



## Research Article

# IMPACT ASSESSMENT OF MIXED BIOFERTILIZERS ON SOIL PHYSICO-BIOCHEMICAL PROPERTIES UNDER POT CULTURE STUDIES

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**Abstract-** In the present investigation, a greenhouse pot culture experiment was carried out in maize to assess the soil physio-biochemical properties changes such as pH, EC, available N, available P, available K, soil organic carbon (SOC), microbial biomass carbon (MBC), substrate induced respiration (SIR), metabolic quotient (MQ), dehydrogenase, alkaline and acid phosphatase due to addition of mixture of commercial biofertilizers viz., *Azospirillum brasilense* (Sp7), *Bacillus megaterium* (Pb1) and *Azotobacter chroococcum* (Ac1) in the form of carrier based and liquid formulations, comparing it with uninoculated control at 15, 30, 45 days intervals after sowing. The analyzed parameters suggested that the application of biofertilizers recorded significantly higher available nutrients in the soil than the uninoculated control soil.

**Keywords-** Biofertilizers, Soil Physico-biochemical Properties, Soil health

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## Introduction

India Soil quality is an integrated characteristic of physical, chemical and biological properties. Monitoring the changes in these properties affected by the nutrient management systems is very much essential to keep the soil sustainability. Biofertilizers would be the viable option for farmers to increase productivity per unit area because of its eco-friendly nature to sustain the agriculture by reducing the chemical inputs and improving the soil health. Hence the effect of mixed biofertilizers with carrier and liquid formulations of *Azospirillum brasilense*, *Azotobacter chroococcum* and *Bacillus megaterium* on physico-chemical and biochemical properties viz., pH, EC, available N, available P, available K, soil organic carbon, microbial biomass carbon, substrate induced respiration, metabolic quotient, dehydrogenase, alkaline and acid phosphatase were estimated in the present study.

## Materials and methods

### Biofertilizers

The commercial biofertilizers of solid and liquid formulations of *Azospirillum brasilense* (Sp7), *Azotobacter chroococcum* (Ac1) and *Bacillus megaterium* var. Phosphaticum (Pb1) were obtained from Biofertilizer Production and Quality Control Laboratory, Department of Agricultural Microbiology, TNAU, Coimbatore-3.

### Treatment details

Three treatments each with six replications were followed as detailed below.

T1 - Mixture of liquid based commercial bioinoculants Sp7+ Ac1+Pb1

T2 - Mixture of carrier based commercial bioinoculants Sp7+ Ac1+Pb1

T3 - Uninoculated Control

### Pot culture Experiment to assess Physico-chemical analysis of soil

A pot culture in maize (cv.CO HM6) was carried out in order to assess the impact of mixed biofertilizers (Sp7, Pb1 and Ac1) on physico-chemical analysis of soil due

to its inoculation at different intervals. The maize plants were grown in pots upto 45 days after sowing (DAS) as per the treatments. The soil was then collected from all the treatments pots at four different intervals of period viz., 0, 15, 30 and 45 DAS and the analyses for physico-chemical and biochemical properties were analyzed. The physico-chemical analysis viz., Soil pH [1]; Electrical conductivity (EC) Electrical conductivity was determined in 1:2.5 soil-water suspension using "ELICO" conductivity bridge.; Available nitrogen [2], Available phosphorus [3], Available potassium [4], Dehydrogenase (EC 1.1.1.1) [5], Acid phosphatase (E.C. 3.1.3.2) [6], Alkaline phosphatase (E.C. 3.1.3.1) [6], Soil organic carbon [7], Soil microbial biomass carbon (MBC) [8], Substrate induced respiration (SIR) [9] and Quantification of soil metabolic quotient ( $qCO_2$ ) [10] was estimated as per the standard procedure.

### Statistical analyses

All the data were subjected to statistical analysis with the software and statistical packages AGRES, AGDATA [11] and Microsoft Excel for Windows 2007 add-ins with XLSTAT Version 2010.5.05 (XLSTAT, 2010). Statistically significant differences between the treatments were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5 percent significance level.

