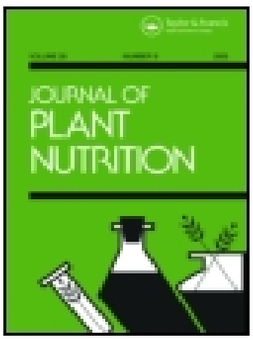

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Biosolubilization of rock phosphate by *Pleurotus ostreatus* with brewery sludge and its effect on the growth of maize (*Zea mays* L.)

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ABSTRACT

Rock Phosphate (RP) is a natural phosphorus source which can be an alternative to chemical fertilizers; but unfortunately, we lack technologies to make it applicable in alkaline soils. Therefore the aim of this work was to study the effects of phosphorous solubilizing fungus *Pleurotus ostreatus* on rock phosphate solubilization and its effect on growth of *Zea mays* L. incorporated with brewery sludge. The activity of the selected strain indicates the formation of the halo zone supplemented with rock phosphate. The maximum concentration of soluble phosphorus (P) was found at the 9th day of incubation in Pikovskaya's broth containing rock phosphate as P source. The inoculation of *P. ostreatus* with RP and brewery sludge significantly increased the root length (49%), shoot length (27%), root fresh weight (20%), root dry weight (46%), shoot fresh weight (89%), shoot dry weight (87%), chlorophyll content (79%) and nutrient accumulation, i.e., P (2.13), K (4.48), Ca (134.21), Mg (38.09), Cu (1.70), Mn (5.15), Zn (12.08), S (78.25) times greater than control in *Zea mays* L. The phosphatic residues after microbial solubilization showed structural and elemental changes confirmed by SEM and FE-SEM attached with energy-dispersive X-ray. The XRD and FTIR pattern shows that the mineral constituent of the treated sample signifies the proper P solubilization. Though few reports are available on *P. ostreatus* but biosolubilization with rock phosphate in the presence of brewery sludge is not yet reported. Overall, these findings suggest a possible application of the abundant waste materials on the sustainable growth of the plants.

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KEYWORDS
brewery sludge; maize; nutrient uptake; plant growth; pleurotus ostreatus; rock phosphate (RP)

Introduction

Phosphorous (P) is an essential macronutrient for plant growth and development (Vance 2011). It is the world's second largest nutritional supplement for crops after nitrogen (Azziz et al. 2012) and played an important biochemical role in respiration, cell division, photosynthesis, cell enlargement, synthesis of nucleic acids, proteins and adenosine triphosphate (ATP) (Yadav and Pandey 2018; Calle-Castañeda, Márquez-Godoy, and Hernández-Ortiz 2018). Most of the soils have a large reserve of total P, but the amount of P, which is actually available to the plants to support optimum plant growth is very small. Therefore, the continuous application of phosphatic

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fertilizers is essential for increasing crop yield. However, the manufacture of conventional P fertilizer includes chemical treatment of high-grade phosphate ore with sulfuric acid at an elevated temperature that is expensive and potentially leads to environmental damage (Vassilev et al. 2006). To increase the accessibility of P, rock Phosphate (RP) enriched with organic waste and phosphate solubilizing microorganisms (PSMs) could be an excellent approach to develop sustainable agriculture in an economically efficient and eco-friendly way. The total phosphate reserves in India is estimated to be 260 Mt, while only 142 Mt can be recoverable (Biswas 2011) as most of them are not suitable for the manufacture of phosphatic fertilizer due to low P content. The low pH of the acidic soil enhances the RP solubilization while they perform poorly in alkaline soil due to high pH. Therefore the direct application of RP is not suitable for neutral soil (Fertilizer Statistics, 2015–2016). One of the appropriate technologies to enhance the insoluble phosphates solubilization is microbial-based mechanism with strong chelation and complexing properties on the production of organic acids (Vassilev et al. 2014). These organic acids (e.g., oxalic acid, citric acid, acetic acid, succinic acid, etc.) produced by phosphate solubilizing microbes solubilize insoluble P by lowering of pH, the hydroxyl and carboxyl groups present in the organic acids chelating the cations (mainly calcium) bound to phosphate leading to increased solubility and availability of mineral phosphates (Mander et al. 2012; Khan, Zaidi, and Wani 2007; Trabelsi et al. 2017; Wei et al. 2017; Reyes, Valery, and Valduz 2006).

However, to ensure the microbial growth, organic acid production, and RP solubilization metabolizable C compounds must be applied (Vassilev and Vassileva 2003). A wide range of carbohydrates has been tried, i.e., glucose, sucrose, fructose, xylose, starch (Cerezine, Nahas, and Banzatto 1988), many agro-industrial waste, i.e., sugar-beet wastes, olive cake, dry olive cake, and olive mill wastewaters. The application of brewery sludge as alternative carbohydrate sources can also be taken into consideration. The sludge is usually rich in organic matter and essential nutrients, which has the potential for use as fertilizers and soil conditioners and can substitute mineral fertilizers (Silva, Resck, and Sharma 2002). According to a previous study, the aerobic sludge of winery, used as the soil amendment and the availability of N, P, K, S was found to increased significantly. It was also found to enhance the organic and mineralizable carbon and total microbial activity of soil (Thomas and Rahman 2006). Likewise, the amount of sludge has increased drastically as a consequence of the aerobic biological treatment of brewery effluent coming out from the United Brewery situated at eastern Odisha. It is therefore a need for the development of appropriate waste management strategy to reutilize it as fertilizer for improvement of soil fertility and crop production. Keeping this in view the present study was therefore conducted to assess the effect of RP and organic sludge from the brewery industry on growth and nutrient uptake of *Zea mays* L. inoculated with P-solubilizing fungus. These materials could give an inexpensive source of phosphatic fertilizer for crop production in India.

