

## Utilization of Perishable Plant Waste for Biofertilizer Production

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### ABSTRACT

The study focuses on converting plant waste into biofertilizer using an eco-friendly method. Perishable plant waste from sources like arrow bamboo, mango trees, and pigeon wood was collected from Uka Tarsadia University and decomposed with a microbial blend of *Bacillus subtilis*, *Lactobacillus* spp., and *Aspergillus niger*. This blend efficiently breaks down organic waste into nutrient-rich compost. The compost was analysed for macronutrients—nitrogen, phosphorus, and potassium and applied to Kalmegh (*Andrographis paniculata*) plants in field trials. The experimental setup included a control group (Positive and Negative Control) and five treatment groups, each replicated three times, totaling 21 units. Soil analysis 15 days after applying the Plant-Based Biofertilizer (PBB) showed significant improvements compared to controls. Treatment 2 (Soil + PBB at 7 kg/25 gm) had the highest nitrogen content (208 kg/ha), while Treatment 3 (Soil + PBB at 7 kg/35 gm) had the highest phosphorus (302 kg/ha). Treatment 3 also showed the best plant height (88 cm), number of branches (19), and days to flowering (70), while Treatment 2 had the highest fresh (3.92 gm) and dry leaf weights (0.65 gm). Two-way ANOVA confirmed Treatment 2 as most effective for optimizing Kalmegh growth and soil fertility.

**Key words:** Plant waste, composting, microbial decomposition, biofertilizer, anova, waste management

### INTRODUCTION

Plant waste from agriculture can be categorized into harvesting residues (e.g., wheat straw, rice husks), pruning waste (e.g., branches, leaves from maintenance), and processing byproducts (e.g., fruit peels, vegetable trimmings) (Koul *et al.*, 2022). Proper management of these wastes through composting or green fertilizers reduces environmental impact and supports sustainable agriculture (Ajila *et al.*, 2012).

Composting, vermicomposting, and biochar production, each offering unique benefits for soil enhancement and waste management (Raza *et al.*, 2022; Ahmed *et al.*, 2019). Composting is a natural biological process that transforms plant waste into nutrient-rich compost. Aerobic composting involves maintaining an oxygen-rich environment by regularly turning the compost pile, which promotes the activity of aerobic microorganisms and produces high-quality compost relatively quickly, often within a few months (BEFFA, 2002). In contrast, anaerobic composting occurs in the absence of oxygen, typically within sealed environments or landfills. This method decomposes plant waste more slowly and generates methane, making it more suitable for large-scale waste

management scenarios. Vermicomposting utilizes earthworms, particularly red wigglers (*Eisenia fetida*), to convert organic matter into high-quality compost (Thakur *et al.*, 2021). This process involves managing earthworms in bins or beds with a mix of plant waste, ensuring adequate moisture and aeration, and results in vermicompost rich in nutrients and beneficial microorganisms (Singh and Sinha, 2022). Biochar production involves pyrolysis, a process that heats plant waste in the absence of oxygen to produce biochar (Sharma *et al.*, 2009). This method can be adjusted through various technologies, such as kilns and retorts, to create different types of biochar based on temperature and feedstock. Biochar enhances soil fertility by improving nutrient retention, adjusting soil pH in acidic conditions, and fostering beneficial microbial activity (Saleem *et al.*, 2022). Additionally, it plays a role in carbon sequestration, contributing to climate change mitigation efforts.

Perishable plant waste, often discarded after harvest or food processing, can be repurposed as biofertilizer, offering a sustainable solution for waste reduction and soil enrichment (Li *et al.*, 2023). This waste is rich in essential nutrients like nitrogen, phosphorus, and potassium. Converting it into biofertilizer recycles these nutrients into the soil, enhancing plant growth and improving soil

structure, water retention, and microbial activity. Unlike synthetic fertilizers, biofertilizers from plant waste are eco-friendly, reducing disposal costs and environmental pollution (Diacono *et al.*, 2019). They can be produced locally, supporting sustainable agriculture and decreasing reliance on external inputs.

However, untreated plant waste can emit methane, a potent greenhouse gas, and produce leachate that contaminates soil and water, posing environmental hazards (Reay *et al.*, 2018). The continuous use of chemical fertilizers can deplete soil fertility and lower crop quality, affecting market prices (Sarker *et al.*, 2024). Conversely, organic manures improve soil texture and structure, promoting better growth and yield, particularly in crops like Kalmegh. This highlights the role of organic fertilizers in sustainable agriculture. Moreover, the traditional practice of burning perishable plant waste exacerbates air pollution, releasing harmful particulate matter and greenhouse gases into the atmosphere, further aggravating environmental degradation (Matu, 2010; Vurukonda *et al.*, 2024).

