EFFECTIVENESS OF DIAMMONIUM PHOSPHATE IMPREGNATED WITH *PSEUDOMONAS PUTIDA* FOR IMPROVING MAIZE GROWTH AND PHOSPHORUS USE EFFICIENCY

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ABSTRACT

Use efficiency of soil applied phosphatic fertilizers in calcareous soils is less than 25%. Phosphorus from these fertilizers becomes fixed or precipitated by Ca^{+2} and Mg^{+2} in such soils. This efficiency can be improved by using phosphorus-solubilizing bacteria (PSB). A pot study was conducted to investigate the comparative impact of different levels of diammonium phosphate (DAP) fertilizer impregnated with *Pseudomonas putida* biotype A (Q_7) on growth attributes and phosphorus use efficiency (PUE) of maize in comparison to conventionally used DAP fertilizer. The culture of pre-isolated phosphate solubilizing bacterial strain Q_7 was used to impregnate phosphatic fertilizers with the help of molasses used as carbon source. In general microbial inoculation is known to be effective for enhancing nutrient use efficiency. However, results showed that impregnated phosphatic fertilizer (DAP) improved maize growth and dry matter yield up to 12% over conventional DAP fertilizer. Use efficiencies of impregnated DAP i.e. up to 62% increase of agronomic efficiency and 8% increase of physiological efficiency over control. Similarly, phosphors uptake was also increased with impregnated DAP by 33% over conventional DAP application. Results may imply that impregnation of DAP fertilizer could be a novel approach for improving growth and P - use efficiency of maize crop.

Keywords: Impregnation, maize, DAP, PUE, *Pseudomonas putida.*

INTRODUCTION

Low use efficiency (UE) is the major drawback of phosphorus in all soils particularly in calcareous soils. In these soils, applied inorganic P immediately precipitated with Ca^{+2} and Mg^{+2} (Pradhan and Sukla, 2005; Aziz *et al*., 2016) and thus lead to low productivity of crops. Without use of inorganic P, it is impossible to get target yield from a cereal crop. This inefficient use of inorganic P will emerge as root cause of persistent low yield. A decrease in the use of inorganic P is taken as an alarming sign for low production by all researchers (Yaseen *et al*., 2014).

In Pakistan, maize is the 3rd most important cereal crop and mostly cultivated on calcareous soils. Due to high pH and low organic matter, about 90% of these soils are deficient in P (Ahmad and Rashid, 2004). Maize production per unit area in Pakistan is still less than the neighboring countries. This is due to low PUE (<25%) because major portion of applied inorganic P become unavailable. Therefore, there is need to make P fertilizers more efficient and protective so that potential yield of maize cultivars can be achieved. By 2020 more than 70% of the grain yield will have to depend on inorganic fertilizers (Tilmen *et al*., 2011). Without phosphatic fertilizer application, it will be difficult to achieve potential yield, while the prices of inorganic fertilizers are increasing. There is no proportional increase in yield as increase recorded in fertilizers rates. This is all because of low effectiveness of applied fertilizers (Bumb and Baanante, 1996; Timilsena *et al*., 2015).

Possible strategies to increase crop production include the use of high yielding varieties or to increase the crop potential by genetic engineering techniques. An alternative technique for increasing crop production is to increase the efficiency of applied fertilizers. Among the strategies by which P fertilizers can be used more effectively include: band placement of phosphatic fertilizers; use of organic matter; acidic phosphatic fertilizers and use of microbes to enhance efficiency of applied fertilizers (Trolove *et al*., 2003). P-solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB (phosphorus solubilizing bacteria) strains is associated with the release of low molecular weight organic acids, through which their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Rodriguez *et al*., 2004). In addition, some PSB produce phosphatase like phytase that hydrolyse organic forms of phosphate compounds efficiently (Riggs *et al*., 2001). The PSB have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield. The use of PSB as inoculants increases the P uptake by plants. Simple inoculation of seeds with PSB gives crop yield responses equivalent to 50 percent of the need for phosphatic fertilizers (Shaharoona *et al*., 2008). Currently, different strains of these bacteria has been identified and used in fertilizer, among them *Pseudomonas* spp. strains are highly efficient in solubilizing insoluble phosphate (Jilani *et al*., 2007; Shaharoona *et al*., 2006b, 2007). Previously information is available on the application of microbes to improve phosphorus use efficiency (PUE) with reduced rates of fertilizers, but the method of application was seed inoculation (Shaharoona *et al*., 2008). We introduced a novel approach to improve PUE in maize crop. In this technique, the P-solubilizing bacterium *P. putida* Q7 is added to soil with impregnation of phosphatic fertilizers (fertilizers coated with microbes) using some organic materials. This method of microbe's delivery is more convenient because this is less laborious for farmers and use efficiency of fertilizer is also increased. So, the effect of applied impregnated DAP attaining potential yield of maize crop is evaluated under alkaline/calcareous with aim of efficient use of inorganic P nutrient.

