The Bio-Efficacy of R1&R2 Biofertilizers in Organic Cotton Cultivation

GRAMEENA VIKAS KENDRAM – Cotton Field Report

Location : **ASR & Manyam Districts of Andhra Pradesh**

Trial Partner : **Grameena Vikasa Kendram (GVK), Vishakhapatnam**

Conducted by : **FIB-SOL Life Tech. Pvt. Ltd., and 1000 Farms Agritech Pvt. Ltd.**

Trial Product : **Raddis Cotton I & Raddis Cotton II**

Region covered : **GL Puram, Salur and Addateegala**

Total acre Coverage : **3000**

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STUDY HIGHLIGHTS

- This report presents an **Impact Assessment of R1R2** application in the 2024-25 cotton season, compared to the previous season, focusing on the impact of introducing an additional dosage.
- R1R2 was implemented across **three rainfed clusters** GL Puram, Salur and Addateegala - mirroring conditions from the prior season for effective comparison.
- In 2023-24, a **single dose** of FIB-SOL was applied over 476 acres, marking the initial phase of adoption.
- Encouraged by **30-40% yield improvements** and positive farmer feedback, the area under FIB-SOL expanded significantly in the current season.
- Two modes of application were used:
  - **First Dose**: Seed treatment at sowing.
  - **Second Dose**: Soil drenching during vegetative phase.
- For seed treatment, 900 grams of Seed and 100 ml of R1R2 were used per acre; seeds were shade-dried for 30 minutes post-treatment.
- The second dose involved 100 ml of R2 diluted in 200 litres of water or alternatively applied with sand and water as a broadcast or pour near the root zone.
- Enhanced Vegetative and Reproductive traits were observed: increased Plant height, Stem girth, Leaf area, Chlorophyll content, Monopodial & Sympodial Branches, Leaf Weight, Number of squares and Bolls per plant, and Boll size and weight.
- Metagenomic assessments confirmed synergistic microbial activity and the persistence of applied strains, improving soil ecological function.
- 14. Statistical testing using ANOVA revealed significant improvements in multiple traits including Boll count, Chlorophyll content and Stem girth.
- Soil microbial analysis showed increased beneficial microbial populations post-treatment, supporting nutrient mobilization and uptake.
- Cotton fibre quality was assessed across R1R2 plots with control fields showing consistently better Ginning %, Fibre Strength, Micronaire value, and lower Trash content.
- Treated crops showed reduced pest incidence and nutrient deficiency symptoms, further supporting yield stability.
- Economic yield gains ranged between 14% and 120%, with benefit-cost ratios up to 7:1, directly benefiting small and marginal farmers.

- ❖ R1R2 proved to be a climate-resilient solution, performing reliably even under heavy rainfall conditions and in degraded soils.
- ❖ Partnering with GVK Society enabled effective training and distribution, ensuring standard application practices and farmer capacity building.
- ❖ R1R2 treated plots consistently outperformed control plots across various socio-economic and geographical settings.

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ACKNOWLEDGEMENT

We extend our deepest gratitude to GVK Society for their unwavering support, visionary partnership, and commitment to empowering farming communities through sustainable agricultural innovations. The successful implementation and expansion of FIB-SOL during the 2024-25 cotton season would not have been possible without their relentless dedication and on-ground presence.

Special acknowledgment is due to the GVK field team members at the grassroots level, whose tireless efforts in farmer engagement, training, and field-level coordination ensured the standardization of application protocols and maximized the impact of the intervention. Their work has been instrumental in translating a promising biological solution into tangible improvements in crop productivity, resilience and farmer livelihoods.

We also extend our heartfelt thanks to the FIB-SOL team, whose hands-on execution and involvement at the ground level played a pivotal role in ensuring the smooth execution of trials, effective implementation of protocols and systematic collection of scientific data. Their dedication and technical support were critical in maintaining research integrity and generating impactful insights throughout the season.

This collaboration has not only driven significant economic gains and enhanced cotton yield across diverse geographies but has also laid a strong foundation for scalable, climate-resilient and inclusive farming practices. Together, we have taken a meaningful step toward transforming cotton farming into a more sustainable and prosperous enterprise for small and marginal farmers.

We look forward to continuing this impactful journey with GVK Society and our dedicated field teams, further strengthening our shared mission to innovate, uplift and create lasting value in Indian agriculture.

1. Introduction

Cotton is a key crop for small-scale farmers in the rainfed, dryland regions of GL Puram, Salur, and Addateegala. However, erratic rainfall in these areas leads to moisture stress, limiting nutrient and water availability, reducing seed germination, and lowering yields. To overcome this, farmers can adopt drought-tolerant cotton varieties and use biological inputs like Plant Growth Promoting Rhizobacteria. These beneficial microbes boost nutrient uptake, enhance soil health, and protect plants from pathogens, leading to better crop resilience, lower input costs, and sustainable productivity.

FIB-SOL, renowned for its expertise in Nano Biotechnology and Bioengineering has achieved a groundbreaking milestone in agriculture with the development of the **World's First Patented Gel-based Biologicals**. These eco-friendly fertilizers, derived from natural ingredients like microorganisms, present a sustainable alternative to traditional chemical fertilizers, boasting increased crop yields by up to 20% and promoting soil health, FIB-SOL's biofertilizers utilize a unique gel carrier, made from biodegradable polymers, ensuring slow and controlled nutrient release for prolonged plant nourishment.

1000 Farms, an Agritech startup, is a beacon for struggling rural farmers, providing eco-friendly inputs and services to enhance agricultural efficiency. It empowers farmers with sustainable practices, aiming to boost crop yields and overall agricultural sustainability to the marginal and below marginal farmers.

Grameena Vikas Kendram (GVK) Society is a linchpin in promoting sustainable agriculture and rural development. Employing a holistic approach, it tackles the roots of poverty and inequality in agricultural communities. Through community-led development, GVK collaborates closely with farmers, empowering them to drive their own initiatives. With a focus on capacity building and promotion of sustainable practices and modern technologies, GVK Society is a catalyst for enhancing agricultural productivity, income and environmental sustainability in rural areas.

Objectives:

- To evaluate the impact of R1R2 application on Quantitative and Qualitative traits in rainfed Raddis Cotton.
- To quantify the increase in yield and yield-contributing traits in R1R2 treated plots compared to control plots.

- To assess nutrient uptake efficiency and its effect on enhancing the Soil microbial population.
- To compare the Yield performance between Single dose and Double dose applications of R1R2 under the same field conditions.

About Technology:

FIB-SOL innovation offers an efficient solution for delivering bio-fertilizers, which are soil microbes, using advanced Encapsulation Technology. This technology enables the compact (1000X) and effective delivery of soil bacteria, enhancing the availability of macronutrients like nitrogen, phosphorus, and potassium to plants. The result is improved nutrient absorption, better soil quality, and higher cotton yields. Specifically, FIB-SOL R1 & R2 are combinations of soil bacteria encapsulated in a biodegradable gel, containing 10^{11} CFU/ml. These formulations boost crop growth, are water-soluble, and are easy to apply.

This study aimed to evaluate the impact of Raddis Cotton I and Radis Cotton II biofertilizers (R1 & R2) on cotton crop yield and soil microbiome in three regions: GL Puram, Salur, and Addateegala. Data on critical agronomic parameters—such as chlorophyll content, leaf area, stem girth, boll development, and final yield per acre—were collected at 90 and 150–180 days after sowing (DAS). Comparative analysis was done between control plots (without R1 & R2) and treated plots (with R1 & R2), where the product was applied as a seed treatment and through soil drenching. Additionally, microbial population dynamics and cost-benefit analysis were performed to understand the broader agronomic and economic benefits.

2. Regional Overview

The field experiment was conducted across three different areas, like GL Puram, Salur, and Addateegala region (Fig.1) of GL Puram, Vizayanagaram, and Alluri Settaramarau district, respectively, Andhra Pradesh, India, from June 2024 to January 2025, covering 3000 acres to assess the impact of R1 & R2 treatment on cotton crop growth compared to a control (Without R1 & R2) group.

The trial product given was Raddis Cotton I applied through seed treatment in the month of June and July 2024, and Raddis Cotton II applied through soil drenching in the month of September and October 2024 (Table 1).

Fig 1: Study Location of Regional Distribution

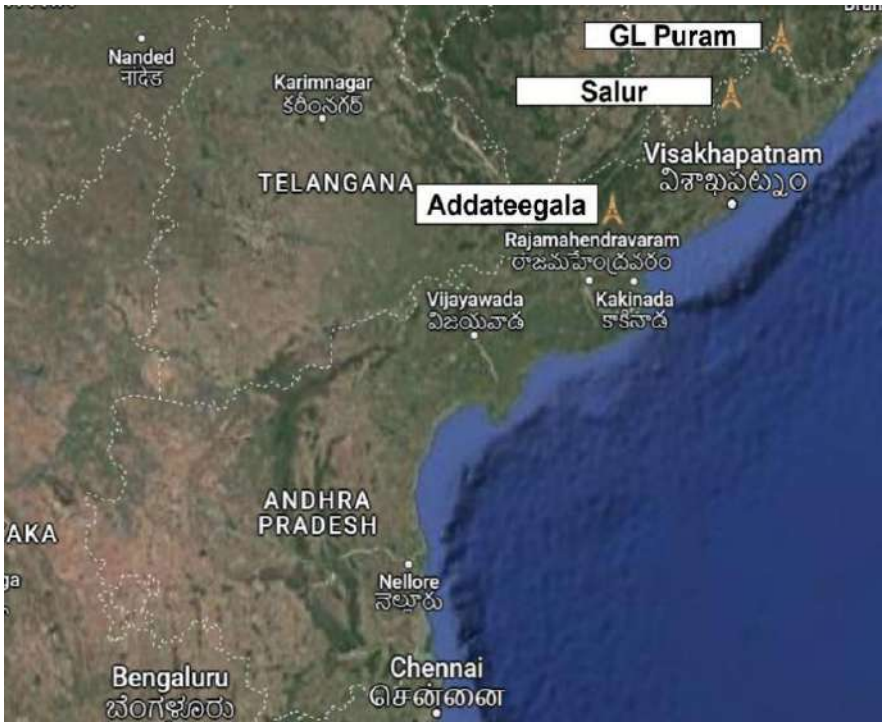


Table 1: Regional Overview

		GL PURAM	SALUR	ADDATEEGALA
Crop		Cotton		
Varieties		Non Asha Non Bunny Non Bunny Asha Non Mallika Bunny Asha Non Partech	Native variety	Asha Asha - Bunny Gaysal seed Tulasi Bunny Non Asha Partec Tulasi
No. of Varieties		7	2	9
Method of Application	Control	Farmer Practice Followed		
	R1R2	June/July - Seed Treatment (Raddis cotton I) August/September - Soil Drenching (Raddis cotton II)		
Total No. of farmers		53	74	63
Seed Treatment & Sowing (R1 Duration)		18/06/2024 - 13/07/2024	05/06/2024 - 30/06/2024	05/06/2024 - 30/06/2024
Data Collection date (30 - 80 days)		01/08/2024 - 14/09/2024	29/07/2024 - 30/08/2024	29/07/2024 - 30/08/2024
Data Collection Date (100 - 150 days)		18/10/2024 - 18/11/2024	21/10/2024 - 21/11/2024	23/10/2024 - 26/11/2024

3. Sample Distribution:

Given the constraints of a large implementation area (3000 acres) and the need to optimize costs while ensuring statistically significant results, the sampling approach is based on the different types of analyses (Table 2, Fig.2). Metagenomic analysis and Post Harvest analysis

were performed with 6 farmers whose details are given in Table 3. Here's a logical approach to fixing the ratios for sample sizes:

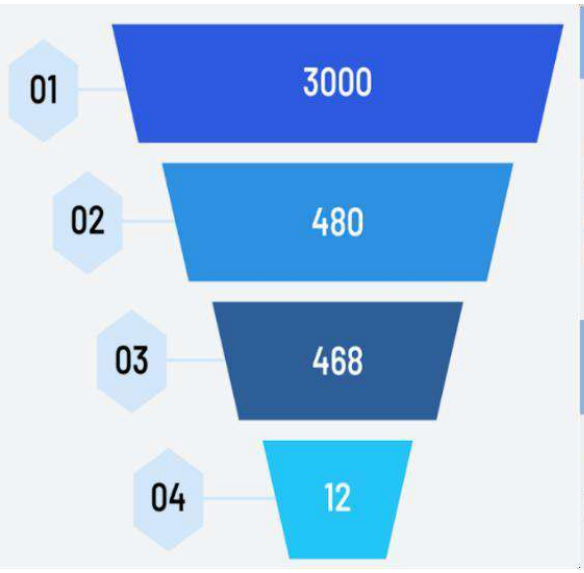
3.1. Field Plot Distribution

- The 3000 acres are divided into 3 types to ensure representation from different areas like GL Puram, Addateegala, and Salur
- For large-scale trials, a common practice is to use 1-2% of the total area for intensive sampling (Table.2).