Kalmegh, a key ingredient in the West Bengal medicine 'Alu', is used for general debility, dyspepsia, and bowel complaints. Known for its efficacy against chronic malaria, it is often a substitute for *Swertia chirata* (Gupta and Prakash, 2020). Given the economic value and environmental benefits, optimizing organic nutrient management for kalmegh is essential. Identifying the best organic sources and application rates can enhance both yield and quality of this vital medicinal herb (Vijaya and Nanavati, 1978).

## MATERIALS AND METHOD

### Collection and Preparation of Plant Waste, Maintaining Carbon-Nitrogen Ratios, and Composting with Microbial Cultures

Perishable plant waste, including arrow bamboo leaves, mango tree leaves, and pigeon wood stem, was collected from the university premises. The collected waste consisted of leaves and decomposable stems. The waste materials were chopped into smaller pieces to facilitate decomposition. Equal proportions of the collected waste, leaves, and decomposable stems were mixed. This blend ensured a balanced carbon-to-nitrogen (C: N) ratio, essential for efficient composting. Stems and leaves are valuable organic materials that significantly enhance composting processes by providing essential carbon and nitrogen

sources for microbial activity and nutrient cycling. Research indicates that these plant parts are abundant in carbon compounds such as cellulose and lignin, which serve as primary energy sources for decomposer organisms like bacteria and fungi during composting (Sánchez *et al.*, 2017). Additionally, they contain nitrogen in the form of proteins and amino acids, vital for microbial growth and enzyme production necessary for decomposition. Maintaining an adequate ratio of carbon to nitrogen (typically around 30:1) is crucial for promoting efficient composting, as it supports microbial metabolism and facilitates the breakdown of organic matter into stable humic substances and nutrients that benefit soil health (Tiwari *et al.*, 2023). Integrating stems and leaves into composting not only reduces organic waste sent to landfills but also enhances the resulting compost with nutrients essential for plant growth and soil fertility (Bernal *et al.*, 2009; Garcia-Gil *et al.*, 2000; Gajalakshmi *et al.*, 2001). A plastic container with drilled holes for aeration was selected. The container was filled with 4-5 alternating layers of the waste blend and microbial culture, consisting of *Bacillus subtilis*, *Lactobacillus* spp., and *Aspergillus niger*, selected for their efficacy in organic waste degradation. Approximately 20 ml of waste decomposer containing microbial culture was added in 200 litres of water containing container. To enhance microbial activity, 2 kg of jaggery (a carbon source) was also added. The culture underwent twice-daily stirring over a period of seven days to foster microbial growth, preparing it for the degradation process of perishable plant waste (Figure 1).



Fig. 1. Visually outlines the process involved in utilizing perishable plant waste as a biofertilizer

### Composting, Post-Harvest Analysis, NPK

### Level Evaluation, and Field Trials

The composting process involved covering the container to maintain controlled conditions. Over 50-60 days, plant waste decomposed into nutrient-rich compost, monitored for optimal moisture, aeration, and temperature. The mature compost, characterized by its dark color, crumbly texture, and earthy aroma, was analyzed for NPK content to evaluate its suitability as a biofertilizer. Methods used included the Modified Kjeldahl for nitrogen, Flame Photometry for potassium, and Bray P2 for phosphorus. Kalmegh plant growth was then evaluated according to DUS criteria set by the Ministry of Agriculture and Farmers Welfare, Government of India (Mishra *et al.*, 2023; Sharma *et al.*, 2020; Yahaya *et al.*). Kalmegh (*Andrographis paniculata*) (Burm. f.) Nees., a medicinal herb, was utilized in the field trials of our study. The seeds for Kalmegh were procured from the Zandu Foundation for Health Care, Ambach, Vapi, India. Subsequently, field trials were initiated using Kalmegh plants, where the microbial compost was applied to the soil around the plants. These trials aimed to evaluate the effectiveness of the compost as

a plant-based biofertilizer, considering its impact on growth, yield, and overall plant health parameters specified under the DUS guidelines (Table 1). The comprehensive approach from composting to field trials underscored the sustainable utilization of organic resources for enhancing soil fertility and agricultural productivity (Ministry of Agriculture and Farmers Welfare, 2024; Chouhan *et al.*, 2023; Khan *et al.*, 2015; Gautam *et al.*, 2021).

