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Integration of Phosphate Solubilising Bacteria, Sulphur Oxidizing Bacteria with NPK on Maize (*Zea mays*)

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Abstract. Deficiency of phosphorus can reduce the yield up to 15-20% and its availability to crop plants is the main issue, particularly in calcareous soils of Pakistan. Micro-organisms, phosphorus solubilizing bacteria (PSB) and sulphur oxidizing bacteria (SOB) have the ability to make P accessible for optimum plant growth, under conditions of nutrients disparity. Therefore, six treatments *i.e.* control, ½ dose of NPK, full recommended dose of NPK, ½ NPK+ SOB, ½ NPK + PSB and ½ NPK + SOB+ PSB were applied in a field experiment to investigate the integrated role of SOB and PSB with NPK fertilizers for enhancing the maize production. The treatment was concluded to have major impact on agro-morphological traits, seed quality and growth parameters of maize. Results depicted that the use of ½ NPK + SOB + PSB gave maximum germination count/plot (151.33), plant height (189.03 cm), number of ears/plant (1.60), grains/ear (472.33), 1000-grain weight (305.67 g), grain yield (5350.50 Kg/ha), harvest index (31.23%), leaf area (379.77 cm^2), total dry matter accumulated (181.43 g/plant), crop growth rate (30.60 g/day), net assimilation rate (9.31 g/day) and protein contents (8.49%).

Keywords: maize, PSB, SOB, NPK, growth, quality parameters

Introduction

Maize (*Zea mays* L.) has an important role in Pakistan's current cropping scheme and ranks third after wheat and rice (Farhad *et al*., 2009). Maize is one of the major export crops and with great economic and social importance has an important role in human and animal nutition. It contributes 2.1% in agriculture and 0.4% in gross domestic products (Chandio *et al*., 2016). The growing demand for increased yield in agriculture, also grows the demand for new technologies with less impact on natural resources. The use of micro-organisms that promoter plant growth can suport or even satisfy the demand for nitrogen (N) and phosphorus (P) in different crops (Baldotto *et al*., 2010; Baldani *et al*., 2009).

Phosphorus (P) is a most important growth limiting nutrient (Sharma *et al*., 2013) and plays important role in nearly all phases of plant cycle including root growth, photosynthesis, anthesis, seed production and maturation. Its deficiency causes stunted growth and severe yield losses. Its concentration in soil solution is very low, because soluble forms of P are fixed by soil solid phase, making less than 0.01% of total P available to plants (Niazi *et al*., 2015). Therefore, one phosphorous is one of the least mobile nutrients in soil (Balemi and Negisho, 2012). Most of the P that is present in soil and supplied to crops by inorganic fertilizers becomes unavailable by precipitation by reacting in acidic soils with Fe^{+3} and Al^{+3} and with Ca^{2+} in calcareous soils, respectively (Abbasi *et al*., 2015). Different genera of bacteria have the ability to mineralize and solubilize P pools in soil and these bacteria significantly promot their bioavilability. This process not only compensates the input of high cost fertilizers but it can also enhance the mobilisation of insoluble P already added to soil from the fertilizers. Such group of bacteria is termed as phosphate solubilizing bacteria (PSB) and inoculation with PSB as bio-fertilizers enhances P accumulation and biomass production of plants (Abbasi *et al*., 2015). Phosphates solubilizing bacterial (PSB) species have capacity to mineralize the both natural and inorganic (p) phosphorus (Khiari and Parent, 2005). Phosphorus accessibility from the soil can be extended by phosphate solubilizing bacteria (PSB) with microscopic organisms oxidi-sing sulphur studied by (Turan *et al*., 2007). The immunization of phosphorus solubilizing micro-organisms (PSB) enhanced the yield of crop by solubilizing the phosphate that was associated on soil and stable in soil Gull *et al*. (2004).