Materials and methods

Sample collection and characterization

Rock phosphate (RP) was obtained from Hindustan Zinc Limited, Udaipur, Rajasthan, India. It is having 7.45 total P, 29.89% total Ca, 0.59% total Mg, 3.32% total Fe, 0.83% total Na, 2.83% total Al, 16.65% total Si, 1.56% total S, 0.15% total K, 0.22% total Ti, 11.2 mg kg^{-1} total V, 40.6 mg kg^{-1} total Mn, 47 mg kg^{-1} total Cu, 220.5 mg kg^{-1} total Zn, 100.9 mg kg^{-1} total Ba and 23.7 mg kg^{-1} total La respectively. The heavy metal concentrations of Co, Pb, and Ni are 13.6, 6.6 and 78.1 mg kg^{-1} respectively. The XRD analysis shows the presence of tricalcium phosphate $Ca_3(PO_4)_2$, Calcite ($CaCO_3$), quartz (SiO_2), lime (CaO), Dolomite $CaMg(CO_3)_2$, Chlorapatite ($Ca_5(PO_4)_3$ Cl), alumina (Al_2O_3) and fluorapatite ($Ca_5(PO_4)_3F$) as the main minerals. This rock phosphate can be classified as low-grade rock phosphate based on P content.

Table 1. Physico-chemical properties of the soil and sludge used in this study.

Property	Soil	Sludge
pH	5.62	6.27
Electrical conductivity (EC) (dSm^{-1})	0.11	0.65
Organic carbon (%)	0.08	21.6
Total N (ppm)	0.98	45000
Total P (as P_2O_5) (%)	0.1	3.3
Total K (as K_2O) (%)	ND	0.2
Manganese (mg/kg)	21	48
Magnesium (mg/kg)	27	1232
Zinc (mg/kg)	32	79
Copper (mg/kg)	12	46

The soil used for pot experiment was collected from the premises of CSIR-Institute of minerals and materials technology, Bhubaneswar, India and the organic sludge collected from United Breweries Limited, Khurda, Bhubaneswar, India. The high organic carbon content is the most important factor for the selection of the sludge for the present study. The maize seed was procured from the Odisha state seeds corporation, Bhubaneswar, India. All the samples (soil, RP, and sludge) were air-dried properly and passed through a 2 mm sieve prior analysis. The physico-chemical properties of the soil and sludge are presented in Table 1.

P solubilization test in PVK agar medium

The fungal strain *Pleurotus ostreatus* (NCIM-1200) was collected from the National Collection of Industrial Microorganism, Pune, India. The phosphate solubilizing activity of the strain was analyzed by plate assay method using pikovskaya (PVK) agar replacing tricalcium phosphate ($Ca_3(PO_4)_2$) with 20 mg P_2O_5 of rock phosphate (Nautiyal 1999). Here, insoluble RP represents the only source of Phosphorus. *P. ostreatus* was inoculated on the Pikovskaya agar plate and were incubated at 37°±0.1 °C for 24–48 hrs (Nagar 2012). The extent of P solubilization by the fungal strain was determined by measuring the clear zone (in mm) around the colonies by taking the halo zone formation into consideration.

P solubilization test in PVK broth medium

The ability of *P. ostreatus* to solubilize insoluble RP has been tested in 250 ml Erlenmeyer flasks containing 50 ml of PVK broth amended with rock phosphate equivalent to 20 mg P_2O_5. Later, the flask was incubated in a rotary orbital shaker (Remi Orbital Shaking Incubators, CIS-24 BL) at 120 rpm in 30 °C for 15 days as the previous studies reported the optimum temperature for maximum microbial phosphorus solubilization was to be 30° C (Kim, Jordan, and Kirshanan 1997; Rosado et al. 1998; Fasim et al. 2002). All the experiments were conducted in triplicate. The aliquot was withdrawn after different growth periods (first day and then after two days interval) followed by centrifugation at 10,000 rpm for 15 min (Sagervanshi et al. 2012). The pH of the medium was measured by Water Analyzer Model 371 Systronics and the soluble P by vanadium ammonium molybdenum colorimetric method (Jiang, Zhao, and Zhu 2001). After leaching the solid residues were recovered and washed with distilled water followed by drying. Later, the sample was homogenized to fine powder and analyzed by scanning electron microscopy (SEM, JEOL, JSM-6510), FESEM equipped with energy dispersive X-ray (EDX, Carl Zeiss, SUPRA GEMINI55), X-ray diffraction (XRD, Rigaku, Ultima IV) and Fourier Transform Infrared spectrometry (FTIR, Perkin Elmer, Spectrum-GX) to know their surface character, structural morphology, chemical composition and surface functional groups present in newly synthesized products.

Organic acid production by the isolates

To determine the organic acids released by *P. ostreatus*, the strain was grown in Pikovskaya broth supplemented with RP equivalent to 20 mg P_2O_5, and incubated at 30 °C for six days. Later, the sample was centrifuged at 5000 rpm for 10 min (Sagervanshi et al. 2012). Supernatant of blended cultures were filtered through 0.20-μm syringe filters (cellulose acetate) and the organic acid was detected using HPLC (shimadzu SPD-M20A) with PDA detector by using methanol 5% and 0.1% TFA 95% as mobile phase with flow rate of 1 ml per minute with column particle size 5 µm, length 25 cm and column diameter 4.6 mm (Merck C18 column).

Inoculant preparation and application

For preparation of inoculum, single isolated colony of *P. ostreatus* was suspended into 50 ml PVK broth (Glucose 10 g, $(NH_4)_2$ SO_4 0.5 g, $MgSO_4.7H_2O$ 0.1 g, Yeast Extract 0.5 g, KCl 0.2 g, NaCl 0.2 g, $FeCl_3$(trace), $FeSO_4.7H_2O$ 0.002 g, $MnSO_4.7H_2O$ 0.002 g, $MgCl_2.6H_2O$(trace), $Ca_3(PO_4)_5$ 5 g) per liter of deionized water and incubated at 28 °C, 150 rpm, for 3 days. The fungal cultures were then centrifuged at 10,000 rpm for 3 min, followed by washing the cells with sterile distilled water (Gurdeep and Reddy 2015). The fungal cells were reharvested in sterile distilled water and utilized as an inoculums (Bhuvaneswari, Turgeon, and Bauer 1980).

