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INFLUENCE OF *BACILLUS MEGATERIUM* AND PH ON THE SOLUBILITY OF SOKOTO ROCK PHOSPHATE IN SOIL

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ABSTRACT

A research was carried out to determine the role of *Bacillus megaterium* and soil pH in relation to phosphorus availability in soil using Sokoto Rock Phosphate. The experiment was laid out in completely randomized design (CRD) in the laboratory using three (3) treatments 0, 5 and 10ml of *Bacillus megaterium* replicated three times. The results obtained shows that there is no significance difference at ($p < 0.05$) in phosphorus concentration in relation to inoculants and uninoculated treatments at 0ml, 5ml and 10ml using Phosphorus solubilizing bacteria, significant difference was only observed in relation to soil pH at 4th, 5th and 6th weeks after inoculation with the highest available phosphorus of 0.8gkg^{-1} at 4th week with a mean pH of 7.5. The study suggest that although, the trend and relative effectiveness of microorganisms in the soil are very complicated and unpredictable, the *B. megaterium* is not always effective at phosphorus solubilization as was observed in so many research elsewhere which may be affected by many factors, such as Phosphate Solubilizing Bacteria (PSB) used, nutritional status of soil and environmental factors. Therefore it was concluded that pH is important in improving the activities of phosphate solubilizing organisms. More research is needed to identify, screen and characterize more PSB for their ultimate application under field conditions.

INTRODUCTION

Phosphorus (P) is second only to nitrogen as the most essential macro-nutrient required by plants (Srinivasan *et al.*, 2012). It is a key nutrient for sustainable agricultural productivity which limits plant growth in many soils (Sceveno *et al.*, 2011). Despite the considerable addition of phosphorus to soil, the amount available for plant is usually low because, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils (El-Gizawy and Mehasen 2009). Phosphorus is important in plants, especially in photosynthesis, membrane formation, carbon metabolism, energy generation, glycolysis, nucleic acid synthesis, respiration, enzyme activation and nitrogen fixation (Leidi and Rodriguez-Navarro, 2000; Wu *et al.*, 2005). Low phosphorus availability of many tropical and subtropical soils in combination with insufficient P fertilizer application has been identified as one of the major factors responsible for low yields. (Kretzchmar *et al.*, 1991). Its deficiency affects root architecture, seed development and normal crop maturity (Borch *et al.*, 1999, Williamson *et al.*, 2001). It is a vital component of ATP, the "energy unit" of plants and of DNA, the genetic "memory unit" of all living things (Griffith, 1999). Phosphorus (P) is an essential macronutrient, being required by plants in relatively large quantities (approximately 0.2 to 0.8%) (Mengel and Kirkby, 1987; Mills and Jones, 1996).

Therefore, many soils throughout the world are deficient in P because P-concentrations available to

plants are generally low even at pH 6.5 where it is most soluble (Gyaneshwar *et al.*, 2002). Thus P availability to crops in adequate amounts is a global issue and 30–40% crop yield of the world's arable land is limited by P availability (Vance *et al.*, 2003).

Inorganic forms of P are solubilized by a group of heterotrophic microorganisms by excreting organic acids that dissolves phosphatic minerals directly, releasing P into soil solution (He *et al.*, 2002). Phosphate solubilizing bacteria (PSB) are being used as biofertilizers since 1950s in order to release phosphorus availability in soil (Krasilnikov, 1957).

Rock phosphate is being considered as another source of phosphorus for reversing soil fertility depletion (Ghosal *et al.*, 1998; White *et al.*, 1999). Although, on one hand, insoluble organic compounds of phosphorus are largely unavailable to plants and many microorganisms can bring the phosphate into solution (Prosenjit *et al.*, 1999; GuangLong *et al.*, 1999). Therefore, the P-content in average soils is about 0.5% but only 0.1% of the total P is available to plants (Zou *et al.*, 1992). It is recognized that the availability of phosphate in soils is a major factor limiting the productivity of many ecosystems (Daniels *et al.*, 2009).

