INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–1117/2019/22–1–65–72
DOI: 10.17957/IJAB/15.1034
http://www.fspublishers.org

Full Length Article

Phosphorus Solubilization through Acidified Organic Extract Improved Growth, Yield and Phosphorus Uptake of Maize Grown in Calcareous Soil

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Abstract

Deficiency of plant available phosphorus (P) is prevailing across the globe. Farming community in Pakistan is also facing the issue of poor availability of P due to high soil pH and calcareous nature of soils resulting in lower P-fertilizer use efficiency (FUE) of expensive fertilizers. Cow manure and elemental sulfur (S°) were bio-augmented with sulfur oxidizing bacteria (SOB) and the resultant solid product was diluted with water to get acidified extract (AE). SOB oxidize S° to SO_4^{-2} and generate sulfuric acid, having the potential to solubilize insoluble P compounds to simple plant available P ($H_2PO_4^-$ or HPO_4^{2-}). Maize was grown in pots with recommended dose (82.5 mg kg^{-1}) and half (41.25 mg kg^{-1}) dose of P by using three types of P fertilizers *viz*., diammonium phosphate (DAP), single superphosphate (SSP) and rock phosphate (RP) with and without 10% acidified extract (AE) at the rate of 247 L ha^{-1}. The results revealed that combined use of P fertilizers and AE improved growth, yield and related traits and P uptake of maize compared to alone application of P fertilizers. Combined application of DAP with AE proven to be most significant followed by SSP and RP, each with AE, respectively. DAP and AE improved dry shoot biomass, spade value, cob length, grains per cob and P uptake in shoots by 57.45, 12.96, 57.39, 74.56 and 92.42% over sole application of DAP, respectively. In conclusion, combined application of DAP and AE was advantageous in improving P acquisition and maize productivity.

Keywords: Sulfur oxidizing bacteria; Elemental sulfur; Acidulated extract; High pH soils; Phosphorus mobilization

Introduction

Global boost in human population requires amplified agricultural production and for that, phosphorus (P) availability in the soil is crucial (Cordell *et al*., 2009; Gilbert, 2009). It is an essential macronutrient for plant growth (Sharma *et al*., 2011; Satyaprakash *et al*., 2017) due to its involvement in many plant processes such as carbon metabolism, photosynthesis, membrane formation (Wu-Wong *et al*., 2006) as well as in the transfer and storage of energy (Griffith and Ryan, 1999). It is an important part of "DNA" and structural component of many phospho-proteins, co-enzymes and phospholipids (Juneja *et al.*, 2013; Yang *et al.*, 2017). Additionally, P has a vital influence on root elongation, propagation (Borch *et al*., 1999) and maintenance of root architecture (Williamson *et al.,* 2001), seed formation and ordinary crop maturity. Poor P bio-availability and mobility in soil (Elser *et al*., 2007; Chen *et al*., 2008) is the prime cause of 30-40% reduction in crop yield (Vance *et al.,* 2003). In calcareous soils, main reason behind the limited availability of P is calcium carbonate concentration which serves as a sink for phosphate precipitation (Hopkins and Ellsworth, 2005). Although, total soil P contents in Pakistan, are ranging from 163 to 1050 mg kg^{-1} that seems to be more than enough (Memon *et al*., 2011). However, only 1.0 mg kg^{-1} is available to plants (Vassilev *et al.,* 2001; Solangi *et al.,* 2006) to cope up optimum crop production.

In order to maximize plant growth and yield, external source of P fertilizers is required. Unfortunately, the overall P fertilizer use efficiency in our country is very low (Vassilev and Vassileva, 2003) and only 10–25% of the applied fertilizer is taken up by plants (Vance, 2001). The rest comes in contact with soil colloids to be converted into insoluble form of tri-calcium phosphate (Dobermann *et al.*, 1998) and becomes unavailable (Gill *et al.*, 2004). Further, the price of P fertilizers, such as DAP and nitrophos, is too high compared to their efficiency. However, rock phosphate is the cheapest source of P which was little used in agriculture sector.