Pot experiments

The pot experiment was conducted to assess the effect of RP and sludge inoculated with *P. ostreatus* on growth and nutrient uptake by maize (*Zea mays* L.). The experiment was a completely randomized block design of four treatments with three replications. Before sowing, the seeds were surface sterilized using 0.1% Mercuric chloride solution for 5 min, followed by washing with autoclaved distilled water. Each pot was 20 cm height and 25 cm in diameter. The treatments used in this experiment were as follows: T0 (only soil); T1 (soil + RP); T2 (soil + RP+*Pleurotus ostreatus*); T3 (soil + RP +*Pleurotus ostreatus*+Sludge). Before sowing the soil was amended with (200 mg P_2O_5 kg^{-1} substrate) in all treatments except control and in T3 sludge was amended in 1:1(soil: sludge) ratio followed by microbial inoculation. The soil was irrigated properly before sowing to maintain moisture for seed germination. Inorganic fertilizer has not been applied during the experiment. The plants were harvested after the 45 days of treatment and were properly washed to clear all adhering soil particles. From each treatment five randomly selected plants were uprooted and length of the root and shoot measured followed by fresh weight of root and shoot. The plant material was dried at 70 °C for root dry weight and shoot dry weight determination. Chlorophyll contents were measured with MC-100 chlorophyll concentration meter by Apogee Instruments. Shoot P content was determined by the molybdo-vanado method described by Reuter et al. (Reuter, Robinson, and Dutkiewicz 1997). Plant samples then ground to a fine powder (0.5 mm) and were analyzed for total nitrogen by Kjeldahl apparatus. Total potassium, calcium, magnesium, copper, iron, manganese, zinc and sulfur were measured in the dry ash digestion using the AA-6300 SHIMADZU Atomic Absorption Spectrophotometer (Chapman and Pratt 1961).

Statistical analysis

Statistical analysis was performed using Origin 8.0 and IBM SPSS Statistics 20. The data on root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, chlorophyll content and nutrient accumulation were subjected to analysis of variance (ANOVA) and the means were compared with Tukey's test at $P < 0.05$ considered significant with a

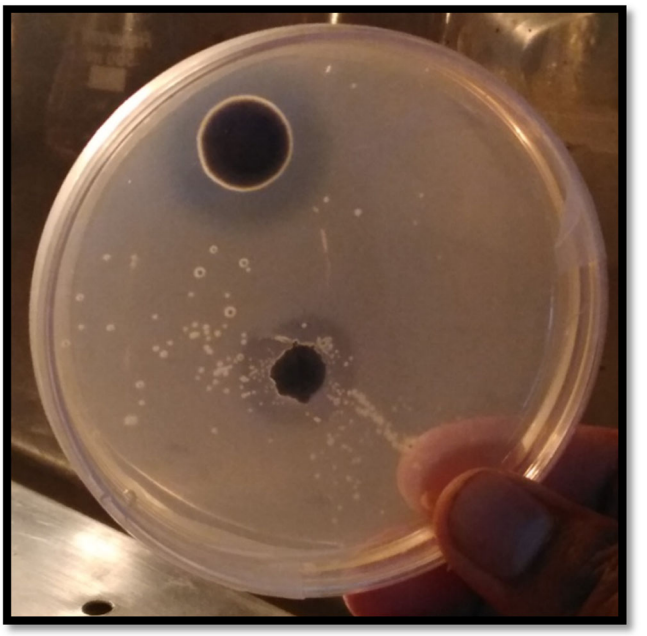


Figure 1. Clear halo zone formed by Pleurotus ostreatus grown on modified PVK plates.

confidence limit of 95%. All the data are graphically presented as mean ± standard deviation of triplicates (n = 3).

Results

P solubilization test in PVK agar medium

A clear halo zone (13 mm) with diametric extension was appeared on the 5th day of incubation as the zone of RP solubilization (Figure 1). Thus, the results demonstrated that the *P. ostreatus* could solubilize rock phosphate to produce soluble P.

P solubilization test in PVK broth medium

It was observed that the dissolved phosphate concentration increases by a decrease in pH of the medium (Figure 2). The decrease of pH is due to the secretion of the organic acid by *P. ostreatus* in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and enhance the inorganic P solubilization (Stevenson 2005). Hence pH plays a major role in RP solubilization and its strong inverse relationship with solubilized P in RP amended liquid medium is reported by many authors and supports the present study (El-Tarabily, Nassar, and Sivasithamparam 2008; Narsian and Patel 2000). The solubilization of RP (20 mg P_2O_5) was maximum (72.76 µg ml^{-1}) at 9th day by *P. ostreatus* and sudden decrease was observed at 12th day, which might be due to the precipitation reactions with cations such as Fe-P and Al-P in acidic medium (Bayer et al. 2001; Yadav et al. 2017). In addition, it was known that Al and Fe solubility increases below the pH 5.5 (Haynes and Mokolobate 2001; Stumm and Morgan 2012) and reabsorption of the solubilized P could be possible since the fungus has the capacity to solubilize a specific quantity of P which is in equilibrium with fixed P (Duponnois, Kisa, and Plenchette 2006).

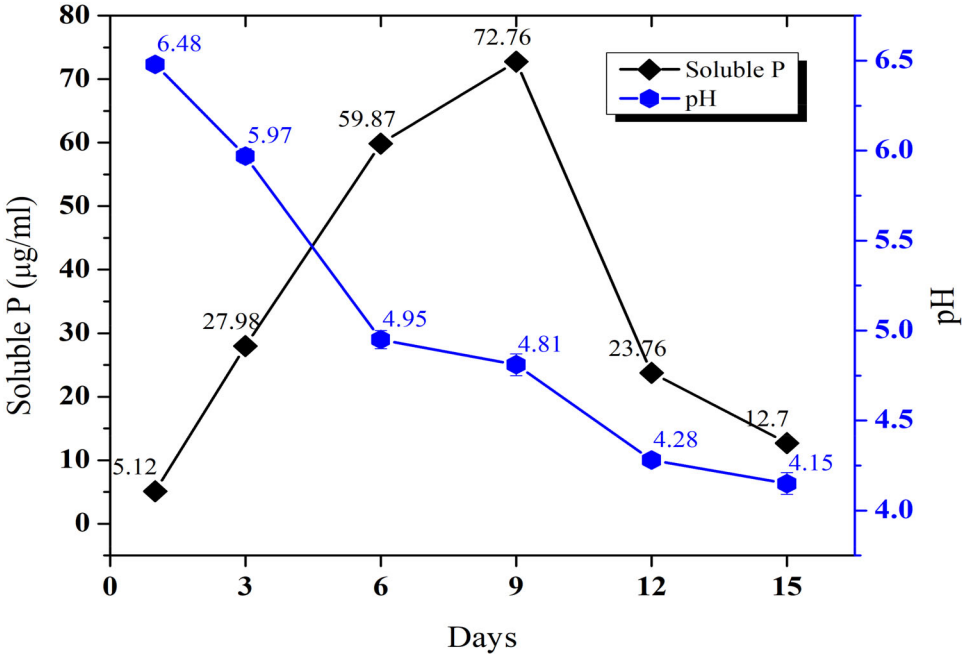


Figure 2. Effect of incubation period on the solubilization of RP and pH by *Pleurotus ostreatus*.