Anaerobic digestion of organic waste, shown in Figure 2, involves microorganisms breaking down waste to produce compost, releasing carbon dioxide and methane gases. This compost, when applied to soil, enhances mineralization and improves soil properties such as aggregate stability, bulk density, infiltration rate, water holding capacity, nutrient levels, electrical conductivity, pH, and cation exchange capacity (CEC). CEC is vital for soil fertility as it measures the soil's ability to supply essential nutrients, boosting crop growth and biological activities (Altomare and Tringovska, 2011; Jacoby *et al.*, 2017; Prakash *et al.*, 2015; Kumar *et al.*, 2017; Hossain *et al.*, 2016).

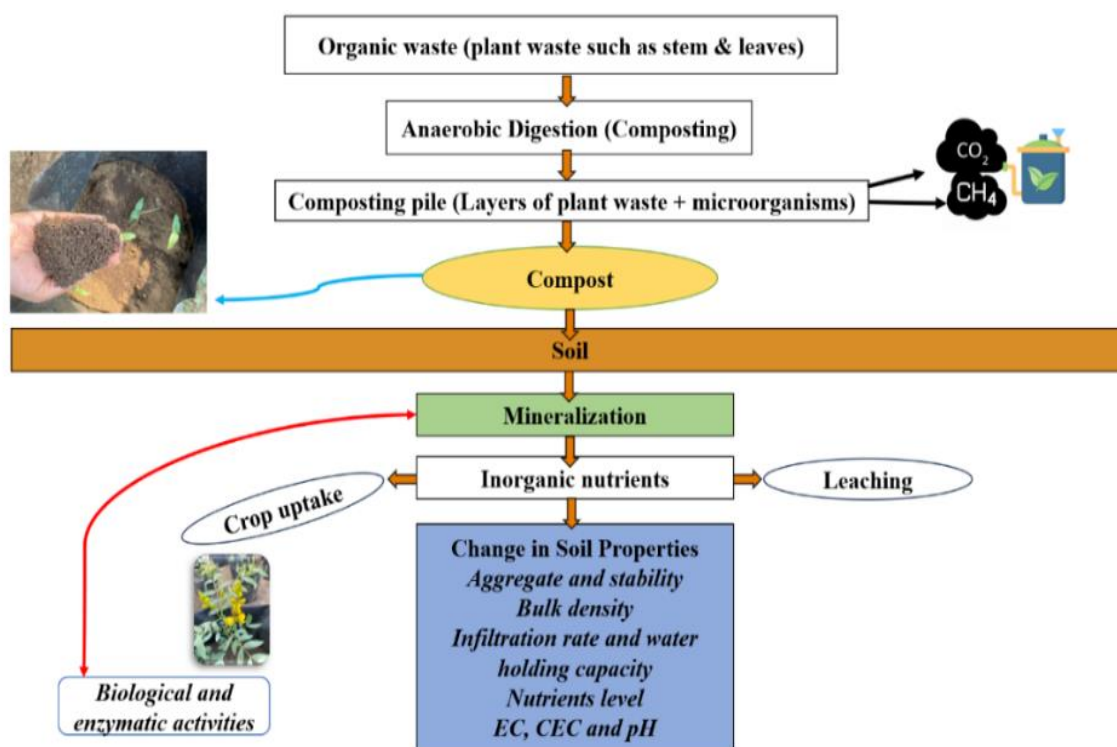


Fig. 2. Anaerobic composting process utilizing microorganisms and the effects of biofertilizers on soil quality.

**Table 1.** The following are the Distinctness, Uniformity, and Stability (DUS) characteristics noted for kalmegh.

No.	Character	How data was recorded
1.	Plant height (cm)	Plant height was measured in centimeter from soil surface to top of the plant at the time of maturity.
2.	Number of branches	Number of primary branches per plant were recorded by counting primary branches on each selected plant.

3.	Leaf length	Length of leaves measured by using vernier caliper and converted to centimeter at the end of growing period when full length of leaves achieved.
4.	Leaf breadth	Width of leaves measured by using vernier caliper and converted to centimeter at the end of growing period when full length of leaves achieved.
5.	Days to flower initiation	Number of days required for flowering of each observational plant in each pot was recorded from the date of sowing.
6.	Fresh leaves weight/plant	At the time of harvesting, fresh weight of each observational plant was recorded.
7.	Dry leaves weight/plant	After sun drying, dry weight of each observational plant was recorded.

