# Unlocking the potential plant growth-promoting properties of chickpea (Cicer arietinum L.) seed endophytes bio-inoculants for improving the soil health and crop production

Arpan Mukherjee[1], Anand Gaurav[1], Amit Patel[1], Saurabh Singh[1], Gowardhan Chouhan[1], Arthur Pereira[2], and JAY PRAKASH VERMA[3]

[1]Banaras Hindu University Institute of Environment & Sustainable Development
[2]Federal University of Ceara
[3]Institute of Environment and Sustainable Development

March 8, 2021

**Abstract**

Sustainable agronomic practices are tried all over the world to promote safe and eco-friendly crop production. Therefore, in the present study, the effect of seed endophytic bacteria and its consortia on soil biochemical property, soil nutrient, and yield of chickpea (Cicer arietinum L.) under field and pot conditions are investigated. Both the experimental results proved a significant increase in total soil organic carbon (OC), electric conductivity (EC), organic matter (OM), soil nutrient like available N, P and K content and important soil enzymes like dehydrogenase (DHA), beta glucosidase, alkaline phosphate, and urease was observed under the Enterobacter hormaechei BHUJPCS-15 (T1), Enterobacter cloacae BHUJPCS-21 (T2) and combined T3 (consortia of T1 and T2) treatments. Similarly, a significant increase in the grain yield (27-45% and 57-73%) in microbial treatment was found in pot and field experiments, respectively than control. In addition, whereas the higher plant biomass (14-38% and 42-78%) was recorded in the treated plant over the control plant. Similarly, the plant photosynthetic pigment (Chl a, b, total Chl) were also increased in the microbial treated plant than the control untreated chickpea plant. Our present study highlights the significance of sustainable agronomic practices for improving the soil quality and agricultural yield while reducing adverse impacts of chemicals by the use of seed endophytic microbes and their consortia.

Unlocking the potential plant growth-promoting properties of chickpea (*Cicer arietinum* L.) seed endophytes bio-inoculants for improving the soil health and crop production

Arpan Mukherjee[1], Anand Kumar Gaurav[1], Amit Kumar Patel[1], Saurabh Singh[1], Gowardhan Kumar Chouhan[1], Arthur Prudêncio de A. Pereira[2], Jay Prakash Verma[1*]

[1]Institute of Environment and Sustainable Development, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India

[2]Soil Science Department, Soil Microbiology Laboratory, Federal University of Ceará, Fortaleza, Ceará, Brazil

[*]Corresponding Email address: verma_bhu@yahoo.co.in, jpv.iesd@bhu.ac.in, Mobile No. +91-9452762725 (India)

**Abstract**

Sustainable agronomic practices are tried all over the world to promote safe and eco-friendly crop production. Therefore, in the present study, the effect of seed endophytic bacteria and its consortia on soil biochemical property, soil nutrient and yield of chickpea (*Cicer arietinum* L.) under field and pot conditions are investigated. Both the experimental results proved a significant increase in total soil organic carbon (OC), electric conductivity (EC), organic matter (OM), soil nutrient like available N, P and K content and important soil enzymes like dehydrogenase (DHA), beta glucosidase, alkaline phosphate and urease was observed under the *Enterobacter hormaechei* BHUJPCS-15 (T1), *Enterobacter cloacae* BHUJPCS-21 (T2) and combined T3 (consortia of T1 and T2) treatments. Similarly, a significant increase in the grain yield (27-45% and 57-73%) in microbial treatment was found in pot and field experiment, respectively than control. In addition, whereas the higher plant biomass (14-38% and 42-78%) was recorded in treated plant over the control plant. Similarly, the plant photosynthetic pigment (Chl a, b, total Chl) were also increased in microbial treated plant than the control untreated chickpea plant. Our present study highlights the significance of sustainable agronomic practices for improving the soil quality and agricultural yield while reducing adverse impacts of chemicals by the use of seed endophytic microbes and its consortia.

**Key words**

Seed Endophytes, Chickpea, Plant Growth Promoting Microbes (PGPM), Soil Health and Enzyme, Soil Nutrient, Microbial Consortia

## Introduction

Recently, the main problem around the world agriculture is the loss of soil quality and fertility. Due to loss of organic matter (OM) and decomposition of different chemicals to the agricultural soil and productivity of agricultural crops reduces. Currently, other major problems are random industrialisation, urbanization and cutting of natural forest. Additionally, the poor agro-waste management practices increase the greenhouse gas emissions, random chemical use directed to loss of soil quality and fertility along with the loss of soil biodiversity (Abhilash et al., 2016). Furthermore, the overgrowth of population around the world required more quality food in limited fertile soils. For achieving this target to feed the human being needs change in current agricultural process and practices around the world which highly dependents on the chemical fertilizers and chemical pesticides **(** Bhardwaj et al., 2014**).** The uses of chemical directly affect human health and global environment as well as soil fertility (Mukherjee et al., 2020a). Therefore, priority should be given to the use of plant growth promoting microorganisms (PGPM) as biofertilizers both for the food security and sustainable crop production by improving soil health and fertility as an eco-friendly approach to conserve degraded land. Application of the organic manures along with the inorganic fertilizers help to increase the soil organic matter, soil structure, improved soil nutrient, helps to maintain the soil cation exchange capacity and soil biological activity and diversity, and restoration of soil (Dubey et al., 2020; Singh et al., 2020; Saha et al., 2008). Though chemicals fertilizers and pesticides are important input for agricultural sector to get higher yield, but unbalance and excess application of these agrochemical affects the soil properties, crop productivity and causes a serious health issue, land problems such as soil degradation (Hepperly et al., 2009).

Therefore, an integrated application of biofertilizer as microbial inoculum in the agriculture field to minimise the agrochemical use under sustainable agricultural practices must be needed. Application of PGPM either with seed treatment, soil application, and foliar use help to reduce the pathogenic infection as well as induce plant growth promoting activities (Mukherjee et al., 2020b). Till date a lot of microbial inoculants are available in the market and the number of commercial microbial agent's increased spontaneously as different studies have been conducted to verify their effectiveness (Berg, 2009). Among all the microbial cultures, the plant growth-promoting microbes (PGPM) are well studied and known to promote the soil health development, induce available soil nutrient and help in plant disease suppression (Mukherjee et al., 2020c and 2019; Verma et al., 2014).

