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Characterization of Microbial Community of Turmeric (*Curcumin longa*) Rhizosphere Treated with Diverse Organic Waste and Effect on Yield

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Abstract
In this study, the impact of the addition of various organic manure on soil microbial community during turmeric growth was investigated. The organic treatments were poultry manure, swine waste, cocoa husk and cow dung applied at the rate of 6 t/ha, while NPK (15:15:15) fertilizer applied at the rate of 400 kg/ha was used as the standard to compare the organic waste and absolute control (no fertilization). The results showed that each organic treatment had a distinct rhizosphere microbial community and improved the physiochemical properties of the soil. The soil treated with poultry manure had 6 bacterial and 3 fungal isolates, swine waste gave 4 bacterial and 2 fungal, soil treated with cow dung had 6 bacterial and 2 fungal, while cocoa husk had 4 bacterial and 3 fungal isolates. NPK, absolute control and pre-cropping had only one bacterial isolate (E. coli) and no fungi. The result of the yield showed poultry manure (22.28 t/ha), cow dung (20.66 t/ha), cocoa husk (20.39 t/ha), swine waste (16.17 t/ha), NPK (10.61 t/ha) and zero application (3.11 t/ha). A positive correlation was observed between microbial load and soil mineral content and microbial load and plant yield.
Keywords: Microbial community, Rhizosphere, Organic wastes, and Turmeric

Introduction

Bacteria live in the soil. The number and type of bacteria found in different soils are influenced by the type of plants grown on the soil, the soil conditions including temperature and moisture as well as the mineral content of soil. The concentration of bacteria that colonize the roots of plants, the rhizosphere, is much greater than those in the rest of the soil. This is because of the nutrients from root exudates which include sugars, amino acids, organic acids, and other small molecules. A particular bacterium can affect different plants disparately. The interaction between soil bacteria and plants may be beneficial, harmful, or neutral to the plant. One of the mechanisms of bacterial plant growth promotion is providing plants with nutrients that they lack such as fixed nitrogen, iron, and phosphorus. All those bacteria inhabiting plant roots and influencing plant growth positively by any mechanism are referred to as plant growth-promoting rhizobacteria (PGPR).

Plant Growth-Promoting Rhizobacteria (PGPR) play a crucial role in enhancing plant growth and health through both direct and indirect mechanisms. Directly, PGPR can boost plant growth by aiding in resource acquisition and regulating plant hormone levels. Indirectly, they act as biocontrol agents, counteracting the negative impact of pathogenic organisms on plant development. The significance of PGPR in agriculture has been widely recognized, with various bacterial species such as Pseudomonas, Azospirillum, Azotobacter, Bacillus, Klebsiella, Enterobacter, Xanthomonas, and Serratia being identified as potent promoters of plant growth.

The ability of PGPR to influence plant growth has been extensively studied and documented in scientific literature. For instance, Glick (1995) and Glick *et al*. (1999) have highlighted the diverse mechanisms through which PGPR can positively impact plant physiology. By understanding the complex interactions between PGPR and plants, researchers and farmers can harness the potential of these beneficial bacteria to optimize agricultural practices and improve crop yield.

In the context of turmeric cultivation, the role of PGPR in the rhizosphere microbiome is of particular interest. The turmeric rhizosphere, the region surrounding the plant's roots, harbours a diverse community of microorganisms that interact with the plant and influence its growth and development. Identifying the specific microorganisms present in the turmeric rhizosphere and elucidating their functions is essential for developing strategies to enhance plant growth and overall crop health.

Therefore, a comprehensive analysis of the turmeric

rhizosphere microbiome is crucial for unlocking the full potential of PGPR in promoting plant growth. By exploring the interactions between PGPR and turmeric plants at the microbial level, researchers can uncover novel insights into how these beneficial bacteria contribute to plant health and productivity. This knowledge can pave the way for the development of sustainable agricultural practices that leverage the power of PGPR to ensure optimal plant growth and yield in turmeric cultivation.