There are various mechanisms by which microorganisms solubilize inorganic phosphate. It can be by secretion of organic acids (Goldstein, 1995) or by production of siderophores (Vassilev *et al.*, 2006). Fortunately, various kinds of bacteria (Harris *et al.*, 2006; Zaidi *et al.*, 2009; Khan *et al.*, 2010) and fungi (Whitelaw, 2000; Wakelin *et al.*, 2007) have been

isolated and characterized for their ability to solubilize unavailable phosphate (PO₄) to available forms. Such transformations increase P availability and promote plant growth (Whitelaw, 2000; Harris *et al.*, 2006). Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al.*, 2002). Phosphorus solubilizing bacteria use different mechanisms to bring about the insoluble forms of the phosphate into soluble forms, but it is generally believed that the major mechanism of the mineral phosphate solubilization is the release of microbial metabolites such as organic acids (Singh and Amberger, 1997; Whitelaw, 2000; Lin *et al.*, 2006).

Recently, phosphate solubilizing microorganisms have attracted the attention of agriculturists as soil inocula to improve the plant growth and yield (Whitelaw, 2000; Harris *et al.*, 2006). Considerable success was earlier claimed, particularly by Russian workers, in increased yields and quality of crops by inoculating seeds with pure and efficient strains of *Bacillus megaterium* commonly called "Phosphobacterin" (Menkina 1963). Therefore, one of the approaches would be to increase the number and activity of efficient PSM in the root zone of plants by use of microbial inoculants for increasing phosphorus availability to the plants from the soil as well as added phosphate.

In Nigeria, one of the major problems faced by farmers is poor soil fertility that is detrimental for sustainable agricultural productivity (Mutsaers, 1990). Phosphorus deficiency is one of the most common nutritional stress in many regions of the world, affecting 42% of the cultivated land over the world (Liu *et al.*, 1994) which result in low yielding of crops (Khan *et al.*, 2013). Therefore, the scarcity of Phosphorus as fertilizer and the consequences of climate change can dramatically influence the food security for future generations (Mäder *et al.*, 2011). Hence with increasing demand of agricultural production, phosphorus (P) is receiving more attention because it is the least mobile element in plants and soil contrary to other macronutrients (Sharma *et al.*, 2011). Plants take P in soluble form but soil P is present as insoluble phosphate form thereby not utilized by plants. The maintenance of high levels of soil available phosphorus has been a major challenge to agricultural scientists, ecologists and farm managers. Even in phosphorus rich soils, due to its insolubility, only a small proportion (0.1%) is available to plants (Madhi *et al.*, 2011).

As the cost of the chemical fertilizer is very high and its availability and uses are also becoming imperative, new options are needed to better exploit soil P resources, selection of efficient cultivars or using alternative strategies of management of soil and agro ecosystems to optimize P bioavailability. However, a large quantity of available phosphorus is needed to achieve maximum productivity. Therefore, a soil should provide a sufficient concentration of phosphorus for optimum plant growth. This will reduce the ever increasing prices of Phosphorus fertilizers. As a consequence of these constraints, there seems no option but to exploit strategies/approaches to enhance availability of indigenous (non-available) soil P for sustainable agricultural production. The main objective of the study is to determine the influence of *Bacillus megaterium* and pH on the Solubility of Sokoto rock phosphate in soil.

MATERIALS AND METHODS

STUDY AREA

A screen house study was conducted in Usmanu Danfodiyo University Sokoto, Nigeria. Sokoto State situated between latitude 13°05'N and longitude 05°15'E, 315 above sea level. It has a land area of 692km² and a population of 2,208,874 males and 2,261,302 female (NPC, 2006). The climate of Sokoto state is hot, semi arid, tropical type AW in the koppen classification (Sombroek and Zonneveld, 1971). The mean annual rainfall is about 400-700mm, which is often erratic in distribution (Singh, 1995) with minimum and maximum temperature of 15°C and 40°C (Arnborg, 1988). FAO (1969) described the common types of land use in the areas as numerous transhumance herds of cattle owned by Fulani graze extensively in both the fallow farmland and uncultivated areas. Ojanuga (2006) found that crops mainly cultivated in floodplain areas are vegetables like onion, okra, pepper, tomatoes, cassava, carrot, garden eggs, in the dry season where water is pumped either from tube well, rivers, stream to crops field, while in upland areas usually mixed cropping of cereals and legumes is common.

Sampling Procedure

The soils used for the experiment were collected from teaching and research farm of Usmanu Danfodiyo University, Sokoto randomly from a depth of 0-30cm furrow slice under cultivation using soil auger. Composite sample collected were thoroughly mixed to make a representative sample, and heated to about 105°C to avoid any contamination before finally used in the pot trials. The experiment was laid out in completely randomized design (CRD) using three (3) treatments replicated three times.

