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Research Article

Study on the Role of Phosphate Solubilizing Fungi in Phosphorous Bioavailability and Growth Enhancement of Potato

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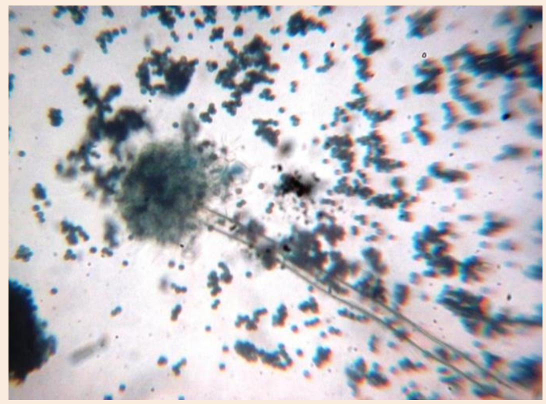
Abstract

Phosphorus is an essential plant nutrient for plant growth. Chemical P fertilizers are required for crop production, but only a small part of P is utilized by plants, while the rest is converted into insoluble fixed forms. Phosphate solubilizing microbes(PSMs) are known to improve the solubilization of fixed soil phosphorus and applied phosphates, resulting in higher crop yield. Soil samples collected from different areas analyzed to know their properties which support the microbial community to survive and form their beneficial action. Strains from the genera *aspergillus* is the most powerful phosphate solubilizers. In our research *A. niger* is identified as phosphate solubilizing fungi by their morphological characteristics. On applying the inoculation of phosphate solubilizing fungi, decrease in pH was observed in liquid medium ranging from 4.0 to 3.2 from initial pH of 7.5±0.2. The decrease in pH of the culture medium indicated the production of various organic acids by the culture. The results revealed that phosphate solubilizing fungi *A. niger* performed significant improvement in plant productivity and quality. Hence the application of biofertilizer prepared by above mentioned fungi should be helpful to improve the physico-chemical, biochemical and biological properties of rock-P amended soil.



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Introduction

Phosphorus is an essential plant nutrient for plant growth. Most soils contain considerable reserves of total P, but a major portion of it remains comparatively immobile and only less than 10% of soil P enters the plant-animal cycle. P fertilizers are required for crop production, but only a small part of P is utilized by plants, while the rest is converted into insoluble fixed forms. P deficiency is usually the consequence of low intrinsic P fertility due to weathering, in mixing with demanding, nutrient-extracting agricultural practices [1]. Plant root-associated phosphate solubilizing bacteria (PSB) have been considered as one of the possible alternatives for inorganic phosphate fertilizers for promoting plant growth and yield. Seed or soil inoculation with PSB is known to improve the solubilization of fixed soil phosphorus and applied phosphates, resulting in higher crop yield [2]. In fact, PSB render more phosphates into the soluble form than required for their growth and metabolism by secreting organic acids or enzymes (*e. g.* phosphatases), the surplus get the plants [3]. The interest in PSB has increased due to the prospective use of efficient strains as bio-inoculant (biofertilizer) components in organic farming, which is emerging as an alternative to chemical inputs in intensive agriculture [4]. However, their root colonization, persistence and performance in the rhizosphere are severely affected by environmental factors, especially under stressful soil conditions. The principle mechanism for mineral phosphate solubilization is the production of organic acids and acid phosphatases play major role in the mineralization of organic phosphorus in soil. It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganism. Production of organic acids results inacidification of the microbial cell and its surroundings. PSMs are a low-cost solution that enriches the soil giving a thrust to economic development without disturbing ecological balance. The inoculation of P-solubilizing microorganisms is a promising technique because it can increase P availability in soils fertilized with rock phosphates [5)]. Several authors reported yield increasing on wheat[6], onion [7], alfalfa and soybean through simple inoculation of P-solubilizing fungi (PSF) [8]. Inoculation of phosphate solubilizing fungi and mycorrhizal fungi improves the physico-chemical, biochemical and biological properties of rock-P amended soil [9].

