Full text open access online (Since 2007)
© Kamla-Raj S-EM 2026
Ethno Med, 20(3): 151-169 (2026)
PRINT: ISSN 0973-5070 ONLINE: ISSN 2456-6772
DOI: 10.31901/24566772.2026/20.03.730

Pergularia daemia as a Novel Bioindicator of Soil Fertility: A Comprehensive Phytochemical and Agronomic Study

Saranya Chinnadurai[1*]

[1]Department of Microbiology, Prince Shri Venkateshwara Arts and Science College, Chennai, Tamil Nadu, India*
E-mail: saranyamicro@princescience.in

KEYWORDS Bioindicator. Microbial Biomass. *Pergulariadaemia*. Soil Fertility. Sustainable Agriculture

ABSTRACT Soil fertility degradation threatens sustainable agriculture, especially in developing countries. This study evaluated *Pergularia daemia* as a bioindicator of soil fertility and examined its combined effect with termite soil on crop productivity. Phytochemical screening of leaves, stems, roots, and flowers using different solvent systems showed that ethanolic extracts contained the highest levels of alkaloids, flavonoids, and phenols. HPLC analyses confirmed the presence of important bioactive compounds. A 30-day pot experiment using *Pennisetum glaucum* compared sterile soil, termite soil, and treatments with *P. daemia.* The combination of termite soil and *P. daemia* produced the greatest plant growth and improved nitrogen and phosphorus levels. Beneficial microorganisms such as *Citrobacter freundii*, *Paenibacillus sp*. and *Lactococcus sp*. were also identified. The findings highlight a low-cost, eco-friendly strategy for improving soil fertility and sustainable agriculture.

INTRODUCTION

Soil degradation represents one of the most pressing environmental challenges of the 21st century, affecting agricultural productivity and food security globally. Recent studies have highlighted the increasing extent of land degradation and declining soil quality associated with intensive agricultural practices and excessive chemical inputs (Chaudhuri et al. 2023). According to recent surveys, soil pollution affects approximately 78 percent of Indian agricultural land, with 34 percent attributed to chemical fertilisers and synthetic amendments (Environmental Protection Agency 2016). This alarming trend necessitates the development of sustainable, cost-effective solutions for soil fertility assessment and enhancement.

Bioindicators offer a practical approach to monitoring soil health, responding rapidly to environmental changes compared to conventional chemical analyses (Jones et al. 2018). Plants capable of indicating soil fertility status while simultaneously improving soil quality represent an ideal solution for resource-limited agricultural communities. *Pergulariadaemia* (Forssk.) Chiov., belonging to the family Asclepiadaceae, has emerged as a promising candidate for such dual functionality.

P. daemia is a perennial, latex-producing climbing herb distributed across tropical and subtropical regions of India, Africa, and Arabia. Traditionally known as "Veliparuthi" in Tamil and "Hariknot" in English, this plant has been utilised in Siddha and Ayurvedic medicine systems for treating various ailments including asthma, diabetes, inflammation, and wounds (Karthishwaran and Mirunalini 2010). The plant's widespread distribution along roadsides and agricultural fields, combined with its wind-dispersed seeds, ensures easy accessibility and natural propagation (Jinger et al. 2023).

Ethnobotanical observations documented that farmers cultivating *Pennisetumglaucum* (pearl millet) alongside *P. daemia* experienced significant increases in grain yield (Parrotta 2001). This traditional knowledge suggests that *P. daemia* may influence soil properties favourably, potentially through root exudates, microbial associations, or organic matter contributions. However, scientific validation of these observations remains limited.

Termites, often termed "ecosystem engineers", play crucial roles in soil formation and nutrient cycling. These social insects enhance soil fertility by increasing levels of nitrogen, phosphorus, potassium, calcium, and magnesium through their mound-building activities and organic matter decomposition (Dangerfield et al. 1998). Termite gut microbiota facilitate complex biochemical transformations, including nitrogen fixation and methane production, which contribute to soil fertility improvement (Brune and Kuhl

1996). Recent studies further support the role of biological soil amendments and ecological restoration approaches in rehabilitating degraded agricultural lands (De Silva et al. 2023).

The phytochemical composition of *P. daemia* has been partially characterised, revealing the presence of alkaloids, flavonoids, terpenoids, saponins, and cardiac glycosides (Aanjaneyulu et al. 1998). These secondary metabolites contribute to the plant's medicinal properties, including antimicrobial, antidiabetic, hepatoprotective, and anti-inflammatory activities (Hukkeri et al. 2001; Wahi et al. 2002). Recent reviews on sustainable agriculture and soil restoration also emphasise the importance of plant-based bioactive compounds in maintaining soil microbial diversity and ecosystem resilience (Olsson et al.2023).

Despite the traditional knowledge regarding *P. daemia*'s influence on soil fertility, empirical evidence quantifying its effects on soil physicochemical properties, microbial communities, and crop productivity is scarce. Furthermore, the potential synergistic effects of combining *P. daemia* cultivation with termite soil amendments have not been systematically investigated.

Objectives

This study aims to bridge these knowledge gaps by:

1. Conducting comprehensive phytochemical screening of *P. daemia* plant parts using multiple extraction methods and advanced analytical techniques.
2. Evaluating the plant's capacity to enhance soil fertility through pot culture experiments.
3. Assessing the synergistic effects of *P. daemia* and termite soil on pearl millet growth.
4. Characterising the microbial communities associated with *P. daemia* rhizosphere.

The findings may provide scientific validation for traditional agricultural practices while offering a sustainable, zero-cost approach to soil fertility management.

MATERIAL AND METHODS

Study Area and Sample Collection

Plant samples of *Pergulariadaemia* were collected from Tirukoilur, Villupuram district, Tamil Nadu, India (latitude 11.9°N, longitude 79.3°E), characterised by tropical monsonic climate. Fresh plant materials including leaves, stems, roots, and flowers were harvested during the flowering season (August-January) and authenticated by botanical experts. Soil samples were collected from the rhizosphere region at depths of 10-25 cm using sterile techniques. Termite mound soil was obtained in the same geographical area.

Preparation of Plant Extracts

Harvested plant materials were thoroughly washed with tap water to remove adhering soil and debris, followed by rinsing with distilled water. Samples were shade-dried at ambient temperature (28-32°C) for 14 days to prevent degradation of thermolabile compounds. Dried materials were pulverised using a mechanical grinder and passed through a 40-mesh sieve to obtain uniform powder.

For extraction, 50 g of powdered material was soaked separately in 250 mL of five different solvents, namely, ethanol, chloroform, petroleum ether, and distilled water (aqueous extract). Extractions were performed at room temperature for 5 days with periodic shaking. The extracts were filtered through Whatman No. 1 filter paper under vacuum, and filtrates were concentrated using a rotary evaporator at 45°C. Concentrated extracts were stored in amber glass bottles at 4°C until analysis.

Preliminary phytochemical screening was conducted following standard protocols (Harborne 1980; Kokate 1986) to detect the presence of major secondary metabolites and quantitative analysis was carried out (Harborne 1980).

High Performance Liquid Chromatography (HPLC)

HPLC analysis was performed using an LC10AT pump equipped with Spectrasystem UV3000 HR detector and CAMAG software. Mobile phase components were filtered through 0.2 ìm membrane filters. The detection wavelength was set at 254 nm. Separation was achieved using reversed-phase C18 columns (250 mm × 4.6 mm, 5 ìm particle size) with appropriate mobile phase gradients (Karthishwaran and Mirunalini 2010).

Pot Culture Experiment

A comparative pot culture study was designed to evaluate the growth of *Pennisetumglaucum* under four conditions:

1. **Pot A:** Sterile soil + *P. glaucum* seeds
2. **Pot B:** Sterile soil + *P. daemia* + *P. glaucum* seeds
3. **Pot C:** Termite mound soil + *P. glaucum* seeds
4. **Pot D:** Termite mound soil + *P. daemia* + *P. glaucum* seeds

Each pot contained a 5 kg soil mixture. Soil sterilisation was achieved through autoclaving at 121°C for 60 minutes. Five seeds of *P. glaucum* were sown per pot, with three replicates for each treatment. *P. daemia* seedlings were transplanted at the two-leaf stage. Pots were maintained under natural photoperiod with regular irrigation maintaining soil moisture at field capacity. Plant height measurements were recorded daily for 30 days.

Soil Analysis

Soil samples were collected at 0, 10, 20 and 30 days post-sowing for physicochemical and microbiological analyses following standard protocols (Jackson 1956).