In order to improve the P nutrition for optimum plant growth, a wide range of strategies have been adopted in alkaline and calcareous soils (Curtin and Syers, 2001). High P fertilizer rates, slow-release fertilizers (Thonar *et al.,* 2017), acid producing fertilizers, sulfur containing fertilizers are used to increase P acquisition efficiency of plants. Apart from this, organic amendments (Mitran *et al.,* 2018), microbial activity (Novo *et al.,* 2018), SOB (Sattar *et al.,* 2017), SOB and phosphate solubilizing bacteria (PSB) (Solanki *et al*., 2018), acidified fertilizers and acid producing materials (Pedersen *et al*., 2017) are also promising approaches. These sources can be applied alone or in various combinations to improve P availability to plants. Among the acid producing materials, S° is found effective and economical to enhance P solubility and uptake in calcareous soils (Kaplan and Orman, 1998). In soil, SOB oxidize S° through biochemical process and produce H_2SO_4 (Jaggi *et al*., 2005) which lowers the rhizosphere pH and solubilize $CaCO_3$ (Cifuentes and Linderman, 1993; Brahim *et al*., 2017; Andrade *et al*., 2018) and enhance the nutrient bioavailability especially of P (Deluca *et al.,* 1989). However, low organic matter content in the soil restricts the activity of these microbes as it is required as a food source. Therefore, addition of organic matter can accelerate biological oxidation of elemental sulfur (Chun *et al.,* 2007), reason being, an increase in heterotrophic microbial population that can oxidize S° substantially.

Nowadays, combined use of organic matter as a food source with S° for heterotrophic SOB is preferred (Sattar *et al.,* 2017). Previously, large quantities of organic matter with S° have to be used to attain the required benefits, therefore, making this practice uneconomical for small-scale farmers. Keeping in view the above-mentioned constraints, the study was conducted to formulate an acidified extract from cow manure and S°, bio-augmented with SOB. This product was evaluated to enhance the growth, yield and P use efficiency using various P fertilizers in maize.

Materials and Methods

This pot study was conducted in the wire house of the Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad-Pakistan. Soil was collected from field and was air dried, grounded and then passed through 2 mm sieve. Same soil was analyzed for physico-chemical characteristics prior to sowing *i.e*., organic matter 0.77%, EC 1.89 dS m^{-1}, pH 7.89, total nitrogen 0.085%, available phosphorus 4.5 mg kg^{-1}, extractable potassium 82 mg kg^{-1} and plant available micronutrients Fe 4.5 mg kg^{-1}, Zn 0.51 mg kg^{-1} and Mn 0.43 mg kg^{-1}.

Acidified Extract Formulation

Different ecologies were targeted for the isolation of SOB strains. Parameters like pH lowering in minimum time and sulfate ion production in broth were tested to screen the most efficient strains. An acidified product (elemental sulfur and SOB amended low pH cow dung) with minimum pH was formulated by bio-augmenting selected SOB to Sº added cow dung. The prepared product was optimized for temperature, moisture, nutritional source and then low pH amendment was finally obtained after 21 days in the presence of most competent SOB isolate ARS-4. The strain ARS-4 was identified from Macrogen (Seoul, Korea) as *Lysinibacillus* spp. through 16S rRNA gene (1351 bp) sequencing which was then deposited in the GenBank database under the accession number MH924824.1. Keeping in view the economical aspect, the formulated solid product was then diluted with water in different ratios (5, 10 and 15%) for soil application (lab incubation study, data not shown in this study) to determine temporal P release pattern. Based on attaining minimum pH and maximum P release in soil, the most economical dilution ratio (10%) was applied to pot grown maize.