### Results and discussion

Physico and biochemical properties of the soil imply the health and fertility which in turn reflect the crop growth and yield. The results of soil physico-chemical properties done in this research has been given in the [Fig-1] The soil physico-chemical properties are highly influenced by crop management, fertilizer application, tillage practices and organic carbon content [12]. Soil fertility and productivity are mainly directed by the soil microbiome which drive most of the biological processes including nutrients availability, organic matter decomposition, SOC build-up [13] and improved the physical properties such as structure, Impact

## Assessment of Mixed Biofertilizers on Soil Physico-biochemical Properties under Pot Culture Studies

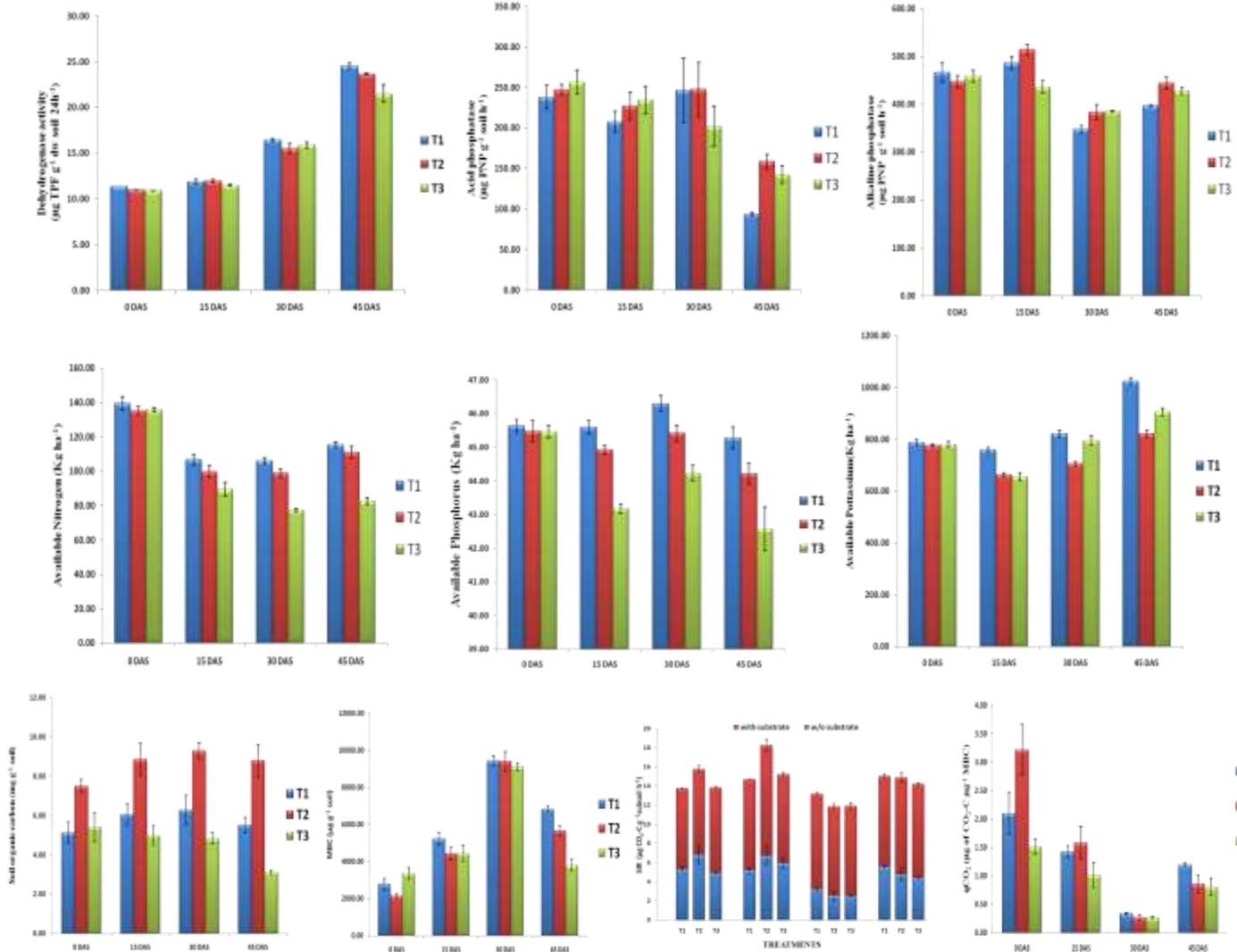


Fig-1 Impact of bioinoculants application on [A] Dehydrogenase ; [B] Acid phosphatase; [C] Alkaline phosphatase; [D] Available N; [E] Available P; [F] Available K; [G] SOC; [H] MBC; [I] SIR & [J] qCO<sub>2</sub> in maize (cv. CO HM6) under pot culture conditions at different intervals.

porosity, aeration and water infiltration by stabilizing the soil aggregates [14]. Hence, the present investigation was carried out to assess the changes in soil physico-chemical properties with special reference to biological processes such as soil enzymes, microbial biomass carbon, substrate induced respiration etc. due to addition of bioinoculants.

In general, it is widely accepted that application of both biofertilizers and inorganic fertilizers significantly affected the soil pH, available macro (NPK) nutrients and micronutrients (Fe, Zn, Mo, Mn). The soil here used in study is alkaline with pH ranged from 8.10 to 8.42. In the present investigation also, the analyzed physico-chemical parameters suggested that the application of bioinoculants recorded significantly higher available nutrients in the soil than the uninoculated control soil. Results revealed that decrease of available