Research Article

Phosphorus solubilizing bacteria in combination with organic supplements improve phosphorus and chlorophyll content and growth parameters of maize

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Abstract

Sustainable crops production coupled with environmental safety measures has become a center of attention at current global canvas of science and research. Phosphorus solubilizing bacteria (PSB) are known to solubilize fixed phosphorus. Biochar is not only a nutrients rich organic amendment but also a very relevant input in relation to present climate change trend. Compost has been reported for a long time to improve soil physical properties in addition to having sufficient quantity of essential nutrients to sustain higher crops yield. Therefore, the application of two PSB strains, Achromobactor xylosoxides (S1) and Pseudomonas aeruginosa (S3), were tested in combination with and without a mixture of biochar and compost to improve some selective growth parameters of maize. The data obtained depicted that the mixture of biochar and compost significantly improved maize growth parameters as compared PSB strains and the control. Combination of PSB, biochar and compost remained more effective than their separate application. The PSB strain, Achromobactor xylosoxides was observed to be better than the Pseudomonas aeruginosa with and without organic supplements. Conclusively, the efficiency of PSB strains increases when used in conjunction with organic supplements like biochar and compost with respect to phosphorus and chlorophyll content and maize growth indices.

Keywords: Corn. Rhizobacteria, Biochar, Compost, Productivity

Introduction

Phosphorus is a primary macro nutrient essential for plant growth. Using soil microbes like phosphorus solubilizing bacteria (PSB) or mycorrhizae is a friendly approach to increase the availability of phosphorus. Soil organic amendments like a biochar improve the plant growth by changing the soil physical and chemical properties. It is estimated that less than 1% soluble orthophosphate out of total phosphorus ratio absorbed by plant [1]. The quantity of phosphorus available for plant uptake is very low in Pakistani soils. Phosphorus is present in both organic and inorganic forms in soil. The largest portion of total phosphorus in soil is in organic from [2] e.,g., nucleotides and phospholipids have been reported to improve the plant growth. Phosphorus is bound in soil organically and inorganically [3].

A vast range of micro-organisms solubilize the inorganic phosphorus [4]. The soil pH decreases and bound phosphorus with calcium dissociates through release of citrate and gluconate by the microbes [5]. The plant growth promoting rhizobacteria (PGPR) produce various biochemical substance such as hydrogen sulfide, humic substances, carbon dioxides, protons, mineral acids, organic acids and siderophores [6-7]. Certain organic acids like acetic, glycolic, lactic, isobutyric, malonic, isovaleric, 2-keto-gluconic and succinic acids etc., are produced by PSB, which cause acidification in surrounding soil and release soluble orthophosphate ions [8]. The orthophosphate ions solubilized by organic acids become available to plant [9]. The siderophores play an important role to solubilize the insoluble phosphates [10]. The rhizosphere bacteria release different types of exudates which solubilize the phosphorus and potassium. Moreover, they also produce various growth regulators which improve the plant growth [11]. The PSB release the fixed phosphorus and make it available for plant uptake .The beneficial bacteria including Arthrobacter, Bacillus, Pseudomonas, Rhizobium, Erwinia, and Azospirillum are used to formulate bio-fertilizers on industrial scale.

Biochar is a charcoal like substance that is prepared after burning the different organic residues such as feed stocks by limited supply of O2 [12]. High surface area and porosity are key characteristics of biochar [38]. Use of biochar in soil improves the soil physio chemical properties, such as soil porosity, CEC and soil structure [5]. The biochar application significantly stabilizes the ecosystem, sequesters carbon thus leading to the mitigation of climate change effects and improving soil fertility [13-14]. The biochar makes the nutrient cycling more beneficial for plant growth [15]. The other benefits of biochar addition in soil include the increase in C, N and P level and water absorbing capacity [16-17], that of Ca, Mg and Zn [18] and decrease in the leaching losses of some macro nutrient [19-20]. Addition of biochar renders the water free from the heavy metals [21].

Composts are formed from raw organic material. Compost increases organic matter content and macro and micro nutrients in soil, which improve the plant growth and yield [22].

Maize (*Zea mays L.*) is a cereal crop that is grown all over the world. The maize oil is also used for edible purpose. So an experiment was designed with the hypothesis that the PSB, biochar and compost would improve phosphorus and chlorophyll content and

growth indices of maize.

