DOI : 10.58993/ijh/2025.82.2.6

Indian J. Hortic. 82(2), June 2025: 165-170

Beneficial effect of microbial bioformulations on nutrient availability, microbial biomass carbon and key enzymatic activities in citrus orchard soil

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ABSTRACT

The current investigation is designed to illustrate the beneficial effect of different microbial bioformulations on nutrient availability, microbial biomass carbon and key enzymatic activities in a citrus orchard soil. Three different microbial bioformulations namely Biogreen-5, Bioveer and Biosona were arranged in seven different treatments including T_1: Biogreen (soil + foliar application); T_2: Biogreen (foliar application); T_3: Bioveer (soil + foliar application); T_4: Bioveer (foliar application); T_5: Biosona (soil + foliar application); T_6: Biosona (foliar application); T_7: Control (water spray). Biogreen when applied as soil and foliar application increased the soil pH by 6.18%, organic carbon by 27.27%, and cationic exchange capacity by 6.24% over control. Like-wise, Biogreen applied both as soil and foliar application increased the availability of N, P, K, Mn, Zn, Fe and B content by 20.17%, 22.19%, 31.74%, 52.81%, 40.76%, 33.60% and 51.47%, respectively over control. Furthermore, the application of Biogreen (soil + foliar application), demonstrated noteworthy enhanced in microbial biomass carbon, and key enzyme activities including dehydrogenase, phosphomonoesterase, and fluorescein diacetate hydrolysis, with significant improvements up to 39.58%, 23.39%, 30.21%, and 36.15%, respectively as compared to control. The findings of the current investigation indicated that Biogreen followed by bioveer serves as an effective biological formulation, for sustaining microbial biomass carbon, cationic exchange capacity, essential plant nutrients and key enzymatic activities.

Key words: Microbial population, Trichoderma, rhizospere, dehydrogenase, soil health.

INTRODUCTION

The origin of citrus can be traced back to Southeast Asia, where its cultivation began around 4000 years ago. The vast genus citrus features several important cultivars as a result the taxonomy of the genus remains quite complex (Kausik, 11). Citrus is acknowledged as a perennial fruit crop, and its rhizosphere microbiome is considered essential for promoting the growth and well-being of citrus plants. Yield potential of citrus cultivars are, however, dependent on how effectively that variety can adapt to a specific soil environment, nutrient allocations, and the related segregation of rhizosphere microbial community dynamics (Ngullie *et al.*, 15). The composition of soil nutrients directly impacts on various aspects of citrus cultivation including the growth, fruit development, retention, yield, and the overall enhancement of orchard production and quality parameters. Although chemical fertilization seems to be useful in citrus cultivation, rapid and unlawful usage of chemicals are not desirable, as the chemicals are associated to soil toxicity and quality deterioration, creation of maximum residue limits (MRLs) and environmental degradation (Bhattacharyya *et al.*, 4; Bhattacharyya *et al.*, 3). This necessitates an emergent concern for the use of non-chemical and environment-friendly alternatives that would lead to develop climate resilient agriculture. Microbial bioformulations are the products of living organisms that pose no or significantly less amount of threat to the soil and environment, hence can be rapidly used for improving the physicochemical and biological properties of agricultural soil for enhanced sustainability. Plant growth promoting rhizobacteria (PGPR) are considered as pivotal components of microbial bioformulations that possess substantial benefits to soil and plant sustainability either directly or indirectly (Bora *et al.*, 5; Nath *et al.*, 14). PGPRs facilitate to enhancement of soil health by promoting sustainability in plant growth, mineralizing soil nutrients, and improving nutrient absorption by plants. According to Hasan *et al.* (8) PGPRs are crucial players in agriculture as they assist in performing lots of vital biochemical processes associated to plant growth and nutrition. Nitrogen fixation, phosphate and potassium solubilization, heavy metal mitigation, and

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production of phytohormones like auxin, gibberellin, and cytokinin falls under the category. Revisiting plant-microbe interactions in citrus rhizosphere and citing their importance in crop production presents an effective way to promote these green and cleaner tools for improvement of citrus fruits. The current experiment has, therefore, been designed to study the application potential of different types of microbial bioformulations in improving sustainability of citrus cultivars of Assam, North-east India through enhancing soil nutrient status and quality production potential. Further, the current approach has also obtained its prominence in utilizing microbial consortium-mediated bioformulations in engineering citrus rhizosphere through enhanced nutrient mining and key enzyme activities.

Table 1. The initial physicochemical properties of the soil at the experimental site.

