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Full Length Research Paper

A Study of Rhizospheric Bacteria of *Jatropha curcas* and their PGPR Properties

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Abstract

The present study deals with the rhizospheric microflora of Jatropha curcas plant and the plant growth promoting properties of the rhizospheric bacteria of Jatropha curcas. The samples were collected from three different sites viz., Crop Research Centre of GBPUAT Pantnagar Udhamsinghnagar (244 amsl), Dungri-Chapad, Rudraprayag distt at (891 amsl) and Jatropha plantation HNBGU Chauras campus, Distt Tehri-Garhwal at (560 amsl). The samplings were done in three different seasons namely summer, rainy and winter. The bacteria isolated from the soil samples followed by PGPR tests like HCN production, Phosphate solubilisation, siderophores and ammonium production. The selected isolates were estimated quantitatively for inorganic phosphate solubilised and indole acetic acid produced in 3 days at 28°C. The highest amount of phosphate solubilised was recorded to be 65.83 µg/ml with a corresponding pH of 2.92 by strain S3R3C1 and highest level of IAA was by isolate S2R3C2 (48.46 µg/ml). A negative correlation between phosphate solubilised by bacteria and corresponding pH was also recorded.

Keywords: *PGPR, Siderophore, Jatropha curcas Linn.*

Introduction

Jatropha (*Jatropha curcas* L.) is a hardy non-edible oil-seed plant that can sustain harsh environments, adapt well to semi-arid marginal, and wastelands and hence has been treated as a potential alternative energy source (Foidl *et al*. 1996; Gubitz *et al.* 1999). In India, to reduce dependence on the crude oil and to achieve energy independence by the year 2012, *Jatropha* and *Pongamia* have been promoted under the National Biodiesel Mission.

Plant-growth-promoting rhizobacteria (PGPR) can enhance plant growth either directly by producing phytohormones or indirectly by producing siderophores for sequestering iron or solubilizing phosphorus or over expression of indole acetic acid (Xie Hong *et al.* 1996; Cattelan *et al.* 1999 and Mayak *et al.* 1999). Identification of promising PGPR for *Jatropha* helps to enhance production. It was likewise observed that *Jatropha* plants with mycorrhiza (i.e., mycorrhizal plants) grown in acidic soils had significantly thicker stems and longer shoots compared to non-mycorrhizal plants. Mycorrhizal plants generally have wider and thicker leaves and absorbed more NPK, and Cu compared to non-mycorrhizal ones. Given Jatropha's ability to thrive in hostile environments while sustaining the population of mycorrhizae and other beneficial soil microbes, farmers need not fear when planning to venture into large-scale *Jatropha* production. Some bacteria support plant growth indirectly, by improving and/or eliminating the growth-restricting conditions either via production of antagonistic substances or by inducing resistance against plant pathogens (Castric, 1975). Apart from rhizobial symbionts, the rhizosphere-associated beneficial bacteria (RABB) consist of either biocontrol agents such as *Pseudomonas* and *Bacillus* group, which antagonize pathogenic or deleterious microorganisms and/or bioenhancer agents such as *Azospirillum, Herbaspirillum*, *Enterobacter*, *Acetobacter*, *Azotobater*, and *Pseudomonas* group which directly enhance plant growth (Sarwar and Kremer, 1995).

Materials and Method

Bacterial Isolation and maintenance

The bacterial strains were isolated from the rhizospheric soil of *Jatropha* plants from three different plantations, viz., Crop Research Centre of GBPUAT Pantnagar Udhamsinghnagar (244 amsl), Dungri-Chapad , Rudraprayag Distt at (891 amsl) and *Jatropha* plantation HNBGU Chauras campus situated in Srinagar Valley Distt Tehri-Garhwal at (560 amsl). The samples were collected periodically i.e., summer, rainy and winter seasons. The composite sample of soil were prepared, serially diluted and plated in nutrient agar and incubated at 30˚C. The Isolated axeinic cultures were maintained on nutrient agar slants and glycerol stocks, respectively

Assay for siderophore production

Production of siderophore was determined by Chromazurol S (CAS) agar method (Schwyne and Neilands 1987). Briefly, the bacterial inoculum was spotted into the center of a CAS agar plate. After incubation at 28°C for 5 days, siderophores production was assayed by the change in the colour of the medium from blue to orange.

