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ORIGINAL PAPER

Acidithiobacillus thiooxidans IW16 and Sulfur Synergistically with Struvite Aggrandize the Phosphorus Bioavailability to Wheat in Alkaline Soil

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Abstract

Environmentally hazardous wastewaters from various origins could prove an impending source for phosphorus (P) recovery as struvite. This study aimed to employ an eco-friendly approach for P utilization from struvite, and to neutralize its alkaline effect in the soil through supplementation of sulfur-oxidizing bacteria (SOB) *Acidithiobacillus thiooxidans* IW16. Struvite precipitated and recovered from wastewater was tested for P release and bioavailability to grow wheat in alkaline soil under greenhouse conditions. Treatments were control (no P application), P from single superphosphate (SSP) fertilizer, P from struvite, P from struvite + sulfur (100 mg kg^{-1} of soil), and P from rock phosphate; and all these treatments were compared with and without SOB inoculation through irrigation water. Struvite application, especially with sulfur and/or SOB, maintained an adequate P level (as with SSP fertilizer) in both wheat plants and soil throughout the growing period. Wheat plant agronomic attributes were also improved with struvite as for SSP fertilization. Moreover, supplementation of SOB inoculum with struvite and other P sources significantly improved the P bioavailability and crop yield through increased phosphate solubility in alkaline soil. In conclusion, inoculation of SOB especially with sulfur (S) supplementation in struvite treatment caused the pH reduction of alkaline soil through S oxidation (H_2SO_4 formation), which solubilized the fixed-P in struvite as well as soil and thus improved P bioavailability to wheat plants. These findings strengthen the concept of struvite scavenging from wastewater for environmental safety, and to introduce it as an alternative resource for P fertilization.

Keywords Wastewater · Struvite · Sulfur-oxidizing bacteria · Phosphorus use efficiency

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1 Introduction

Phosphorus (P) is crucial to crop productivity, while phosphate rock holds a pivotal role as a major parenting source in supplementing P fertilizers to agriculture. The limiting and non-renewable reserves of rock phosphate contribute to 90% of P demands and may end up in next 70–100 years (Reinhard et al. 2017; Li et al. 2018), leaving us with no natural P substitute (Kim et al. 2018). Further, cadmium build up in agricultural soils is an associated issue with rock phosphate (Pizzol et al. 2014). Large-scale wastewater generation is correlated with rapidly increasing urbanization and industrialization, and has become a preordained concern of modern era around the globe. Release of extensive nutrients into the environment is a major consequence of discharged wastewaters, resulting in eutrophication and algal blooms at receiving repositories (Yetilmezsoy et al. 2017). However, conjugative aspects of the predicted future P supply limitations and increased population with high wastewater production, having substantial nutrients, could prove base for an alternate and sustainable resource development.

Futuristic approach for explorative research towards sustainability of P sources in agricultural production has provoked scientists to nutrients recovery approaches from wastewaters (Desmidt et al. 2015: Huang et al. 2018), shifting the focus from protection to recovery and sustainability (Wei et al. 2018). Nutrients recovery from wastewater is considered as a cost-effective practice for crop productivity (Yetilmezsoy et al. 2011). Excessive P disposed in municipal wastewaters could be recovered as struvite and used as fertilizer for crop production (Plaza et al. 2007). Struvite ($MgNH_4PO_4.6H_2O$) is considered as an alternate source of P, nitrogen (N) and magnesium (Mg) for crop productivity (Gilbert 2009). Ionic strength, pH and agitation levels control the struvite crystallization process (Wang et al. 2006; Kozik et al. 2013; Desmidt et al. 2013). However, the metal ions concentration in the substrate would hinder the struvite formation, and associated metal phosphates may develop (Pastor et al. 2008). Though with low solubility, the reported researches found struvite as an effective fertilizer source for better plants growth, and increased biomass production with higher P uptake (Plaza et al. 2007; Uysal et al. 2010).

Struvite precipitation process and conditions have been extensively reviewed, reproduced, and optimized for different wastewater sources by several scientists at both in vitro and pilot scales (Kataki et al. 2016). The crystallized product shows compositional shifts depending upon the conditions used; mostly the molar ratio for N, P and Mg ions has extensively been altered to achieve the best precipitates in a number of studies (Negrea et al. 2010; Michałowska-Kaczmarczyk and Michałowski 2014; Michałowska-Kaczmarczyk et al. 2015). The P content usually ranges as 11–26% in struvite based on the crystallization method (Johnston and Richards 2003), and only 1–2% is considered water-soluble (Bridger et al. 1962). This is associated with its slow-release nature making it advantageous over other P sources (Li and Zhao 2002; Negrea et al. 2010).

