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EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ON THE PHOSPHORUS AVAILABILITY AND YIELD OF COTTON (GOSSYPIUM HIRSUTUM).

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Abstract: Phosphate solubilizing bacteria (PSB) and plants have symbiotic relationship, as bacteria provide soluble phosphate for the plants and plants supply root borne carbon compounds which can be metabolized for bacterial growth. PSB solubilize the applied and fixed soil phosphorus resulting in higher crop yield. Intensive cropping has resulted-in wide spread deficiency of Phosphorus in our soils and situation is becoming more serious because of a drastic increase in the cost of phosphatic fertilizers. Keeping in view the capabilities of microbes (Bacillus sp.), a field experiment was conducted on cotton at farmer field district Faisalabad in 2008. Effect of PSM (Bacillus spp.) was studied at three phosphorus levels i.e.20, 40 and 60 kg ha-1 while N was applied at recommended dose (120 kg ha-1). Bacillus spp. was applied as seed coating to the cotton crop (Var.BT-121).Recommended plant protection measures were adopted. Results revealed that Bacillus spp. significantly increased the seed cotton yield, number of boll plant-1, boll weight, plant height, GOT (%), staple length, plant P and available P in the soil. Maximum seed cotton yield 4250 kg ha-1 was obtained with Bacillus inoculation along with 60 kg of P followed by 4162 kg ha-1 with Bacillus inoculation and 40 kg of P compared with their respective controls i.e.4093 and 3962 kg ha-1respectively. Soil P was improved from 8.1 to 9.5 ppm by Bacillus inoculation. Phosphorus in plant matter was also higher (0.39%) as compare with control (0.36%). Rhizosphere soil pH was found slightly decreased (8.12 to 8.0) by Bacillus inoculation compare with control. It is concluded that PSB inoculation not only exerts beneficial effect on crop growth but also enhances the phosphorus concentration in the plant and soil.

Key words: Cotton, Phosphate solubilization, Bacillus, Available phosphorus

Introduction

Phosphorus is not deficient in most of the soils but crops show the symptom of its deficiency (3). It is due to the fact that almost 75–90% of added P-fertilizer is precipitated by metal cation complexes (40) like calcium in calcareous soils of Pakistan (22). Phosphate solubilizing bacteria are used as biofertilizers since 1950,s (26,28).Soil micro organisms are responsible to intervene the soil processes such as decomposition, nutrient mobilization, mineralization, solubilization, nitrogen fixation and growth hormone production (10,25). Phosphate solubilizing bacteria (PSB) have been reported to increase P-concentration by converting insoluble forms to soluble ones through the production of organic acids (9, 29) and hence increased the crop yields (17, 45). Inoculation of soil with P-solubilizing microorganisms is a promising approach that may alleviate the deficiency of phosphorus (8). This bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species and nutritional status of soil (23). Species of the genus Bacillus and Pseudomonas have been identified by many workers as P-solubilizers (36, 43).Bacillus solublizes the phosphorus in the rhizosphere and makes it available to plants (16, 18,35). These microbes

1-For Proof and Correspondence: **Naseem Akhtar** lowers the soil pH (19) by secreting different types of organic acids like carboxylic acid and thus dissociate the strong bonds of phosphate in the calcareous soil. Use of microbial inoculants, enhance the nutrient availability and nutrient use efficiency (18).Biofertilizer are cheaper, easy to handle and have no hazardous effect on the environment (12). It has also been assumed that the amount of total phosphorus, being added to arable soils, is sufficient to sustain maximum crop yields worldwide for about 100 years (15, 16). This situation has certainly brought the subject of phosphate solubilization to the revolutionary edge and it will result in less dependence on costly mineral fertilizers in future.

Present study was designed to explore the potential of Bacillus for its P-solubilizing capacity and effect on the yield parameters of cotton.

Materials and methods

Soil samples and Isolation of Bacillus

Bacillus was isolated from the rhizosphere soil of cotton growing area of Fiber Crop Section, in the laboratory of Soil Bacteriology Section AARI, Faisalabad, by dilution plate technique. For the isolation of Bacillus, rhizosphere soil suspension was placed in the oven for heat shock at 80 °C for 10 minutes and on cooling inoculated on the selective medium (30). Plates were incubated at 28 ± 2 °C for seven days. The growth of Bacillus was purified and screened out on the Pikovskaya medium (33). From each plate, the growth was picked and sub-cultured repeatedly to get a pure culture. After preliminary screening following the standard methods as summarized in Bergey's Manual of Systematic Bacteriology (27) the pure culture was predicted as Bacillus megaterium.

