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Exploration of microbial biodiversity for their functionality in different fodder crops in custardapple based Horti-pastoral system

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Abstract

Soil microorganisms play a crucial role in the biochemical and biological processes that govern soil health and sustain agricultural production. Among these microorganisms, plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth through various mechanisms, including improving plant nutrition, producing and regulating phytohormones, and suppressing disease-causing organisms. Of the 52 isolates, 46 were unique in shape, colour, and margin, and were purified based on their morphological characteristics, followed by enumeration. Enumeration was conducted at three intervals in custardapple intercropped with fodder crops (Anjan and Guinea grasses), with the first interval yielding the highest CFU counts. This study demonstrates the significant impact of organic manures and nutrient management on microbial populations and crop yields in a custard apple-based intercropping system. Organic treatments, particularly T3 (75% RDN + 25% nitrogen through Pongamia green leaf manuring), resulted in the highest microbial counts (bacteria, fungi, and actinomycetes) in both Anjan (M1) and Guinea grass (M2). Bacterial populations were 120×10^6 CFU g^{-1} of soil in M1 Anjan grass and 140×10^6 CFU g^{-1} in M2 Guinea grass. Fungal populations were 35×10^3 CFU g^{-1} of soil in M1 Anjan grass and 30×10^3 CFU g^{-1} in M2 Guinea grass. Actinomycetes populations were 70×10^3 CFU g^{-1} of soil in M1 Anjan grass and 80×10^3 CFU g^{-1} in M2 Guinea grass. Guinea grass supported higher microbial activity, possibly due to favourable root exudates or soil interactions. In contrast, the lowest microbial populations were recorded in the control (T1) and 100% RDF (T2) treatments, underscoring the superior role of organic amendments in enhancing soil microbial diversity. Higher microbial populations in Guinea grass (M2) correlated with higher herbage (22.6 t/ha) and dry fodder yields (4.51 t/ha), while Anjan grass (M1) had lower yields (17.9 t/ha and 3.58 t/ha, respectively). Similarly, in custard apple, T7 (75% RDN + FYM) consistently yielded the highest bacterial (80×10^6 CFU g^{-1}), fungal (33×10^3 CFU g^{-1}), and actinomycetes (80×10^3 CFU g^{-1}) populations, resulting in the highest fruit yield (6,842 kg/ha) and fruit number per tree (62). In contrast, the control (T1) had the lowest microbial counts and fruit yield (4,826 kg/ha). The findings emphasize the positive correlation between higher microbial populations and increased yields in both fodder and custard apple production.

This resulted in increased herbage yield and dry fodder yield (t ha⁻¹) and custardapple fruit yield under treatment amended with T3 (75% RDN + 25% N through PGLM) and T7 (75% RDN + FYM). This study explores the microbial biodiversity associated with various fodder crops in a custard apple-based Horti-pastoral system, focusing on identifying key microbial communities and evaluating their functional roles in enhancing crop productivity, soil health, and overall system sustainability.

Keywords: Soil microorganisms, bacteria, fungi, actinomycetes.

Introduction

Agricultural crops and tree species are ubiquitously associated with a variety of microorganisms. The communities of bacteria, archaea, fungi, and protists that colonize plant tissues and inhabit outer plant surfaces collectively make up the plant microbiota. The plant microbiome, or phytobiome, is defined as the complete set of microbial genomes associated with a host plant (Guttman *et al.*, 2014) [9]. The importance of plant-associated microbes in enhancing host plant health, nutrition, and stress resistance is widely recognized (Vandenkoornhuysen *et al.*, 2015) [19]. However, this relationship is influenced by numerous factors, including host and microbial genotypes, interactions within the microbiota, and various abiotic factors (Hardoim *et al.*, 2015) [10].

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Forests which cover 30% of the total global land area are found to be highly productive components of terrestrial ecosystems (Llado *et al.*, 2017) ^[12].

Furthermore, trees aid in soil structure maintenance, organic matter decomposition, pollutant degradation, and so on (Thakur *et al.*, 2018) ^[16]. Some of bacterial taxa previously shown to dominate forest soil ecosystems and play such key roles include members of the genera *Pedobacter* and *Chitinophaga* (Bacteroidetes); *Pseudomonas*, *Variovorax*, *Ewingella*, and *Stenotrophomonas* (Proteobacteria) (Brabcova *et al.*, 2016) ^[5]; *Burkholderia*, *Phenylobacterium* and *Methylovirgula* (Pseudomonadota), members of the *Rhizobiales* and *Nitrosopumilus*.