Struvite has been proven technically feasible and economically beneficial (Shu et al. 2006). It has excellent fertilizer quality as compared with other standard chemical fertilizers (Liu et al. 2011). Companies have been supplying struvite as an additive and substitute raw material for P fertilizer production (Rafie et al. 2013). Struvite use is more evident in potted plants and turf grasses because of its characteristic slow-release nature (Yetilmezsoy and Sapci-Zengin 2009; Li and Zhao 2003; Antonini et al. 2012). Fertilizer effect of dairy industry's waste-produced struvite on maize plants was investigated, and in comparison with chemical fertilizer dosages, struvite enhanced the fresh and dry matter yield (Uysal and Kuru 2015). Although many researchers have evaluated struvite's fertilizer ability, the applicability in major cereals over the whole growing period along with its solubility in alkaline conditions is still a concern. Solubility is only 0.04 mM in alkaline conditions (Le Corre et al. 2009), which may reach up to 65–100% in acidic and neutral soil pH conditions (Cabeza et al. 2011).

Elemental S° is oxidized to sulfates by SOB to fulfill their energy needs (Pokorna and Zabranska 2015). Among SOB, *Acidithiobacillus thiooxidans* bacterial species is more influential for biological S oxidation in soil (Yang et al. 2010), which produces sulfuric acid thus lowers soil pH. It helps to dissolve insoluble calcium-bounded P minerals like fluorapatite ($Ca_5(PO_4)_3F$), calcite ($CaCO_3$) and lime (CaO) and converts them into plant accessible P forms (Stamford et al. 2003; Arai and Sparks 2007). Phosphate solubilization through bacterial S oxidation phenomenon is established and very effective (Aria et al. 2010). Biological sulfur oxidation by *Acidithiobacillus thiooxidans* releases phosphate from rock phosphate (Besharati et al. 2007), which has a high positive correlation with the quantity of bacterially generated sulfuric acid (Bhatti and Yawar 2010). However, there is insufficient information available about phosphate solubilization in alkaline and calcareous soils through bacterial S oxidation. The amalgamation of elemental sulfur with SOB enhanced sorghum fresh and dry biomass at 600 ppm of S addition (Kandil et al. 2011). To our knowledge, no supplementation to counter the pH rise under alkaline soils with struvite application is known. We propose acidophilus bacterial inoculation along with various P sources in alkaline conditions to estimate the efficiency of struvite, majorly as a P source, for *Triticum aestivum* L. over the whole growing period. Further, no information about the interactive effect of SOB and struvite exists. Therefore, for undertaking this study, we hypothesized that struvite is a slow-release P resource as alternate to P fertilizer, and SOB inoculation in alkaline soil enhances P bioavailability.

Main objectives of this experiment were to study the suitability of struvite as P source for wheat in alkaline soil, evaluate the effect of SOB on struvite solubility and P bioavailability from it, and determine the P-releasing pattern of struvite and its effect on plant growth and yield.

2 Materials and Methods

2.1 Wastewater Characterization

Municipal wastewater was collected from sewerage water filtration plant I-9, Islamabad, stored at 4 °C and analyzed for pH, NH_4^+-N, $PO_4^{3-}-P$ and Mg^{2+} contents for its viability as a struvite production source (APHA 2005). The pH was slightly alkaline (7.81), NH_4^+-N was estimated as 46 mg L^{-1}, $PO_4^{3-}-P$ was 59.5 mg L^{-1} and the Mg^{2+} content was measured as 32 mg L^{-1}. Interference of metal ions in struvite precipitation is well understood; therefore, metal ions (Cd, Ca, K, Ni, Co, Cr, Fe, Hg and Cu) were also determined in wastewater following APHA (2012). The samples were digested using concentrated nitric acid on a hot plate at temperature of about 105 °C for 2 h. The samples were not allowed to boil; more nitric acid was added when required until the formation of a clear solution. The heavy metals were then analyzed using atomic absorption spectrophotometer (AA-6300, Shimadzu) and in addition cold vapor generator (CVG) for Hg (Muhmood et al. 2018), analysis report is presented in Table 1. Most of the metal ions were below detection limit, and fewer amounts of Ca and K were recorded as reported in some previous studies for municipal wastewater (Forrest et al. 2008; Latifian et al. 2012).