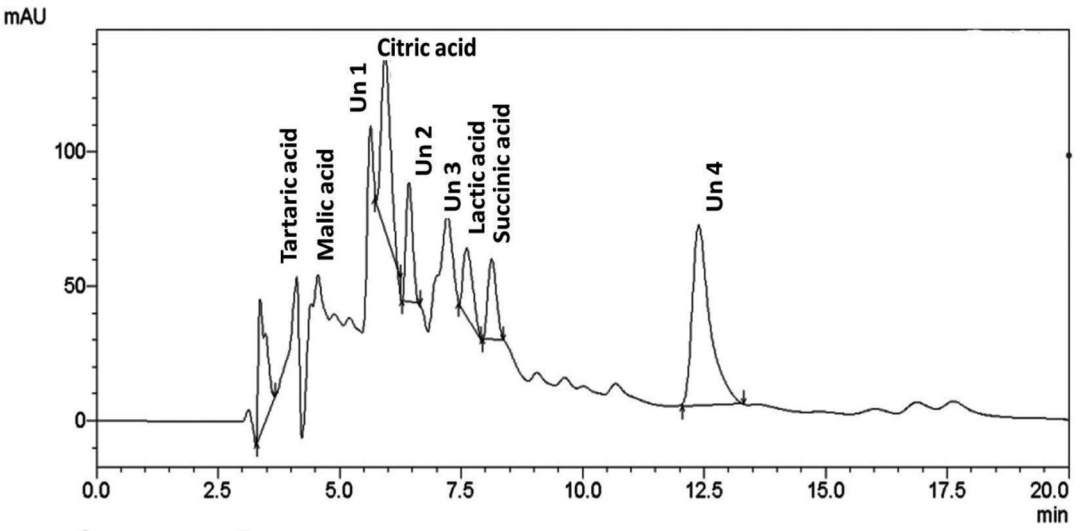


Figure 3. HPLC analysis of the known and unknown organic acids produced by *Pleurotus ostreatus*.

The major organic acids produced by the fungus were tartaric acid (95.92 ppm), malic acid (80.06 ppm), citric acid (741.46 ppm), lactic acid (4394.12 ppm), succinic acid (95.71 ppm) along with four unknown acids Un 1, Un 2, Un 3 and Un 4 (Figure 3). This mixture of organic acid was successful in decreasing the pH and induces higher P-solubilization. Lactic acid was predominant among five organic acids, followed by citric acid in the culture broth of *P. ostreatus*. It was reported that solublization of phosphate is mediate by organic acid produced by PSM which further support our study (Cunningham and Kuiack 1992).

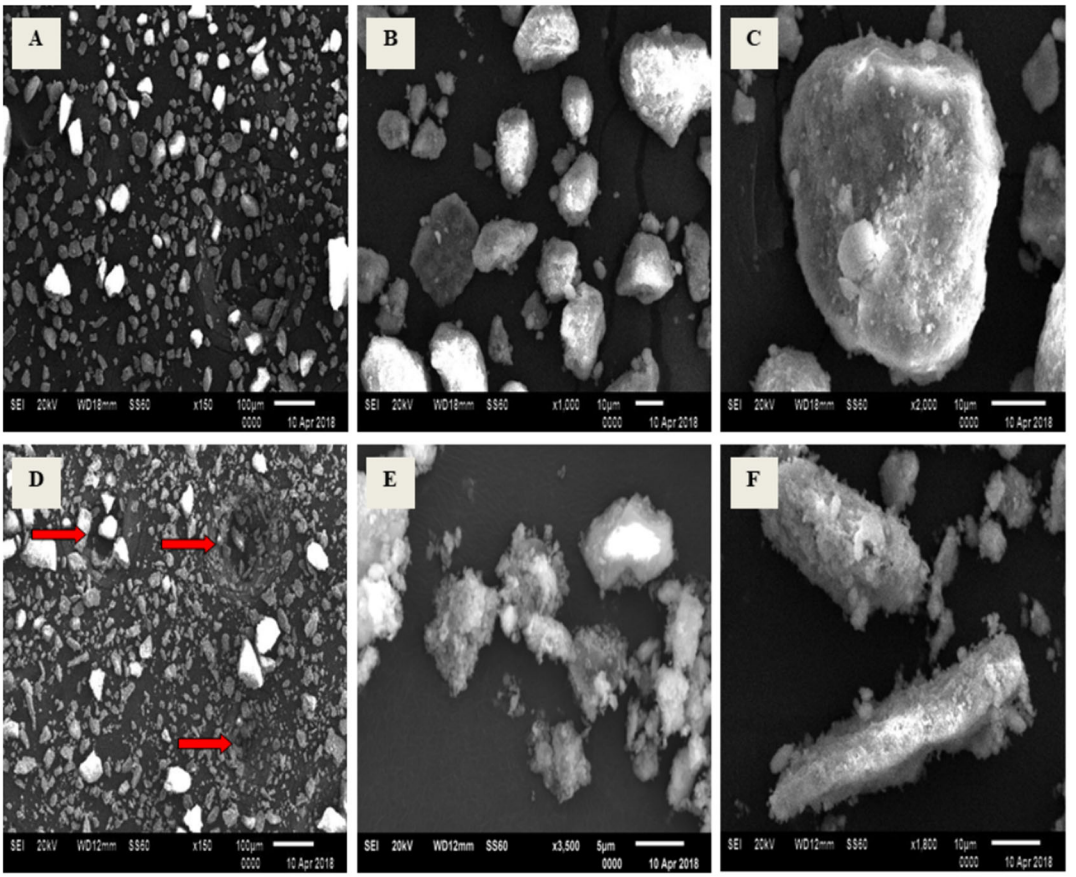
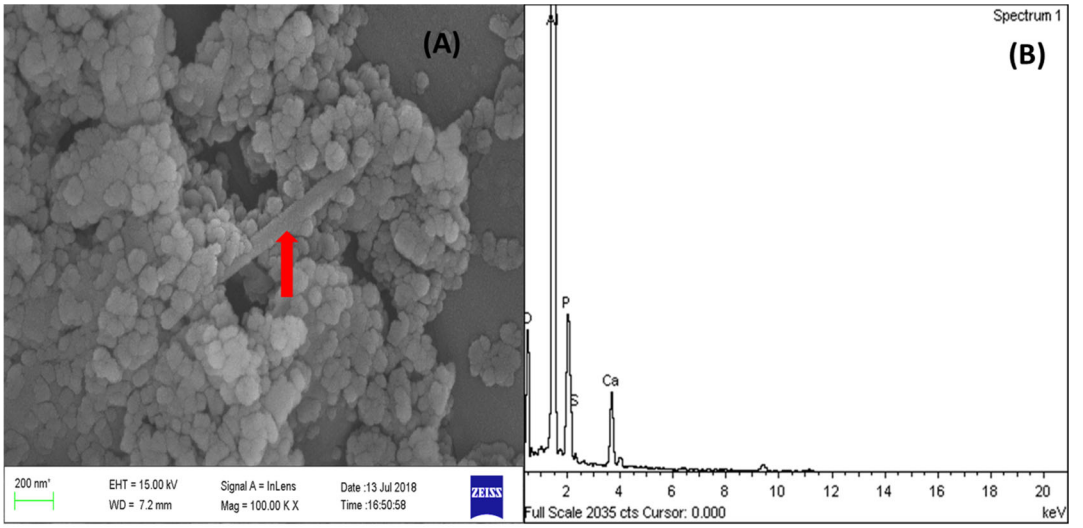