The application of seed endophytic bacteria in sustainable agriculture is a burning approach but very limited studies are conducted on it (Mukherjee et al., 2020b). Harnessing seed microbiome is considered as a viable emerging approach to sustainably increase agriculture productivity, soil sustainability and land development. While research studies showed that the use of bio-inoculants didn't show good results in field conditions due to lack of indigenous strains and different agro-climatic zone. Recently, it was proposed that if the bio-inoculants were developed either from plant endophytes, core microbiota or with the crop microbiome, the result showed significant outcomes with the application of bioinoculants, (Mukherjee et al., 2020b; Qiu et al., 2019). However, most of the evidence for seed microbiome came from the molecular approaches and very few extensive field level works have been done with the isolated seed microbes. The main functional role of these seed endophytic microbiota is very poorly known. Currently, such types of evidence are very less and needs systematic evaluation of the seed microbes to support this argument. In order to manage all the agricultural resources along with the improving soil health and quality, yield and stability of agri-products, the present research study was conducted on chickpea (*Cicer arietinum* L.) at pot and field condition in Varanasi district of Uttar Pradesh, India.

This study was conducted with common market available chickpea (variety P-362) which is a very important daily consumable protein, nutrient rich pulse crop worldwide. In India, it use as a multipurpose crop for human consumption as well as fodder (Yadav et al., 2017). Therefore the sustainable management of chickpea crop in India is very essential to attain the target of environmental sustainability. In this present study we use chickpea seed endophytes for improving the crop production and soil quality. Our study tried to investigate the potential role of chickpea seed endophytes on (1) *Chickpea (C. arietinum* L.) crop growth, yield, (2) the soil health and nutrients (both physicochemical, biological properties), (3) the relation between the crop production and soil fertility.

## 2. Materials and methods

### 2.1 Microbial consortia development for pot and field experiments

Isolation of chickpea seed endophytic bacteria and their biochemical, molecular characterization and primary plant growth promoting test was done previously (Mukherjee et al., 2020b). Depending on the all plant growth promoting property we have selected two strains and prepared their consortia depending on the compatibility. We have checked all plant growth promoting biochemical property of microbial consortia by the methods followed by Mukherjee et al. (2020b).

### 2.2 Experimental layout

Research was carried out (2019–2020) in two different area i) Experimental field of Institute of Environment and Sustainable Development (IESD), Banaras Hindu University, Varanasi (25°15´44´´ N; 82°59´41´´E) and (ii) Ratanpur, Babatpur (25°26´38" N; 82°48´ 14" E), the season was winter with the temperature ranging from 7°C - 25°C and the relative humidity was 67–84% in Uttar Pradesh, India. The experiment was conducted in pot and field respectively with triplicate.

### 2.3 Cropping system

This study was conducted with the use of common variety in local market chickpea seed P-362. The pot contained 2kg of sterilised soil, and the field study was conducted on 6ft × 4ft plot in triplicates, the spacing of plantation was done as recommended in local standard agricultural practices. The control (T0) plots contained seeds without microbial inoculant where treatment plot (T1, T2 and T3) contained chickpea seed endophytic bacteria treated seeds (*Enterobacter hormaechei*BHUJPCS-15 (T1), *Enterobacter cloacae* BHUJPCS-21 (T2), combined T3 is consortia of T1 and T2 microbes), each plot contained equal number of germinated chickpea seeds.

### 2.4 Soil sampling and analysis

#### 2.4.1. Soil physico-chemical properties, soil essential nutrients and soil enzyme study

Five soil sub-samples were collected from chickpea plant rhizosphere area of each plot of the experiment field and mixed together to form a composite soil and it considered as one sample from each replicated plot of field. After collecting the soil immediate put in the sterilised plastic bag in ice box. The pot soils were collected in same process only single pot soil collected as single replication. Soil physicochemical property like pH (Jackson, 1958), electrical conductance (EC) (Chopra and Kanwar, 1982), organic carbon (OC) (Walkley and Black, 1934); organic matter (OM) were measured. The soil essential nutrient like available N (Subbiah and Asija, 1956); available P (Olsen et al., 1954) and available K (Hanway and Heidel 1952), important soil enzyme activity like dehydrogenase (Casida et al. 1964); urease (Kandeler and Gerber 1988); alkaline phosphatase (Eiazi and Tabatabai 1977) and Beta-glucosidase (Eivazi and Tabatabai, 1988), were measured from the soil sample that were collected from the experimental field and pot during flowering time.

### 2.5 Plant growth and yield for sustainable agriculture

Plant growth-related data from each treatment plot were recorded at the regular time interval. For plant growth measurement, 10 randomly selected plants from each treatment and control plot were selected (from 15 days after sowing) and aboveground shoot, branching, number of flower parameter were observed and recorded. After the grain yield the matured plants were cut just above the root collar the shoot length, fresh and oven dried shoot weight per plant and per plot were recorded. For grain yield, total mature plants in each plot were cut and harvested and grains separated from plant to measure the weight. Total cleaned chickpea seeds were measured for seed yield/plot and, and take 10 plants grain that were previously selected to measure the yield/plant. Same procedure followed for pot experiments for grain and plant growth measurements.

### 2.6 Statistical analysis

All experiment were conducted in triplicates, and the results were prepared as the mean± standard deviation (SD) of different independent replicates by using Analysis of variance (ANOVA) followed by the Duncan post hoc tests (SPSS software version 16.0). The values of P [?] 0.05 were considered as statistically significant.

## 3. Result

### 3.1. Response of endophytic microbial inoculum on soil physico-chemical properties

From the previous experimental result and compatibility test, we have selected two seed endophytic bacteria for preparing the consortia (T3). These are *Enterobacter hormaechei* BHUJPCS-15 (Accession number MN078052) (T1) and *Enterobacter cloacae* BHUJPCS-21 (Accession number MN078055) (T2). We have found that microbial consortia have the ability to produce IAA (61.04 µg/ml), ammonia (105.45 µg/ml), siderophore (18.06) and solubilize essential nutrient like phosphate (871.14 µg/ml) and potassium, and showed antagonistic activity against chickpea pathogen *Fusarium* sp. *in-vitro* condition**(Table. 1)** . After showing positive result in PGP biochemical activity we set an experiment for chickpea growth promotion with the use of these two seed endophytes and its consortia. Experiment was done in pot and field condition to check microbial effect on soil health and yield **(Fig. 1).**