3.2. Sampling Strategy

- Random sampling (Fig.2) to ensure samples are representative of the entire area.
- Allocate a higher proportion of samples to the most critical analyses (e.g., plant yield and quality) and fewer samples to supporting analyses (e.g., soil microbial analysis).

Fig 2: Study Framework



01 TOTAL AREA OF STUDY	02 TOTAL SAMPLE SIZE
Field experiment Area: 3,000 acres. Three regions: GL Puram, Salur, and Addateegala each covering 1,000 acres	The study's total sample size: 480 Plant and Yield Analysis:50% Soil Physicochemical Analysis:25% Soil Microbial Analysis:15% Soil Metagenomic Analysis: 5% Post-Harvest Analysis: 5%
03 PARAMETERS STUDY	**04 METAGENOMICS-STUDY**
13 Plant, soil, and yield parameters were studied and analyzed	Abundance and distribution of microbial community were studied and analyzed

3.3. Proposed Ratios for Sample Sizes

❖ **Plant and Yield Analysis**

- **Importance:** High, as it directly measures the impact on crop performance.
- **Sample Size Ratio:** 50%
- **Calculation:**

i. **Area for intensive sampling:** 3000 acres * 0.02 = 60 acres (20 from each region)

ii. **Number of plots (assuming 1 acre per plot):** 60 plots

iii. **Number of plants per plot:** 3 plants

iv. **Total plants: 60 plots * 3 plants =** 180 plants

Table 2: Summary of Sampling Proportion

Analysis Type	No. of R1R2 Treated Plots	Samples Per R1R2 Treated Plot	Total R1R2 Treated	No. of Control Field Plots	Samples per Control Field Plot	Total Control Field Samples	Total Samples
Plant and Yield Analysis (50%)	60	3	180	60	3	180	360
Soil Physicochemical Analysis (25%)	30	1	30	30	1	30	60
Soil Microbial Analysis (15%)	18	1	18	18	1	18	36
Soil Metagenomic Analysis (5%)	6	1	6	6	1	6	12
Post-Harvest Analysis (5%)	6	1	6	6	1	6	12

❖ **Soil Physicochemical Analysis**

- **Importance:** Medium, as it supports understanding of soil health and nutrient status.
- **Sample Size Ratio:** 25%
- **Calculation:**

i. **Number of plots:** 30 plots (same plots as plant analysis, 10 from each region)

ii. **Soil samples per plot:** 1 (collected from at least 5 spots)

iii. **Total soil samples:** 30 plots * 1 samples = 30 samples

❖ **Soil Microbial Analysis**

- **Importance:** Medium to High, critical for understanding microbial health but can be cost-intensive.
- **Sample Size Ratio:** 15%
- **Calculation:**

i. **Number of plots:** 18 plots (a subset of the 30 plots, 6 from each region)

ii. **Soil samples per plot:** 1 (collected from at least 5 spots)

iii. **Total soil samples:** 18 plots * 1 sample = 18

❖ **Soil Metagenomic Analysis**

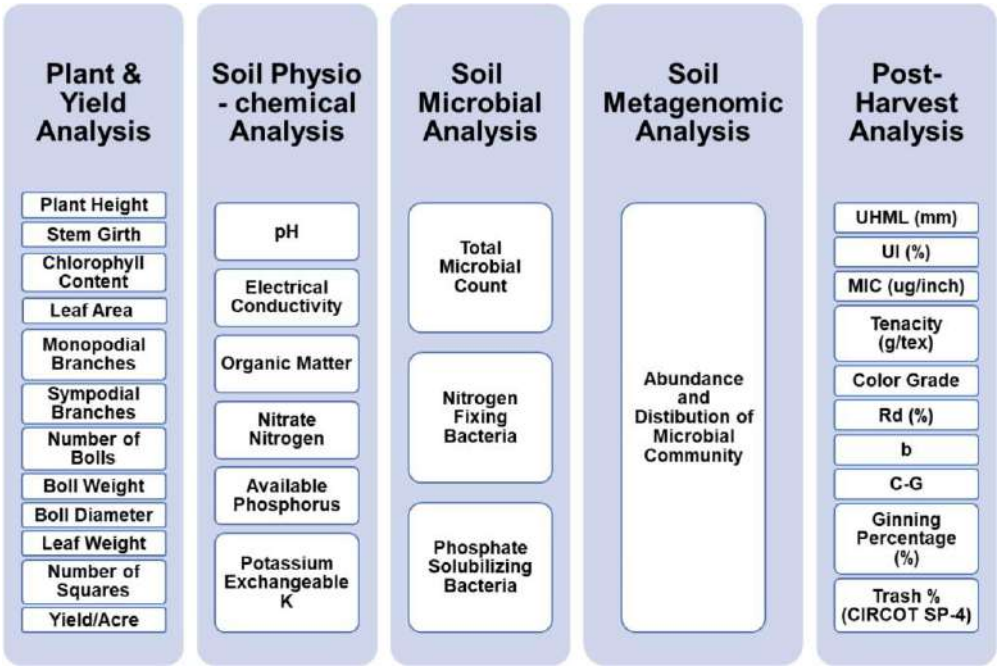
- **Importance:** Medium to High, provides detailed microbial community structure but is highly cost-intensive.
- **Sample Size Ratio:** 5%
- **Calculation:**

i. **Number of plots:** 6 plots (a subset of the 30 plots, 2 from each region)

ii. **Soil samples per plot:** 1 (collected from the rhizospheres of 5 plants)

iii. **Total soil samples:** 6 plots * 1 = 6 samples

Fig 3: Overall Study Parameters



❖ **Post-Harvest Analysis**

- **Importance:** High, assesses the quality of the harvested product.
- **Sample Size Ratio:** 5%
- **Calculation:**

i. **Number of plots:** 6 plots (a subset of the 30 plots)

Table 3: Summary of Farmer Plots and Agronomic Practices

S No	Farmer Details	Field No: 1	Field No: 2	Field No: 3	Field No: 4	Field No: 5	Field No: 6
1	Name	Neerasa Padma	Bondapalli Dalemma Nagaraj	Kattula Buchayya	Pedakapu Venkati	Murla Jangamma	Kopuri Laxmi
2	R1R2 Plot Area (Acre)	3	6	1	2	4	2
3	Control Field Plot Area (Acre)	1	1	1	3	1	1
4	Crop	Cotton	Cotton	Cotton	Cotton	Cotton	Cotton
	a)Seed Variety	Bunny	Partech	Native Variety	Native Variety	Asha Bunny	Asha Bunny
5	Location	Bithrapadu, Jiyyammavalasa	Rajyalaxmipuram, Komarada	Jammuvalasa, Ramabadrapuram	Panasalapadu, Pachipenta	Chavitidiball, Y Ramavaram	Chavitidiballu, Y Ramavaram
6	District	Parvatipuram	Parvatipuram	Vizayanagaram	Parvatipuram	Alluri Settaramarau	Alluri Settaramaraju
7	Date of Sowing	28/06/2024	03/07/2024	23/06/2024	28/06/2024	23/06/2024	28/06/2024
8	Date of Data Collection	19/08/2024 26/10/2024	12/08/2024 15/11/2024	22/08/2024 22/10/2024	20/08/2024 08/11/2024	05/09/2024 15/11/2024	20/09/2024 08/01/2025

4. Vegetative Crop Parameters

4.1. Plant Height

In GL Puram, the plant height was consistently improved in the R1 & R2 treated field than the control field with notable increases of 18.66% at 30 - 50 DAS and 14.03% at 130 - 150 DAS. In Salur, improvements were also observed at 50 - 80 DAS (10.65%) and 150 - 180 DAS (Fig.4) (8.06%).

Addateegala showed the most significant response to R1 & R2, with dramatic increases in later stages including 50.92% increases at 130 - 150 DAS and 70.82 % increase at 150 - 180 DAS, indicating enhanced late-stage vegetative growth. These results (Table 4) suggest that R1 & R2 enhances plant height by improving nutrient uptake and stress resilience**.**

Fig 4: Regional Plant Height Comparison (R1 & R2 vs Control plots)

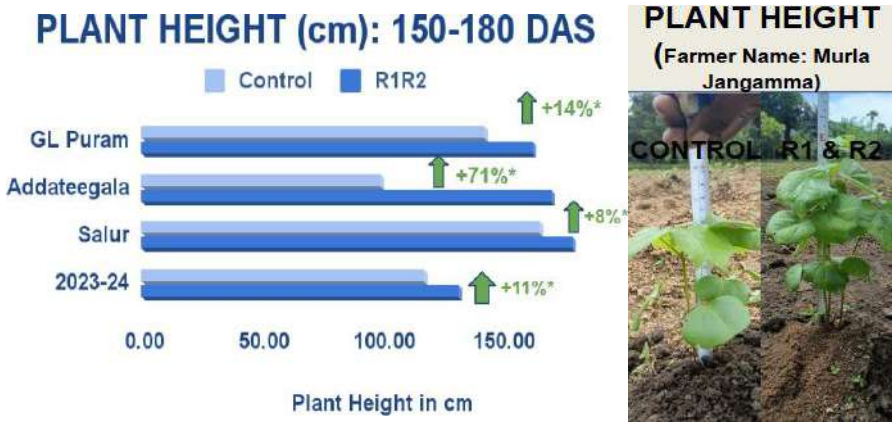


Table 4: Comparative Analysis of Plant Height in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
30 - 50	Control (cm)	56.10±15.22	77.20±6.11	45.30±54.91
	R1 & R2 (cm)	66.56±9.46	79.53±9.85	45.93±18.18
	Increase (%)	18.66	3.01	1.39
50 - 80	Control (cm)	83.01±16.33	87.80±13.98	42.71±16.65
	R1 & R2 (cm)	81.97±11.43	97.15±14.46	58.98±20.47*
	Increase (%)	-1.25	10.65	38.08
100 - 130	Control (cm)	144.80±15.76	159±9.60	126.10±24.24
	R1 & R2 (cm)	161.10±8.66	169.40±17.42	149.51±20.72*
	Increase (%)	11.26	6.54	18.57
130 - 150	Control (cm)	143.30±24.47	161.44±11.40	96.48±21.62
	R1 & R2 (cm)	163.40±7.89	171.87±19.46	145.61±21.17*
	Increase (%)	14.03	6.46	50.92
150 - 180	Control (cm)	-	166.30±12.38	100.03±28.22
	R1 & R2 (cm)	-	179.70±10.16	170.87±20.36*
	Increase (%)	-	8.06	70.82

NOTE: * indicates p-Value ≤0.05 is significant

4.3. Stem Girth

In 30 - 50 DAS of Addateegala, the stem girth shows the most significant improvement of about 79% in the R1 & R2 treated field. GL Puram, Salur also showed notable increases of 23% and 7% respectively indicating that it enhances stem girth of the cotton plant (Table 5). Addateegala region showed continued increases in the stem girth of about 84% showing that the R1 & R2 treated plot shows consistent increased performance in 50-80 DAS.

