دراسة تأثير البكتريا المحللة للفوسفات في إنتاجية محصول العدس وبعض خواص التربة في محافظة درعا / سورية

Study the Effect of Phosphate-Solubilizing Bcteria on Lentil Yield and Some Soil Properties in Deraa Governorate/Syria

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الملخّص

دُرست فعالية البكتيريا المحللة للفوسفات في إنتاجية العدس وبعض خصائص التربة، وذلك ضمن تجربة حقلية في محطة بحوث ازرع (مركز بحوث درعا ،الهيئة العامة للبحوث العلمية الزراعية السورية) خلال موسمي 2010 و 2011. تضمنت التجربة 8 معاملات (شاهد، 50 % من التوصية السمادية، و 75 % من التوصية السمادية، وكامل التوصية السمادية، وملقح البكتريا المحللة للفوسفات، و 50 % من التوصية السمادية + بكتريا ، و 75 % من التوصية السمادية + بكتريا ، و 100 % من التوصية السمادية + بكتريا) بثلاثة مكررات.

حُللت التربة قبل الزراعة وبعد الحصاد، وتمت دراسة بعض مؤشرات نمو وإنتاجية محصول العدس. لوحظت زيادة في تركيز الآزوت الكلي والفوسفور المتاح في التربة، وكذلك في محتوى الأوراق من الآزوت والفوسفور عند التلقيح بالبكتريا في جميع المعاملات مقارنةً بالمعاملات غير الملقحة وكذلك بالشاهد، وذلك في كلا الموسمين. وقد أثرت البكتريا معنوياً في عدد الأيام اللازمة للإنبات والإزهار والنضج، ونسبة الإنبات وارتفاع النبات. كما ازدادت إنتاجية العدس معنوياً في المعاملات المخصبة بالبكتريا المحللة للفوسفات مقارنةً بالشاهد وذلك في الموسمين. وبشكل عام كانت المعاملة المسمدة بـ 75 % من التوصية السمادية مع البكتريا أفضل معنوياً (الإنتاج البيولوجي 4.250 طن/هكتار في الموسم الأول و 6.633 طن/هكتار في الموسم الثاني) من المعاملة المسمدة بكامل التوصية (4.038 و 6.383 طن/هكتار في الموسم الأول والثاني على التوالي).

الكلمات المفتاحية : البكتريا المحللة للفوسفات، العدس، الفوسفور، الرايزوبيوم.

Abstract

A field experiment was conducted in order to study the effects of phosphate- solubilizing bacteria on lentil yield and some soil properties. The experiment included eight treatments (control, 50% mineral fertilizers as per the recommendations of Ministry of Agriculture and Agrarian Reform (MAAR), 75% mineral fertilizers, 100% mineral fertilizers, phosphate-solubilizing bacteria, 50% mineral fertilizers + bacteria, 75% mineral fertilizers + bacteria, 100% mineral fertilizers + bacteria)] with three replications at Izraa Research station in Deraa in the growing seasons 2010 and 2011.

The soil was analyzed pefor-cultivation and upan harvesting. N and P were estimated in both soil and plant. Also, some plant parameters were identified in addition to the lentil yield.

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Soil total N, available P, leaves N content and leaves P content were increased by inoculation with PSB in all treatments compared to those non-inoculated in the two seasons. PSB significantly affected no. of days to germination, no. of days to flowering, no. of days to maturity, percent germination and plant height.
In addition, lentil yield significantly increased in treatments amended with PSB in the two seasons compared to the control. Moreover, treatment amended with 75% of chemical fertilizers and PSB (Biological yield was 4.25 ton/ha at season1 and 6.633 ton/ha at season2) was significantly better than that amended with full dose of chemical fertilizers (4.038, 6.383 ton/ha at the two season respectively).
Keywords: Phosphate-solubilizing bacteria , Lentil, Phosphorus, *Rizobium*.