Materials & Methods

Sample collection

Soil samples (500 g) were collected from the forest area of four districts (Guna, Sagar, Hoshangabad and Rajgarh) of central India. Soils of Madhya Pradesh vary as per the physico-chemicals parameters and composition in the different region. The major soil groups are alluvial black (deep, medium and shallow) mixed red and black, red and yellow and skeletal soils. Black soils cover about 47.6 % of the total area of the state followed by red and yellow soils about 36.5 per cent [10]. The soil types of a particular area play critical role is determining the fertility status and cropping pattern[11].The samples were taken up to a depth of 15 cm, after removing approximately 3 cm of the soil surfaces. The soil samples were placed in a sterile poly ethylene bags, closed tightly to avoid external contamination and transported to the laboratory and maintained at 4°C until further use.

Physico-chemical analysis of soil

Samples were analyzed for physicochemical parameters such as pH, EC, Nitrogen, organic carbon, bulk density, colour, moisture and phosphorous content, and organic matter, by employing standard methods [12].

Screening of phosphate solubilizing fungi

To screen for PSF, one gram of each soil sample was suspended in 9 ml of sterilized distilled water andmixed vigorously. Serial dilutions were prepared and plated on Pikovaskaya medium composed of 10 g glucose, 0.5 g yeast extract, 0.1 g $CaCl_2$, 0.25 g $MgSO_4.7H_2O$ supplemented with 2.5 g $Ca_3(PO_4)_2$ and15 g agar per liter. After 48 hours incubation at room temperature, plates were examined for clear zones around bacterial colonies. The PSF colonies were picked and sub cultured as above, until a pure culture was obtain.

Identification

For the identification of fungi lactophenol cotton blue and slide culture techniques were performed and structures were observed under microscope. The Characters of each isolates were differentiated based upon the colony morphology and spores presentation.

Quantitative estimation of solubilized phosphate

The amount of phosphorous present in the isolates was determined by Subba Rao and Fiske method. 1 mL of sample was taken in two test tubes and its volume was made up to 8.6 mL with distilled water. 1 mL of ammonium molybdate was added to the tubes and vortexed. The color intensity was read out after 10 min in samples at 660 nm. Concentration of phosphorous in sample was calculated [13].

Plant growth regulators production

The bacterial isolates were inoculated to Czapek's solution supplemented with 0.005 M L-tryptophan. They were incubated at 37°C for seven days in dark. After incubation, the cultures were centrifuged at 6000 rpm to remove the bacterial cells and the supernatant was collected in a conical flask. The amount of Indole Acetic Acid and Gibberellic Acid (GA) were determined using the supernatant.

Bioassays
After in-vitro testing of solubilization of Rock Phosphates by PSM in shake-flask fermentation conditions. These test cultures were amended in soils and evaluated on potato, in terms of shoot height, root length, dry weight, no. of branches and no. of flowers.

Results and Discussion
Physicochemical analysis of Soil

The Physico-Chemical values of the soil sample collected at different sampling sites were calculated (**Figure 1**). The result (**Table 1**) indicates that the quality of soil considerably varies from location to location and sample to sample. Soil samples collected from deferent areas of M.P. (India), show the different colors that is important for the soil diversity on behalf of chemical variation and responsible for the diversity of vegetation. The analysis of chemical properties indicates that the black soils of Hoshangabad and Rajgarh were generally slightly acidic and other show alkaline pH. The ranges of pH of soil were 5-8. The pH values of the impacted soils lies within the acidic range and may not support the growth of most crops that thrive on alkaline soil, this may lead to loss of macro minerals needed for plant growth. Thus acidification of soil depletes important nutrient elements such as potassium, calcium and magnesium [14]. Electrical conductivity showed variation between .39ds/m to .49 ds/m for red soil and .16ds/m to .33ds/m for black and yellow. Generally it is believed that higher the concentration of ions in the soil solution more is its electrical conductance. Organic carbon of soil samples varied as .31-.51% in red soil and .57-1% in black or yellow soil respectively. Bulk densities of red soils have low bulk density, while black soils have high bulk density. High bulk density may be due to high clay and that too of well shrink type over burden leading to compaction. Phosphorous in the present investigation vary from 26±2.1 Kg/hectare to 47±2.8 Kg/hectare **(Figure 1G)**. The highest value of P was found in black soil sample of Rajgarh and Hoshangabad may be due to use of excessive phosphorous fertilizers. Application of phosphorus (P) is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crop. Previous researches have already reported the importance of phosphorus [15]. The available nitrogen was measured for all locations, its value ranges from 172±2.1 to 193.3 Kg/hectare for red, and brown soil and 197±4.9 to 215±21 Kg/hectare for black soil, 183±19 Kg/hectare nitrogen investigate in yellow soil.