N were 21.05 percent for T1 and 21.84 percent for T2 and 48 percent for T3 from 0 to 45 DAS. This indicated that the inoculation of biofertilizers either in liquid or carrier based has increased the available N content over the uninoculated control. The highest available P recorded for T1 at 30 DAS i.e., 45.60 Kg ha<sup>-1</sup> soil and the lowest was seen for T3 at 45 DAS with 42.58 Kg ha<sup>-1</sup>. The results showed that the pot culture soil was highly rich in potassium. The highest available K was observed in T1 at 45 DAS with 1024.39 Kg ha<sup>-1</sup> and lowest occurred in T3 at 15 DAS of 655.38 Kg ha<sup>-1</sup>. There was an overall increase in the values of available nitrogen, phosphorus and potassium content in the soils of the treated pots with bioinoculants compared to uninoculated control. Similarly, [15] also obtained the results when applied with biofertilizer treatments (*Azospirillum brasilense* + *Azotobacter chroococcum* + *Bacillus polymyxa* + *B. circulans*) + 15% aqueous extract of compost recorded available N, P and K (1.84, 0.35 and 2.02 percent) highest, when compared to

(1.29, 0.16 and 1.00 percent) the control plants, respectively. In the present investigation, the increase in available nitrogen concentration could be due to the high ability of *Azospirillum brasilense* (Sp7) and *Azotobacter chroococcum* (Ac1) in N<sub>2</sub>-fixation and the increase in the availability of phosphorus (P) and potassium (K) may be attributed to the production of organic acids by phosphorus solubilising bacteria (Pb1) strain applied. These organic acids adhered to mineral surface and extracted the nutrients non-specifically from the mineral particles through electron transfer; break the oxygen links in the minerals and released the nutrients and chelated ions present in the solution through carboxyl and hydroxyl groups and thereby indirectly accelerating the dissolution rate of minerals [16]. These findings are in agreement with the findings of [17] and [18].