Fig 5: Regional Stem Girth in Control vs. R1 & R2 Treated Cotton Plots

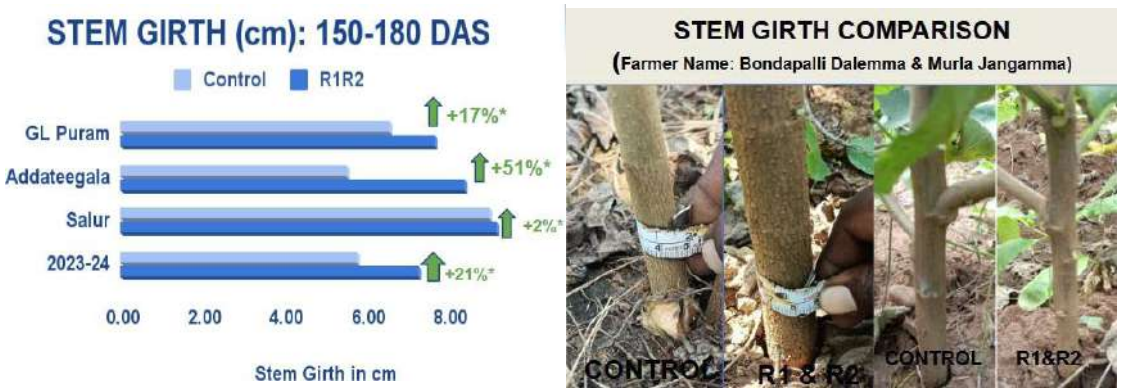


Table 5: Regional Stem Girth in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
30 - 50	Control (cm)	2.95±0.70	4.69±0.47	1.40±0.66
	R1 & R2 (cm)	3.62±0.58*	5.01±0.70*	2.50±0.92*
	Increase (%)	22.72	6.88	78.57
50 - 80	Control (cm)	3.82±0.47	5.16±0.80	2.25±1.28
	R1 & R2 (cm)	4.15±0.71*	5.65±0.85*	4.14±3.13*
	Increase (%)	8.71	9.5	84
100 - 130	Control (cm)	6.45±0.93	7.47±0.30	7.57±1.12
	R1 & R2 (cm)	7.57±0.76*	8.21±1.17*	8.51±1.54*
	Increase (%)	17.36	9.91	12.42
130 - 150	Control (cm)	6.57±1.33	23.39±3.49	6.02±1.20
	R1 & R2 (cm)	7.69±0.62*	26.01±3.37*	8.08±1.51*
	Increase (%)	17.05	11.2	34.22
150 - 180	Control (cm)	-	9.01±8.96	5.55±0.80
	R1 & R2 (cm)	-	9.19±7.79	8.40±1.32*
	Increase (%)	-	2	51.35

NOTE: * indicates p-Value ≤0.05 is significant

However, the GL Puram and Salur also showed an increase of 9% and 10% respectively. In 100-130 DAS, 17%, 10%, 12% increases were observed in GL Puram, Salur and Addateegala region respectively (Fig.5). In cotton, stem girth reflects the plant structural strength and

physiological capacity. A thicker stem girth supports better water and nutrient transport contributing to the yield increment of the plant and better boll retention.

4.4. Chlorophyll Content

R1 & R2 treatment consistently shows increases in chlorophyll content across all stages with higher percentage increase of 23% at 150-180 DAS (Fig.6) in Addateegala thereby it results in enhancing plant health, photosynthesis capacity, greater boll development and yield (Table 6).

Fig 6: Regional Chlorophyll Content in Control vs. R1 & R2 Treated Cotton Plots

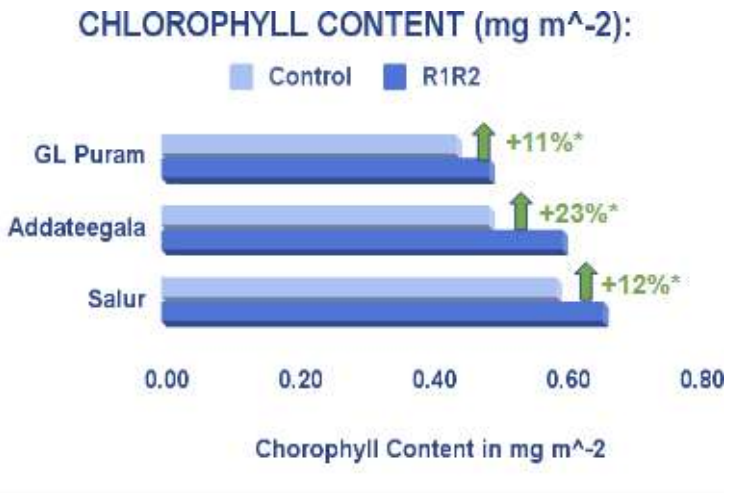


Table 6: Regional Chlorophyll Content in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
30 - 50	Control (mg/m2)	0.51±0.06	0.60±0.01	0.47±0.08
	R1 & R2 (mg/m2)	0.57±0.04*	0.62±0.01*	0.54±0.03*
	Increase (%)	12.76	3.33	14.29
50 - 80	Control (mg/m2)	0.53±0.04	0.59±0.02	0.50±0.07
	R1 & R2 (mg/m2)	0.58±0.03*	0.63±0.02*	0.56±0.06*
	Increase (%)	9.43	6.78	10.12
100 - 130	Control (mg/m2)	0.47±0.05	0.60±0.03	0.50±0.05
	R1 & R2 (mg/m2)	0.53±0.05*	0.65±0.04*	0.57±0.04*
	Increase (%)	12.77	8.33	12.9
130 - 150	Control (mg/m2)	0.44±0.06	4.79±1.29	0.52±0.10
	R1 & R2 (mg/m2)	0.49±0.05*	5.24±1.30*	0.56±0.05*
	Increase (%)	11.36	9.39	7.69
150 - 180	Control (mg/m2)	-	0.59±0.04	0.49±0.15
	R1 & R2 (mg/m2)	-	0.66±0.04*	0.60±0.03*
	Increase (%)	-	11.86	23.09

NOTE: * indicates p-Value ≤0.05 is significant

4.5. Leaf Area

Leaf area is a vital growth parameter because larger leaves are equal to more surface area for photosynthesis and enhance boll formation. The below data shows that the R1 & R2 treatment significantly increased leaf area across all stages and locations, especially in Addateegala (Table 7 & Fig.7).

Fig 7: Regional Leaf Area in Control vs. R1 & R2 Treated Cotton Plots

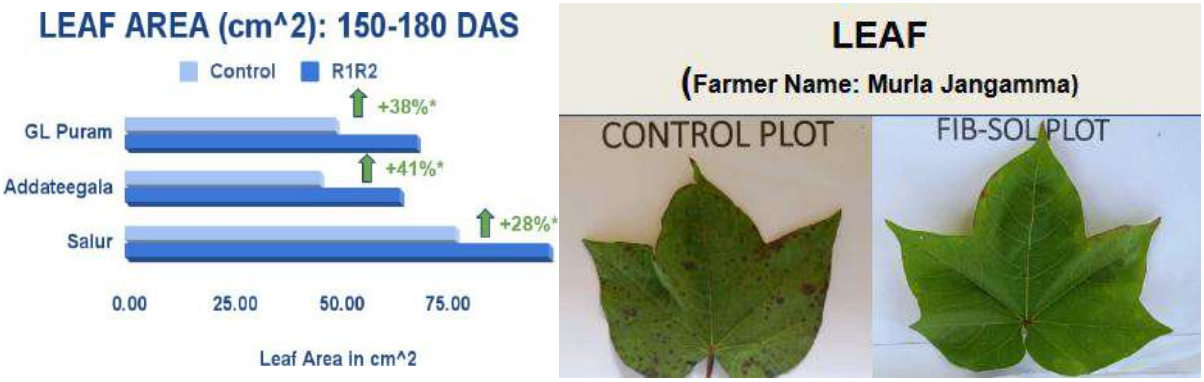


Table 7: Regional Leaf Area in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
30 - 50	Control (cm2)	85.29±22.84	76.90±10.71	48.50±0.57
	R1 & R2 (cm2)	108.84±16.21*	98.62±14.70*	78.48±21.58
	Increase (%)	28	28	62
50 - 80	Control (cm2)	102.84±17.19	102.82±389.46	62.65±23.31
	R1 & R2 (cm2)	111.79±20.37*	101.18±50.13*	98.45±8.6*
	Increase (%)	9	-2	57
100 - 130	Control (cm2)	53.32±17.19	71.36±21.28	61.15±19.32
	R1 & R2 (cm2)	72.25±16.48*	86.92±26.34*	76.64±24.40*
	Increase (%)	36	22	25
130 - 150	Control (cm2)	49.80±18.40	68.96±17.78	57.42±17.94
	R1 & R2 (cm2)	68.70±18.90*	78.63±20.61*	66.32±21.16*
	Increase (%)	38	14	15
150 - 180	Control (cm2)	-	77.96±24.19	45.97±15.78
	R1 & R2 (cm2)	-	99.91±97.85*	64.82±23.25*
	Increase (%)	-	28	41

NOTE: * indicates p-Value ≤0.05 is significant

4.6. Monopodial Branches

Monopodial branches form the main structural framework of the cotton plant, supporting vertical growth and serving as the base for sympodial (fruit-bearing) branches. An

increase in monopodial branches enhances canopy development, improves light interception, and supports a higher reproductive load.

Table 8 represents the comparative performance of control and R1 & R2 treated plots across different locations and growth stages. Results (Fig.8) indicate a consistent and significant improvement in monopodial branching due to R1 & R2 application.

Table 8: Regional Monopodial branches in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
100 - 130	Control	17.54±2	21.67±3.85	23.30±4.6
	R1 & R2	20.01±2.04*	24.04±3.96*	27.77±4.29*
	Increase (%)	14	11	19
130 - 150	Control	17.54±2.79	50.97±5.99	22.01±5.6
	R1 & R2	19.94±2*	56.22±5.54*	26.13±5.4*
	Increase (%)	14	10	19
150 - 180	Control	-	23.93±2.9	16.65±3.7
	R1 & R2	-	26.70±2.8*	22.65±3.4*
	Increase (%)	-	12	36

NOTE: * indicates p-Value ≤0.05 is significant

Across all locations and stages, the number of monopodial branches increased under R1 & R2 treatment, with the highest increase (36%) observed at Addateegala during 150–180 DAS. This indicates that R1 & R2 enhances vegetative architecture and supports robust plant development through critical growth phases.

4.7. Sympodial Branches

Sympodial branches are the fruit-bearing branches in cotton and directly influence boll setting and yield potential. The number of sympodial branches is a key agronomic trait indicating reproductive vigor. The data in Table illustrates the effect of R1 & R2 treatment on the development of sympodial branches at different growth stages and locations. R1 & R2 treatment consistently increased the number of sympodial branches across all locations and time intervals. The most significant improvement was observed in Addateegala during 150–180 DAS (Fig.8), with a 69% increase compared to control. These results (Table.9) demonstrate R1 & R2's positive impact on enhancing reproductive growth and potential boll load in cotton.

Fig 8: Regional Monopodial branches & Sympodial Branches in Control vs. R1 & R2 Treated Cotton Plots

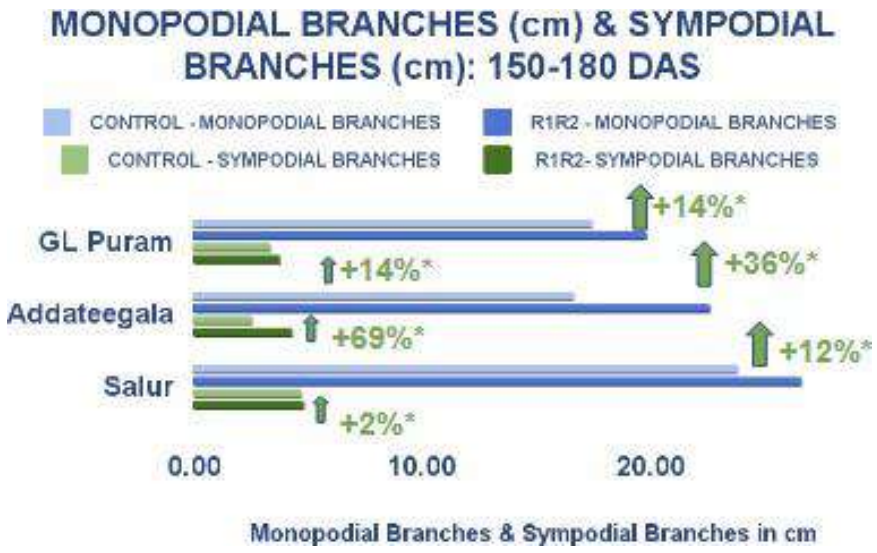


Table 9: Regional Sympodial Branches in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
100 - 130	Control	3.03±0.9	4.3±1	3.2±1.1
	R1 & R2	3.54±0.8*	4.8±1.2*	4.4±1.2*
	Increase (%)	17	13	38
130 - 150	Control	3.38±0.9	20.20±2	2.74±0.8
	R1 & R2	3.87±1	22.30±2*	4.16±1.1*
	Increase (%)	14	10	52
150 - 180	Control	-	4.74±1	2.55±0.6
	R1 & R2	-	4.83±1	4.30±1.1*
	Increase (%)	-	2	69

NOTE: * indicates p-Value ≤0.05 is significant

4.8. Leaf Weight

Leaf weight is a key physiological parameter indicating overall plant health and photosynthetic capacity. Heavier leaves suggest increased biomass accumulation, which supports higher photosynthetic efficiency and nutrient storage. The following data (Table 10) compares leaf weight in control and R1 & R2 treated cotton plants across different locations and growth stages.