Soil property	Value
i) Soil reaction (pH)	5.30
ii) Organic carbon (%)	0.56
iii) Cation exchange capacity (CEC) (meq/100g)	5.60
iii) Available N (kg/ha)	244.12
iv) Available P_2O_5 (kg/ha)	21.45
v) Available K_2O (kg/ha)	140.78
vi) Available Manganese (ppm)	2.10
vii) Available Iron (ppm)	4.76
viii) Available Zn (ppm)	0.90
ix) Available B (ppm)	0.33

MATERIALS AND METHODS

The bioformulations like Biogreen-5 (Consortium of *Trichoderma viride* and plant growth promoting microbes), Bioveer (*Trichoderma viride*) and Biosona (*Beauveria bassiana*) were collected from the Biocontrol Laboratory of the Department of Plant Pathology under DBT- North East Centre for Agricultural Biotechnology, Assam Agricultural University, Jorhat, Assam, India. The effectiveness of the microbial bioformulations in enhancing the soil nutrient content in Assam Lemon plants (*Citrus limon* L.) was assessed under field conditions. The study was conducted at the Experimental Farm, Department of Horticulture, AAU in Jorhat (latitude 26º47'N and longitude 94º12'E), Assam, India during April to November for two consecutive years 2021 and 2022, respectively. The experiment followed a randomized block design (RBD) and consisted of seven different treatments: T1: Biogreen (soil + foliar application), T2: Biogreen (foliar application), T3: Bioveer (soil + foliar application), T4: Bioveer (foliar application), T5: Biosona (soil + foliar application), T6: Biosona (foliar application), and T7: control. The initial chemical properties of soil are enlisted in Table 1.

Rhizospheric soil samples were collected from the citrus rhizosphere from a depth of 0–30 cm during November 2021 and 2022 from each treatment, followed by air drying and passing through a 2.0 mm sieve and stored in polypropylene bags for further analysis. Soil pH and cation exchange capacity (CEC) were determined in accordance to Jackson (9). Rapid titration procedure of Walkley and Black (19) was followed for the determination of organic carbon content (%) of soil. The alkaline potassium permanganate method, as described by Subbiah and Asija (16), the Bray and Kurtz No. 1 method, and neutral normal ammonium acetate (Jackson, 9) were used to determine the available nitrogen, phosphorus, and potassium content in the soil, respectively. Micronutrient availability in soil like zinc, iron, copper and manganese content were determined by atomic absorption spectrophotometer using DTPA extraction method (Lindsay and Norvell, 12). The available boron content was determined by Azomethine-H method (Wolf, 20). The rhizospheric soil samples were maintained at 4 ^{o}C and subsequently brought to ambient temperature before the estimation of individual biological parameters. Microbial biomass carbon (MBC) was determined by chloroform fumigation-extraction technique as per Vance *et al.* (18). The soil enzymatic activities such as dehydrogenase activity (DHA), phosphomonoesterase (PEMese) and fluorescein diacetate (FDA) were determined in accordance to Casida *et al.* (6), Tabatabai and Bremner (17) and Adam and Duncan (1), respectively.

RESULTS AND DISCUSSION

Significant variations in soil pH, organic carbon (OC) and CEC in soil were observed due to application of different microbial bioformulations in citrus orchard soil. Results (Table 2) revealed that the Biogreen applied both as soil and foliar treatment could increase the soil pH by 6.18%, OC by 27.27%, and CEC by 6.24% (Fig. 1) over control. It may be due to the fact that inoculation of plants with microbial symbionts, mycorrhizal fungi and PGPR helps in plant establishments and further improvement of physicochemical and biological properties of rhizosphere soil.

Considering the nutrient status of soil after application of microbial bioformulations (Biogreen, Bioveer, Biosona), there were significant improvements in available N, P and K content. Biogreen followed

Table 2. Effect of bioformulations on per cent increase of pH, CEC, organic C, available NPK content in soil over control.

Treatment	pH	CEC (meq $100g^{-1}$)	Organic C (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
T_1: Biogreen (soil + foliar application)	5.66	6.09	0.75	310.41	30.46	226.55
T_2: Biogreen (foliar application)	5.62	5.98	0.70	298.30	28.58	216.13
T_3: Bioveer (soil + foliar application)	5.58	5.96	0.72	297.37	28.20	192.98
T_4: Bioveer (foliar application)	5.49	5.92	0.64	290.05	27.00	185.72
T_5: Biosona (soil + foliar application)	5.43	5.92	0.61	274.85	25.05	162.24
T_6: Biosona (foliar application)	5.32	5.81	0.57	265.22	24.00	160.43
T_7: Control (water spray)	5.31	5.71	0.55	247.81	23.70	153.65
S. Ed. (±)	0.213	0.043	0.070	4.92	2.27	3.34
CD ($_{P=0.05}$)	NS	0.056	0.032	10.09	4.64	6.84