Figure 4. SEM images of rock phosphate surfaces without (A, B, C) and with (D, E, F) *Pleurotus ostreatus*.

Characterization of treated rock phosphate

The SEM micrograph of rock phosphate residue showed noticeable structural changes after microbial treatment which is shown in Figure 4. Rock phosphate particles were found to have smooth surfaces (Figure 4A). The fungus was found to make a rugged structure on the surface of the rock phosphate particles (Figure 4D). Compared with control sample, treated RP surfaces were markedly corroded by the microbes, and some asymmetrical holes were observed which were shown in red arrow on the RP surface. This could be due to the proton attack from acid compounds secreted by microbes and leaching of phosphatize (Figures 4A,D), which was also similar with the result found by Hui He et al. 2014). There were many clusters of regular, round and sharp edges particles appeared in RP with no treatment (Figure 4B). While, the microbes treated residue have irregular round structure without having sharp edges, which were non-uniformly distributed (monodispersed) with significant deformation (Figure 4E). RP particle form rod-shaped and de-agglomerated structure which were different from control sample having a round and distinct boundary (Figures 4F,C) which verified the solubilization of fluoroapatite to soluble phosphate confirmed further by XRD analysis.

The structural change in the treated sample was investigated by FESEM attached with Energy-dispersive X-ray (EDX) in different places of the sample. It was shown that the phosphate rock consists of two different structural phases (Figure 5) with spherical and elongated having estimated sizes of 800 nm, which was disappeared in microbes treated sample (Figure 6) with more



Element	App Conc.	Intensity Corrn.	Weight %	Weight % Sigma	Atomic %
OK	2.52	0.8287	22.03	0.85	30.42
Al K	8.91	1.1485	56.17	1.15	45.99
P K	0.82	1.1021	5.41	0.39	3.85
S K	0.07	0.8269	0.62	0.17	0.42
Ca K	1.02	0.9793	7.53	0.32	4.15

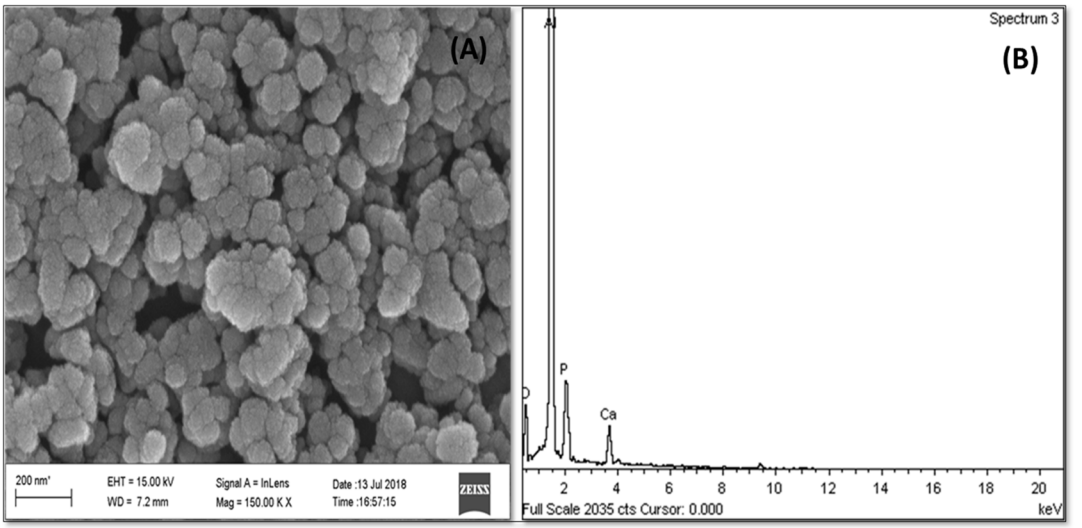
Figure 5. FE-SEM (A) and EDX (B) analysis of rock phosphate without *Pleurotus ostreatus* and the corresponding table represents the intensity and % weight of different element present in the respective sample.

weathered structure. However, the surfaces of the raw RP exhibit a compressed configuration with only little porosity (Figure 5).