Soil organic carbon (SOC) equilibrium is governed by a number of interacting factors such as temperature, moisture, texture, quantity and quality of organic matter, methods of organic matter application, soil tillage and cropping system [19]. The change in SOC contents are also directly associated with changes in microbial biomass carbon and biological activity in the soil. Besides living plant roots and organisms, soil microbial biomass is a living portion of soil organic matter. The response to changes in inputs of organic material is quicker in soil microbial biomass than in soil organic matter [20]. In the present study, the highest organic carbon was found in T2 at 30 DAS having 9.29 mg g<sup>-1</sup> soil and lowest was recorded in T3 at 45 DAS with 3.12 mg g<sup>-1</sup> soil. Overall, the carrier-based formulation of mixed bioinoculums (T2) showed the highest SOC at all intervals than T1 and uninoculated control (T3), because the carrier material used here was lignite, which contains higher organic carbon content. The soil microbial biomass (MBC), which represents about 1 - 5 percent of total soil organic carbon, can

provide an effective early warning of the improvement or deterioration of soil quality as a result of different management practices [21]. Information on changes in microbial biomass is valuable to study soil microbial activity, because it not only provides an indication of slower, less easily detectable soil organic matter changes, but also it represents an important labile pool of available nutrients to plant [22]. In accordance with the findings of [23], in the present study it was observed that the microbial biomass carbon was significantly enhanced in the treatment T1 over uninoculated control at all interval periods. This was a clearly indicative of the microbial build up in the soil as a result of application of mixture of bioinoculants.

Most microorganisms in the soil are dormant due to limited nutrient conditions [24], so their rate of respiration is low. However, their respiration can be stimulated by adding an easily decomposable substrate. Higher SIR indicates the presence of metabolically active microorganisms including r and K strategists with the former growing faster when substrate is abundant, whereas the K strategic microbes can grow when resources are limited [25]. Maximum SIR was recorded at 15 DAS of T2 treatment with  $11.59 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ , followed by the same treatment T2 at 45 DAS with  $10.15 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$  and least was recorded at 0 DAS of T1 with  $8.42 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ , followed by T3 at 0 DAS with  $8.91 \mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ . Hence, this indicates that the soil which did not receive any nutrient source had not shown any significant difference in soil respiration rate whereas the soil amended with readily available nutrient source like glucose had increased the respiration rate two folds when compared to basal respiration. The data on soil microbial biomass, organic-C build up due to application of bioinoculants addition as reported in the present investigation supported this finding. SIR activity of soil enhanced by soil microbial biomass and higher microbial counts due to organic amendments highlighted the importance use of application of bioinoculants.

The metabolic quotient has been used as a bioindicator of environmental stress on microbial communities, disturbances and ecosystem development. When soil environment comes in stress or disturbed conditions, soil microbes need more energy to maintain survival and result in metabolic quotient augment [26]. The  $q\text{CO}_2$  reflects metabolically active fraction of soil microbiome, which provides a measure of specific metabolic activity that varied according to the composition and physiological state of the microbial community, carbon and energy sources and abiotic factors. The low  $q\text{CO}_2$  reflects a more efficient use of substrates by the soil microbial biomass, in other words energy and carbon required for biomass maintenance in such soil is less [27]. A high  $q\text{CO}_2$  reveals a high maintenance carbon demand and if the soil system cannot replenish the carbon which is lost through respiration, microbial biomass decline [28]. In the present study, the  $q\text{CO}_2$  was decreased from initial stage *i.e.* 0 day to the 30 days after sowing and a slight increase was observed from 30 to 45 days after sowing irrespective of the treatments. This indicated that upto 30 day, the growth of maize plants was slow which resulted in weak ability of roots to absorb soil nutrients and reducing the stress to soil microbiome development. But after that at 45 day, the expanding roots with strong absorbing ability of soil nutrients by competing with the soil microorganisms, reduced the microorganisms inhabiting the rhizosphere [29]. The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils [30].