Assay for hydrogen cyanide production

Hydrogen cyanide production was assayed by the method suggested by Castric (1975). For the production of HCN, bacteria were streaked into King's B agar plates supplemented with glycine. After this, petriplates were inverted and a piece of filter paper soaked with 0.5% picric acid and 2% of sodium carbonate, and dried was placed on the lid. Petri plates were sealed with para film and incubated at 28°C for 96 h. Colour change of the filter paper form yellow to brown after incubation was considered as microbial production of cyanide.

Qualitative estimation of phosphate solubilisation and ammonium production

To determine the solubilisation of phosphate, the bacterial strains were streaked into Pikovskaya agar medium (Pikovskaya, 1948) and incubated at 28°C for 3 days. Thereafter, the colonies showing the clear zones around them were considered as positive. For detection of production of ammonium by isolates peptone water was inoculated by isolates and after 3 days incubation at 28°C presence of ammonium was detected by adding few drops of Nessler's reagent. Appearance of yellow color shows positive result.

Quantitative estimation of phosphate solubilisation and IAA production

Quantitative estimation of P was done at 28ºC, by inoculating 1 ml of bacterial suspension (3 x 10^7 CFU /ml) in 50 ml of NBRIP broth (Mehta and Nautiyal 2001) in Erlenmeyer flasks (150 ml), and incubating for 7days. At the end of the incubation period, the culture suspension was centrifuged at 10 000 g for 10 min and the P content in the supernatant was spectrophotometrically estimated by the ascorbic acid method (Murphy and Riley 1962). The IAA production was estimated by inoculating a bacterial suspension (3 x 10^7 CFU/ ml) in 10 ml Luria Bertani (LB) broth containing l-tryptophan (100 lg /ml), and incubating it for 48 h. The IAA concentration in the culture supernatant was estimated by the procedure of Gordon and Weber (1951).

Results and discussion

In this study 144 axenic cultures were isolated through serial dilution method and maintained on nutrient agar slants and glycerol stocks. These isolates were then subjected to various qualitative PGPR tests namely ammonium production, HCN production, siderophores production and phosphate solubilisation. The result has been provided in Table no 1.

Table 1: Performance of Isolates when qualitatively screened for PGPR properties namely HCN production, Ammonium production, Siderophore production and Phosphate solubilisation

Isolates	HCN Production	Ammonium Production	Siderophore Production	Phosphate solubilization
S1P1C1	(-)ve	(+)ve	(-)ve	(-)ve
S1P1C2	(+)ve	(+)ve	(-)ve	(-)ve
S1P1C3	(+)ve	(+)ve	(+)ve	(-)ve
S1P1C4	(-)ve	(+)ve	(-)ve	(-)ve
S1P1C5	(+)ve	(-)ve	(-)ve	(-)ve
S1P1C6	(-)ve	(+)ve	(-)ve	(-)ve
S1P1C7	(+)ve	(+)ve	(+)ve	(-)ve
S1P2C1	(-)ve	(+)ve	(-)ve	(-)ve
S1P2C2	(+)ve	(-)ve	(-)ve	(-)ve
S1P2C3	(-)ve	(+)ve	(-)ve	(-)ve
S1P2C4	(+)ve	(-)ve	(-)ve	(-)ve
S1P2C5	(-)ve	(+)ve	(-)ve	(-)ve
S1P2C6	(-)ve	(-)ve	(-)ve	(-)ve
S1P2C7	(-)ve	(-)ve	(-)ve	(-)ve
S1P2C8	(+)ve	(+)ve	(+)ve	(+)ve
S1P2C9	(-)ve	(-)ve	(-)ve	(-)ve
S1P2C10	(-)ve	(+)ve	(-)ve	(-)ve
S1P3C1	(-)ve	(-)ve	(-)ve	(-)ve
S1P3C2	(-)ve	(+)ve	(-)ve	(-)ve
S1P3C3	(-)ve	(-)ve	(-)ve	(-)ve
S1P3C4	(-)ve	(+)ve	(-)ve	(-)ve
S1P3C5	(-)ve	(-)ve	(-)ve	(-)ve
S1P3C6	(-)ve	(-)ve	(-)ve	(-)ve