R1 & R2 treatment resulted (Fig.9) in a significant increase in leaf weight across all observed locations and time periods. The highest increase was observed in GL Puram at

100–130 DAS (42%), followed closely by Addateegala at the same stage (41%). This increase in leaf biomass suggests improved nutrient uptake and retention, which contributes to better plant vigor, higher photosynthetic efficiency, and ultimately greater cotton yield potential.

Fig 9: Regional Leaf Weight in Control vs. R1 & R2 Treated Cotton Plots

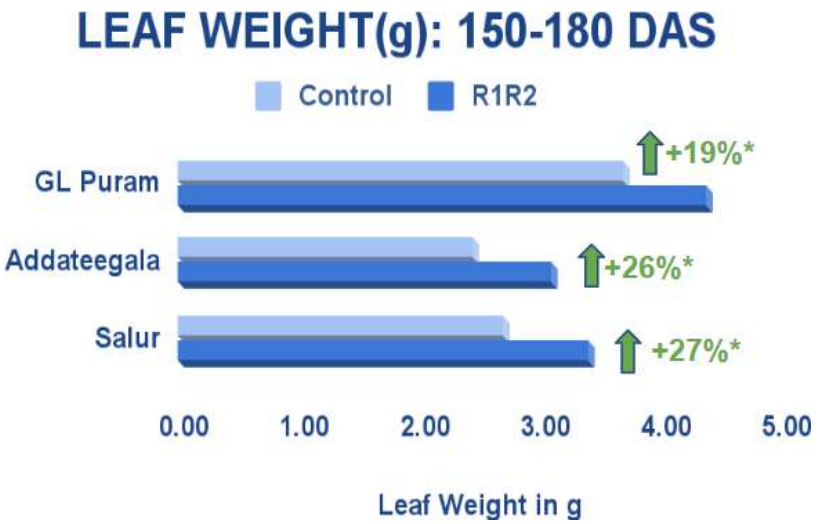


Table 10: Regional Leaf Weight in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
100 - 130	Control (g)	3±0.6	2.33±0.28	2.33±0.47
	R1 & R2 (g)	4±1*	3.02±0.54*	3.3±0.74*
	Increase (%)	42	30	41
130 - 150	Control (g)	3.7±0.88	2.53±0.51	2.47±0.6
	R1 & R2 (g)	4.38±1.2*	3±1.4*	3.21±0.83*
	Increase (%)	19	15	30
150 - 180	Control (g)	-	2.69±0.9	2.45±0.5
	R1 & R2 (g)	-	3.41±2*	3.10±0.89*
	Increase (%)	-	27	27

NOTE: * indicates p-Value ≤0.05 is significant

5. REPRODUCTIVE CROP PARAMETERS

5.1. Number of Bolls

The number of bolls per plant is a critical yield-contributing parameter in cotton. Bolls are the primary fruiting bodies that house the cotton lint and seeds. A higher number of bolls generally translates into a better yield, assuming boll size and retention are consistent. This section (Fig.10) compares boll development between control and R1 & R2 treated cotton crops across different growth stages and locations.

Fig 10: Regional No. of Bolls in Control vs. R1 & R2 Treated Cotton Plots

Table 11: Regional No. of Bolls in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
100 - 130	Control	40.65±12	53.53±4	28.30±4
	R1 & R2	53.40±10*	58.64±6*	41.53±7*
	Increase (%)	31	10	47
130 - 150	Control	42.51±12	9.72±0.38	27.34±5.2
	R1 & R2	54.42±9*	10.18±0.38*	39.21±7*
	Increase (%)	28	5	43
150 - 180	Control	-	53.51±6	25±4
	R1 & R2	-	58.84±5*	40.85±8*
	Increase (%)	-	10	63

NOTE: * indicates p-Value ≤0.05 is significant

R1 & R2 application consistently increased the number of bolls per plant across all observed locations and time intervals. The increase was particularly pronounced in Addateegala at 150–180 DAS (Table.11), where boll count increased by 63% compared to the control. This consistent improvement in boll formation under R1 & R2 treatment demonstrates its effectiveness in enhancing reproductive performance and yield potential in cotton crops.

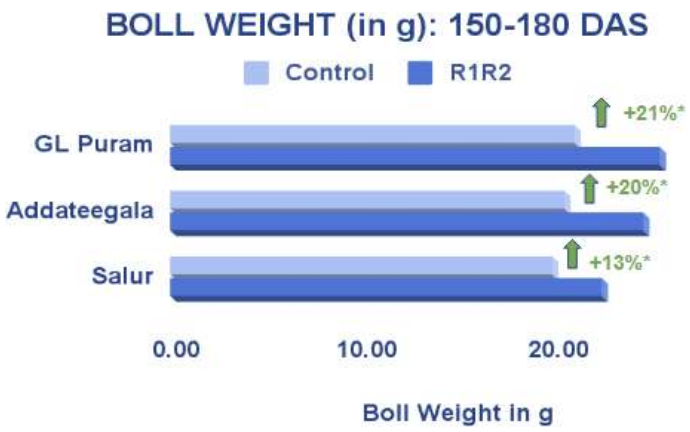
5.2. Boll Weight

Boll weight is a direct determinant of cotton yield. Heavier bolls imply greater seed cotton per plant, thereby enhancing total productivity. The following data (Fig.11) presents the comparative effect of R1 & R2 application on boll weight in cotton plants across different locations and crop growth stages.

Across all three locations and time periods, R1 & R2 application consistently improved boll weight in cotton plants. The maximum increase (21%) was observed both at Addateegala during 100–130 DAS and GL Puram during 130–150 DAS. These results (Table.12) highlight

the role of R1 & R2 in improving the mass of cotton bolls, thereby contributing to higher yield potential alongside increased boll count.

Fig 11: Regional Boll Weight in Control vs. R1 & R2 Treated Cotton Plots



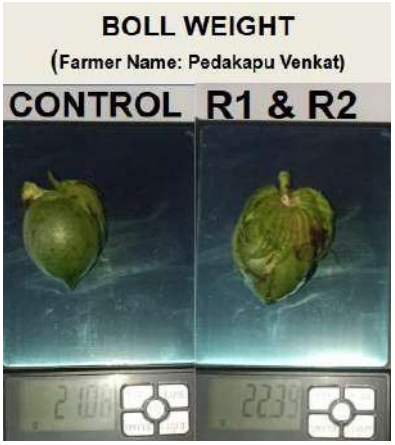


Table 12: Regional Boll Weight in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
100 - 130	Control (g)	21.98±2	19.6±2	20.43±3.2
	R1 & R2 (g)	25.18±3*	22.8±3*	24.80±4*
	Increase (%)	15	16	21
130 - 150	Control (g)	21.19±2	7.75±0.6	21.8±3
	R1 & R2 (g)	25.62±3*	7.95±0.7*	24±3.3*
	Increase (%)	21	3	10
150 - 180	Control (g)	-	20±3	20.65±3.5
	R1 & R2 (g)	-	23±3*	24.75±2.8*
	Increase (%)	-	13	20

NOTE: * indicates p-Value ≤0.05 is significant

5.3. Boll Diameter

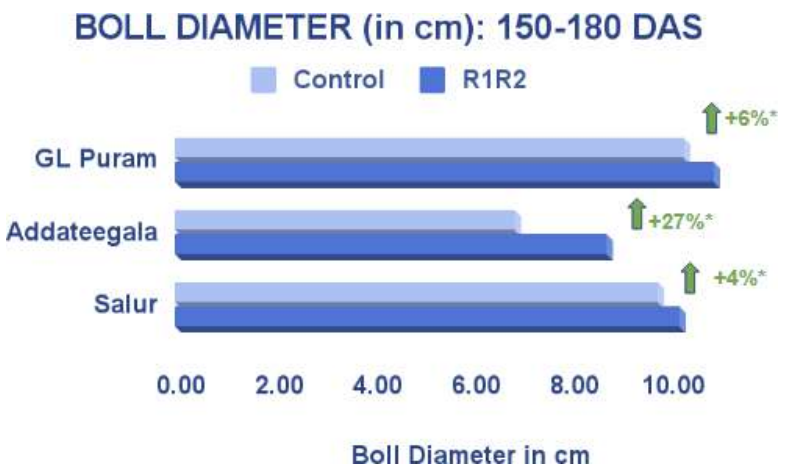
Boll diameter is an important trait that influences cotton yield, as it directly relates to the size and weight of the boll. Larger bolls typically indicate better development and higher lint content. The data below (Fig.12) illustrates the comparative performance of R1 & R2 treated plants versus control in terms of boll diameter across different locations and growth stages. The application of R1 & R2 enhanced boll diameter in cotton plants across all locations and developmental stages (Table.13). Notably, the highest increase was observed in Addateegala at 130–150 DAS (31%), followed by 29% at 100–130 DAS. This improvement suggests that R1 & R2 not only supports boll formation but also contributes to increased boll girth, which is vital for improving seed cotton output and overall plant productivity.

Table 13: Regional Boll Diameter in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
100 - 130	Control (cm)	10.7±0.75	9.65±0.33	7.27±1.36
	R1 & R2 (cm)	11.3±0.76*	10.16±0.35*	9.34±1.26*
	Increase (%)	6	5	29
130 - 150	Control (cm)	10.32±0.92	0.6±0.04	6.86±1.3
	R1 & R2 (cm)	10.93±0.72*	0.65±0.03*	8.97±1.2*
	Increase (%)	6	10	31
150 - 180	Control (cm)	-	9.83±0.52	7±1.1
	R1 & R2 (cm)	-	10.25±0.54*	8.78±1.3*
	Increase (%)	-	4	27

NOTE: * indicates p-Value ≤0.05 is significant

Fig 12: Regional Boll Diameter in Control vs. R1 & R2 Treated Cotton Plots



5.4. Number of Squares

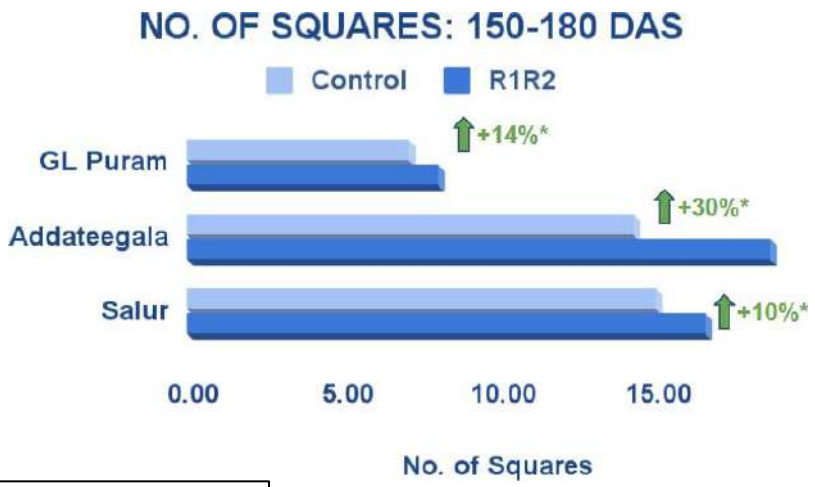
The number of squares is a key indicator of reproductive potential in cotton. A higher number of squares directly contributes to an increased number of bolls, thereby improving yield. The following table.14 compares the number of squares in control and R1 & R2 treated cotton plants at various growth stages across three locations. R1 & R2 treatment significantly increased the number of squares across all locations and stages (Fig.13). The highest increase was observed in GL Puram during 100–130 DAS (51%), indicating early and robust reproductive activity. Enhanced square formation under R1 & R2 treatment suggests improved nutrient availability and hormonal balance, which supports stronger yield potential through increased boll formation.

