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Research Article

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Evaluation of Rock Phosphate and Manures with Phosphorus Solubilizing Bacteria for Growth and Yield Attributes of Okra (*Abelmoschus esculentus*)

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Abstract

Phosphorus (P) is one of the most important plant nutrient for increasing soil productivity and sustainable crop production. A pot experiment was conducted to study the effect of seed inoculation by phosphorus solubilizing bacteria (PSB) and organic and mineral P fertilizers on growth, yield and P uptake of okra. Phosphorus was applied at the rate of 90 kg ha^{-1} in the form of poultry manure (PM); sheep manure (SM) and rock phosphate (RP). Nine treatments were allocated for the experiment having PM, SM and RP alone, organic manures with RP in combination, or consortium of all the three along with PSB. The result revealed that sole application of SM at three growth stages gave maximum root and shoot growth, which was 75 and 78% more from control respectively. It was concluded that Leaf area (36%), chlorophyll contents (62%), yield (60%) and P uptake (31%) of okra were significantly enhanced by the integrated use of PM+SM+RP+PSB at all growth stages over control. Complementary application of this inoculants with manures and RP most favored okra growth and yield and can be considered as an appropriate substitute for chemical P fertilizer in organic and sustainable agricultural system.

Keywords: Phosphorus; Organic and inorganic P fertilizers; Phosphorus solubilizing bacteria; P uptake; Okra

Introduction

In order to face the future challenges of increasing population necessitate to increases the crop production. A major constraint to overcome in the crop production is the maintenance of soil productivity. Highly utilization of inorganic fertilizers leads to P fixation in soil, nutrient imbalance and soil acidity, so improvements of environmental conditions as well as the need to reduce the cost of fertilizing crops are reason for advocating use of organic materials. Phosphorus (P) is a necessary macronutrient for biological growth and development of plants (Fernandez *et al*., 2007). It plays a key role in the growth and development of roots, crop maturity, photosynthesis, cell division, grain formation and transfers of heredity (Sharma, 2002). Phosphorus mainly occurs in soil as apatite, like fluor-chlor-hydroxy, carbonate, apatite and rock phosphate (Paul and Clark, 1989). The mobility of this element is very slow in the soil and cannot respond to rapid uptake by plants and they only utilize a small amount of P from applied sources (Salehrastin, N., 1999). It is easily transformed into insoluble complexes with calcium, aluminum and magnesium (Sample *et al*., 1980). Phosphate ions are fixed and precipitated in soil, because of their large reactivity with soil compounds (Rodriguez and Fraga, 1999; Fernandez *et al*., 2007). Mineral fertilizer increased vegetative growth, yield and quality of crop (Nkoa *et al*., 2001; Babik and Elkner, 2002). Rock phosphate (RP) is a cheap alternative source of P has been applied which becomes familiar throughout the world (Zapata and Axmann, 1995). Pakistan RP reserves are 7.45 million tones located at Kakul (0.75 milllion tones) and Lagarban (6.7 million tons) in Hazara division of Khyber Pakhtunkha (Khan and Khan, 1988). Rock phosphate is not readily available to plants due to the slow release from the minerals (Rajan *et al*., 1996). Direct use of RP may be agronomically more constructive and environmentally more feasible than soluble P (Singh and Amberger, 1998). Organic manures improves the soil structure, aeration, reduce water and wind erosion, increase water-holding capacity, support root development leading to higher yield and better quality of plants (Abou *et al*., 2005). On the other hand, soil microorganisms release P from organic pools of total soil P by mineralization such as phosphate solubilizing bacteria (PSB), which are mostly present near the plant roots, and metabolically more active (Vazquez *et al*., 2000). To improve plant nutrient contents and crop production, the sustainable solution of PSB as bio-fertilizer was used (Vessey, 2003). By solubilization PSB can help in increasing the availability of accumulated phosphates for plant growth (Richardson, 1994). P solubilization by microorganisms is one of the most important traits associated with P availability in soils (Chen *et al.*, 2006). Okra is a flowering plant in the family Malvaceae (*Abelmoschus esculentus*), originating from tropical, subtropical and West Africa (Tindal and Rice, 1983). It is now grown in all parts of the tropics and during summer in the warmer parts of the temperate regions. It is grown from March to June in Pakistan and is very delicious vegetable (Abid *et al.*, 2002). For improving the fruit yield and balance nutrient supply to the crop, organic and inorganic fertilizers can also be given in combinations (Mario *et al.*, 1989). The combination of ground RP with PM appreciably enhanced growth and yield of okra compared to application of each material separately (Akande*et al.,* 2003). Okra is grown in various ecologies around the world; however there was not a great deal with P sources effect on its growth, yield and P uptake. The present investigation was carried out to check the effect of organic manures as well as rock phosphate and PSB on the growth, yield and P uptake of okra.

