Nano World Journal

https://doi.org/10.17756/nwj.2022-s1-006

Research Article

Open Access

# Analysis of Culturable Rhizospheric Microbial Population in Black Chickpea and Mustard Crops Suggesting the Application of Nano Fertilizers in the Field

**Banty Meena, Austin Mathew, and Rakesh Kumar Sharma***

*Department of Biosciences, Manipal University Jaipur, Jaipur, Rajasthan, India*

***Correspondence to:**
Rakesh Kumar Sharma
Department of Biosciences, Manipal University Jaipur, Jaipur, Rajasthan, India

**Received:** August 23, 2022
**Accepted:** October 07, 2022
**Published:** October 09, 2022

**Citation:** Meena B, Mathew A, Sharma RK. 2022. Analysis of Culturable Rhizospheric Microbial Population in Black Chickpea and Mustard Crops Suggesting the Application of Nano Fertilizers in the Field. *NanoWorld J 8(S1):* S28-S31.



Published by United Scientific Group

## Abstract

Black chickpea and mustard are important Rabi crops in Rajasthan, India. This study explored comparative culturable rhizospheric bacterial population associated with black chickpea and mustard plants in the sandy soils of Bagru, Jaipur. The experiment was conducted from sowing to harvesting for the full crop season i. e. 110 days, and total 4 samples were collected in 30 days interval with NPK fertilizer. Plant growth was measured along with colony forming units (CFU) from the rhizosphere of both the crops. Rhizospheric soil showed higher CFU/g as compared to bulk soil. Bulk soil contained $8.7 \times 10^4$ CFU/g and mustard rhizospheric soil contained $3.6 \times 10^7$ CFU/g, while black chickpea rhizospheric soil had $2.6 \times 10^6$ CFU/g. The CFU count was constantly increasing in the case of mustard, while it initially increased and then significantly decreased in the case of black chickpea at the harvesting time. The plant growth was positively correlated with CFU count. These results establish a correlation among soil, microbes, and plant. It is further, recommended that the application of nano fertilizers will be useful instead of traditional chemical fertilizer used in the sandy loam soil of this region. As these nano fertilizers may assist diverse plant growth promoting microbial population.

## Keywords

Culturable microbes, Rhizospheric populations, Soil, Plant

## Introduction

The word "rhizosphere" was coined by scientist L. Hiltner. It's the area that's a few millimetres (2 - 80 mm) away from the root system. It can also be defined as a zone that favours microorganisms' physical and chemical activity and is responsible for high microbial activity [1]. The soil adhering to plant roots is called rhizospheric soil. This soil shows exceptionally high number of bacterial populations. Rhizosphere contains a great diversity of microorganisms, which utilize amino acids, sugars, nucleotides and other organic contents from the plant root and provide the plants with inorganic salts and nutrients like phosphate, nitrogenous compounds, phenols, and growth hormones (indole acetic acid, gibberellin , etc.) [2, 3].

Root exudates are the organic and inorganic wastes of the root system. The rate of microbial activity has a significant impact on root exudation [4]. Thus, plant root exudates and microorganisms share a common habitat, where each other are influenced by the presence and activity of their partner. However, the rhizosphere zone is separate from the bulk soil and is also known as Edaphosphere or Non-rhizosphere. Bulk soil exhibits lower microbial activity as compared to rhizospheric soil as it completely lacks the microbe-plant association.

There are different types of microorganisms found in the rhizospheric soil including several beneficial microorganisms like mycorrhizal fungi, protozoa, nitrogen fixing bacteria, and other microorganisms that do not directly affect the root system [4]. Besides, there are some deleterious microorganisms are also found in the soil that harm the root system, which includes pathogenic fungi, bacteria, nematodes, etc. [5]. Rhizospheric soil also contains some neutral microorganisms, which show a neutral effect and includes actinomycetes, algae, etc.

Plants produce several biologically active chemicals into the rhizosphere to combat infection and give tissue-specific resistance. Indeed, root exudates are known to perform a variety of roles in ecological interactions with microbial soil communities, including serving as signaling molecules, attractants, and stimulants, as well as inhibitors and repellents [4]. By providing metabolizable organic materials and safe havens, plant roots support the colonization bacterial microflora in the rhizospheric zone. In exchange, these bacteria enhance their hosts' ability to absorb water and nutrients, grow more quickly, generate more biomass and yield, as well as abiotic stresses including salt stress, drought, low temperatures, heavy metals, and organic pollutants [6]. By solubilizing the bound form of phosphate into an absorbable form, the rhizospheric microbial communities can improve plant development (JS Singh, 2013) [2]. These beneficial microbial communities have a favorable outcome on the health and yield of the host plant therefore they are also referred to as plant probiotics [7].