- A Soil sample + grinded rock phosphate + 0ml of *B. megaterium*
- B Soil sample + grinded rock phosphate + 5ml of *B. megaterium*
- C Soil sample + grinded rock phosphate + 10ml of *B. megaterium*

Soil samples were mixed thoroughly with in plastic pots. The isolate were inoculated on plastic pots of sterilized soil of 10kg pot⁻¹ (0, 5, and 10ml respectively). All pots were irrigated regularly in order to provide sufficient moisture for microbial activities at 2days interval. The isolate were Standardized using Macfarland turbidity standard sub cultured on nutrient agar plate and incubated at 37°C for 24hours before inoculating the organism in the soil.

Physical and Chemical Analysis

Particle size analysis was determined by hydrometer method (Bouyoucos, 1951). Moisture content was determined gravimetrically. Soil pH was determined using 1:1 soil to water. Using pH meter. Organic carbon was determined using walkley and Black method (Walkley and Black, 1934). Available phosphorus was determined using Bray No 1 (Bray and Kurtz, 1945). Total nitrogen was determined using micro- kjedhal digestion distillation method. Sodium and potassium was determined using flame photometer. Calcium and magnesium was determined by EDTA titration methods. The data was subjected to Analysis of Variance (ANOVA) using Stat View Statistical Package (2002).

RESULTS AND DISCUSSION

Table 1 shows the physical and chemical properties of the soil before the experiment. The textural class of the soil was sandy loam with moderately acid pH, organic carbon, organic matter content and total nitrogen of the

soil were low. Calcium and available phosphorus were low, potassium and sodium were high, cation exchange capacity and magnesium were medium according to the ratings of Esu (1991).

Table 1. Soil physical and chemical properties before inoculation

Physical properties	Mean (%)
Sand (gkg ⁻¹)	639
Silt (gkg ⁻¹)	302
Clay (gkg ⁻¹)	59
Texture	Sandy loam
Chemical properties	
pH	5.9
Organic carbon (gkg ⁻¹)	2.8
Organic matter (gkg ⁻¹)	4.8
Total nitrogen (gkg ⁻¹)	0.03
Available phosphorus (mgkg ⁻¹)	0.28
Cation exchange capacity (cmolkg ⁻¹)	10.1
Exchangeable bases (cmolkg ⁻¹)	
Calcium (Ca ²⁺)	0.54
Magnesium (Mg ²⁺)	0.43
Potassium (K ⁺)	0.40
Sodium (Na ⁺)	0.43

Table 2 shows the comparison of phosphorus concentration and treatment with *B. megaterium*. In the first week the concentration of phosphorus in the 0ml treatment was found to be 0.32gkg⁻¹ which is highest compared with concentration in 5ml and 10ml which are 0.30gkg⁻¹ and 0.31gkg⁻¹ respectively. In the second week, highest concentration of phosphorus was observed in the 5ml treatment with concentration of 0.31gkg⁻¹, followed by 10ml treatment with concentration of 0.28gkg⁻¹. The lowest concentration occurs in 0ml treatment with concentration of 0.26gkg⁻¹

¹. In the third week the concentration in 0ml, 5ml, and 10ml treatment was found to be 0.63 gkg⁻¹, 0.68gkg⁻¹ and 0.64gkg⁻¹ respectively. There was no significant difference in the 1st and 2nd between all the treatment. But on the 3rd week there was significant difference in all the treatments with the first and second weeks. This could be as a result of increase in soil pH across all the experimental weeks. Table 3 no significance difference was observed between the treatment means at 0, 5 and 10ml of *Bacillus megaterium* inoculation respectively.