Materials and Methods

Potting media: Soil was collected, air dried, ground and passed through a 4 mm sieve. Sandy clay loam with with composition of sand 47.74%, silt 26.13% and clay 26.13% having pH 7.85, EC (dS m^{-1}) 0.065, organic matter content 0.91%, N 0.072%, and available P (1.03 mg kg^{-1}) was filled in clay pots with 10kg capacity.

Bacterial strain: An agro-bacterium strain of PSB (Ca-18) was obtained from National Institute of Biology and Genetic Engineering (NIBGE), Faisalabad. Inoculum of PSB was prepared in LB broth media, containing about 10^8 CFU ml^{-1}. Experimental Layout: A pot experiment was conducted to evaluate the effect of different P sources on growth, yield and P uptake of okra. Inoculation of seed was done by dipping the seeds in inoculum for half an hour. Five healthy seeds of okra were sown in each pot and three seedlings were maintained after thinning.

Treatments: Nitrogen was applied at a rate of 120 kg/ha as basal dose in the form of urea. Phosphorus was applied at the recommend rate of 90 kg P ha^{-1} in the form of RP (30% P), PM (1.71% P) and SM (0.42% P) on the basis of their P content. The experiment was arranged in a complete randomized design with 9 treatments replicated 3 times. Treatments were: T1 (control), T2 (PM), T3 (SM), T4 (RP), T5 (RP+PM50:50), T6 (RP+SM50:50), T7 (RP+PM+PSB 50:50), T8 (RP+SM+PSB 50:50), T9 (RP+PM+SM+PSB 50:25:25).

Data collection: Data was recorded for root and shoot growth characteristics, leaf area, chlorophyll contents (Wintermans and Demots, 1965) to assess the plant growth. Pod length was measured with a meter rod and plant samples were taken at each growth stage (vegetative, flowering and maturity) for determination of total P according to the procedure of Soltanpur and Workman (1979). Post-harvest soil samples were collected and analyzed for nitrogen (%) by the method of (Bremner and Mulvaney, 1982); while potassium (mg kg^{-1}) and phosphorus (mg kg^{-1}), were determined by (Soltanpour and Workman, 1979) method. Organic matter (%) was determined by Nelson and Sommers, (1982) method while, pH was measured with Mc Lean, (1982) method.

Statistical analysis: The data obtained in the study were subjected to analysis of variance using Statistix (8.01) computer software. The analysis was carried out in Complete Randomized Design and treatments mean were compared using least significance difference test (LSD) at 5% level of probability (Steel *et al*., 1997).

Results

Shoot Growth Characteristics: Nutrient supplied from PM, SM and RP alone or in combination with PSB significantly affected shoot dry weight (SDW) of okra. Application of SM produced the highest SDW at all three growth stages. The degree of increase in SDW was 50, 70 and 75% at vegetative (1.660 g), flowering (3.09 g) and maturity (5.46 g) stages respectively over control. Shoot length (SL) of okra was positively affected by PM, SM and RP alone or in combination with PSB, however highest shoot length was observed in SM application at three growth stages. An increase in SL was 29, 48 and 47 % recorded at vegetative (28.80 cm), flowering (33.33 cm) and maturity (45.83 cm) stages respectively over control (Table 1). There a positive relationship between shoot length and shoot dry weight and improvement in shoot growth by SM might be due to improving the physical characteristics of the soil by increasing the porosity and being an organic matter source in soil.