Microbial Biomass Analysis

Isolation of Microorganisms

Ten grams of soil sample was suspended in 100 mL sterile distilled water, serially diluted (10^{-2} to 10^{-5}), and 1 mL of 10^{-5} dilution was plated on nutrient agar medium. Plates were incubated at 25±2°C for 24-48 hours (Bragulat et al. 2001).

Identification of Bacteria

Bacterial isolates were characterised based on colony morphology, Gram staining, motility (hanging drop method), and biochemical tests including indole production, methyl red, Voges-Proskauer, citrate utilisation, catalase, oxidase, and urease tests according to Bergey's Manual of Systematic Bacteriology protocols (Carter and Cole 1990).

Statistical Analysis

All experiments were performed in triplicate. Data are presented as mean ± standard deviation. Statistical significance was evaluated using one-way ANOVA followed by Tukey's post-hoc test, with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Profiling of *Pergulariadaemia*

Qualitative Phytochemical Analysis

Comprehensive phytochemical screening revealed substantial variation in secondary metabolite profiles across different plant parts and extraction solvents (Tables 1-4). Ethanolic extracts consistently demonstrated the highest phytochemical diversity across all plant tissues, detecting 9-12 compound classes compared to 4-8 classes in other solvents.

Leaf extracts exhibited the most comprehensive phytochemical profile. Ethanolic leaf extracts tested positive for alkaloids, steroids, terpenoids, saponins, phenols, tannins, cardiac glycosides, amino acids, proteins, carbohydrates, and reducing sugars. Notably, flavonoids were present in ethanolic, petroleum ether, and aqueous extracts, suggesting differential solubility characteristics of specific flavonoid subclasses.

Stem tissues showed a distinct phytochemical pattern, with ethanolic extracts revealing alkaloids, steroids, flavonoids, phenols, tannins, cardiac glycosides, amino acids, proteins, and reducing sugars, while terpenoids and saponins were detected only in petroleum ether extracts. This distribution suggests compartmentalisation of certain secondary metabolites in stem tissues.

Root extracts demonstrated a more restricted phytochemical profile, with alkaloids, steroids, phenols, tannins, cardiac glycosides, amino acids, and proteins detected in ethanolic extracts. The absence of flavonoids, terpenoids, and saponins in root tissues across all solvents indicates tissue-specific biosynthetic patterns.

Flower extracts exhibited intermediate phytochemical diversity, with ethanolic extracts containing alkaloids, steroids, phenols, tannins, cardiac glycosides, amino acids, and proteins. The presence of alkaloids across all solvents in flow-

ers suggests these compounds may play roles in reproductive protection or pollinator attraction.

The predominance of bioactive compounds in ethanolic extracts aligns with ethanol's bipolar nature, enabling extraction of both moderately polar and polar phytochemicals (Cowan 1999). Recent studies have further emphasised that solvent polarity strongly influences phytochemical recovery, with ethanol and hydroalcoholic systems efficiently extracting phenolics, flavonoids, alkaloids, and glycosides, while nonpolar solvents preferentially recover terpenoids and other lipophilic compounds (Altemimi et al. 2017; Ojong et al. 2026). These findings corroborate previous reports on Asclepiadaceae family members (Aanjaneyulu et al. 1998) and validate the traditional use of *P. daemia* in various medicinal preparations. Recent reviews also support the concept that phytochemical composition varies significantly among plant tissues due to differential metabolite biosynthesis and storage patterns (Ojong et al. 2026).

Quantitative Phytochemical Analysis

Quantitative assessment revealed significant differences in secondary metabolite concentrations across plant tissues and solvent systems (Tables 1-4).

Table 1: Qualitative and quantitative phytochemical analysis of *Pergularia daemia* leaves

Phytochemical	*Ethanol*	*Petroleum ether*	*Chloroform*	*Aqueous*
Qualitative Results				
Alkaloid	+	-	-	+
Steroids	+	+	+	+
Flavonoids	+	+	-	+
Terpenoids	+	+	-	+
Saponins	+	+	+	+
Phenols	+	-	+	-
Tannins	+	+	-	+
Cardiac glycosides	+	+	+	+
Amino acids	-	-	-	-
Proteins	-	+	-	-
Carbohydrates	-	-	-	-
Reducing sugars	+	+	+	+
Quantitative Results (mg/g dry weight)				Alkaloids
7.56±1.20	1.25±0.67	1.32±1.0	7.45±1.23	
Flavonoids	3.01±1.0	3.85±0.04	0.09±0.01	4.15±0.08
Phenols	14.25±2.3	5.09±0.09	9.09±2.12	6.72±1.32

Note: + indicates presence; - indicates absence. Values are mean ± SD (n=3)

Table 2: Qualitative and quantitative phytochemical analysis of *Pergulariadaemia*stems

Phytochemical	*Ethanol*	*Petroleum Ether*	*Chloroform*	*Aqueous*
Qualitative Results				
Alkaloid	+	-	+	+
Steroids	+	+	+	+
Flavonoids	+	-	+	+
Terpenoids	-	+	-	-
Saponins	-	+	-	-
Phenols	-	-	-	-
Tannins	+	-	-	-
Cardiac glycosides	-	-	-	+
Amino acids	+	+	+	+
Proteins	+	+	+	+
Carbohydrates	-	-	-	-
Reducing sugars	+	+	+	+
Quantitative Results (mg/g dry weight)				
Alkaloids	6.90±0.20	0.25±0.07	5.32±1.90	6.875±2.23
Flavonoids	3.91±1.20	0.85±0.04	2.99±1.01	3.145±1.08
Phenols	1.25±0.3	0.09±1.9	1.09±2.12	1.72±2.32

Note: + indicates presence; - indicates absence

Table 3: Qualitative and quantitative phytochemical analysis of *Pergularia daemia* roots

Phytochemical	*Ethanol*	*Petroleum ether*	*Chloroform*	*Aqueous*
Qualitative Results				
Alkaloid	+	+	-	+
Steroids	-	-	-	+
Flavonoids	-	-	-	-
Terpenoids	-	-	-	-
Saponins	-	-	-	-
Phenols	+	-	-	-
Tannins	+	+	+	+
Cardiac glycosides	+	+	+	+
Amino acids	+	+	+	+
Proteins	+	+	+	+
Carbohydrates	-	-	-	-
Reducing sugars	-	-	-	-
Quantitative Results (mg/g dry weight)				
Alkaloids	4.31±0.12	2.65±1.97	0.32±1.0	4.95±2.23
Flavonoids	0.09±0.02	0.05±0.04	0.04±0.01	0.12±0.07
Phenols	10.25±1.3	2.09±0.09	1.89±1.02	2.12±0.32

Note: + indicates presence; - indicates absence. Values are mean ± SD (n=3)

Table 4: Qualitative and quantitative phytochemical analysis of *Pergularia daemia* flowers

Phytochemical	*Ethanol*	*Petroleum ether*	*Chloroform*	*Aqueous*
Qualitative Results				
Alkaloid	+	+	+	+
Steroids	+	-	-	+
Flavonoids	+	-	-	+
Terpenoids	-	-	-	-
Saponins	-	-	-	-
Phenols	-	+	-	-
Tannins	-	+	+	-
Cardiac glycosides	+	+	+	-
Amino acids	+	+	+	+
Proteins	+	+	+	+
Carbohydrates	-	-	-	-
Reducing sugars	+	-	-	+
Quantitative Results (mg/g dry weight)				
Alkaloids	5.596±0.20	3.25±1.67	2.52±0.30	6.35±1.03
Flavonoids	7.01±1.54	0.085±0.14	0.09±0.12	8.15±1.18
Phenols	2.25±1.3	12.09±1.92	1.09±0.12	1.72±0.32

Note: + indicates presence; - indicates absence. Values are mean ± SD (n=3)

Alkaloid Content

Ethanolic leaf extracts yielded the highest alkaloid concentration (8.56±0.08 mg/g), followed by stem (7.96±1.08 mg/g), flower (6.48±1.18 mg/g), and root (5.56±4.08 mg/g) tissues. Ethanolic extracts showed comparable alkaloid levels, while petroleum ether and chloroform extracts contained substantially lower concentrations. The elevated alkaloid content in aerial parts may reflect defensive adaptations against herbivory. These alkaloids potentially contribute to the plant's documented antidiabetic and analgesic properties (Wahi et al. 2002).