MATERIALS AND METHODS

Preparation of impregnated fertilizers:

Preparation of inoculum: Inoculum of the selected bacterial strain *P. putida* biotype A (Q7) was prepared in 200 mL Erlenmeyer flask by using National Botanical Research Institute's Phosphate (NBRIP) medium (Nautiyal, 1999) as broth culture. The inoculated broth was incubated in a shaking incubator (Firstek Scientific, Tokyo, Japan) at 100 rpm for 48 hours at 28±1ºC. An OD of 0.5, measured with an optical density meter (Biolog® Model-21907; Biolog Inc.) at a wavelength of 535 nm, was achieved by dilution to maintain a uniform cell density (10^7–10^8 CFU · mL^{-1}) prior to fertilizer grain coating.

Impregnation of *P. putida* culture on DAP fertilizer granules: Slurry was prepared by mixing one litter volume of *P. putida* biotype A Q7 broth culture and organic materials (compost and molasses). To prepare impregnated DAP, slurry containing bacterial strain Q7 and organic materials @ 20.0 g/kg of fertilizer, was coated on DAP fertilizer. The impregnated DAP grains was dried under controlled conditions and stored it till used in polythene bag.

Pot experiment: A pot experiment was conducted to investigate the potential of the *P. putida* biotype A (Q_7) impregnated diammonium phosphate (DAP) for improving growth and phosphorus use efficiency of maize plants. The experiment was planned according to completely randomized design (CRD) with three replications. The soil used during the experiment was taken from the research area of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. It was ground and passed through sieve with a pore size of 2 mm and analyzed for various physico-chemical characteristics before filling in the pots. The soil was sandy clay loam (Bouyoucos, 1962), showed pH 7.7, organic matter 0.64% (Walkley and Black, 1934), phosphorus 6.7 mg kg^{-1} of soil (Olsen *et al*., 1954) and field capacity was measured by Topp *et al*. (1993). Ten kilogram soil was filled in each pot and weighed on top loading electrical balance after the addition of water and fertilizers. Recommended rate of N, P and K fertilizers were applied at the concentration of 175, 120, 90 kg ha^{-1} in the form of urea, diammonium phosphate (DAP) and sulfate of potash (SOP). N level was fluctuated with variant levels of DAP; the remaining was balanced with N fertilizer (Urea). In all treatments, 100% of recommended N and K were applied. Control was without any fertilizer application. Three levels of impregnated DAP i.e. 100, 75 and 50% of recommended P were used. A treatment without microbial impregnated DAP (alone DAP) was also included for comparison between impregnated DAP and conventional DAP.

Three seeds of maize cv. F-1 Monsanto 6525 were sown in each pot. Two plants of same vigor in each pot were retained after two weeks of successful germination. Uprooted seedlings were chopped and incorporated in the same pot. The moisture of each pot was maintained at field capacity after 24 h on weight basis. Other agronomic and protective measures were followed as recommended for maize production.

The crop was harvested 80 days after sowing. Data regarding growth attributes were recorded at different periods of plant growth. Root and shoot length was measured by measuring scale. Root volume was determined by water displacement method (Harrington *et al*., 1994). Root and shoot oven dried weight was recorded by using top loading digital balance (MJ-3000). For chemical analyses, the harvested plants were washed with distilled water, dried till constant weight at 65°C in an oven and ground. The ground plant materials (0.5 g) was digested in a di-acid (2:1 HNO_3:$HClO_4$) mixture (Wolf, 1982). Nitrogen was analyzed according to Jackson (1962) while P was determined by vanadate-molybdate spectrophotometric procedure (Jones *et al*., 1991). However, K was determined with flame photometer following the method of Chapman and Pratt (1961). The N, P and K uptakes by root and shoot were calculated by multiplying N, P or K concentration in root and shoot with its dry weight. Chemical analysis for root and shoot were done separately. Nutrient uptakes by plant were calculated by shoot uptake plus root uptake.