Root Growth Characteristics: Okra root dry weight (RDW) was significantly ($P \leq 0.05$) affected by PM, SM and RP alone or in combination with PSB. The higher RDW at vegetative, flowering and maturity stages were recorded where sole application of SM was applied. The degree of increase in RDW was 58, 53 and 78 % at vegetative (0.22 g), flowering (0.55 g) and maturity (1.96 g) stages respectively over control. There was a significant difference for root length (RL) of okra at all growth stage, while maximum RL was recorded where SM was individually applied. An increase in RL was 55, 36 and 50 % recorded at vegetative (18.50 g), flowering (17.50 g) and maturity (14.60 g) stages respectively over control (Table 2).

Table 1. Shoot Dry Weight (SDW) and shoot length (SL) of okra at vegetative, flowering and maturity stages as influenced by different P sources on okra.

	Shoot dry weight (g)			Shoot length (cm)		
Treatments	Vegetative	Flowering	Maturity	Vegetative	Flowering	Maturity
Control Control	0.833e	0.90f	1.36e	20.53e	17.23de	24.43f
PM	1.196c	2.07c	4.10a	26.16b	22.23cd	38.43c
SM	1.660a	3.09a	5.46a	28.80a	33.33a	45.83a
RP	0.840e	1.10ef	1.93d	21.00de	19.36e	30.16e
RP+PM	1.366b	2.49b	2.46d	27.25ab	27.00b	34.53d
RP+SM	1.190c	1.34de	3.43c	22.1cd	24.76e	41.83bc
RP+PM+PSB	1.373b	1.53d	3.43c	27.25ab	22.37cd	39.06c
RP+SM+PSB	0.950d	1.50d	2.06d	23.50c	27.66b	34.50d
RP+PM+SM+PSB	0.843e	1.53d	4.06b	22.95cd	23.33c	44.23ab
LSD ($p\leq0.05$)	0.049	0.25	0.56	1.96	2.73	3.52

T1= Control: T2= PM: T3= SM: T4= RP: T5= RP+PM: T6= RP+SM: T7= RP+PM+PSB: T8= RP+SM+PSB: T9= RP+PM+SM+PSB

Table 2. Root Dry Weight (RDW) and root length (RL) of okra at vegetative, flowering and maturity stages as influenced by different P sources.

	Root dry weight (g)			Root length (cm)		
Treatments	Vegetative	Flowering	Maturity	Vegetative	Flowering	Maturity
Control Control	0.093e	0.26e	0.43e	8.30f	11.20f	7.36h
PM	0.16bc	0.32cd	0.73cd	12.83cd	13.00de	13.23c
SM	0.22a	0.55a	1.96a	18.50a	17.50a	14.60a
RP	0.096e	0.30d	0.53de	12.20de	16.00b	8.75g
RP+PM	0.17bc	0.45b	0.90c	10.96e	15.40bc	11.33e
RP+SM	0.15cd	0.34c	1.23b	18.00a	13.06de	7.50h
RP+PM+PSB	0.17b	0.32cd	0.90c	14.16bc	14.43cd	13.86b
RP+SM+PSB	0.14d	0.35c	1.26b	14.83b	11.80ef	12.50d
RP+PM+SM+PSB	0.098e	0.30d	1.30b	17.66a	11.30f	10.30f
LSD ($p\leq0.05$)	0.022	0.037	0.24	1.59	1.45	0.30

Leaf Characteristics: Leaf area (LA) of okra plant was significantly affected ($P\leq0.05$) by organic manures and RP alone or in combination with PSB. Highest leaf area was measured where PM+SM+ RP+PSB were applied in combination. The degree of increase in LA was 27.2, 42 and 38 % recorded at vegetative (7.50 cm^2), flowering (12.16 cm^2) and maturity (13.70 cm^2) stages respectively over control. Organic manures application with RP and PSB was effective in chlorophyll contents (CC) of okra and produced the highest CC of okra compared to other treatments. The degree of increase in CC was 56, 54 and 75 % recorded at vegetative (14.8 mg cm^{-2}), flowering (13.59 mg cm^{-2}) and maturity (25.82 mg cm^{-2}) stages respectively over control (Table 3).