### Statistical Analysis

The data from the field trials and the soil analysis after various treatments were analysed statistically using two-way ANOVA and Tukey's pairwise test, with both analyses performed using Microsoft Excel software (Shruthi and Shivashankara, 2022).

### RESULTS

The NPK analysis evidently shows a difference in the amounts of N, P, and K in the treatments and control groups (Table 2). This suggests that the plant-based biofertilizer (PBB) has a significant impact on increasing the nutrient efficacy of the soil. A controlled experiment was established to investigate the growth of Kalmegh plants. A control group was maintained with five treatment groups, each with three replications, resulting in a total of 21 experimental units. For each treatment, we prepared a mixture of soil and plant-based organic manure, using a ratio of 15 grams of manure per 7-8 kg soil pot, after 15 days of pre-treatment of soil with plant based biofertilizer, we then sowed 20 seeds (pretreated with systemic fungicide composed of 8% Metalaxyl and 64% Mancozeb, this treatment is typically done to protect the seeds from fungal diseases and promote healthy growth) (Reddy, 2010). of Kalmegh in each pot. There is a growing market for herbal medicines, especially those with Kalmegh. But there are concerns about their safety, effectiveness, quality, and availability.

It's important to grow medicinal plants commercially to supply natural drugs to pharmaceutical companies. However, there's a problem with the lack of standard farming practices, especially when it comes to managing nutrients (Jat and Gajbhiye, 2019). The objective of this experiment is to observe and analyze the impact of plant-based organic manure on the growth and development of Kalmegh plants. The results of this study could provide valuable insights into sustainable and effective agricultural practices.

Groups in the experiment were divided into Negative Control, Positive Control, and five treatments (T<sub>1</sub> to T<sub>5</sub>) (Figure 3). Analysis showed treatment (T<sub>2</sub>) had the highest values for N (208), P (40), and K (298). Treatments (T<sub>1</sub>) and (T<sub>3</sub>) also performed well, while (T<sub>4</sub>) and (T<sub>5</sub>) were moderate. The Positive Control outperformed the Negative Control but was less effective than Treatments. The Negative Control had the lowest values. Error bars indicated significant differences, with Treatments (T<sub>1</sub>), (T<sub>2</sub>), and (T<sub>3</sub>) showing notable effectiveness compared to controls and other treatments.

Analysis of nutrient levels (Nitrogen, Phosphorus, Potassium) across treatments (T<sub>1</sub>-T<sub>5</sub>) shows variability, with an F-statistic of 3.83 indicating differences among groups. The P-value of 0.02, below the 0.05 threshold, provides sufficient evidence to reject the null hypothesis, confirming significant differences in nutrient levels among the treatment groups (Table 3).

**Table 2.** Comparative data of control and treatments for N, P, K analysis

Groups	Soil/plant based biofertilizer (PBB) (kg/gm)	N kg/ha	P kg/ha	K kg/ha
Negative Control	Only Soil (7 kg)	175.10	27	231
Positive Control	Soil + FYM (7 kg/15 gm)	197.00	34	295
Treatment (T <sub>1</sub> )	Soil + PBB (7 kg/15 gm)	205.90	32	284
Treatment (T <sub>2</sub> )	Soil + PBB (7 kg/25 gm)	208.00	40	298
Treatment (T <sub>3</sub> )	Soil + PBB (7 kg/35 gm)	202.60	36	302
Treatment (T <sub>4</sub> )	Soil + PBB (7 kg/45 gm)	198.50	42	298
Treatment (T <sub>5</sub> )	Soil + PBB (7 kg/55 gm)	192.10	37	291

Keys: FYM: Farm Yard Manure, PBB: Plant based bio fertilizer, N: Nitrogen, P: Phosphorous, K: Potassium.

**Table 3.** Two-way ANOVA for comparative data of control and treatments for N, P, K Analysis.

Source of variation	SS	df	MS	F	P-value	F crit
Rows (Treatment groups)	3002.211	6	500.3686	3.837547	0.02	2.99612
Columns (Nutrient levels)	225227	2	112613.5	863.6824	0.00	3.885294
Error	1564.651	12	130.3876			
Total	229793.8	20				

