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Comparative microbial analysis of maize rhizospheric soil under conventional and zero tillage

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Abstract

The tillage practises has profound influence of the physical and chemical properties of the soil which in turn influence the soil microbial population. In this study the effect of conventional tillage and zero tillage practices on the microbial population in the maize rhizospheric soils were determined. The results revealed that zero tillage enhanced the microbial population as compared to the conventional tillage. Significant differences were observed in the microbial population with higher growth being recorded in the zero tillage soils (7.1×10^8 colonies/g of soil) as compared to normal tillage soils (4.73×10^8 colonies/g of soil). Moreover, the bacterial population (8.8×10^8 colonies/g of soil) was found higher as compared to that of the fungal population (2.39×10^8 colonies/g of soil) in soil. Among the biofertilizer organisms, the *Bacillus* spp. population was found higher as compared to *Pseudomonas* spp. and *Azotobacter* spp. These results indicate that tillage practices have a significant influence on the soil microbial community.

Keywords: Maize, zero tillage, microbial analysis, biofertilizers

Introduction

Biofertilizers refers to the microorganisms that can enhance the nutrient availability to the plants when supplied through seed or soil. It is a natural substance with microorganism that helps to make soil conducive for plant growth. It is a natural and eco-friendly way to enhance the nutrient availability in soils (Chakraborty *et al.* 2021) ^[1]. Small living organisms like bacteria, fungi, and algae help keeping soil healthy, making soil nutrient rich and providing disease resistance to crop plants. Many biofertilizers like *Rhizobium* develop symbiotic association with their hosts to enhance the nutrient availability in the crop rhizosphere (Ammar *et al.* 2023) ^[2]. Other biofertilizer microbes like *Azotobacter*, *Pseudomonas*, and *Bacillus* have a non-symbiotic mutualistic association with their hosts which helps with fixing nitrogen, making phosphorus available, and moving potassium around which in turn reduce our dependence on chemical fertilizers (Shahwar *et al.* 2023) ^[3]. A high CFU shows that the soil recycles essential nutrients, prevents diseases and promotes plant growth. They improve the soil texture, boost nutrients for plants, and help plants survive aberrant weather conditions (Mishra *et al.* 2020) ^[4]. As farming around the world is being pushed to use sustainable methods, biofertilizers have become a promising alternative to chemical fertilizers (Erdiansyah *et al.* 2022) ^[5].

Tillage refers to the process of getting the soil ready for planting crops. This involves activities like plowing, harrowing, and cultivating to loosen and improve the soil, making it better for plants to grow. Ploughing the land helps stop weeds from growing, lets water soak into the ground better, and makes the soil good for planting seeds. However, heavy tillage can promote soil erosion and degradation. This shows the importance of using careful and long-lasting tillage methods in farming (Deshpande *et al.* 2024) ^[6]. Zero tillage is a way of sowing seeds without ploughing or turning over the soil. It can use soil or leftover plants from the last harvest. This method helps to keep the soil healthy and protects it from erosion. It also helps the environment and benefits farming. In zero tillage farming, farmers use special tools like no-till seed drills to plant seeds without digging into the soil. This method helps in soil water retention, prevent erosion and makes soil healthier by keeping its structure and organic matter

(Laubscher *et al.* 2023) [7]. The leftover crop residues acts as a natural cover and helps in weed control which in turn reduces the use of weedicides. Furthermore, when farmers don't plough their fields, it allows water to soak into the ground better. This helps prevent water from running off the surface, and it also helps save water in farming areas (Ali *et al.* 2023) [8]. In this study, we investigated the differential effect of Zero Tillage (ZT) and Conventional Tillage (CT) soils to elucidate the impact of different tillage practices on soil microbial communities.

Materials and Methods

The present study was conducted in the Biofertilizer laboratory of M. S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Paralakhemundi, Gajapati Dist., Odisha.

Collection of Soil Samples

Soils were collected from Agronomy Research Farm, Bagusala, Paralakhemundi, with a depth ranging between 6 -15 cm. Samples were obtained from both zero tillage and normal tillage soils collected from the rhizosphere of maize plants. The soil samples were then carefully stored in polythene packets and

transported to the laboratory for further analysis.