Experimental Design

Seeds of maize hybrid Hycorn were obtained from ICI, Pvt. Ltd., Pakistan. Five seeds were sown in each pot under three sources of P *viz*., DAP, SSP and RP applied at 100 and 50% of recommended rate with and without 10% AE while AE alone was used for comparison. Moreover, 82.5 mg P kg^{-1} were used as 100% recommended rate and 10% AE was applied at the rate of 247 L ha^{-1}. The experiment was laid out following completely randomized design (CRD) with three replications. Recommended dose of NK (87.5 and 62.5 mg kg^{-1} of soil) was applied by using urea and sulfate of potash (SOP), respectively. The Sº and SOB amended low pH product was fertigated with tap water at four critical stages of maize as leaf blade formation, stalk formation, cob/husk/tassel formation and grain formation.

Data Collection

Harvesting of maize was done at physiological maturity. Plant height was measured by using meter rod. Plants were initially sun dried and then in the oven at 70ºC till constant weight. Dry shoot biomass and 100-grains weight were determined by using digital electric balance. The cobs were separated from plants and length was measured. For grains per cob determination, the grains were separated from the piths manually and counted.

Gaseous Exchange Measurements

Gaseous-exchange measurements *i.e*., photosynthetic rate (A) and transpiration rate (E) were analyzed using CIRAS-3 (PP System, Amesbury, M.A., U.S.A.). Gas exchange was measured from the top third, fully matured leaf of each plants (Fifty days after sowing,). The water use efficiency of plants was calculated as follows:

$$\text{Water use efficiency (WUE)} = \text{Photosynthetic rate(A)}/\text{Transpiration rate(E)}$$

Phosphorus Analysis in Roots and Shoots of Plants

The plant materials were digested to estimate P following the process of Wolf (1982) and P was calculated by Olsen method (Olsen and Sommers, 1982).

Phosphorus uptake in shoot was calculated as follow:

P uptake in shoots (mg/pot)=P concentration in shoots (%)*Dry matter(mg/pot)/100

Statistical Analysis

All collected data were subjected to analysis of variance (ANOVA) (Steel *et al.*, 1997) by using Statistix 8.1 software (computer software) and means were compared by Tukey HSD test.

Results

Plant Growth Parameters

Combined application of acidified extract and recommended dose of P fertilizers significantly enhanced dry biomass and height of maize as compared to their respective controls (Fig. 1a and b). Maximum increase in dry biomass 112.13% was recorded through combined application of AE and RP as compared to separate treatment of RP. Acidified extract along with DAP and SSP showed 57.45 and 53.22% increase in dry biomass than their individual application, respectively.

In case of plant height, maximum value of 200.7 cm was recorded through combined application of AE and recommended dose of P as DAP followed by treatment where half dose of P as DAP and AE were applied. Maximum 100-grains weight of maize was observed in treatments where 100% P was applied as DAP with AE, which differed non-significantly from 100% P as SSP with AE, followed by treatment where 100% P was applied as SSP and RP without AE (Fig. 2).

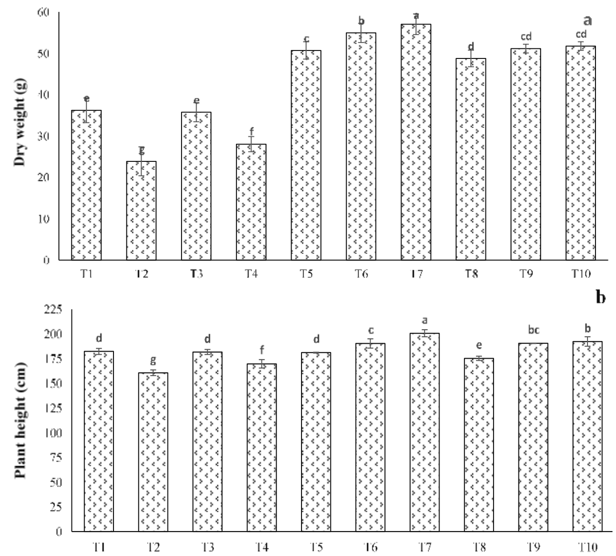

Fig. 1: Dry weight (**a**) and plant height (**b**) of maize plant after applying acidified extract and different sources of P
Here T_1: 100% P as DAP; T_2: 100% P as RP; T_3: 100% P as SSP; T_4: 10% AE; T_5: 100% P as RP+10% AE; T_6: 100% P as SSP+10% AE; T_7: 100% P as DAP+10% AE; T_8: 50% P as RP+10% AE; T_9: 50% P as SSP+10% AE; T_{10}: 50% P as DAP+10% AE