Nutrient use efficiencies: Similarly, different forms of fertilizer use efficiency were calculated by using following formulae.

$$PE\ (g/g) = \frac{\text{Fertilized plant biomass} - \text{unfertilized plant biomass}}{\text{Uptake by fertilized plant} - \text{Uptake by unfertilized plant}}$$

$$AE\ (g/g) = \frac{\text{Fertilized plant biomass} - \text{unfertilized plant biomass}}{\text{Amount of fertilizer applied}}$$

$$ARE\ (\%) = \frac{\text{Uptake by fertilized plant} - \text{Uptake by unfertilized plant}}{\text{Amount of fertilizer applied}} \times 100$$

Whereas PE, AE and ARE stands for physiological use efficiency, agronomic use efficiency and apparent recovery efficiency, respectively.

Statistical analysis: Statistical procedures were applied to analyze the data (Steel *et al*., 1997) using factorial completely randomized design and means were compared using HSD test at 5% probability level.

RESULTS

Effectiveness of fertilizer coated phosphate solubilizing strain *P. putida* biotype A (Q_7) for improving root dry weight of maize in this trial is evident from the data given in Table 2. Results showed that due to coating of this strain on fertilizer grain significantly increased root dry weight; however, the efficacy of impregnation decreased with increasing fertilizers doses. The coating of strain *P. putida* biotype A (Q_7) on fertilizer was more promising than conventional fertilizer (without coating), as the former significantly increased the root dry weight by 49%, 42% and 34% at 100%, 75% and 50% of recommended rate of fertilizer over respective uninoculated control (conventional DAP) respectively. Shoot dry weight was also significantly increased in response to application of impregnated fertilizer. All rates of impregnated phosphatic fertilizers performed better then control while, full recommended (100%) dose of impregnated fertilizer caused 12% increase in shoot dry weight over conventional DAP. The effect of impregnation with microbial strain on number of leaves per plant in the presence of different doses of fertilizers is evident from the data summarized in Table 1. Results revealed that impregnation caused significant increases in number of leaves per plant at most of the fertilizer doses. Microbial strain was found to be more effective in promoting number of leaves over control (without any amendment). Similarly, chlorophyll content of flag leaf was increased in response to applied impregnated fertilizers over conventional fertilizer application. Impregnation with microbial strain *P. putida* biotype A (Q_7) caused significant increase in chlorophyll content at all fertilizers levels that were 44%, 34% and 24% higher than conventional fertilizer application at 100%, 75% and 50% rate of impregnated fertilizer respectively (Table 1).

Root volume of maize was also significantly increased in response to *P. putida* biotype A (Q_7) impregnated fertilizers. Impregnation with microbial strain caused maximum increase in root volume (37%) over conventional fertilizer application without coating. There was 24 and 18% increase in root volume by 75 and 50% of recommended fertilizer rate over full dose of conventional fertilizer, respectively. Impregnated fertilizer also caused significant increase in leaf length and shoot length (Table 1-2) at all levels of fertilizers, but there is non-significant increase in root length.

P. putida biotype A (Q_7) significantly influenced the P directly, however affected uptake of N and K indirectly. The results given in Figure 1 showed the impact of microbial strain on the uptake of N, P and K. Maximum increase in P uptake was observed in treatment with *P. putida* biotype A (Q_7) impregnation at 100% of recommended rate i.e. 33% over conventional DAP application. Similarly, there was 45, 32 and 11% increase in N uptake; while, 43, 30 and 8% increase in K uptake by 100, 75 and 50% of recommended rate of impregnated DAP, respectively over conventional DAP.

Table 1. Growth response of maize to application of different rates of *P. putida* impregnated DAP and P from commercial source under pot conditions.