The physiochemical property of experimental soil was checked and recorded, the pH of pot and field soil were alkaline and the range varied from 7.09 to 8.08 and 8.01 to 8.52 respectively, there was no significant change in pH of pot and field trial. Other physiochemical property such as EC ranged from 15.04 to 19.12 (us/cm) and 25.00 to 28.14 (us/cm) in pot and field soil respectively, and the significant change was showed in all three treatment Control (T0)>*E cloacae* BHUJPCS-21 (T2)> *E hormaechei*BHUJPCS-15 (T1)> Consortia (T3) in compare with untreated control both in pot and field in same manner **(Table: 2).**Organic matter (OM) and organic carbon (OC) also recorded and the rage was varied from 1.86 to 2.18 and 1.07 to1.32 respectively in pot soil and the significant increase in % OM and % OC was observed in Control > *E cloacae* BHUJPCS-21 > *E hormaechei* BHUJPCS-15 > Consortia order, and % OM and % OC was recorded 1.28 to 1.87 and 25.00 to 28.14 in experimental field in Control> *E hormaechei* BHUJPCS-15>*E cloacae* BHUJPCS-21> Consortia and Control> *E cloacae* BHUJPCS-21> *E hormaechei* BHUJPCS-15> Consortia respectively

**(Table: 2).**

Soil essential nutrient such as available N, P and K were significantly increased in microbial seed treated pot and field soil in comparison with the untreated control soil. In case of the pot soil the amount of available nitrogen (AN) varied from 38.12 kg/ha to 49.47 kg/ha, and all the treatments showed significant increase compared with untreated control pot, where the consortia treatment showed maximum amount of AN (49.47 kg/ha) followed by *E. cloacae* BHUJPCS-21 (44.74 kg/ha),*E. hormaechei* BHUJPCS-15 (42.17 kg/ha) and control (38.12 kg/ha). Available phosphate (AP) was significantly increased in consortia (25.41 kg/ha) followed by the *E. hormaechei* BHUJPCS-15 (22.14 kg/ha) and*E. cloacae* BHUJPCS-21 (20.47 kg/ha) in compare with untreated control (19.98 kg/ha) soil. Available potassium (AK) also significantly increased in consortia (43.01 kg/ha) than *E. cloacae*BHUJPCS-21(41.84 kg/ha) and *E. hormaechei* BHUJPCS-15 (36.14 kg/ha) in comparison with control (30.35 kg/ha) soil. On the other hand the available N, P, K of the experimental field also recorded and observed that the all treatments showed significant increase in the AN observed in consortia (49.47 kg/ha) followed by *E. cloacae*BHUJPCS-21(44.74 kg/ha) and *E. hormaechei* BHUJPCS-15 (42.17 kg/ha) in comparison with the untreated control (38.12 kg/ha) site. Consortia treated plot recorded highest amount of AP (25.41 kg/ha) followed by *E. hormaechei* BHUJPCS-15 (22.14 kg/ha), *E. cloacae* BHUJPCS-21 (20.47 kg/ha) and control (19.98 kg/ha), all three treated site showed significant increase in AP in compare with untreated control plot. Available K (AK) observation showed that the maximum AK was found in consortia (43.01 kg/ha), *E. cloacae* BHUJPCS-21 (41.84 kg/ha), *E. hormaechei* BHUJPCS-15 (30.35 kg/ha) and all three treatments were significantly increased than control plot

**(Figure. 2 and 3).**

A significant increase in soil essential enzymes were observed in all three treatment both in pot and field experiment. We observed that highest DHA, urease, alkaline phosphatase, and β-glucosidase enzymes activity was observed in consortia (71%, 64%, 37% and 72%) followed by *E. cloacae* BHUJPCS-21 (61%, 51%, 31%, and 62%) and*E. hormaechei* BHUJPCS-15 (50%, 24%, 28%, and 52%) than control treatments in pot trial. Similar types of results were observed in field soil during field soil enzymes activity study, all four soil enzyme activity increased in consortia (64%, 82%, 29%, 78%)> *E. cloacae* BHUJPCS-21 (36%, 34%, 23%, 39%)> *E. hormaechei* BHUJPCS-15 (29%, 55%, 22%, 54%) than the untreated control plot. Conclusively, our field and pot study revealed the impact of seed endophytic bacterial culture to the adaptive agro-practices on several soil biochemical characteristics and puts forward the relative valuable and relationships between microbial consortia with the soil parameters that responded significantly to increase soil fertility and soil health **(Fig. 4, 5).**

From this study, maximum plant length after one month of germination was recorded as Consortia> *E. cloacae*BHUJPCS-21> *E. hormaechei* BHUJPCS-15> control, and the data showed that length increased was maximum both in pot and field experiment in consortia (35% and 28%) followed by*E. cloacae* BHUJPCS-21 (19% and 8%) and *E. hormaechei*BHUJPCS-15 (17% and 25%) in compare with the control plant. Where the average plant dry weight was increase in consortia (36% and 66%) followed by *E. cloacae* BHUJPCS-21 (14% and 62%) and *E. hormaechei* BHUJPCS-15 (10% and 45%). The plant dry weight per plot was increased in same manner both in the pot and field i.e. consortia> *E. cloacae* BHUJPCS-21>*E. hormaechei* BHUJPCS-15> control. Maximum increase in yield was recorded and found that average seed weight/ plant was in consortia (52% and 54%) followed by *E. hormaechei* BHUJPCS-15 (35% and 38%), *E. cloacae* BHUJPCS-21 (30% and 28%), and the seed weight/ plot was significantly increased in consortia (45% and 73%) > *E. hormaechei* BHUJPCS-15 (27% and 57%)> *E. cloacae* BHUJPCS-21 (24% and 42%) than control plant respectively both in pot and field. Pigment of plant such as chlorophyll a, b and total chlorophyll was also increased in consortia> *E. cloacae* BHUJPCS-21>*E. hormaechei* BHUJPCS-15> control both in pot and field (**Fig. 6 to 9).**

