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Evaluation of Rock and Super Phosphates Effects on Yield and Yield Components of Rice (*Oriza sativa* L.) With and Without Phosphate Solubilizing Bacteria

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Abstract: Impact of phosphorus application which is one of the major nutrient elements that could limit agricultural production was studied in form of phosphorus solubilizing bacteria (PSB), *Bacillus coagulans*, with and without various amounts of phosphorous fertilizer on rice under both pot and field experiments. The filed experiment was conducted in spring season of 2005 at the Rice Research Institute located in Tonekabon- Iran at 50.53, N and 36.49, E. A RCBD design with three replications was used. Fertilizer treatments included of granule bio-phosphate (100 kg ha^{-1}), phosphate solubilizing bacteria (*Bacillus coagulans*), rock phosphate (A or apatite, 150 kg ha^{-1}), super phosphate(150 kg ha^{-1}), super phosphate(75 kg ha^{-1}), super phosphate(75 kg ha^{-1}) + phosphate solubilizing bacteria, super phosphate(150 kg ha^{-1}) + phosphate solubilizing bacteria and rock phosphate + phosphate solubilizing bacteria. Some of studied experimental variables were comprised grain yield, full filled grain, effective tiller (fertile tiller), 1000-grain weight and length of panicle. The results of this study indicated that, there were no significant differences in fertile tillers and length of grain in both pot and field conditions. But significant differences in 1000-grain weight and number of filled grain were observed in both pot and field experiments. The results also showed that grain yield was increased by combined treatments PSB inoculation with rock phosphate or TSP. In the meantime the most grain yield per panicle was observed in the PSB and the least in the control treatments. The lowest and the highest dry weights were obtained in plants treated with A+PSB and TSP, respectively at the anthesis.

Key words: Phosphate solubilizing bacteria · Rock phosphate · Super phosphate · Rice

INTRODUCTION

Phosphorus (P) is one of the major nutrient elements which limits agricultural production in the word. It is added to the soil in form of fertilizers, apart from which is utilized by plants the rest will rapidly be converted into insoluble complex in the soil [1].The P-content in average soils is about 0.05% (w/w) but only 0.1% of the total P is available to plants [2]. This leads to the need of frequent application of fertilizers, but its use on a regular basis has become a costly affair and also environmentally undesirable [3]. Therefore, the necessity to develop economical and eco-friendly technologies is steadily increasing [1]. Natural phosphate rocks have been recognized as a valuable alternative for P fertilizer [3]. Traditional P fertilizer production is on chemical processing of insoluble mineral phosphate high-grade ore, which includes an energy intensive treatment with sulfuric acid at high temperature. This process is environmentally undesirable, not only because of the release of contaminants into the main product but also gas streams and by-products [1]. Many soil microorganisms, including bacteria and fungi, are able to mobilize sparingly soluble inorganic and organic phosphates and they have an enormous potential in providing soil phosphates for plant growth [4,5]. However, use of P fertilizer has become a need for plant production. These phosphate-solubilizing microorganisms render insoluble phosphate into soluble form through the process of acidification, chelation and exchanges reactions [6]. An increase in P availability to

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plants through the inoculation of PSB has also been reported previously in pot experiments and under field conditions [7-9].

Recent study [10-12] have shown that rock phosphate may be a suitable substitute for chemical phosphatic fertilizer in aquaculture, especially in acidic soils.

Recently some researchers have shown that by inoculating wheat seeds with pock phosphate solubilizing microorganisms under field conditions it is possible to obtain the some amount of wheat grain yields compare to those produced by the expensive diammonium phosphate (DAP) fertilizer [13,14]. *Bacillus sp*. has been identified to be the most effective agent in the process [15], which has been clearly demonstrated in aquatic systems with rock phosphate as an insoluble source of P [16].

In the present work we describe how the efficient rock phosphate and phosphate solubilizing bacteria were obtained from Iranian soils and selected for based their potential as the plant growth promoting for rice yield in Iran.

