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Rhizosphere Effect of Microorganisms on Green Gram Cultivated Soil - A Field Study

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ABSTRACT

The quantitative comparative analysis of the rhizosphere effect between rhizosphere soil and non-rhizosphere soil of the green gram cultivated soil was done based on R : S ratio (Root-Soil ratio). The results revealed that high rhizosphere effect was seen in the rhizosphere soil of green gram also the quantification studies explored that the rhizosphere effect among the microbes was more in bacteria than in fungi and actinomycetes during the plate count method on various media used. The result of the isolation and enumeration of rhizosphere bacterial culture by selective and decimal dilution method revealed that among the bacterial culture, the quantity of *Rhizobium*sp($140x10^6$±2.056CFU/ml) was more followed by Phosphate Solubilizing Bacteria(PSB) ($89x10^6$±3.080CFU/ml) and Cellulolytic bacteria ($54x10^6$±3.012CFU/ml). The lowest count was observed in the TCBS agar medium).

Keywords
Rhizosphere effect, green gram soil, R:S ratio, Bacteria, fungi

INTRODUCTION

Microbial communities play a pivotal role in the functioning of plants by influencing their physiology and development and are beneficial to plant growth (Rodrigo Mendes, et al.2013). Plants are colonized by an astounding number of microorganisms that can reach cell densities much greater than the number of plant cells (Johnson and Graham, 2013).Microbial population interacts with plants through a series of complex mechanism and the interactions can be beneficial, neutral or detrimental depending upon the nature of microbiome in the plant also the microbial activity is high due to the secretion of bioactive compounds from roots (Gaurav Yadav, et al.2017).

The root microbiota provides indirect pathogen protection, and to serve additional host functions through the acquisition of nutrients from soil for plant growth. Thus, the plant microbiota emerge mutualism through diverse biochemical mechanisms by plant growth–promoting and plant health–promoting bacteria (DavideBulgarelli, et al.2013). The study of microbiomes helps in the identification of new groups involved in plant diseases from the rhizospheremicrobiome and the number of studies have revealed that many plant-associated microorganisms have profound effects on seed germination, seedling vigor, plant growth and development, nutrition, diseases, and productivity (Inceoglu, et al.2013).

The microbial community at the seedling stage was distinct from the other developmental time and the phylum such as Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria and specific generaare associated with plant development and root exudation (Jacqueline Chaparro, et al.2014). Recently, it was reported that the composition of root exudates can change a plant developmental gradient (Chaparro et al., 2013). Cumulative secretion levels of sugars and sugar alcohols were higher in early time points and decreased through plant growth (Badri et al., 2013). As the plant ages it releases specific substrates and potentially antimicrobial compounds in an effort to select for particular microbial inhabitants of the rhizosphere (Selvakumar et al., 2012).

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Symbiotic nitrogen-fixing *Rhizobium* species utilize the flavonoid compounds secreted by the roots of leguminous plants and produce nod factors that induce the formation of root nodules by the plants and the bacteria in these nodules are nourished by the plants, in turn, these microbes convert nitrogen gas to nitrate that can be used by the plant(Tian, et al.2012).With this background, this study quantified the rhizosphere bacteria, fungi, and actinomycetes with non-rhizosphere region of green gram field based on R:S ratio and various selective media were used to identify the types of bacteria present in rhizosphere region.

MATERIALS AND METHODS

Sample collection

The rhizosphere and non-rhizosphere soil were collected aseptically and separately in sterile conical flasks from the green gram field of Thiruvengadam. These collected soil samples were placed in an ice container and transported immediately to the lab for further microbiological analysis

Quantification of rhizosphere and non rhizospheremicroflora

The quantity of one gram rhizosphere and non-rhizosphere soil was dissolved in 100ml of sterile water separately and were kept in a shaker for about 15 minutes. Then one ml of sample was taken and serially diluted up to 10^{-7} dilutions. From the respective selected dilution, one ml of sample was plated by pour plate method in the nutrient agar medium, Rose Bengal agar medium, and Glycerol yeast agar medium separately. These plates were incubated at 37^{o} C for 48hrs for bacteria and 7 days for fungi and actinomycetes. The following selective media were used to find out which bacteria have the highest levels in the rhizosphere region.