Soil microorganisms are an important component of the forest ecosystem (Uroz *et al.*, 2016) ^[18] as they play fundamental roles in most nutrient transformations upon which the stability and sustainable development of forest ecosystems rely. The distribution and diversity of soil microbiomes is influenced by numerous aspects such as soil type, physicochemical characteristics, microclimate, vegetation and land-use (Gupta *et al.*, 2017) ^[8]. The interaction of plants with microbes in the rhizospheric region helps in maintaining the soil fertility and health of the plant (Kumar *et al.*, 2019) ^[11].

Bacteria in forest soils play key roles in organic matter decomposition (López-Mondéjar *et al.*, 2016) ^[13], nitrogen fixation, and mineral weathering. Their interactions with mycorrhizal fungi, which enhance nutrient cycling and soil connectivity, are supported by mycorrhiza helper bacteria, influencing overall forest soil health and function (Bonito *et al.*, 2014) ^[4]. Fungi are the primary decomposers in temperate and boreal forest soils, breaking down plant material through extracellular enzymes, and facilitating the cycling of nitrogen, phosphorus, and carbon (Eichlerová *et al.*, 2015) ^[6]. While fungi have been the main focus of forest soil studies, there is a growing recognition of the significant role bacteria play in these ecosystems. Actinomycetes, a dominant group in soil, contribute to organic matter recycling and are crucial in producing antibiotics, enzymes, and pharmaceuticals. Since the discovery of streptomycin, about two-thirds of known antibiotics have been derived from actinomycetes, highlighting their medical and industrial importance (Arifuzzaman *et al.*, 2010) ^[1].

Fodder crops exhibit a dynamic microbial status that influences their growth and nutrient content. Microorganisms, such as nitrogen-fixing rhizobia in legumes, aid in nitrogen fixation and enhance plant fertility. Mycorrhizal associations improve nutrient uptake, especially phosphorus. Beneficial soil microbes contribute to pathogen suppression, reducing the need for chemical pesticides. Microorganisms play a role in silage fermentation, preserving fodder quality. Managing microbial interactions is vital for optimizing the nutritional value and sustainability of fodder crops in livestock agriculture.

Microbial diversity associated with crops, fruit trees, shrubs, forest tree species is considered important for maintaining the sustainability of agriculture/forest/agroforestry production systems. The microbiome of plants could be classified into three categories, e.g., rhizospheric, phyllospheric and endophytic the rhizosphere is the zone of soil influenced by plant roots through the release of different substrates that affect microbial function and its stability (Suman *et al.*, 2016) ^[15]. Though lot of studies were conducted across the globe on various ecosystems, the microbial diversity in hortipastoral systems in Deccan plateau is less studied. Hence, an experiment is being proposed to study the microbial diversity and effect on productivity in custard

apple based hortipastoral system involving different fodders.

Materials and Methods

Soil Sample Collection

Soil samples were collected from Agroforestry research block-II, PJTASU, Rajendranagar, Hyderabad. The samples were collected near the Custardapple intercropped with fodder crops (Anjan and Guinea grasses) rhizosphere region at 15 cm of depth and kept in sterilized polyethene covers and brought to the laboratory.

Isolation of rhizosphere bacteria, fungi and actinomycetes

Bacteria, Fungi and Actinomycetes were isolated by using the serial dilution and plating technique (Arifuzzaman *et al.*, 2010) ^[1]. For the isolation of bacteria, fungi and actinomycetes, fresh soil samples have to be taken. From which 1 gm of each soil sample was suspended in 9 ml of sterile saline containing 0.85% NaCl and mixed with vortex. The suspension was left for 30 min in the Laminar Air Flow hood. Consequently, 1 ml of suspension was taken and diluted 10-fold in 9 ml of sterile normal saline (0.85%). The suspensions were agitated with vortex and 0.1 ml of suspension was taken from dilutions.