Energy-dispersive X-ray (EDX) analysis indicated that (Figure 5) RP contained Al, O, P, S and Ca prior the treatment, whereas after treatment S was disappeared and the peak of O, P and Ca has been reduced (Figure 6). The quantitative chemical composition (Figure 6) showed that after treatment the RP contained less O, P and Ca element, whereas, S was completely vanished. This sudden change indicated that solubilization by the fungus had a significant impact in altering RP composition.

Phase and crystal structure of the natural rock phosphate before and after the bio-solubilization process were investigated by X-ray diffraction (XRD) study and the results were shown in Figure 7. This data was in well conformity with the Joint Committee on Powder Diffraction Standards (JCPDS) card no 36-1451. The strongest peak was observed at 2Θ values of 14.59, 28.04 the lattice planes (031), (101) respectively indicating the presence of quartz SiO_2 in the sample. Some other peaks were also observed at 2Θ values 11.82, 18.20, 22.28, 33.41, 35.96 which confirmed the presence of Calcium phosphate, lime CaO, carbonates which are in the form dolomite Ca Mg $(CO_3)_2$, Chlorapatite $Ca_5(PO_4)_3$ Cl, alumina Al_2O_3 respectively. Another major peak of calcite $CaCO_3$ at 2Θ: 13.32 corresponding to the plane (112) was found, which was eventually decreased in length in bio-solubilized sample indicating the enrichment of crystallinity after the process. The peak of Fluorapatite $Ca_5(PO_4)_3F$ was disappeared in the treated sample signifying a certain level of impurities were removed during the solubilization process of phosphate rock.

The FT-IR spectra of rock phosphate before and after solubilization treatment illustrated within the section of 4000 – 400 cm-1 in Figure 8. It was noticed that the process had a notable



Element	App Conc.	Intensity Corrn.	Weight %	Weight % Sigma	Atomic %
OK	0.66	0.9063	6.46	0.56	10.55
Al K	12.75	1.2970	87.37	0.70	84.63
P K	0.27	0.9759	2.49	0.38	2.10
Ca K	0.28	0.9797	2.52	0.24	1.64

Figure 6. FE-SEM (A) and EDX (B) analysis of rock phosphate with *Pleurotus ostreatus* and the corresponding table represents the intensity and % weight of different element present in the respective sample.

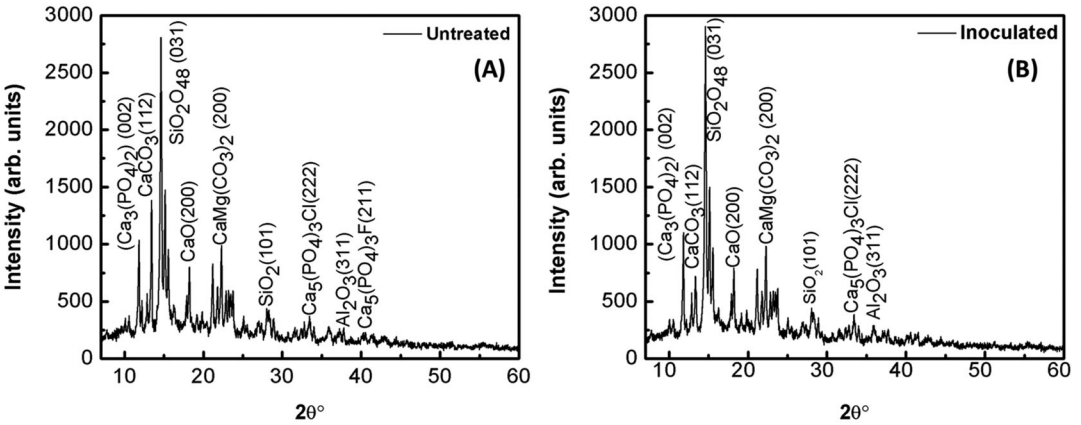


Figure 7. X-Ray diffraction (XRD) patterns of Rock phosphate before (A) and after (B) inoculation of *Pleurotus ostreatus*.

and significant effect on the intensity and positions of the vibrational bands; moreover there was presence and vanishing of some peak occurs. The disappearance of the bands related to calcite at 471 cm-1 as well as carbonate situated at 1559 cm-1 implied that carbonate and calcite substitutions induce vacancies at the OH sites, and we assumed that the treatment was responsible of the total decomposition of carbonate bands and intensities decreases (Fahami, Nasiri-Tabrizi, and Ebrahimi-Kahrizsangi 2012). Moreover, after the solubilization process, the band sites and their intensities were somewhat affected. The change in the number of phosphate bands were observed as the bend intensely shifted from 694 cm^{-1} to 798 cm^{-1}. The shift could be happened due to the

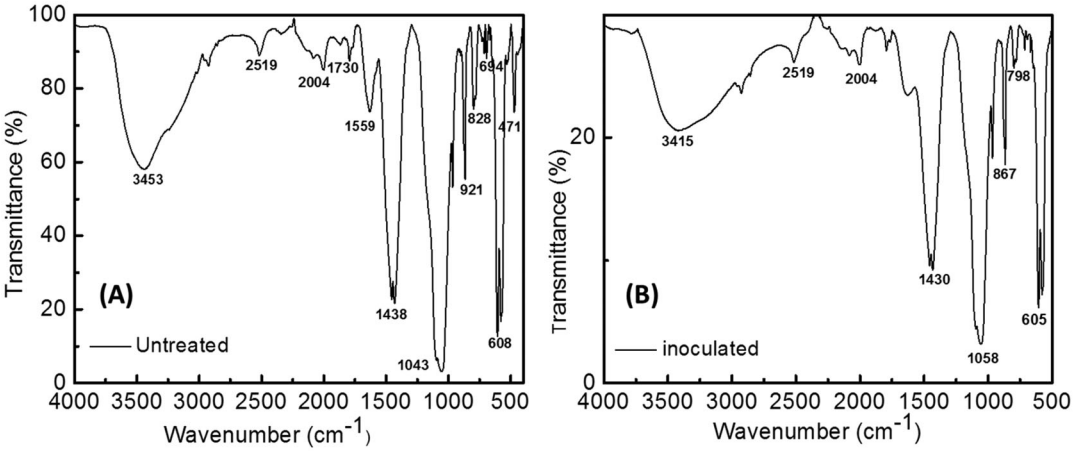


Figure 8. Fourier transform infrared spectroscopy (FTIR) of Rock phosphate before (A) and after (B) inoculation of *Pleurotus ostreatus*.

variation repulsion potential of the contracted or dilated crystal lattice which was confirmed by XRD analysis. However, other structures were also decreased in the order of magnitude to a lesser extent.