MATERIALS AND METHODS

Field Experimental Design: Field experiment was conducted with nine treatments, including of control (without fertilizer), granule bio-phosphate (*Bacillus coagulans*+ZnSO4+organic matter), phosphate solubilizing bacteria (*Bacillus coagulans*), rock phosphate (A or apatite, 100 kg ha^{-1}), super phosphate (150 kg ha^{-1}), super phosphate% 50(75 kg ha^{-1}), super phosphate%50+phosphate solubilizing bacteria, super phosphate+phosphate solubilizing bacteria and rock phosphate+phosphate solubilizing bacteria. Field experiment was conducted at the Rice Research Institute located in Tonekabon- Iran at 50.53, N and 36.49, E, in spring season of 2005.

A randomized complete block design with three replications was also used. Each plot area was 3×4 m (12 rows of 4m spaced 25cm apart) for the plant crop. The seedling were planted in May 2005 and harvested 3 months later in August 2006. All plants received 100 kg K ha^{-1} as KCl and 100 kg N ha^{-1} as urea.

Some physicochemical properties of field's soil (from 0-20 cm depth), were: pH 7.4, P 10.2 mg ha^{-1}, K 490 mg ha^{-1}, electrical conductivity (saturation extract) 1.28 mmhos/cm, sand 48%, silt 42.4% and clay 9.6%.

Bacterial Strain: One bacterial strain was isolated from the field-grown crop's rhizosphere and identified as *Bacillus coagulans* in Soil and Water Research Institute located in Tehran. On 5 randomly chosen plants in the two central rows of each plot, the number of tillers per shrub was counted 40days after seedling.

At different growth stages, dry weight of stem with leaves (top dry weight) was measured. At the time of final harvest five panicles from each treatment were randomly taken for counting the numbers filled grains per panicle. Yield components and morphological characteristics were measured as Standard Evaluation System (SES): INGER [17]. Plants were dried at 70^{0C} air oven for 72h [9]. The grain yield was measured from the collected forty plants for each replication.

Pot Experiment: Pot experiment was carried out in the greenhouse of University of Tehran, Aboureihan College during 2006 cropping season.

Pots were filled with 4.5kg soil first and the same treatments as field experiment with four replicates were used. Therefore, 36 pots (nine treatments 1 replicate) were arranged in a randomized complete block design with four replicates. Twenty seeds were sown in each pot. Rice seeds were placed at the same depth (approximately 2.5cm below the soil surface) in each pot. Cycle of 10-15h natural light, 20-23°C temperature and 70% humidity were supplied during growth period. Plants were thinned at the earliest to maintain the desirable number of uniform plants (seven seedlings per pot). To maintain the plants, under flooded conditions the pots were watered was slowly over the top soil. Watering was maintained at this moisture content every 1-2 days.

Dry weight of plant samples were recorded at anthesis. Remaining plants were harvested at full maturity. Plants were dried at 70°C air oven for 72h. After harvesting grain yield, straw yield and yield components were also recorded.

Rice Cultivar: Shiroodi cultivar with 120-130 days growth and photoperiod sensitive was used. Seeds were obtained from the collection of the Rice Research Institute located in Tonekabon, North of Iran.

An analysis of variance (ANOVA) and Duncan's multiple rang test (at $P<0.05$) were performed using SAS 7.9 to analyze the statistical differences and to discriminate between means [18].

RESULTS

Field Experiment: The results showed that significant differences were observed in the total number of tillers

Table 1: Mean comparison of yield components, HI and grain length of rice cv. Shiroodi affected by different rates of super phosphate (TSP), rock phosphate (Apatite) and phosphate solubilizing bacteria (PSB)