Introduction

The efficiency of phosphate fertilizers is so low especially in arid and semi arid regain (5-35%); however, one part of P is fixed in non-dissolved inorganic forms, other is fixed in organic forms and the rest is dissolved in soil (Hagin and Tucker, 1982; Wild, 1988). Phosphorus can be found in soil as $CaHPO_4$, $Ca(H_2PO_4)_2$ and unavailable forms such as apatite and hydroxyapatite. Mineral phosphate can be also found associated with the surface of hydrated oxides of Fe, Al and Mn, which are poorly soluble (Rodriguez and Fraga, 1999). Bio-fertilizers are useful for reducing the pollution rate of soil and water (El-Assiouty and Abo Sedera, 2005). Many rhizobacteria are able to solublilize sparingly soluble phosphates, usually by releasing chelating organic acids (Vessey et *al*., 2004). These microorganisms secrete different types of organic acids e.g., carboxylic acid (Deubel and Merbach, 2005), thus lowering the pH in the rhizosphere (He and Zhu, 1988) and consequently dissociate the bound forms of phosphate like $Ca_3 (PO_4)_2$ in calcareous soils.
Phosphate transformation is influenced by soil microorganisms and thus influence the subsequent availability of phosphate to plant roots (Richardson, 2001).These microbes also play a significant role as plant growth-promoting rhizobacteria (PGPR) in the bio-fertilization of crops (Aftab and Bano, 2008). Young et *al*., (2003) reported P-solubilizing bacteria not only improved the growth and quality of crops but also drastically reduced (1/3-1/2) the usage of chemical or organic fertilizers. Lentil is able to fix atmospheric nitrogen and enrich soil with nitrogen when included in different crop rotations by farmer (Shah et *al*., 2000).This crop is valued as a high protein source and for its residues (Hoque et *al*.,2002; Mishra et *al*., 2001).
In 2010, lentil was grown over an area of 131 thousand hectares in Syria, so the aim of this paper is to study the effect of phosphate-solubilizing bacteria on lentil yield and certain soil chemical properties under Syrian soil conditions.

Material and Methods

1. Soil description

The top 30cm-layer of soil was collected from GCSAR's Izraa station (Derra/Syria). Soil samples were air dried and ground to pass a 2 mm sieve before their analyses. Composite sample was taken to specify the following nutrient characteristics of soil: Total N was estimated by Kjeldahl's method (Richards,1962), Organic matter was determined by potassium dichromate ($K_2Cr_2O_7$) method (Ryan et *al*., 2001), Available P by spectrophotometer (Olsen and Sommers, 1982), Available K by flame photometer (Richards, 1954).

2. PSB preparation

The local isolate *Bacillus megaterium* (P.S.B 43 Source: Bio-fertilization Section, GCSAR) was grown on Pikovskaya culture for 3 days at 28±2C˚. The produced culture (inoculate) contained 10^9 cells /1 ml.

3. Experimental design

A complete randomized-block design (CRBD) was used, with three replicates. The experiment contained eight treatments namely:
Control (N0P0)
50% mineral fertilizers as per MAAR's recommendations (N_1P_1).
75% mineral fertilizers as per MAAR's recommendations (N_2P_2).
100% mineral fertilizers as per MAAR's recommendations (N_3P_3).

Phosphate solubilizing bacteria (PSB).
50% mineral fertilizers as per MAAR's recommendations + bacteria (PSB +N_1P_1).
75% mineral fertilizers as per MAAR's recommendations + bacteria (PSB +N_2P_2).
100% mineral fertilizers as per MAAR's recommendations + bacteria (PSB +N_3P_3).
Statistical analyses were performed using one-way ANOVA and comparisons of means were performed by using LSD test at $P \leq 0.05$.

4. Seed inoculation and cultivation

The lentil seeds (lentil variety Edleb3) in treatments (5, 6, 7 and 8) were inoculated with PSB and cultivated at Izraa station for the two seasons on 29/12/2009 and 6/1/2011 respectively. Lentil was drilled at 30 cm apart. Lentil was grown under rainfed conditions

Total rainfall during cropping season (October to May) was 299.3 and 327.9 mm during two seasons, respectively.