Yield Characteristics

Pod Fresh Weight: Okra pod fresh weight (PFW) was significantly affected by sole application or combined application of organic and inorganic sources. At first picking SM+RP+PSB application produced the highest PFW over control. At second picking combined application of PM+RP+PSB showed higher PFW. At third picking higher PFW was recorded with integration of PM+SM+RP+PSB. The degree of increase 44, 76 and 63 % were recorded at first, second and third pickings over control respectively. There is a vital relationship between okra growth and yield attributes indicated that treatments higher LA and CC lead to higher pod weight (Figure1).

Pod length: Pod length (PL) at first picking was significantly increased, where SM+RP+PSB was applied, while at second picking higher PL was measured where PM+RP+PSB was used. However, higher PL was measured at third picking with combined application of PM+SM+RP+PSB. There was a 46, 70 and 57% increase in PL at first, second and third picking over control, respectively (Figure 2).

Okra P uptake: Total P uptake was significantly increased by combined application of PM+SM+RP+PSB at first, second and third pickings, while increase in P uptake was up to 24, 25 and 44 % by the same treatment at first, second and third pickings respectively over control. The increase in P uptake might be due to the effect of PSB on other P organic and mineral fertilizers by enhancing the P release in mineral pool (Figure 3).

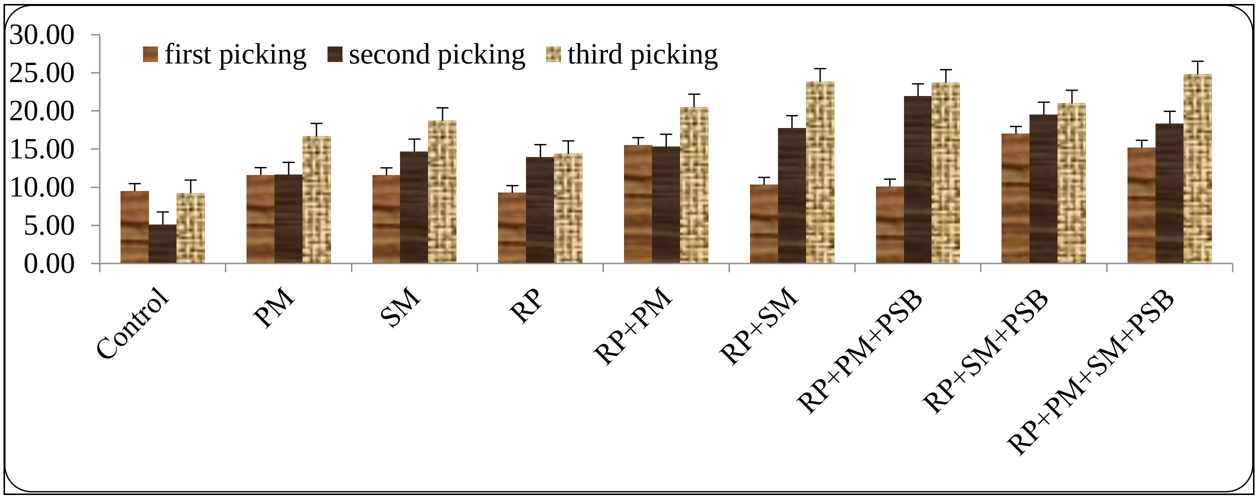

Figure 1. Pod fresh weight at first, second and third pickings as influenced by different P sources on okra.

