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EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA (*BACILLOUS MEGA THERIUM*) AND PHOSPHATE FERTILIZER ON YIELD AND YIELD COMPONENTS OF WHTEAT

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ABSTRACT

A field study was conducted to investigate the effects and significance of phosphate solubilizing bacteria (*Bacillus megatherium*) and different levels of phosphate fertilizer on yield and yield components of wheat. Different microbial inoculums levels were used alone and combined with P fertilizer. The experiment was laid out in randomized complete block design (RCBD) in three replications using seven different treatments (bacteria alone and with various P combinations). Data was recorded on plant height (cm), number of spikelets/spike, number of grains/spike, plant yield (g), 1000-grain weight (g), biological yield (kg/ha) and grain yield (kg/ha). The bacteria were counted in one gram of soil at the depth of 0-15 and 15-30 (cm) after two week sowing and after harvesting of the crop. The results showed that the grain yield was significantly increased (3450 kg/ha) at T4 (90 kg p/ha, whereas, non-significant difference for grain yield (2385 and 2390 kg/ha respectively) was observed among T2 (40 kg/ha) and T5 (phosphobacteria 25 ml/kg of seed). The lowest grain yield (1180 kg/ha) was recorded in control. The results suggested that at T5 (phosphobacteria 25 ml/kg of seed) could produce reasonable and economic yield.

Keywords: phosphate solubilizing bacteria, *Bacillus megatherium, Yield and yield components, Wheat.*

INTRODUCTION

Soil microorganisms are playing a key role in soil P dynamic and subsequent availability of phosphate to plants (Richardson, 2001). The soil bacteria are capable of transforming soil P to the forms available to plant. Microbial biomass assimilates soluble P, and prevents it from adsorption or fixation (Ghadeer *et al.*, 2012; Khan and Joergesen, 2009). Release of P by PSB from insoluble and fixed/adsorbed forms is an import aspect regarding P availability in soils. Microbes influence soil fertility through soil decomposition, mineralization and strong/release of nutrients. Microorganisms enhance the availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphate (Chen *et al.*, 2006; Kang *et al.*, 2002; Pradhan and Sukla, 2005). Wheat is an important cereal food crop of Pakistan. Wheat yield can be improved through the improved varieties, improved agronomic practices and soil fertility (Sial *et al.*, 2013, Ahmed *et al.*, 2014; Safdar *et al.*, 2014; Sonya et al., 2005; Sial *et al.*, 2007). Microorganism involved in phosphorus acquisition includes mycorrhizal fungi and (PSMS) (Fankem *et al.*, 2006). *Bacillus megatherium* can be referred as the most important (Subbarao, 1988; Kucey *et al.*, 1989). Phosphorus (P) is a major growth limiting nutrient and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezwa *et al.*, 2002). The root development, stalk and stem strength, flower and seed formation, crop maturity and production, nitrogen fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition. Although microbes are in use to improve soil fertility during the last century, however, a limited work has been reported on P solubilizing bacteria as compared to nitrogen fixation bacteria. Phosphate solubilizing bacteria (PSB) are being used as biofertlizer since 1950s (Kudashev, 1956; Krasilinikov, 1957). Soil P dynamic is characterized by physiochemical (sorption-desorption) and biological (mobilization-mineralization) process. Large amount of P applied as fertilizer enter into the immobile pools thorough precipitation reaction with highly reactive $A1^{3+}$ and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soil (Gyaneshwar *et al.*, 2002; Hao *et al.*, 2002). Efficiency of P fertilizer through out the world is around 10-25% (Isherword, 1998) and the concentration of bioavailable P in soil is very low reaching the level 1.0mg kg^{-1} soil (Goldstein, 1994). Inorganic form of P are solubilized by a group of heterotrophic microorganisms excreting organic acids that dissolve phosphate minerals and/or chelate cationic partners of the P ions i.e. PO_4^{3-} directly, releasing P into solution (He *et al.*, 2002). These bacteria in the presence of labile carbon serve as sink for P by rapidly immobilzing it even in low P soils (Bunemann *et al.*, 2004). Subsequently PSB become a source of P to plants upon its release from their cells. The PSB and plant growth promoting rhizobacteria (PGPR) together could reduce P fertilizer application by 50% without any significant reduction of crop yield (Jilani *et al.*, 2007; Yazdani *et al.*, 2009). It infers that PSB inoculants /biofertilizers hold great prospects for sustaining crop production with optimized fertilization. Evidence of naturally occurring rhizospheric solubilizing microorganism (PSM) was reported date back to 1903 (Khan *et al.*, 2007). Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al.*, 2002). Bacillus megatherium, B. circulans, B. subtilis, B. polymyxa, B. sircalmous, Psendomonas striata, and Enterobacte r could be referred as the most important strain (Subbaroa, 1988; Kucey et al., 1989. A nemato fungus Arthrobotrys oligospora also has the ability to solubilize the phosphate rocks, Duponnois et al., 2006. Among the whole microbial population in soil PSB constitutes 1-50%, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5% in P solubilization potential (Chen *et al.,* 2006). A nematofungus *(Arthrobotrys oligospora)* also has the ability to solubilize the phosphate rocks (Duponnois *et al.,* 2006). Other studies have shown the vesicular arbuscular (VA) mycorrhiza increase phosphate up take (Mosse *et al.,* 1976). Mycorrhizal dependency of tropical forage plants may vary greatly among species of the same genus. Similar differences in mycorrhizal depending of other plants species such as citrus, wheat, forest, mints, hard wood trees and crop plants have been reported by other workers (Azcon and Ocampo; 1981; Janos; 1980, Menga *et al.*, 1978 Plenchette *et al.*, 1983, Pope *et al.*, 1983. The aim of our study was to determine the role of P-solubilizing microbes (Bacteria and Mycorrhiza) in the soil fertility and to estimate its application and cost benefit ratio as compared to chemical fertilizers.