Materials and Methods

Turmeric (*Curcuma longa*) was grown with different organic fertilizers. The fertilizers consisted of four organic waste matter (poultry manure, cow dung, pig waste and cocoa husk) applied at the rate of 6 t/ha one week before planting. NPK fertilizer applied at the rate of 400 kg/ha was used as the standard to compare the organic waste and absolute control (zero fertilizer). The experimental design used was RCBD with 4 replications. The rhizospheres of the different treatments were evaluated for microbial diversity and microbial count, and the yield of the rhizome was also determined.

Analysis of physicochemical properties of the study soil

Soil samples were taken from a depth of 20 – 40 cm at the beginning of the experiment before planting and after harvesting. The macro-Kjeldahl digestion method (Bremner and Mulvaney, 1982) was used to determine total N, organic carbon (OC) was determined by the dichromate oxidation method of Walkley and Black method (Nelson and Sommers, 1982), Organic matter (OM) determination was by multiplying OC expressed in percentage with the conventional Van Bernmeller factor of 1.724. Soil pH (H_2O) was measured (soil/water ratio of 1:2.5) with a digital pH meter (McLean, 1982), available P was determined by the Bray 2 method (Bray and Kurtz, 1945), exchangeable K in extract estimated by flame photometry, exchangeable acidity was determined by the titrimetric method after extraction with1.0 M KCl (McLean, 1982) and effective cation exchange capacity (ECEC) was determined by the sum of the exchangeable bases and the exchangeable acidity.

Isolation of bacteria from soil samples

The soil microorganisms were isolated by adopting the method by Fawole and Oso (2007). One gram of sieved soil was weighed into a 50 ml sterile beaker to which 10 ml of sterile water was added, the beaker was swirled to mix until a homogenous solution was obtained. The solution was allowed to settle, and a tenfold serial dilution was performed with 1 ml of the supernatant. Simply, 1 ml of the supernatant was transferred into the first beaker containing 9 ml of sterile water and labelled 10^{-1}, the same process was repeated for the second test tube by collecting 1 ml from the tube labelled 10^{-1} and transferring it into the tube labelled 10^{-2}. The process was repeated up to the last test tube labelled10^{-10}.

To obtain disease colonies of 30 – 300 cfu (colony forming units) bacteria, only tubes labelled 10^{-4} - 10^{-7} dilutions were used. Five plates of nutrient agar were prepared into which (with the use of a micropipette) 100µl (0.1 ml) of the required diluent (10^{-4}, 10^{-5}, 10^{-6} or 10^{-7}) was dropped at the center of the appropriately labelled plate. The plate was left for a while until diluent was well absorbed into the nutrient agar, the plate was tapped, inverted, and kept in an incubator at 37^0C for 18 - 24 hrs. After incubation, the number of each bacterial isolates were counted and recorded. The colony forming units (cfu) were calculated thus:

$$\text{Cfu/gm (wet soil)} = \frac{\text{No. of colonies x dilution factor}}{\text{Volume of the sample plated}}$$

Data Analysis

All data in the study were subjected to ANOVA and significant means were separated at a 5% level.

Results and Discussion

The nutrient composition analysis of various organic wastes, as presented in Table 1, reveals that all the evaluated organic wastes are abundant in essential nutrients necessary for plant growth. Among them, poultry manure and swine waste stand out as particularly rich sources of Nitrogen, with notably high pH levels. When these organic materials are applied to the soil, they not only provide valuable nutrients but also contribute to improving soil properties and increasing soil pH, as illustrated in Table 3. This elevation in soil pH can have significant implications for enhancing soil microbial activities. Studies, such as the one conducted by Keum *et al*. (2015), have indicated that bacterial populations tend to thrive in environments with neutral pH levels. Therefore, the application of organic wastes rich in nutrients and with the potential to raise soil pH can create favourable conditions for the proliferation of beneficial soil bacteria. This, in turn, can lead to increased microbial activities in the soil, further enhancing nutrient cycling, organic matter decomposition, and overall soil health. The synergistic effects of nutrient-rich organic wastes and elevated soil pH on soil microbial communities can have profound impacts on crop production. By creating a conducive environment for beneficial bacteria to flourish, farmers can potentially improve nutrient availability to plants, enhance soil structure, and promote overall plant health. The enhanced microbial activities resulting from the application of organic wastes can contribute to better soil fertility, increased nutrient uptake by plants, and ultimately, higher crop yields.