2.2 Struvite Precipitation Setup

Struvite crystallization conditions have been optimized under various scenarios by scientists. A wide range of ionic molar concentration ratios have been applied but the most effective ratio is 1.2:1:1 for N, P and Mg (Zhang et al. 2012), which was adopted in this study. The precipitation conditions for struvite were ratio of NH_4^+-N: $PO_4^{3-}-P$: $Mg^{2+} = 1.2:1:1$ as maintained by using magnesium chloride hexahydrate ($MgCl_2.6H_2O$) and trisodium phosphate (Na_3PO_4), pH 9.6, and stirring speed of 250 rpm at room temperature. The precipitated struvite was filtered through Whatman No. 42, oven dried at 60 °C, weighed, and evaluated for its nutrient and heavy metal contents (Table 1).

2.3 Evaluation of Struvite as P Source

Besides the nutrient content analysis of struvite, it was tested for its effectiveness as phosphatic fertilizer in a greenhouse pot experiment using wheat (*Triticum aestivum* L.) as the test crop. Alkaline nature of both the soil and struvite was studied for the interesting reactions occurring in soil for P release. For testing of P release, struvite was tested among these five treatments on 5 kg soil in plastic pots, viz., (i) control (no P application), (ii) P from SSP fertilizer, (iii) P from struvite, (iv) P from struvite + sulfur (100 mg kg^{-1} of soil), and (v) P from rock phosphate. Phosphorus was applied at the rate of 100 mg kg^{-1} soil in all treatments irrespective of sources. Nitrogen and potassium were applied at recommended doses (150 and 100 mg kg^{-1} of soil, respectively) in all treatments including control. In addition, all five treatments were applied with and without SOB inoculation at the rate of 1 mL (of 10^6 cells fresh culture) kg^{-1} of soil. The SOB inoculum was applied twice in whole growing period through irrigation water; once after sowing, and other at tillering stage. *Acidithiobacillus thiooxidans* (SOB strain IW16), proved the best P solubilizer in our previously reported study (Ullah et al. 2013), was used in this experiment. Soil and plant samples were collected at tillering, booting and maturity stages of the crop. Data were recorded and analyzed for different soil and plant parameters. Plant fresh and dry matter biomass yield, plant height, spike length, number of grains per spike, and grain yield were recorded. The soil was analyzed for pH and available P using $NaHCO_3$ extraction method (Olsen and Sommers 1982) at tillering, booting and maturity stages. The plant samples were analyzed for P content through dry ashing (Chapman and Pratt 1961), followed by developing vanadomolybdo-phosphoric acid yellow color (Cottenie

Table 1 Wastewater characterization and precipitated struvite analysis

Parameter	Wastewater Concentration (mg L^{-1})	Struvite % Weight
EC	2.18 (dS m^{-1})	–
pH	7.81	–
TDS	540	–
N	46 (NH_4^+-N)	4.6
P	59.5 ($PO_4^{3-}-P$)	9.58
Mg	32	8.35
K	30	0.07
Ca	26	0.28
Cl	44	–
Cr	ND	–
Cd	ND	–
Cu	ND	–
Pb	ND	–
Hg	ND	–
Ni	ND	–
Co	ND	–
Zn	0.8	–

*ND, not in detectable amount

1980), and the color was read at 410 nm after 30 min (Estefan et al. 2013). Phosphorus uptake was calculated as follows:

$$\text{P uptake}\left(\text{mg kg}^{-1}\right) = \frac{\text{P conc.(ppm)} \times \text{Biomass yield (g)}}{\text{Weight of soil per pot (g)}}$$

2.4 Statistical Analysis

The data collected for various soil and plant parameters at each sampling stage were analyzed statistically under two factor factorial design, and treatment means obtained were compared by LSD at 5% level of significance (Steel et al. 1997). Statistical software employed to analyze data was Statstix 8.1, and graphs were drawn with MS Excel 2007.