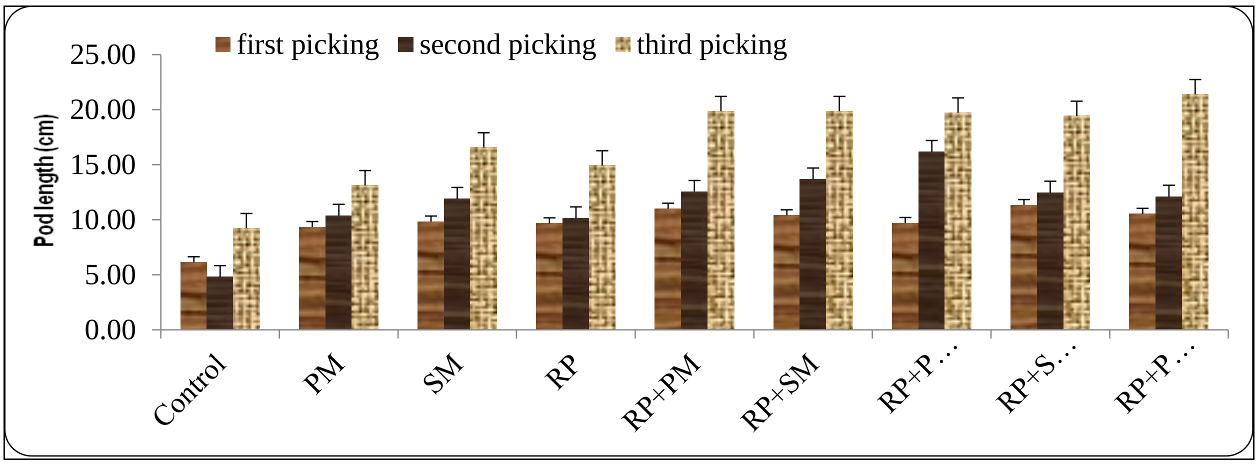

Figure 2. Pod length at first, second and third pickings as influenced by different P sources on okra.

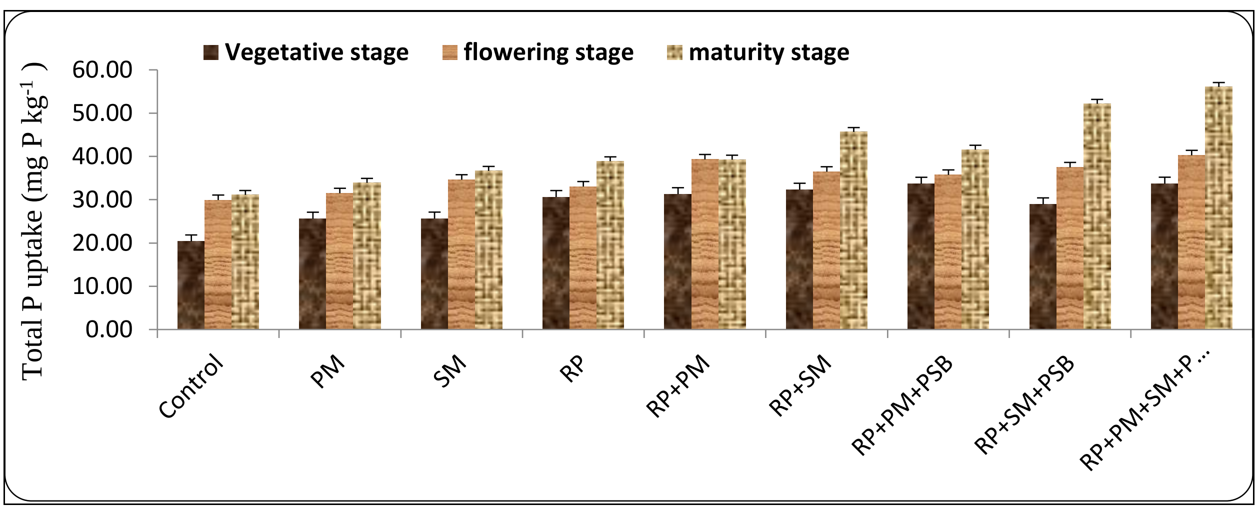

Figure 3. Total P uptake at vegetative, flowering and maturity stages as influenced by different P sources on okra.

Table 3. Leaf area (LA) and chlorophyll contents (CC) of okra at vegetative, flowering and maturity stages as influenced by different P sources.

	Leaf area (cm^2)			Chlorophyll contents (mg cm^{-2})		
Treatments	Vegetative	Flowering	Maturity	Vegetative	Flowering	laturity
Control Control	5.46c	7.00c	8.56f	6.58e	6.23d	6.586f
PM	7.00ab	12.00a	12.73b	11.61b	6.56d	18.74c
SM	5.50bc	11.16ab	11.83cd	12.30b	6.56d	19.92bc
RP	5.60bc	10.46ab	10.76e	6.90de	8.29c	10.01e
RP+PM	7.00ab	10.23b	11.26de	7.38cde	8.88c	11.79de
RP+SM	7.10ab	11.20ab	12.70b	7.38cde	6.48d	19.27c
RP+PM+PSB	7.00ab	11.03ab	12.43bc	8.38cd	8.24c	22.85ab
RP+SM+PSB	5.72bc	11.20ab	11.53de	8.64c	9.69b	14.62d
RP+PM+SM+PSB	7.50a	12.16a	13. 70 a	14.8a	13.59a	25.82a
LSD ($p \leq 0.05$)	1.53	1.731	0.80	1.72	0.77	2.99