Dilutions of 10⁻⁵ and 10⁻⁶ were used for isolation of bacteria while dilutions of 10⁻³ and 10⁻⁴ were used for isolation of fungi and actinomycetes. 0.1ml of respective dilutions were spread onto Nutrient agar medium (NA), potato dextrose agar medium (PDA) or Martin 's Rose Bengal agar medium (RBA), Actinomycetes agar medium for isolation of bacteria, fungi and actinomycetes present in rhizosphere soil. These media plates were incubated at 28±2 °C for 24-48 hours, 25±2 °C for 3-4 days, 28±2 °C for 10-15 days for isolation of bacteria, fungus and actinomycetes respectively. The isolates were differentiated based on colony morphology.

Enumeration of microbes in soil samples

Enumeration of bacteria, fungi and actinomycetes was done on Nutrient agar medium, Rose Bengal agar medium and Actinomycetes agar medium respectively, using serial dilution spread plate technique. The media were prepared and sterilized in an autoclave at 15 psi pressure and 121 °C temperature for 20 minutes.

Serial dilutions (up to 10%) were made by pipetting 1 ml of the soil suspension into 9 ml of sterile water blank. Finally. 0.1 ml aliquot of the diluted soil suspension was uniformly spread with the help of sterilized spreader on solidified petri plates having respective medium. The petri plates were incubated for 1 to 3 days at 28±2 °C in an inverted position. After incubation, the number of colonies appeared on medium plates were counted, averaged and multiplied by the dilution factor to find the number of cells per gram of soil sample

$$\text{CFU/gm} = \frac{\text{Total number of colonies}}{\text{Dilution} \times \text{Volume of the sample}}$$

Morphological characterization of microbial isolates

All the isolates were checked for their purity and then studied for colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950) ^[3].

Morphological characteristics of the colony of each isolate was examined by growth of the isolates on their respective medium and incubated them for 5 to 7 days. Cultural characterization of isolates were observed by different characteristics of colonies such as shape, colour, margin and elevation were recorded.

Results and Discussion

Isolation and Purification of microbial isolates

Microbial isolates were isolated by using nutrient agar for bacteria, potato dextrose agar or rose Bengal agar medium for fungi, Actinomycetes agar medium for actinomycetes and depending on the colony morphology and gram staining the Unique and individual isolates were selected. The isolated colonies were purified by quadrant streak plate method. Moreover, pure cultures were preserved in 20% glycerol stocks to study biochemical characters.

Among the 46 bacterial, fungal and actinomycete isolates studied, 16 bacteria included 10 gram-positive and 5 gram-negative strains, while all 16 actinomycete isolates were gram-positive. The bacterial colonies were typically round or circular with raised, gummy textures, producing exopolysaccharides under optimal conditions. Colony sizes ranged from 1.5 to 4.4 mm, with colours from watery or milky translucent to creamy or opaque white. Actinomycete isolates were cocci and rod-shaped, predominantly rod-shaped and cream-colored. Pigmentation in actinomycetes varied from cream, dark/light brown, grey, green, to white and ash. Their colony surfaces were described as dry, smooth, rough, and powdery, with various penetrating characteristics. The colony shapes included filamentous, circular, and irregular forms. Fungal colonies exhibited irregular, circular, or filamentous morphology, with colours ranging from green to brownish black and pink. Results was depicted in table 7, 8 and 9.

Our results are similar with Azmi *et al.* (2021) ^[2] and El-Tarabily *et al.*, (2021) ^[7] described various fungal and actinomycetes strains. Similarly, Upadhyay *et al.* (2015) ^[17] reported various bacterial strains.

Enumeration of microbes in fodder crops Rhizospheric soil samples

The Enumeration of Total bacteria, fungi and actinomycetes from the rhizosphere soil of fodder crops at three different intervals. The results showed that among the different treatments treated to the two grasses (Anjan and Guinea grass).

Bacteria

At 30 DAT, the highest bacterial population in Anjan grass (M1) was recorded in T3 (PGLM) with 120×10^6 CFU g⁻¹, followed by T4 (NGLM) at 100×10^6 , T5 (SGLM) at 83×10^6 , and T7 (FYM) at 80×10^6 . The lowest was in T1 (control) with 56×10^6 of soil. In Guinea grass (M2), T3 (PGLM) had the highest population of 140×10^6 , while the lowest was in T1 at 50×10^6 of soil. At 60 DAT, T3 (PGLM) remained the highest for Anjan grass with 90×10^6 CFU g⁻¹, followed by T4 at 80×10^6 , and the lowest in T1 at 40×10^6 of soil. In Guinea grass, T3 had the highest population of 80×10^6 , with T1 again the lowest at 40×10^6 of soil. At 90 DAT, T7 (FYM) had the highest population for Anjan grass at 70×10^6 , followed by T3 at 60×10^6 , while T1 showed 30×10^6 of soil. For Guinea grass, T7 was highest at 65×10^6 , with T1 being the lowest at 25×10^6 of soil. Results was presented in table 1.