S.O.V.	Number of fertile tiller per plant	Panicle length (cm)	1000 - grain weight (g)	Number of filled grain per panicle	Number of empty grain per panicle	Grain length (cm)	Harvest index (HI%)
Pot experiment							
Control	2.5a	25.5a	28.85bc	85.62ab	15.62abc	1.1a	40.9a
Bio-phosphate	2.25a	25.75a	27.00c	83.37ab	17.62abc	1.1a	38.2a
TSP	2.37a	26.37a	29.97ab	80.16b	20.25ab	1.1a	37.3a
PSB	2.5a	26.00a	28.92bc	88.37ab	16.12abc	1.1a	40.0a
TSP50%+PSB	2.0a	26.00a	30.92a	81.12ab	22.17a	1.1a	39.8a
Apatite	2.12a	26.00a	28.00bc	88.25ab	14.5bc	1.1a	41.9a
TSP50%	2.5a	26.37a	28.47bc	91.00ab	12.62c	1.1a	42.2a
TSP+PSB	2.0a	26.37a	28.42bc	86.50ab	12.75bc	1.1a	38.0a
A+PSB	2.37a	26.00a	28.07bc	94.50a	12.12c	1.1a	40.1a
CV%	2.3	5.40	4.50	6.40	7.5		3.2
Field experiment							
Control	25.0a	27.9ab	30.8ab	105.7a	19.0a	1.1a	53.4a
Bio-phosphate	24.0a	27.7b	28.9d	102.7a	19.3a	1.1a	53.1a
TSP	25.0a	27.8ab	29.5dc	103.8a	19.5a	1.1a	52.6a
PSB	22.0a	29.4ab	29.9abcd	112.3a	21.1a	1.1a	53.6a
TSP50%+PSB	23.0a	29.7a	30.9ab	118.3a	19.1a	1.1a	51.6a
Apatite	23.3a	27.9ab	31.1a	109.0a	21.6a	1.1a	55.4a
TSP50%	25.3a	28.9ab	30.6abc	112.3a	18.5a	1.1a	51.0a
TSP+PSB	25.3a	29.1ab	29d	115.5a	22.7a	1.1a	56.1a
A+PSB	23.7a	28.2ab	29.8bdc	113.5a	22.7a	1.1a	56.3a
CV%	10.97	3.32	2.19	12.94	26.29		6.14

1-Within a column, means followed by the same letters are no significantly difference at the Duncan's multiple range tests

(Table 1). This had ranged from 19.3 in TSP50%+PSB to 33 in the rock phosphate (apatite) treatment (Fig. 3). Comparing bacterial treatments indicated that the bio-phosphate had more tillers than PSB. No significant differences were observed for fertile tillers between different treatments.

The longest panicle was obtained from TSP50%+PSB and the shortest one was from the granule bio-phosphate Therefore, the length of panicle was higher in treatments where *Bacillus coagulans* such as PSB, TSP50%+PSB and A+PSB were applied. Addition of TSP did not provide the length panicle than in control treatment.

Data in Table 1 showed that there were significant differences between treatments 1000-grain weight, so that the lowest grain weights were obtained from the granule bio-phosphate and TSP+PSB. Super phosphate fertilizer decreased 1000-grain weight compare to control treatment. The highest 1000-grain weight was produced with apatite under field conditions. When TSP50% was used along with PSB, a much greater effect was observed compare to TSP (150 kg ha^{-1}) and PSB combination.

The highest grain yield in hectare of (7593.7 kg h^{-1}) was recorded in treatment, which received TSP50%. Apparently, the P applied as the TSP50% was sufficient for the grain yield (Fig. 1). The lowest grain yield (5647.5 kg h^{-1}) was recorded in TSP50%+PSB. In general, the bacterial treatments had desirable grain yield. For example, A+PSB was produced more grain yield than control.

There were no differences in harvest index under field conditions (Table 1). Data also showed that dry weight at 20 (day after seedling) DAS or 276.1°C GDD (growing degree day) was no significantly different between treatments (Fig. 5), but at 56 DAS (GDD=857.5) obvious differences were seen among treatments. In this period the super phosphate fertilizer increased dry weight more than other treatments. This policy maintenance to final anthesis period (GDD=1153.3). At anthesis, the lowest and the highest weights were observed in A+PSB and TSP, respectively. This trend was different at the maturity (GDD=1381.7). So that biomass (dry weight) ranged from 918.8gram in apatite to 1263.3grm per m^2 in A+PSB (Fig. 6).