5. Fertilization

Mineral N and P were added to treatment 4 and 8 according to soil analyses and as per MAAR's recommendations (50 kg urea/ha and 110 kg superphosphate/ha for the first season, and 50 kg urea /ha and 110 kg superphosphate/ha for the second season), whereas 75% from fertilizer recommendations was added to treatment 3 and 7, and finally 50% from fertilizer recommendations was added to treatment 2 and 6.

Results and Discussion

1. Soil properties

It is worthy to mention that the physical analysis of the experimental soil indicated that mechanical analysis is clay with 26% sand, 20% silt and 54% clay so soil texture is clay. The soil was calcareous with 27.1% $CaCO_3$, pH 7.6 and EC_e 1.6 dS/m. Some soil chemical analysis are presented in table 1.

Table. 1. Some chemical analyses for some selected variables before plantation.

%		mg/kg	
Organic matter	Total N	Available P	Available K
0.09	0.05	14.3	450

2. The effect of PSB on total N and available P

Table 2 presents total N and available soil P content at the end of the experiment. PSB increased total N in soil up to 10 and 8% comparing to the control in the two seasons respectively. It also shows significant differences in TN in treatment amended with chemical fertilizers and PSB (PSB + N_3P_3) comparing to that amended with chemical fertilizers without PSB (N_3P_3). However, TN increased by inoculation with PSB in all treatments comparing to non-inoculated. This increase may be due to the interaction between PSB and lentil Rizobium (Alshater et *al*., 2007).

At the same time, available P significantly increased in soil for treatment (PSB) up to 18 and 28% comparing to the control in the two seasons respectively. Moreover, the above table shows significant differences in soil Av.P at treatment amended with 75% dose of chemical fertilizers and PSB (PSB + N_2P_2) comparing to full dose of chemical fertilizers (N_3P_3). Nevertheless, treatment amended with full dose of chemical fertilizers and PSB (PSB + N_3P_3) gave the highest Av.P in soil comparing to other treatments. Consequently, Av.P significantly increased by inoculation with PSB in all treatments comparing to those non-inoculated. These differences in available phosphorus are due to the effect of PSB (Richardson, 1994) by exuding phosphates enzyme that contribute to hydrolyze organic P. In addition, it exude organic acids such citric acid and oxalic acid, and produce CO_2 that forming H_2CO_3 with soil water (Bagyaraj et *al*., 2000).

Table. 2. Total N and available soil P content.

Treatments	TN (%)		Av.P (mg/kg)	
	Season 1	Season 2	Season 1	Season 2
N_0P_0	0.029^{f}	0.024^{e}	8.3^{h}	3.8^{f}
N_1P_1	0.036^{de}	0.029^{cd}	10.2^{f}	5.2^{e}
N_2P_2	0.04^{cd}	0.032^{bc}	10.9^{e}	6.1^{d}
N_3P_3	0.043^{bc}	0.034^{b}	11.6^{c}	6.8^{c}
PSB	0.032^{ef}	0.026^{de}	9.8^{g}	4.9^{e}
PSB + N_1P_1	0.039^{cd}	0.031^{bc}	11.3^{d}	6.6^{c}
PSB + N_2P_2	0.045^{ab}	0.035^{ab}	12.2^{b}	7.4^{b}
PSB + N_3P_3	0.048^{a}	0.039^{a}	13.4^{a}	8.9^{a}
$LSD_{0.05}$	0.004	0.0046	0.28	0.43

Means in the column followed by the same small letter are not significantly different at 0.05 level.

3. The effect of PSB on plant content of nitrogen and phosphorus

Table 3 shows total N and total P concentration in lentil leaves. Lentil leaves in treatments inoculated with PSB have significantly higher N content compared to the control and other treatments without inoculation. Total N increased in the two seasons up to 40% and 39% compared to the control respectively in treatment amended with PSB and full dose of mineral fertilizers. This table also shows significant differences in leaves total P at treatments amended with PSB comparing to the control and to other treatments without inoculation. Total P increased in treatment (PSB + N_3P_3) at the two seasons up to 29 % and 46 % compared to the control respectively. Also table shows no significant differences in P concentration at treatment (PSB + N_2P_2) compared to treatment (PSB + N_3P_3) this due to the effect of PSB in solubilizing the soil P and playing a significant role as plant growth-promoting rhizobacteria (PGPR).