## 4. Discussion

Till now most studies have focussed on the PGPR on soil and plant performance **(Berg and Zachow, 2011).** As far as we know, there have been no such studies in the field of the chickpea seed endophytic bacterial interactive effects on the soil health as well as plant productivity. Our previous research studies showed the effect of chickpea seed bacterial endophytes on plant defence and plant growth in lab condition (Mukherjee et al., 2020b). The results of our present study revealed the effects of endophytes both on the plant growth and yield as well as in soil nutrients and health (Table. 2; Fig. 1 to 5). Among all the soil chemical property, soil pH did not show any significant change in our experiment both in pot and field condition, as the soil are slightly alkaline and didn't show any significant effect on the yield. But other biochemical properties of soils like EC, OC and OM were significantly increased in all three treatments in consortia> *E. cloacae* BHUJPCS-21>*E. hormaechei* BHUJPCS-15> control order. This result indicates that the microbial consortia along with the microbe help to induce the soil quality through the change of EC, OC and OM, as these are important indicators of soil health and crop productivity. Where the electrical conductivity (EC) is an important physicochemical property directly linked with the concentration of soil ions, from the experimental data we can hypothesize that our microbial strains have the potential property to improve the EC of soil. OM is also an important factor for soil health and plant productivity and it helps to improve the soil water holding capacity, serve as raw source of soil and plant nutrients. Data also indicates that the seed endophytes application increase the nutrient availability (Available N, P and K) in experimental soil both in the pot and field condition. Soil nutrient is an essential property for the crop production. Thus N, P, K are also very crucial elements for several enzymes, proteins, hormones, amino acids and building block of genetic materials both in plants and microbes **(** Maathuis 2009; Krouk et al., 2010, Mukherjee et al., 2019**).** During our experiment we observed that the consortia treatment showed highly significant response followed by *E. cloacae* BHUJPCS-21 and *E. hormaechei* BHUJPCS-15 under both pot and field conditions. Similar response was supported by Raklami et al., (2019) during soil analyses of microbial consortia treated experiment. We have observed a linear correlation with the increased plant dry weight and length, seeds production, pigments synthesis in microbial consortia treatments with increase in available soil N, P, K content. We have also observed increase in essential soil enzyme like alkaline phosphatase, urease, beta glycosidase and dehydrogenase (DHA) upon application of seed endophytic microbes and its consortia. The soil enzymes are known to be a function of microbial activity and our results clearly showed increase in soil enzyme that were directly linked that our microbial culture have some effect on the soil enzyme activity. Same result was observed by Guo et al., (2019) and showed that the application of microbes in soil enzymes in can increase the soil enzymes activity than the untreated soil. Increase in the plant health in terms of growth, dry weight, height and yield supported that the endophytic bacterial consortia helps to increase the crop health and productivity (Yadav et al., 2017). Increase in the crops' productivity and soil health in the terms of essential enzymes and nutrients (Available N, P and K) was due to the fact that microbes are known to be functional as community in the soil ecology. It explains that high number of different property containing strains in microbial consortia led to the better alternative in terms of plant biomass, shoot length and crop productivity. To best of our knowledge this is the first-time report of chickpea seed endophytic microbial consortia for improving the soil health and increasing the crop productivity. Similar types of studies were done in tomato plant by the use of microbial consortia by Akintokun et al., (2016). Malik and Sindhu (2011) used another microbial consortium (*Pseudomonas* sp. and *Mesorhizobium* sp.) to increase the plant growth. Several different research works have been reported on the application of double microbial consortia in different plants to check the growth and yield of different plants such as potato (*B. cereus* , *B. subtilis* , *Azotobacter* ), brinjal (*Azospirillum* , *Azotobacter* ) and Radish (*B. subtilis* and *P. fluorescens* ) (Singh et al., 2013; Sood and Sharma2001; Patel et al., 2011; Mohamed and Gomaa 2012).

Increase in productivity is further supported by our finding of higher level of photosynthetic pigments such as chlorophyll a, b and total chlorophyll in the consortia treated test plants as compared with control. In the consortia treatments the chlorophyll contains in terms of chl a, chl b, total chlorophyll in chickpea both in pot trial and field trial significantly increased than the untreated control plants followed by other treatments. Eleiwa et al. (2012) showed similar results in wheat plant in field trial on the application of *B. polymyx* and*A.brasilinseas* which dramatically increase the amount of chlorophyll a, b and carotenoids in treated plants. The photosynthetic activity is directly proportional to the enhanced productivity (Mbarki et al., 2018), the pigments help to accumulate more energy and also helps in higher photosynthate assimilation in plants.

Some other previous reports have suggested the effect of PGPR using single-strain inoculations (Lucy et al., 2004), but the microbial consortia also showed more beneficial effects than single strain (Ryu et al., 2007). Seed endophytic microbial consortium possibly mimics the natural soil environmental conditions where the important soil microbial community can leave. Hence, in the present study enhanced soil nutrient, other physiochemical content and growth and yield of chickpea plant in the endophytic microbial consortium treatment could be an attribute to the natural and synergistic environmental effects of the two-chickpea seed endophytic bacteria.

## 5. Conclusions

The seed endophytic bacterial strains (*E. hormaechei* BHUJPCS-15 and *E. cloacae* BHUJPCS-21) and its consortia have the potentiality to improve the soil health and yield of chickpea crops. This research work is a preliminary study on chickpea seed endophytes as plant growth promoting agent in pot and field conditions. Overall, we have provided an empirical evidence for the presence of chickpea seed endophytic microbiome and demonstrated that these microbes and its consortia have a number of plants growth and soil health and fertility inducing traits and it support the theory of the co-evolution of host and its core microbiota. These two isolates were seed endophytes and upon re-introduction with its host seed enhanced its growth and soil health to helpful for soil conservation by re-charging of this indigenous strain. This microbial seed endophytic consortium is an environment friendly, economically viable and socially acceptable that means complete fulfil the sustainable development goal. This current research study advocates that the use of seed endophytic microbial consortia to improve nutrient content in soil, which also enhances plant health and yield. We demonstrated that a useful seed endophytic microbial consortium can increase the physiochemical property, nutritive value of soil rhizosphere of plants whose intake or consumption could be an effective way to improve the plant yield.

## Author contributions

This research practical work, data collection, data analysis and manuscript writing were done by AM. AKG, AKP and GKC help in data collection, SS helped in data analysis. Experiment designed and final editing of the whole manuscript was done by JPV.

## Ethical approval

This article does not contain any studies with animals performed by any of the authors.

## Conflict of interest

Authors do not have any conflict of interest.

## Acknowledgments

Authors want to thanks Head of the Department, Institute of Environment and Sustainable Development, Banaras Hindu University, AM especially grateful to Science and Engineering Research Board (Ref. File No. EEQ/2017/000775) Government of India, for financial assistance. We would like to thank Mr. Ram Bilas Patel (Ratanpur, Babatpur) for providing his agricultural land for our experiment.