Influence of inoculants on growth and nutrient accumulation in maize plants

The inoculation of *P. ostreatus* combined with RP and sludge enhanced plant growth parameters such as root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight and chlorophyll content than control (Figure 9). The application of microbial inoculant showed promoting effects on maize plant compared to non inoculated soil.

RP, when applied alone increased root fresh weight (20%), shoot fresh weight (30.88%), shoot dry weight (25%) and chlorophyll content (26.77%) significantly ($P < 0.05$) than control. The response of root length, shoot length and root dry weight was non-significant ($P > 0.05$).

The effect of rock phosphate with fungal strain further increased plant growth. The shoot length was 1.06 times higher than non inoculated soil (T1) and 1.15 times higher than control soil. The application of rock phosphate with *P. ostreatus* significantly ($P > 0.05$) increased all other growth response such as root length, root fresh weight, and root dry weight 1.11, 1.4 and 1.16 times, respectively than only RP amended soil except chlorophyll content, which was non-significant ($P < 0.05$). The shoot fresh weight, chlorophyll content and shoot dry weight was increased up to 60.29%, 38.79%, and 81.25% respectively, in RP amended soil inoculated with *P. ostreatus* compared to control soil.

The effects of experimental treatments on plants growth were more prominent in soil treated with sludge, RP and *Pleurotus ostreatus*. Most of the growth traits were significantly ($P < 0.05$) higher in this combined treatment except root fresh weight which was highest in RP+*P.ostreatus* with no sludge-treated soil. Overall the maximum stimulatory effect was obtained in soil with all substrate i.e., RP, sludge and *P. ostreatus* whereas 49.20% increase in root length, 27.45% increase in shoot length, 46.66% increase in root dry weight, 89.70% shoot fresh weight, 87.5% shoot dry weight, and 78.68% chlorophyll content were increased as compared to the un-inoculated control.

The treatment also had a significant effect on accumulation of macro- and micro-nutrients (Table 2). The accumulation of P by plant inoculated with RP, *P. ostreatus* and sludge yielded significantly ($P < 0.05$) highest P than all the treatment (Table 2). The increase in P accumulation in the plant was 2.13, 2.09 and 1.06 times greater than the control, soil + RP and soil + RP+*P.*

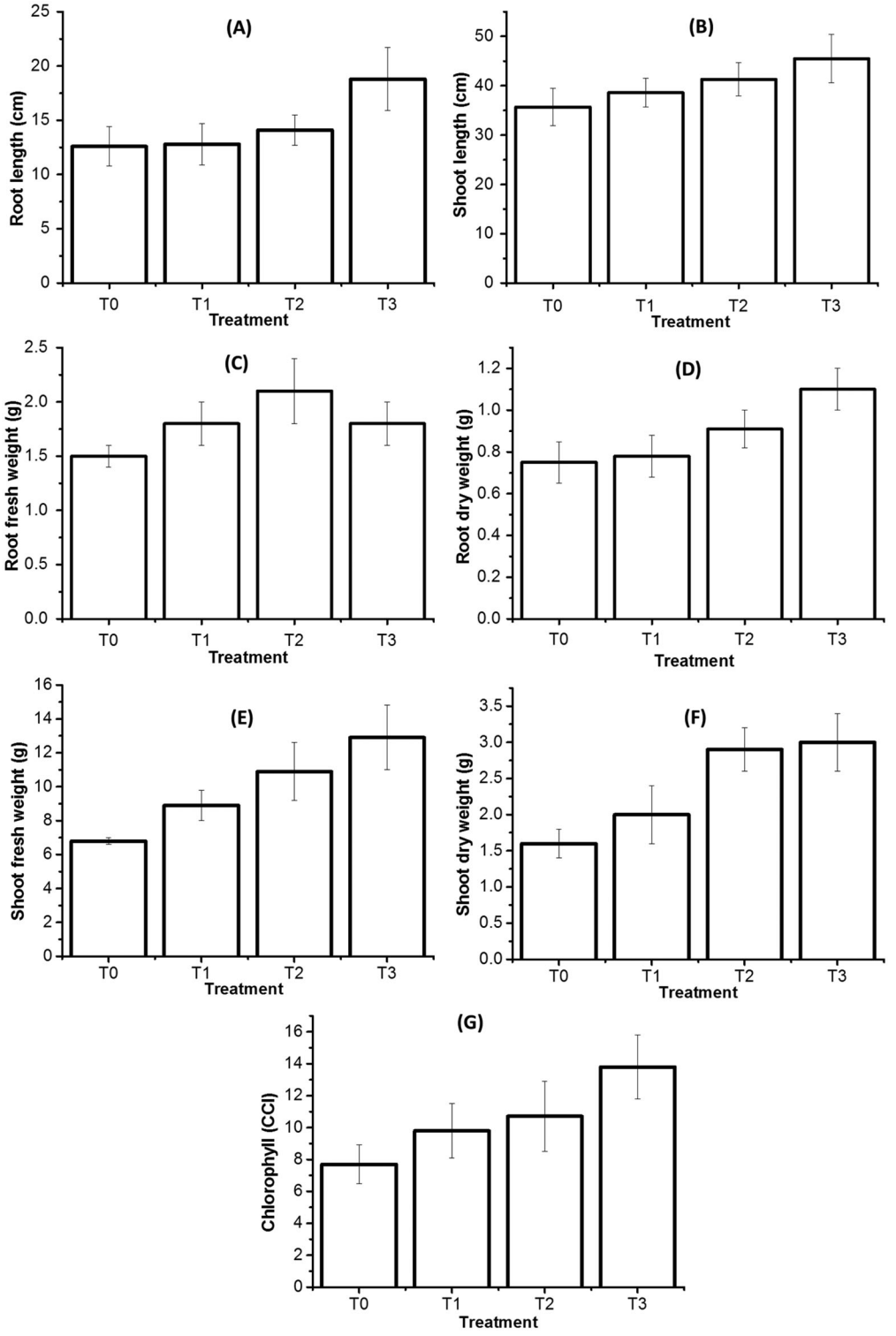


Figure 9. Root length (A), Shoot length (B), Root fresh weight (C), Root dry weight (D), Shoot fresh weight (E), Shoot dry weight (F), Chlorophyll (G) of maize grown under different conditions after 45 days. T0(Only soil); T1(soil + RP); T2(soil + RP+*Pleurotus ostreatus*); T3(soil + RP + Sludge+*Pleurotus ostreatus*).