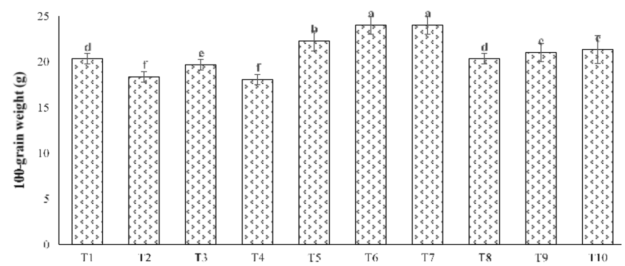

Fig. 2: 100-grains weight of maize plant after applying acidified extract and different sources of P
Here T_1: 100% P as DAP; T_2: 100% P as RP; T_3: 100% P as SSP; T_4: 10% AE; T_5: 100% P as RP+10% AE; T_6: 100% P as SSP+10% AE; T_7: 100% P as DAP+10% AE; T_8: 50% P as RP+10% AE; T_9: 50% P as SSP+10% AE; T_{10}: 50% P as DAP+10% AE

Gas Exchange Traits

Considerably good results of spade value were recorded from treatments where P fertilizers were applied with AE. The separate application of RP showed minimum result however, combined application of RP and AE showed 22.46% increase in spade value over respective control. The 100% P as DAP and SSP showed 12.96% and 11.72% increase when combined with AE compared to their respective controls (Fig. 3a).

Different treatments of P and AE showed variable response to photosynthetic activity of maize plants (Fig. 3b). Recommended dose of P as DAP along with AE showed maximum photosynthetic activity followed by 100% P as SSP and RP with AE application. The 50% P as DAP, SSP and RP combined with AE showed significant response than their individual application. Acidified extract application along with 100% DAP showed maximum response in transpiration rate (2.59 mmol H_2O m^{-2} s^{-1}) that differed non-significantly from AE + 100% P as SSP (Fig. 3c). Combined application of AE and 100% P as RP showed transpiration rate *i.e.*, 2.56 mmol H_2O m^{-2} s^{-1}. Data regarding water use efficiency showed that application of AE with recommended dose of P fertilizers significantly boosted the WUE (Fig. 3d). Maximum WUE was recorded where DAP, SSP and RP were applied in combination with AE followed by the treatments, where half of the recommended dose of P fertilizers with AE were applied (Fig. 3d).

P Contents in Plant Shoots and Roots

The combined application of AE and P fertilizers significantly improved P contents in maize as compared to sole application of P fertilizers. Maximum P content 42.3% increase in shoot was observed where AE was applied with recommended dose of P as SSP, followed by AE + 100%P