Table 14: Regional No. of squares in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
100 - 130	Control	7±5	13.60±2.1	17.27±5.3
	R1 & R2	10.50±4.6*	16.13±2.6*	19.50±4
	Increase (%)	51	19	13
130 - 150	Control	7.16±4.4	14.18±2.27	12.81±4
	R1 & R2	8.14±4.13*	14.93±2.28*	17.74±4*
	Increase (%)	14	5	38
150 - 180	Control	-	15.12±3	14.45±3.47
	R1 & R2	-	16.72±3*	18.85±3.31*
	Increase (%)	-	11	30

NOTE: * indicates p-Value ≤0.05 is significant

Fig 13: Regional No. of squares in Control vs. R1 & R2 Treated Cotton Plots



6. Yield Parameters (Yield/Acre)

Yield per acre is the most critical agronomic and economic indicator of cotton crop performance. It is directly influenced by plant growth, number and weight of bolls, and overall plant health. The following table.15 & Fig.14 presents the impact of R1 & R2 application on cotton yield across different developmental stages and locations. R1 & R2 application led to a substantial improvement in cotton yield across most locations and growth stages. The highest gain (135%) was observed in Addateegala at 150–180 DAS. Notably, Salur recorded a 111% yield boost during the 130–150 DAS period. The only reduction (-11%) occurred in Addateegala during the 100–130 DAS stage, which may be attributed to environmental or physiological stress factors. Overall, the data demonstrate R1 & R2's effectiveness in

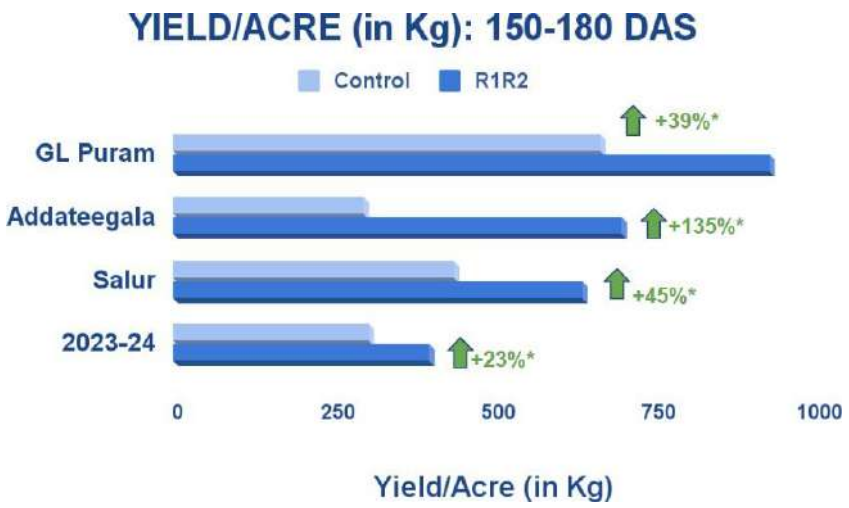
enhancing yield performance, likely through improved plant vigor, boll development, and nutrient use efficiency.

Table 15: Comparative Analysis of Yield/acre in Control vs. R1 & R2 Treated Cotton Plots

DAS	Fields	GL Puram	Salur	Addateegala
100 - 130	Control (kg)	609±214	609±214	1155±296
	R1 & R2 (kg)	910±327*	910±327	1029±382
	Increase (%)	50	50	-11
130 - 150	Control (kg)	668±221	683±212	361±73
	R1 & R2 (kg)	931±364*	1441±788*	614±248
	Increase (%)	39	111	70
150 - 180	Control (kg)	-	441±224	299±98
	R1 & R2 (kg)	-	640±341*	701±242
	Increase (%)	-	45	135

NOTE: * indicates p-Value ≤0.05 is significant

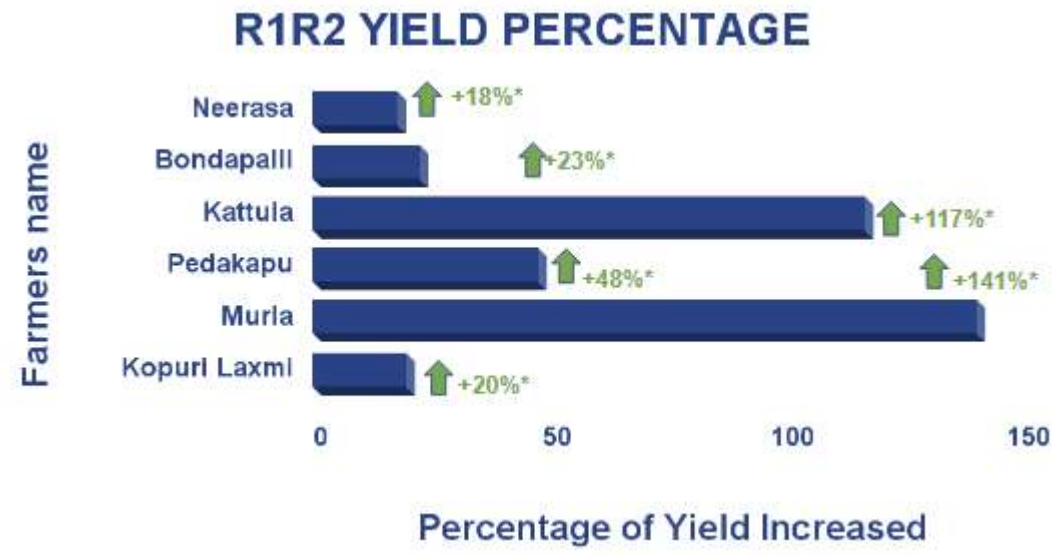
Fig 14: Regional Yield/acre Comparison: Control vs. R1 & R2 Treated Cotton Plots



6.1. Yield/acre of Metagenomics Farmers

- All six farmers reported an increase in yield after R1&R2 application (Fig.15), confirming its consistent positive effect.
- The maximum yield improvement was observed in Murla's field (75%), suggesting optimal responsiveness or favorable field conditions.
- Farmers Bondapalli and Kattula each observed a 41% increase, while Pedakapu recorded a 36% gain.
- Neerasa and Kopuri Laxmi recorded more modest increases at 25% and 20%, respectively.

Fig 15: Yield/acre Comparison of Metagenomics Farmers



7. METAGENOMICS STUDIES

7.1. Stacked Bar Plot

Microbial DNA was extracted from 250 mg of soil samples using a modified DNeasy PowerLyzer PowerSoil Kit (Cat. No: 12855-100, Qiagen GmbH, Germany) to maximize DNA yield and purity. DNA concentration was quantified using Nanodrop to ensure precise measurement of nucleic acid content. For sequencing, 25 nanograms of DNA was utilized as a template to amplify the hypervariable V3-V4 region of the 16S rRNA gene on the Illumina NextSeq platform. All samples met rigorous quality control standards (Q20 > 95%) and were subsequently processed for downstream analysis. Initial quality control of raw reads was conducted with FastQC, followed by adapter and barcode trimming using TrimGalore Sv0.6.10.

For downstream analysis, relative abundance calculations were conducted to determine the composition of the top 15 most abundant species (Fig.16), with comparisons made between the study groups with pre- and post-treatment. In the stacked bar plot, a similar post-treatment trend is observed in both groups. However, two species exhibit inverse responses to treatment. Candidatus Korobacter versatilis increased at 180 DAS in both groups, whereas Dokdonella koreensis decreased. The overall plant growth- promoting rhizobacteria (PGPR) composition remained largely consistent at 90 DAS and 180 DAS, two genera exhibited contrasting trends: Frankia declined at 180 DAS, whereas Rhizobium increased (Fig.17).

Fig 16: Species Abundance Distribution Across Control vs R1 & R2

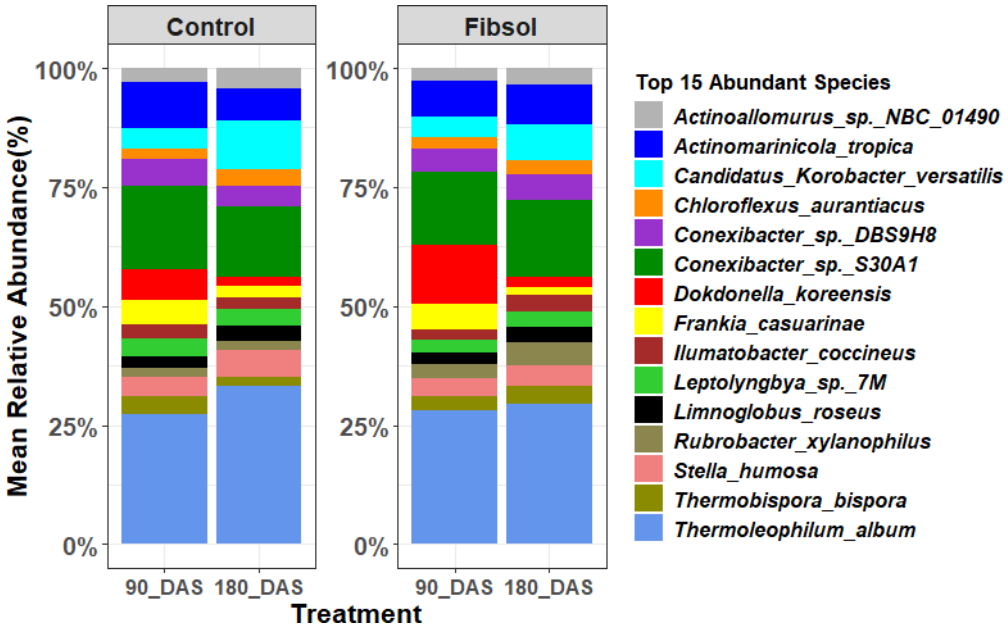
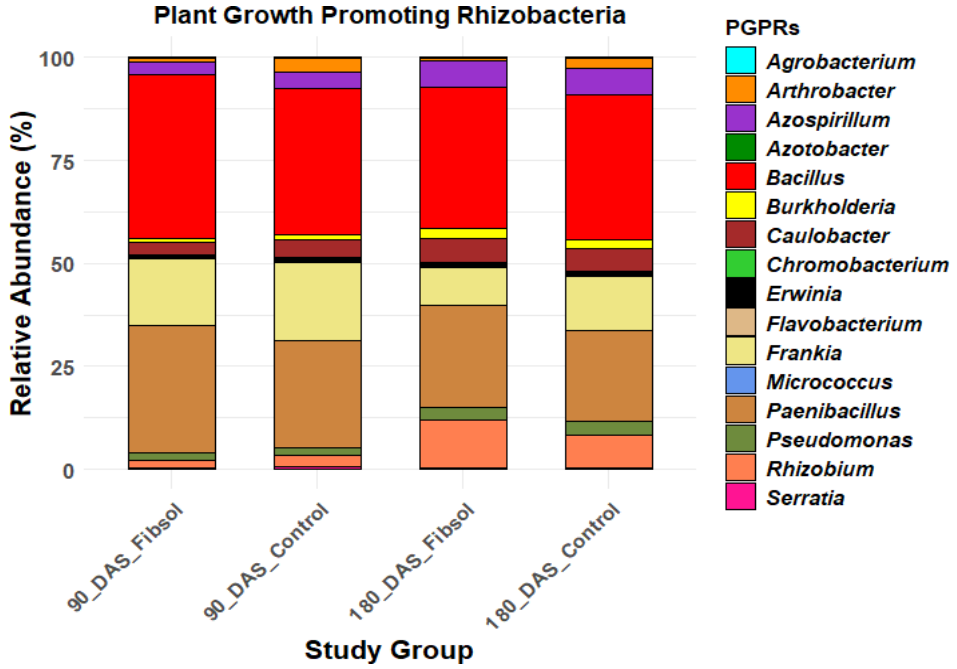