Table 2. Accumulation of Macro and micro nutrient in maize plants (*Zea mays L.*) T0 (Only soil); T1 (soil + RP); T2 (soil + RP+*Pleurotus ostreatus*); T3(soil + RP + Sludge+*Pleurotus ostreatus*).

Nutrient accumulation (mg kg^{-1})	T0	T1	T2	T3
Total P	70.62 (0.36)^{a}	71.98 (0.85)^{a}	141.87 (0.65)^{b}	150.65 (0.45)^{c}
K	124.98 (0.08)^{a}	345.98 (0.41)^{b}	364.98 (0.50)^{b}	560.9 (0.02)^{c}
Ca	0.76 (0.02)^{ab}	78 (0.005)^{bc}	98 (0.35)^{d}	102 (0.65)^{d}
Mg	2.97 (0.006)^{a}	89.65 (0.011)^{b}	95.98 (0.84)^{bc}	112.76 (0.45)^{c}
Cu	4.09 (0.010)^{a}	4.39 (0.81)^{ab}	6.89 (0.65)^{b}	6.99 (0.43)^{bc}
Mn	1.9 (0.35)^{d}	7.9 (0.08)^{e}	9.6 (0.45)^{f}	9.8 (0.55)^{f}
Zn	4.7 (0.007)^{a}	37.6 (0.14)^{b}	62.8 (0.02)^{c}	56.8 (0.76)^{d}
S	0.87 (0.06)^{b}	48.65 (0.47)^{c}	57.98 (0.54)^{d}	68.08 (1.12)^{e}

Mean values with standard deviation (in enclosed bracket) of each parameter are given in the table for the triplicate set of experiment. Different superscript letters in each row represent the significant difference of means at $p < 0.05$ as per Tukey's test.

ostreatus treated soil, respectively. Most notably, the soil treated with only RP yielded the P accumulation that was similar to the P accumulation resulting from control.

The significant accumulation of other macro and micronutrients (K, Ca, Mg, Cu, Mn, Zn, and S) in the plants were also observed in the treatment inoculated with RP + sludge+*P. ostreatus* together with the exception for Zn which was highest in RP with only *P. ostreatus* amended soil (Table 2).

Discussion

In the present investigation, *P. ostreatus* was selected for RP solubilizing efficiency and could be utilized as biofertilizers. The P solubilizing activity was detected on plates having RP as sole P source by the formation of halo zone around their colonies, which confirms the solubilization of rock phosphate. The *P. ostreatus* indicated high solubilization and decreased the pH of the broth at the same time. The remarkable drop in pH commonly accompanied by phosphate solubilization (Henri et al. 2014; Kloeppe et al. 1999; Gupta et al. 2007). The drop in pH was caused by the acidification of the medium, which might be happened because of: [1] the proton and carbon dioxide released due to the biological activity during the growth of fungi (Marschner 1998), [2] the production and exudation of low molecular weight organic acids (Gadd 1999), [3] the absorption of nutrients in exchange for protons (Burgstaller and Schinner 1993). Medium acidification is one of the well-known mechanisms of mineral P solubilization which was demonstrated in many previous studies conducted with a variety of microorganisms including bacteria and fungi (. Rashid et al. 2004; Perez et al. 2007; Halder et al. 1990; Marra et al. 2011). The morphological and chemical changes with functional groups alteration in newly synthesized products further confirm the relationship between the organic acids and P transformation after analytical characterization.

This activity was analyzed on the maize as the test plant and different growth parameters were measured. The application of RP and brewery sludge with the inoculation of *P. ostreatus* prominently improved the growth parameters such as root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, chlorophyll content and nutrient accumulation than control. The total P uptake of the maize crops was also increased significantly compared to the un-inoculated control. In the rhizosphere, microbes secrete organic acids which resulted in phosphate solubilization from insoluble complexes, making it available for plant uptake (Richardson et al. 2009). Phosphate solubilizing microbes release H^{+} ions into the soil (Alvarez et al. 2004) and consequently increased the solubility/mineralization of P from added naturally occurring RP sources. This above phenomenon completely agrees with our findings.

As brewery sludge is rich in nutrients and organic matter, it enhanced microbial activity (Thomas and Rahman 2006). There were scientific evidence of combined application of mineral

phosphates with organic sludge inoculated with phosphate solubilizing microbes affect the circulation of P fractions (Wei et al. 2016) which, increase the productivity of different crops (Basta, Ryan, and Chaney 2005), which further supports our findings. Numerous reports were also available on P solubilization by various microorganisms. However, the efficiency of rock phosphate solubilization followed by maize plant growth study with *P. ostreatus* amended with brewery sludge is a new approach for utilization of solid waste in a sustained manner to get an eco-friendly agricultural approach. The outcomes clearly demonstrated that application of low-grade RP, brewery sludge with *P. ostreatus* can enhance the plant growth and release the P in the soil in a cost effective way.

Conclusion

From the above results, it was confirmed that low-grade rock phosphate can be utilized as a P source fertilizer by direct application to soils along with brewery industry sludge and P solubilizing fungus *P. ostreatus*. These outcomes was having an agronomic significance for crop development and yield which may help with tackling issues experienced with food shortage and crop production. The investigation of P solubilization in the broth confirmed the morphological, elemental and phase alteration by analytical characterization. The growth response further confirmed the P solubilization within the soil while further studies are required to investigate the exact mechanism of P solubilization in the vicinity of the rhizosphere and other plant growth factor by the fungus.

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Disclosure statement

All the authors in this manuscript declare that they have no conflict of interest.

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