## Reference

Abhilash, P. C., Dubey, R. K., Tripathi, V., Gupta, V. K., & Singh, H. B. (2016). Plant growth-promoting microorganisms for environmental sustainability. *Trends in Biotechnology* , 34(11), 847-850. https://doi.org/10.1016/j.tibtech.2016.05.005

Akintokun, A. K., & Taiwo, M. O. (2016). Biocontrol potentials of individual specie of rhizobacteria and their consortium against phytopathogenic *Fusarium oxysporum* and *Rhizoctonia solani* .*International Journal of Scientific Research in Environmental Sciences* , *4* (7), 0219-0227. doi.org/10.12983/ijsres-2016-p0219-0227

Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology* ,*84* (1), 11-18. doi.org/10.1007/s00253-009-2092-7

Berg, G., & Zachow, C. (2011). PGPR interplay with rhizosphere communities and effect on plant growth and health. In *Bacteria in Agrobiology: Crop Ecosystems* (pp. 97-109). Springer, Berlin, Heidelberg. doi.org/10.1007/978-3-642-18357-7_4

Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity.*Microbial Cell Factories* , *13* (1), 1-10. doi.org/10.1186/1475-2859-13-66

Casida Jr, L. E., Klein, D. A., & Santoro, T. (1964). Soil dehydrogenase activity. *Soil Science* , *98* (6), 371-376.

Chopra, S.L. and Kanwar, J.S. (1982). Analytical Agri-cultural Chemistry, Kalyani Publishers, NewDelhi.

Dubey, R. K., Dubey, P. K., Chaurasia, R., Singh, H. B., & Abhilash, P. C. (2020). Sustainable agronomic practices for enhancing the soil quality and yield of *Cicer arietinum* L. under diverse agroecosystems. *Journal of Environmental Management* , 262, 110284. https://doi.org/10.1016/j.jenvman.2020.110284

Eivazi, F., & Tabatabai, M. A. (1977). Phosphatases in soils.*Soil Biology and Biochemistry* , *9* (3), 167-172. doi.org/10.1016/0038-0717(77)90070-0

Eivazi, F., & Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry* , *20* (5), 601-606. doi.org/10.1016/0038-0717(88)90141-1

Eleiwa, M. E., Hamed, E. R., & Shehata, H. S. (2012). The role of biofertilizers and/or some micronutrients on wheat plant (*Triticum aestivum* L.) growth in newly reclaimed soil. *Journal of Medicinal Plants Research* , *6* (17), 3359-3369. doi.org/10.5897/JMPR12.216

Guo, J. H., Zhang, L., Wang, D., Hu, Q., Dai, X., Xie, Y., ... & Liu, H. (2019). Consortium of plant growth-promoting rhizobacteria strains suppresses sweet pepper disease by altering the rhizosphere microbiota.*Frontiers In Microbiology* , *10* , 1668. doi.org/10.3389/fmicb.2019.01668

Hanway JJ, Heidel H (1952) Soil analysis methods as used in Iowastate college soil testing laboratory. Iowa Agric 57:1–31

Hepperly, P., Lotter, D., Ulsh, C. Z., Seidel, R., & Reider, C. (2009). Compost, manure and synthetic fertilizer influences crop yields, soil properties, nitrate leaching and crop nutrient content. *Compost Science & Utilization* , *17* (2), 117-126. doi.org/10.1080/1065657X.2009.10702410

Jackson, M.L. (1958). Organic matter determination forsoils. In: Soil chemical analysis. Prentice-Hall,Englewood Cliffs, NJ, pp 205-226.

Kandeler, E., & Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils* , *6* (1), 68-72. doi.org/10.1007/BF00257924

Krouk, G., Crawford, N. M., Coruzzi, G. M., & Tsay, Y. F. (2010). Nitrate signaling: adaptation to fluctuating environments. *Current Opinion in Plant Biology* , *13* (3), 265-272. doi.org/10.1016/j.pbi.2009.12.003

Lucy, M., Reed, E., & Glick, B. R. (2004). Applications of free living plant growth-promoting rhizobacteria. *Antonie Van Leeuwenhoek* ,*86* (1), 1-25. doi.org/10.1023/B:ANTO.0000024903.10757.6e

Maathuis, F. J. (2009). Physiological functions of mineral macronutrients. *Current Opinion in Plant Biology* , *12* (3), 250-258. doi.org/10.1016/j.pbi.2009.04.003

Malik, D. K., & Sindhu, S. S. (2011). Production of indole acetic acid by Pseudomonas sp.: effect of coinoculation with Mesorhizobium sp. Cicer on nodulation and plant growth of chickpea (Cicer arietinum).*Physiology and Molecular Biology of Plants* , *17* (1), 25-32. doi.org/10.1007/s12298-010-0041-7

Mbarki, S., Sytar, O., Cerda, A., Zivcak, M., Rastogi, A., He, X., ... & Brestic, M. (2018). Strategies to mitigate the salt stress effects on photosynthetic apparatus and productivity of crop plants. In*Salinity Responses and Tolerance in Plants, Volume 1* (pp. 85-136). Springer, Cham. doi.org/10.1007/978-3-319-75671-4_4

Mohamed, H. I., & Gomaa, E. Z. (2012). Effect of plant growth promoting Bacillus subtilis and Pseudomonas fluorescens on growth and pigment composition of radish plants (Raphanus sativus) under NaCl stress.*Photosynthetica* , *50* (2), 263-272. doi.org/10.1007/s11099-012-0032-8

Mukherjee, A., & Patel, J. S. (2020a). Seaweed extract: biostimulator of plant defense and plant productivity. *International Journal of Environmental Science and Technology* , *17* (1), 553-558. doi.org/10.1007/s13762-019-02442-z

Mukherjee, A., Singh, B., & Verma, J. P. (2020b). Harnessing chickpea (*Cicer arietinum* L.) seed endophytes for enhancing plant growth attributes and bio-controlling against *Fusarium* sp.*Microbiological Research* , 126469. doi.org/10.1016/j.micres.2020.126469

Mukherjee, A., Chouhan, G. K., Gaurav, A. K., Jaiswal, D. K., & Verma, J. P. (2020c). Development of indigenous microbial consortium for biocontrol management. In: *New and Future Developments in Microbial Biotechnology and Bioengineering* -Phytomicrobiome for Sustainable Agriculture (Eds. Verma, J.P., Macdonald, C., Gupta, V.K., Podile, A.R.) (pp. 91-104). Elsevier. doi.org/10.1016/B978-0-444-64325-4.00009-2

Mukherjee, A., Gaurav, A. K., Singh, S., Chouhan, G. K., Kumar, A., & Das, S. (2019). Role of Potassium (K) Solubilising Microbes (KSM) in Growth and Induction of Resistance against Biotic and Abiotic Stress in Plant: A Book Review. Climate Change and Environmental Sustainability, 7(2), 212-214.

Olsen, S. R., Cole, C. V., Watanake, F. S., & Dean, C. A. (1954). Estimation of available P in soil by extraction with sodium bicarbonate: Circ US. *Dept Agric* , *939* .

Patel, B. N., Solanki, M. P., Patel, S. R., & Desai, J. R. (2011). Effect of bio-fertilizers growth, physiological parameters, yield and quality of brinjal cv. Surati Ravaiya. *Indian Journal of Horticulture* , *68* (3), 370-374.