S1P3C8	(-)ve	(-)ve	(+)ve	(-)ve
S1P3C9	(-)ve	(-)ve	(-)ve	(-)ve
S2P1C1	(-)ve	(-)ve	(-)ve	(-)ve
S2P1C2	(-)ve	(+)ve	(-)ve	(-)ve
S2P1C3	(+)ve	(-)ve	(-)ve	(-)ve
S2P1C4	(-)ve	(-)ve	(-)ve	(-)ve
S2P1C5	(-)ve	(+)ve	(-)ve	(-)ve
S2P1C6	(-)ve	(-)ve	(-)ve	(-)ve
S2P2C1	(+)ve	(+)ve	(+)ve	(-)ve
S2P2C2	(-)ve	(+)ve	(-)ve	(-)ve
S2P2C3	(-)ve	(-)ve	(-)ve	(+)ve
S2P2C4	(+)ve	(-)ve	(-)ve	(-)ve
S2P2C5	(-)ve	(-)ve	(+)ve	(-)ve
S2P2C6	(-)ve	(+)ve	(+)ve	(-)ve
S3P1C1	(-)ve	(-)ve	(-)ve	(-)ve
S3P1C2	(-)ve	(+)ve	(-)ve	(-)ve
S3P1C3	(-)ve	(+)ve	(-)ve	(-)ve
S3P1C4	(+)ve	(+)ve	(+)ve	(-)ve
S3P2C1	(-)ve	(+)ve	(-)ve	(-)ve
S3P2C2	(-)ve	(-)ve	(-)ve	(-)ve
S3P2C3	(-)ve	(-)ve	(+)ve	(-)ve
S3P2C4	(-)ve	(+)ve	(+)ve	(-)ve
S3P2C5	(-)ve	(-)ve	(-)ve	(-)ve
S3P2C6	(+)ve	(+)ve	(+)ve	(-)ve
S3P2C7	(-)ve	(-)ve	(-)ve	(-)ve
S3P3C1	(-)ve	(-)ve	(-)ve	(-)ve
S3P3C2	(-)ve	(+)ve	(-)ve	(-)ve
S3P3C3	(-)ve	(-)ve	(-)ve	(-)ve
S3P3C4	(+)ve	(-)ve	(-)ve	(-)ve
S3P3C5	(-)ve	(+)ve	(-)ve	(-)ve
S1R1C1	(-)ve	(+)ve	(-)ve	(-)ve
S1R1C2	(+)ve	(+)ve	(+)ve	(-)ve
S1R1C3	(-)ve	(-)ve	(-)ve	(-)ve
S1R2C1	(+)ve	(+)ve	(+)ve	(-)ve
S1R2C2	(+)ve	(+)ve	(+)ve	(-)ve
S1R2C3	(+)ve	(+)ve	(+)ve	(+)ve
S1R2C4	(+)ve	(+)ve	(+)ve	(+)ve
S1R2C5	(-)ve	(-)ve	(-)ve	(-)ve
S1R2C6	(+)ve	(+)ve	(+)ve	(-)ve
S1R2C7	(+)ve	(+)ve	(+)ve	(-)ve
S1R3C1	(+)ve	(-)ve	(-)ve	(-)ve
S1R3C2	(+)ve	(+)ve	(+)ve	(-)ve
S1R3C3	(+)ve	(-)ve	(-)ve	(-)ve
S1R3C4	(-)ve	(+)ve	(-)ve	(-)ve
S1R3C5(A)	(-)ve	(-)ve	(+)ve	(-)ve
SIR3C5(B)	(+)ve	(-)ve	(-)ve	(-)ve