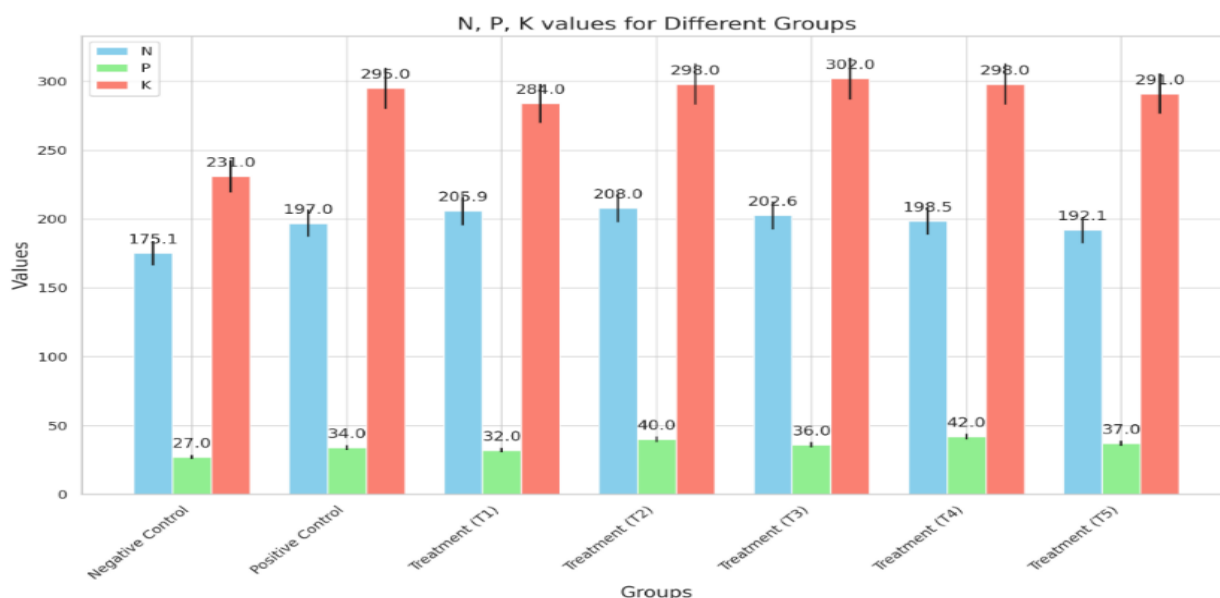


Fig. 3. Provides a visual representation of the N, P, and K values for the Negative Control, Positive Control, and treatments (T<sub>1</sub> to T<sub>5</sub>).

### Impact of Nutrient Levels on Plant Characteristics

The analysis assesses how nutrient levels impact plant traits, aiming to determine their significance. With an F-statistic of 863.8584 and a P-value < 0.0001, there is strong evidence against the null hypothesis. This indicates that nutrient levels (Nitrogen, Phosphorus, and Potassium) significantly affect plant characteristics, showing notable differences among them.

### Summary of Variability Analysis for Nutrient Levels Impact on NPK Content

The within-group variability, or error, has a sum of squares (SS) of 1564.651 with 12 degrees of freedom (df), resulting in a mean square (MS) of 130.3876. This captures variability within each group not explained by the treatment. The total variability, with SS of 229793.8 and 20 df, includes both explained and unexplained variation. ANOVA results show that nutrient levels significantly impact NPK content, indicating meaningful influence on these components.

### Analysis of Variance (ANOVA) Results for

### Nutrient Levels across Treatment and Control Groups

ANOVA results reveal significant differences in NPK levels among treatment groups (T<sub>1</sub>-T<sub>5</sub>), indicating that nutrient levels impact these elements. The Negative Control had lower N (175.1), P (27), and K (231) levels compared to the Positive Control, which had higher levels (N: 197, P: 34, K: 295). Treatment groups (T<sub>1</sub>-T<sub>5</sub>) also showed varying nutrient levels. Post hoc tukey's pairwise tests (N vs. P, P vs. K, N vs. K) confirmed significant differences (p-value: 0.0001494) among nutrient levels, highlighting their distinct roles in plant growth (Table 4).

Field trials demonstrated that plant-based biofertilizer (PBB) positively affected Kalmegh growth, increasing plant height, flowering time, number of branches, and pod yield (Table 5). These findings suggest that PBB significantly enhances Kalmegh growth and productivity, supporting its use as a sustainable agricultural strategy.

Fresh leaf weight reflects nutrient transport, while dry leaf weight indicates nutrient concentration. In our field trial data, the graph (Figure 4) shows fresh leaf weight (Y-axis left, 2.98 to 3.92 grams) and dry leaf weight (Y-axis right, 0.50 to 0.65 grams) for NC (Negative Control), PC (Positive Control), and T<sub>1</sub> to T<sub>5</sub>

(Treatment Groups). Blue bars represent fresh leaf weights, and an orange line denotes dry leaf weights. Error bars show variability or standard error.