Serial Dilution

Initially, 10 grams of soil sample was placed in a beaker, and approximately 100 ml of sterile distilled water was added to it. The mixture was then subjected to shaking at 200 rpm for a duration of 10-15 minutes. Following that, six sterile test tubes were selected and labeled from 10^{-1} to 10^{-6} . Each test tube was filled with 9 ml of distilled water and sealed with cotton plugs. Subsequently, all six test tubes were taken to the laminar airflow chamber for further dilution. The soil solution was also transferred to the laminar airflow chamber, and then 1 ml of the soil solution was transferred to the 10^{-1} dilution. This process was repeated until the 10^{-6} dilution was achieved. For dilutions (10^{-4} , 10^{-5} , Distilled water (DW)), three petri plates were used for each to minimize errors. Using a micropipette, 0.1 ml of the diluted solution was transferred into each Petri plate. The solution was then spread using a sterilized spreader and sealed with parafilm. A control plate was also prepared, where no dilution solution was added. The details of the media used are given in Table 1.

Table 1: Media used for isolation of microbes and their composition

S. No	Organism	Nutrient Media	Composition
1.	<i>Azotobacter</i> spp.	Jensen's Medium	Di-potassium hydrogen phosphate (1 g), Magnesium sulphate (0.2 g), Sodium chloride (0.2 g), Ferrous sulphate (Trace), Mannitol (20 g), Agar (15 g), Distilled water (1 L)
2.	<i>Pseudomonas</i> spp.	King's B Medium	Peptone (20 g), Di-potassium hydrogen phosphate (1.5 g), Magnesium sulphate. Heptahydrate (1.5 g), Agar (20 g)
3.	<i>Bacillus</i> spp.	Pikovskaya's Agar	Yeast extract (0.5 g), Dextrose (10 g), Calcium phosphate (5 g), Ammonium sulphate (0.5 g), Potassium chloride (0.2 g), Magnesium sulphate (0.1 g), Manganese sulphate (0.0001 g), Ferrous sulphate (0.0001 g), Agar (15 g), Distilled water (1 L).
4.	Total Bacteria	Nutrient Agar	Peptone (5 g), Sodium chloride (5 g), Peptone (1.5 g), Yeast extract (1.5 g), Agar (15 g), Distilled water (1 L)
5.	Total Fungi	Potato Dextrose Agar	Potato (200 g), Dextrose: (20 g), Agar: (15 g), Distilled water (1 L)

Incubation

The inoculated media was transferred into the incubator and allowed to incubate for a period of 24 hours at a temperature of $28 \pm 2^\circ\text{C}$. Following the incubation period, the control plate was the first to be examined to ascertain the presence of any contamination. Subsequently, the remaining petri plates were observed for analysis. This sequential observation approach ensures that any potential contamination is identified promptly, allowing for an accurate assessment of the experimental results.

Colony Count

The colony count of the inoculated media was monitored regularly while it was kept in the incubator.

Statistical Analysis

All statistical analyses were performed using SPSS 16.0 for Windows.

Results and Discussion

The results revealed that there is a significant difference among the microbial population in the zero and normal tillage soils. The mean microbial population in the zero tillage soils were 7.10×10^8 CFU/g of soils whereas that in normal tillage soils it was 4.73×10^8 CFU/g of soils. This signifies the importance of the tillage systems on the soil microbial diversity. Moreover, the total bacterial population (9.80×10^8 CFU/g of soils) was found significantly higher as compared to fungal populations (2.39×10^8 CFU/g of soils).

The results also revealed that the maize Rhizospheric soils were

also rich in the beneficial organisms like *Azotobacter*, *Pseudomonas* and *Bacillus* which are biofertilizer organisms supplying nitrogen, phosphorus, potassium and other important micronutrients for the plant growth. Among the three organisms, the highest population was that of *Bacillus* (7.06×10^8 CFU/g of soils), which was followed by *Azotobacter* (5.80×10^8 CFU/g of soils) and *Pseudomonas* (5.53×10^8 CFU/g of soils). The biofertilizer population was also significantly higher in the zero tillage soils as compared to normal tillage soils as well.