Materials and Methods

A pot trial was conducted in the Department of Soil Science, BZ University Multan, Pakistan to study the effect of two PSB strains in combination with biocahr and compost. Following treatments were applied; Strain 1 (*Achromobactor xylosoxides*), Strain 3 (*Pseudomonas aeruginosa*), compost 0.5% + biochar 0.5%, strain 1 (PSB) + compost 0.5% + biochar 0.5% and strain 3 (PSB) + compost 0.5% + biochar 0.5%. The maize seeds were grown in clay pots having 10 kg soil. Seven seed were sown in each pots

PSB Strains

The PSB strains, S1 and S3 were collected from the Laboratory of Soil and Environmental Microbiology of the Department of Soil Science, BZ University. The inocula were prepared in Erlenmeyer flask with DF salt minimal medium [23]. The flask were kept in shaking 2 days at 28 ± 1 °C, setting the shaker speed at 100 rpm. Uniform optical density was achieved with the help of spectrophotometer at 440 nm.

Seed Coating

The seeds of maize variety, 85S45 were dipped in prepared culture medium of PSB strains for 2 hours. Clay, peat and sugar solution (10%) in the ration of 1:1:1 were used as carrier material for seeds coating.

Collection of feedstock and preparation of biochar

Sawdust was dried in sunlight. Biochar was prepared through pyrolysis [24] at 450 °C for 2 hours. The prepared biochar was cooled down finally.

Growth attributes

Different growth parameters were measured like plant shoot length (cm), root length (cm), shoot and root fresh weights (g), shoot and root weights (g), and chlorophyll content (SPAD).

Phosphorus content

Plant samples of maize were cleaned and dried in an oven. Ground samples were subjected to digestion [25] for recovery of P. A 0.1g of the sample was taken in a digestion flask, two ml of H2SO4 were added and left for 24 hour. This incubated samples were continued to be heated until fumes became visible. 1ml of H2O2 was added after an interval of every 20min until the material turned transparent. The volume was raised to 10 ml with distilled water. The phosphorus content were found out in plant through malachite green method [26] at spectrophotometer.

Chlorophyll content

Mature enough green leaves were picked up from each plant and chlorophyll contents were found out [27-28]. A 0.1gram sample was weighed and dipped in 10ml of acetone solution (80%) and kept for 24 hour with covers on the tubes. The readings were recorded at 645 and 663 nm by spectrophotometer. Actual chlorophyll contents were calculated by the formula.

Statistical analysis

The data recorded were statistically analyzed [37]. Computer

based software "Statistix 8.1" was used.

Results

Plant fresh shoot weight, root length and shoot length

Both the strain and organic amendments improved maize growth indices. Maximum and significantly improved fresh shoot weight, root length and shoot length were observed, when the strain 1 was used in combination with biochar and compost. The strain I proved more effective than the strain 3. Though compost and biochar mixture improved growth indices as compared to the PSB strains, but when efficacy of organic amendments and biochar increased while used combined.

Elements/Components	Units	Saw Dust Biochar
Nitrogen	%	1.60
Phosphorus	%	0.40
Potassium	%	2.10
рН	-	8.30
Volatile Matter	%	51.42
Fixed Carbon	%	22.69
Ash Content	%	14.39

Table 2: Chemical Properties of Compost

%	10
	1.0
%	0.53
%	55
-	7.5
dS m-1	2.07
	% - dS m-1



Figure 1: Effect of PSB with and without compost and biochar mixture on maize shoot fresh weight (g)



Figure 2: Effect of PSB with and without compost and biochar mixture on maize root length (cm)



Figure 3: Effect of PSB with and without compost and biochar mixture on maize shoot length (cm)



Figure 4: Effect of PSB with and without compost and biochar mixture on maize plant height (cm)



Figure 5: Effect of PSB with and without compost and biochar mixture on

phosphorus content in maize root



Figure 6: Effect of PSB with and without compost and biochar mixture on phosphorus content in maize shoot



Figure 7: Effect of PSB with and without compost and biochar mixture on phosphorus content in maize chlorophyll content

Plant Height

The same treatment, strain1 + compost + biochar proved more effective than the other treatments as far as maize plant height is concerned. Both the strains are effective but the strain 1 is more effective when applied alone or in combination with compost and biochar. The mixture of compost and biochar remained more effective than the PSB strains.

Shoot and root phosphorus concentration

Both the PSB strains increased the concentration of phosphorus in shoot and root of the plants. In the same way the combination, strain 1 + compost + biochar remained better than other treatments. The compost and biochar mixture exerted greater effect than the PSB strains. The strain 1 resulted in more and significantly improved P content than the strain 3, when used in separate and combination.

Chlorophyll Content

The same trend was observed in case of chlorophyll content. The chlorophyll content increased with the PSB strain 1 and 3 over the control. Compost and biochar mixture further improved chlorophyll as compared to the control as well as both the strains. When the strains were applied along with the compost and biochar, further significant improvement was noticed. The strain 1 remained more effective than the strain 3 when used either alone or in combination with compost and biochar.