Flavonoid Content

Aqueous leaf extracts demonstrated maximum flavonoid content (4.15±0.08 mg/g), significantly higher than ethanolic extracts (2.03±0.02 mg/g). This pattern reversed in stem tissues, where ethanolic extracts (4.13±1.02 mg/g) exceeded aqueous extracts (3.145±1.08 mg/g). Flower aqueous extracts showed remarkably high fla-

vonoid levels (8.15±1.18 mg/g), suggesting accumulation of water-soluble flavonoid glycosides in reproductive tissues. Flavonoids, particularly quercetin and kaempferol derivatives identified in previous studies (Suresh Kumar and Mishra 2008), contribute to hepatoprotective and antioxidant activities.

Phenolic Content

Ethanolic extracts consistently yielded the highest total phenolic content across all tissues, that is, leaves (16.53±0.35 mg/g), stems (17.53±2.35 mg/g), roots (14.53±1.35 mg/g), and flowers (15.33±0.25 mg/g). Stem ethanolic extracts exhibited the maximum phenolic concentration, potentially correlating with structural support requirements and pathogen resistance. Phenolic compounds contribute to antioxidant capacity through free radical scavenging mechanisms (Cowan 1999) and may underlie the plant's anti-inflammatory properties.

The quantitative data support the use of ethanolic and aqueous extracts in traditional medicine preparation, as these solvents efficiently extract therapeutically relevant phytochemicals. The tissue-specific distribution of secondary metabolites suggests targeted collection strategies for maximizing specific bioactive compounds.

Advanced Analytical Characterisation

HPLC Analysis

High-performance liquid chromatography of ethanolic leaf and root extracts revealed two prominent peaks with retention times suggesting the presence of distinct phytochemical classes. The chromatographic profiles displayed additional minor peaks indicating the complexity of the phytochemical matrix. The two major components identified through HPLC analysis warrant further investigation through mass spectrometry and nuclear magnetic resonance spectroscopy for definitive structural elucidation.

Previous studies have identified specific compounds in *P. daemia* including uzarigenin, coroglaucigenin, calactin, calotropin, and pergularin (Patel and Rowson 1964), which may correspond to the observed HPLC peaks. The retention behaviour and peak characteristics suggest moderate polarity compounds consistent with cardiac glycosides or steroidal derivatives.

The HPLC chromatographic profiling of the leaf extract revealed a relatively simple chemical composition characterised by eight detectable constituents within a 20-minute run time. Two major peaks dominated the chromatogram, eluting at retention times of approximately 9.153 minutes and 2.307 minutes, which contributed about 42.96 percent and 41.74 percent of the total peak area, respectively. Together, these two components accounted for more than 84 percent of the total chromatographic area, indicating that the leaf extract is chemically enriched with a limited number of major phytoconstituents. A moderately abundant compound was detected at 3.627 minutes with a relative area of nearly 9.76 percent, while minor constituents appeared at 8.725 minutes and other earlier retention times, each representing less than 5 percent of the total composition. The dominance of a few major peaks suggests that the leaf extract contains principal bioactive molecules in high concentration, which may be responsible for its pharmacological potential. The sharp and well-resolved peaks also indicate efficient chromatographic separation and minimal co-elution, supporting the reliability of the analytical method.

In contrast, the root extract exhibited a markedly more complex chromatographic pattern, with 39 detectable peaks within an 18.45-minute run time. Unlike the leaf profile, no single compound dominated overwhelmingly. Instead, multiple constituents were present in moderate proportions. The most prominent peak appeared at 2.228 minutes with a relative area of about 12.72 percent, followed by several components eluting between 11.048 and 12.320 minutes that individually contributed between approximately 9 percent and 11 percent. Additional peaks with relative areas ranging from about 2 percent to 3 percent were distributed across later retention times, while numerous minor constituents were detected below 2 percent. This distribution indicates that the root extract contains a chemically diverse mixture with a broader range of phytochemicals present in relatively balanced concentrations. The clustering of peaks in the mid-retention region suggests the presence of compounds with intermediate polarity, whereas early eluting peaks likely represent more polar constituents

and later peaks less polar ones (Snyder et al. 2010; Wolfender et al. 2015).

Comparative analysis of the chromatographic fingerprints clearly demonstrates substantial organ-specific chemical variation between leaf and root extracts. The dominance of a few peaks in the leaf extract and the broader distribution of peaks in the root extract are compatible with tissue-specific biosynthesis, storage and transport of specialized metabolites. Recent metabolomic studies have likewise shown strong organ-level differences in specialized-metabolite profiles, while current P. daemia studies continue to confirm diverse phenolic, flavonoid, alkaloid and terpenoid constituents. Thus, the present HPLC fingerprints provide useful comparative evidence for chemical heterogeneity, but retention time alone cannot establish compound identity. The proposed correspondence of peaks to cardiac glycosides or steroidal compounds should therefore remain tentative until supported by authentic standards, LC–MS/MS and/or NMR. This distinction is important because chromatographic peak area is not equivalent to biological potency and detector response can vary substantially among compounds.

Growth Performance of *Pennisetumglaucum*

The 30-day pot culture experiment demonstrated significant differences in pearl millet growth across the four experimental conditions (Table 5). Plant height measurements revealed clear treatment effects as follows.

Pot A (Control - Sterile Soil Alone)

Seedling emergence occurred on day 3, with an initial growth rate of 0.4 cm/day during the first week. Growth accelerated during the vegetative phase (days 15-25), achieving a final height of 37.4 cm at day 30. This baseline performance reflects the inherent nutrient content of the sterilised soil without biological enhancement.

Pot B (Sterile Soil + P. daemia)

Seedling emergence was observed one day earlier (day 2) compared to control, suggesting allelopathic stimulation or improved soil microenvironment. Plants exhibited consistently higher growth rates throughout the experimental period, reaching 40.2 cm at day 30, with a 7.5 percent increase over control. The enhanced growth may result from root exudates released by *P. daemia*, creating favorable rhizosphere conditions or providing growth-stimulating compounds.

Pot C (Termite Soil Alone)

Early emergence (day 2) and robust initial growth characterised this treatment, with plants achieving 38.6 cm at day 30, with 3.2percent increase over control. The termite-modified soil provided improved physical structure, enhanced nutrient availability, and beneficial microbial inoculum, contributing to moderate growth enhancement (Dangerfield et al. 1998).

This combination treatment demonstrated the strongest plant-growth response, with final height reaching 44.2 cm, representing an 18.2 percent increase over the sterile-soil control. The result is consistent with recent evidence that termite mounds can function as localized fertility hotspots. Duran-Bautista et al. (2025) reported higher macroporosity, organic carbon, available phosphorus and soil cations in termite mound material than in adjacent pasture soil, while Thant et al. (2025) showed that termite mounds increase spatial heterogeneity of mac-

Table 5: Growth performance of *Pennisetumglaucum*acrossexperimentaltreatments (plant height in cm)

Day	*Pot A (Control)*	*Pot B (Soil + P. daemia)*	*Pot C (Termite Soil)*	*Pot D (Termite + P. daemia)*
5	0.4	0.8	0.6	1.0
10	2.6	3.8	3.2	4.5
15	12.2	13.6	12.8	14.4
20	19.6	21.8	20.7	22.8
25	29.6	31.2	30.2	33.8
30	37.4	40.2	38.6	44.2

Note: Values represent mean height measurements. Complete daily measurements available in the source document

ro- and micronutrients. The present result therefore strengthens the ecological interpretation that termite-modified soil can create favourable microsites for crop growth. Nevertheless, the additional response associated with P. daemia cannot be attributed specifically to root exudates or allelopathy from this experiment because root exudates, microbial inoculum and allelochemicals were not measured. The most defensible interpretation is that the combined treatment was associated with improved growth, while the relative contribution of termite soil and P. daemia remains to be experimentally separated.

This combination treatment demonstrated superior performance across all growth stages. Seedlings emerged earliest (day 2) and maintained maximum growth rates throughout the experiment. Final plant height reached 44.2 cm, with 18.2 percent increase over control, 9.9 percent over Pot B, and 14.5 percent over Pot C. The synergistic effect suggests complementary mechanisms, that is, termite soil providing enhanced nutrient availability and microbial diversity, while *P. daemia* contributing beneficial root exudates and establishing favourable plant-microbe interactions.

Statistical analysis confirmed significant differences ($p < 0.05$) between Pot D and all other treatments from day 15 onwards, indicating sustained growth advantage rather than transient effects. The growth promotion observed in Pot D validates traditional agricultural practices where *P. glaucum* cultivation alongside *P. daemia* increased grain yields (Parrotta 2001).