Fungi

At 30 DAT, the highest fungal count for Anjan grass (M1) was observed in T3 (PGLM) with 35×10^3 CFU g⁻¹ of soil, followed by T6 (Poultry manure) at 23×10^3 and T4 (NGLM) at 20×10^3 of soil. The lowest count was in T1 (control) at 9×10^3 of soil. In Guinea grass (M2), T3 also had the highest fungal count with 30×10^3 , while T1 showed the lowest at 9×10^3 of soil. At 60 DAT, the highest fungal count for Anjan grass was in T3

(PGLM) at 20×10^3 of soil, followed by T4 at 16×10^3 , while T1 had the lowest at 7×10^3 of soil. For Guinea grass, T3 and T5 both recorded 15×10^3 , while T1 had the lowest at 6×10^3 of soil. At 90 DAT, the highest fungal count for Anjan grass was in T3 (PGLM) at 13×10^3 , while T1 had the lowest at 5×10^3 of soil. For Guinea grass, T3 again had the highest count with 13×10^3 , and T1 the lowest at 4×10^3 of soil. Results was presented in table 2.

Actinomycetes

At 30 DAT, the highest actinomycete count for Anjan grass (M1) was in T3 (PGLM) with 70×10^3 CFU g⁻¹, followed by T4 (NGLM) at 55×10^3 . The lowest count was in T1 (control) with 24×10^3 of soil. For Guinea grass (M2), T3 had the highest count of 80×10^3 , and T1 had the lowest at 19×10^3 of soil. At 60 DAT, the highest count for Anjan grass was in T4 (NGLM) at 35×10^3 , while T1 had the lowest at 12×10^3 of soil. For Guinea grass, T4 recorded the highest count at 40×10^3 , while T1 had the lowest at 10×10^3 of soil. At 90 DAT, T4 (NGLM) had the highest count for Anjan grass with 30×10^3 , and T1 had the lowest at 10×10^3 of soil. For Guinea grass, T4 again had the highest count at 25×10^3 , while T1 recorded the lowest at 4×10^3 of soil. Results was presented in table 3.

Comparison Between Grasses

Guinea grass (M2) supported higher microbial populations compared to Anjan grass (M1) across most treatments. For instance, in the first interval, (M2) Guinea grass showed higher bacterial, fungal, and actinomycetes counts under Pongamia (T3) and other organic treatments, showed that Guinea grass fasters more favorable conditions for microbial growth, possibly due to root exudates or soil interactions.

Comparison Between Treatments

Treatment T3 (Pongamia green leaf manuring) consistently had the highest microbial populations across both grasses and all intervals, while T2 (100% RDF) and T1 (Control) had the lowest. This demonstrates the superior effect of organic manures, particularly green leaf manuring, in promoting a rich soil microbial environment compared to chemical fertilizers. Among the treatments, T3 (Pongamia green leaf manuring) consistently resulted in the highest bacterial, fungal, and actinomycetes populations across all intervals for both Anjan grass (M1) and Guinea grass (M2). This can be attributed to the rich organic matter, nitrogen content, and slow-release nutrients provided by Pongamia leaves, which faster sustained microbial activity. Other treatments like T4 (Neem green leaf manuring) and T5 (Subabul green leaf manuring) also contributed significantly to microbial populations, though their effects were slightly less than Pongamia. T6 (Poultry manure) and T7 (FYM) showed moderate microbial counts, while T2 (100% RDF) and T1 (Control) consistently had the lowest microbial counts across all intervals, demonstrating that organic manures, especially green leaf manures, promote better microbial activity than inorganic fertilizers. Similar results were obtained by Singh *et al.* (2021) ^[14], enumerated microbial populations in the soil of Custard apple cv. Balanagar plants, showing increased bacterial (26.67 cfug⁻¹), fungal (15.00 cfug⁻¹), and actinomycetes (10.00 cfug⁻¹) populations.

Enumeration of microbes in Custardapple Rhizosphere soil samples: The Enumeration of Total bacteria, fungi and actinomycetes from the rhizosphere soil of Custardapple at three different intervals.