Additionally, rhizospheric microorganisms act as biocontrol agents using various mechanisms such as production of antibiotics, siderophores, extracellular hydrolytic enzymes and other secondary metabolites such as hydrogen cyanide. They protect the host plant against the attacks of various root borne bacteria, fungi, and nematodes [2, 8]. *Pseudomonas putida*, *P. alcaligenes*, and *Pseudomonas* strain (Ps28) isolated from chickpea showed potential for the biocontrol of the root-rot disease complex of chickpea caused by *Meloidogyne incognita* and *M. phaseolina*, according to earlier reports [8].

Similarly, the native population of rhizosphere of chickpea, wheat and mustard grown in Haryana showed that the microfloral communities were placed into 4 generas – *Pseudomonas, Aeromonas,* Klepbsiella, and *Enterobacter*. Additionally, they noted that the isolates from the chickpea rhizosphere were more numerous and diverse than the isolates from the wheat and mustard [9]. Another study showed that the bacterial isolates obtained from the rhizospheric soil of chickpea grown in the vicinity of Allahabad belonged to *Bacillus, Pseudomonas,* Azotobacter, and *Rhizobium*. Most bacterial isolates showed the ability to produce IAA and ammonia. On evaluating their stress tolerance, *Bacillus* spp. were tolerant to all heavy metals while *Pseudomonas* spp. were tolerant to selected heavy metals [10].

Crop nutrition requirement is primarily fulfilled by providing chemical fertilizers. However, in future, there is vast scope for greener nano-nutrition of crops as for environmental sustainability, the green nano fertilizers as a source of nutrients may play a key role. Nano fertilizers are cost effective in comparison to chemical fertilizers because of their specific mechanisms of actions, efficiency, and minimum nutrients loss. Regarding mechanisms, the small size of these fertilizers may help in efficient absorption due to the increased surface area. These nano-fertilizers may also influence the microbial activity in the rhizosphere [11].

Thus, rhizospheric soil habitats several beneficial microorganisms, which are highly specific to plant and soil. In this regard, we intend to understand the diversity of culturable soil rhizospheric microorganisms that how the microbial population is influenced by specific plant.

# Material and Methods

## Site of study

Study site for mustard crop was and agriculture farms near Bagru (26.801, 75.545) Jaipur, Rajasthan, India, while for black chickpea it was Gadota (26.788, 75.416), Jaipur, Rajasthan, India. It falls in agro-climatic zone 3-A semi-arid eastern plain zone and the soil is sandy loam in texture.

## Crops and sampling

Mustard (*Brassica nigra*) and Black chickpea (*Cicer arietinum*) crops were monitored from sowing to harvesting during Nov 2021 – April 2022. The field was supplied with NPK Fertilizer as standard procedure, while for water it was dependent on rain. Plant and soil samples were collected in 3 sessions at the interval of 30 days.

## Plant growth parameters

Ten plants from each crop were uprooted at the decided interval and their root length, shoot length, and total length were monitored.

## CFU enumeration

Rhizospheric soil was collected as adhered with roots of the plants, while bulk soil sample was collected from other surrounding area. Soil samples were brought to the lab and serial dilution was done to the factor of $10^7$ using standard protocol. One hundred µl of sample from last three dilutions were plated on nutrient agar medium and the plates were incubated at 30 °C for 48-72 hours in an incubator.

# Results and Discussion

## Mustard plant growth

Mustard plants were uprooted at different intervals and physical parameters were observed. The full plant length was observed to be 29 cm, 86, cm and 170 cm at intervals of 30, 65 and, 105 days from sowing (Figure 1). The stem width of mustard plant when observed at these intervals was found out to be 0.82 cm, 1.4 cm, and 2.98 cm respectively, the root length was observed as 6.58 cm, 8.84 cm, and 10.84 cm while the stem length 22.46 cm, 77.34 cm and, 159.52 cm. While considering all the parameters, it was observed that maximum growth occurred between 65 to 105 days. In this time interval, full plant length increased by 84 cm and stem length increased by 82.18 cm (Figure 1).