Qiu, Z., Egidi, E., Liu, H., Kaur, S., & Singh, B. K. (2019). New frontiers in agriculture productivity: Optimised microbial inoculants and in situ microbiome engineering. *Biotechnology Advances* ,*37* (6), 107371. doi.org/10.1016/j.biotechadv.2019.03.010

Raklami, A., Bechtaoui, N., Tahiri, A. I., Anli, M., Meddich, A., & Oufdou, K. (2019). Use of rhizobacteria and mycorrhizae consortium in the open field as a strategy for improving crop nutrition, productivity and soil fertility. *Frontiers in Microbiology* , *10* , 1106. doi.org/10.3389/fmicb.2019.01106

Ryu, C. M., Murphy, J. F., Reddy, M. S., & Kloepper, J. W. (2007). A two-strain mixture of rhizobacteria elicits induction of systemic resistance against Pseudomonas syringae and Cucumber mosaic virus coupled to promotion of plant growth on Arabidopsis thaliana.*Journal of Microbiology and Biotechnology* , *17* (2), 280-286.

Saha, S., Prakash, V., Kundu, S., Kumar, N., & Mina, B. L. (2008). Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean–wheat system in NW Himalaya. *European Journal of Soil Biology* , *44* (3), 309-315. doi.org/10.1016/j.ejsobi.2008.02.004

Singh, S. P., Singh, H. B., & Singh, D. K. (2013). *Trichoderma harzianum* and *Pseudomonas* sp. mediated management of sclerotium rolfsii rot in tomato (*Lycopersicon esculentum* mill.). *Life sciences* , *8* (3), 801-804.

Singh, S., Jaiswal, D. K., Krishna, R., Mukherjee, A., & Verma, J. P. (2020). Restoration of degraded lands through bioenergy plantations.*Restoration Ecology* , *28* (2), 263-266. doi.org/10.1111/rec.13095

Sood, M. C., & Sharma, R. C. (2001). Value of growth promoting bacteria, vermicompost and Azotobacter on potato production in Shimla hills. *Journal of Indian Potato Association* , *28* (1), 52-53.

Subbiah, B., & Asija, G. L. (1956). Alkaline permanganate method of available nitrogen determination. *Current Science* , *25* , 259.

Verma, J. P., Yadav, J., Tiwari, K. N., & Jaiswal, D. K. (2014). Evaluation of plant growth promoting activities of microbial strains and their effect on growth and yield of chickpea (*Cicer arietinum* L.) in India.

*Soil Biology and Biochemistry* , *70* , 33-37. doi.org/10.1016/j.soilbio.2013.12.001

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* , *37* (1), 29-38.

Yadav, S. K., Singh, S., Singh, H. B., & Sarma, B. K. (2017). Compatible rhizosphere-competent microbial consortium adds value to the nutritional quality in edible parts of chickpea. *Journal of Agricultural and Food Chemistry* , *65* (30), 6122-6130. doi.org/10.1021/acs.jafc.7b01326

**List of figures:**

**Fig.1.** Pictures highlights the details of the experimental sites (Pot and field), test crop (Chickpea/ *Cicer arietinum* L. variety P-362) grown with the inoculation of chickpea seed endophytes, plants were grown on the field at Ratanpur (Babatpur) and on pot trial was conducted at experimental field of institute of environment and sustainable development, Banaras Hindu University district Varanasi, of Uttar Pradesh, India. **A-** Control (Without inoculation/ treatment), **B-** T1 (*Enterobacter hormaechei* BHUJPCS-15 treatment), **C-** T2 (*Enterobacter cloacae* BHUJPCS-21 treatment) and **D-** T3 contain consortia of T1 and T2 organism treatment.

**Fig. 2:** Measurement of available soil essential nutrients (N, P and K) in pot experiment of chickpea rhizosphere soil (Data are the mean value of different treatments, and the letter a-c showed the level of significant).

**Fig. 3:** Measurement of available soil essential nutrients (N, P and K) in field experiment of chickpea rhizosphere soil (Data are the mean value of different treatments, and the letter a-c showed the level of significant).

**Fig. 4:** Measurement the % of soil essential enzymes activity increased in pot experiment of chickpea rhizosphere soil then the untreated control.

**Fig. 5:** Measurement the % of soil essential enzymes activity increased in field experiment of chickpea rhizosphere soil then the untreated control.

**Fig. 6.** Diagrammatic presentation of the effect of seed endophytes and its consortia on plant growth and crop production in the pot trial.

**Fig: 7.** Diagrammatic presentation of the effect of seed endophytes and its consortia on plant growth and crop production in field trial.

**Fig: 8.** Measurement of the plant pigment (chlorophyll a, b and total chlorophyll) in the pot experiment.

**Fig: 9.** Measurement of the plant pigment (chlorophyll a, b and total chlorophyll) in the field experiment.

**Table 1:** Microbial plant growth promoting biochemical activity (T1 and T2 microbial biochemical test was conducted previously Mukherjee et al., (2020b); and T3 biochemical test for this experiment purpose.

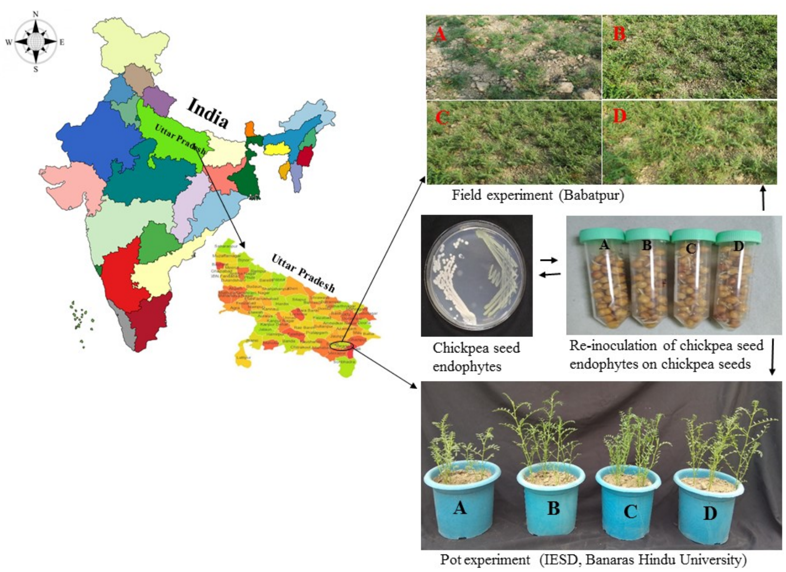
| Treatment | IAA (72 hrs) µg/ml | P- solubilisation (7 day) µg/ml | Protease activity | Siderophore (%) | Potassium solubilisation | Antagonistic effect against *Fusarium* sp. | Cellulase | Ammonia Production (72hrs) µg/ml | Ca act |
|---|---|---|---|---|---|---|---|---|---|
| T1 | 58.91 ±0.77[b] | 997.00±17.85[c] | - | 3.86±0.59[a] | ++ | - | - | 96.11±1.13[a] | + |
| T2 | 55.49 ±0.17[a] | 787.00±15.04[a] | | 16.01±0.3[b] | - | +++ | - | 100.39±0.75[b] | + |
| T3 (T1 + T2) | 61.04 ±0.87c | 871.14±10.77[b] | | 18.06±0.77[c] | ++ | +++ | - | 105.45±2.01[c] | + |