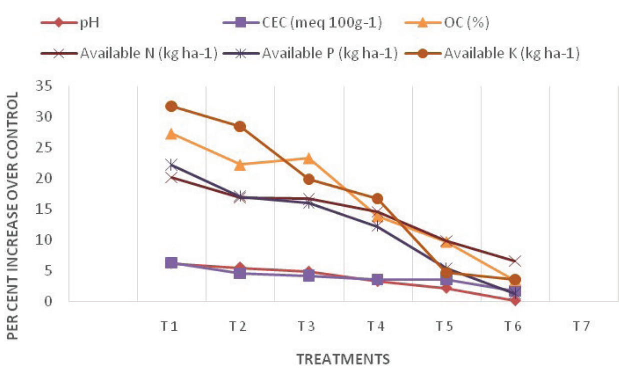

Fig. 1. Effect of bioformulations on per cent increase of pH, CEC, organic C, Available NPK content in soil over control (T_1: Biogreen (soil + foliar application); T_2: Biogreen (foliar application); T_3: Bioveer (soil + foliar application); T_4: Bioveer (foliar application); T_5: Biosona (soil + foliar application); T_6: Biosona (foliar application); T_7: Control (water spray).

by Bioveer and Biosona could effectively increase the soil nutrient contents in comparison to control experiments. Biogreen applied both as soil and foliar application could increase the available N, P and K content by 20.17, 22.19 and 31.74%, respectively over control. Similarly, Bioveer and Biosona were also effective in increasing the available nutrient contents over control. The finding was aligned to Jamal *et al.* (10), recorded significant increase in total N and P content in plants after inoculation of PGPR, *Bacillus amyloliquefaciens* in soil resulting in higher crop yield and quality. According to the researchers, enhanced biological N_2 fixation and solubilising fixed soil P and increased inorganic P availability to plants by PGPR results in agricultural sustainability and plant protection. Similarly, biogreen applied could increase the availability of micronutrients like Mn, Zn, Fe and B content by 52.81, 40.76, 33.60 and 51.47%, respectively over control (Table 3). Halifu *et al.* (7), observed that inoculation of *Trichoderma* spp. significantly increases the N, P and K content and soil enzyme activity in rhizosphere soil. Significant progress has been made in enhancing the biological characteristics in plant rhizosphere treated with microbial bioformulations like biogreen, followed by bioveer and biosona (Table 3). The application of biogreen, through both soil and foliar methods, significantly enhanced soil health indicators. Notable increases were observed in microbial biomass carbon (MBC) by 39.58%, dehydrogenase activity (DHA) by 23.39%, phosphatase activity (PHM) by 30.21%, and fluorescein diacetate (FDA) hydrolysis by 36.15% compared to the control (Fig. 2).

Similarly, bioveer and biosona also effectively increased the biological properties of soil in

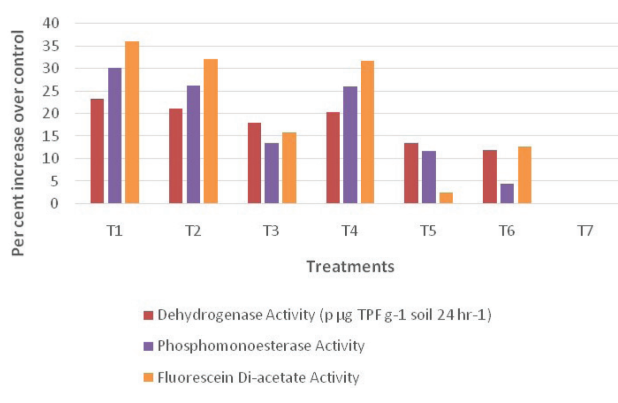

Fig. 2. Effect of bioformulations on per cent increase of different enzyme activities in soil over control (T_1: Biogreen (soil + foliar application); T_2: Biogreen (foliar application); T_3: Bioveer (soil + foliar application); T_4: Bioveer (foliar application); T_5: Biosona (soil + foliar application); T_6: Biosona (foliar application); T_7: Control (water spray).

Table 3. Effect of microbial bioformulations on micronutrient content and enzyme activity in citrus orchard soil.