S1R3C6	(-)ve	(+)ve	(-)ve	(-)ve
S1R3C7	(-)ve	(-)ve	(-)ve	(-)ve
S1R3C8	(+)ve	(+)ve	(+)ve	(-)ve
S1R3C9	(-)ve	(-)ve	(-)ve	(-)ve
S1R3C10	(+)ve	(-)ve	(-)ve	(-)ve
S1R3C11	(+)ve	(+)ve	(+)ve	(-)ve
S1R3C12	(+)ve	(-)ve	(-)ve	(-)ve
S2R1C1	(+)ve	(+)ve	(+)ve	(-)ve
S2R1C2	(-)ve	(+)ve	(-)ve	(-)ve
S2R1C3	(-)ve	(+)ve	(-)ve	(-)ve
S2R1C4	(-)ve	(-)ve	(+)ve	(-)ve
S2R2C1	(+)ve	(-)ve	(-)ve	(-)ve
S2R2C2	(+)ve	(-)ve	(-)ve	(-)ve
S2R2C3	(-)ve	(-)ve	(-)ve	(-)ve
S2R3C1	(+)ve	(+)ve	(+)ve	(+)ve
S2R3C2	(+)ve	(+)ve	(+)ve	(+)ve
S3R1C1	(-)ve	(-)ve	(-)ve	(-)ve
S3R1C2	(+)ve	(-)ve	(-)ve	(-)ve
S3R1C3	(+)ve	(+)ve	(+)ve	(-)ve
S3R1C4	(-)ve	(-)ve	(-)ve	(-)ve
S3R1C5	(-)ve	(-)ve	(+)ve	(-)ve
S3R2C2	(-)ve	(-)ve	(-)ve	(-)ve
S3R3C3	(+)ve	(+)ve	(+)ve	(-)ve
S1C1C1	(-)ve	(-)ve	(-)ve	(-)ve
S1C1C2	(-)ve	(-)ve	(-)ve	(-)ve
S1C1C3	(+)ve	(+)ve	(+)ve	(-)ve
S1C1C4	(-)ve	(-)ve	(-)ve	(-)ve
S1C1C5	(-)ve	(-)ve	(-)ve	(-)ve
S1C1C6	(-)ve	(-)ve	(-)ve	(-)ve
S1C1C7	(-)ve	(+)ve	(-)ve	(-)ve
S1C2C1	(-)ve	(-)ve	(-)ve	(-)ve
S1C2C2	(-)ve	(-)ve	(-)ve	(-)ve
S1C2C3	(+)ve	(+)ve	(+)ve	(-)ve
S1C2C4	(-)ve	(-)ve	(-)ve	(-)ve
S1C2C5	(-)ve	(+)ve	(-)ve	(-)ve
S1C2C6	(-)ve	(+)ve	(-)ve	(-)ve
S1C3C1	(+)ve	(-)ve	(-)ve	(-)ve
S1C3C2	(+)ve	(+)ve	(+)ve	(+)ve
S1C3C3	(-)ve	(-)ve	(-)ve	(-)ve
S1C3C4	(+)ve	(+)ve	(+)ve	(-)ve
S1C3C5	(-)ve	(+)ve	(-)ve	(-)ve
S1C3C6	(-)ve	(-)ve	(-)ve	(-)ve
S1C3C7	(-)ve	(+)ve	(-)ve	(-)ve
S1C3C8	(-)ve	(-)ve	(-)ve	(-)ve
S1C3C9	(+)ve	(+)ve	(+)ve	(+)ve
S2C1C1	(-)ve	(-)ve	(-)ve	(-)ve

S2C1C2	(+)ve	(-)ve	(-)ve	(-)ve
S2C1C3	(-)ve	(+)ve	(-)ve	(-)ve
S2C1C4	(-)ve	(-)ve	(-)ve	(-)ve
S2C2C1	(-)ve	(+)ve	(-)ve	(-)ve
S2C2C2	(-)ve	(+)ve	(-)ve	(-)ve
S2C2C3	(+)ve	(+)ve	(+)ve	(-)ve
S2C3C1	(-)ve	(-)ve	(-)ve	(-)ve
S2C3C2	(-)ve	(-)ve	(-)ve	(-)ve
S2C3C3	(-)ve	(-)ve	(-)ve	(-)ve
S2C3C4	(+)ve	(-)ve	(-)ve	(-)ve
S2C3C5	(-)ve	(+)ve	(-)ve	(-)ve
S2C3C6	(-)ve	(-)ve	(-)ve	(-)ve
S2C3C7	(+)ve	(+)ve	(+)ve	(-)ve
S2C3C8	(-)ve	(-)ve	(-)ve	(-)ve
S2C3C9	(+)ve	(+)ve	(+)ve	(-)ve
S2C3C10	(-)ve	(+)ve	(-)ve	(-)ve
S2C3C11	(+)ve	(+)ve	(+)ve	(-)ve
S3C1C1	(-)ve	(-)ve	(-)ve	(-)ve
S3C1C2	(-)ve	(+)ve	(-)ve	(-)ve
S3C2C1	(-)ve	(+)ve	(+)ve	(-)ve
S3C2C2	(-)ve	(+)ve	(-)ve	(-)ve
S3C2C3	(-)ve	(+)ve	(-)ve	(-)ve
S3C2C4	(-)ve	(-)ve	(-)ve	(-)ve
S3C3C1	(+)ve	(-)ve	(-)ve	(-)ve
S3C3C2	(-)ve	(+)ve	(-)ve	(-)ve
S3C3C3	(-)ve	(+)ve	(-)ve	(-)ve
S3C3C4	(-)ve	(+)ve	(-)ve	(-)ve
S3C3C5	(-)ve	(+)ve	(-)ve	(-)ve
S3C3C6	(-)ve	(+)ve	(-)ve	(-)ve

