	0.22	0.64
SSP (½ R)	21.93	19.87	23.90	265	2.62	0.17	0.63
SSP (R)	24.72	22.66	26.44	299	2.70	0.22	0. 65
LSD at 5%	0.38	0.38	0.06	3.40	0.00	0.01	0.01

Table (14): Effect of single super phosphate (SSP) (½ R & R) or rock phosphate (RP 120 & 240 Kg/ha) on nutritional value of flax cultivars (Letwania-1 and Sakha) in absence (-) or presence (+) of phosphate solubilizing bacteria.

Cult	Treatment	Oil%		Protein %		Flavonoids		Phenol		N%		P%		K%	
						mg /100g dry wt									
		-	.+	-	.+	-	+	-	+	-	+	-	+	-	+
Letwania-1	**Control**	19.45	19.97	15.06	16.51	16.75	17.73	177	189	1.07	1.15	0.13	0.15	0.43	0.48
	RP (120 Kg ha^{-1})	22.76	23.16	18.31	20.30	18.29	21.19	182	206	1.63	2.08	0.13	0.16	0.67	0.68
	RP (240 Kg ha^{-1})	22.15	22.72	18.31	19.94	22.18	25.83	192	241	2.07	2.71	0.21	0.24	0.58	0.74
	SSP (½ R)	22.03	22.06	17.41	19.16	21.80	25.27	208	224	2.32	2.69	0.16	0.20	0.48	0.72
	SSP (R)	22.78	23.93	20.22	20.43	22.63	27.69	230	262	2.38	2.73	0.19	0.22	0.48	0.52
Sakha	**Control**	17.93	19.09	18.49	19.75	17.98	20.88	236	281	1.39	1.64	0.10	0.11	0.50	0.54
	RP (120 Kg ha^{-1})	20.36	21.12	20.34	21.49	23.69	23.96	232	355	1.61	2.09	0.11	0.17	0.54	0.68
	RP (240 Kg ha^{-1})	21.58	23.15	19.22	22.89	24.81	25.93	294	349	2.73	3.18	0.20	0.22	0.56	0.62
	SSP (½ R)	21.76	21.88	21.23	21.71	23.43	25.10	299	327	2.62	2.85	0.14	0.18	0.58	0.66
	SSP (R)	24.88	27.29	23.07	26.95	26.12	29.31	319	387	2.39	3.28	0.21	0.24	0.58	0.68
LSD at 5%		0.881		0.73		0.165		6.03		0.117		0.012		0.012	

Effect of interaction between flax cultivars, SSP, RP and phosphate solubilizing bacteria:

Results in Table (11) illustrate the behavior of flax cultivars grown under applications of SSP or RP in absence and presence of PSB on yield and yield components. Different treatments with or without PSB soil inoculation increased yield and yield components as compared with the corresponding controls. The maximum increases in the yield and yield components were obtained by using single super phosphate (R dose) regardless the absence or the presence of PSB followed by RP at 240 Kg/ha in the presence of PSB in both cultivars . However, in the soil inoculation with PSB rock phosphate at (R dose) led to an increase in all yield and yield components in both cultivars as compared to the un- inoculation. As the percentage of increases in response to (R) single super phosphate reached to 31 % and 56 % as plant height, 95 % and 54 % as number of branches, 15 % and 74 % as flowering zone length 167 % and 140 as seeds weight /plant, 102 % and 88 % as 1000 seed wt (g), 192 and 220% as straw yield/ fed., 179 and 174 as seed yield/fed. as compared to the untreated plants in both flax cultivars (Letwania-1 and Sakha) respectively.

Nutritional value of the yielded seeds:

Effect of flax cultivars:

The nutritional value (oil, protein, flavonoids, phenols, N, P, and K) of the yielded seeds of both flax cultivars (Letwania-1 and Sakha) are presented in Table (12). Data show marked differences between cultivars under study. Sakha cultivar significantly surpassed Letwania-1 in most of the studied nutritional value.

Effect of super phosphate and rock phosphate:

Table (13) represents the effect of different concentrations of single super phosphate (1/2 R and R dose) and rock phosphate (RP120 or 240 Kg/ha) on nutritional value of flax cultivars. Treating flax cultivars with different treatments increased all on nutritional value. The maximum increases were obtained by using SSP (R dose) followed by RP (R dose) and SSP (1/2 R).

Effect of interaction between flax cultivars, SSP, RP and phosphate solubilizing bacteria:

Data in (Table 14) show that application of RP and SSP led to significant increases in the nutritional value of the yielded seeds of both flax cultivars (Letwania-1 and Sakha) as compared to control. Meanwhile, the maximum increases in oil (23 & 52%), protein (36 & 46 %), flavonoids (65% & 63%), phenol (48 & 64 %), N (155 & 136 %), P (69 & 140 %) and K (19 & 34%) of the yielded flax seeds of both flax cultivars (Letwania-1 and Sakha) respectively were obtained when single super phosphate was applied at (R) in presence of PSB. Rock phosphate at high dose in the presence of PSB resulted in an increase in all studied nutritional values. The soil inoculated with PSB led to an increase in all studied nutritional components when compared to the corresponding treatments in absence of bacteria in both cultivars.