MATERIALS AND METHODS

To investigate the effects of phosphate solubilizing bacteria (*Bacillus megatherium*) and different levels of phosphate fertilizer on yield and yield components of wheat, an experiment was conducted randomized complete block design (RCBD) in three replications using seven different treatments (bacteria alone and with various P combinations) at experimental farm of Nuclear Institute of Agriculture (NIA), Tandojam. Different microbial inoculums were used alone and combined with P fertilizer. The treatments were T_1 = control, T_2= 40 kg phosphorus P/ha, T_3= 60 kg P/ha, T_4= 90 kg p/ha, T_5=phosphobacteria (25ml/kg of seed), T_6= Mycorrhiza (25 ml/kg of seed), T_7= Phosphobacteria + Mycorrhiza.The field experiment was conducted on wheat variety Kiran-95 evolved by Nuclear Institute of Agriculture, NIA, Tandojam. Pre-Isolated phosphate solubilizing bacteria and mycorrhiza (*Bacillus megatherium* and *Glomus fasciculatum*) were used. The strains were obtained from experimental farm of Nuclear Institute of Agriculture (NIA) Tanodjam. Phosphobacterial species was subcultured on fresh calcium phosphate media for further confirmation. The colony of phosphobacteria inoculated in to the 25ml of calcium phosphate broth and incubated for 48 hours at the temperature 35^{O}C, then inoculated with the one kg seed of wheat. The sowing was done in normal sowing time (November). The Mycorrhizae (*Glomus fasciculatum*) were isolated from the soil in which the previous crop was maize. The isolated mycorrhiza was cultured on the fresh media of Melin-Norkran agar and incubated at 23-30^{0}C for two weeks, then the pure culture was inoculated to the Melin Norkran broth for 1 week, then the culture was mixed with the seed of wheat at the rate of 25ml/kg and grown in soil. Soil samples were collected at the depth of 0-15 and 15-30 (cm) for bacterial enumeration after two weeks of sowing. At maturity of crop, randomly five plants were selected from each middle line to record data on various agronomic traits. Data was recorded on plant height (cm), number of spikelets/spike, number of grains/spike, plant yield (g), 1000-grain weight (g), biological yield (kg/ha) and grain yield (kg/ha). The bacteria were counted in one gram of soil at the depth of 0-15 and 15-30 (cm) after two week sowing and after harvesting of the crop. The data were subjected to analysis of variance and DMR test (Duncan's Multiple Range Test) for to compare the mean values.