P sources P percent	Leaves plant^{-1}	Leaf length (cm)	chlorophyll contents (SPAD)	Root volume (cm^3)
Control	10±0.50b	42.7±0.38e	15.7±0.58e	33.3±0.29e
$CD_{p\ (1.74\ g)}$	12.7±0.29a	61.5±0.25d	21.2±0.38d	60±0.50d
$PID_{p\ (1.74\ g)}$	13.3±0.58a	73.8±0.38a	30.5±0.60a	82±0.25a
$PID_{p\ (1.31\ g)}$	13±0.01a	68.2±0.14b	28.4±0.43b	74.5±0.43b
$PID_{p\ (0.87\ g)}$	11.3±1.04ab	63.8±0.38c	26.3±0.40c	70.8±0.14c
HSD	2.15	1.17	1.76	1.27

Values sharing same letter in each column do not differ significantly at p = 0.05 according to HSD test. $CD_{P\ (1.74\ g)}$ = 100% of rec. P from commercial DAP, $PID_{P\ (1.74\ g)}$ = 100% of rec. P from *P. putida* impregnated DAP, $PID_{P\ (1.31\ g)}$ = 75% of rec. P from *P. putida* impregnated DAP and $PID_{P\ (0.87\ g)}$ = 50% of rec. P from *P. putida* impregnated DAP

Effect of impregnation on nutrient use efficiency is reflected from the data given in table 3. Results showed that agronomic and physiological efficiency of P was increased with decreasing rates of impregnated phosphatic fertilizers; while, P recovery efficiency was decreased with decreasing rates of impregnated phosphatic fertilizers. Maximum increase in P agronomic efficiency was recorded in case of impregnation with *P.*

putida biotype A (Q_7) where 50% of recommended dose of DAP was applied, and it was 62% higher than conventional DAP application. Similarly, agronomic use efficiency of N and K was decreasing with decreasing the recommended rate of P fertilizer, same is the case was observed in recovery and physiological efficiencies of N and K. These were decreasing with reduced rates of recommended fertilizers.

Table 2. Variations in shoot and root growth of maize on the basis of length and weight as influenced by full and reduced rates of *P. putida* impregnated DAP and P from commercial source under pot conditions.

P sources	length (cm)			dry biomass (g)		
P percent	Root	Shoot	RL: SL	Root	Shoot	RW:SW
Control	51.2±0.25c	43.8±0.80d	1.2±0.05a	3.9±0.52d	13.4±1.43d	0.29±0.02b
$CD_{p\ (1.74\ g)}$	71.3±0.56a	60.8±0.76bc	1.2±0.03a	5.4±0.98c	21.1±5.02b	0.26±0.03c
$PID_{p\ (1.74\ g)}$	71.5±0.59a	71.4±0.48a	1.0±0.01c	8.0±1.03a	23.7±5.22a	0.34±0.01a
$PID_{p\ (1.31\ g)}$	67.5±0.38b	61.5±0.73b	1.1±0.01b	7.7±0.25ab	22.7±0.19a	0.34±0.01a
$PID_{p\ (0.87\ g)}$	66.2±0.56b	58.7±0.43c	1.1±0.11ab	7.2±1.44b	19.6±2.75c	0.37±0.05a
HSD	1.77	2.4		0.63	1.29	

Values sharing same letter in each column do not differ significantly at p = 0.05 according to HSD test. $CD_{P\ (1.74\ g)}$ = 100% of rec. P from commercial DAP, $PID_{P\ (1.74\ g)}$ = 100% of rec. P from *P. putida* impregnated DAP, $PID_{P\ (1.31\ g)}$ = 75% of rec. P from *P. putida* impregnated DAP and $PID_{P\ (0.87\ g)}$ = 50% of rec. P from *P. putida* impregnated DAP

Table 3. Comparison between fertilizers use efficiency affected by application of commercial and impregnated DAP under pot conditions.