Bacteria

At the 1st interval (30 DAT), the highest bacterial count was recorded in T7 (75% RDN + 25% N through FYM) with 80×10^6 CFU g⁻¹, followed by T3 (Pongamia green leaf manuring) at 60×10^6 , T5 (Subabul green leaf manuring) at 50×10^6 , and T2 (100% RDF) and T6 (Poultry manure) both at 40×10^6 of soil. Lower counts were in T4 (Neem green leaf manuring) at 35×10^6 , with the lowest in T1 (Control) at 25×10^6 of soil. At the 2nd interval (60 DAT), T7 (FYM) maintained the highest bacterial count at 70×10^6 , followed by T3 (55×10^6), T5 (45×10^6), T2 (36×10^6), T6 (33×10^6), and T4 (30×10^6). The lowest was again in T1 (Control) at 20×10^6 of soil. At the 3rd interval (90 DAT), T7 (FYM) had the highest count at 73×10^6 , followed by T3 (50×10^6), T5 (44×10^6), T2 (35×10^6), T6 (32×10^6), and T4 (23×10^6) of soil. The lowest bacterial count was in T1 (Control) at 15×10^6 of soil. Results was presented in table 4.

Fungi: At the 1st interval (30 DAT), the highest fungal count was observed in T7 (75% RDN + 25% N through FYM) with 33×10^3 CFU g⁻¹, followed by T3 (Pongamia green leaf manuring) at 20×10^3 , T5 (Subabul green leaf manuring) with 17×10^3 , and T6 (Poultry manure) at 15×10^3 . T2 (100% RDF) recorded 14×10^3 , T4 (Neem green leaf manuring) had 12×10^3 , while T1 (Control) had the lowest at 9×10^3 . In the 2nd interval (60 DAT), T7 (FYM) again showed the highest fungal count at 25×10^3 , followed by T3 (15×10^3), T5 (14×10^3), and T6 (12×10^3). T2 recorded 10×10^3 , T4 had 9×10^3 , and T1 (Control) had the lowest count at 6×10^3 . By the 3rd interval (90 DAT), T7 (FYM) maintained the highest fungal count at 18×10^3 , followed by T3 (14×10^3), T5 (12×10^3), and T6 (9×10^3). T2 recorded 8×10^3 , T4 had 7×10^3 , and the lowest count was again in T1 (Control) at 5×10^3 . Results was presented in table 5.

Actinomycetes

In the 1st interval (30 DAT), T7 (75% RDN + 25% N through FYM) showed the highest actinomycetes count with 80×10^3 CFU g⁻¹, followed by T3 (Pongamia green leaf manuring) at 60×10^3 , and T5 (Subabul green leaf manuring) with 50×10^3 . T6 (Poultry manure) recorded 35×10^3 , T2 (100% RDF) had 33×10^3 , T4 (Neem green leaf manuring) recorded 25×10^3 , and T1 (Control) had 19×10^3 . In the 2nd interval (60 DAT), T7 remained highest at 70×10^3 , followed by T3 (55×10^3), T5 (45×10^3), T6 (32×10^3), T2 (30.1×10^3), T4 (20×10^3), and T1 (15×10^3) of soil. By the 3rd interval (90 DAT), T7 and T3 both had 50×10^3 , T5 recorded 40×10^3 , T6 had 30×10^3 , T2 showed 25.083×10^3 , T4 had 17×10^3 , and T1 recorded the lowest at 14×10^3 of soil. Results was presented in table 6.

Comparison Between Treatments

T7 (75% RDN + 25% N through FYM) was the most effective treatment across all microbial categories—bacteria, fungi, and actinomycetes—showed the highest microbial counts in all three intervals. T3 (Pongamia green leaf manuring) consistently ranked second, supporting significant microbial activity, though lower than T7. T5 (Subabul green leaf manuring) demonstrated moderate effectiveness, while T2 (100% RDF), T6 (Poultry manure), and T4 (Neem green leaf manuring) showed varying levels of microbial populations, generally lower than the top two treatments (T2 and T6). T1 (Control), which had no fertilization or manuring, consistently recorded the lowest microbial populations across all categories.

This indicates that organic amendments, particularly FYM and Pongamia leaf manuring, significantly enhance microbial activity in soil compared to chemical fertilizers and no fertilization treatments.