Table. 3. Total N and P concentration in lentil leaves as affected by fertilization treatments.

Treatments	N (%)		P (%)	
	Season 1	Season 2	Season 1	Season 2
N_0P_0	2.96^{g}	2.83^{g}	0.37^{g}	0.318^{f}
N_1P_1	3.55^{e}	3.24^{e}	0.42^{e}	0.38^{d}
N_2P_2	3.65^{de}	3.53^{d}	0.43^{d}	0.4^{c}
N_3P_3	3.8^{c}	3.72^{bc}	0.45^{c}	0.45^{a}
PSB	3.22^{f}	3.09^{f}	0.38^{f}	0.35^{e}
PSB + N_1P_1	3.76^{cd}	3.6^{c}	0.45^{c}	0.42^{b}
PSB + N_2P_2	3.98^{b}	3.8^{b}	0.47^{b}	0.46^{a}
PSB + N_3P_3	4.14^{a}	3.94^{a}	0.48^{a}	0.46^{a}
$LSD_{0.05}$	0.11	0.095	0.008	0.015

Means in the column followed by the same small letter are not significantly different at 0.05 level.

The results show a combined activity of Rizobium and phosphate-solubilizing bacteria by increasing the total nitrogen in plants amended with PSB, so inoculation with PSB not only increased P absorption from soil but also activated N fixation by lentil root nodules. Ponmurugan and Gopi (2006) reported that phosphate solubilizing potential increases the availability of soluble phosphates and can enhance plant growth by increasing the efficiency of biological nitrogen fixation, and by production of plant growth promoting regulators.
Aftab et *al*., (2005) reported treatments resulted in a statistically significant increase in seed phosphorus content. Also, Kucey et *al*., (1989) reported phosphorus bio-fertilizers could help increase the availability of accumulated phosphate (by solubilization), efficiency of biological nitrogen fixation and increase the availability of Fe, Zn etc., through production of plant growth promoting substances.

4. The effect of PSB on some plant growth parameters

Table 4 presents the efficiency of PSB application on no. of days to germination, percent germination, no. of days to flowering, no. of days to maturity and plant height. The table also reveals a slightly significant increase in all treatments amended with PSB comparing to treatments without inoculation. Thus, no. of days to germination, no. of days to flowering and days to maturity were significantly decreased in treatments amended with PSB comparing to treatments without PSB, whereas percent germination and plant height were significantly increased in treatments amended with PSB.

Table. 4. Effect of fertilization treatments on some of plant growth parameters.

Treatments	No. of days to germination		Germination (%)		No. of days to flowering		No. of days to maturity		Plant height	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
N_0P_0	17^{a}	20^{a}	89^{f}	90^{e}	100^{a}	98^{a}	138^{a}	140^{a}	29^{e}	32^{e}
N_1P_1	16^{b}	19^{b}	91.3^{d}	92.3^{c}	98^{c}	96.3^{b}	136^{c}	138^{b}	31^{d}	33.6^{d}
N_2P_2	15.3^{bc}	18.3^{bc}	91.6^{d}	92.6^{c}	97^{d}	95^{c}	135^{d}	137^{c}	32^{c}	35^{c}
N_3P_3	14.6^{cd}	17.6^{cd}	93^{c}	94^{b}	96^{e}	94^{cd}	134^{e}	136^{cd}	33^{b}	36^{b}
PSB	16^{b}	19^{b}	90^{e}	91^{d}	99^{b}	97^{b}	137^{b}	139^{b}	29.6^{e}	32.6^{e}
PSB + N_1P_1	14.3^{d}	17.3^{d}	93.3^{c}	94.3^{b}	96^{e}	94d^{e}	134^{e}	136^{de}	33^{bc}	36^{bc}
PSB + N_2P_2	13.3^{e}	16.3^{e}	94^{ab}	94.6^{b}	95.3^{f}	93.3^{e}	133^{f}	135^{e}	33.3^{b}	36.3^{b}
PSB + N_3P_3	12.6^{e}	15.6^{e}	94.6^{a}	95.6^{a}	94.3^{g}	92.3^{f}	132^{g}	134.3^{f}	34.3^{a}	37.6^{a}
$LSD_{0.05}$	0.84	0.84	0.65	0.77	0.52	0.72	0.005	0.71	0.72	0.82

Means in the column followed by the same small letter are not significantly different at 0.05 level.