3 Results

3.1 Soil Reaction Influenced by P Source and SOB Inoculation

Data regarding the soil pH dynamics during crop growth is presented in Fig. 1. Efficacy of SOB in regulating soil pH was investigated at different crop growth stages, viz., tillering, booting and harvesting under different P sources. Relationship between soil buffering effect for pH against applied P sources and SOB was established over the growing period. Supplementation of SOB significantly reduced the soil pH over the growing period. At all the crop growth stages, treatments supplemented with SOB had lower soil pH than non-supplemented treatments. Rock phosphate was not significantly different from control (no P application) with respect to soil pH change, irrespective of SOB inoculation. Struvite itself had higher initial pH (9.5) and dissolution of struvite resulted in higher soil pH environment. The sulfate in SSP may have reduced soil pH and lower pH values were recorded in SSP without SOB. However, the least values for soil pH were attained in sulfur and SOB inoculated treatments, where elemental sulfur served as the substrate for SOB.

3.2 Available Phosphorus in Soil

In order to understand the P availability and P source solubility over the growing period in alkaline soil, the extractable P was assessed at three different growth stages of wheat crop (Fig. 2). Different P sources and influence of SOB addition on P availability over time in alkaline soil pointed towards some key reactions occurring in soil. The SSP fertilized soil provided P irrespective of SOB supplementation. The high initial extractable P content for SSP indicated towards the rapid solubilization of acidic chemical fertilizer in the alkaline soil environment. However, at booting and maturity stages of the crop, available P in the SSP amended soil declined abruptly and failed to provide an ample amount of P to plants. Higher initial P concentration after application and then the decline indicated towards the P fixation process in soil. A gradual decrease in P supply over the growing period was recorded for struvite and struvite along with sulfur; the decrease was more profound where no SOB was inoculated. The SOB supplementation resulted in continued solubilization and microbial dissolution of applied P source and thus maintained an adequate P supply throughout the growing period. Raw phosphate rock shows minimal dissolution and P supply in both with and without SOB addition did not differ from control where no P source was added. Struvite and sulfur addition boosted P supply and the slow-release nature was confirmed through maintained adequate P level throughout the growing season of wheat.

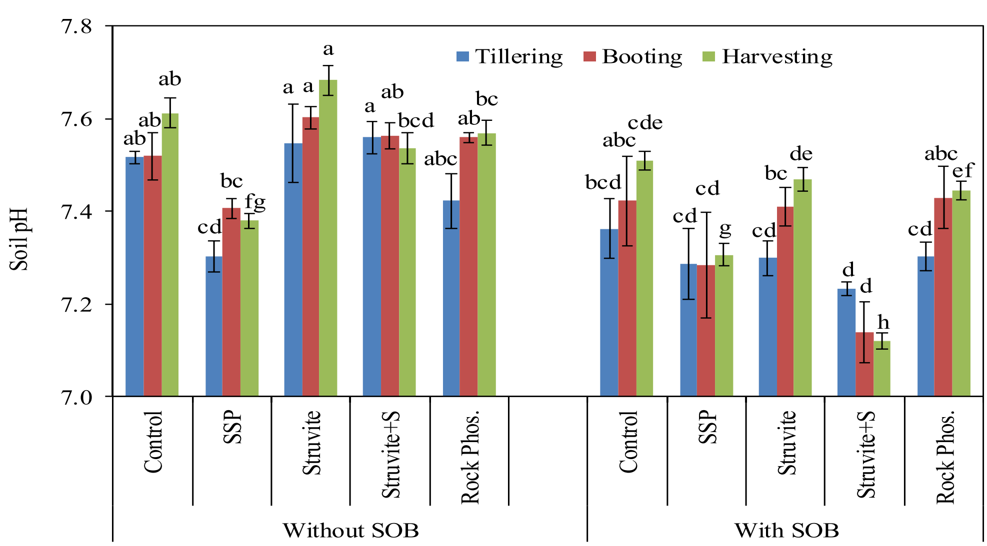

Fig. 1 Effect of different P sources, with and without SOB (*Acidithiobacillus thiooxidans* IW16) on soil pH at different growth stages. Different letter(s) on various treatment bars show statistically significant difference ($P \leq 0.05$) among them. Error bars represent 95% confidence intervals for $n = 3$