RESULTS AND DISCUSSION

The result indicated the differential response of wheat variety Kiran-95 for its various yield and yield associated traits at different treatments (Table 1).

Table-1: Response of phosphobacteria and mycorrhiza in wheat

Treatment	Biological yield kg/ha	Grain yield kg/ha
T1 = Control	4550 e	1180 e
T2= 40 kg p/ha	7310 c	2385 c
T3= 60kg p/ha	8060 b	2955 b
T4=90kg p/ha	8560 a	3450 a
T5=Phosphobacteria 25 ml/kg of seed	7375 c	2390 c
T6=Mycorrhiza, 25ml/kg of seed	6230 d	2000 d
T7=Phosphobacteria + mycorrhiza	6200 d	2000 d

Biological and grain yield (kg/ha): The biological and grain yield was significantly enhanced (8560 and 3450 kg/ha respectively) at treatment No.4=90kg p/ha) followed by T3= 60kg p/ha (8060 and 2955 kg/ha respectively), whereas T2= 40 kg p/ha and T5=Phosphobacteria 25 ml/kg of seed were not significantly different with each other (7310, 2385 and 7375, 2390 kg/ha respectively). The minimum grain yield (1180 kg/ha) was recorded in control (Table 1). Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al.*, 2002; Khan *et al.*, 2007). The PSB solubilize the fix soil p and applied phosphates resulting in higher crop yields (Gul *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). Higher crop yields result from solubilization of fixed soil p and applied phosphates by PSB (Zaidi, 1999). *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al*., 2006).

Plant height (cm): The maximum plant height (68.0 cm) was noted at T4=90kg p/ha, whereas, the lowest plant height was recorded in control. Results showed that the T2= 40 kg p/ha and T2= 40 kg p/ha were similar in plant height followed by T6=Mycorrhiza, 25ml/kg of seed and T6=Mycorrhiza, 25ml/kg of seed.

Number of spikelets/spike: Similarly, number of spikelets/spike were significantly increased (16.0) at T4 and (15.0) T3, whereas T2 and T5 were similar with each other. The number of spikelets/spikes was significantly reduced in control, T6 and T7. Fertilization improves nitrogen nutrition and increases the number of fertile spikelets and flowers, and consequently increases the number of kernels per spike (Singh and Parsad, 2011).

Number of grains/spike: The highest number of grains per spike was recorded in T4 (47.0) followed by T3 (45.0). The lowest number of grain/spike were recorded in control (27.0) followed by T6 (40.0 respectively) and T7 (Table 2). The increase (6.1%) increase in number of kernels per spike with inoculation treatments were observed by Diaz-Zorita and Fernandez-Canigia (2008).

Table-2: Performance of yield and its attributes of wheat

Treatments	Plant height, (cm)	No. of spikelets/spike	No. of grains/ spike
T1 = Control	50 e	12 e	27 e
T2= 40 kg p/ha	63 c	14 c	43 c
T3= 60kg p/ha	66 b	15 b	45 b
T4=90kg p/ha	68 a	16 a	47 a
T5=Phosphobacteria 25 ml/kg of seed	63 c	14 c	43 c
T6=Mycorrhiza, 25ml/kg of seed	61 d	13 d	40 d
T7=Phosphobacteria + mycorrhiza	61 d	13 d	40 d

Total plant yield (g): The maximum total plant yield (3.60g) was recorded in T4 followed by T3 (3.32g). The minimum total plant yield (1.23g) was recorded in control (Table 3). The non significant difference for plant yield was recorded among T2 and T5 and between T6 and T7. Inoculation increases organic matter content of soils and improves physical, chemical, and biological characteristics of soils, and consequently enriches the availability and intake of plant nutrients (Singh and Prasad, 2011, Mahboob, et al., 2010).