Determination of auxin biosynthesis and Phosphate solubilization of isolates

Screening of Bacillus megaterium (ten samples) was carried out for its auxin biosynthesis potential. The isolates of Bacillus were inoculated on Pikovskaya's broth culture for 72 hours. The auxin biosynthesis potential was determined as Indole-3-acetic acid (IAA) equivalents using Salkowski's reagent (2 mL of 0.5M $FeCl_3$+98 mL of 35% $HClO_4$) as described by Sarwar et al. (36). Bacillus isolates, exhibiting the highest auxin biosynthesis were selected for the study of phosphate solubilization.

The Phosphate solubilization capacity of Bacillus isolates (5 samples) were checked on the Pikovskaya's medium (33). Out of five, two isolates were proficient to solubilize insoluble phosphates in the Pikovskaya's medium by forming the halos. The growth and solubilization diameter were determined after incubation at 28 ± 2 °C for seven days. On the bases of diameter of clearing halo zones, solubilization index (SI) (13, 31, 43) was calculated using the following formulae.

$$SI = \frac{\text{colony diameter} + \text{halozone diameter}}{\text{Colony diameter}}$$

Auxin biosynthesis of Bacillus isolates ranges from 2.1-3.7 ppm. Isolates of Bacillus with highest auxin biosynthesis potential and phosphate solubilization were selected for experiment (Tab 1). Inoculum of Bacillus was prepared in Pikovskaya broth medium (33) and incubated at 28 + 2 °C under shaking at 100 rpm for three days. Leaf mold as carrier was processed and sterilized at 121 °C and 15 psi pressure for one hour and inoculated with broth cultures of B. megaterium (10 ml per 100g of peat) and incubated at 28 ± 2 °C. It carries 10^8 CFU g^{-1} of carrier.

Field experiment

Experiment was conducted at farmer's field district Faisalabad during the year 2008, having medium textured soil with pH 8.2, EC 1.3 dSm-1, N 0.030% and available P 7.0 mg kg-1. Three levels of phosphorus viz. 20, 40 and 60 kg ha-1and recommended doze of N (120 kg ha-1) was applied as per treatment i.e.

1: 120-20 kg NP ha-1
2: 120-40 kg NP ha-1
3: 120-60 kg NP ha-1
4: T1 + Bacillus inoculation
5: T2+ Bacillus inoculation
6: T3 +Bacillus inoculation

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Data regarding seed cotton yield was recorded in three picking. Plant height and no. of bolls were recorded before picking while staple length, seed of cotton and ginning turnout were determined after the picking, pH, plant P and available P were find out after harvesting the crop. Phosphorus was determined by modified Olsen method (32). Data were subjected to statistical analysis by following RCBD using standard procedures (39). The difference among the treatment means were compared by applying the Duncan's multiple range tests (11).

Results and discussion

Seed cotton yield and plant height

Inoculation of seed with B. megaterium significantly effects the plant growth. Seed cotton yield kg ha-1(4213) was significantly higher (Table 2) by inoculation compared to control(4022).Results are in line with the finding that PSB containing biofertilizer make the soil phosphorus available for plant growth(18). Results are further confirmed by previous work that inoculation of Bacillus sp. increased the yield of wheat (34) and use of PGPR having P-solubilizing capacity is an effective approach for enhancing yield of crops (41,46,47).Increase in seed cotton yield was 4.7%. Maximum yield was observed by inoculation (4250 kg ha-1) at full dose of fertilizer. Significantly higher plant height (133.3cm) was observed by inoculation over control (130.3). Maximum height was with Bacillus (135.8cm) at full dose of fertilizers (120-60 NP kg ha-1) which is higher than its respective control (134.4 cm). Results are in line with previous finding (4, 5, 20, 21, 24), who observed increase in plant height by microbial inoculation.

Staple length and ginning turnout

Staple length was affected by the inoculation of seed with B. megaterium at sowing. Significant increase in staple length (5.8 cm) and ginning turnout (38.6) was observed by inoculation (Table 3) compared to their respective controls. Increase in staple length was 3.6% while GOT was increased by 2.3%. Maximum staple length was 6.1% while GOT was 39.6% by inoculation at full dose of fertilizer. Results are corroborating by that PSB containing biofertilizer make the accumulation of phosphorus in plant and enhance the yield of pea and barley (7). According to Asea et al., (2) Bacillus megaterium is the most effective PSM with reference to field experiments.