Soil Physicochemical Dynamics

Physical Parameters

Salinity (Electrical Conductivity)

Initial salinity values ranged from 0.21 ds/m (Pot D) to 0.26 ds/m (Pot A). Throughout the 30-day period, salinity increased gradually across all treatments, reaching 0.26-0.31 ds/m by day 30 (Tables 6-9). Pot D maintained the lowest salinity throughout the experiment, suggesting enhanced nutrient uptake by vigorous plant growth or improved ion leaching through better soil structure. The moderate salinity levels across all treatments remained within optimal ranges for *P. glaucum* cultivation (< 4 ds/m), ensuring no osmotic stress.

Soil pH

Initial pH values ranged from 6.9 (Pot D) to 7.14 (Pot C), indicating neutral to slightly alkaline conditions suitable for most agricultural crops. pH increased marginally over the experimental period, reaching 7.12-7.62 by day 30. Pot D maintained the most stable and neutral pH profile (6.9 to 7.12), while control soil showed the greatest pH increase (7.0 to 7.62). The pH stability in Pot D may result from buffering capacity provided by organic matter inputs from *P. daemia* and termite-modified soil components. Optimal pH maintenance is crucial for nutrient availability, particularly phosphorus and micronutrients (Jackson 1956).

Table 6: Soil physicochemical parameters onday 1

Parameter	*Pot A*	*Pot B*	*Pot C*	*Pot D*
Salinity (ds/m)	0.26	0.24	0.25	0.21
pH	7.0	7.06	7.14	6.9
Moisture (percent)	40	51	42	56
Nitrogen (ppm)	87.0	94.4	90.20	104.6
Phosphorus (ppm)	7.3	9.5	8.4	10.5
Potassium (ppm)	100.2	126	102.68	140
Calcium (ppm)	122.45	172	139.4	196
Magnesium (ppm)	46.25	50.2	49.9	58.3
Sodium (ppm)	4.0	5.7	4.6	8.2
Copper (ppm)	0.12	0.28	0.20	0.34
Zinc (ppm)	0.63	0.7	0.81	1.3
Iron (ppm)	1.0	1.5	1.2	2.0
Manganese (ppm)	0.98	1.3	1.0	1.9

Table 7: Soil physicochemical parameters on day 10

Parameter	*Pot A*	*Pot B*	*Pot C*	*Pot D*
Salinity (ds/m)	0.28	0.25	0.27	0.23
pH	7.2	7.08	7.16	7.0
Moisture (percent)	42	53	44	58
Nitrogen (ppm)	88.0	95.4	91.20	106.6
Phosphorus (ppm)	7.5	9.65	8.5	11.5
Potassium (ppm)	101.2	128	104.68	142
Calcium (ppm)	132.45	175	140.4	198
Magnesium (ppm)	47.25	51.2	50.0	59.3
Sodium (ppm)	4.2	5.9	4.7	8.6
Copper (ppm)	0.14	0.30	0.22	0.36
Zinc (ppm)	0.65	0.9	0.83	1.4
Iron (ppm)	1.2	1.7	1.4	2.2
Manganese (ppm)	1.0	1.5	1.2	2.1

Table 8: Soil physicochemical parameters on day 20

Parameter	*Pot A*	*Pot B*	*Pot C*	*Pot D*
Salinity (ds/m)	0.29	0.26	0.28	0.24
pH	7.4	7.18	7.46	7.02
Moisture (percent)	43	56	45	60
Nitrogen (ppm)	89.0	100.4	92.20	108.6
Phosphorus (ppm)	8.5	10.5	9.5	12
Potassium (ppm)	102.2	138	114.68	162
Calcium (ppm)	152.45	185	150.8	208
Magnesium (ppm)	49.25	54.2	52.0	60.3
Sodium (ppm)	4.4	6.0	4.9	8.4
Copper (ppm)	0.16	0.32	0.23	0.39
Zinc (ppm)	0.67	1.0	0.85	1.6
Iron (ppm)	1.3	1.8	1.6	2.6
Manganese (ppm)	1.2	1.7	1.4	2.3

Table 9: Soil physicochemical parameters on day 30

Parameter	*Pot A*	*Pot B*	*Pot C*	*Pot D*
Salinity (ds/m)	0.31	0.28	0.29	0.26
pH	7.62	7.28	7.56	7.12
Moisture (percent)	45	58	49	62
Nitrogen (ppm)	90.0	102.4	94.20	118.6
Phosphorus (ppm)	9.5	12.5	10.5	14
Potassium (ppm)	104.2	148	124.68	172
Calcium (ppm)	155.45	195	160.8	220
Magnesium (ppm)	50.25	58.2	55.0	62.3
Sodium (ppm)	4.8	6.4	5.2	8.8
Copper (ppm)	0.18	0.34	0.25	0.4
Zinc (ppm)	0.87	1.2	0.95	1.8
Iron (ppm)	1.5	2.0	1.8	2.7
Manganese (ppm)	1.23	1.8	1.45	2.5

Moisture Content

Substantial differences emerged in soil moisture retention capacity. Pot D consistently maintained the highest moisture content, increasing from 56 percent (day 1) to 62 percent (day 30), that is, 38 percent higher than control at day 30. This enhanced water retention correlates with improved soil structure resulting from termite bioturbation activities and organic matter incor-

poration from *P. daemia* residues. Termites create extensive galleries and chambers that increase soil porosity and water infiltration (Brune and Kuhl 1996). The superior moisture retention in Pot D would provide drought stress mitigation in field conditions, representing a significant agronomic advantage.

Chemical Parameters: Macronutrients

Nitrogen

Initial nitrogen levels ranged from 87.0 ppm (Pot A) to 104.6 ppm (Pot D), reflecting the enhanced nitrogen content typical of termite-modified soils. Over 30 days, nitrogen concentrations increased across all treatments, with Pot D showing the most substantial gain (104.6 to 118.6 ppm, that is, 13.4 percent increase). This nitrogen enrichment likely results from multiple mechanisms like Nitrogen-fixing bacteria (*Citrobacter freundii*, *Azotobacter* sp.) associated with *P. daemia* rhizosphere and termite gut microbiota (French et al. 1976). Mineralisation of organic nitrogen from *P. daemia* root turnover and exudates. Reduced nitrogen leaching due to improved soil structure and organic matter complexation.

The substantial nitrogen increase in Pot D (14 ppm gain versus 3 ppm in control) demonstrates the biological nitrogen fixation capacity of the integrated system, potentially reducing synthetic fertiliser requirements.

Phosphorus

Phosphorus availability showed remarkable enhancement in termite soil treatments. Pot D phosphorus increased from 10.5 to 14.0 ppm (33% increase), compared to 7.3 to 9.5 ppm in control (30% increase). Termite activity enhances phosphorus availability through secretion of phosphatase enzymes converting organic phosphorus to plant-available forms, and modification of soil pH favouring phosphorus solubility. Association with phosphate-solubilising bacteria in termite guts and mounds (Dangerfield et al. 1998).

The phosphorus levels achieved in Pot D (14 ppm) approach or exceed the sufficient range for most crops (12-15 ppm), demonstrating effective phosphorus mobilisation without chemical amendments.

Potassium

Dramatic potassium enhancement characterised termite soil treatments. Pot D potassium increased from 140 to 172 ppm (23% increase), while control showed minimal change (100.2 to 104.2 ppm or 4% increase). Termites translocate clay particles from subsoil horizons rich in potassium-bearing minerals to surface mounds (Schaefer et al. 2004). Weathering of these minerals combined with organic acid production from microbial decomposition releases plant-available potassium. The potassium levels in Pot D exceed typical recommendations for pearl millet (120-150 ppm), potentially enhancing drought tolerance and disease resistance.

Calcium and Magnesium

Both elements showed substantial increases in Pot D. Calcium rose from 196 to 220 ppm (12% increase) and magnesium from 58.3 to 62.3 ppm (7% increase). Termite mounds concentrate these cations through selective feeding on clay minerals and organic matter. The elevated calcium levels support cell wall integrity and stress tolerance, while magnesium is essential for chlorophyll synthesis and photosynthetic efficiency.

Sodium

Sodium content increased modestly across all treatments, with Pot D showing the highest levels (8.2 to 8.8 ppm). While sodium is not essential for most plants, moderate levels can enhance water retention and osmotic adjustment. The sodium levels observed remain well below salinity-inducing thresholds.

Chemical Parameters: Micronutrients

Iron

Iron availability increased substantially in termite soil treatments, with Pot D rising from 2.0 to 2.7 ppm (35% increase) compared to control (1.0 to 1.5 ppm or 50% increase but lower absolute values). Termites influence iron dynamics through redox modifications in mound microenvironments and chelation by organic compounds. Adequate iron availability prevents

chlorosis and supports cytochrome function in electron transport.