Table 1 Physicochemical Analysis of soil samples collected from different districts (Guna, Hoshangabad, Sagar and Rajgarh) of Madhya Pradesh

S. no.	Study Area / Parameters	Guna			Hoshangabad		Sagar		Rajgarh	
		G1	G2	G3	H1	H2	S1	S2	R1	R2
1	Bulk density (mg/m)	1.6±.007	1.61±.12	1.2±.14	1.1±.14	1.4±.28	1.2±.07	1.5±.21	1.9±.07	1.2±.07
2	pH	6.8±.21	8.1±.14	7.3±.14	5.3±.28	6.4±.49	5.7±.28	6.7±.35	7.9±.42	5.2±.35
3	EC (ds/m)	.39±.12	.72±.02	.23±.02	.16±.01	.28±.17	.18±.02	.19±.07	.49±.05	.33±.05
4	OC%	.31±.02	.51±.01	1.0±.07	65±.07	64±.12	.58±.02	.37±.01	.39±.04	.57±.06
5	N (Kg/hac.)	175.3±14	193.3±3.5	183±19	215±21	197±4.9	178.6±13	175.3±16	172±2.1	201.6±3.5
6	P (Kg/hac.)	26±2.1	32.6±.7	39.6±1.4	44.6±3.5	38.6±2.1	38±.7	32.6±6.3	28.6±2.8	47±2.8
7	Moisture %	6±.7	16.6±8.4	64.6±9.5	43.2±12.2	44.3±2.8	52±9.1	69.3±1.4	9.3±1.4	44.6±12