The findings from the pre-cropping and pre-treatment analysis presented in Table 2 indicate that the soil initially exhibited acidic conditions with a pH of 4.9 and was deficient in essential nutrients such as nitrogen and potassium. However, following the treatment and nutrient utilization by the turmeric crop, as shown in Table 3, significant improvements in soil nutrient levels were observed compared to the control. Additionally, the application of organic matter led to an increase in soil pH, further enhancing soil nutrient availability. The enhancement of soil properties, including nutrient levels and pH, can be primarily attributed to the nutrient content of the organic matter used in the treatment.

Organic materials, such as poultry manure and swine waste, are rich sources of nutrients that can replenish the soil and support plant growth. By incorporating organic matter into the soil, farmers can not only supply essential nutrients to the crops but also improve soil structure, water retention, and microbial activity. The influence of organic matter on soil nutrients and microbial status, as highlighted by Keum *et al*. (2015), underscores the importance of sustainable soil management practices in agriculture. The application of organic amendments can have a profound impact on soil health by enriching nutrient content, stimulating microbial populations, and promoting overall soil fertility. The increase in soil pH resulting from organic matter application further contributes to creating a favourable environment for soil microbes to thrive and facilitate nutrient cycling processes.

The data presented in Table 4 indicates that the addition of organic matter to soil has a positive impact on soil microbial diversity. This observation aligns with the findings reported by Lee *et al*. (2019), who noted an increase in microbial activity following the application of organic matter. Specifically, the study revealed that soil treated with poultry manure exhibited the highest microbial diversity, followed by cow dung. In contrast, soil treated with NPK fertilizer and the untreated control soil showed the lowest microbial populations, with only one bacteria isolate and no fungus detected. Furthermore, the microbial count results illustrated in Figure 1 support these findings, with poultry manure and cow dung treatments resulting in higher microbial counts compared to NPK fertilizer and the control group. The presence of a diverse and abundant microbial community in soils treated with organic matter underscores the role of these amendments in promoting microbial activity and diversity, which are essential for nutrient cycling, organic matter decomposition, and overall soil health. The comparison of different treatments for crop yield, as depicted in Figure 2, further highlights the benefits of organic matter application. The data shows that soil treated with poultry manure resulted in the highest yield, indicating a positive correlation between microbial diversity, soil health, and crop productivity. Cow dung also contributed to increased yield, albeit to a slightly lesser extent. In contrast, NPK fertilizer and the untreated soil exhibited lower yields, underscoring the importance of organic amendments in supporting plant growth and improving agricultural outcomes.

Table 1 shows that the different organic manure treatments affected the microbial diversity at the rhizosphere. The soil treated with poultry manure had 6 bacterial and 3 fungal isolates, swine waste gave 4 bacterial and 2 fungal, cow dung had 6 bacterial and 2 fungal, while cocoa husk had 4 bacterial and 3 fungal isolates. The diversity of microorganisms shows that there could be available macronutrients present in the soil because of the treatments. Soil treated with NPK fertilizer, and soil with zero treatment (control) contained only 1 bacterial isolate each and zero fungi suggesting that NPK does not encourage microbial growth. Pre-cropping soil microbial analysis showed the presence of 2 bacterial isolates and zero fungi. Among already identified plant growth-promoting rhizobacteria (PGPR) (Vejan *et al.,* 2016; Glick, 2012), the bacteria Klebsiella spp. and Pseudomonas aeruginosa were identified in turmeric rhizosphere in all the organic matter treated soil samples. Pseudomonas aeruginosa was found in all except in swine waste, while Klebsiella spp. was present in all the organic matter treated soil. Comparative analysis of microbial colonization with rhizome yield (fig 2) showed poultry manure gave the highest yield while cocoa husk gave the list among the treated soils. This can be attributed not just to the levels of the microbial loads (Fig 1), but also to the nutrient composition of the organic matter (table 1) which showed coca husk having the least amount of nutrients.