Phosphorus source /rate	Fertilizer use efficiency (mg mg^{-1})		
	Nitrogen	Phosphorus	Potassium
		Agronomic use efficiency (mg mg^{-1})	
Control	-	-	-
$CD_{p\ (1.74\ g)}$	5.50 ±0.67ab	6.01±0.73b	9.63±1.17ab
$PID_{p\ (1.74\ g)}$	7.35±0.92a	8.04±1.01ab	12.86±1.61a
$PID_{p\ (1.31\ g)}$	6.65±1.25ab	9.69±1.82a	11.63±2.18ab
$PID_{p\ (0.87\ g)}$	4.46±0.75b	9.74±1.64a	7.80±1.31b
HSD	2.22	3.3	3.89
		Apparent recovery efficiency (%)	
Control	-	-	-
$CD_{p\ (1.74\ g)}$	22.62±0.49c	4.71±0.07d	28.84±1.33b
$PID_{p\ (1.74\ g)}$	38.28±1.78a	9.56±0.38a	49.16±2.93a
$PID_{p\ (1.31\ g)}$	33.53±2.62b	8.66±0.34b	43.02±4.29a
$PID_{p\ (0.87\ g)}$	25.14±0.79c	7.08±0.12c	32.11±1.78b
HSD	3.97	0.63	6.79
		Physiological efficiency (mg mg^{-1})	
Control	-	-	-
$CD_{p\ (1.74\ g)}$	24.27±2.4a	127.83±17.3a	33.3±2.5a
$PID_{p\ (1.74\ g)}$	19.13±1.9ab	84.07±10.4b	26.07±2b
$PID_{p\ (1.31\ g)}$	19.7±2.1ab	111.57±16.3ab	26.9±2.3b
$PID_{p\ (0.87\ g)}$	17.7±2.5b	137.6±23.5a	24.2±2.9b
HSD	5.4	4.12	5.87

Values sharing same letter in each column do not differ significantly at p = 0.05 according to HSD test. $CD_{P\ (1.74\ g)}$ = 100% of rec. P from commercial DAP, $PID_{P\ (1.74\ g)}$ = 100% of rec. P from *P. putida* impregnated DAP, $PID_{P\ (1.31\ g)}$ = 75% of rec. P from *P. putida* impregnated DAP and $PID_{P\ (0.87\ g)}$ = 50% of rec. P from *P. putida* impregnated DAP

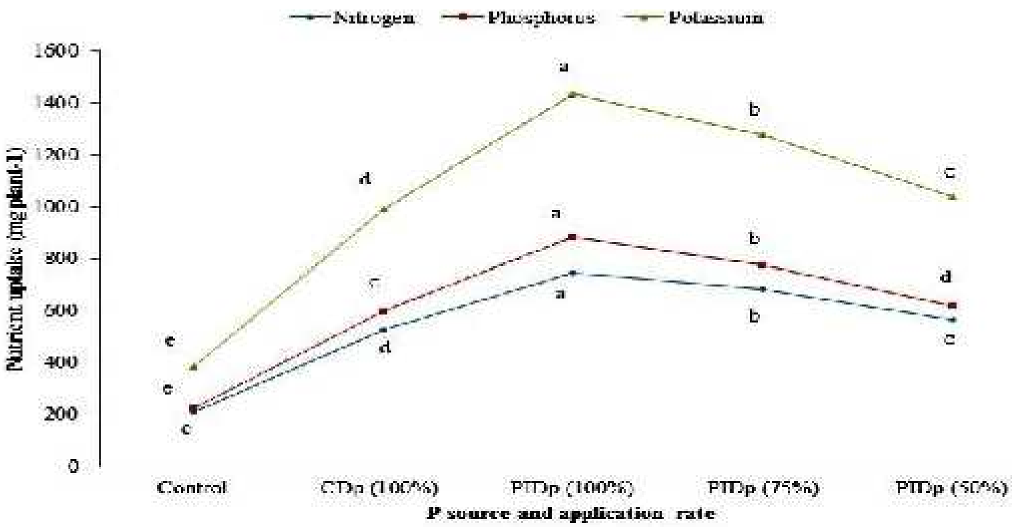

Figure 1. Comparison between uptakes of primary essential macronutrient in maize plants under the influence of DAP impregnated with and without *P. putida*. CD_P (1.74 g) = 100% of rec. P from commercial DAP, PID_P (1.74 g) = 100% of rec. P from *P. putida* impregnated DAP, PID_P (1.31 g) = 75% of rec. P from *P. putida* impregnated DAP and PID_P (0.87 g) = 50% of rec. P from *P. putida* impregnated DAP