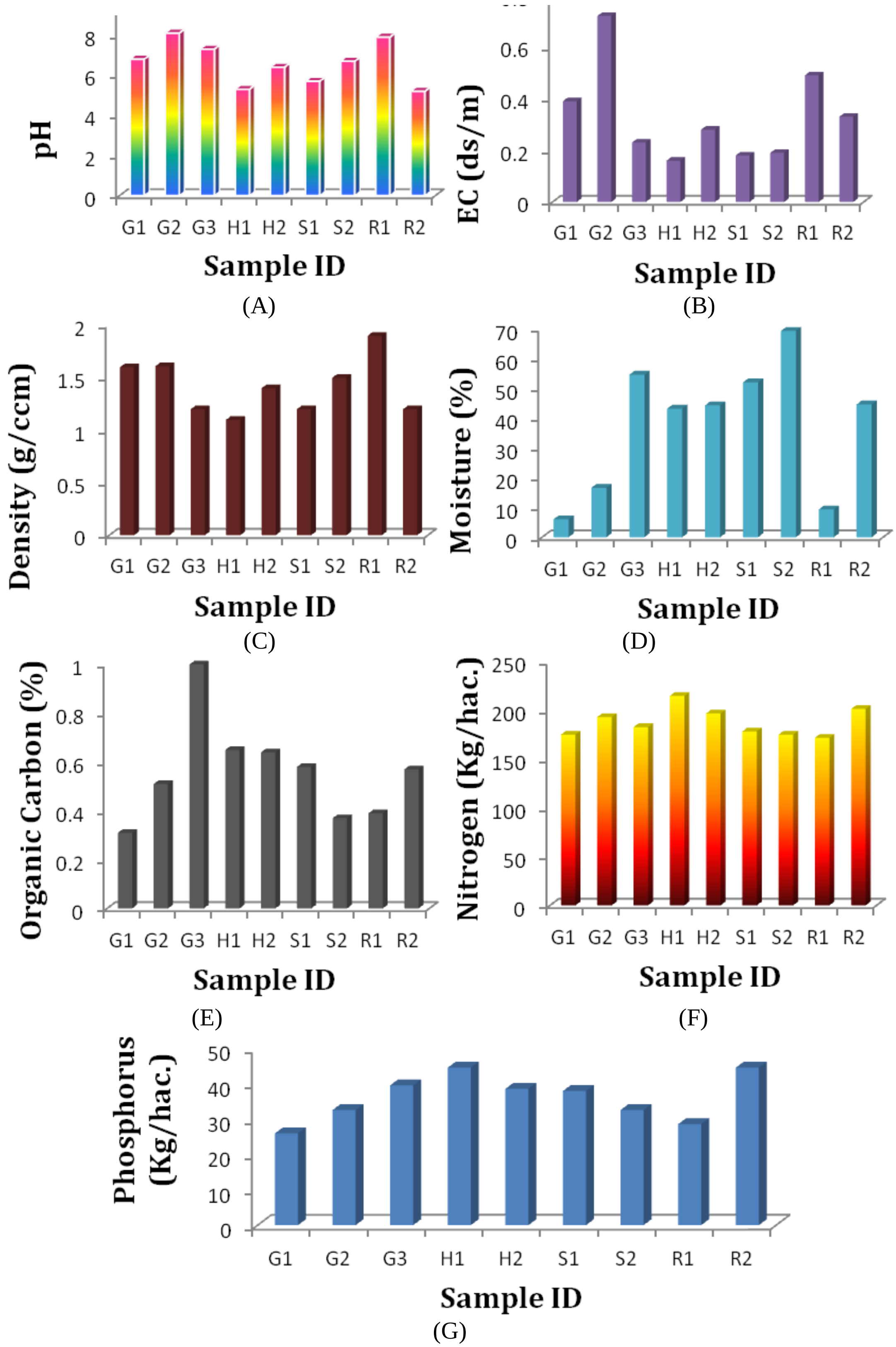
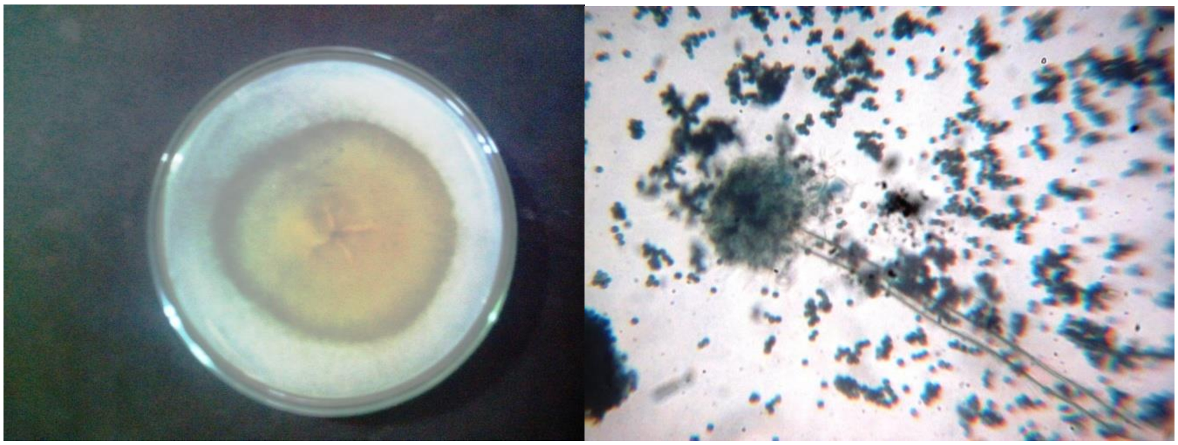


Figure 1 Analysis of different physicochemical parameters of soil (A) pH (B) EC (C) Density (D) Moisture (E) organic carbon (F) nitrogen (G) phosphorus.