A study conducted by Sang-Mo Kang, *et al.*, in the year

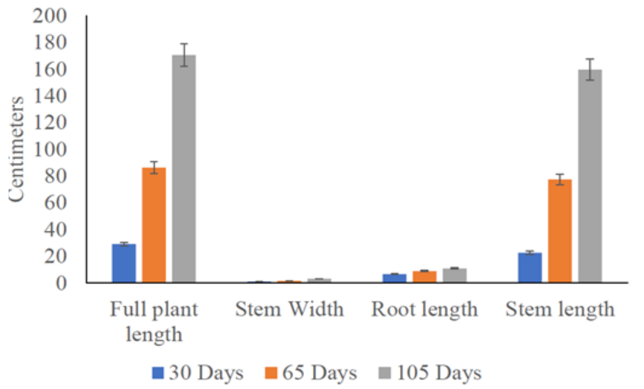


**Figure 1:** Mustard plant growth parameters at different time interval.

2014 showed that a rhizospheric bacteria, *B. megaterium* mj1212 enhanced the growth of mustard plant. The length of the shoots, the length of the roots, and the fresh weight of mustard plants were all significantly increased in plants treated with *B. megaterium* mj1212. In addition, the biochemical study showed that *B. megaterium* mj1212-treated plants had greater levels of chlorophyll, sucrose, glucose, fructose, and amino acids than the control [12].

## Culturable rhizospheric microbial population in mustard

The colony counting, characteristics and morphology was recorded after incubation. The colonies showed variations based on their morphological characteristics. The number of colonies were counted, and colonies were observed for size, shape, color, edge, texture, and transparency. During second session after 65 days, most of the colonies appeared exhibited high amount of exo-polysaccharide production as evident from their shiny and gloss appearance. The colony diameters were in the range of 1 to 3 mm, while the largest colony diameter of 5 mm was observed.

CFUs of the rhizospheric soil were measured 30, 65, and 105 days after sowing and were found to be 244.75 × $10^5$ CFU/g, 210.75 × $10^5$ CFU/g, and 349 × $10^5$ CFU/g, respectively. From Day 30 to Day 65, the CFU seems to drop from 244.75 × $10^5$ CFU/g to 210.75 × $10^5$ CFU/g before rising to 349 × $10^5$ CFU/g when observed at Day 105 (Figure 2).

## Black chickpea plant growth

The physical parameters of Black Chickpea were observed at different time intervals. At Day 30 from sowing, the full stem length was 28.94 cm, stem width was observed as 0.64 cm and root and stem length was observed to be 14.56 cm and 14.38 cm, respectively. The results on Day 70 showed that the full plant length was 40.32 cm with 16.64 cm root length and 23.8 cm stem length. The stem width was 1 cm. After 110 days of sowing, the full plant length did not show a significant increase. It increased from 40.32 cm (at day 70) to 41.36 cm. While the stem length showed an increase from 23.8 cm (at Day 70) to 25.16 cm (at Day 110), the stem width decreased from 1 cm (at day 70) to 0.84 cm. Similarly, root length also decreased from 16.64 cm to 16.2 cm (Figure 3).