Treatment	Mn (ppm)	Zn (ppm)	Fe (ppm)	B uptake (ppm)	Dehydrogenase Activity (*p* µg TPF g^{-1} soil 24 hr^{-1})	Phosphomonoesterase Activity (µg *p* nitrophenol g^{-1}soil hr^{-1})	Fluorescein Di-acetate Activity (µg fluorescein $g^{-1}hr^{-1}$)
T_1: Biogreen (soil + foliar application)	4.09	1.57	7.59	0.68	180.82	341.02	8.03
T_2: Biogreen (foliar application)	3.90	1.36	6.91	0.58	175.82	323.24	7.57
T_3: Bioveer (soil + foliar application)	2.84	1.27	6.74	0.41	169.00	275.44	6.09
$T_{4:}$ Bioveer (foliar application)	2.46	1.23	5.90	0.41	174.12	322.29	7.51
T_5: Biosona (soil + foliar application)	2.25	1.15	5.77	0.50	160.29	269.69	5.26
$T_{6:}$ Biosona (foliar application)	2.15	1.03	5.42	0.38	157.30	249.06	5.88
T_7: Control (water spray)	1.93	0.93	5.035	0.33	138.52	238.00	5.13
S. Ed.(±)	0.050	0.024	0.162	0.050	2.35	4.91	0.17
CD ($_{P=0.05}$)	0.070	0.047	0.310	0.064	4.80	10.06	0.34

comparison to the control. Application of microbial inoculants could increase the microbial population dynamics in rhizosphere, resulting in increased MBC up to 39.58% in T_1: Biogreen (soil + foliar application) followed by 34.87% in T_2: Biogreen (foliar application) as compared to control (Fig. 3) and thus, can be considered as a non-chemical and clear alternative for improving soil health and quality parameters. Moraes *et al.* (13) reported that the application of *Pseudomonas* sp. and *Trichoderma aureoviride*, individually or in combination exerted superior and beneficial effects in improving microbial population dynamics and functional attributes, thus favouring soil biomass carbon of lettuce plants. They also observed positive consequences in

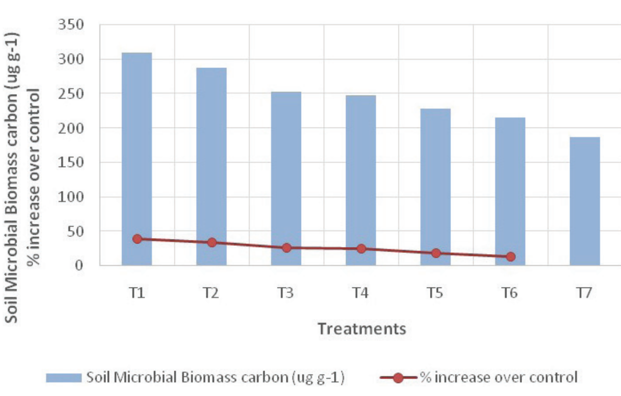

Fig. 3. Effect of bioformulations on soil microbial biomass carbon and its per cent increase over control (T_1: Biogreen (soil + foliar application); T_2: Biogreen (foliar application); T_3: Bioveer (soil + foliar application); $T_{4:}$ Bioveer (foliar application); T_5: Biosona (soil + foliar application); $T_{6:}$ Biosona (foliar application); T_7: Control (water spray).

applying *Pseudomonas* as microbial inoculants in agriculture as the microbe can effectively enhance the biochemical quality of soil through the production of specific enzymes in addition to improving the quality of crop growth and production. PGPMs such as *Pseudomonas* spp. possess many beneficial traits that make them suitable candidates in plant growth promotion and disease protection. Arif *et al.* (2) found that the activity of dehydrogenase was significantly higher in soyabean soils treated with phosphorous enriched compost (PEC) and *B. cereus* GS6. The researchers obtained that PEC in combination with *B. cereus* GS6 could induce phosphomonoesterase activity up to 43.18 ± 1.03 µg p-nitrophenol g^{-1} soil/hr which was significantly higher as compared to the uninoculated control.

The findings of the current investigation concluded that citrus plants treated with microbial bioformulations could effectively increase the physicochemical properties, nutrient status and biological properties of soil. The microbiological transformation of the citrus rhizosphere is apparent through alterations in both soil microbial population numbers and microbial biomass carbon. Simultaneously, there are chemical changes evident in the form of modifications in extractable available nutrients. Together, these transformations enhance the development of diverse rhizosphere properties and hold substantial potential for the development of microbial consortia to enhance rhizosphere quality and mitigate the depletion of nutrients.

AUTHORS' CONTRIBUTION

Conceptualization of the original idea (BCN, SS); experiment designing (BCN, SS); field/ lab experiments operation and data collection (SS, BCN); statistical analysis and interpretation (SS, BCN, PNB & BG); manuscript preparation (SS, BCN, PNB & BG).

DECLARATION

The authors don't have any conflict of interests/ competing interests.

ACKNOWLEDGMENTS

The corresponding author is thankful to the Director of Research (Agri.), and the Director of Post Graduate Studies, Assam Agricultural University, Jorhat, Assam, India, for providing all necessary facilities and support for the successful completion of the study. The authors are also indebted to the Department of Horticulture, Department of Soil Science and the Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University for providing necessary facilities and logistics to conduct lab and field experimentations and data collection.

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(Received : November, 2024; Revised : May, 2025;
Accepted : June, 2025