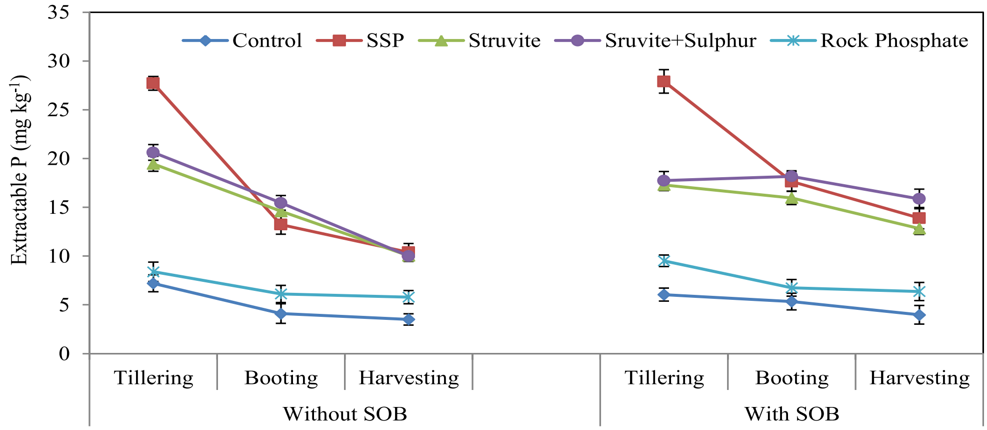

Fig. 2 Effect of different P sources, with and without SOB (*Acidithiobacillus thiooxidans* IW16) on P availability over the growing season. Each treatment line represents the available P level during growing period of crop. Error bars represent 95% confidence intervals for $n = 3$

3.3 Phosphorus Uptake by Wheat

Temporal P uptake for the crop was determined to comprehend the best combination of P source with the microbial inoculum (Table 2). The P uptake from soil by crop plants increased with the progression of growing period, and at the final stage of sampling, i.e., harvesting, the P uptake from stalk ranged from 7.96 to 1.34 mg kg^{-1}. Maximum P uptake was under the SOB inoculated treatments with struvite + sulfur which showed the higher P uptake of 7.96 mg kg^{-1} followed by struvite and SSP which had 6.71 and 6.43 mg kg^{-1}, respectively. Inoculation of SOB was positively correlated with the P uptake by grains in a different set of treatments (Table 2). The P uptake for grains ranged from 1.97 to 13.29 mg kg^{-1}. The inoculation of SOB positively affected the P uptake in grains. Phosphorus content of wheat grains indicated that struvite alone and in combination with sulfur could provide adequate P supply as did the conventionally used SSP. However, phosphate rock could not provide adequate P to crop plants and resulted in decreased growth and P uptake under both with and without SOB.

3.4 Crop Growth and Yield Attributes

Application of wastewater precipitated struvite and its ability to provide nutrients in comparison with SSP was tested by evaluating different crop growth parameters (Table 3). Grain production and formation were better where P availability and uptake remained higher at critical growth stages. Biomass yield estimated at each sampled stage shows a direct relationship with P supply. The application of SOB significantly improved plant growth parameters and yield attributes. Struvite alone and in combination with sulfur-enhanced biological yield and plant height was comparable with chemical fertilizer SSP. However, raw rock phosphate could not boost P availability and thus, growth attributes in the tested crop. Adequate supplementation of P helps improve not only plant height but biological yield as well. The measured per pot grain yield was higher for SSP and struvite supplemented treatments.

4 Discussion

4.1 Impact of P Source and SOB Inoculation on Soil Reaction

Nature and characteristics of P sources played a vital role in controlling soil pH and nutrients availability over the crop growth period. Soil pH changed non-significantly over time in control where no P source was applied, without SOB. Initial higher pH values for struvite-incorporated treatments could be correlated with the fact that the struvite itself had a very high initial pH (Rahman et al. 2011). Stamford et al. (2003) reported pH reduction by SOB due to biological sulfur oxidation and H_2SO_4 production. The SSP addition also supplemented some sulfate along with P, which helped control soil pH and resulted in the lower pH values at all the sampling stages, compared with control. Struvite incorporation resulted in an increased pH level which kept on increasing significantly from initial to final crop stage, with increased solubility of struvite with the time (Kataki et al. 2016). Inoculation of SOB influences the production of acidic soil environment through sulfur oxidation (Ullah et al. 2013). Elemental S is an important substrate for SOB and its microbial transformation prevails over abiotic conversion, maintaining soil pH to lower levels (Yavuz et al. 2007). Alkaline nature of both struvite and soil enhanced sulfur oxidation rate in the presence of SOB (Zhao et al. 2015), and improved availability of other nutrients as well (Soliman et al. 2006). Availability of all the nutrients is generally governed by the soil environment, and it increases with the increase in nearness to the neutrality of soil pH (Eman and Taalab 2004). The proton (H^+) released during biological oxidation of elemental sulfur has been well described by Tang et al. (2009).