Table 1 Selective media and rhizospherebacteria.

Name of the Hi-media	Name of the Rhizosphere bacteria
YEMA (Yeast Mannitol Agar)	*Rhizobium*sp
Bacillus M1383	*Bacillus* sp
Pseudomonas Isolation Agar 406	*Pseudomonas*sp
Cellulose Powder- Peptone Medium	Cellulolytic sp
Hydroxy Apatite (HA) Medium	Phosphate solubilizing sp
KF Streptococcal Agar M248	*Streptococcus*sp
TCBS Agar M 189	*Vibrio cholera*
Phenolphthalein phosphate Agar M652	*Staphylococcus*sp
S.S.AgarM108	*Salmonella*sp and *Shigella*sp

The amount of one ml of sample was serially diluted and was plated on the above mentioned selective agar plates adopting the pour-plating technique. The plates were incubated for 24-48 hours at 37^{0}C. Bacterial outgrowth in countable plates was selected and enumerated. The bacterial populations were expressed as the number of Colony Forming Units per ml (CFU/ml).

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RESULTS

Microbial counts of rhizosphere and non-rhizosphere soil colonies were counted and the R:S value was calculated by the following formula.

$$\text{R:S ratio} = \frac{\text{Number of microorganisms (bacteria or fungi) in the rhizosphere soil}}{\text{Number of microorganisms (bacteria or fungi) in the non-rhizosphere soil}}$$

$$\text{Number of microorganisms/gram of soil} = \frac{\text{Number of colonies/plate} \times \text{dilution factor}}{\text{The dry weight of the soil taken}}$$

The results revealed that the rhizosphere soil consists of more microbial populationthan non-rhizosphere soil (Table 1) and among the microbial population bacterial population influenced (3.2×10^4) more than other microbial population in the soil in both rhizosphere and non-rhizosphere soil.

Table 2: Microbial Plate Counts (CFU/ml)

S.No	Microbes	Number of colonies present in rhizosphere soil CFU/ml	Number of colonies present in non rhizosphere soil CFU/ml
1.	Bacteria	$3.2\times10^4 \pm 0.07$	$2.2\times10^2 \pm 0.06$
2.	Fungi	$6.9\times10^6 \pm 0.10$	$5.5\times10^4 \pm 0.09$
3.	Actinomycetes	$3\times10^4 \pm 0.02$	$1.8\times10^3 \pm 0.05$

Table 3: R:S ratio of rhizosphere microbes

S.No	Microbes	R:S ratio
1.	Bacteria	66:1±0.08
2.	Fungi	22:8±0.01
3.	Actinomycetes	16:6±0.06