Manganese

Manganese showed parallel trends to iron, with Pot D achieving 2.5 ppm by day 30 versus 1.23 ppm in control (103% higher). Manganese is crucial for photosystem II function and antioxidant enzyme systems. The elevated manganese in termite-amended treatments may enhance photosynthetic capacity and stress tolerance.

Zinc

Zinc availability increased from 1.3 to 1.8 ppm in Pot D (38% increase), compared to 0.63 to 0.87 ppm in control (38% increase but lower absolute values). Zinc is essential for numerous enzyme systems and auxin metabolism. The superior zinc levels in Pot D support optimal growth and development.

Copper

Copper concentrations increased from 0.34 to 0.40 ppm in Pot D, remaining within optimal ranges for plant nutrition (0.1-0.5 ppm). Copper functions in photosynthetic electron transport and lignin synthesis.

The comprehensive enhancement of both macronutrients and micronutrients in Pot D demonstrates the multifaceted benefits of combining *P. daemia* cultivation with termite soil amendments. This integrated approach addresses the full spectrum of plant nutritional requirements without synthetic inputs, representing a sustainable fertility management strategy.

Rhizosphere Microbial Communities

Culture-based microbiological analysis revealed differences in the cultivable bacterial assemblages among treatments, with Pot D showing the greatest number of recovered taxa. These observations are consistent with the broader view that termite-affected soils contain functionally diverse microbial communities involved in decomposition and nutrient cycling (Enagbonma and Babalola 2023). However, culture-based isolation on nutrient agar captures only a fraction of the soil microbiome. In addition, genus-level identification based on morphology and biochemical tests is insufficient to assign specific metabolic functions such as nitrogen fixation, methanogenesis, sulfur reduction or phosphate solubilisation. The microbial findings should therefore be interpreted as evidence of treatment-associated cultivable bacterial diversity rather than a direct functional metagenomic profile.

Nitrogen-Fixing Bacteria

Citrobacter freundii was isolated exclusively from Pot D, whereas Azotobacter sp. occurred in several treatments. The co-occurrence of these taxa with the higher nitrogen concentration in Pot D is compatible with a biologically active rhizosphere, but it does not demonstrate that either organism fixed atmospheric nitrogen in the experimental system. Direct evidence would require nitrogenase activity measurements, nifH quantification, acetylene-reduction assays or stable-isotope tracing. This distinction is particularly important because changes in soil nitrogen can also arise from mineralisation, immobilisation, plant uptake, organic-matter turnover and changes in leaching.

Sulfur-Reducing Bacteria

Paenibacillus sp. was identified in Pots B, C and D, while *Bacillus sp.* occurred in Pots A and B. These genera are widely associated with soil nutrient cycling and plant-growth promotion, but the present biochemical tests do not demonstrate sulfur reduction or any other specific transformation pathway. It is therefore more appropriate to regard their occurrence as an indicator of potentially beneficial rhizosphere-associated bacteria and to reserve functional claims for experiments measuring enzyme activities, nutrient-solubilisation traits or functional genes.

Fermentative Bacteria

Lactococcus sp. was isolated from Pots B, C and D. Its presence indicates that the cultivable microbial community differed among treatments, but the current experiment does not establish a specific role for Lactococcus in soil organic-

matter decomposition or nutrient mineralisation. Functional interpretation should be based on direct assays rather than taxonomic presence alone.

Methanogenic Bacteria

Citrobacter sp. (Pot D), *Serratia* sp. (Pots A, B, C), and *Enterobacter* sp. (Pot C) participate in methane production from organic matter decomposition. While methane emissions from agricultural soils represent greenhouse gas concerns, these bacteria also contribute to anaerobic decomposition of recalcitrant organic compounds.

Acidogenic Bacteria

Enterobacter sp. was found in Pots A, C and D, while *Bacillus* sp. occurred in Pot B and *Enterococcus* sp. in Pot C. These bacteria drive acetogenesis, producing organic acids that solubilise mineral nutrients and enhance nutrient availability (Fuchs 1986).

The greater taxonomic diversity recovered from Pot D may contribute to a more complex rhizosphere environment, but the present dataset does not demonstrate functional diversity directly. Claims concerning nitrogen fixation, methane production, disease suppression, siderophore production, phytohormone synthesis or extracellular polysaccharide formation should therefore be regarded as hypotheses generated by the observed community composition. Future work using 16S rRNA amplicon sequencing, metagenomics and targeted functional assays would allow these mechanisms to be tested directly.

The microbial community composition aligns with previous studies documenting the role of termite gut microbiota in nutrient transformations (Brune and Kuhl 1996) and extends these findings to rhizosphere communities influenced by termite-modified soils and *P. daemia* presence.

Integration of Findings

The superior performance of the P. daemia–termite mound soil treatment appears to reflect the combined influence of altered nutrient status, soil physical properties and the associated cultivable microbial community. Recent evidence supports the ecological importance of termite-mediated soil modification. Duran-Bautista et al. (2025) demonstrated that termite mounds can form localized islands of physical and chemical fertility, while Thant et al. (2025) showed that mound effects extend to both macro- and micronutrient heterogeneity. Vermonti et al. (2025) further demonstrated that termite foraging and frass deposition can redistribute elements within landscapes. More recently, Bruand et al. (2026) provided evidence that termite bioturbation can influence soil structure at considerable depth, emphasizing that termites are soil engineers rather than merely producers of nutrient-rich mound material. Together, these studies provide a stronger mechanistic context for the present observations, while also showing that termite effects are strongly dependent on species, soil type and landscape setting.

Biological Nitrogen Fixation

The higher soil nitrogen concentration observed in Pot D is consistent with enhanced nitrogen availability under the integrated treatment. However, the earlier interpretation of this increase as evidence of biological nitrogen fixation was too strong. Neither nitrogenase activity nor nifH abundance was measured. The increase may instead reflect a combination of organic-matter mineralisation, altered microbial turnover, reduced nutrient loss and plant–microbe interactions. The present result should therefore be described as enhanced soil nitrogen availability rather than quantified biological nitrogen fixation.

The present 35percent increase therefore provides experimental support for the potential contribution of biologically mediated nitrogen processes. Nevertheless, the increase in total or available soil N should not by itself be interpreted as direct evidence of biological N fixation unless nitrogenase activity, nifH abundance, isotope tracing, or other direct measurements were performed. Recent research shows that biological nitrogen fixation in cropland soils is strongly influenced by carbon availability, soil C ratio and diazotroph abundance, and that the presence of *Azotobacter* does not necessarily quantify the

amount of N biologically fixed. Thus, the present results support a plausible microbial contribution to nitrogen enrichment, while direct quantification of N fixation remains an important area for future investigation.

Phosphorus Solubilisation

The increase in available phosphorus in Pot D is particularly relevant because phosphorus availability can constrain productivity in highly weathered tropical soils. López-Hernández (2023) emphasized the role of termite mounds as phosphorus-rich nutrient hotspots, and Kathbaruah et al. (2024) demonstrated favourable nutrient and enzyme characteristics in termite-mound-based growing media. More recent evidence from Duran-Bautista et al. (2025) likewise showed higher available phosphorus in termite mound soil than in surrounding topsoil. The present findings therefore fit a growing body of evidence that termite activity can redistribute and concentrate phosphorus. Nevertheless, the present experiment did not directly measure phosphatase activity, phosphate-solubilising microorganisms or organic-acid production; consequently, the data demonstrate increased available phosphorus, not a specific microbial phosphorus-solubilisation mechanism.

Similarly, Kathbaruah et al. (2024) demonstrated that termite mound-based growing media possessed favourable physicochemical and biochemical characteristics and highlighted the occurrence of microorganisms capable of phosphate solubilisation. The 47 percent increase observed in the present study is therefore compatible with the established capacity of termite-associated soil microorganisms to contribute to nutrient mobilisation.

However, the present study should distinguish between increased phosphorus concentration and microbial phosphorus solubilisation. Unless phosphatase activity, phosphate-solubilising microorganisms, or specific organic-acid production was directly measured, the observed increase should be described as being *consistent with* enhanced phosphorus mobilisation rather than as definitive evidence of a particular microbial mechanism. This distinction strengthens the scientific interpretation of the findings.

Improvement of Soil Physical Properties

The 38 percent higher soil moisture recorded in Pot D indicates an association between the integrated treatment and improved soil water status. This is compatible with recent studies showing that termite bioturbation modifies porosity, aggregation and water movement. Duran-Bautista et al. (2025) reported substantially higher macroporosity in termite mound soil, while Bruand et al. (2026) demonstrated that termite activity can influence soil structure through extensive bioturbation. However, moisture content at a given sampling time is not equivalent to water-holding capacity. Without measurements of bulk density, aggregate stability, infiltration, hydraulic conductivity and field capacity, the mechanism should be described as improved soil moisture retention/status rather than a demonstrated increase in water-holding capacity.