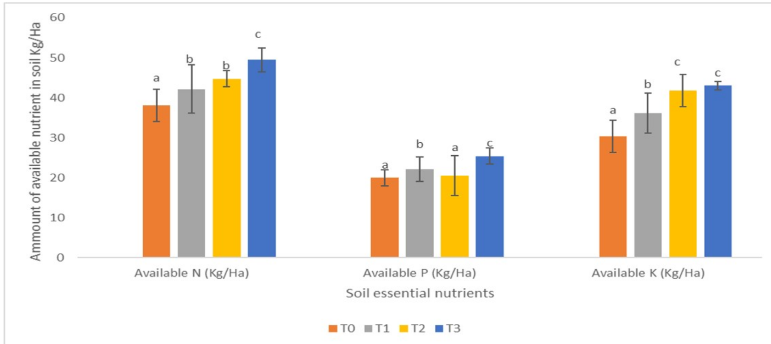
*Note T1 = *Enterobacter hormaechei* BHUJPCS-15(MN078052), T2=*Enterobacter cloacae* BHUJPCS-21 (NMN078055), and T3= consortia of T1 and T2 (Data Values are the mean ±SD, mean values in the each columns with the same superscript do not differ significantly by the Duncan multiple post hoc test ($P$ [?]0.05).

**Table 2:** Pot and field soil physiochemical property

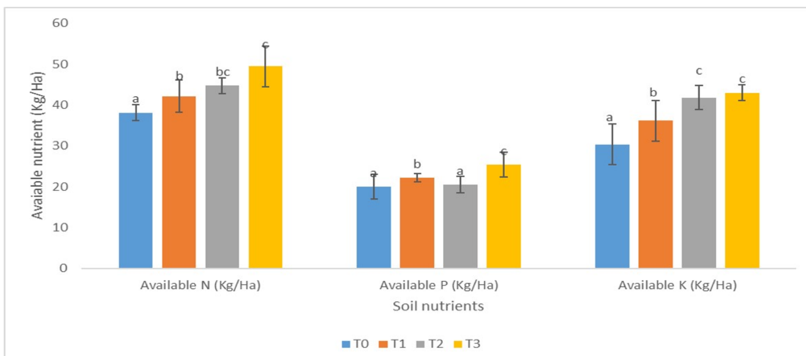
| Treatments **Pot trial** | **pH** | **EC (us/cm)** | **% OC** | **% OM** |
|---|---|---|---|---|
| T0 | 8.05 ±0.20$^{a}$ | 15.04 ±0.51$^{a}$ | 1.07 ±0.74 $^{a}$ | 1.86 ±0.21$^{a}$ |
| T1 | 7.90 ±0.14$^{a}$ | 18.14 ±0.40$^{bc}$ | 1.26 ±0.35 $^{b}$ | 2.18 ±0.34 $^{b}$ |
| T2 | 8.08 ±0.54$^{a}$ | 17.41 ±0.04$^{b}$ | 1.11 ±0.12 $^{a}$ | 1.93 ±0.14$^{a}$ |
| T3 | 8.01 ±0.71$^{a}$ | 19.12 ±0.14$^{c}$ | 1.32 ±0.22$^{b}$ | 2.10 ±0.35$^{b}$ |
| **Field trial** | **Field trial** | **Field trial** | **Field trial** | **Field trial** |
| T0 | 8.52 ±0.40$^{a}$ | 25.00 ±0.20$^{a}$ | 0.74±0.01$^{a}$ | 1.28±0.01$^{a}$ |
| T1 | 8.01 ±0.20$^{a}$ | 27.41±1.20$^{bc}$ | 0.85±0.02$^{b}$ | 1.48±0.20$^{b}$ |
| T2 | 8.11 ±0.10$^{a}$ | 26.01±0.90$^{b}$ | 0.78±0.01$^{a}$ | 1.35±0.04$^{b}$ |
| T3 | 8.02 ±0.20$^{a}$ | 28.14±0.80$^{c}$ | 0.94±0.01$^{c}$ | 1.87±0.06$^{c}$ |

# Note EC= electrical conductivity, OC= Organic carbon; OM= Organic matter, T0 – Control, T1 - *Enterobacter hormaechei* BHUJPCS-15, T2- *Enterobacter cloacae* BHUJPCS-21, T3 - consortia of T1 and T2. Data Values are the mean ±SD, mean values in the each columns with the same superscript do not differ significantly by the Duncan multiple post hoc test ($P$ [?]0.05).

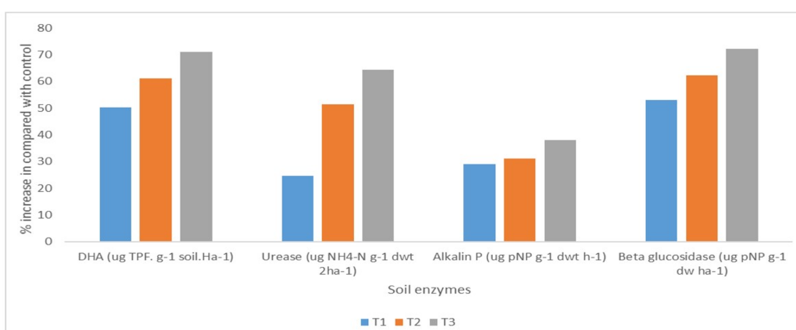




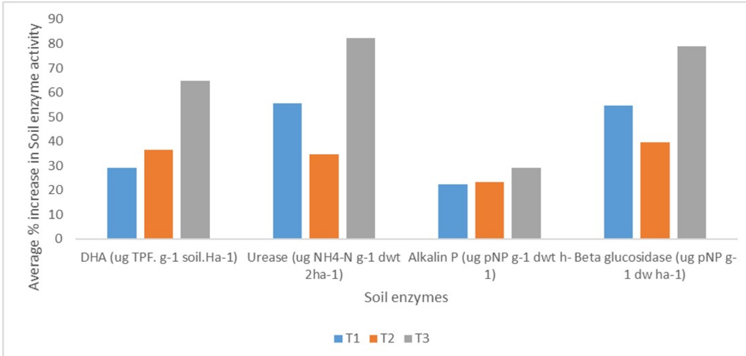

*Note T0= Control (without inoculation), T1 = *Enterobacter hormaechei* BHUJPCS-15, T2= *Enterobacter cloacae* BHUJPCS-21, and T3= consortia (T1 + T2)
(Data Values are the mean ±SD, mean values in the each columns with the same superscript do not differ significantly by the Duncan multiple post hoc test (P≤0.05).