Monika et al., (2009) reported plant height increases with the treatment of PSB as compared to the control. No. of flowers/ plant is more in treatments inoculated with PSB as compared to the control. This may due to producing Plant growth promoting rhizobacteria (PGPR) by PSB.

5. The effect of PSB on lentil yield

Table 5 shows a significant increase in treatments inoculated with PSB in the two seasons with superiority to the treatment (PSB + N_3P_3). The lentil yield increased in the two seasons in treatments amended with PSB up to 22% and 33% compared to the control respectively, and up to 26% and 32% in treatment (PSB + N_3P_3) compared to treatment (N_3P_3). Moreover, the table shows significant differences in lentil yield in treatment amended with 75% dose of chemical fertilizers and PSB (PSB + N_2P_2) comparing to full dose of chemical fertilizers (N_3P_3).

Table. 5. Effect of fertilization treatments on lentil productivity (ton/ha).

Treatments	Biological yield		Straw		Grain	
	Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
N_0P_0	3.022[h]	5.170[h]	2.842[f]	4.450[f]	0.18[h]	0.72[h]
N_1P_1	3.327[f]	5.647[f]	3.037[e]	4.517[de]	0.29[f]	1.130[f]
N_2P_2	3.538[e]	5.837[e]	3.213[d]	4.547[d]	0.325[e]	1.290[e]
N_3P_3	4.038[c]	6.383[c]	3.658[b]	4.763[bc]	0.38[c]	1.620[c]
PSB	3.166[g]	5.447[g]	2.946[ef]	4.487[ef]	0.22[g]	0.96[g]
PSB + N_1P_1	3.772[d]	6.116[d]	3.422[c]	4.706[c]	0.35[d]	1.410[d]
PSB + N_2P_2	4.250[b]	6.633[b]	3.839[a]	4.793[b]	0.41[b]	1.840[b]
PSB + N_3P_3	4.416[a]	7.150[a]	3.936[a]	5.000[a]	0.48[a]	2.150[a]
$LSD_{0.05}$	0.119	0.083	0.104	0.058	0.021	0.074

Means in the column followed by the same small letter are not significantly different at 0.05 level.

Kumar et *al*.,(1999) and Chabot and Antoun (1996) were concluded that maximum grain yield and biological yield were recorded when PSM (phosphate-solubilizing microorganisms) was used with phosphorus alone or along with organic matter. Saad and Hammad (1998) also reported that the highest straw yield was obtained with inoculation of phosphate-solubilizing bacteria along with the application of calcium superphosphate.

Figure 1 shows the Effect of fertilization treatments on 100-grain weight. The results are also in line with table 5. The superiority was treatment (PSB + N_3P_3). This agree with Aftab *et al*.,(2005) who reported that phosphate-solubilizing bacteria increased the 100-grain weight due to inoculations and enabling P to become available for plant uptake after solubilization.