Values in Mean±Standard Deviation

Fig 1Microbial load in both rhizosphere and non-rhizosphere green gram soil

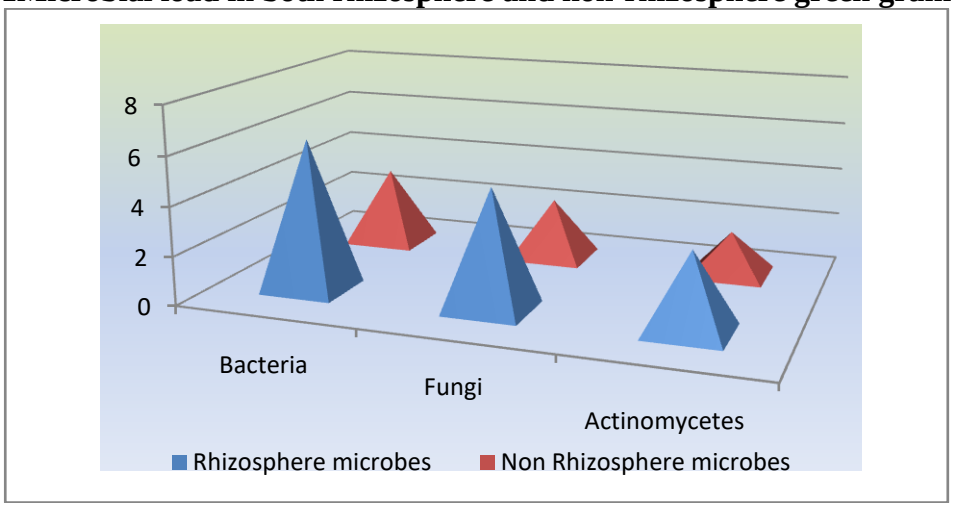

Fig 2Total heterotrophic bacterial population in the rhizosphere region

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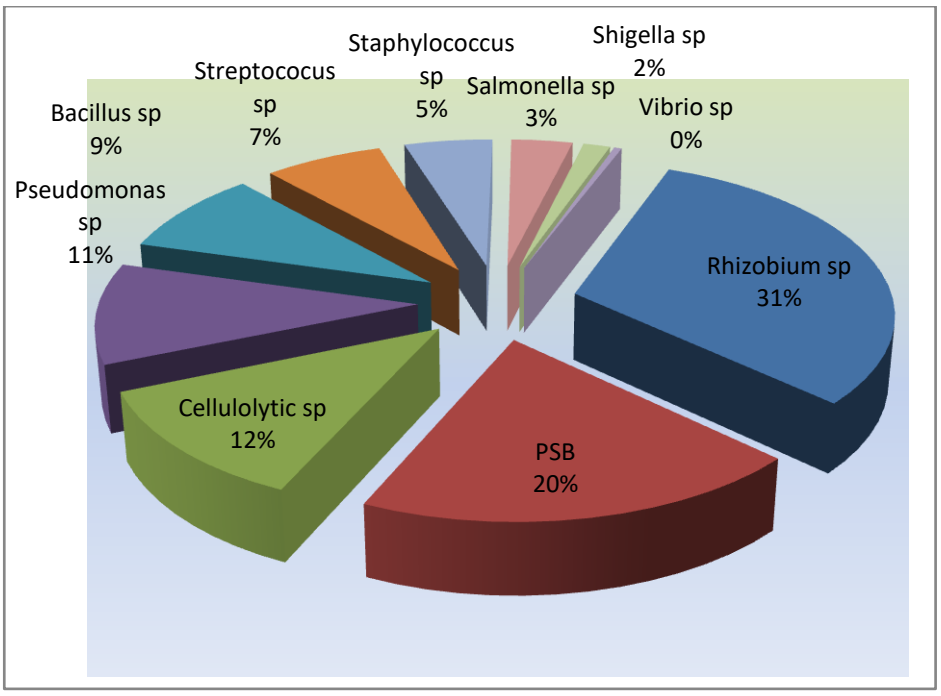

DISCUSSION

The diversity of microbes associated with plant roots are enormous, in the order of tens of thousands of species (Barata, et al.2012). This statement coincided with the present investigation that the greater microbial population were observed in the rhizosphereregion than the non-rhizosphere region also the rhizosphere effect was greater in the bacteria than in the actinomycetes and fungi (Table 2). The rhizosphere effect increased with the age of the plant and normally reached its maximum at the stage of greater vegetative growth. Following the death of the plant, the microbial population reverted gradually to the level as that of the surrounding soil. The viable nature of Rhizobium in the soil is more when compared to other soil microbes and its sustainability mainly depends on the physical and chemical nature of the soil (Chaparro, et al. 2013). This was true in this study because, during rhizosphere bacterial analysis, the quantity of Rhizobium is more when compared to other bacterial counts (Fig 3) also, the microbial load is more in the rhizosphere region compared to the count of non-rhizosphere bacterial load (Fig2).
The flourishing nature of the rhizosphere could be increased by the addition of either biofertilizer or by carrier-based inoculum in the rhizosphere region of the plant (Owen, et al. 2015).The presence of efficient phosphate solubilizing microorganisms in the rhizosphere of crops and soil increases the availability of phosphorous from an insoluble source of phosphates and make it readily available to plants (Sahu and Brahmaprakash, 2016). In this study, next to Rhizobium the concentration of PSB is more when compared to its following microbial count. Most of the phosphates solubilizing organisms are *Pseudomonas*sp and *Bacillus* sp. Rhizosphere effect not only enhances the microbial population but also acted as antierodibility by improving soil physical processes such as aggregate stability (Zhenhong, et al.2020). Fromthis study, it is concludedthat the plants and microorganisms release exudates which improve the soil environment and provide food for animalsandmicrobes in the soil.

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Ethics Statement: This article does not contain any studies with human participants or animals.

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