T1= Control: T2= PM: T3= SM: T4= RP: T5= RP+PM: T6= RP+SM: T7= RP+PM+PSB: T8= RP+SM+PSB: T9= RP+PM+SM+PSB

Soil characteristics affected by different P sources: Soil pH was non-significantly affected in all treatments where PM, SM, RP and PSB applied either alone or in combination. Addition of organic manures significantly increased ($P \leq 0.05$) organic matter contents of soil. Soil organic matter contents were 0.91 % before sowing and 1.89 % after harvesting where SM was applied. Increase in organic matter contents indicated that SM not only enhanced the nutrients supply to plants but also improved the physical condition of the soil. Soil potassium was 80 mg kg^{-1} before sowing while after harvesting highest soil K (116.17 mg kg^{-1}) was recorded in SM application. The degree of increase in K contents by SM application was 43% over control. Lower K contents (76 mg kg^{-1}) were observed in RP application; however 17.10 % more K contents were recorded over control (Table 4).

Available Soil P contents were 0.89 mg kg^{-1} before sowing however highest P contents were observed (6.07 mg P kg^{-1}) where integrated use of organic manure and RP was applied with PSB. Combined application of manures with RP and PSB significantly enhanced the P contents up to 83% over control. Lower P contents were recorded in RP application; however 33.11% higher P contents were recorded over control. Total N contents were 0.072% before sowing however after harvesting highest total N (0.095%) was recorded in SM application. SM application significantly increased the total N contents up to 45% over control (Table 4).

Table 4. Post harvest soil analysis.

Treatments	pH	Organic Matter (%)	Potassium (mgkg^{-1})	Soil P (mgk^{-1})	Total O. Carbon (%)	Total N (%)
Control Control	8.17a	1.04d	63.00f	1.03e	0.62e	0.052e
PM	8.12ab	1.38bc	96.00cd	1.73d	0.85cd	0.073cd
SM	8.10ab	1.89a	116.17a	3.81c	1.10a	0.095a
RP	8.12ab	1.26cd	76.00e	1.54d	0.75d	0.064d
RP+PM	8.08bc	1.39bc	90.66d	4.54b	0.80d	0.069d
RP+SM	8.12ab	1.66ab	105.83bc	1.91d	1.02ab	0.088ab
RP+PM+PSB	8.16a	1.38bc	106.00b	3.48c	0.84cd	0.073cd
RP+SM+PSB	8.08bc	1.43bc	101.17bc	4.64b	0.92bc	0.079bc
RP+PM+SM+PSB	8.00c	1.66ab	88.00d	6.07a	0.99ab	0.085b
LSD ($p \leq 0.05$)	0.083	0.30	9.88	0.46	0.11	00.55

Discussion

Although some research showed progress in plant growth and yield by the use of organic manures. In this experiment, combined application of organic and inorganic P fertilizers with PSB improved the growth, yield and P uptake characteristics of okra at three growth stages; however to some extent sole application of organic manures also showed good response. Jesu and Adekayode, (2010) reported that application of manures increased the shoot fresh and dry weight of Africans nut cherry through more P availability and biological activity in soil. These manures are high in nutrients (Taha *et al.,* 2011) especially nitrogen and phosphorus. Application of these phosphorus fertilizer and FYM (Powon *et al.,* 2005) had significant ($p < 0.05$) effect on SDW and positive interaction between P and organic sources. Increase in SL by SM which in turn increase the uptake of nutrients and maximum SL was recorded in rice and wheat crops by the application of P fertilizers (Khan *et al.,* 2005; Khan *et al.,* 2007). A pronounced increase in SDW and SL with the application of SM indicated that it is highly important in improving growth of okra at various growth stages.