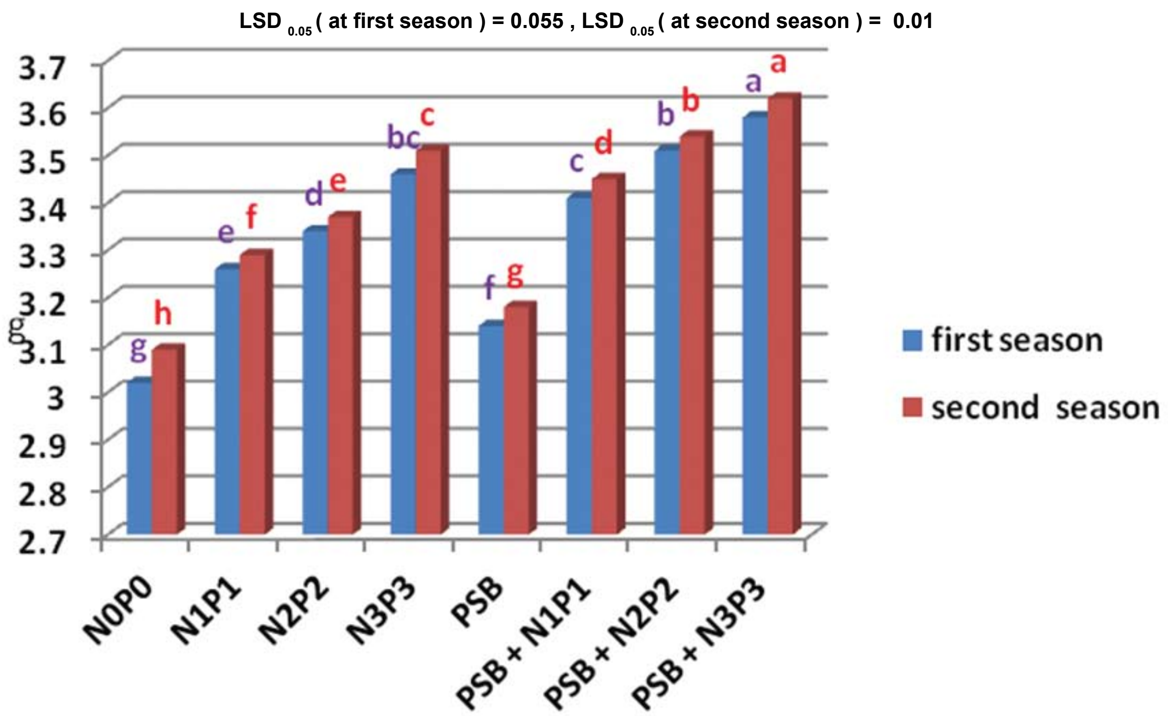

Figure. 1. Effect of fertlization treatments on 100-grain weight (gram).

The single and dual inoculation with fertilizer (P_2O_5) significantly increased root and shoot weight, plant height, spike length, grain yield, seed P content, leaf protein and leaf sugar content of the tested crop (Aftab and Bano, 2008).