The correlation analysis presented in Tables 4 and 5 indicates a positive relationship between soil mineral content, microbial load, and crop yield, which is consistent with the findings reported by Olojede *et al*. (2011). This suggests that higher levels of soil minerals and microbial populations are associated with increased crop yields, highlighting the importance of soil health and microbial activity in supporting plant growth and productivity. One of the key mechanisms through which plant growth-promoting rhizobacteria (PGPR) enhance plant growth is by making nutrients more accessible to plants. Organic fertilizers and manures, which are rich sources of nutrients and organic matter, have been shown by various researchers, including Olojede *et al*. (2011), Senobi (2010), and Njoku *et al*. (2014), to improve soil health, increase nutrient use efficiency in root and tuber crops, and ultimately boost yields. By supplying essential nutrients and fostering a favourable soil environment, organic amendments play a crucial role in supporting plant growth and development. Based on these observations, we can hypothesize that the application of organic matter to soil enhances microbial growth and diversity, which in turn facilitates nutrient uptake by plants. The presence of a diverse microbial community in the soil, including plant growth-promoting rhizobacteria, can help improve nutrient availability to plants, promote root development, and enhance overall plant health. Additionally, certain beneficial rhizobacteria have been reported to exhibit antimicrobial properties, which can help suppress the growth of pathogenic organisms in the rhizosphere, further supporting plant growth and health.

Conclusion

The analysis of treated turmeric rhizosphere has identified Pseudomonas spp. and Klebsiella as prevalent plant growth-promoting rhizobacteria (PGPR) across all treatments. The consistent presence of these beneficial microbes suggests their potential role in enhancing rhizome yield in turmeric cultivation. These findings indicate that Pseudomonas and Klebsiella species hold promise as effective PGPR that can positively impact turmeric seed production. Given the

observed correlation between the presence of Pseudomonas and Klebsiella species in the rhizosphere and increased rhizome yield, it is recommended that further evaluation of these PGPR be conducted in the context of organic farming practices. By incorporating Pseudomonas and Klebsiella species as part of organic farming strategies, farmers may be able to leverage the beneficial effects of these microbes to improve turmeric seed production. The identification of Pseudomonas and Klebsiella species as potential PGPR highlights the importance of understanding and harnessing the beneficial interactions between plants and soil microbes in agricultural systems. By focusing on the promotion of these specific PGPR, organic farmers can potentially enhance the growth, health, and yield of turmeric crops in a sustainable and environmentally friendly manner.

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Table 1: Average Nutrient Composition of organic waste used in the experiment

Property	Poultry manure	Swine waste	Cow dung	Cocoa husk
Nitrogen (%)	2.88	2.19	1.88	1.46
Phosphorus (%)	1.94	1.70	2.02	1.80
Potassium (%)	1.44	1.43	1.39	1.50
C: N ratio	11.44	13.74	15.11	19.58
pH	8.5	8.8	6.9	7.7
Organic matter (%)	56.54	51.90	48.97	49.31

Table 2. Soil physicochemical properties before cropping and treatment application

Soil properties	Value
Nitrogen (%)	0.093
Phosphorous (mg/kg)	14.8
Calcium (cmol/kg)	3.6
Magnesium (cmol/kg)	1.0
Potassium (cmol/kg)	0.211
Sodium (cmol/kg)	0.103
Exchangeable acidity (cmol/kg)	1.72
pH	4.9
Organic matter (%)	1.64
Microbial load (x 10^6 cfu/ml)	2.0