4.2 Influence of P Sources and SOB on P Availability over Growing Season

The P fixation phenomenon is one of the key hindrances to an adequate supply of P over the growing period. Soils with the

Table 2 Phosphorus uptake by wheat stalk and seed under different P sources, and with and without supplementation of SOB

		*P uptake by stalk (mg kg^{-1})			*P uptake by grain
		α Tillering	β Booting	γ Harvesting	δ (mg kg^{-1})
Without SOB	Control	0.02 e	0.28 e	1.34 f	1.97 f
	SSP	0.13 c	2.96 c	5.90 c	9.90 c
	Struvite	0.16 bc	2.87 c	5.09 d	8.87 d
	Struvite + sulfur	0.16 bc	3.16 c	4.94 d	9.64 c
	Rock Phosphate	0.05 d	0.71 d	1.98 e	3.31 e
With SOB	Control	0.05 de	0.89 d	1.98 e	3.40 e
	SSP	0.23 a	3.03 c	6.43 b	11.13 b
	Struvite	0.21 a	3.86 b	6.71 b	11.53 b
	Struvite + sulfur	0.22 a	6.18 a	7.96 a	13.29 a
	Rock phosphate	0.04 de	0.94 d	2.47 e	3.87 e

*LSD values indicated as $\alpha = 0.0315$, $\beta = 0.4011$, $\gamma = 0.5215$ and $\delta = 0.6597$ are for tillering, booting, harvesting and grain P uptake values. Values in each column with the same alphabet(s) differ non-significantly ($P \leq 0.05$)

dominance of 1:1 clay mineral like kaolinite have high P fixation. Moreover, the pH holds an important position in controlling nutrients availability to crop plants. Most of the plant essential nutrients approach their maximum availability when the soil pH approaches neutrality (Stamford et al. 2003). The P fertilizer source precipitated from wastewater (struvite) under high pH values showed some interesting phenomena upon its application in alkaline soils. Effectiveness of struvite under moderate or low pH soils is better as compared with high pH soils (Kataki et al. 2016). Struvite alone and in conjunction with sulfur maintained the adequacy level for P from germination till maturity of the crop (Yetilmezsoy et al. 2011; Gilbert 2009). The P bioavailability improved with organic amendments in soil as compared with inorganic P fertilization (Ara et al. 2018). The elemental S incorporation also contributes towards the conversion of insoluble soil P pool to soluble one (Abdel-Fattah et al. 2005). Struvite proved to be an excellent P fertilizer when compared with other standard chemical fertilizers (Massey et al. 2009; Perez et al. 2009; Dalecha et al. 2012). The fact that struvite is considered as a slow-release fertilizer was well established from our findings as well (Negrea et al. 2010). The P availability altered non-significantly from tillering to harvesting in struvite-amended treatments. Supplementation of SOB inoculum raised microbial activity in the soil and increased organic acid production thus reduced the soil pH (Yang et al. 2010). Struvite-applied treatments increased soil pH with the time as its solubility increased. However, struvite when added with sulfur and SOB enhanced P availability over crop growth period and maintained a sufficient level of P bioavailability (Cabeza et al. 2011; Ullah et al. 2013). The applied elemental sulfur acted as a substrate source for an added SOB and resulted in improved nutrients availability by increased microbial activity (Abdel-Fattah et al. 2005). Rock phosphate and control where no P source was used differed non-significantly and supplied the least P to plants throughout the growing season.

4.3 Phosphorus Uptake by Wheat Stalk

Wastewater precipitated P source (struvite) displayed interesting results regarding P uptake. Treatments having SSP, struvite, and struvite + sulfur were statistically non-significant, showing the effectiveness of struvite as a good nutrient source for crop plants (Gell et al. 2011). Since the use of struvite in the alkaline soil environment might elevate soil pH, the SOB inoculum was used as a remedial measure for the proposed increase. Struvite alone and in combination with sulfur without SOB inoculation was not significantly different from the chemical P source, SSP (Yetilmezsoy et al. 2013; Uysal and Kuru 2015). The tested and reported P uptake from struvite is 100% (Westerman 2009). Addition of SOB improved crop growth, nutrient availability and uptake in all treatments as reported by previous work (El-Assiouty and Abo-Sedra 2005; Soliman et al. 2006). However, the use of SOB better responded in sulfur added struvite treatment where suitable growing conditions and food source helped bacterial proliferation and increased P bioavailability. Rock phosphate is used for most of phosphatic fertilizers production. However, the use of sole rock phosphate was not helpful in providing P to plants and did not differ significantly from control where no P source was added.