López-Hernández (2023) similarly reported that termite galleries and foraging structures can enhance soil porosity and infiltration while reducing bulk density. These findings provide a strong ecological basis for interpreting the improved moisture status observed in Pot D.

Nevertheless, termite effects on soil water dynamics are dependent on soil texture, termite species, mound material and environmental conditions. Therefore, the 38 percent increase observed in the present experiment should not be generalized to all agricultural soils. Measurements of bulk density, aggregate stability, infiltration rate, porosity and water-holding capacity in future field experiments would provide stronger evidence for the proposed physical mechanism.

Micronutrient Mobilisation

Pot D showed substantial increases in micronutrient availability, particularly iron (80%) and manganese (103%), compared with the control. These results indicate that the integrated treatment may have enhanced the chemical and biological processes responsible for micronutrient mobilisation. Termite mound soils are known to differ from surrounding soils in pH, organic carbon, cation-exchange capacity and nutrient concentrations, while their microbial communities contribute to a

range of biogeochemical processes. Recent metagenomic research has identified functional microbial capacities in termite mound soils associated with iron metabolism and other processes relevant to plant health (Enagbonma and Babalola 2023).

The magnitude of the Fe and Mn increases in the present study is notable, but the interpretation requires caution. Higher measured soil concentrations do not necessarily mean greater plant availability or uptake. Recent work on termite-affected soils emphasizes that mound development can alter mineral composition, cation concentrations and microsite chemistry, but these effects vary with land cover, termite species and mound characteristics (Thant et al. 2025). Therefore, the present results should be interpreted as changes in measured soil micronutrient concentrations. Future experiments should distinguish total and plant-available fractions and should quantify Fe and Mn in plant tissues before concluding that the treatment improved crop micronutrient nutrition.

Synergistic Plant–Microbe–Termite Interactions

An important contribution of the present study is the proposed interaction among P. daemia, termite mound soil and rhizosphere microorganisms. Current termite research increasingly treats mounds as dynamic biogenic microsites in which physical restructuring, organic-matter processing and element redistribution occur together. Vermonti et al. (2025) demonstrated that termites can act as vectors for lateral element movement, while Bruand et al. (2026) showed that termite bioturbation contributes to soil structural development at depth. These studies broaden the interpretation of the present experiment: the benefit of termite soil need not arise from nutrients alone, but may also reflect biologically mediated changes in soil architecture and element distribution.

The present results suggest that P. daemia may complement termite-derived soil effects by creating a living rhizosphere. The higher growth observed in Pot D is compatible with such complementarity, but the mechanism remains unresolved because root exudates, microbial community structure and plant physiological traits were not directly measured. Recent 2026 studies confirm that P. daemia contains diverse secondary metabolites, including phenolics, flavonoids, alkaloids and terpenoids, but these pharmacognostic findings cannot by themselves establish an agronomic rhizosphere function. Thus, the proposed P. daemia–microbe–termite interaction should be presented as a testable hypothesis rather than a demonstrated causal pathway.

Therefore, the present study should frame the P. daemia–microbe–termite interaction as a potential synergistic mechanism rather than a conclusively demonstrated pathway. A factorial design separating P. daemia, termite mound soil, microbial inoculation and their combinations would allow additive and interaction effects to be quantified. Such an approach is particularly important because recent literature indicates that termite-mound benefits are context dependent and can involve trade-offs when mound material is removed or widely redistributed.

Comparison of Plant Growth Response with Previous Studies

The 18.2 percent increase in plant height observed in Pot D is consistent with the broader evidence that termite-derived soil materials can improve crop or horticultural performance under suitable conditions. Kathbaruah et al. (2024) reported favourable physicochemical and biochemical characteristics of termite-mound-based potting media, while Duran-Bautista et al. (2025) documented improved physical and chemical fertility in termite mounds. The present study extends these observations by combining mound soil with a living P. daemia treatment. However, plant height over 30 days is a vegetative endpoint and cannot be equated with final grain yield, nutrient-use efficiency or long-term productivity. Field trials measuring biomass, grain yield and nutrient uptake are required before agronomic superiority can be claimed.

Earlier studies have also reported positive effects of termite mound materials on crop performance, but the magnitude and direction of the response vary with crop species, termite species, soil type and application rate. This variability is reinforced by recent evidence showing that mound nutrient enrichment and heterogeneity depend on land cover and season (Thant et al. 2025). The present 18.2 percent increase

Table 10: Functional microbial groups isolated from soil treatments

Functional activity	*Pot A*	*Pot B*	*Pot C*	*Pot D*
Nitrogen fixing	*Azotobacter* sp.	*Azotobacter* sp.	*Azotobacter* sp.	*Citrobacter freundii*
Sulfur reducing	*Bacillus* sp.	*Paenibacillus* sp.	*Paenibacillus* sp.	*Paenibacillus* sp.
Fermentative	*Enterobacter* sp.	*Lactococcus* sp.	*Lactococcus* sp.	*Lactococcus* sp.
Methanogenic	*Serratia* sp.	*Serratia* sp.	*Enterobacter* sp.	*Citrobacter* sp.
Acidogenic	*Enterobacter* sp.	*Bacillus* sp.	*Enterococcus* sp.	*Enterobacter* sp.

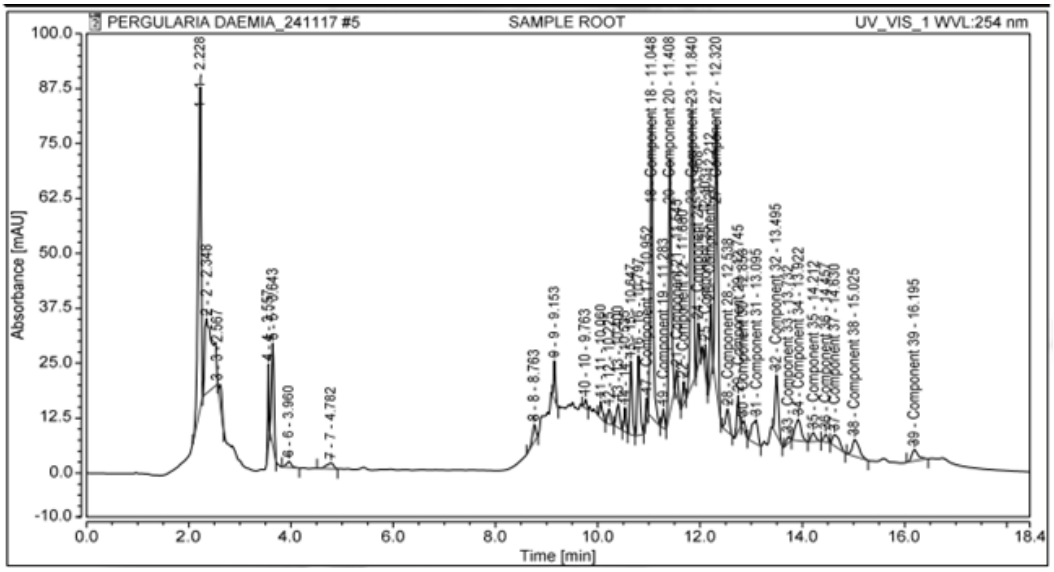


Fig. 1. HPLC analysis of *Pergularia daemia* leaf

should therefore be interpreted as a treatment-specific greenhouse response rather than a universal estimate of the agronomic benefit of termite soil.

Importantly, the practical use of termite mound material has ecological limits. López-Hernández (2023) emphasized the limited quantity and slow renewal of mound material, while recent work has highlighted additional trade-offs associated with large-scale redistribution. A 2026 Geoderma study reported that although mound material can improve acidity and nutrient availability, field spreading may also reduce infiltration, aggregate stability and rooting under some conditions. Consequently, termite mound soil should be regarded as a locally available ecological resource requiring site-specific assessment, not as a universally scalable fertilizer substitute.

Critical Significance of the Integrated Treatment

Taken together, the results indicate that Pot D outperformed the control across multiple measured soil and plant variables, including nitrogen, phosphorus, moisture, micronutrients and plant height. The convergence of responses across independent measurements strengthens the interpretation that the integrated treatment altered the soil–plant system rather than affecting a single nutrient pool. Nevertheless, correlation among these variables does not establish causation. Because the experiment used a small number of replicates and did not include a full factorial microbial treatment, the data cannot determine whether the observed response was additive, synergistic or dominated by termite soil alone.