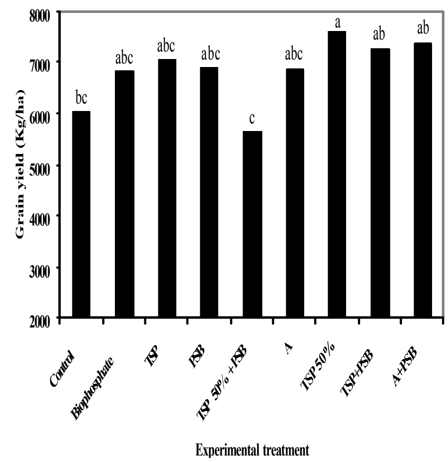

Fig. 1: Mean of grain yield per hectare affected by different rates of TSP, rock phosphate and PSB in field

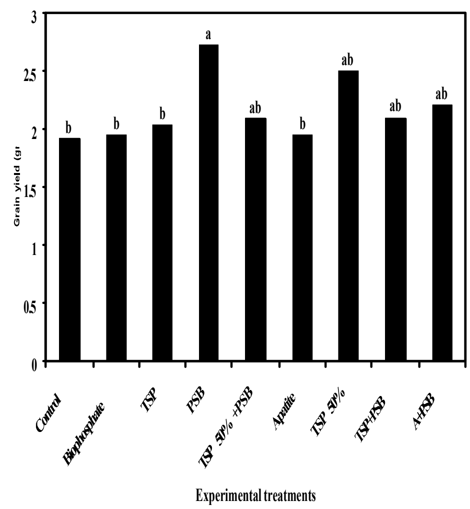

Fig. 2: Mean of grain yield per panicle affected by different rates of TSP, rock phosphate and PSB in greenhouse

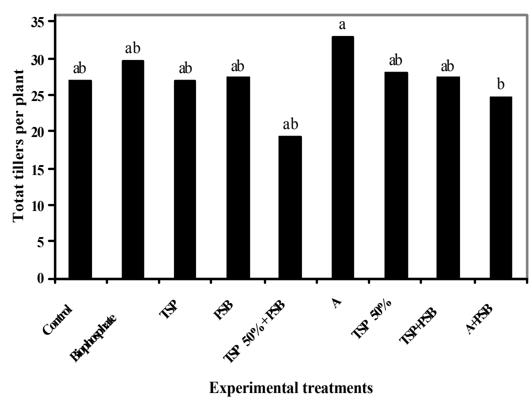

Fig. 3: Mean of total tillers affected by different rates of TSP, rock phosphate and PSB in field

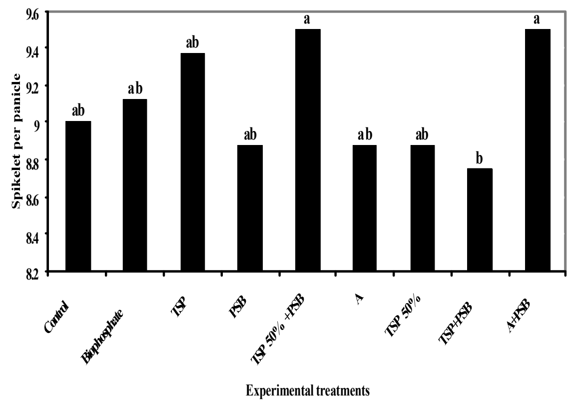

Fig. 4: Mean of spikelet number per panicle affected by different rates of TSP, rock phosphate and PSB in greenhouse

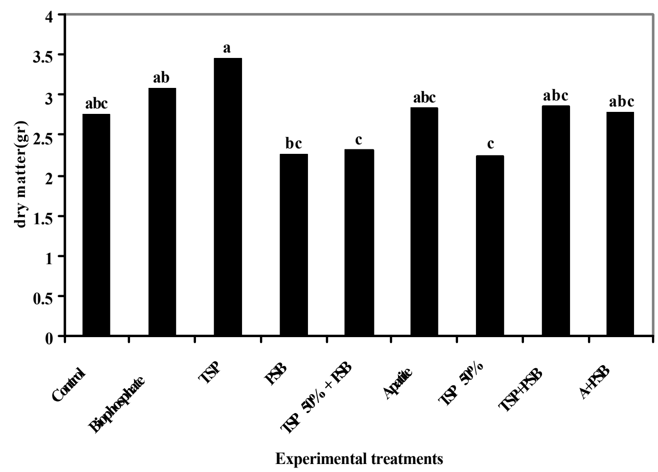

Fig. 5: Means of grain dry matters affected by different rates of TSP, rock phosphate and PSB in greenhouse at (anthesis period)