Table 3: Post-cropping soil physiochemical properties with treatments

Soil properties	Poultry manure	Swine waste	Cow dung	Cocoa husk	Control
Nitrogen (%)	0.217	0.241	0.200	0.184	0.081
Phosphorous	23.43	20.63	19.67	18.57	12.43
Calcium (cmol/kg)	7.0	6.26	5.56	5.2	2.9
Magnesium (cmol/kg)	1.77	1.53	2.1	1.03	0.50
Potassium (cmol/kg)	0.397	0.350	0.310	0.427	0.068
Sodium (cmol/kg)	0.306	0.287	0.274	0.268	0.082
Exchangeable acidity (cmol/kg)	9.82	8.91	8.93	7.72	5.26
pH	6.5	6.7	5.9	5.8	4.3
Organic matter	3.81	3.11	3.53	3.04	1.01

Table 4: Effect of application of organic matter on soil microbial diversity

Microorganisms	Poultry manure	Swine waste	Cow dung	Cocoa husk	NPK	Control	Pre-cropping
Bacterial isolate (24hrs incubation)	E. coli Staphylococcus aureus Klebsiella spp. Enterococci spp. Pseudomonas aeruginosa Actinomycetes	E. coli Staphylococcus aureus Klebsiella spp. Actinomycetes	E. coli Staphylococcus -Klebsiella spp. Enterococci spp. Pseudomonas aeruginosa Actinomycetes	Klebsiella spp. Enterococci spp. Pseudomonas aeruginosa Actinomycetes	E. coli	E. coli	E. coli Pseudomonas aeruginosa
Fungal isolate (72 hrs. incubation)	Aspergillus spp. Rhizopus spp. Fusarium spp.	Rhizopus spp. Fusarium spp.	Aspergillus spp. Rhizopus spp.	Aspergillus spp. Rhizopus spp. Fusarium spp.	Nil	Nil	Nil