Improvement in the availability and mobility of nutrients lead to higher RDW by organic P source, which in-turn enhanced the uptake of nutrients in okra (Adewole and Ilesanmi, 2011). Mineralization rate was increased up to 20 % by PSB in tomato plants, which is more than soluble P from the insoluble forms and resulted in significantly higher RDW (Turan *et al.,* 2006). Organic manures presence was responsible for higher RL through the activation of living organisms which release phyto-hormones and stimulate plant growth by the absorption of nutrients through roots (Arisha *et al.,* 2003). Organic manures application increased plant height, leaf area, stem girth, leaf number, root length and shoot fresh weight of African cherry nut seedlings (Jesu and Adekayode, 2010). Higher RDW and RL by the application of SM indicated that organic manures like SM is the best reservoir of essential nutrients and are responsible for the better growth of plants.

Plant height and LA was significantly enhanced by the applied inoculants compared to un-inoculated plants (Shaikh and Mohammad 2009). Combined application of organic manures with RP enhanced the yield of French bean compared to sole application of inorganic fertilizer (Manjunath *et al.,* 2006; Nagaraju *et al.,* 1995). Plots amended with the higher organic P level application in maize crop produced highest mean LA (Amanullah *et al.,* 2010). Chlorophyll contents and leaf photosynthesis were significantly increased by PSB inoculation in aerobic rice over control treatments (Panhwar *et al.,*2011). Leaf chlorophyll contents were enhanced by application of mycorrhiza along with PSB (*Pseudomonas putida*) in barley (Mehrvarz and Chaichi, 2008). Co-application of PSB with organic manures and RP enhanced LA and CC of okra indicated that integrated use produced better response over control; hence microbes played a key role in supplying of essential nutrients to plants.

Combined application of RP, organic manures with PSB inoculation gave significantly higher number of pods and pod weight as compared to un-inoculated treatments in French bean (Manjunath *et al.,* 2006). Sheep manure increases not only fruit yield but also improve the moisture and nutrient supply of crops (Aliyu, 2003; Yahaya, 2008). Integrated use of manures and RP with PSB showed highest PFW indicated that it is highly remarkable in terms of growth and yield of okra. Okra yield was significantly enhanced by the application of RP in combination with organic manures (Akande *et al.,* 2006). It was evident from the results; that PFW directly related to the PL hence co-application of manures with RP and PSB produced better response to okra crop.

Combined application of RP and PSB showed increase in the P uptake by plants (Sharma *et al.,* 2010). A significant increase in dry matter yield and P uptake in beans, maize and rice were recorded by RP application (Menon *et al.,* 1991). The application of PSB significantly increased soluble P and plant P uptake in aerobic rice (Ponhwar*et al.,* 2011). Inoculation of arbuscular mycorrhiza with PSB enhanced the P uptake of both native P from the soil and P from the RP (Cabello *et al.,* 2005). Biological P fertilizers increased root P uptake through root development (Kaya and Heggs, 2002). From organic and inorganic P fertilizers PSB release essential nutrients by acting upon them indicated that P uptake was highly depends upon these microbes which enable P to remain mobile in nutrient pool for better uptake by the plants.

Soil pH and organic matter contents were non-significantly differs among all the treatments at the end of the experiment by the application of SM (Zahra and Tahboub, 2008). Higher amounts of K contents were recorded by using sheep manure or mixture of manures showed a significant difference with other treatments (control, cattle and poultry) (Zahra and Tahboub, 2008). Soil total N and P contents were increased with increasing rates of organic manures application (Maerere *et al.,* 2001). Organic manures (PM and CM) improved the soil organic carbon and N status of soil (Ewulo, 2005). All the parameters were affected by the application of SM indicated that it is responsible for increasing the nutrient contents in soil.

Conclusion: It can be concluded from the study that the effect of organic manures, P solubilizer and rock phosphate was comparably high over control. However, organics like poultry and sheep manures played a tremendous role in nutrient uptake when usually used with rock phosphate and PSB. Sheep manure comparatively more reliable P source for okra crop followed by poultry manure, however the highest P uptake by combine application of PSB with organic manure and rock phosphate, indicated that integrated use could be best choice regarding to okra yield and P uptake.

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