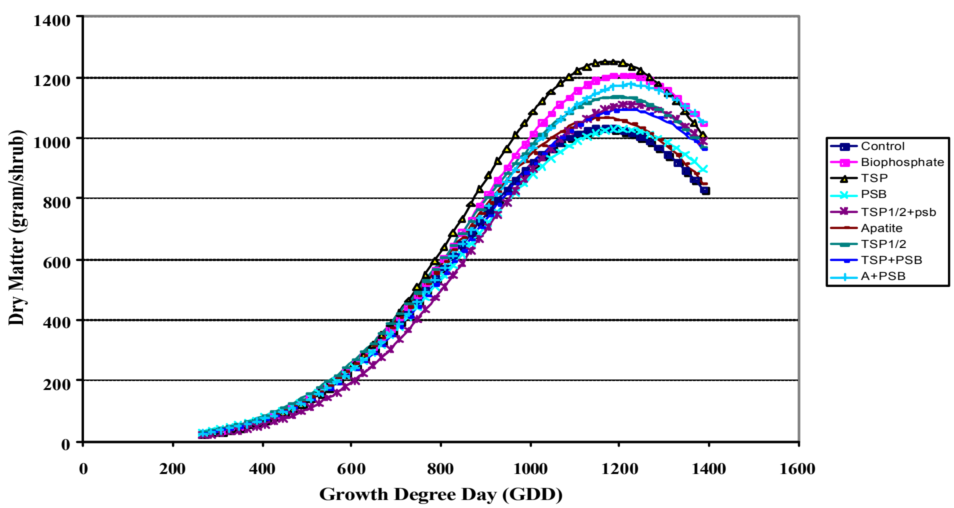

Fig. 6: Mean grain Dry Matter as affected by different rates of TSP, rock phosphate and PSB in field condition

Pot Experiment: The obtained data did not show significant differences between treatments for fertile tillers in pot experiment. The number of spikelet per panicle ranged from 8.75 in TSP+PSB to 9.5 in the A+PSB and TSP50%+PSB (Fig. 4). This trait was the same at most treatments. In addition, for panicle length no significant differences were observed among treatments (Table 1).

The applied treatments significantly varied the 1000-grain weight (Table 1). The lowest weights obtained from bio-phosphate, apatite, A+PSB, control and PSB, respectively. The highest 1000-grain weight was observed in the TSP50%+PSB. Data also showed that rock phosphate (Apatite) and PSB incorporation did not increase 1000-grain weight (Table 1). When TSP applied in pots more grain weight was generally seen than control treatment. Considering the harvest index (HI%), the results showed that there was no differences among treatments, Whereas the highest and the lowest harvest index were observed in TSP50% and TSP, respectively.

The results mentioned that the highest and the lowest filled grains per panicle were in A+PSB and TSP respectively (Table 1 and Fig. 2). Further, apatite and PSB incorporation had the lowest empty grains per panicle. TSP50% had filled grains as well as A+PSB and also had a few empty grains. Comparing the control with other treatment, there were some increases for empty grains. When the bacterial treatments were compared with each other, no significant differences were observed between filled and empty grains per panicle in bio-phosphate and (Table 1).

The maximum grain yield per panicle was recorded (2.73 g) in PSB and the minimum (1.92 g) in control. The inoculated plants with PSB produced more grain weight per panicle than bio-phosphate treatment but the grown plants with rock phosphate (apatite) had the same as bio-phosphate. Substitution of TSP50% with rock phosphate caused significant reduction in grain yield as compared to the supplied TSP50%. But no such reductions were noticed when rock phosphate was used along with PSB.

DISCUSSION

It was apparent from the results that the various treatments had a different inherent potential for improving plant yield, yield components and dry weight. The changes observed, regarding growth values by rock phosphate and/or bacterial inoculation was sometimes comparable to those of P-application.