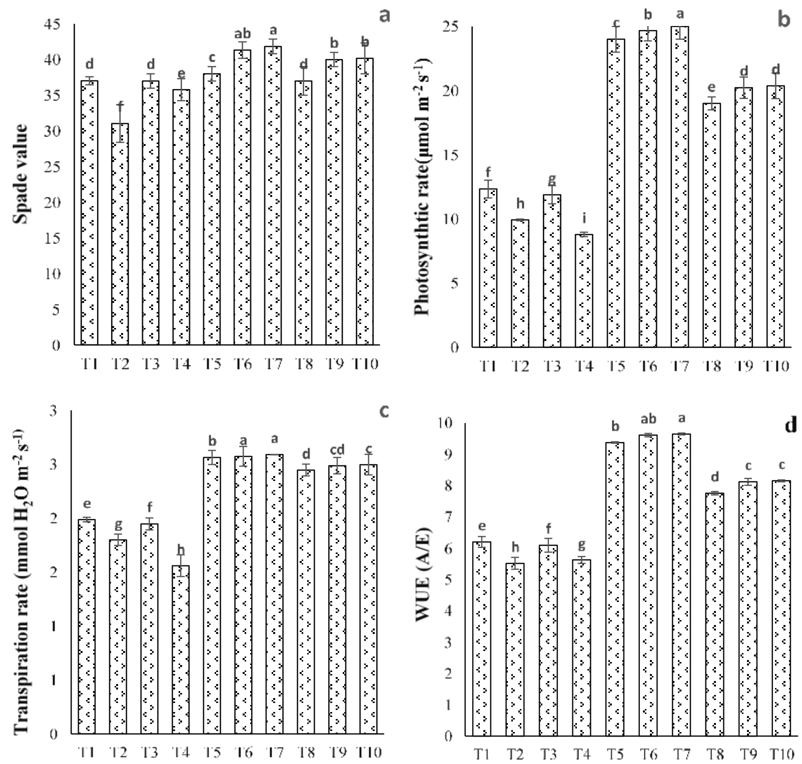

Fig. 3: Spade value (**a**), photosynthetic rate (**b**), transpiration rate (**c**), water use efficiency (**d**) of maize plant after application of acidic extract and different sources of P

Here T_1: 100% P as DAP; T_2: 100% P as RP; T_3: 100% P as SSP; T_4: 10% AE; T_5: 100% P as RP+10% AE; T_6: 100% P as SSP+10% AE; T_7: 100% P as DAP+10% AE; T_8: 50% P as RP+10% AE; T_9: 50% P as SSP+10% AE; T_{10}: 50% P as DAP+10% AE

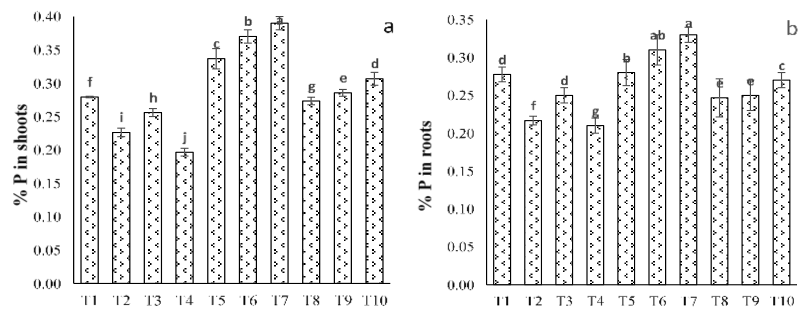

Fig. 4: P contents in maize shoot (**a**) and root (**b**) after application of acidified extract and different sources of P

Here T_1: 100% P as DAP; T_2: 100% P as RP; T_3: 100% P as SSP; T_4: 10% AE; T_5: 100% P as RP+10% AE; T_6: 100% P as SSP+10% AE; T_7: 100% P as DAP+10% AE; T_8: 50% P as RP+10% AE; T_9: 50% P as SSP+10% AE; T_{10}: 50% P as DAP+10% AE

as DAP (39.28%), compared to alone use of SSP and DAP (Fig. 4a). Alone application of acidified extract showed minimum response in P uptake, however, when it was applied with half and full dose of P-fertilizers showed prominent outcomes. In case of P contents in root, treatments where P fertilizers were applied alone showed good results, however, these results increased significantly when AE was applied with P fertilizers. Recommended dose of P as DAP, SSP and RP showed P content as 0.28, 0.25 and 0.22%, respectively whereas when AE was applied with these P fertilizers, P values were observed as 0.33, 0.31 and 0.28% (Fig. 4b).