Table 2: Mean pH and Available Phosphorus with *B. Megaterium* at 1st, 2nd and 3rd weeks after inoculation

Treatments	Week	Mean pH	Mean available P (gkg ⁻¹)
Soil sample + grinded rock phosphate + 0ml of <i>B. megaterium</i>	1 st	5.9	0.32 ^b
	2 nd	6.2	0.26 ^b
	3 rd	6.4	0.63 ^a
	Mean		0.40
Soil sample + grinded rock phosphate + 5ml of <i>B. megaterium</i>	1 st	5.8	0.30 ^b
	2 nd	6.2	0.31 ^b
	3 rd	6.4	0.68 ^a
	Mean		0.43
Soil sample + grinded rock phosphate + 10ml of <i>B. megaterium</i>	1 st	5.9	0.31 ^b
	2 nd	6.3	0.28 ^b
	3 rd	6.5	0.64 ^a
	Mean		0.41

Values with letters across the column are statistically different at (p<0.05).

Table 3. Treatment means comparison of available P at 1st, 2nd and 3rd weeks after inoculation

Treatment	Mean
0ml	0.40
5ml	0.43
10ml	0.41
	Ns.

Table 4 shows mean soil pH and available phosphorus. In the fourth week there was an increased in soil pH recorded in the 5ml treatment with soil pH of 7.5 which also shows the highest concentration of available phosphorus 0.8gkg⁻¹. While in the 0ml and 10ml treatments they were having the same pH 7.4 and the

same p availability of 0.7gkg⁻¹ respectively. In the fifth week the treatment have the same pH value of 7.3 and available phosphorus of 0.62 gkg⁻¹, 0.61gkg⁻¹ and 0.61gkg⁻¹ respectively, this could be as a result of pH decline. In the sixth week the pH value in the 0ml, 5ml and 10ml treatments were 7.1, 7.3 and 7.3 respectively.

The corresponding phosphorus concentrations in the different treatments are 0.60gkg⁻¹, 0.64gkg⁻¹ and 0.64gkg⁻¹ respectively. This could be attributed to decline pH values. Therefore it was shown that Soil pH is one of the determinant factor in the plant nutrient availability in the soil and this was seriously observed especially at 4th weeks were the pH is within the range of 7.4-7.5 with the highest p availability in soil which

may tend to favors' the activities of this organisms. This result contradicts the findings of Omar, (1998) who reported a greatest soluble phosphorus following inoculation with bacteria. The findings do not conform to the work carried out by Mullins *et al.* (2001), who reported that P concentrations were not affected by soil pH. Table 5 no significance difference was observed between the treatment means.

Table 4. Mean pH and available Phosphorus with *B. Megaterium* at 4th, 5th and 6th weeks after inoculation

Treatments	Week	Mean pH	Mean available P (gkg ⁻¹)
Soil sample + grinded rock phosphate + 0ml of <i>B.megaterium</i>	4 th	7.4	0.70 ^a
	5 th	7.3	0.62 ^b
	6 th	7.1	0.60 ^b
	Mean		0.64
Soil sample + grinded rock phosphate + 5ml of <i>B.megaterium</i>	4 th	7.5	0.80 ^a
	5 th	7.3	0.61 ^b
	6 th	7.3	0.64 ^b
	Mean		0.68
Soil sample + grinded rock phosphate + 10ml of <i>B.megaterium</i>	4 th	7.4	0.70 ^a
	5 th	7.3	0.61 ^b
	6 th	7.3	0.64 ^b
	Mean		0.65

Means followed by the same letter(s) within the same column are not significantly difference at (p<0.05).

Table 5: Mean Available phosphorus at 4th, 5th and 6th weeks after inoculation

Treatment	Available P
0ml	0.64
5ml	0.68
10ml	0.65
	Ns

CONCLUSION

Phosphorus is one of the major nutrient element of plant and also most limiting element in the tropics. This research was carried out to determine the role of *Bacillus megaterium* in solubilizing phosphorus. The result obtained in this study shows that there is no significant difference in the concentration of phosphorus in relation to inoculants and non inoculants treatments. Significant difference was only observed at high pH values of 7.5 with available phosphorus of 0.8gkg⁻¹. The study suggested that although; the trend of using *B. megaterium* is not always effective as

phosphorus solubilization as was observed in so many research elsewhere. However, phosphate solubilization by bacteria is a complex phenomenon affected by many factors, such as PSB used, nutritional status of soil and environmental factors most likely soil pH. Hence, it needs further studies to understand the characteristics and mechanisms of phosphate solubilization by PSB. Efforts should be made to identify, screen and characterize more PSB for their ultimate application under field conditions. So that, the successful implementation of PSB to better exploit soil P resources can be an alternative sustainable strategy for management of soil to optimize P bioavailability.

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