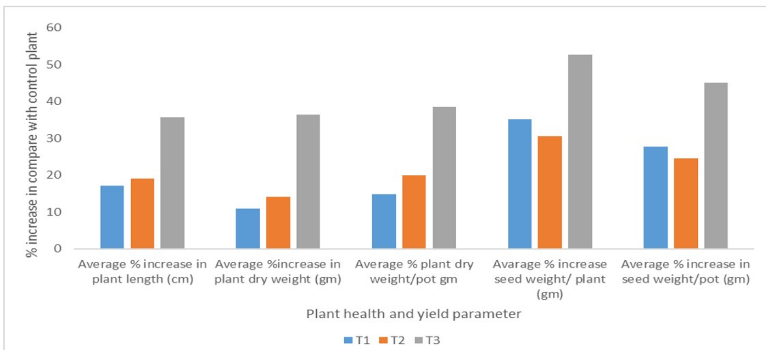

*Note T0= Control (without inoculation), T1 = *Enterobacter hormaechei* BHUJPCS-15, T2= *Enterobacter cloacae* BHUJPCS-21, and T3= consortia (T1 + T2).
(Data Values are the mean ±SD, mean values in the each columns with the same superscript do not differ significantly by the Duncan multiple post hoc test (P≤0.05).



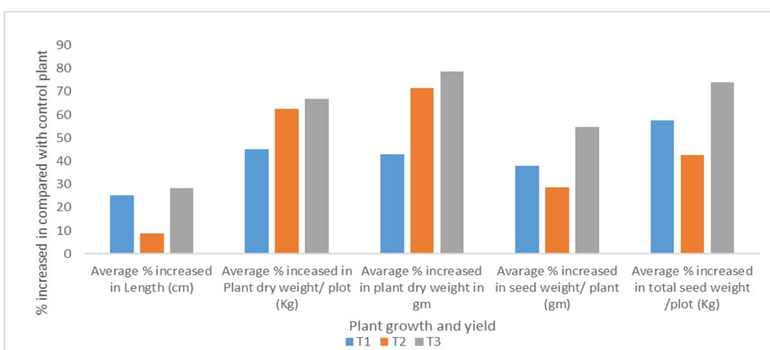

*Note T0 = Control (without inoculation), T1 = *Enterobacter hormaechei* BHUJPCS-15, T2= *Enterobacter cloacae* BHUJPCS-21, and T3= Consortia (T1 + T2)



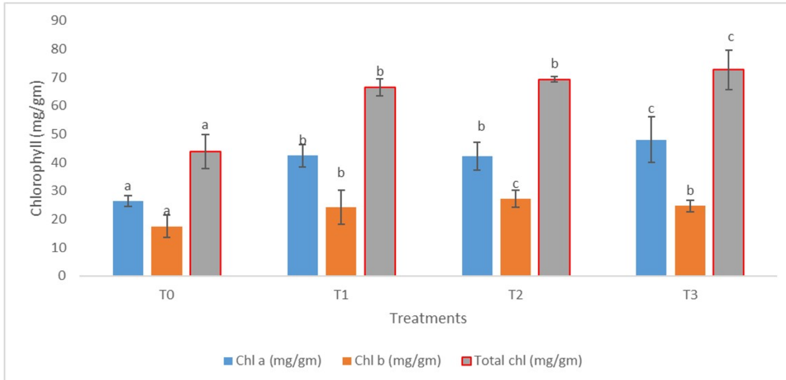

*Note T0 = Control (without inoculation), T1 = *Enterobacter hormaechei* BHUJPCS-15, T2= *Enterobacter cloacae* BHUJPCS-21, and T3= Consortia (T1 + T2)



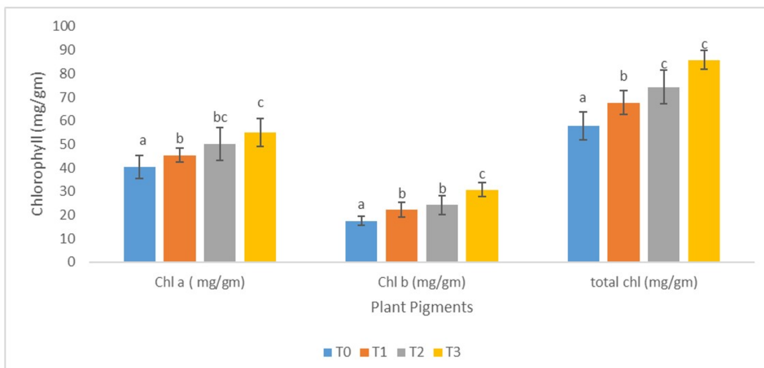

*Note, T1 = *Enterobacter hormaechei* BHUJPCS-15, T2= *Enterobacter cloacae* BHUJPCS-21, and T3= Consortia (T1 + T2)




*Note, T1 = *Enterobacter hormaechei* BHUJPCS-15, T2= *Enterobacter cloacae* BHUJPCS-21, and T3= Consortia (T1 + T2)




*Note T0= Control (without inoculation), T1 = *Enterobacter hormaechei* BHUJPCS-15, T2= *Enterobacter cloacae* BHUJPCS-21, and T3= consortia (T1 + T2).
(Data Values are the mean ±SD, mean values in the each columns with the same superscript do not differ significantly by the Duncan multiple post hoc test (P≤0.05).




*Note T0= Control (without inoculation), T1 = *Enterobacter hormaechei* BHUJPCS-15, T2= *Enterobacter cloacae* BHUJPCS-21, and T3= consortia (T1 + T2).
(Data Values are the mean ±SD, mean values in the each columns with the same superscript do not differ significantly by the Duncan multiple post hoc test (P≤0.05).