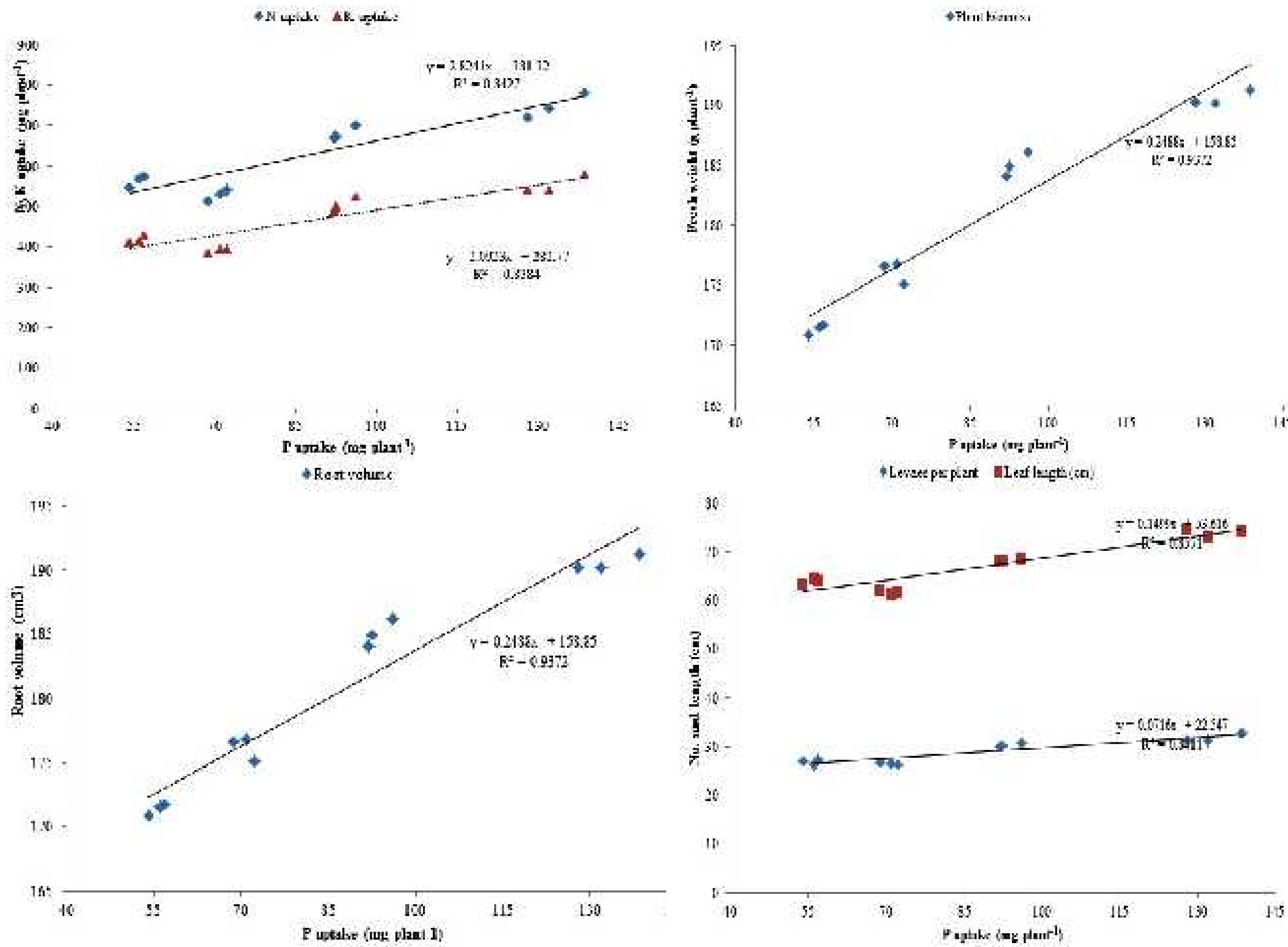

Figure 2. Correlation between (a) P uptake and other fertilizer uptake, (b) P uptake and fresh weight, (c) P uptake and root volume, (d) P uptake and leaf number and length in maize under the influence of different rates of P from various sources.

DISCUSSION

The low efficiency of a phosphatic fertilizer is one of the important issues in the calcareous soils particularly in Pakistani soils. Pakistani soils (>90% of arable lands) are calcareous in nature with pertaining situations of high pH, low organic matter (< 1%), extensive farming without proper crop rotation (NFDC, 2003; Aulakh, 2010). All these factors are the big constrains for low productively potential of maize in Pakistan as P is the 2nd most critical macronutrients and any reduction in its application causes an incredible reduction in yield. So, the only way to put off such great losses is to make chemical P fertilizers more efficient. Microbes can play important role in increasing nutrient use efficiency (Trolove *et al*., 2003, Lucy *et al*., 2004). Phosphorus solubilizing bacteria (PSB) have the potential to solubilize unavailable soil P mainly by chelation-mediated mechanism (Shahroona *et al*., 2007a). These bacteria enhance the P availability to plants by mineralizing organic P in the soil or by solubilizing precipitated phosphates (Parani and Saha, 2012). In the present study we evaluated the potential of *P. putida* for improving growth and P use efficiency of fertilizer and making P fertilizers.