This study demonstrated that application of bioinoculants exhibited greater biological activity (*i.e.* assayed soil enzymes) than uninoculated soil, agreeing with several previous works [31-33]. It also revealed that, dehydrogenase activity was affected not only by bioinoculants application but also at different interval periods of growth of plant. In the present study, there was a continuous increase in dehydrogenase activity occurred from 0 day to 45<sup>th</sup> day, which might be due to maximum rhizosphere effect of root system that augmented the native microflora of the root system. Higher dehydrogenase activity was observed on 45 DAS where T1 recorded  $24.54 \mu\text{g TPF released g}^{-1} \text{ dw soil day}^{-1}$ , followed by T2 having  $23.68 \mu\text{g TPF released g}^{-1} \text{ dw soil day}^{-1}$ . Phosphatases play an important role in P cycling where organic P is more due to limited biological mineralization of organic matter as a result of formation of complexes of organic P with active aluminum and iron [34] and the amount of available P is low. P transformation and cycle also depend on soil reaction. In this study, the least acid phosphatase was recorded in T1 with  $93.93 \mu\text{g pNP released g}^{-1} \text{ dw soil h}^{-1}$  followed by T3 and T2 with  $142.66$

and  $158.69 \mu\text{g pNP released g}^{-1} \text{ dw soil h}^{-1}$  respectively. In case of alkaline phosphatase activity, the highest was found at 15 DAS and lowest was recorded at 30 DAS for all treatments. The T2 and T1 recorded highest alkaline phosphatase activity with  $514.92$  and  $487.01 \mu\text{g pNP released g}^{-1} \text{ dw soil h}^{-1}$  during 15 DAS respectively and were on par with each other over uninoculated control T3 with  $436.88 \mu\text{g pNP released g}^{-1} \text{ dw soil h}^{-1}$ , whereas least activity was recorded at 30 DAS for T1 with  $347.62 \mu\text{g pNP released g}^{-1} \text{ dw soil h}^{-1}$ , followed by T3 and T2 with  $385.58$  and  $383.38 \mu\text{g pNP released g}^{-1} \text{ dw soil h}^{-1}$  respectively. The present result showed that the acid phosphatase activity was much lower than alkaline phosphatase due to alkaline reaction of the soil (pH 8.1 - 8.4). Earlier studies also proved that phosphatases were strongly influenced by pH [35]. Further, there was no significant difference shown among the treatments in the present study. This might be due to difficulty in differentiating between root- and Phosphorus solubilising microorganisms (PSM) produced phosphatases [36], even though some evidence suggests that phosphatases of microbial origin possess a greater affinity for organic phosphate compounds than those derived from plant roots [37]. Moreover, the relationship between PSM introduced into soil, phosphatase activity and the subsequent mineralization of organic phosphates still remains poorly understood [38].

### Conclusion

The physico-biochemical characterization of the pot culture soil was analysed, which showed that application of bioinoculants will improve the soil health and fertility compared to uninoculated soil. There was an overall increase in the values of available nitrogen, phosphorus and potassium content in the soils of the treated pots with biofertilizers compared to uninoculated control. The carrier based formulation of mixed biofertilizers showed the highest SOC at all intervals than liquid formulation and uninoculated control. The results of remaining parameters like MBC, SIR, MQ, dehydrogenase, alkaline and acid phosphatase was significantly higher in the liquid based followed by carrier-based formulation of biofertilizers in comparison with uninoculated control. In conclusion, the physico-biochemical characterization of the pot culture soil showed that application of biofertilizers will improve the soil health and fertility compared to uninoculated soil.

**Application of research:** This research results can be suggested for the application of mixed biofertilizers to increase the health and fertility status of the soil at the field level.

**Research Category:** Agricultural Microbiology

**Abbreviations:** N-Nitrogen, P-Phosphorus, SOC-Soil organic carbon, K- Potassium, MBC-Microbial biomass carbon, SIR-Substrate induced respiration,  $q\text{CO}_2$  - Metabolic quotient, DAS -Days after sowing.

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**Cultivar / Variety / Breed name:** Maize (cv.CO HM6)

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

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