Table-3: Total plant yield and 1000- grain yield in different treatments

Treatments	Total plant yield(g)	1000-grain weight (g)
T1 = Control	1.23 e	16.9 e
T2= 40 kg p/ha	2.40 c	29.2 c
T3= 60kg p/ha	3.32 b	31.9 b
T4=90kg p/ha	3.60 a	40.06 a
T5=Phosphobacteria 25 ml/kg of seed	2.38 c	29.9 c
T6=Mycorrhiza, 25ml/kg of seed	2.00 d	20.00 d
T7=Phosphobacteria + mycorrhiza	2.00 d	20.00 d

1000-grain weight: The data revealed that the highest (40.06g) 1000-grain weight was found at T4 followed by T3 (31.9g), whereas the lowest weight (16.09g) was recorded in control followed by T6 and T7 (20.0 g respectively). Phosphate rock minerals are often too insoluble to provide sufficient p for crop uptake. Use of PSMs can increase crop yields up to 70 percent (Verma, 1993). Combined inoculation of arbuscular mycorrhiza and PSB give better uptake of both native p from the soil and p coming from the phosphate rock (Goenadi *et al.*, 2000; Cabello *et al*., 2005).

Bacterial counts at the depth of 0-15 and 15-30 cm after sowing of two week: The highest bacteria per gram of soil were recorded in T4 followed by T3, whereas control showed the lowest number of bacteria per gram of soil. There was not significantly different in T2 and T5, whereas T6 and T7 were identical at the depth of 0-15cm and same trend was found as at the depth of 15-30cm (Table 4).

Bacterial counts at the depth of 0-15 and 15-30 (cm) after harvesting of the crop: The bacteria were significantly increased at T4 followed by T3. The control was lowest in number of bacteria per gram of soil, whereas T6 and T7 were similar. Same trend was recorded as at the depth of 15-30cm after harvesting of the crop (Table 5).

Table-4: Bacteria in one gram of soil after sowing of one week at the depth of 0- 15 and 15-30 cm

Treatments	0-15cm	15-30 cm
T1 = Control	$128x10^2$ e	96×10^2 e
T2= 40 kg p/ha	$158x10^4$ c	131×10^4 c
T3= 60kg p/ha	172×10^6 b	137×10^6 b
T4=90kg p/ha	188×10^8 a	143×10^8 a
T5=Phosphobacteria 25 ml/kg of seed	158×10^4 c	131×10^4 c
T6=Mycorrhiza, 25ml/kg of seed	172×10^3 d	120×10^3 d
T7=Phosphobacteria + mycorrhiza	172×10^3 d	120×10^3 d

Table-5: Bacterial counts after harvesting of the crop at the depth of 0-15 and 15-30 cm

Treatments	0-15 cm	15-30 cm
T1 = Control	$130\text{-}10^2$ e	80×10^2 e
T2= 40 kg p/ha	163×10^4 c	122×10^4 c
T3= 60kg p/ha	179×10^6 b	142×10^6 b
T4=90kg p/ha	200×10^8 a	170×10^8 a
T5=Phosphobacteria 25 ml/kg of seed	163×10^4 c	122×10^4 c
T6=Mycorrhiza, 25ml/kg of seed	150×10^3 d	100×10^3 d
T7=Phosphobacteria + mycorrhiza	149×10^3 d	100×10^3 d

CONCLUSION

Our results suggested that the *Bacillus Megatherium* can significantly established in the soil at the depth of 0-15 and 15-30 cm after sowing of one week and after harvesting of the crop. Phosphobacteria significantly enhanced the biological and grain yield as compared to control. Phosphorus solubilizing bacteria at the rate of 25 ml/kg of seed solubilize the 40 kg p/ ha which were not available for crop utilization.

Due to the higher rates of chemical fertilizers and low income, the farmers limits the fertilizer use and their interest in biological fertilizers is increasing day by day for sustainable agriculture. In our case, the grain yield is increased in T4 and T3 but when we calculate the cost-benefit ratio, the microbial fertilizers could produce reasonable yield. Therefore, the economic yield from wheat could be obtained from wheat with the use of Phosphobacteria alone or in combination with P fertilizers.

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