Boll weight and number of bolls

Number of bolls and boll weight was affected by the inoculation of B. megaterium. Inoculum non-significantly affects the no. of boll. Significant increase in boll weight (9.2 g) was observed by inoculation. (Table 4) compared to its respective control (8.8). Increase in boll weight was 4.5% by inoculation. Maximum boll weight was 9.2 g while number of boll plant-1 was 140 by inoculation at full dose of fertilizer. Similar results were found by

Turan et al 2006 (42) who observed the increase in plant growth by Bacillus inoculation. Results are further confirmed by previous finding that phosphorus uptake efficiency increased with phosphorus application along with inoculation (38).

Seed of cotton and soil pH

Effect of inoculation was observed (Tab. 5) non-significantly on seed percentage of cotton with all the treatments. However inoculation showed low percentage of seed (61.4) compared to control (62.3%). Inoculation lowers the soil pH non significantly by producing organic acids. The release of organic acids by PSB reduces the pH in the rhizosphere (1, 13, 46).

Plant and available Phosphorus

Phosphorus contents in the plants were increased non-significantly by the inoculation of B. megaterium. Plant phosphorus(Tab 6) was increased in plant matter (0.39%) compared to control (0.36%) while the available phosphorus in the soil increased significantly by inoculation(8.6mg kg-1) than control(7.8mg kg-1). Maximum plant Phosphorus was observed by inoculation (0.43%) at full dose of fertilizer and increase in available Phosphorus was 10.3% by inoculation over control. Previous finding confirmed the results that PSB give better uptake of both native P from the soil and P coming from the phosphatic rock (6, 14).

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Table 1. Some important features of isolate tested during the investigation.

Isolates	IAA equivalents ($\mu g\ mL^{-1}$)	Gram reaction	Solubilization Index (SI)
Bacillus megaterium	3.1	+ve	3.7

Tab 2. Effect of phosphate solublizing microorganism on seed cotton yield and plant height

Treatments	Seed cotton yield (kg ha^{-1})			Plant height (cm)		
Kg NPha^{-1}	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
120-20	3880	3991	3935.5	124.5	130.3	127.4
120-40	3962	4162	4062.5	131.9	133.9	132.9
120-60	4093	4250	4171.5	134.5	135.8	135.2
Mean	4022 B	4213 A		130.3B	133.3A	
LSD	185			2.670		

*Means followed by different letter(s), differ significantly using LSD test ($p<0.05$)

Tab 3. Effect of phosphate solublizing microorganism on Staple length and GOT of cotton.

Treatments	Staple length (cm)			GOT (%)		
Kg NP ha^{-1}	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
120-20	5.3	5.4	5.35	37.1	37.6	36.85
120-40	5.7	5.8	5.75	37.6	38.7	38.15
120-60	5.8	6.1	5.95	38.5	39.6	39.05
Mean	5.6B	5.8A		37.7B	38.6A	
LSD	0.1877			0.6522		

*Means followed by different letter(s), differ significantly using LSD test ($p<0.05$)

Tab 4. Effect of phosphate solublizing microorganism on boll weight and No. of bolls of cotton.

Treatments	Boll weight (g)			No. of bolls plant^{-1}		
Kg NP ha^{-1}	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
120-20	8.7	8.8	8.75	133	133	133.0
120-40	8.8	9.0	8.9	135	134	134.5
120-60	9.0	9.2	9.1	136	140	138.0
Mean	8.8B	9.2A		135	136	
LSD	0.3017			NS		

*Means followed by different letter(s) differ significantly using LSD test ($p<0.05$)

Tab 5. Effect of phosphate solublizing microorganism on Seed percentage and pH of post harvest soil of cotton.

Treatments	Seed (%)			pH		
Kg NP ha^{-1}	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
120-20	62.9	62.4	62.65	8.12	8.06	8.09
120-40	62.4	61.3	61.85	8.15	7.97	8.06
120-60	61.5	60.4	60.95	8.08	7.98	8.03
Mean	62.3	61.4		8.12	8.0	
LSD	NS			NS		

*Means followed by different letter(s) differ significantly using LSD test ($p<0.05$)

Tab 6. Effect of phosphate solublizing microorganism on plant matter of cotton and available Phosphorus

Treatments	Plant P (%)			Available P (mg kg^{-1})		
Kg NP ha^{-1}	Un-inoculated	Inoculated	Mean	Un-inoculated	Inoculated	Mean
120-20	0.32	0.36	0.34	7.5	7.8	7.65
120-40	0.35	0.37	0.36	7.9	8.6	8.25
120-60	0.40	0.43	0.42	8.1	9.5	8.8
Mean	0.36	0.39		7.8B	8.6A	
LSD	NS			0.791		

*Means followed by different letter(s) differ significantly using LSD test ($p<0.05$)