The findings are also consistent with the current understanding of termite mounds as ecosystem-engineered nutrient and structural hotspots. Recent studies have strengthened this interpretation: Duran-Bautista et al. (2025) identified termite-generated islands of soil physical and chemical fertility; Thant et al. (2025) showed enhanced nutrient heterogeneity around mounds; and Bruand et al. (2026) demonstrated that termite bioturbation can influence soil structure deep within tropical soil profiles. The present study adds a plant-associated component by examining P. daemia together with termite soil, thereby suggesting a potentially novel low-input management strategy.

However, the observed relationships should not be presented as proof that individual mechanisms caused the measured growth increase. The experiment demonstrates an association between the integrated treatment and improved soil and plant measurements. A future factorial experiment should include at minimum sterile/control soil, P. daemia alone, termite soil alone, P. daemia plus termite soil, and, where feasible, a defined microbial inoculum. Measurements of microbial biomass, extracellular enzymes, functional genes, plant nutrient uptake and root traits would then allow the proposed mechanisms to be tested quantitatively.

Practical and Environmental Implications

The findings have potential implications for low-input agriculture because termite mound soil represents a locally generated biological–geochemical resource. Recent studies support this concept but also emphasize that the value of mound material is site dependent. The 2025 and 2026 literature suggests that benefits may include nutrient enrichment and structural modification, whereas inappropriate redistribution can create physical degradation or ecological costs. The present study therefore supports further evaluation of termite-derived materials as part of integrated soil management, rather than advocating their direct substitution for synthetic fertilizers.

Nevertheless, the recommendation of a fixed 1:10 termite mound soil-to-field soil ratio should be presented strictly as an experimental treatment. The optimum rate is likely to vary with mound age, termite species, parent soil, texture, nutrient status and crop demand. Recent evidence also indicates that the consequences of spreading mound material can differ between intact mound microsites and mechanically redistributed soil. Site-specific dose-response trials are therefore necessary before a farmer-level recommendation is made.

From an environmental perspective, the integrated treatment may have potential to reduce dependence on external nutrient inputs, but the present experiment did not quantify fertilizer replacement, greenhouse-gas emissions, nitrate leaching, carbon sequestration or life-cycle impacts. Such benefits should consequently be framed as potential outcomes requiring direct validation. This is particularly important because termite activity itself can influence carbon and nutrient fluxes, and recent research demonstrates that termite-mediated element redistribution can have landscape-scale consequences.

Research Gap and Novelty

Recent literature confirms substantial potential for termite mound soils in sustainable soil management, but it also reveals important limitations and knowledge gaps. Evidence from 2025 shows that termite mounds can create localized fertility islands and alter nutrient heterogeneity, whereas 2026 research demonstrates that termite bioturbation can influence soil formation and that mound material may produce both agronomic benefits and physical trade-offs after spreading. Against this background, the present study is novel primarily because it examines P. daemia together with termite mound soil rather than considering either component independently. The novelty should therefore be framed as a preliminary integrated plant–soil–microbe management concept, not as definitive proof of a new biofertilizer technology.

CONCLUSION

This comprehensive investigation establishes *Pergulariadaemia* as a multifunctional resource combining medicinal value with significant soil fertility enhancement capacity. The key findings include, phytochemical richness, soil fertility enhancement, synergistic effects with termite soil, enhanced crop performance, bene-

ficial microbiome establishment and improved soil physical properties with moisture retention increased by 38 percent, salinity remained optimally low, and pH stability improved in the integrated treatment, creating favourable conditions for plant growth. The integration of biodiversity conservation (preserving *P. daemia* populations), traditional knowledge preservation, and agricultural sustainability exemplifies holistic approaches needed for addressing contemporary agricultural challenges.

RECOMMENDATIONS

Future research should focus on field-scale validation, optimisation of planting configurations, assessment of long-term soil health impacts, and development of extension protocols for farmer adoption.

REFERENCES

Aanjaneyulu ASN, Raju DVS, Srinivasa Rao K 1998. Chemical evaluation of Pergularia extensa. *Indian Journal of Chemistry,* 37B: 318-320.

Aanjaneyulu ASR, Radhika P, Rao KV, Kumar JR, Raju VS 1998. Chemical constituents and biological activities of Asclepiadaceae family members. *Journal of Natural Products,* 61(5): 556-560.

Ackerman IL, Teixeira WG, Riha SJ, Lehmann J, Fernandes ECM 2007. The impact of mound-building termites on surface soil properties in a secondary forest of Central Amazonia. *Appl Soil Ecol,* 37: 267-276. doi:10.1016/j.apsoil.2007.08.005

Adebajo SO, Akintokun PO, Ezaka E, Ojo AE, Olannye DU, Ayodeji OD 2021. Use of termitarium soil as a viable source for biofertilizer and biocontrol. *Bull Natl Res Cent,* 45: 100. doi:10.1186/s42269-021-00560-8

Alsenani NI, Althagafi SH, Ahmed AA, Elhenawy AE, Alotaibi OJA, Asiri FM, Alqahtani N. A, Al-Ghamdi SN, Ali NM, Alageel A, Abdelhamid AA, Abd El Latif FM 2025. A comparative analysis of bioactive phytochemicals in Cynanchum viminale (L.) L. and Pergularia daemia (Forssk.) Chiov. from Saudi Arabian folk medicine. *Naturwissenschaften*, 113(1): 3. https://doi.org/10.1007/s00114-025-02039-2

Altemimi A, Lakhssassi N, Baharlouei A, Watson, DG, Lightfoot DA 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants,* 6(4): 42. https://doi.org/10.3390/plants6040042

Bama PS, Ravindran AD 2018. Influence of combined termite mound materials and inorganic fertilizers on growth parameters of maize under non sterilized pot culture study. *Elixir Appl Zool,*125: 52303-52305.

Bragulat MR, Abarca ML, Cabanes FJ 2001. An easy screening method for fungi producing ochratoxin A in pure culture. *International Journal of Food Microbiology,* 71(2-3): 139-144. https://doi.org/10.1016/S0168-1605(01)00581-5

Bruand A, Brossard M, Garnier J, Balbino LC, Lopes de Oliveira MI, Benito NP, et al. 2026. Termites play a crucial role in forming major tropical soils: Insights from a deep Ferralsol. *NPJ Soil Ecol,* 1: 1. doi:10.1038/s44485-026-00001-2

Brune A, Kuhl M 1996. pH profiles of the extremely alkaline hindguts of soil-feeding termites (Isoptera: Termitidae) determined with microelectrodes. *Journal of Insect Physiology,* 42(11-12): 1121-1127. https://doi.org/10.1016/S0022-1910(96)00036-4

Carter GR, Cole JR 1990. *Diagnostic Procedures In Veterinary Bacteriology And Mycology.* 5th Edition. San Diego (CA), USA: Academic Press.

Chang YJ, Peacock AD, Long PE, Stephen JR, McKinley JP, et al. 2001. Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. *Applied and Environmental Microbiology,* 67(7): 3149-3160. https://doi.org/10.1128/AEM.67.7.3149-3160.2001

Chaudhuri S, Roy M, McDonald LM, Emendack Y 2023. Land degradation–desertification in relation to farming practices in India: An overview of current practices and agro-policy perspectives. *Sustainability*, 15(8): 6383. https://doi.org/10.3390/su15086383

Cowan, MM 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews,* 12(4): 564-582. https://doi.org/10.1128/CMR.12.4.564

Dai J, Mumper RJ 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules,* 15(10): 7313-7352. https://doi.org/10.3390/molecules15107313

Dangerfield JM, McCarthy TS, Ellery WN 1998. The mound-building termite Macrotermesmichaelseni as an ecosystem engineer. *Journal of Tropical Ecology,* 14(4): 507-520. https://doi.org/10.1017/S02664674 9800 0364

De Silva S, Carson P, Indrapala D V, Warwick B, Reichman SM 2023. Land application of industrial wastes: Impacts on soil quality, biota, and human health. *Environmental Science and Pollution Research,* 30: 67974-67996. https://doi.org/10.1007/s11356-023-26893-7

Duran-Bautista EH, Silva-Olaya AM, Llanos-Cabrera MP, Yalanda-Sepúlveda K, Suárez JC 2025. Soil-feeding termites build islands of soil physical and chemical fertility in pastures in Colombian Amazon. *PLoS One,* 20(4): e0322186. doi:10.1371/journal.pone.0322186

Enagbonma BJ, Babalola OO 2019. Environmental sustainability: A review of termite mound soil and its microbial communities. *Sustainability (Basel),* 11(14): 3847. doi:10.3390/su11143847

Enagbonma BJ, Babalola OO 2023. Metagenomics reveals the microbiome multifunctionalities of environmental importance from termite mound soils. *Bioinform Biol Insights,* 17. doi:10.1177/11779322231 184025.