Isolation & Identification

Out of all bacteria and fungus isolated from the soil only one fungi show significant zone of phosphate solubilization. A clear halo zone was formed around the colonies after 5 days of incubation on solidified PVK medium supplemented with calcium phosphate, indicating phosphate-solubilizing ability of the fungal isolates (**Figure 2A).** The fungal strain was identified as *Aspergillus niger* based upon their colony morphology, spore characteristics and microscopic studies (**Figure 2B**). *A. niger* showed black surface pigmentation with a suede like surface consisting of a dense felt of conodiophores. Conodiophores are long, smooth walled and have conical shaped terminal vesicle which support a single row of phial ides on the upper two third of the vesicle (**Table 2**).



A B

Figure 2 Isolation and identification of fungi by (A) holozone and (B) microscopic observation

Table 2 Morphological observation of isolated fungi

S. no.	Parameters	Observations
1	Size	1500
2	Surface	Smooth
3	Colour	Black
4	Shape	Glubose
5	Conodiphore	Long smooth

After confirming the phosphorus solubilizing ability on solid medium, the phosphorus solubilization in liquid medium was carried out. Different researchers have used different media for studying phosphorus solubilization in liquid medium. Fungus species show best result on PKV medium. Phosphate solubilization rate is depending on time of incubation. In this study the pH in the supernatant of the bacterial incubations was observed to decrease during the release experiments while being almost stable in the control incubation. The obtained results (**Figure 3**) show that the pH of the culture media of the *A. niger* in the presence of TCP, gradually decreased during the first few days of incubation up to values in the range 7.5 to 3.2 that were subsequently maintained. Phosphate solubilization was accompanied by a decrease in the pH of the medium.

A fall in pH during the growth of *Aspergillus* species in liquid medium containing insoluble phosphates has often been reported due to production of dicarboxylic (oxalic, succinic); dicarboxylic hydroxyl (malic) and tricarboxylic hydroxyl (citric) acids from simple carbohydrates [16]. Assimilation of ammonium present in Pikovskaya's medium may be one of the causes of lower pH due to higher acid production. Higher acid production and P solubilization from ammonium assimilation has a been reported for the solubilization of fluoroapatite by *Aspergillus niger* [17] and tri calcium phosphate by *A. aculeatus* [18].

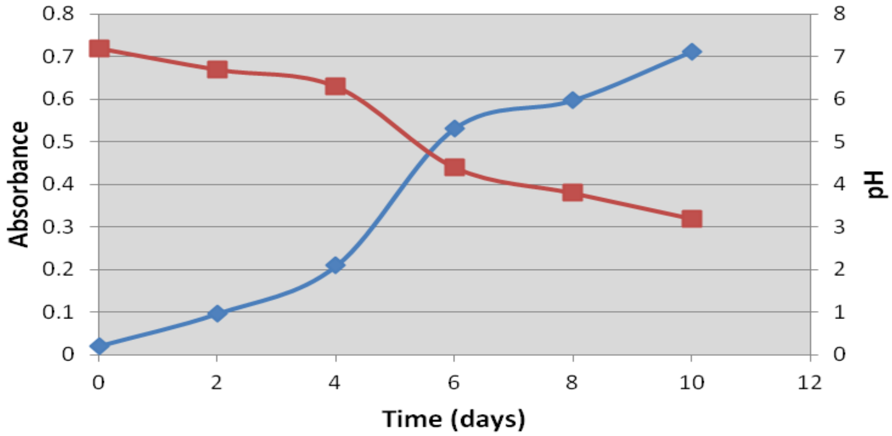


Figure 3 Quantitative analysis of phosphate solubilized by *A. niger*

The growth and yield parameters of potato such as plant height, fresh and dry weight, total flowering, branching and no. of root per plant were significantly increased by phosphate solubilizing microbes. The increase in height was 21.90%, diameter of shot 12.75%, leaf area 26.86%, and leaf number per plant 29.0%. Dry weight, shoot length, root length and no. of flower are represented in **Table 3& Figure4**.