Calcareous soils owing to high calcium concentrations results in decreased solubility of rock phosphate. However, the struvite solubility was more than rock phosphate under alkaline calcareous conditions. Moreover, P uptake increased sharply from tillering to booting, which further increased till maturity. In the set of treatments without microbial inoculation, SSP proved effective in supplying P to plants as the

Table 3 Influence of different P sources, with and without supplementation of SOB on crop growth parameters

		*Biomass yield (per plant in g)			**Plant height (cm)			*Grain yield
		α Tillering	β Booting	γ Harvesting	α Tillering	β Booting	γ Harvesting	δ g Pot^{-1}
Without SOB	Control	0.03 e	0.56 h	3.69 g	19.47 f	37.25 e	51.65 d	2.66 g
	SSP	0.13 c	2.53 cd	7.20 c	33.87 cd	60.11 c	75.35 c	5.76 c
	Struvite	0.17 b	2.59 cd	6.63 d	38.94 b	60.96 c	74.51 c	5.01 d
	Struvite + sulfur	0.16 b	2.65 c	6.60 d	44.03 a	61.80 c	75.35 c	5.81 c
	Rock phosphate	0.07 d	1.32 g	5.06 f	28.78 e	49.11 d	54.19d	4.05 e
With SOB	Control	0.07 d	1.62 f	5.23 f	28.79 e	60.11 c	70.27 c	4.03 f
	SSP	0.22 a	2.37 d	7.26 c	37.25 bc	75.35b	84.67 b	6.07 b
	Struvite	0.20 a	2.97 b	7.80 b	39.79 b	79.58 b	88.05 b	6.17 b
	Struvite + sulfur	0.21 a	4.54 a	8.85 a	47.41 a	88.9 a	101.6 a	6.83 a
	Rock phosphate	0.06 d	1.89 e	5.71 e	31.33de	64.34 c	75.35 c	4.50 e

*LSD values for biomass yield at tillering, booting and harvesting stages, and grain yield are α 0.0278, β 0.2668, γ 0.4459 and δ 0.1851, respectively

**LSD values for plant height at tillering, booting and harvesting stages are α 4.2269, β 5.8557 and γ 7.1656, respectively

Values in the column with the same alphabet are not different significantly ($P \leq 0.05$)

sulfate contained in SSP helped in moderating soil pH. Struvite provided enough nutrients for uptake, and the results were in accordance with those reported in the VUNA project (2015). Ganrot et al. (2007) reported that P uptake in the wheat crop was not affected by the change in P source, and struvite along with other chemical P fertilizers proved a good source of P for the plant (Desmidt et al. 2015). The SSP amended treatment without SOB addition, and struvite supplemented with sulfur and SOB helped greater P uptake at the maturity stage of the crop (Gonzalez-Ponce and Garcıa-Lopez-de-Sa 2007). However, the use of rock phosphate did not aid in high P uptake because of low solubility and dissolution in the alkaline soil environment (Massey et al. 2007; Kataki et al. 2016). Rock phosphate was closely related to control and was far below from SSP and struvite in both with and without SOB inoculated set of treatments.

4.4 Phosphorus Uptake by Wheat Grain

Struvite alone and SSP showed comparable results and hence proved struvite as an effective nutrient source for crop plants (Perez et al. 2009; Liu et al. 2011; Dalecha et al. 2012). However, when struvite supplemented with sulfur and SOB, the highest P uptake and plant growth was recorded which could be correlated to the role of SOB in sulfur oxidation and enhancing P bioavailability by acidifying the soil environment through the production of sulfuric acid (Kandil et al. 2011). The lowest P uptake was recorded in the treatments having rock phosphate and those having no P fertilizer, control. Results for total P uptake were accredited by previous studies (Simons 2008; Ryan et al. 2008; Antonini et al. 2012).