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A field survey was directed to determine the influences and impact of (PSB) and diverse levels of (P) fertilizer on yield and quantities of the crop by Sial *et al*. (2015). Diverse microbial inoculums levels were utilized alone and joined with phosphorus fertilizer. Uzma *et al*. (2014) concluded that the levels of inorganic fertilizers from 60-100% and recommended dose of NPKZn increased the cob^{-1} length, 1000-grain weight, grain protein and starch content of maize. (Azotobacter+PSB) showed similar results when dual inoculation applied to the seed. Abbas *et al*. (2013) resulted that the co-inoculation of PGPR and PSB indicated maximum plant height. The treatment with the combination of Iple Iple (II) + PSB + recommended K + ¾ N + ¾ P gave maximum plant phosphorous content. Amanullah and Khan (2015) concluded that phosphorous applied at the higher rates and compost applied at sowing time increased maize yield and maize yield components significantly. The yield and yield components of maize were strongly increased under semi aride conditions with maize seeds. Baloach *et al*. (2014) were studied the combine effects of PSB and humic compounds to improve the yield of maize. The humic acid at 10 Kg/ha + PSB bio-fertilizer at 2 Kg/ha showed maximum results in that parameters biological yield, harvesting index, grain yield and stover yield as equated to control. Amanullah *et al*. (2010) studied the effect of phosphorus on maize yield and growth. Phosphorus has a significant impact on crop growth rate (CGR), dry matter (DM), leaf area index (LAI) and the biological yield from leaf area ratio (LAR), relative growth rate (RGR), net assimilation rate (NAR) has no important effect.(NAR), absolute growth rate (AGR) and (RGR) show the negative effect due to increasing the plant density. Realizing the importance of PSB and SOB in an integrated manner with NPK for improving the nutrients availability especially P, a field experiment was executed to investigate the initial growth and mineral nutrition of maize (*Zea mays* L.) in response to application of NPK rates combined with the inoculation PSB and SOB.

Materials and Methods

A field experiment was conducted at National Agriculture Research Centre, Islamabad (Maize Program) during the autumn, 2016. The treatments were arranged in Randomized Complete Block Design (RCBD) with three replications. Seeds of maize Cv. Islamabad Gold were sown on ridges maintaining. The treatments were controled (without fertilizer), ½ dose of NPK, full recommended dose of NPK, ½ NPK + SOB, ½ NPK + PSB and ½ NPK + SOB+ PSB. PSB and SOB are collected from the microbiology laboratory, Land Resources Research Institute, National Agricultural Research Centre, Islamabad. Maize seeds were inoculated with phosphorus solubilising bacteria (PSB) and sulphur oxidizing bacteria (SOB). Data were collected on growth and yield chrematistics; germination count/plot, Plant height in (cm), ear number/plant, grain/ear, 1000-grain weight (g), grain yield (Kg/ha), harvest index (%), leaf area (cm^2), total dry matter accumulation/ plant (g), crop growth rate (g/m^2/day), net assimilation rate (g/m^2/day) and grain protein contents (%). Data collected were subjected to analysis of variance and the means obtained was compared by LSD at 5% level of probability (Montgomery, 2001).

Results and Discussions

Statistical analysis of the maize germination count revealed a substantial difference between various treatments (Table 1). The maximum germination count was recorded (151.33 plants/plot) by the application of ½ NPK + PSB + SOB, which was statistically equal to

Table 1. Effect of NPK with PSB and SOB on agro-morphological, growth and quality parameters of maize

Treatments	Germination count/plot	Plant height (cm)	Ear number/ plant	Grain/ Ear	1000-grain weight (g)	Grain yield (Kg/ha)	Harvest index (%)	Leaf area (cm^2)	Total Dry matter accumulation/ plant (g)	CGR/g/ day	NAR/g/ day
Control (T_1)	79f	155e	1.07 c	261e	280f	3114f	23f	199f	151f	27e	4c
½ NPK(T_2)	95e	166d	1.27abc	298d	285e	4114e	26e	218e	159e	28d	5c
Full NPK(T_3)	114d	172c	1.20bc	334c	290d	4324d	27d	244d	163d	28d	6b
½ NPK+PSB(T_4)	127c	78b	1.33abc	352c	293c	45324c	29c	302c	171c	29c	7b
½ NPK+SOB(T_5)	140b	182b	1.47ab	429b	297b	4739b	30b	326b	174b	29c	7b
½ NPK+PSB+SOB(T_6)	151a	189a	1.60a	472a	306a	5022a	31a	380a	181a	31a	9a
LSD	6	5	0. 5	21	3	7	1	14	3	0.2	1

Means with different letters are significantly different at 5% level of probability.