DISCUSSION

Plant Growth:

Application of rock phosphate and single super phosphate led to significant increases in the growth criteria (plant height, fresh and dry weight of shoot and root) of both flax cultivars (Letwania-1 and Sakha) (Table 4&5). The soil inoculated with (PSB) leads to a significant increase in all studied morphological parameter when compared to the corresponding treatments in absence of PSB in both cultivars Table (5). The obtained results are in harmony with those of Akram et al .,(2017), in which they observed that, application of a high dose of phosphorus, promotes plant height of chili crop as compared to low rate of phosphorus. Many of the calcium phosphates, including rock phosphate are insoluble in soil with respect to the release of inorganic P (Pi) at rates necessary to support agronomic levels of plant growth (Goldstein, 2000). However, application of RP and inoculation with PSB produced organic acids helps to dissolve the rock phosphate and increase the availability of phosphorus. Their solubility increases with a decrease of soil pH. Phosphate solubilization is the result of combined effect of pH decrease and organic acids production (Fankem et al., 2006).The soil inoculation with PSB significantly enhanced shoot and root growth of both flax cultivars. That may be due to not only P but also some other nutrient elements in plant tissues were increased in PSB treated flax seedlings indicating better nutrient uptake by the plants due to bacterial inoculation. Islam & Hossain, (2012) showed that phosphate solubilizing rhizobacteria improve the growth and yield of several economically essential crops**.** P solubilization, by the PSB inoculants involved in nitrogen fixation and excretion of phytohormones such as (IAA) for encouragement of growth and nutrient uptake of wheat seedlings (Panhwar et al 2013).

Photosynthetic pigments contents:

The different treatments of phosphorus in the present and absent of PSB effectively increased the photosynthetic pigments in both cultivars Table (8). In this respect, Dutt et al., (2013) found that adding phosphorus to the soil lead to an increase in the rate of photosynthesis in apricot seedlings. Phosphorus plays a vital role in photosynthetic process may be due to increase significantly plant CO_2 assimilation capacity (Qiu and Israel, 1994). Stimulation of photosynthesis by P has often been explained by stimulating the Calvin cycle activity, in particular, by increasing the amount and activity of Rubisco (Lauer et al., 1989) and the regeneration of ribulose-1,5-bisphosphate (RuBP) (Pieters et al., 2001).

Photosynthesis is the most important photochemical sink for energy absorbed by leaves, and therefore the photosynthetic apparatus is susceptible to be exposed to harmful excess light energy in plants evoked by P insufficiency. Li et al., (2004) demonstrated that, P induces possible photo stimulation to PSII. P deficiency might boundary photosynthesis through affecting translocation of carbon from the chloroplasts (Sivak and Walker1986).

Singh et al., (2018) observed that, application of nitrogen and phosphorus (15 and 30 Kg/ha) in lentil plant and inoculated with the plant growth promoting phosphate solubilizing *Chryseobacterium* sp. enhancing photosynthesis This can be correlated with enhanced plant growth and yield due to better photosynthesis capability of the plants.

Phenol contents, total soluble sugars and free amino acids.

Total soluble sugar, free amino acids and phenol contents of flax cultivars increased by phosphorus applications in the presence and absence of PSB. Plant utilizes light energy in the presence of chlorophyll to combine carbon dioxide and water into simple sugars, with the energy source for ATP, which is then available as an energy source for many other reactions that occur within the plant, while the sugars are used as building blocks to produce other cell structural and storage components (Epinosa et al., 1999). Adebooye and Oloyede (2007) noticed that P had a stimulating effect on the TSS content of *Trichosanthes cucumerina* L.

Nitrogen and phosphorous nutrients played a significant role in regulating the concentrations/contents and components of amino acids in roots and grains, and accordingly, affect the grain yield and nutrient quality of rice (Changer et al., 2008).

The increase in phenolic compounds by increasing the levels of nitrogen and phosphorus fertilizer may belong to the role of both N and P elements in activation of phenolic compounds biosynthesis path ways such as shikimic acid and Acetyl CoA, (Hayfau, 2014).