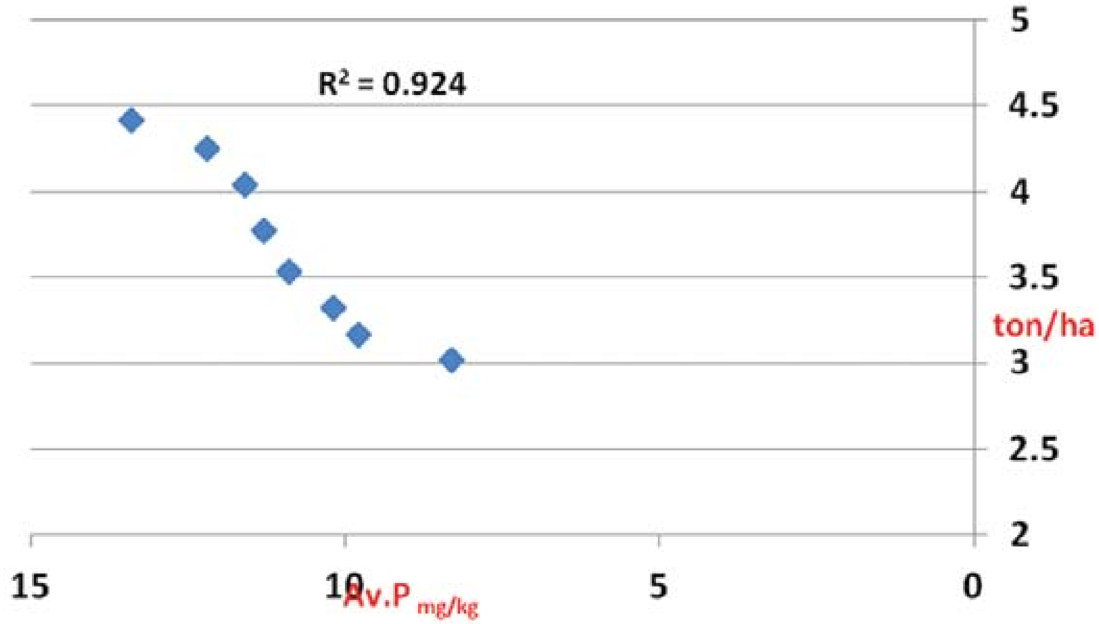

Figure. 2. The correlation between biological yield of the first season and soil available P at P ≤0.05 and P ≤0.01 .

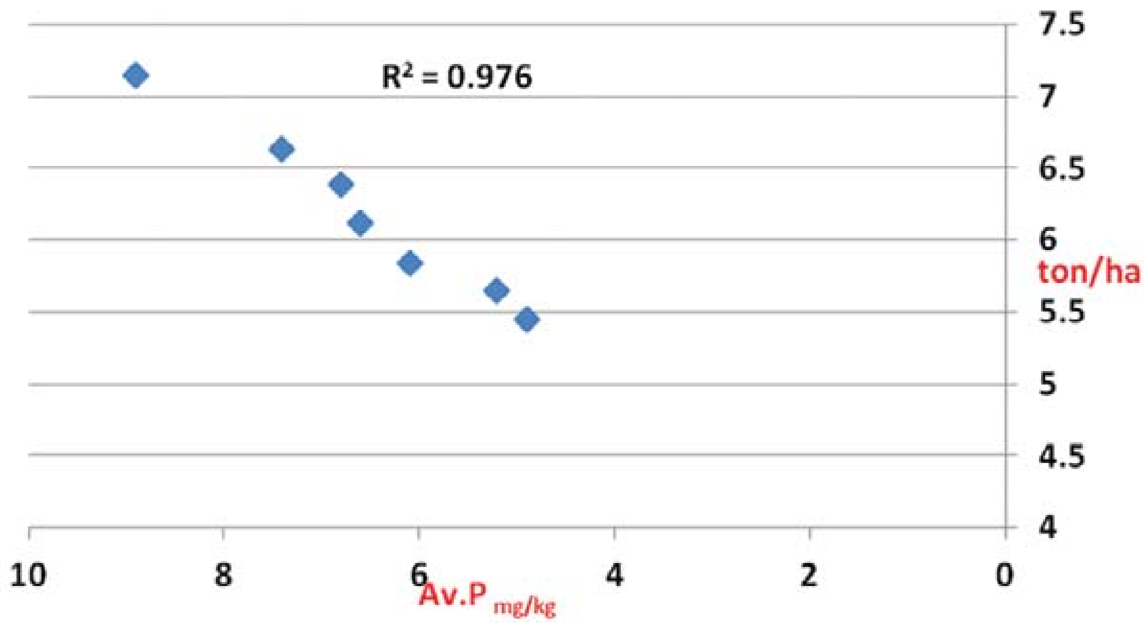

Figure. 3. The correlation between biological yield of the next season and soil available P at P ≤0.05 and P ≤0.01.

Figures 2 and 3 show the positive correlation at P ≤0.05 and P ≤0.01 between soil available P and lentil yield in the two seasons, the significant positive correlation (R^2= 0.92 and 0.97, respectively) between available P (x) and yield (y). This positive slope shows that when available P increases by amending the soil with PSB and NP, lentil yield will increased due to increasing of available P and producing PGPR by PSB.

Conclusion

It is inferred from the results that PSB positively affected soil fertility, plant nutrition and lentil yield by increasing phosphorous solubility, N fixation efficiency and plant growth enhancement.

Consequently, treatment amended with 75% dose of chemical fertilizers + PSB gave yield more than treatment amended with full dose of chemical fertilizers, clarifying the possibility of reducing chemical fertilizers use in lentil cultivation by inoculation with phosphate-solubilizing bacteria.

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