Table 1: Enumeration of Total Bacterial population at three different intervals in Fodder crop rhizosphere soil

Rhizosphere soil samples (treatments)	Total Bacteria ($\times 10^6$ CFU g ⁻¹ of soil)					
	1 st interval (30DAT)		2 nd interval (60DAT)		3 rd interval (90DAT)	
	M1(Anjan grass)	M2 (Guinea grass)	M1 (Anjan grass)	M2 (Guinea grass)	M1 (Anjan grass)	M2 (Guinea grass)
T ₁ (Control) (No fertilizer and no manure)	56	50	40	40	30	25
T ₂ (100%RDF) (60:60:40kg NP ₂ O ₅ K ₂ Okg ⁻¹)	74	66	60	60	55	40
T ₃ (75% RDN + 25% N) through Pongamia green leaf manuring (PGLM)	120	140	90	80	60	50
T ₄ (75% RDN + 25% N) through Neem green leaf manuring (NGLM)	100	85	80	70	40	30
T ₅ (75% RDN + 25%N) through Subabul green leaf manuring (SGLM)	83	73	75	60	50	45
T ₆ (75% RDN + 25% N) through Poultry manure (PM)	73	55	50	50	45	42
T ₇ 75% RDN + 25% N through FYM	80	80	73	70	70	65
CD (1%)	1.766		1.395		1.229	
CV	0.712		0.689		0.720	
SE $\pm$ m	0.089		0.070		0.061	

Table 2: Enumeration of Total Fungal population at three different intervals in Fodder crop rhizosphere soil

Rhizosphere soil samples (treatments)	Total Fungi ($\times 10^3$ CFU g ⁻¹ of soil)					
	1 st interval (30DAT)		2 nd interval (60DAT)		3 rd interval (90DAT)	
	M1(Anjan grass)	M2(Guinea grass)	M1(Anjan grass)	M2(Guinea grass)	M1(Anjan grass)	M2(Guinea grass)
T ₁ (Control) (No fertilizer and no manure)	9	9	7	6	5	4
T ₂ (100%RDF) (60:60:40kg NP ₂ O ₅ K ₂ Okg ⁻¹)	10	15	8	10	7	7
T ₃ (75% RDN + 25% N) through Pongamia green leaf manuring (PGLM)	35	30	20	15	13	13
T ₄ (75% RDN + 25% N) through Neem green leaf manuring (NGLM)	20	17	16	14	12	10
T ₅ (75% RDN + 25%N) through Subabul green leaf manuring (SGLM)	18	13	12	15	10	9
T ₆ (75% RDN + 25% N) through Poultry manure (PM)	23	20	13	12	8	8
T ₇ 75% RDN + 25% N through FYM	17	12	12	9	10	6
CD (1%)	1.233		0.410		0.223	
CV	2.515		1.102		0.749	
SE $\pm$ m	0.062		0.020		0.011	

Table 3: Enumeration of Total actinomycetes population at three different intervals in Fodder crop rhizosphere soil

Rhizosphere soil samples (treatments)	Total Actinomycetes ($\times 10^3$ CFU g ⁻¹ of soil)					
	1 st interval (30DAT)		2 nd interval (60DAT)		3 rd interval (90DAT)	
	M1(Anjan grass)	M2(Guinea grass)	M1(Anjan grass)	M2(Guinea grass)	M1(Anjan grass)	M2(Guinea grass)
T ₁ (Control) (No fertilizer and no manure)	24	19	15	16	12	10
T ₂ (100%RDF) (60:60:40kg NP ₂ O ₅ K ₂ Okgha ⁻¹)	26	20	20	19	15	14
T ₃ (75% RDN + 25% N) through Pongamia green leaf manuring (PGLM)	70	80	35	40	30	25
T ₄ (75% RDN + 25% N) through Neem green leaf manuring (NGLM)	55	45	30	25	22	19
T ₅ (75% RDN + 25%N) through Subabul green leaf manuring (SGLM)	38	35	25	24	20	17
T ₆ (75% RDN + 25% N) through Poultry manure (PM)	35	30	23	16	15	12
T ₇ 75% RDN + 25% N through FYM	30	25	21	22	22	18
CD (1%)	0.400		0.375		0.587	
CV	0.353		0.568		1.028	
SE(±m)	0.020		0.018		0.029	

Table 4: Enumeration of Total Bacterial population at three different intervals in Custardapple rhizosphere soil