Yield Parameters

Data regarding cob growth related parameters of maize revealed that acidified extract along with P sources showed statistically significant effect as compared with alone P fertilizers (Fig. 5a). Sole application of DAP and SSP showed 9.53 cm and 9.33 cm cob length, respectively. Application of AE along with DAP and SSP showed 57.39 and 75.02% increase in cob length as compared with DAP and SSP alone, respectively. Almost similar findings were recorded for number of grains per cob where combined application of AE with DAP, SSP and RP showed 74.56,

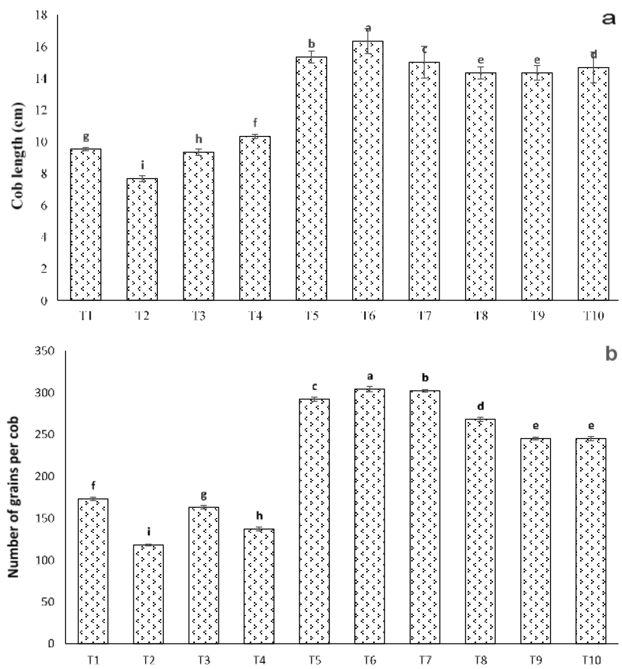

Fig. 5: Cob length (**a**) and number of grains per cob (**b**) after application of acidified extract and different sources of P
Here T_1: 100% P as DAP; T_2: 100% P as RP; T_3: 100% P as SSP; T_4: 10% AE; T_5: 100% P as RP+10% AE; T_6: 100% P as SSP+10% AE; T_7: 100% P as DAP+10% AE; T_8: 50% P as RP+10% AE; T_9: 50% P as SSP+10% AE; T_{10}: 50% P as DAP+10% AE

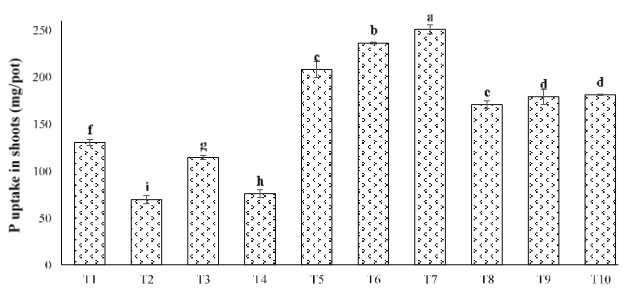

Fig. 6: P uptake of maize shoot after application of acidic extract and different sources of P
Here T_1: 100% P as DAP; T_2: 100% P as RP; T_3: 100% P as SSP; T_4: 10% AE; T_5: 100% P as RP+10% AE; T_6: 100% P as SSP+10% AE; T_7: 100% P as DAP+10% AE; T_8: 50% P as RP+10% AE; T_9: 50% P as SSP+10% AE; T_{10}: 50% P as DAP+10% AE

86.5 and 147% increment over sole DAP, SSP and RP application, respectively (Fig. 5b).

P Uptake in Shoots (mg/pot)

Statistically significant increase in P uptake by maize shoot was recorded from integrated application of P-fertilizers with AE. Maximum P uptake was observed by combined application of AE and recommended dose of P as DAP, followed by treatments where AE + 100% of P as SSP and RP were applied. These treatments improved P uptake by 92.42, 106.6 and 199.79% over their respective control treatments. Treatments where half dose of P fertilizers with AE were applied showed better results than alone application of fertilizers (Fig. 6).