Better root growth is considered as prerequisite for healthy plant growth. Ion uptake, soil nutrient supply and root morphology had consistently indicated the importance of root morphology parameters in the uptake of a variety of nutrients especially N, P and K (Barber and Silverbush, 1984; German *et al*., 2000). The plant root development was affected by the application of phosphate solubilizing bacteria (Shaharoona *et al*., 2006a). External application of PSB with fertilizers enhanced soluble P in the solution and this had a positive impact on root growth (Shaharoona *et al*., 2007a). Root growth is not only sensitive to external concentration of nutrients but also regulated by plant growth regulating substances, such as auxins (Salisbury, 1994) and ethylene (Arshad and Frenkenberger, 2002). The root development and plant biomass were correlated with the higher availability of P; moreover, the beneficial effects of applied bacteria have been attributed to their ability to produce various compounds such as phytohormones, vitamins and siderophores (Arshad and Frankenberger, 1993). Results of this study revealed that pots having addition of impregnated DAP were able to make more biomass by accelerating photosynthesis rate (Cleyet-Marcel *et al*., 2001; Khalid *et al*., 2004) as improved chlorophyll content are the evidence for this regard. The pH of the soil tightly regulated the P availability and whenever PSB are applied in soils, these release organic acids and acid phosphatase that are involved in mineralization of organic P in soils (Reddy *et al*., 2002; Fernandez *et al.*, 2007). The activity of acids and enzymes result in acidification of microbial cell and its surrounding. The temporary acidic surroundings in alkaline soils enhanced availability of P to plants that also influenced the uptake of other nutrients (Grichko and Glick, 2001; Dobbelaere *et al.*, 2003). Thus, it can be assumed that root growth in term of more biomass can be obtained if PSB like *Bacillus* sp. and *Pseudomonas* sp. are inoculated into soil low in P status because these strains are responsible for more P solubilization and more nutrient and water uptake (Khan, 2005). Several researchers have used different *Pseudomonas* species as a bioinoculant for improving crop productivity (Zaida *et al*., 2003; Naveed *et al*., 2008; Shaharoona *et al*., 2008). Phosphorus use efficiency was increased in response to fertilizer impregnation (Table 3), most likely due to increased root growth that exploited more soil volume for efficient uptake of nutrients by plants, resulting in more biomass production. In addition, the maximum PUE was recorded under low level P (i.e. 50% of recommended P), these results are also supported by Shaharoona *et al*. (2007a). This improvement in P use efficiency occurred might be due to P solubilizing potential of *P. putida* biotype A (Q_7) impregnated on DAP fertilizer granules. This strain of *Pseudomonas* made P available to plants for uptake either chelating newly added P or solubilizing precipitated P in the soil. As the *P. putida* biotype A (Q_7) was impregnated over DAP, so the chelating mechanism might be predominant for the higher P use efficiency in maize. This improvement in P efficiency caused an increase in plant growth. This improvement in plant growth (Tables 1 and 2) might be due to P mediated N or K uptake because corresponding increase in uptakes of N and K also occurred as uptake of P improved (Figure 2). When P availability with other essential nutrients is increased due to PSB, then the improvement in growth and developmental processes occurred (Cakmakci *et al.*, 2005), that caused improvement in plant biomass. These PSB caused more nutrient uptake by plants by the action of chelating substances (Puente *et al.*, 2004). The PSB also indirectly influence N uptake by plant by controlling the P concentration in soil (Reed and Glick, 2004). That is why; there was more N concentration in plant shoots where more P concentration in shoot due to addition of PSB with fertilizers (Jodie *et al.*, 2006). In short, impregnation of phosphatic fertilizers was effective even at reduced rates of chemical fertilizers. Further research is needed to explore the potential of impregnated phosphatic fertilizers in different agro ecological zone under field conditions. The research work should be extended for field recommendations and consistent yield increase.

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