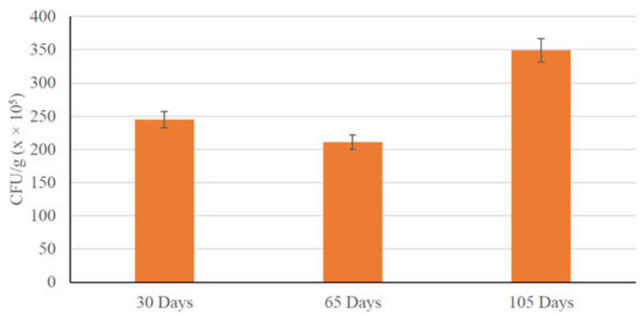


**Figure 2**: Culturable microbial population from Mustard rhizospheric soil during different time interval.

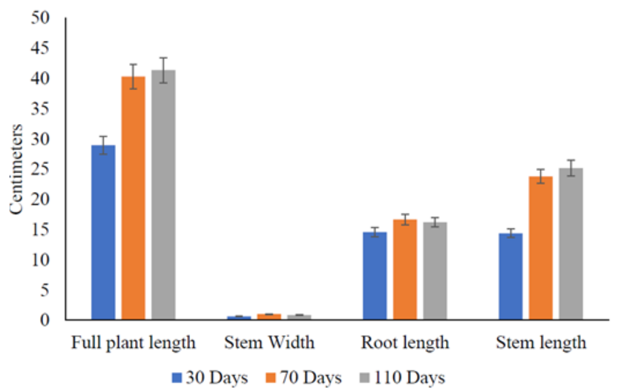


**Figure 3:** Black Chickpea plant growth parameters at different time interval.

On comparing these results with that of Mustard plant, it was observed that mustard plant showed maximum growth between 65 days after sowing to 110 days after sowing while black chickpea plant showed maximum growth in the initial stage, that is, 0 to 30 days after sowing. According to a study, several *B. subtilis* strains significantly improved the growth (root and shoot length) and dry matter of chickpea plants while also reduced the occurrence of wilt [13]. Another research by Aradhana Mishra et al. showed that, in comparison to the uninoculated control, chickpea plants treated with Plant growth promoting *Pantoea agglomerans* NBRISRM (NBRISRM) had increased shoot length, shoot dry weight, number of pods, and weight of 100 seeds by 13.5, 13.5, 12.9, and 13.6 percent, respectively [14].

## Culturable rhizospheric microbial population in black chickpea

At 30, 70, and 110 days after seeding, the CFUs of the rhizospheric soil was measured, and it was found out to be 156× $10^5$ CFU/g, 267 × $10^5$ CFU/g, and 307 × $10^5$ CFU/g, respectively. From Day 30 to Day 70, the CFU increases significantly, however from Day 70 to Day 110, the CFU does not increase as much (Figure 4). This signifies a higher population of rhizospheric bacteria during the initial growth stages of mustard.

The correlation among different plant growth parameters along with CFU revealed that the microbial population was positively correlated by all the plant growth parameters (Table 1). This indicated that plant growth has a positive influence on soil microbial population and vice versa. Microbial population in the rhizosphere of black chickpea plant was most

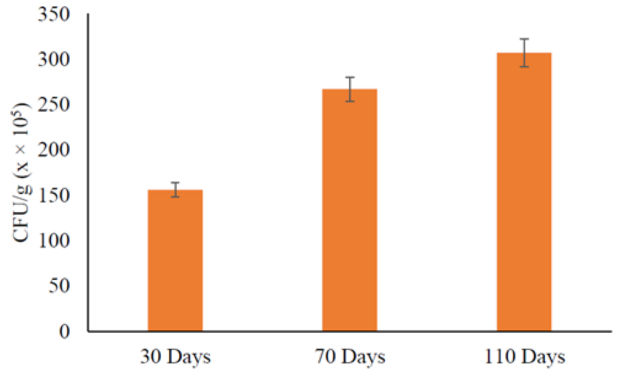


**Figure 4:** Culturable microbial population from black chickpea rhizospheric soil during different time interval.

influenced by the growth of plant in comparison to mustard. This further endorses the specificity of plant with microbial population.

In the present study NPK fertilizer was used by the farmers, however our results suggest that instead of such fertilizers nano-fertilizers comprising macronutrients like P, K, Ca, Mg, and S may be a better option as these fertilizers are showing promising results with increased efficiency in terms of enhanced plant growth, positive influence on rhizospheric microbial population and ultimately the productivity of crops [15].

**Table 1:** Correlation between culturable microbial population and plant growth parameters.

| **Mustard plant and CFU** | | | | |
|---|---|---|---|---|
| | Full plant length | Root length | Stem length | CFU |
| Full plant length | 1.000 | | | |
| Stem Width | 0.989 | | | |
| Root length | 0.989 | 1.000 | | |
| Stem length | 1.000 | 0.989 | 1.000 | |
| CFU | 0.796 | 0.700 | 0.799 | 1.000 |
| **Black chickpea plant and CFU** | | | | |
| Full plant length | 1.000 | | | |
| Stem Width | 0.860 | | | |
| Root length | 0.962 | 1.000 | | |
| Stem length | 0.999 | 0.950 | 1.000 | |
| CFU | 0.983 | 0.896 | 0.990 | 1.000 |

# Conclusion

Roots secrete many organic compounds into the soil, which deposit in the rhizosphere zone. These secreted compounds are called root exudates and have diverse role in plants as well as in soil. The rhizosphere microbiome facilitates communication between the plant and the surrounding soil environment, and they together contribute to improved crop productivity. Analysis of culturable microbial community may help in designing the soil/climate and plant specific biofertilizer. The result of the study recommends that the application of nano-fertilizers will be advantageous in such kind of soil type having lower water holding capacity and the microbial population is less diverse.