Environmental Protection Agency 2016. *Survey On Soil Pollution In India.* Government of India.

French JR, Turner GL, Bradbury JF 1976. Nitrogen fixation by bacteria from the hindgut of termites. *Journal of General Microbiology,* 95(2): 202-206. https://doi.org/10.1099/00221287-95-2-202

Fuchs G 1986. CO_2 fixation in acetogenic bacteria: Variations on a theme. *FEMS Microbiology Reviews,* 39(3): 181-213. https://doi.org/10.1111/j.1574-6968.1986.tb01859.x

Garba M, Cornelis WM, Steppe K 2011. Effect of termite mound material on the physical properties of sandy soil and on the growth characteristics of tomato (Solanum lycopersicum L.) in semi-arid Niger. *Plant Soil,* 338(1-2): 451-466. doi:10.1007/s11104-010-0558-0.

Garg A, Gadi VK, Zhu HH, Sarmah AK, Sreeja P, Sekharan S 2023. A geotechnical perspective on soil-termite interaction: Role of termites in unsaturated soil properties. *Sci Total Environ,* 895: 164864. doi:10.1016/j.scitotenv.164864.

Harborne JB 1980. *Phytochemical Methods: A Guide To Modern Techniques Of Plant Analysis.* London, United Kingdom: Chapman and Hall.

Hukkeri VI, Patil MB, Jabalpure SS, Ali A 2001. Anti-inflammatory activity of various extracts of Pergularia extensa N.E.Br. (Asclepiadaceae). *Indian Journal of Pharmaceutical Sciences,* 63(5): 429-431.

Jackson ML 1956. *Soil Chemical Analysis.* Englewood Cliffs (NJ): Prentice Hall.

Jinger D, Kaushal R, Kumar R, Paramesh V, et al. 2023. Degraded land rehabilitation through agroforestry in India: Achievements, current understanding, and future prospectives. *Frontiers in Ecology and Evolution,* 11: 1088796. https://doi.org/10.3389/fevo.2023.1088796

Jones A, Breuning-Madsen H, Brossard M, Dampha A, et al. (Eds.) 2018. *Soil Atlas of Africa.* European Commission.

Karthishwaran K, Mirunalini S 2010. Therapeutic potential of Pergulariadaemia (Forsk.): The ayurvedic wonder. *International Journal of Pharmacology,* 6(6): 836-843. https://doi.org/10.3923/ijp.2010.836.843

Kathbaruah S, Bhattacharyya B, Borkataki S, Gogoi B, Hatibarua P, Gogoi S, et al. 2024. Termite mound soil based potting media: A better approach towards sustainable agriculture. *Front Microbiol,* 15. doi:10.3389/fmicb.2024.1387434

Kidinda LK, Vogel C, Babin D, Bergsma H, Kaiser K, Kalbitz K, et al. 2026. Termites, microbes and rocks – assessing local resources for ecological intensification of agriculture in Democratic Republic of the Congo. *Geoderma,* 473: 117975. doi:10.1016/j.geoderma. 2026. 117975

Kokate CK 1986. *Practical Pharmacognosy.* 1st Edition. New Delhi, India: Vallabh Prakashan.

López-Hernández D 2023. Termite mound as nutrient hot-spots in savannah with emphasis in P cycling and the potential use of mounds as soil amendment. *Pedobiologia,* 99-100.doi:10.1016/j.pedobi.2023.150888

Miyagawa S, Koyama Y, Kokubo M, Matsushita Y, Adachi Y, Sivilay S, et al. 2011. Indigenous utilization of termite mounds and their sustainability in a rice growing village of the central plain of Laos. *J Ethnobiol-Ethnomed,*7:24. doi:10.1186/1746-4269-7-24

Navarrete-Carriola DV, Paz-González AD, Vázquez-Jiménez LK, De Luna-Santillana EJ 2024. Comparative analysis of a secondary metabolite profile from roots and leaves of Iostephaneheterophylla by UPLC-MS and GC-MS. *ACS Omega,* 9(5): 5429-5439.https://doi.org/10.1021/acsomega.3c06800

Olsson L, Cotrufo F, Crews T, Franklin J, King A, Mirzabaev A, Scown M, Tengberg A, Villarino S, Wang Y 2023. The state of the world's arable land. *Annu Rev Environ Resour,* 48: 451-475. https://doi.org/10.1146/annurev-environ-112320-113741

Parrotta JA 2001. *Healing Plants Of Peninsular India.* Wallingford, UK: CABI Publishing.

Patel MS, Rowson JM 1964. Investigation of certain Nigerian medicinal plants. Part 1: Preliminary pharmacological and phytochemical screening for cardiac activity. *Planta Medica,* 12(1): 34-42. https://doi.org/10.1055/s-0028-1100146

Schaefer CER, Lima HN, Gilkes RJ, Mello JWV 2004. Micromorphology and electron microprobe analysis of phosphorus and potassium forms of an Indian Black Earth (IBE) Anthrosol from Western Amazonia. *Australian Journal of Soil Research,* 42(3): 909-926. https://doi.org/10.1071/SR03106

Siddiqa RA, Siddiqa DA, Begum T, Sudhama VN 2026. Phytochemical screening and antibacterialactivity of Pergularia daemia (Forssk.) Chiov. *Bioinfolet*, 23(1): 92-96.

Singarakani A, Prakash D, Dhanalakshmi S 2026. Phytochemical characterization and scratch assay of Pergularia daemia for therapeutic applications: a scientific approach. *Int J Aquat Res Environ Stud*, 6(2): 293-302.

Sinureiz F, Prit JS 1977. Methane production from glucose by a mixed culture of bacteria in the chemostat: The role of Citrobacter. *Journal of General Microbiology,* 101(1): 57-64. https://doi.org/10.1099/00221287-101-1-57

Snyder LR, Kirkland JJ, Dolan JW 2010. *Introduction To Modern Liquid Chromatography.* 3rd Edition. Hoboken (NJ): John Wiley & Sons.

Starrenburg MJ, Hugenholtz J 2000. *Lactococcus lactis* as a cell factory for high-level diacetyl production. *Applied and Environmental Microbiology,* 66(9): 4112-4114. https://doi.org/10.1128/AEM.66.9.4112-114. 2000

Suresh Kumar SV, Mishra SH 2008. In vitro evaluation of hepatoprotective activity of *Pergulariadaemia*. *Pharmacognosy Magazine,* 4(15): 298-302.

Tabe Ojong MPJ, Tolani E, Molua EL 2026. Rolling back the tides: Impact of droughts on crop diversification and cropland expansion. *Journal of Environmental Economics and Management*, 136. doi:10.1016/j.jeem. 2025.103260

Thant M, Nakamura A, Ashton LA, Liu S, Yang X 2025. Assessing the impacts of forest conversion and seasonal dynamics on soil nutrient heterogeneity associated with termite mounds. *For Ecol Manage*, 589: 122768. doi:10.1016/j.foreco.2025.122768

Vermonti N, Clarke CE, Crous CJ, Miller JA, Nel T, Francis ML 2025. Termites as vectors for the lateral movement of elements in the landscape: Their influence on nutrient cycling and landscape heterogeneity. *Appl Soil Ecol,* 206: 105898. doi:10.1016/j.apsoil.2025.105898

Wahi AK, Ravi J, Hemalatha S, Singh PN 2002. Antidiabetic activity of Daemia extensa. *Journal of Natural Remedies,* 2(1): 80-83.

Wang M, Zhang S, Li R, Zhao Q 2024. Unraveling the specialized metabolic pathways in medicinal plant genomes: A review. *Frontiers in Plant Science,* 15. https://doi.org/10.3389/fpls.2024.1459533

Wolfender J-L, Marti G, Thomas A, Bertrand S 2015. Current approaches and challenges for the metabolite profiling of complex natural extracts. *Journal of Chromatography A,* 1382: 136-164. https://doi.org/10.1016/j.chroma.2014.10.091

Yuan F, Yan S, Zhao J 2024. Elucidating the phytochemical landscape of leaves, stems, and tubers of Codonopsisconvolvulacea through integrated metabolomics. *Molecules,* 29(13): 3193. https://doi.org/10.3390/molecules29133193

Paper received for publication in May, 2026
Paper accepted for publication in September, 2026