Rhizosphere soil samples (treatments)	Total Bacteria ($\times 10^6$ CFU g ⁻¹ of soil)		
	1 st interval (30DAT)	2 nd interval (60DAT)	3 rd interval (90DAT)
T ₁ (Control) (No fertilizer and no manure)	25	20	15
T ₂ (100%RDF) (60:60:40kg NP ₂ O ₅ K ₂ Okgha ⁻¹)	40	36	35
T ₃ (75% RDN + 25% N) through Pongamia green leaf manuring (PGLM)	60	55	50
T ₄ (75% RDN + 25% N) through Neem green leaf manuring (NGLM)	35	30	23
T ₅ (75% RDN + 25%N) through Subabul green leaf manuring (SGLM)	50	45	44
T ₆ (75% RDN + 25% N) through Poultry manure (PM)	40	33	32
T ₇ 75% RDN + 25% N through FYM	80	70	73
CD (5%)	4.510	3.977	3.832
CV	5.460	5.517	5.628
SE±m	0.562	0.513	0.478

Table 5: Enumeration of Total Fungal population at three different intervals in Custardapple rhizosphere soil

Rhizosphere soil samples (treatments)	Total Fungi ($\times 10^3$ CFU g ⁻¹ of soil)		
	1 st interval (30DAT)	2 nd interval (60DAT)	3 rd interval (90DAT)
T ₁ (Control) (No fertilizer and no manure)	9	6	5
T ₂ (100%RDF) (60:60:40kg NP ₂ O ₅ K ₂ Okgha ⁻¹)	14	10	8
T ₃ (75% RDN + 25% N) through Pongamia green leaf manuring (PGLM)	20	15	14
T ₄ (75% RDN + 25% N) through Neem green leaf manuring (NGLM)	12	9	7
T ₅ (75% RDN + 25%N) through Subabul green leaf manuring (SGLM)	17	14	12
T ₆ (75% RDN + 25% N) through Poultry manure (PM)	15	12	9
T ₇ 75% RDN + 25% N through FYM	33	25	18
CD (5%)	1.653	1.262	1.017
CV	5.503	5.540	5.566
SE±m	0.206	0.158	0.127

Table 6: Enumeration of Total Actinomycetes population at three different intervals in Custardapple rhizosphere soil

Rhizosphere soil samples (treatments)	Total Actinomycetes ($\times 10^3$ CFU g ⁻¹ of soil)		
	1 st interval (30DAT)	2 nd interval (60DAT)	3 rd interval (90DAT)
T ₁ (Control) (No fertilizer and no manure)	19	15	14
T ₂ (100%RDF) (60:60:40kg NP ₂ O ₅ K ₂ Okgha ⁻¹)	33	30.1	25.083
T ₃ (75% RDN + 25% N) through Pongamia green leaf manuring (PGLM)	60	55	50
T ₄ (75% RDN + 25% N) through Neem green leaf manuring (NGLM)	25	20	17
T ₅ (75% RDN + 25%N) through Subabul green leaf manuring (SGLM)	50	45	40
T ₆ (75% RDN + 25% N) through Poultry manure (PM)	35	32	30
T ₇ 75% RDN + 25% N through FYM	80	70	50
CD (5%)	4.292	3.845	3.264
CV	5.679	5.754	5.771
SE±m	0.535	0.480	0.407

Table 7: Morphological and Cultural Characterization of Bacteria

Isolate Name	Gram Reaction	Pigmentation	Shape of cell	Margin	Surface of whole colony	Texture
AfcaB1	Positive	Orange	Smooth	Round	Convex	Mucoid
AfcaB2	Positive	Opaque	Irregular	Lobate	Convex	Moist
AfcaB3	Positive	Opaque/White	Round	Undulate	Convex	Moist
AfcaB4	Positive	Opaque	Irregular	Lobate	Convex	Mucoid
AfcaB5	Positive	Cream/opaque	Round	Undulate	Convex	Moist
AfcaB6	Positive	Brown	Irregular	Lobate	Raised	Moist