## References

1. Hinsinger P, Marschner P. 2006. Rhizosphere--perspectives and challenges--a tribute to Lorenz Hiltner 12-17 September 2004--Munich, Germany. *Plant Soil* 283(1-2): 7-8. https://doi.org/10.1007/s11104-006-0057-5
2. Singh JS. 2013. Plant growth promoting rhizobacteria. *Reson* 18(3): 275-281. https://doi.org/10.1007/s12045-013-0038-y
3. Zaidi A, Khan MS, Ahemad M, Oves M, Wani PA. 2009. Recent advances in plant growth promotion by phosphate-solubilizing microbes. In: Khan M, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin, Heidelberg, Germany, pp 23-50. https://doi.org/10.1007/978-3-642-01979-1_2
4. Meena VS, Meena SK, Verma JP, Kumar A, Aeronn A, et al. 2017. Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: a review. *Ecol Eng* 107: 8-32. https://doi.org/10.1016/j.ecoleng.2017.06.058
5. Nehl DB, Allen SJ, Brown JF. 1997. Deleterious rhizosphere bacteria: an integrating perspective. *Appl Soil Ecol* 5(1): 1-2. https://doi.org/10.1016/S0929-1393(96)00124-2
6. Fan D, Schwinghamer T, Smith DL. 2018. Isolation and diversity of culturable rhizobacteria associated with economically important crops and uncultivated plants in Québec, Canada. *Syst Appl Microbiol* 41(6): 629-640. https://doi.org/10.1016/j.syapm.2018.06.004
7. Panpatte DG, Jhala YK, Shelat HN, Vyas RV. 2016. *Pseudomonas fluorescens*: a promising biocontrol agent and PGPR for sustainable agriculture. In: Singh D, Singh H, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, India, pp. 257-270. https://doi.org/10.1007/978-81-322-2647-5_15
8. Akhtar MS, Siddiqui ZA. 2009. Use of plant growth-promoting rhizobacteria for the biocontrol of root-rot disease complex of chickpea. *Australas Plant Pathol* 38(1): 44-50. https://doi.org/10.1071/AP08075
9. Kundu BS, Nehra K, Yadav R, Toma M. 2009. Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. *Indian J Microbiol* 49(2): 120-127. https://doi.org/10.1007/s12088-009-0016-y
10. Joseph B, Patra RR, Lawrence R. 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *Int J Plant Prod* 1(2): 141-152. https://doi.org/10.22069/IJPP.2012.532
11. Elemike EE, Uzoh IM, Onwudiwe DC, Babalola OO. 2019. The role of nanotechnology in the fortification of plant nutrients and improvement of crop production. *Applied Sciences* 9(3): 499. https://doi.org/10.3390/app9030499
12. Kang SM, Radhakrishnan R, You YH, Joo GJ, Lee IJ, et al. 2014. Phosphate solubilizing *Bacillus megaterium* mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plaint growth. *Indian J Microbiol* 54(4): 427-433. https://doi.org/10.1007/s12088-014-0476-6
13. Singh RK, Kumar DP, Singh P, Solanki MK, Srivastava S, et al. 2014. Multifarious plant growth promoting characteristics of chickpea rhizosphere associated Bacilli help to suppress soil-borne pathogens. *Plant Growth Regul* 73(1): 91-101. https://doi.org/10.1007/s10725-013-9870-z
14. Mishra A, Chauhan PS, Chaudhry V, Tripathi M, Nautiyal CS. 2011. Rhizosphere competent *Pantoea agglomerans* enhances maize (*Zea mays*) and chickpea (*Cicer arietinum* L.) growth, without altering the rhizosphere functional diversity. *Antonie Van Leeuwenhoek* 100(3): 405-413. https://doi.org/10.1007/s10482-011-9596-8
15. Ditta A, Arshad M. 2016. Applications and perspectives of using nanomaterials for sustainable plant nutrition. *Nanotechnol Rev* 5(2): 209-229. https://doi.org/10.1515/ntrev-2015-0060