½ NPK + SOB (140.67) and ½ NPK + PSB (127.33), followed by complete NPK (114.33 plants/plot) and ½ NPK (95.00 per plot), while the minimum germination was recorded in control treatment (79.00 plants/plot). The results are in confirmatory with Hameeda *et al*., (2008) who described that application of PSB enhanced the germination of maize. Phosphate Solubilizing Bacteria (PSB), which are rhizobacteria that convert insoluble phosphates into soluble forms through acidification, chelation, exchange reactions and production of organic acids.

The maximum plant height (189.03 cm) was recorded in ½ NPK+phosphorous solubilizing bacteria and sulphur oxidizing bacteria followed by treatment ½ NPK + SOB) (182.07) which was found statistically at par with ½ NPK + PSB) (177.90). However, the minimum plant height (155.37 cm) was measured in control. The data reported showed that the application of PSB, SOB or both bio-fertilisers by soil application methods increased height of the plant (Table 1). Experimental findings are in confirmatory with Shafiq and Tahir in (2015).Similar results are also reported by Abbas *et al*. (2013). Release of P by PSB from insoluble and fixed/ adsorbed forms is an import aspect regarding P availability in soils. There are strong evidences that 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 (Khan and Jorgensen, 2009).

The maximum number of ear/plant (1.60) was observed in ½ NPK + PSB + SOB treatment which are statistically at par with ½ NPK + SOB treatment (1.4). The data inferences that ear/plant (Table 1) showed non-significant response of different treatments.. The minimum number of ear/plant (1.06) was taken in treatment (control), whereas T_1, is statistically at par with T_2, ½ NPK (1.27) and treatment T_3, full NPK (1.20) and T4, (1.33) are also statistically at par with each other. Significant difference was found among various treatments on grain/ear (Table 1). The results showed that in treatment, the highest grain/ear (472.33) was counted when ½ NPK + PSB + SOB was applied followed by ½ NPK + SOB (429.33) and treatment ½ NPK + PSB(351.67), which was statistically at par with complete NPK (334.33). It was also cleared from the data that the lowest grain/plant were recorded with T_1 (261.33) followed by T_2 (298.33) respectively. Our findings are similar with Asghar *et al*. (2010), our results are closely in line with Jinjala *et al*. (2016).

1000-grains weight of maize crop was analyzed that significant difference among treatment means shown in (Table 1). The plot which we apply, ½ NPK + PSB + SOB showed maximum 1000-grains weight (305.67) followed by T_5 (297.50), T_4 (293.10), T_3 (289.60) and T_2 (285.10) respectively, whereas the minimum 1000-grain weight (280.33) was detected in that plot which was not use any fertilization T_1 (control). Maize grain weight was increased by the application of NPK due to increased nutrient efficiency. Our findings are in line with Uzma *et al*. (2014). The similar results are also found with Amanullah and Khan (2015).

The statistical data indicated that grain yield was recorded in treatment (Table 1). The maximum grain yield (5022.4 Kg/ha) was observed in the treatment ½ NPK+ PSB + SOB were applied. Followed by T_5 (4739.3 Kg/ha), T_4 (4532.4 Kg/ha), T_3 (4324.7 Kg/ha) and T_2 (3914.4 Kg/ha) respectively. The treatment T_1, (control) shown minimum grain yield (2114.3 Kg/ha). The related results are initiated with Upadhyay *et al*. (2016). Similar judgments were also reported by Baloach *et al*. (2014). The yield is calculated from the 1000 grain weight so, the increasing the grain yield. As we see the significant differences between the weight of 1000 grain and grain yield which are the same. The results are in confirmatory with Amanullah and Khalid (2005) and Singh *et al*. (2004).

Data on the harvest index revealed a substantial difference between the means of treatment (Table 1). The maximum harvest index (31.22) was observed in that plot which we apply both phosphorous solubilizing bacteria and sulphur oxidising bacteria T_6, followed by ½ NPK+SOB treatment T_5 (29.77), T_4 (28.59), T_3 (27.47) and T_2 (25.70), respectively. Whereas, in the plot there is no use of fertilizer (T_1) and which was showed minimum value of control of harvest index (23.45). Results seen indicated in line with Amanullah and Khan (2015). Our findings are similar with Baloach *et al*. (2014).