Table 2: Phosphate solubilisation and IAA Production by selected strains

Isolates	Inorganic Phosphate solubilised (µg/ml)	pH	IAA Production (µg/ml)
S1R3C5	25.87±0.25	4.42±0.03	18.34±0.07
S1R3C8	24.3 ±0.26	4.37±0.02	20.17±0.06
S3R3C1	65.83±0.11	2.98±0.08	40.14±0.11
S3R3C3	28.23±0.15	4.15±0.03	9.25±0.09
S1R2C5	21.83±0.12	4.92±0.03	20.29±0.04
S3R1C3	23.27±0.15	4.75±0.03	25.31±0.11
S1C1C3	24.46±0.25	4.34±0.03	23.37±0.06
S1R2C2	23.36±0.15	4.45±0.03	25.51±0,03
S1R1C2	29.06±0.15	4.13±0.02	20.15±0.09
S1P1C3	25.36±0.21	4.35±0.04	22.18±0.03
S1R2C1	24.3±0.26	4.35±0.03	30.15±0.03
S1C1S3	23.4±0.10	4.69±0.02	15.18±0.04
S1R2C6	25.37±0.15	4.25±0.04	17.17±0.06

S1R2C3	39.35±0.10	3.12±0.09	23.21±0.06
S2C3S2	25.23±0.15	4.88±0.03	12.18±0.04
S2R3C4	50.16±0.25	3.24±0.03	42.17±0.20
S1C3S2	38.4±0.43	3.09±0.04	20.39±0.05
S1P2S8	23.4±0.2	4.53±0.02	18.36±0.21
S1R3C11	27.3±0.2	4.89±0.02	14.41±0.21
S2R3C2	52.1±0.2	3.13±0.020	48.46±0.18
S2R3C1	21.36±0.15	5.12±0.03	20.32±0.11
S1P1C7	23.63±0.25	5.15±0.02	18.3±0.16
S2P2C1	24.36±0.15	4.97±0.03	14.29±0.12
S3P1C4	28.36±0.25	4.36±0.02	20.25±0.07
S3P2C6	26.36±0.15	4.14±0.03	20.37±0.04
S2R1C1	27.2±0.1	5.03±0.08	14.34±0.10
S2R2C2	25.23±0.15	4.35±0.04	19.24±0.03
S1C3C4	25.63±0.30	4.36±0.03	28.30±0.02
S1C3C9	22.47±0.15	5.11±0.03	17.49±0.14
S2C2C3	28.3±0.1	4.16±0.04	20.13±0.05
S2C3C7	28.67±0.25	4.05±0.07	16.44±0.11
S2C3C9	22.37±0.15	4.85±0.02	18.35±0.07
S3C3C6	21.13±0.25	4.95±0.04	15.47±0.17

Table1 depicts that most of the strains were positive for ammonium production and only seven isolates were positive for phosphate solubilisation on Pikovaskya agar plates. Any strain which was positive for three or more of the above mentioned PGPR properties were selected. These selected isolates were further analysed and estimated for Phosphate solubilisation in Pikovaskya's broth along with subsequent drop in pH and for indole acetic acid production in Luria Bertini broth supplemented with tryptophan.

Phosphorus is one of the major essential macro nutrients for growth and development of plants making up about 0.2% of plant dry weight. Phosphorus exists in nature in a variety of organic and inorganic forms which are insoluble or very poorly soluble (Paul and Clark, 1996). Plants acquire phosphorus from soil as phosphate anions which are extremely reactive and get immobilized through precipitation with cations such as Ca^{+2}, Mg^{+2}, Fe^{+3} and Al^{+3} depending on the particular properties of soil (Helford, 1997).