Results are showing that the effect of the granule bio-phosphate on 1000-grain weight in the pot experiment was similar to field experiment. The decrease of 1000-grain weight in inoculated plants by bio-phosphate may be due to inability in solving the insoluble P of soil in aquatic conditions of rice field. Since, P is the most important element it's decline could induce the decreases in grain weight and grain quality [19]. There was a close relationship among 1000-grain weight, filled grain and empty grain under pot experiment. In general, when the number of filled grain increased it could cause a decline in 1000-grain weight in inoculated plants and A+PSB, but TSP50% had the maximum amount of filled grains. These results showed that where had not only the lowest 1000 grains weight but also the highest empty grains. The number of filled grains had a direct relation with available phosphorus rates. Because of decline in P severity, grain production was weak or grains were empty and unfertile [19]. The number of filled grains is more important trait than 1000-grain weight [20,21]. The results of pot experiment showed that TSP had not only the great 1000-grain weight but also the lowest filled grain and many empty grains.

The results of this study indicated that there were no significant differences in grain length among both pot and field experiments. This is an expected result, since rice seeds are covered by interior and exterior glumes. Thus, grains growth is limited by these hard covers [22-24]. No significant differences were observed for fertile tillers in both pot and field experiments. This is evident, because decreases in fertile plants occur only under very low Phosphorus conditions. Kobayasi [25] reported that fertile tillers have the most relationship with nitrogen. Space limitation for plants under pot experiment induced the lower number of fertile tillers compared with what was seen in field experiment.

In greenhouse, the dry matter of inoculated plants was found to be significantly more than un-inoculated plants.

Generally, there are two main ways to reach the more grain yield, increasing in HI and/or total dry matter accumulation [26]. Data of the field experiment showed that changes in dry matter accumulation was in sigmoid form and very slow at the primary stages, but when plants reached the anthesis period it increased in line form (Fig. 6). Finally, at stage very close to maturity, dry matter accumulation was decreased. These results were similar to those obtained by Diepenbrock [27]. Data also indicated that added phosphorus fertilizer had not any effect on dry matter at initial growth stages, because control treatment (without inoculums or any P fertilizer) had desirable dry weight at pre-anthesis period. But decrease of dry weight was observed during GDD=857.5°C to GDD=928.8°C. At maturity period, plants treated with rock phosphate (apatite) and plants in control treatment produced the lowest dry weight, because of lack in available P in apatite treatment. Although apatite had not positive effect on dry weight, but when it was used in conjunction with PSB, a great increase was obtained in dry weight. Probably the reason of increasing dry weight was related in production of specific hormones by *Bacillus coagulans*. This result confirm the finding of Gutierrez *et al*. [28,29] who found that microorganisms have the production capability of oxin and gibberellins in rhizospher and could increase dry weight rate in the plants treated with inoculation. Also Resit *et al*. [30] was declared that PSB could increase plant biomass. Sundara *et al*. [31] found that application of PSB combined with apatite is more effective than phosphorus fertilizer.

Comparing dry weight of plants treated with PSB and bio-phosphate, the results showed that inoculated plant with bio-phosphate increased more greatly dry weight than plants inoculated only by PSB. Medina and Probanza [32] found that *Bacillus Pumullus* and *Glomus deserticola* have higher efficiency than chemical phosphorus fertilizer in production of dry weight. Similar reports which were submitted by Puente *et al*. [33,34], Mustafa and Canbolat [35] confirm the increase of dry weight.

Results of pot and field experiments showed that control treatments in both cultured conditions had the lowest yield. This is evident, since plots of control treatment had lack of P fertilizer. Phosphorus is one of the necessary elements [36], if the rate of soil phosphorus is declined, the yield also will decrease. Inoculated plants with PSB in both pot and field experiments had desirable yield. In the field conditions, when PSB was combined with rock phosphate (apatite), yield was more greatly increased. These results are in line with those obtained by Sundara *et al*. [31], who found that application of rock phosphate with phosphate solubilizing bacteria enriched the rhizospher more than the other treatments.

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