Discussion

The data revealed that the PSB strains showed improvement in all the parameters noted, over the control. The mixture of compost, and biochar remained better than the strains. The effectively of compost and biochar in conjunction with the PSB strains improved. The strain 1 showed better performance than that of the strain 3. Increased fresh weight of maize has been reported through the application of organic amendments. Addition of organic amendments might have improved the soil physical condition and increased availability of nutrients resulting in improved shoot weight [29]. Schulz et al. [30] reported that the biomass of oat plant was not affected by the use of organic amendments. PSB have an important role in improving plants growth as availability of phosphorus might have been promoted [31-32]. Organic amendments result in an improvement in plant height too [33]. The reason behind might be the better soil fertility and provision of nutrients through those amendments. Brennan et al. [29] observed that the addition of organic supplements increased the length of maize roots and shoot significantly. Sgroy et al. [31] reported that PSB also played their role to increase the root length and shoot length. Application of organic substances increase the phosphorus concentration in plants shoot and root [34-35]. The bio-availability of phosphorus might have increased for the plants through the application of organic amendments Phosphorus uptake efficiency might have increased too due to the release of root secretions and the presence of bacteria near the root surface [36]. Ali et al. [33] observed that the supplementation of organic amendments increased the chlorophyll content significantly in wheat crop.

Conclusion

The result showed that application of PSB strains, Achromobactor xylosoxides and Pseudomonas aeruginosa assisted with biochar and compost improved growth and physiological indices of maize crops.

References

1. Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA (1999) Principles and applications of soil microbiology. Prentice Hall Inc, Upper Saddle River.

2. Speir T, Ross D (1978) Soil phosphatase and sulphatase. In: Burns R (ed) Soil Enzymes. Academic Press, New York, pp 197–250

3. Metcalf WW, Wanner BL, Chen C-M, et al (1991) Involvement of the Escherichia coli phn (psiD) Gene Cluster in Assimilation of Phosphorus in the Form of Phosphonates, Phosphite, Pi Esters, and Pi

4. Bucher M (2007) Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytol. 173:11–26

5. Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant Soil 337:1–18

6. Teodosieva R, Yakimova Bojinova D, Ivanova R, et al (2006) Rock phosphate solubilization by soil bacteria. J Univ Chem Technol Metall 41:297–302

7. Ansari FA, Ahmad I (2018) Biofilm Development, Plant Growth Promoting Traits and Rhizosphere Colonization by &It;i>Pseudomonas entomophila&It;/i> FAP1: A Promising PGPR. Adv Microbiol 08:235–251. https://doi.org/10.4236/aim.2018.83016 8. Vazquez P, Holguin G, Puente ME, et al (2000) Phosphatesolubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol Fertil Soils 30:460–468. https://doi.org/10.1007/s003740050024

9. Danish S, Zafar-ul-Hye M (2019) Co-application of ACCdeaminase producing PGPR and timber-waste biochar improves pigments formation, growth and yield of wheat under drought stress. Sci Rep 9:1–13. https://doi.org/10.1038/s41598-019-42374-9

10. Han J-H, Park G-C, Kyoung &, Kim S (2017) Antagonistic Evaluation of Chromobacterium sp. JH7 for Biological Control of Ginseng Root Rot Caused by Cylindrocarpon destructans. Mycobiology 45:370–378

11. Rafique M, Sultan T, Ortas I, Chaudhary HJ (2017) Enhancement of maize plant growth with inoculation of phosphate-solubilizing bacteria and biochar amendment in soil. Soil Sci Plant Nutr 63:460–469. https://doi.org/10.1080/00380768.2017.1373599

12. Amonette J, Joseph S (2009) Characteristics of Biochar - Microchemical Properties. In: Amonette J, Joseph S (eds) Biochar for Environmental Management: Science and Technology. Earthscan, London, UK, pp 33–52

13. Lehmann J, Gaunt J, Rondon M (2006) Bio-char Sequestration in Terrestrial Ecosystems – A Review. Mitig Adapt Strateg Glob Chang 11:395–419

14. Sohi SP, Krull E, Bol R (2010) A review of biochar and its use and function in soil. Adv Agron 105:47–82. https://doi.org/10.1016/S0065-2113(10)05002-9

15. Steiner C, Das KC, Garcia M, et al (2008) Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. Pedobiologia (Jena) 51:359–366. https://doi.org/10.1016/j.pedobi.2007.08.002

16. Thies J, Rillig MC (2009) Characteristics of biochar: Biological properties. In: Biochar for environmental management: Science and technology. Earthscan, London

17. Borchard N, Wolf A, Laabs V, et al (2012) Physical activation of biochar and its meaning for soil fertility and nutrient leaching - a greenhouse experiment. Soil Use Manag 28:177–184. https://doi.org/10.1111/j.1475-2743.2012.00407.x

18. Major J, Lehmann J, Rondon M, Goodale C (2010) Fate of soilapplied black carbon: downward migration, leaching and soil respiration. Glob Chang Biol 16:1366–1379

19. Novak JM, Lima I, Xing B, et al (2009) Characterization of designer biochar produced at different temperatures and their effects on a loamy sand. Ann Environ Sci 3:195–206.