Fig.17: PGPR population Density in Control and Treatment Zones

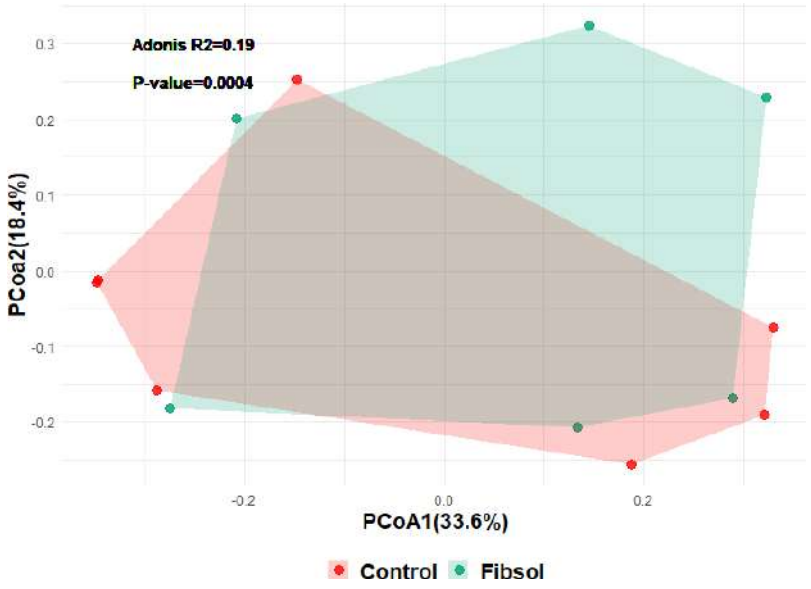


7.2 Beta Diversity

Beta diversity was assessed to evaluate microbial richness and community dissimilarity using the vegan package in R. Beta diversity was analyzed with the Bray-Curtis dissimilarity index and visualized using Principal Coordinate Analysis (PCoA) ordination (Fig.18). The Beta Diversity PCoA plot demonstrates microbial community composition differences between the Control and R1 & R2 treated groups. PCoA1 (33.6%) and PCoA2 (18.4%) explain the major variation in community structure. The Adonis R^2 value of 0.19 suggests that 19% of the observed variation is attributable to R1 & R2 treatment, indicating a moderate effect. The

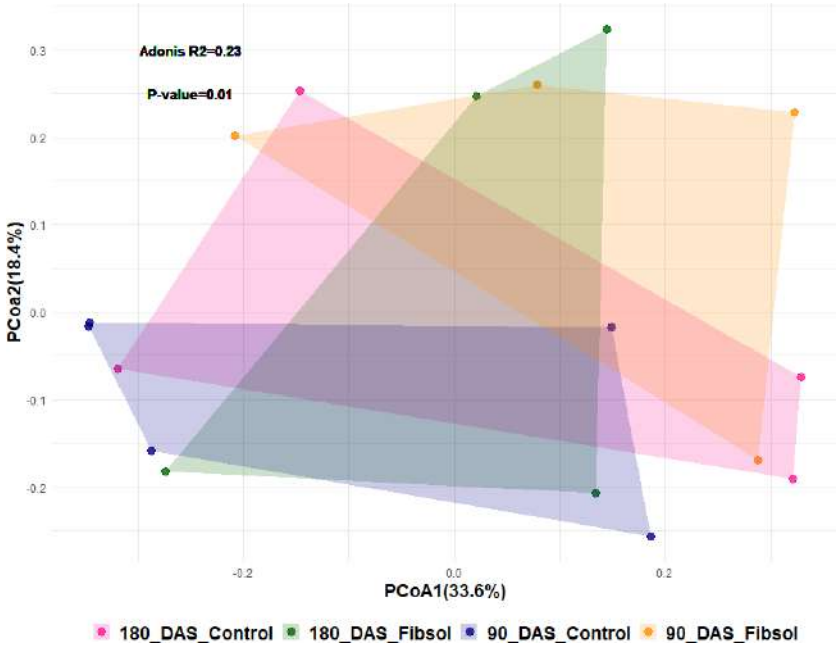
statistically significant p-value (0.0004) confirms distinct microbial composition between the groups, with clear separation in the clusters, signifying that R1 & R2 treatment induced notable microbial shifts.

Fig 18: Beta Diversity Gradients Across Control vs R1 & R2



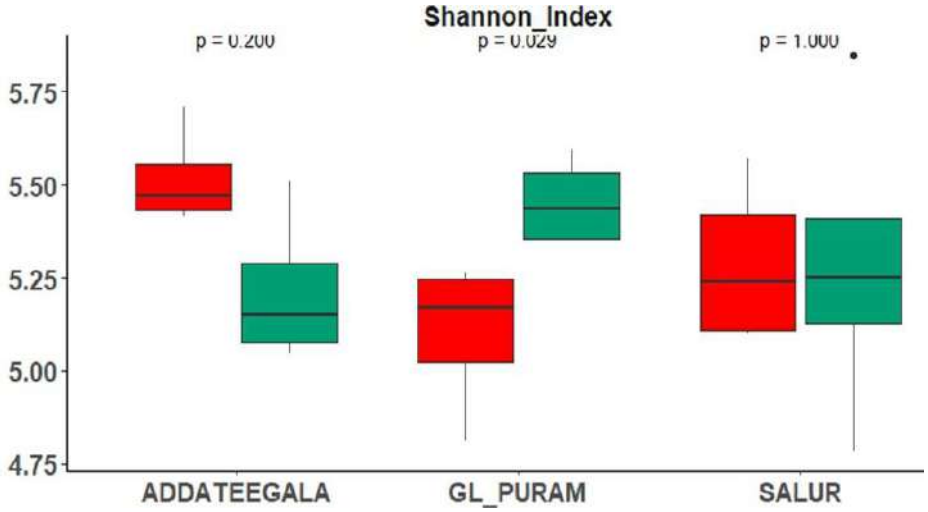
Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity revealed distinct clustering of microbial communities across treatment groups and time points, with the first two axes (PCoA1 and PCoA2) explaining 33.6% and 18.4% of the total variation, respectively. The microbial communities in R1 & R2 treated samples were clearly separated from their respective controls at both 90 and 180 days after sowing (DAS), indicating a significant shift in community structure following treatment (Fig.19).

Fig 19: Beta Diversity Gradients Across 90 DAS & 180 DAS of Control vs R1 & R2



This was supported by PERMANOVA (Adonis) results ($R^2 = 0.23$, $p = 0.01$), confirming that treatment explained a substantial portion (23%) of the observed variation. Notably, the R1 & R2 treated groups showed minimal overlap with controls at each time point, highlighting a consistent and time-independent effect of the treatment on soil microbial composition. These findings suggest that R1 & R2 application led to a sustained restructuring of the soil microbiome over time.

Fig 20: Shannon Diversity Index of Treated field across three regions



In Shannon diversity, Microbial richness (Observed-OTUs) showed significantly increased in GL Puram ($p = 0.029$), indicating enhanced species diversity (Fig.20).

7.3. Pairwise Permanova Analysis

To identify specific taxa significantly differing in composition based on three different regions, a MANOVA analysis was performed. Statistical significance of beta diversity differences between study groups was determined using PERMANOVA (Adonis function). All statistical analyses were conducted with the ggpubr package (https://CRAN.R-project.org/package=ggpubr). Pairwise PERMANOVA (Adonis) was conducted using the vegan package in R to assess differences in microbial community across treatment groups based on Bray- Curti's dissimilarity.

Table 16: Pairwise Adonis Analysis

Pairwise Results

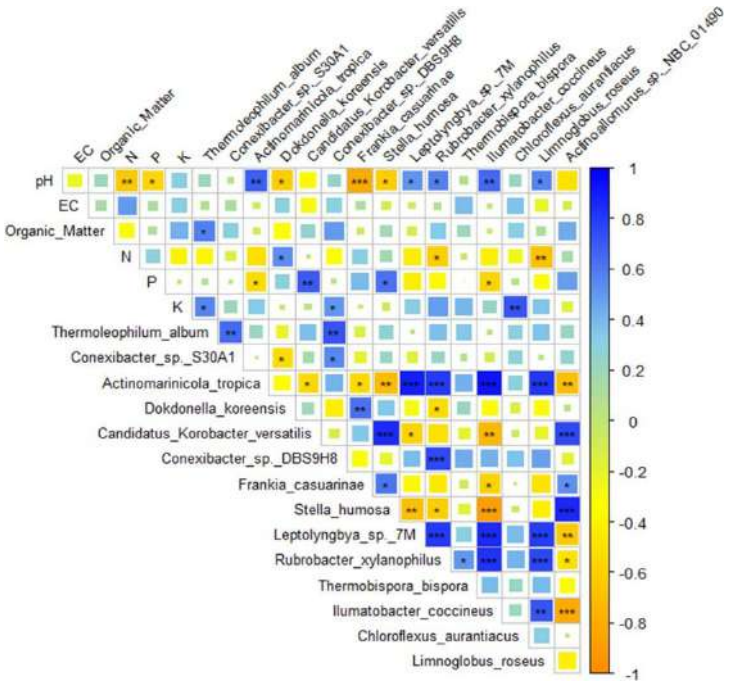
pairs	R2	p.value	p.adjusted	sig
90_DAS_Fibsol vs 180_DAS_Fibsol	0.055099	0.867	0.884	
90_DAS_Fibsol vs 90_DAS_Control	0.162668	0.094	0.141	
90_DAS_Fibsol vs 180_DAS_Control	0.243515	0.004	0.024	*
180_DAS_Fibsol vs 90_DAS_Control	0.192271	0.051	0.102	
180_DAS_Fibsol vs 180_DAS_Control	**0.266898**	**0.008**	**0.024**	*
90 DAS Control vs 180 DAS Control	0.05239	0.884	0.884	

Pairwise Adonis analysis indicated that R1 & R2 treatment induced significant shifts in soil microbial community composition when compared to control conditions. Notably, at 180 DAS, the microbial community in the R1 & R2 treated soil differed significantly from that in the control group (R^2 = 0.267, p.adj = 0.024), suggesting a strong and sustained effect of the treatment over time (Table.16). Interestingly, even at an earlier time point (90 DAS), the R1 & R2 treated soil microbiome was significantly different from the control at 180 DAS (R^2 = 0.244, p.adj = 0.024), reinforcing the notion that R1 & R2 alters the microbial trajectory as early as 90 DAS. While within-treatment temporal shifts (e.g., 90_DAS_ R1 & R2 vs 180_DAS_ R1 & R2) were not significant, the consistent differences compared to control conditions at both time points suggest that R1 & R2 has a stable and lasting influence on community structure, rather than transient or time-driven changes alone.

7.4. Correlation Analysis

Subsequently, Spearman correlation analyses were performed using the Hmisc and corrplot packages to explore associations between key microbial taxa, abiotic soil parameters (e.g., pH, organic matter, EC), and macronutrients such as N, P and K.

Fig 21: Correlation Matrix of Key Growth Variables



The correlation analysis between microbial species and soil abiotic factors revealed (Fig.21) distinct patterns of association that may provide insights into microbial ecological preferences and potential functional roles in soil health. Notably, several species exhibited a positive correlation with soil pH, including Actinomarinicola tropica, Leptolyngbya sp. 7M, Rubrobacter xylanophilus, Ilumatobacter coccineus, and Limnoglobus roseus, suggesting that

these taxa are favoured in more alkaline conditions.

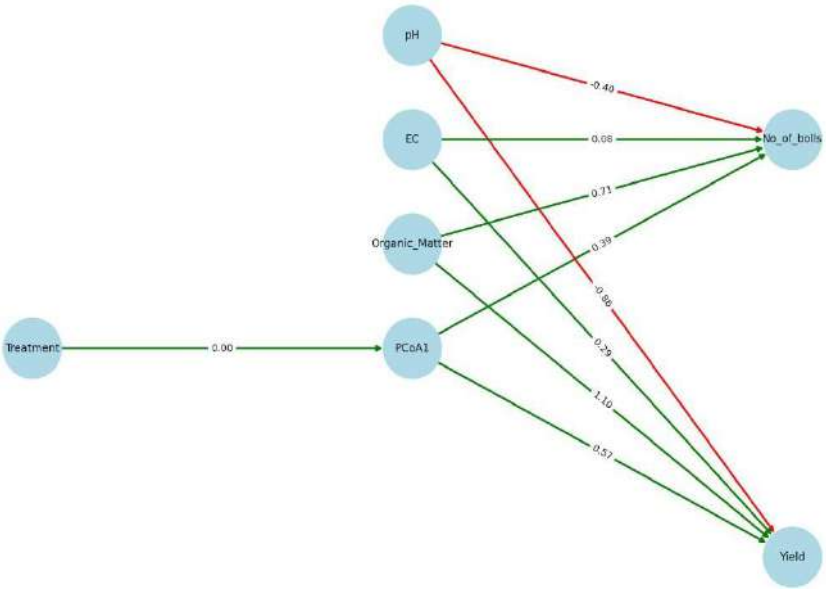
Conversely, species such as Dokdonella koreensis, Frankia casuarinae, and Stella humosa showed a negative association with pH, indicating their preference for more acidic environments. With respect to organic matter, Thermooleophilum album displayed a positive correlation, implying its abundance is enhanced in nutrient-rich soils. The factor nitrate-nitrogen showed mixed associations: Dokdonella koreensis had a positive correlation, while *Rubrobacter xylanophilus* and Limnoglobus roseus were negatively correlated, highlighting varied nitrogen assimilation or tolerance capacities among species. Interestingly, correlations with available phosphorus were also split; Stella humosa and Candidatus Korobacter versatilis had positive correlations, while Actinomarinicola tropica and Ilumatobacter coccineus had negative ones, indicating differential phosphorus utilization strategies.