Utilization of biological fertilizer increased fresh and dry weight, no. of pods per plant that it could be due to increasing other nutrient solubilization and absorption by plant, also biological phosphate fertilizer can be used as a solution for increasing phosphate and micronutrient sorption in the alkaline soil. Its effect already reported by researchers on different crops. In maize both qualitative and quantitative characteristics were significantly increased by phosphate-solubilizing microorganisms and also increased the growth and resistance of plants in water deficit conditions [19]. Hoshang Naserirad et al. [20] and Asad Rokhzadi [21] indicated that inoculation with biofertilizers containing Azotobacter and Azospirillum increased plant height, leaf number per plant, fruit mean weight and yield in compare to control (without biofertilizer).

Table 3 Morphometeric observations of PSM treated potato plant

S.no.	Parameters	Observations
1	Shoot length (cm.)	27.3
2	Root length (cm.)	9.8
3	Dry mass (gm)	15.6
4	Flowers/ plant	7.1
5	Braches/plant	5.4

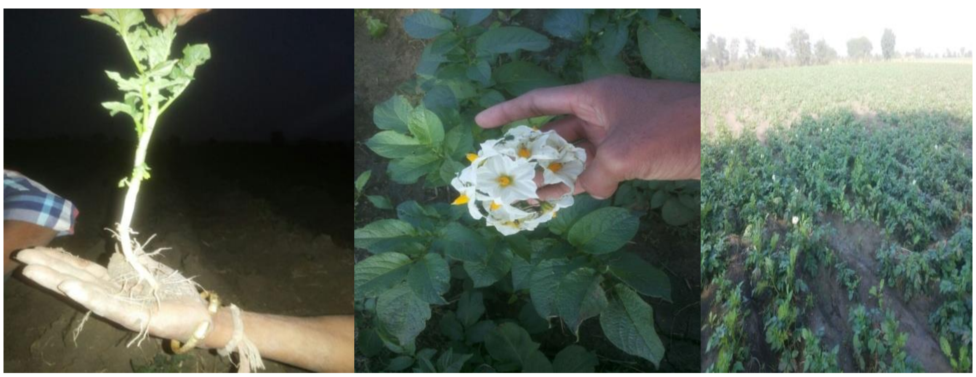


Figure 4 growth pattern of potato crop treated with phosphate solubilizing fungi.

The microorganisms have enormous potential in providing soil P for plant growth. Use of PSMs can increase crop yields up to 70 percent. The phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. The PSB exhibiting multiple plant growth promoting traits on soil-plant system is needed to uncover their efficacy as effective bio inoculants. The inoculation of PSB and plant growth-promoting rhizobacteria (PGPR) together could reduce 50% of P fertilizer application without any significant decrease of crop yield [22]. Combined inoculation of arbuscular mycorrhiza and PSBs give better uptake of both native P from the soil and P coming from the phosphatic rock and enhance plant growth by solublizing P from different fractions of soil [23]. The PSBs are able to synthesize phyto hormones like Indole acetic acid (IAA), Gibberellic acid (GA_3) and siderophore [24]. PSBs are also enhance plant growth by increasing the efficiency of biological nitrogen fixation or enhancing the availability of other trace elements such as iron, zinc, etc.

Conclusion

From the results, it is concluded that red soil is less fertile due to low clay content in soil, low water holding capacity, low organic matter, and poor nutrient status as compared to black soil. The results have shown that the various soil samples differ in many important physical and chemical properties. Therefore, their potential to support various types of land use is expected to differ. This means that if their productivity must be enhanced, then maximized differences should be appreciated and taken into consideration in the planning and management of the various potential use types. According to the results it can be stated that PSB, under specific conditions, mobilizes unavailable forms of soil and fertilizers. *A. niger* fungus, adequately selected, provide soluble nutrients to plants which improved plant nutrition and growth. A more thorough understanding of interactions between soil microorganisms is needed for an optimal utilization of these interactions with respect to growth and development of plants. In the long term, this approach would ensure a cost-effective, sustainable and environmental friendly production system for agriculture.

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