20. Laird D, Fleming P, Wang B, et al (2010) Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158:436–442. https://doi.org/10.1016/j.geoderma.2010.05.012

21. Mohan D, Pittman CU, Bricka M, et al (2007) Sorption of arsenic, cadmium, and lead by chars produced from fast pyrolysis of wood and bark during bio-oil production. J Colloid Interface Sci 310:57–73. https://doi.org/10.1016/j.jcis.2007.01.020

22. Clemente R, Walker DJ, Pardo T, et al (2012) The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions. J

Hazard Mater 223–224:63–71. https://doi.org/10.1016/j.jhazmat.2012.04.048

23. Dworkin M, Foster JW (1958) Experiments with some microorganisms which utilize ethane and hydrogen. J Bacteriol 75:592–603

24. Qayyum MF, Abid M, Danish S, et al (2014) Effects of various biochars on seed germination and carbon mineralization in an alkaline soil. Pakistan J Agric Sci 51:977–982

25. Wolf B (1982) A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. Commun Soil Sci Plant Anal 13:1035–1059. https://doi.org/10.1080/00103628209367332

26. Ohno T, Zibilske LM (1991) Determination of low concentrations of phosphorus in soil extracts using malachite green. Soil Sci Soc Am J 55:892–895

27. Arnon DI (1949) Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol 24:1–15. https://doi.org/10.1104/pp.24.1.1

28. Ravelo-Pérez LM, Hernández-Borges J, Rodríguez-Delgado MÁ (2008) Multi-walled carbon nanotubes as efficient solid-phase extraction materials of organophosphorus pesticides from apple, grape, orange and pineapple fruit juices. J Chromatogr A 1211:33–42. https://doi.org/10.1016/j.chroma.2008.09.084

29. Brennan A, Jiménez EM, Puschenreiter M, et al (2014) Effects of biochar amendment on root traits and contaminant availability of maize plants in a copper and arsenic impacted soil. Plant Soil 379:351–360. https://doi.org/10.1007/s11104-014-2074-0

30. Schulz H, Dunst G, Glaser B (2014) No Effect Level of Co-Composted Biochar on Plant Growth and Soil Properties in a Greenhouse Experiment. Agronomy 4:34–51. https://doi.org/10.3390/agronomy4010034

31. Sgroy V, Cassán F, Masciarelli O, et al (2009) Isolation and

characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte Prosopis strombulifera. Appl Microbiol Biotechnol 85:371–381. https://doi.org/10.1007/s00253-009-2116-3

32. Tiwari S, Lata C, Chauhan PS, Nautiyal CS (2016) Pseudomonas putida attunes morphophysiological, biochemical and molecular responses in Cicer arietinum L. during drought stress and recovery. Plant Physiol Biochem 99:108–117. https://doi.org/10.1016/j.plaphy.2015.11.001

33. Ali K, Arif M, Jan MT, et al (2015) Biochar: A novel tool to enhance wheat productivity and soil fertility on sustainable basis under wheat-maize-wheat cropping pattern. Pak J Bot 47:1023–1031

34. Farooq M, Wahid A, Lee D-J, et al (2010) Drought Stress: Comparative Time Course Action of the Foliar Applied Glycinebetaine, Salicylic Acid, Nitrous Oxide, Brassinosteroids and Spermine in Improving Drought Resistance of Rice. J Agron Crop Sci 196:336–345. https://doi.org/10.1111/j.1439-037X.2010.00422.x

35. Prendergast-Miller MT, Duvall M, Sohi SP (2014) Biochar-root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability. Eur J Soil Sci 65:173–185. https://doi.org/10.1111/ejss.12079

36. Geneva M, Zehirov G, Djonova E, et al (2006) The effect of inoculation of pea plants with mycorrhizal fungi and Rhizobium on nitrogen and phosphorus assimilation. Plant Soil Environ 52:435–440. https://doi.org/10.17221/3463-pse

37. Steel RG, Torrie JH, Dickey DA (1997) Principles and Procedures of Statistics: A Biometrical Approach, 3rd edn. McGraw Hill Book International Co., Singapore

38. Mohan D, Pittman CU, Steele PH (2006) Pyrolysis of wood/biomass for bio-oil: A critical review. Energy and Fuels 20:848–889

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