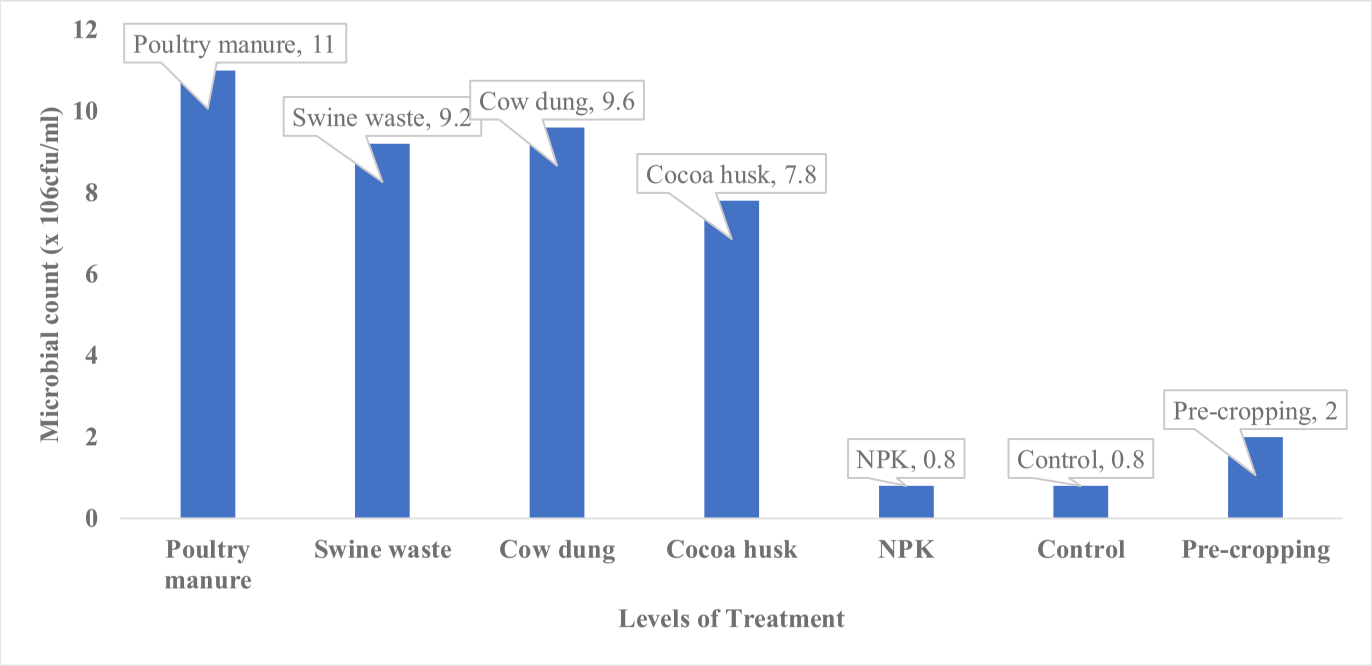

Fig. 1: Effect of the levels of treatment on microbial load at the rhizosphere

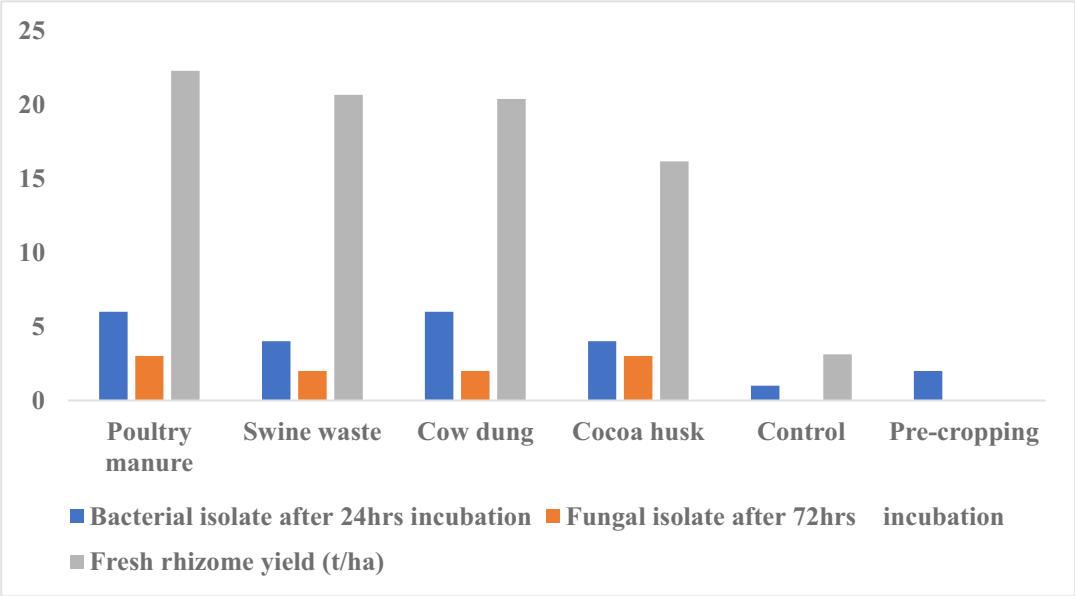

Fig 2: Comparative analysis of microbial colonization with rhizome yield

Table 5: Correlation analysis of Minerals and yield

	Yield
Nitrogen	0.98*
Calcium	0.96*
Magnesium	0.84*
Potassium	0.84*
Sodium	0.99*
Exchangeable acidity	0.93*

**** Mean significant at 0.001***

Table 6: Correlation analysis of microbes and yield

	Yield
Bacterial	0.97*
Fungi	0.91*
Total actinomycete	0.93*

**** Mean significant at 0.001***