Lastly, *Thermooleophilum album, Chloroflexus aurantiacus*, and *Conexibacter sp.* DBS9H8 were positively correlated with potassium exchangeability, suggesting their possible involvement in potassium turnover or adaptation to potassium-rich microenvironments. Overall, these associations highlight the nuanced and species- specific interactions between microbial communities with key soil physicochemical parameters and macronutrients.

7.5. Structural Equation Model

A Structural Equation Model (SEM) was constructed (Fig.22) using the lavaan package to examine the direct and indirect pathways by which treatment, microbiome community composition (PCoA1 scores), and soil properties influence crop yield. This integrative approach allowed for a comprehensive understanding of the interplay between microbiota, soil environment, and crop performance.

Fig 22: Structural Equation Model Path Diagram



Despite suboptimal overall model fit, likely attributable to the limited sample size (N=12), the SEM analysis identified several statistically robust associations that elucidate the primary determinants of crop yield. Microbiome composition, as captured by the first principal coordinate axis (PCoA1), exhibited a significant and positive effect on yield (standardized coefficient = 0.575, p & lt; 0.001), indicating a potentially beneficial role of microbial community structure in enhancing crop productivity.

Among abiotic soil parameters, organic matter demonstrated a markedly strong and statistically significant positive association with yield (standardized coefficient = 1.101, p & lt; 0.001), whereas soil pH exerted a significant negative influence (standardized coefficient = -0.865, p = 0.002), suggesting that acidic conditions may be more favorable for optimal yield under these conditions. The model accounted for 79% of the variance in yield, highlighting the critical roles of both soil health and microbial ecology in driving agricultural performance.

8. Soil Microbial Dynamics

Soil microbial activity indicates the soil health and nutrient cycling. Colony Forming Units (CFU/g) were assessed at 90 DAS (Days After Sowing) and 150 DAS in control and R1 & R2 treated plots to evaluate changes in microbial populations.

Table 17: Comparative Soil Parameter Analysis of control and R1 & R2 field

PARAMETERS	FIELDS	90 DAS	Post Harvest	SOIL TEST RATING			
				LOW	MEDIUM	OPTIMUM	VERY HIGH
pH	Control	6.28	6.27				
	R1R2	6.12	6.5				
Organic Matter (%)	Control	0.47	0.85				
	R1R2	0.53	0.925				
Nitrate Nitrogen (mg/kg)	Control	95.41	58.29				
	R1R2	72.89	49.98				
Available Phosphorus (mg/kg)	Control	62.72	56.07				
	R1R2	26.3	46.78				
Potassium Exchangeable K (mg/kg)	Control	149	199.25				
	R1R2	118.67	178				

Table 18: Comparative Soil Analysis of region Control vs. R1 & R2 Treated Cotton Plots

Region		pH		Organic Matter (%)		Nitrate Nitrogen (mg/kg)		Available Phosphorus (mg/kg)		Potassium Exchangeable K (mg/kg)	
		90 DAS	Post harvest	90 DAS	Post harvest	90 DAS	Post harvest	90 DAS	Post harvest	90 DAS	Post harvest
GL Puram	Control	5.44	6.66	0.51	0.79	181.39	38.955	106.9	28.25	206	245
GL Puram	R1R2	5.85	6.45	0.96	1.13	93.145	51.315	41.43	47.755	143.5	184
Salur	Control	7.55	6.2	0.27	1.06	56.25	48.03	15.31	87.11	124	153
Salur	R1R2	6.99	7.3	0.62	1.03	50.27	42.51	7.52	3.87	152	221
Addateegala	Control	5.86	5.77	0.62	0.84	48.59	80.69	65.95	53.075	117	153.5
Addateegala	R1R2	5.88	5.8	0.41	0.41	58.16	54.79	57.51	87.75	68	123

MICROBIAL COUNTS IN CONTROL VS R1&R2 PLOTS

Fig.23: Quantitative Comparison of Total and Differential Microbial Counts in Control and R1 & R2 Treated Samples

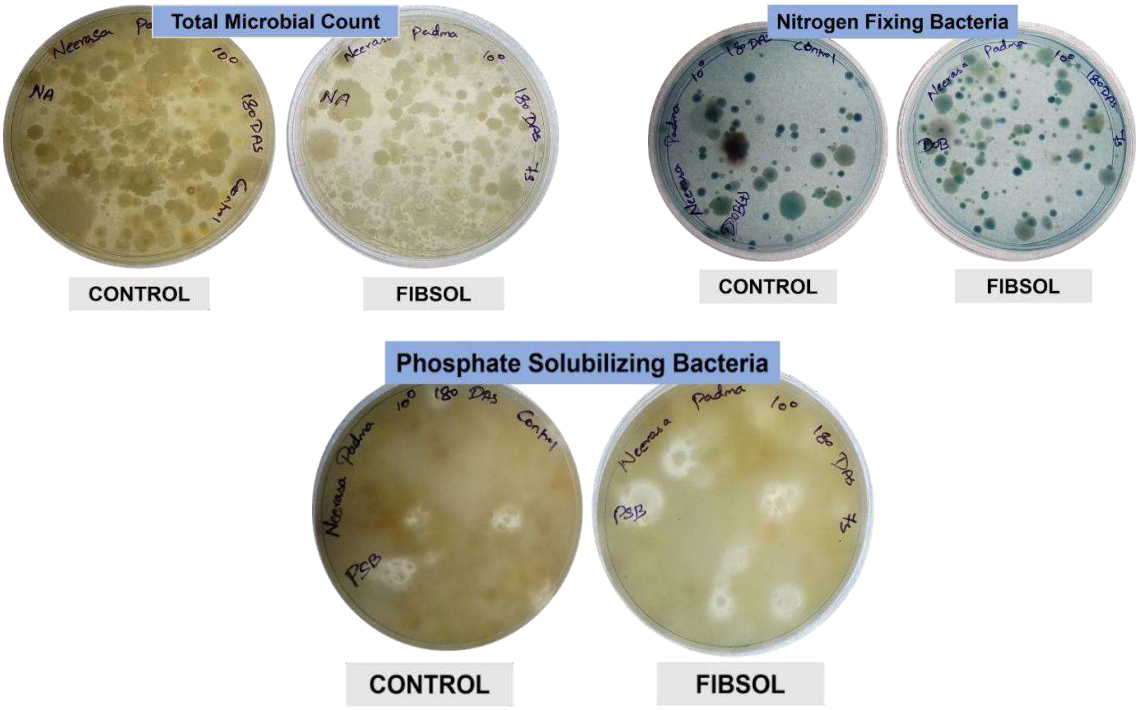


Table 19: Comparison of Total and Differential Microbial Counts in Control vs. R1 & R2 Treated Cotton Plots

S NO	Microbial Analysis	OVERALL				CFU/g
		90 DAS		180 DAS		
		Control	R1R2	Control	R1R2	
1	Total Microbial Count	10^7	10^7	10^6	10^7	≥10^6
2	Nitrogen Fixing Bacteria	10^6	10^7	10^6	10^7*	-
3	Phosphate Solubilizing Bacteria	10^6	10^7	10^6	10^7*	-

NOTE: *p Value ≤0.05 is significant

- **Total microbial count (**Table.19**)** remained within acceptable levels across all stages and treatments, with R1 & R2 consistently maintaining or improving microbial density.
- **Nitrogen-fixing bacteria** saw a notable boost at 90 DAS with R1 & R2, though levels equalized by 150 DAS.
- **Phosphate-solubilizing bacteria** exhibited higher counts in R1 & R2 treatments at both 90 and 150 DAS, suggesting improved phosphorus availability over time.
- These findings suggest that R1 & R2 positively influences soil microbial dynamics, contributing to better soil health and nutrient support for plant growth.

9. POST HARVEST COTTON DATA

Table 20: Effect of R1 & R2 on Cotton Fiber Quality

S.No	Farmer name	Cluster	Field type	UHML (mm)	UI (%)	MIC (ug/inch)	Tenacity (g/tex)	COLOR GRADE			Ginning percentage (%)	Trash % (CIRCOT SP-4)
								Rd (%)	b	C-G		
1	Bondapalli Dalamma	GL Puram	R1&R2	27.2	86	4.8	29.3	81.6	7.5	21-1	30.2	1.34
		GL Puram	Control	28.4	84	4.2	29.9	87.9	4.9	31-1	30	1.82
2	Neevasa padma	GL Puram	R1&R2	30.9	86	4.9	32.7	81.7	7.3	21-2	32.43	2.08
		GL Puram	Control	29.6	88	4.6	31.6	83.9	8.2	11-1	31.57	1.89
3	Pedakapu Venkati	Salur	R1&R2	29.9	86	4.6	31.4	80.7	7.9	21-1	31.47	2.12
		Salur	Control	28.4	85	3.9	30.7	84.7	6.7	21-1	29.4	2.1
4	Kattula Buchayya	Salur	R1&R2	29.7	87	4.8	31.9	78.9	7	31-2	33.4	2.15
		Salur	Control	29.8	88	5	32.9	84.5	7.5	11-1	32.43	2.05
5	Kopuri Laxmi	Addateegala	R1&R2	30	87	3.6	31.3	81.2	7.4	21-2	31.1	1.38
		Addateegala	Control	31.9	87	4.4	34.3	71.3	10.7	33-2	33.4	1.58
6	Murla Jangamma	Addateegala	R1&R2	30.7	88	4.6	31.8	83.8	7.4	11-2	32.5	1.81
		Addateegala	Control	29.9	87	4.4	32.8	83.7	8.7	11-1	32.97	1.74

10. ECONOMICAL STUDIES

To assess the economic viability of R1 & R2 application in cotton cultivation, yield per acre and benefit-to-cost (B: C) ratios were analyzed across different farmers. This section compares the control yields with those under R1 & R2 treatment, highlighting yield improvements and corresponding B: C ratios.

All farmers recorded positive yield improvements under R1 & R2 application, ranging from ~15% to 141%(Table.21).

Kattula Buchayya and Murla Jangamma experienced the highest yield gains, translating to strong economic benefits. Calculation of Benefit cost ratio of Neevasa padma farmer were calculated and cost of cultivation explained elaborately (Table.22).

Table 21: Impact of R1 & R2 on cotton yield and Economic Returns (Control vs. R1 & R2)

S NO	Farmers Name	Control yield/acre	FIB-SOL yield/acre	% Increased
1	Neerasa Padma	846	995	18
2	Bondapalli Dalemma	417	512	23
3	Kattula Buchayya	538	1167	117
4	Pedakapu venkat	254	375	48
5	Murla Jangamma	245	590	141
6	Kopuri laxmi	500	600	20
	Total	2800	4239	51

The **B:C ratios** reflect economic profitability, with values as high as **6.4:1**, indicating that R1 & R2 is a cost-effective input for cotton cultivation.

Farmer Name : Neerasa Padma

Control Yield/acre : 846 Kg

R1 & R2 Yield/acre : 995 Kg

Market Selling Price of 1kg Cotton : Rs 68

Gross Income : Rs.67,660

Cost of Cultivation/Investment : Rs. 14,300

Profit : Gross Income - Cost of Cultivation

= 67,660 - 14,300

= Rs. 53,360

Table 22: Cost of Cultivation

Cultivation	Amount (Rs)
Plowing	3600
Seed	1640
Sowing	1600
Manures	1800
Sprayings	1200
Weeding	1800
Picking	1200
Raddis cotton I	730
Raddis Cotton II	730
Total	**14,300**

Return On Investment: (Profit/Cost of Investment) *100

: (53,360 /14,300) *100

ROI : 373

B:C Ratio : Gross Income / Cost of Cultivation

: 67660/14300 = 4.7

B:C = 5:1

The comparative analysis of gross income per acre (Fig.24) among different farmers reveals a clear economic advantage of using R1R2 treatments over conventional practices. On average, the treated fields recorded a 70% higher gross income compared to the control fields. The most notable improvement was observed in the case of Murla Jangamma, who achieved a 141% increase in income, followed by Kattula Buchayya with 117%, and Pedakapu Venkat with 48%. Other farmers, including Neerasa Padma, Bondapalli Dalemma, and Kopuri Laxmi, experienced moderate-income increases ranging from 18% to 23% and 20%, respectively.