Discussion

Plant vegetative growth reflected the behavior of plants against optimally applied inputs (Tariq *et al.*, 2014). Plant growth and yield attributes were significantly improved where combined AE and P fertilizers were applied than their separate application. It might be attributed to the fact that AE instantly lowers the soil pH by providing it a pH shock (data not shown in this study) that mobilizes P. The same fact was reported by Pedersen *et al.* (2017), the AE induces lowering of pH that ultimately desorbs the locked nutrients especially P to be taken up by plants. Further, the acidification effect can enhance growth promoting effects of the slurry (Amin *et al*., 1989; Lynch *et al.*, 1991) suppress ammonia volatilization (Fangueiro *et al.*, 2015) while increasing the dissolved nutrients pool (Christensen *et al.*, 2009) which regulate the growth of plants. Phosphorus is involved in leaf expansion, leave growth and root growth whereas, deficiency of P causes reduction of average length of the cell division zone in maize leaves, as well as its deficiency can cause reduction of both cell production and cell division rates (Assuero *et al*., 2004). Leaf expansion of plants is strongly related to the expansion of epidermal cells and this might be reduced in P-deficient plants because of shrinkage in root network. P deficiency causes lowering of cytosol Pi concentrations which negatively affect the Calvin cycle (Heldt *et al*., 1977). As a result, level of triose phosphate would reduce and starch would accumulate in leaf while the export of carbohydrates to roots should decrease (Rao and Terry, 1989; Williamson *et al.*, 2001; Wissuwa *et al*., 2005). This reduction in root growth ultimately effects the plant growth and development. Optimum amount of P enhances the plant height (Maqsood *et al*., 2001; Ayub *et al.*, 2002), leaves per plant (Krey *et al.*, 2013). Pedersen *et al.* (2017) reported that application of manure slurry acidified with sulfuric acid enhanced P bioavailability and vegetative growth of maize in sandy soil.

Gas exchange traits of maize (transpiration rate, photosynthetic rate, water use efficiency and spade value) were significantly improved after combined application of acidified extract with recommended sources of P fertilizer. Increased supply of P to plant, promotes root growth that substantially results in higher rate of water loss from aerial parts of plant. Increased P facilitates the initiation and growth of roots and increase in root length promotes the uptake of moisture and crucial elements from soil (Singh and Sale, 2000; Zafar *et al*., 2011). The response may be attributed to role of P in regulation of metabolic pathways in the cytosol and chloroplasts (Woodrow and Rowan, 1979). In vacuolated cells of plants, the vacuole acts as storage pool, and about 85–95% of the total P of the cell is present in the vacuoles as Pi (Lauer *et al.*, 1989). Deficiency of P reduces the carbohydrate translocation to roots which affect the root architecture and growth which minimize the P uptake in plants (Wissuwa *et al.,* 2005). Due to lower uptake of P, Pi concentration in the stromata of chloroplasts is reduced which strongly affects the photosynthesis and carbon partitioning in the light-dark cycle. The optimum range required for maximum photosynthesis is 2.0–2.5 m*M*, whereas the concentration below 1.4–1.0 m*M* strongly inhibits photosynthesis (Heber *et al.,* 1989; Jiang *et al*., 2007).