Organic acids are metabolized two or three times faster in the rhizosphere than in bulk soil, typically with 60% being mineralized and reminder incorporated into microbial biomass. A range of organic acid with phosphate solubilizing activity are produced by rhizobacteria and among them are strains from genera such as *Arthorobacter, Azospirillum, Bacillus, Pseudomonas* and *Rhizobium* but gluconic acid and 2-ketogluconic acid appear to be the most active and important (Goldstein 1986).

A survey of Indian soil revealed that 98% of soil is deficient because the concentration of pre phosphorus, the form available to plants even in fertile soils is generally not higher than 10µm even at pH 6.5 where it is most soluble. These low levels of phosphate are due to high reactivity of soluble P with Ca, Fe or Al that leads to precipitation. In acidic soil inorganic phosphate is associated with Al or Fe compounds where as in alkaline soils, calcium phosphates are the predominant form of inorganic phosphate (Rodriguez *et al*. 2006., Tilak *et al.* 2005). The phosphate solubilizing bacteria help in solubilizing inorganic phosphate, which in turn increases the uptake of phosphate by plant. Thus, phosphate solubilisation by microbes is one of the most important PGPR properties.

In the present study the highest amount of phosphate solubilised was 65.83 µg/ml with a corresponding pH of 2.92 by strain S3R3C1 followed by S2R3C2 (52.1 µg/ml, pH 3.13) and S2R3C4 (50.16 µg/ml, pH 3.24). The correlation coefficient between the amount of phosphate solubilised and pH was -0.7622, a negative value shows that negative correlation existed between the two properties which suggest that lower the pH drops more the value of phosphate solubilised. This fact can be explained by the mechanism of phosphate uptake by plants in soil. As mentioned earlier organic acids produced by soil microorganisms play a crucial part in mineralization along with uptake of phosphate and production of these acids lower the pH of medium that in turn helps in solubilisation of inorganic phosphate.

Indole acetic acid production is another very important PGPR property. Some rhizospheric bacteria have ability to produce Indole acetic acid as secondary metabolites due to rich supply of substrates like tryptophan by plant root exudates. Indole acetic acid helps in the production of longer roots with increased number of root hairs and root laterals which are involved in nutrient uptake (Datta and Basu, 2000). IAA stimulates cell elongation by modifying certain conditions like, increase in osmotic contents of the cell, increase in permeability of water into cell, decrease in wall pressure, an increase in cell wall synthesis, inducing specific RXA and protein synthesis. It promotes embial activity, inhibit or delay abscission of leaves, induce flowering and fruiting. (Zhao, 2010).

In this study we found strains producing IAA in very high amounts. The highest IAA production was recorded by isolate S2R3C2 (48.46 µg/ml) followed by S2R3C4 (42.17 µg/ml).

Conclusion

The present study deals with the plant growth promoting rhizobacteria of *Jatropha curcas, a* pioneering biofuel plant that has shown decent potential, can be grown on wastelands and does not require much attention as a crop. The study of the microflora of this plant is still in its infancy and much remains to be explored about its rhizospheric microflora and possible PGPR for this plant. This study is a contributing step in the same direction.

Three plantations sites situated at different altitudes of Central Himalayan region were chosen for study and composite soil samples were taken on the seasonal basis to nullify any seasonal shift in microbial composition of soil. 144 bacterial strains were isolated and subjected to qualitative PGPR tests namely Siderophore Production, Phosphate solubilisation, HCN Production and Ammonium Production. Isolates positive for three or more of above mentioned tests were further subjected to qualitative tests namely estimation of IAA Production and Inorganic phosphate solubilised. Three strains namely S3R3C1, S2R3C2 and S2R3C4 were found to perform best under laboratory conditions. These strains can further be studied *in vitro* in Pot experiments and field trials to assess their effects on *Jatropha curcas* as separate bioinoculant or in consortia and can be developed in a potential bio fertilizer for this crop. Thus providing an ecofriendly alternative to traditional fertilizers for the same with additional benefit for soil as biofertilizers enhances the natural microflora of the soil which renders soil fertile for future uses.

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