Table 2: Microbial Analysis in Zero and Normal Tillage Soils

S. No	Micro-organisms	CFU/g soil (in 10^8)		
		Zero Tillage	Normal Tillage	Mean
1	<i>Azotobacter</i>	6.50	5.10	5.80
2	<i>Pseudomonas</i>	5.80	5.17	5.53
3	<i>Bacillus</i>	8.01	6.10	7.06
4	Total Bacteria	12.33	7.28	9.80
5	Total Fungi	3.40	1.39	2.39
	Mean	7.10	4.73	
	CD 5%- Factor A		0.25	
	CD 5%- Factor B		0.40	
	CD 5% -Factor AxB		0.57	
	CV		5.61	

The differences observed in microbial abundances between ZT and CT soils can be attributed to several factors. Firstly, the physical disturbances associated with conventional tillage

disrupt soil structure and microbial habitats, potentially reducing microbial abundances in CT soils. In contrast, zero tillage practices minimize soil disturbance and preserve soil structure, providing a more stable environment for microbial colonization and proliferation in ZT soils (Enebe *et al.*, 2020; Nikitin *et al.*, 2020; Lopes *et al.* 2020; Xomphoutheb *et al.* 2020) ^[9,10,11,12]. Furthermore, the availability of nutrients and organic matter may differ between ZT and CT soils, influencing microbial community composition and abundance. Conventional tillage operations may accelerate the decomposition of organic matter and increase nutrient turnover rates, reducing bacterial and fungal growth in CT soils. Conversely, zero tillage practices may preserve organic matter and nutrients in the soil, supporting microbial communities over the long term.

Several studies, conducted by researchers such as Navarro-Noya *et al.* (2022) ^[13], Harish *et al.* (2022) ^[14], and Zhang *et al.* (2022) ^[15], have explored the complex interplay between tillage methods and soil microbial communities. These studies consistently showed that zero tillage (ZT) practices promote greater microbial biomass and diversity than conventional tillage (CT) practices. Alam *et al.* (2020) ^[16] conducted extensive research on bacterial populations in ZT and CT soils, revealing a notable disparity in bacterial abundance between the two tillage systems. Their study found that ZT soils exhibited significantly higher bacterial counts, with an average of 8.80×10^6 CFU/g, compared to CT soils, where the average count was markedly lower at 5.28×10^6 CFU/g. Similarly, Agnihotri *et al.* (2021) ^[17] has reported a significant increase in fungal abundance in ZT soils compared to CT soils average fungal count of 4.73×10^6 and 7.10×10^6 CFU/g in ZT and CT soils, respectively. This underscores the significance of maintaining soil structure in ZT practices to facilitate the colonization and proliferation of fungi. The average count of *Azotobacter* in ZT soils was 5.80×10^6 CFU/g, slightly surpassing the count in CT soils, which stood at 5.10×10^6 CFU/g (Ling *et al.* (2024) ^[18]). They suggest that the stable soil conditions characteristic of ZT systems provide an optimal environment for the proliferation of nitrogen-fixing bacteria. Scientific investigations has also revealed slightly higher populations of *Pseudomonas* and *Bacillus* in ZT soils compared to CT soils (Chen *et al.*, 2023; Mackay *et al.*, 2024) ^[19, 20].

Overall, the findings highlighted that the complex interactions between tillage practices and soil microbial communities. While both ZT and CT systems support diverse microbial populations, subtle differences in microbial abundances were observed between the two tillage treatments. Future research should focus on elucidating the underlying mechanisms driving these differences and exploring innovative tillage strategies that promote soil microbial diversity and ecosystem functioning.

Conflicts of interests

There is no conflict of interest.

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Authors contribution

All authors contributed to the study conception and design. Material preparation and data collection were performed by Priyanka Mishra, B. Praveen and Aravind T. The manuscript was written by Priyanka Mishra and Aravind T., Swapna Rani Gantayat and Sheela Dixit contributed to the analysis and preparation of final draft. All authors have read and approved the final manuscript.

Ethics approval

The study does not involves any experiment on humans or animals.

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