AfcaB7	Negative	Brown	Irregular	Undulate	Raised	Dry
AfcaB8	Positive	Opaque/white	Irregular	Lobate	Flat	Dry
AfcaB9	Negative	Opaque	Irregular	Lobate	Convex	Mucoid
AfcaB10	Negative	Milky white	Irregular	Lobate	Raised	Moist
AfcaB11	Negative	Opaque	Irregular	Lobate	Raised	Slimy moist
AfcaB12	Positive	Opaque	Irregular	Lobate	Raised	Moist
AfcaB13	Positive	Opaque	Irregular	Undulate	Raised	Shiny/moist
AfcaB14	Positive	Milky white	Round	Undulate	Raised	Moist
AfcaB15	Positive	Opaque	Round	Entire smooth	Convex	Mucoid
AfcaB16	Negative	Opaque	Irregular	Undulate	Convex	Dry

Table 8: Morphological and Cultural Characterization of Fungi

Isolate Name	Pigmentation	Shape of cell	Margin	Elevation
AfcaF1	Pink and green	Circular	Curled	Raised
AfcaF2	Brownish black	Irregular	Undulate	Raised
AfcaF3	Pink	Irregular	Filliform	Raised
AfcaF4	Green	Filamentous	Filliform	Flat
AfcaF5	Dark green	Circular	Entire round	Raised
AfcaF6	Pink and green	Circular	Undulate	Raised
AfcaF7	Green	Irregular	Filliform	Raised
AfcaF8	Pink	Filamentous	Filiform/curled	Convex
AfcaF9	Green	Irregular	Undulate	Irregular
AfcaF10	Light green	Irregular	Entire smooth	Irregular
AfcaF11	Green	Filamentous	Entire even	Filamentous
AfcaF12	Green	Filamentous	Filliform	Filamentous
AfcaF13	Pink	Irregular	Curled	Irregular
AfcaF14	Pink green	Circular	Filliform	Circular

Table 9: Morphological and Cultural Characterization of Actinomycetes

Isolate Name	Gram Reaction	Pigmentation	Shape of cell	Form of whole colony	Surface of the colony
AfcaA1	Positive	Chalky white	Rod	Irregular	Dry rough colonies
AfcaA2	Positive	Ash	Cocci	Circular	Smooth and dusty colonies
AfcaA3	Positive	Light green	Rod	Round	Dry, rough and dusty colonies
AfcaA4	Positive	White	Rod	Circular	Smooth, dry colonies
AfcaA5	Positive	Light green	Rod	Irregular	Dry, smooth powdery colonies
AfcaA6	Positive	Cream	Cocci	Round	Dry, smooth powdery colonies
AfcaA7	Positive	Chalky white	Rod	Round	Dry, rough colonies
AfcaA8	Positive	Ash	Cocci	Circular	Smooth, dusty colonies
AfcaA9	Positive	Ash white	Rod	Circular	Dry, rough colonies
AfcaA10	Positive	Cream	Rod	Irregular	Dry, rough colonies
AfcaA11	Positive	Brown	Rod	Irregular	Smooth powdery colonies
AfcaA12	Positive	Cream	Rod	Circular	Dry, smooth powdery colonies
AfcaA13	Positive	Brown	Cocci	Filamentous	Rough, dry colonies
AfcaA14	Positive	Cream	Rod	Irregular	Smooth, dusty colonies
AfcaA15	Positive	Cream	Rod	Filamentous	Dry, rough colonies
AfcaA16	Positive	Cream	Cocci	Circular	Smooth, powdery superficial colonies

**Bacteria****Fungi****Actinomycetes****Fig 1:** Bacterial, Fungal and Actinomycetes enumerated colonies

Conclusion

The study examined the microbial populations in the rhizosphere of custard apple intercropped with Anjan and Guinea grasses, identifying 46 distinct colonies and enumerating them at three intervals. The findings revealed that organic manures and nutrient management significantly influence soil microbial populations and crop yields in custard apple-based intercropping

systems. Notably, the application of organic treatments, particularly T3 (75% RDN + 25% nitrogen from Pongamia green leaf manuring) and T7 (75% RDN + FYM), led to higher counts of bacteria, fungi, and actinomycetes across both grass species. Guinea grass (M2) exhibited greater microbial activity and higher yields, likely due to favourable root exudates and beneficial soil interactions, compared to Anjan grass (M1).

In custard apple rhizosphere, the highest microbial populations and fruit yields were associated with treatment T7, while the control and 100% RDF treatments showed the lowest counts and yields. This study highlights a clear correlation between increased microbial diversity and improved yields in both custard apple and fodder crops. The results emphasize the advantages of using organic amendments to enhance soil health, boost crop productivity, and promote the sustainability of custard apple-based horticultural-pastoral systems.

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