Yield and yield components*:*

The increase in the seeds yield could be the reflection of the effect of phosphorus on growth and development. It might be due to (a) marked increases in growth criteria of plant (Table 4&5) which gave a chance to the plant to carry more flowers, pods and hence more seeds. And (b) marked increases in the photosynthetic pigments content (Table 8), which could lead to an increase in photosynthesis, resulting in greater transfer of photo-assimilates to the seeds and leading to an increases in the weight of grains as well due to transfer of more assimilates into the grains (Sadiq et al., 2017). As it well known, that the phosphorus element is a necessary nutrient which leads to the storage and transfer of chemical energy in plant, acceleration of growth, increase of flowering and early the maturity (Hashemi and Mojaddam 2015). Parhizkar et al., (2012) reported that the highest grain yield of flax has been achieved with 120 kg/ha phosphorus. Hashemi and Mojaddam(2015) showed that triple superphosphate and biological phosphate fertilizer significantly increased the yield, and yield component of sesame. The PSB solubilize the fixed soil P and applied phosphates resulting in higher crop yields (Gull et al., 2004). The PSB in conjunction with single super phosphate and rock phosphate reduce the P dose by 25 and 50 %, respectively (Sundara et al., 2002).

Nutritional value of the yielded seeds:

Application of rock phosphate and single super phosphate led to significant increases in the nutritional value of the yielded seeds of both flax cultivars (Letwania-1 and Sakha) (Table 14). Awad Alla et al., (2013) observed that, the enhancement of essential oil production of coriander fruits by the addition of phosphorus fertilizer. They attributed this enhancement to the development of plant nucleoproteins, transfer of metabolite compounds and the efficiency of its root system leading to more absorbing of water and nutrients, which led to increasing the rate of physiological processes and finally gave better yields.Abou-Zied (2011) found that, the interaction between P fertilizer rates at 150 kg Egyptian rock phosphate (ERP)/fed combined with PDB (*Bacillus megaterium*) led to significant increase in oil production, oil percentage, oil yield/plant and oil yield/fed. of soybean. These increments in oil yield can be explained by the important role of phosphorus as an essential constituent of phospholipids (Devlin, 1975) also, their positive effects may be due to their effects on the enzymes activity and metabolism of essential oil production. These are in accordance with those obtained by Abd-El Kader and Ghaly (2003) on coriander plant. In general, protein concentrations were enhanced with increased P application rates Yin et al., (2016) on soybean. AwadAlla et al., (2013) found that the increase was parallel to the gradual increase in the rate of Egyptian rock phosphate from 0 to 150 kg/fed. The interaction between ERP and PDB gave the highest fruit yield, oil percentage, and oil yield.

Shaheen, et al., (2012) showed that, the protein, of onion bulbs tissue significantly response by the application of phosphorus fertilizer as chemical individual fertilizer and/or as mixed with natural rock phosphate or with bio-fertilizer (phosphorein). Ahmed and El-Abagy (2007) who illustrated clearly that supplied faba bean plants with P-fertilization increased the mean values of protein %.

Flavonoids have high antioxidant activity which plays a number of important roles in stress protection of plants (Hernandez, et al., 2004).

Phosphorus treatments resulted in increases in nitrogen, phosphorus and potassium percentage in the absence and presence of PSB as compared with the corresponding controls (Table 14). Khalid (2012) and Rotaru et al., (2015) found that, P increased the N%, P% and K % of Anise, Coriander, sweet fennel plants. Also, Vyas & Gulati, 2009 found that in wheat increased P uptake also when PSB were applied with rock phosphates. In addition, PSB treatments might increase the organic acids excreted from the roots into the soil and consequently increase the solubility of most nutrients which release slowly into the rhizosphere zone where it may be utilized by the. plants (Fankem et al., 2006). Similar to our work, other studies have also shown that PGPR increased the nitrogen content in the legume plant, ending up with improved plant growth (Garcia et al., 2004 and Rotaru et al., 2015). Significant responses of seed P concentrations to P applications were observed Yin et al., (2016) on soybean**.** Ahmed and El-Abagy (2007) who illustrated clearly that supplied faba bean plants with P-fertilization increased the mean values of N %, P % and K %.

CONCLUSION

As a conclusion: We can also concluded that, application of phosphate solubilizing bacteria, with rock phosphate, play an important role in the availability of phosphorus to plant and in consequences increased plant activities as wells as seed quality and quantity of studied flax cultivars. The results suggested that application of phosphate solubilizing bacteria, with rock phosphate as fertilizer could be saving the utilization of super phosphate and reducing the high cost of chemical fertilizer and also pollution. PSB can be used as an eco-friendly approach to increase production and health, and develop sustainable agriculture. Also it emphasized the prospects and potentials of using bacteria, with rock phosphate biofertilizer as renewable natural phosphorus resources for plant and it's also none polluting, inexpensive and utilize renewable resources.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

ACKNOWLEGEMENT

This work was funded by the National Research Centre through the project entitled “Maximizing the productivity and quality traits of some oil crops (flax, peanut and sunflower) grown under newly reclaimed lands”. The principal investigator Associate professor Bakry A. Bakry.

AUTHOR CONTRIBUTIONS

BAB designed, farming plants and statistical analysis, TNE, MMSA and HMSE designed and performed the experiments, responsible for all the physiological and biochemical analysis. All authors wrote the manuscript, reviewed, read and approved the final version.

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