These consistent gains across various farmers suggest that the R1R2 treatment is universally effective and economically beneficial, presenting a strong case for its wider adoption in cotton cultivation practices.

Fig.24: Regional Comparison of Gross Income/acre: Control vs. R1 & R2

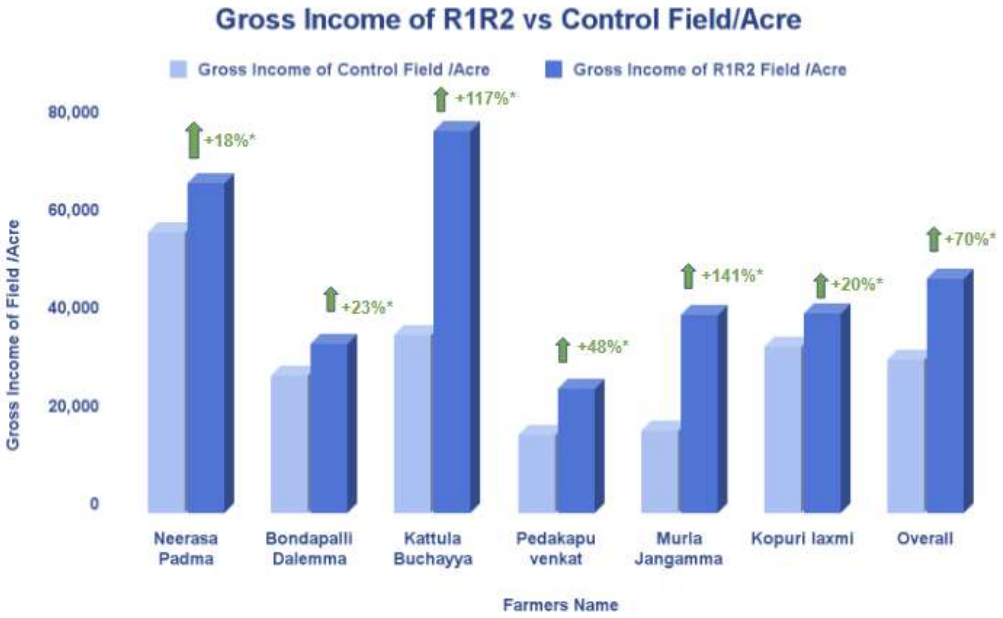


Table 23: Comparative Economic Analysis of R1 & R2 Application Across Farmers

Farmers Name	Control B:C Ratio	R1R2 B:C Ratio
Neerasa Padma	4.4:1	5:1
Bondapalli Dalemma	2.3:1	2.6:1
Kattula Buchayya	3.3:1	6.4:1
Pedakapu venkat	1.3:1	2.3:1
Murla Jangamma	1.4:1	2.8:1
Kopuri laxmi	3:1	3.2:1
Overall	2.7:1	4:1

The Benefit-to-Cost (B:C) ratio analysis (Table 23) highlights the economic efficiency of implementing the R1 and R2 treatments in cotton cultivation. All farmers demonstrated an improvement in B:C ratios when switching from control practices to the R1R2 treatment. The most significant gain was observed in Kattula Buchayya's field, where the B:C ratio nearly doubled from 3.3:1 to 6.4:1, indicating a strong return on investment. Similarly, Murla Jangamma and Pedakapu Venkat recorded notable increases from 1.4:1 to 3:1 and 1.3:1 to 2:1, respectively. Even farmers with already efficient practices like Neerasa Padma and Kopuri

Laxmi experienced meaningful improvements. On average, the overall B:C ratio increased from 2.7:1 under control conditions to 4:1 with R1R2, underscoring the economic viability and scalability of the treatment across different farm environments.

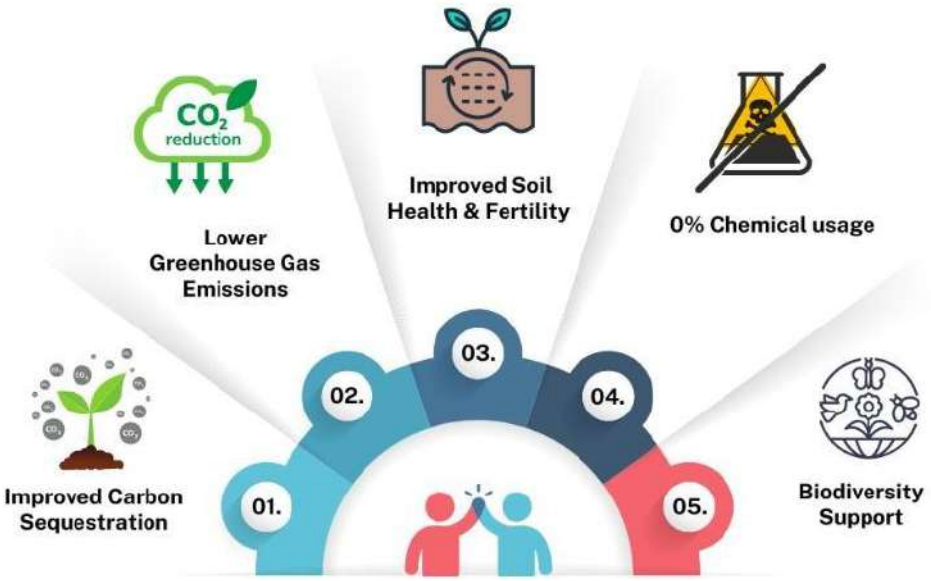
11 Environmental Studies

One of the primary challenges in modern agriculture is the environmental impact of excessive fertilizer use. Especially nitrogen-based fertilizers, which is the largest contributor to greenhouse gas (GHG) emissions globally (Table.24).

Table 24: Conventional vs Organic vs R1R2

	Yield Kg/Acre	CO_2 Kg Released/ Kg of Cotton Fibre	1% Reduction in CO_2 Emission
Conventional	428	1.5	-
Organic	466 +1.08%*	0.9	38 *
FIBSOL Treatment	706 +1.64%*	0.9	57 *

Fig.25: USP of R1R2



Enhancing fertilizer efficiency is the most effective way to reduce cotton's carbon footprint. The R1R2 treatment method cuts CO_2 emissions by 57% per kilogram of cotton fiber, reducing emissions from 1.5 kg to 0.9 kg CO_2/kg, outperforming the organic method's 38% reduction.

Additionally, R1R2 achieves the highest yield at 706 kg per acre, a 1.64% increase over conventional methods, while organic cultivation offers only a 1.08% gain. This makes R1R2 the most sustainable and productive option among the three methods.

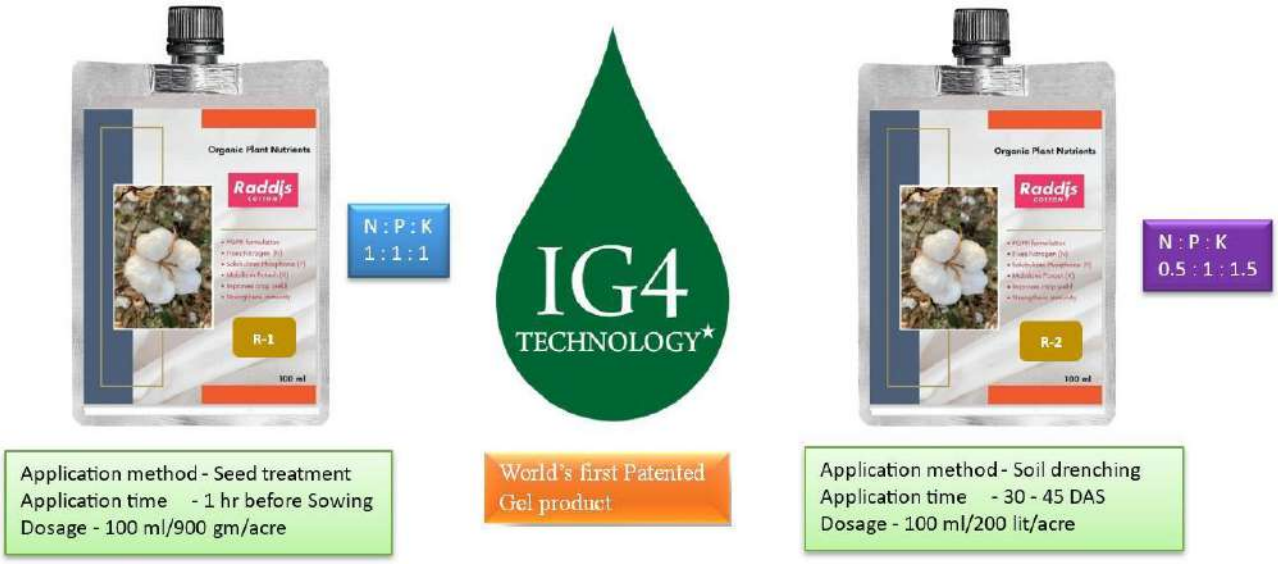
12. R1 & R2 PRODUCTS

R1&R2 is the world's first patented gel product combining soil bacteria in a biodegradable polymer gel. It enhances nutrient availability by fixing nitrogen, solubilizing phosphorus, and mobilizing potassium, with a concentration of 10^{11} CFU/ml. This water-soluble, easy-to-use product boosts yield, fiber quality, boll size, and drought tolerance in cotton, increasing yields by 20–30% while meeting textile industry standards.

12.1 Salient Features:

- Technology : IG4
- Light weight : Bulky biofertilizers are now compact
- Soil : Quality improves from poor to good
- Water Soluble : Easy to apply
- Condensation : High Pay Load > 1011 CFU/ml of Beneficial Bacteria
- Encapsulation : Protected in a gel to increases the durability
- Biodegradable : Easily decomposed in the soil

Fig.26: R1 & R2 Products



12.2 Precautions to be taken:

- Don't mix R1&R2 with any chemical pesticides or Synthetic Fertilizers.
- Apply in cool climate i.e. in early morning or evening times.
- Avoid application/spraying of any chemicals, fertilizers before or after 2 days of

application of R1&R2.

- At the time R1&R2 application maintain some moisture conditions in the fields.
- R1&R2 should be stored only at room temperature.

Table 25: R1R2 Product Application procedure

S. No	Crop	Method of application	Dosage/acre	Time of application
1	Cotton	Seed treatment	100 ml/ 900 grams of seed	1 hr Before Sowing
2		Water drenching	100 ml/400 litres of water	30 - 45 DAS
3		Sand broadcasting	100 ml/20 kgs of sand	30 - 45 DAS

13. Conclusion

In conclusion, the application of Raddis Cotton I and II (R1 & R2) biofertilizers demonstrated a significant positive impact on cotton crop performance across multiple agronomic and ecological parameters. Treated plots consistently outperformed control plots in plant height, stem girth, leaf area, branch development, boll count, and overall yield, with Addateegala region showing the most pronounced improvements. Notably, cotton yield increased substantially, with some regions achieving over 100% gains. The microbial analysis confirmed that R1 & R2 treatments induced clear and sustained shifts in soil microbial communities, promoting beneficial taxa associated with improved soil health and nutrient cycling. Beta diversity and PERMANOVA analyses highlighted statistically significant differences in microbial composition between treated and control soils, affirming the biofertilizers' role in shaping rhizospheric biodiversity. Correlation studies further linked microbial taxa with essential soil properties and macronutrients, suggesting enhanced functional potential in R1 & R2 treated soils. The SEM model reinforced the idea that shifts in microbiome structure positively influence crop productivity, mediating soil-crop interactions. These findings underscore the agronomic and ecological value of integrating biofertilizers like R1 & R2 into cotton cultivation practices, offering a sustainable pathway to increase yield, restore soil fertility, and reduce reliance on chemical inputs.