The present study revealed that yield related parameters were significantly improved by the combined application of AE and P fertilizers. The resultant peak response under the effect of AE is attributed to the instant localized acidity of rhizosphere by oxidation of S° through SOB as well as P-mineral solubilization due to H_2SO_4 and organic acids production from microbes that improved dissolution of P and then its uptake by plants and accumulation in grains. Extract with P fertilizers showed maximum 100 grain weight (Fig. 2) by virtue of the combined application of P fertilizers and acidified extract that improved P availability at critical plant growth stages. Zhao *et al*. (2010) elaborated that nutrient solubility from mineral surfaces, minerals speciation, eventual bioavailability and movement of the essential nutrients within soil is driven and regulated by the most vital factor *i.e*., pH. Applied P and its enhanced bioavailability significantly improved the plant growth, confirming the verdict of Hussain *et al.* (2006) and Balochgharayi (2011) that applying P fertilizers is beneficial and wholesome in enhancing all physiological and yield parameters of maize crop as well as cob related parameters. Amanullah *et al.* (2010) advocated the same fact that yield and yield components of maize increased significantly with increase in P contents. They further stated that application P fertilizers to maize crop increased grower's income by enhancing yield and decrease in P concentration can severely reduce the maize yield. Moreover, optimum concentration of P is most important for maximum yield and yield related parameters (Duggul, 1990) *e.g*., grain weight/cob, cobs/plant and grain rows/cob (Amin *et al.,* 1989) grains/cob (Maqsood *et al*., 2001) and 1000-grains weight (Toor, 1990).

Maximum P contents in shoot and root were recorded where P fertilizers and AE were applied in combination. AE helps to solubilize the minerals due to H_2SO_4 and lowering of soil pH simultaneously. Also, the organic acids produced from microbes aids up in dissolving P in soil to be up taken by plants. Siami *et al.* (2008) and Chaghazardi *et al.* (2014) confirmed these findings that application of acidified materials in soil enhanced the nutrient contents in plants. Oxidation of S° results in decreasing soil pH to solubilize the locked nutrients and improve the characteristics of calcareous soil (Ullah *et al.,* 2014; Havlin *et al.,* 2016). Our findings were in line with the observations made by Iqbal *et al.* (2012) that S° oxidation cause H^+ production that induces the replacement of metal cations from mineral surfaces such as iron hydroxide resulting in co-dissolution of other locked and needful nutrients from minerals that are needed by plants as well. Moreover, negative sites for metal complexation are provided due to sulfate production from S° oxidation process which are easily accessible to plants roots. Other than this, researchers have also manipulated pH by applying of mineral acids as HNO_3 (Schwertmann *et al.,* 1987), acetic acid (Tessier *et al.,* 1979) and organic acids (citric, gallic and oxalic acids) (Renella *et al.,* 2004; Khalid and Fawy, 2011) to find that addition of acids induce solubilization of carbonates to mobilize the mineral nutrients and add up in buffering the soils (Schwertmann *et al.,* 1987; Besharati, 2017). Kunze (1965) reported that acids help to dissolve the calcium minerals in soil and this solubility further depends upon several factors such as percentage and type of carbonate present and its particle size. The buffer capacity of soil is decreased under the effect of acids, thereby improving the nutrients bioavailability and uptake by plants and improving characteristics of calcareous soils as well (Kayser *et al*., 2000; Malakouti and Homaei, 2005; Kalich and Golchin, 2008). But, the major disadvantage of adding mineral acids directly to soil is the health hazard, their application can pose. Also, there is a depressive effect on soil respiration that suppresses overall biological activity in the soil as well as microbial biomass (Popovic, 1984).

However, with the advent of a new strategy, also come few limitations. For this approach, the addition of the extract in soil is a time specific requirement. It should be added at peak nutrient requirement of the crop because pH of soil cannot be changed permanently. So, its addition at crucial stages of crop life cycle when there is maximum nutrient requirement would be critical. For attaining maximum benefit from this approach, it would be beneficial to apply the extract frequently with irrigation. Therefore, the application method of this product needs further R&D to make it easier for farmers.

Conclusion

Application of P fertilizers with acidified extract significantly improved plant growth, gas exchange traits and P uptake in plants than their sole application. The approach is promising in improving P use efficiency and maize productivity, however, multi-sites field trials need to be performed to warrant successful performance in the field.

Acknowledgements

Authors are very much obliged to Environmental Sciences Lab., Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad for provision of excellent research facilities. The first author also acknowledges the financial assistance provided by HEC awarded Indigenous Ph.D. fellowship program Phase II Batch II scholarship.

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[Received 07 Aug 2018; Accepted 12 Feb 2019; Published (online) 26 Apr 2019]