Data regarding leaf area per plant showed a significant difference between the treatments (Table 1). It was concluded that maximum leaf area/plant (LAP) (379.77 cm^2) was found in treatment, where ½ NPK+PSB+SOB (T_6) was applied. Followed by the treatment (T_5) ½NPK + SOB give (325.77 cm^2), (T_4) ½ NPK + PSB (302.30 cm^2), (T_3) Full NPK (244.20 cm^2) and (T_2) ½ NPK (218.37 cm^2) respectively. However, minimum leaf area/plant (199.33 cm^2) was measured in control treatment (T_1).The results are in confirmatory with the

Banerjee *et al*. (2006).Who concluded that N120SSP30-VAM and N120RP30PSB gave higher yield and yield contributing attributes. The similar results were reported Nwanyanwu *et al*. (2015).

Results inference that maximum dry matter/plant was initiate in treatment (T_6), where ½ NPK + SOB + PSB was applied (181.43 g). The following treatments (174.23 g), (171.0 4 g), (162.87g),(159.53 g) are ½ NPK + SOB (T_5), ½ NPK + PSB (T_4), full NPK (T_3) and ½ NPK (T_2) statistical analysis indicated that significant difference among the treatment means shown in (Table 1). However, the treatment (T_1) minimum dry matter/plant were measured in control. Our results are in line with Banerjee *et al*. (2006).

The maximum crop growth rate (CGR) (30.60 g/day) was observed in crop plant which we applied the treatment T_6, ½ NPK+PSB+SOB followed by T_5 (29.30/ g^2/day), T_4 (28.79/ g^2/day) data concerning crop growth rate indicated significant difference among the treatment means (Table 1). T_3 (28.42/g^2/day) are statistically at par with T_2 (28.26/g^2/day). On the other hand the minimum value of CGR (27.16/g^2/day) was observed in treatment T_3 (control) in which no synthetic and bio fertilizer are used. The results are comparable with Banerjee *et al*. (2006).

Net assimilation rate (NAR) of maize crop was inclined by the application of phosphorus solubilizing bacteria and sulphur oxidizing bacteria through soil application and inferences statistically significant difference (Table 1). T_5 (7.10) are at par with T_4 (6.82) and T_3 (6.50).Results indicated that maximum (NAR) was found in treatment T_1 (9.31), where (½ NPK+PSB+SOB) was applied. The minimum value was found in control treatment T_1 (1.71) which is statistically similar with T_2 (4.44). The study results are in coherent with Amanullah *et al*. (2010).

Analysis of variance showed the statistically significant effect of both PSB and SOB along with ½ NPK on grain proteins content (Table 1).Result depicted that maximum grain proteins content was analyzed in the treatment T_6 (8.49), where ½ NPK, phosphorous solubilizing bacteria and sulphur oxidizing bacteria was applied. Followed by T_5 (7.08), T_4 (6.89), T_3 (6.17) and T_2 (5.56) respectively. The minimum protein content was recorded in the control of treatment T_1 (4.85), and this may be ascribed to intense protein synthesis in plants and its efficient storage in the presence of abundant supply of available nutrients through bio-fertilizer and organics. The easy availability of nutrients leads to balanced C: N ratio which enhanced the vegetative growth of plant resulting in high photosynthetic activity. Which finally out yielded better protein content in plant and higher grain yield which in turn improved the protein yield. The results of present investigation corroborate with the findings of few previous studies (Sharma *et al*., 2013a and b; Pathak *et al*., 2002). Findings of this study are similar with Shafiq and Tahir (2015) who was reported increase in grain proteins with the application of (PSB) *Bacillus subtilis* and *Vesicular arbuscular* mycorrhiza (VAM). Similar findings were also conveyed by Jinjala *et al*. (2016).

Conclusion

Conjunction of NPK with PSB and SOB improved maize growth and the yield and quality. Bio-fertilizers could save the environment and improve the economics of maize growers.

Conflict of Interest. The authors declare no conflict of interest.

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