4.5 Crop Growth and Yield Characteristics

Struvite alone and in combination with sulfur was statistically equivalent to the chemical fertilizer SSP in providing nutrients to crop plants, and resulted in better growth and yield (Gonzalez-Ponce et al. 2009). Struvite resulted in better growth and productivity of wheat under alkaline soil conditions, and also resulted in better biological yield due to reduced N losses (Rahman et al. 2011). Chemical fertilizer SSP and struvite alone as well as in combination with sulfur proved better in development of tillers as compared with sole rock phosphate and control, where no P was added. Uysal and Kuru (2015) demonstrated the role of P in plant growth and linked plant growth parameters including the height of the plant with the P availability in soil. The availability of nutrients and proliferation of microbial diversity through SOB inoculation in alkaline soil environment helped in the better response of the applied P sources (McLaughlin et al. 2015). The behavior of alkaline-natured struvite entails its supplementation with SOB inoculum as a pH controlling agent. Use of sulfur which served as a medium for the inoculated SOB, a bio-fertilizer, resulted in the superior outcome with crop growth perspective (Abdel-Fattah and Abd-El-Khader 2004). Sole rock phosphate solubility under alkaline conditions remained a key concern, and P availability coped with stress in these treatments. Better root proliferation under microbial inoculation enhanced nutrients bioavailability, and resulted in better biological and grain yield. Plant biomass increases at a higher level of Olsen P in the soil (Afzal and Bano 2008). Increase in biological yield was correlated with better nutrient uptake by crop especially N and P, which have their role in vegetative growth (Ma et al. 2004; Rasul et al. 2011). Tillers production was better in SSP and struvite-amended treatments as compared with rock phosphate and control, because of low dissolution under alkaline conditions (Gonzalez-Ponce et al. 2009; Yetilmezsoy et al. 2009). Overall growth with respect to plant height and biological yield was improved through supplementation of a good P fertilizer source.

Use of struvite and adequate level of DAP results in better biological yield and better growth and vigor (Yetilmezsoy et al. 2013). Plant growth promoting microbes like SOB enhance the P availability in rhizosphere and accelerate root proliferation, which decrease water stress in wheat (Mutumba et al. 2018). Increased availability of P as P_2O_5 at same N levels increased the number of grains spike^{-1} showing the importance of P for seed formation and grain filling. The crop was applied with the recommended doses of nitrogen and potassium but the P absence in control resulted in lower biomass and yield production. Greater nutrient availability improves soil fertility status thus nutrient uptake by plants, which results in better crop vigor and height (Imtiaz et al. 2003; Uysal and Demir 2013). Crop response was better and improved towards the SOB inoculated treatments, and sulfur application had an immense impact on lowering the soil pH and thus increased nutrients availability, which resulted in better crop production (Eman and Taalab 2004; Magda et al. 2007). Impact of struvite application was not limited to P availability only but the nitrogen and magnesium present in it enhanced the crop growth and productivity (Uysal and Kuru 2015). Villar-Mir et al. (2002) also found increased grain yield of wheat crop when proper P fertilizer doses were used. Chlorophyll contents were measured at each sampling stage and were found non-significantly different in relation to applied P sources and microbial inoculation as explained in previous studies (Prater 2014).

Use of struvite as fertilizer in different crop plants has been accredited by various studies over the past few years (Perez et al. 2009; Gonzalez-Ponce et al. 2009; Ackerman et al. 2013; Uysal et al. 2014). Majorly, struvite has been used on turfgrasses, vegetables and ornamental plants, as the excessive doses are not harmful to the plants due to the slow releasing nature of struvite. However, its use in major crops has also been reported (Yetilmezsoy et al. 2013). The current study suggests struvite can also be applied to major cereals in alkaline soils, as it provides nutrients throughout the growing period due to decreased instant solubility. Moreover, application of SOB aided in a suitable soil pH environment and increased nutrients release, which resulted in better crop growth and production.

5 Conclusions

Utilization of struvite as phosphorus fertilizer resulted in better growth and yield of wheat, and phosphorus availability and uptake were comparable with single superphosphate. Struvite maintained adequate phosphorus availability throughout the growing season via its slow-release mechanism and was found impressive as P source for major cereal crop wheat. Further, the amalgamation of the sulfur-oxidizing bacteria inoculum (*Acidithiobacillus thiooxidans* IW16) and sulfur with struvite resulted in increased phosphorus bioavailability in alkaline soil, and achieved maximum production of wheat. Sole rock phosphate could not prove efficient in supplying phosphorus to wheat crop owing to its decreased solubility under alkaline soil environment. Therefore, acidifying soil amendments like sulfur particularly along with sulfur-oxidizing bacteria could be recommended for alkaline soils.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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