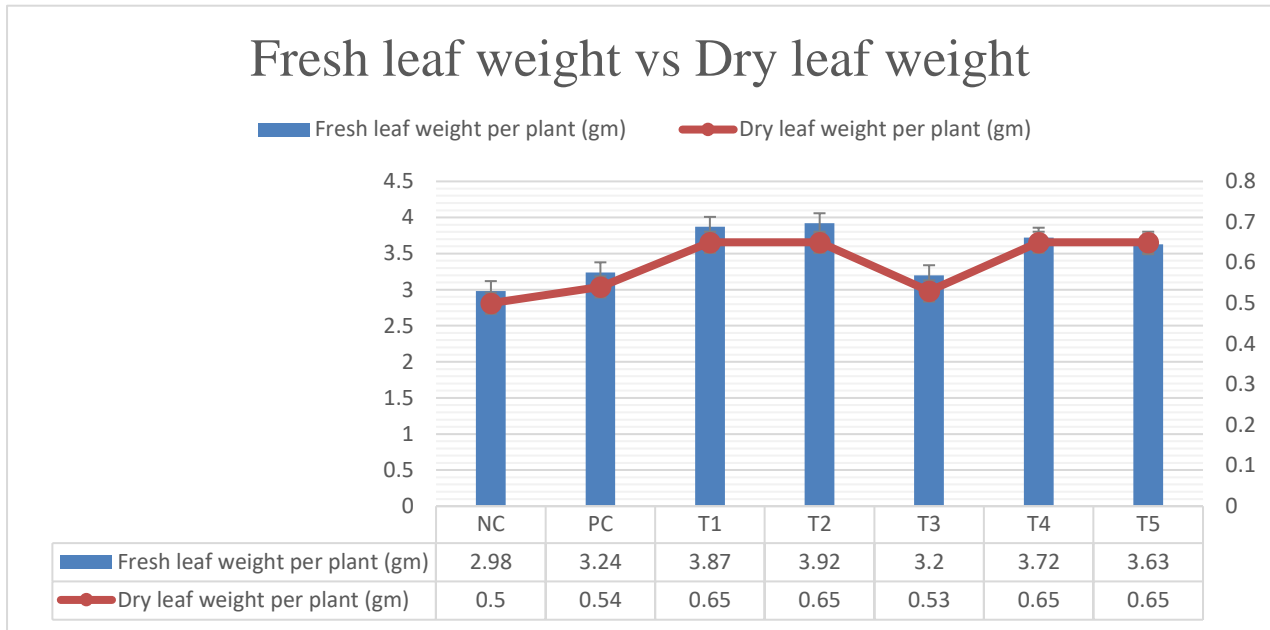


Fig. 4. Illustrates how treatments influence both fresh and dry leaf weights of plants, with treatments T<sub>1</sub>, T<sub>4</sub>, and T<sub>5</sub> showing consistently higher weights compared to controls.

Table 4. Tukey’s pairwise test

	N	P	K
N		0.0001494	0.0001494
P	26.84		0.0001494
K	14.71	41.55	

Table 5. Presents a comparative analysis of kalmegh growth parameters across different groups: Negative Control (NC), Positive Control (PC), and five different treatments (T<sub>1</sub> to T<sub>5</sub>).

Groups	PH (cm)	NOB (nos.)	LL (cm)	LB (cm)	DTFI (nos.)	FLWPP (gm)	DLWPP (gm)
NC	58	12	1.60	0.60	85	2.98	0.50
PC	69	20	2.00	0.90	72	3.24	0.54
T <sub>1</sub>	75	20	2.20	1.10	70	3.87	0.65
T <sub>2</sub>	85	24	2.20	1.80	72	3.92	0.65
T <sub>3</sub>	88	19	2.40	1.82	70	3.20	0.53
T <sub>4</sub>	85	18	2.40	1.50	72	3.72	0.65
T <sub>5</sub>	80	22	2.10	1.10	70	3.63	0.65

Keys: PH: Plant height, NOB: Number of branches, LL: Leaf length, LB: Leaf breadth, DTFI: Days to flower initiation, FLWPP: Fresh leaf weight per plant, DLWPP: Dry leaf weight per plant.

Data shows that the Negative Control (NC) had a fresh leaf weight of 2.98 grams and a dry leaf weight of 0.50 grams, while the Positive Control (PC) had slightly higher values at 3.24 grams and 0.54 grams. Treatment 2 (T<sub>2</sub>) had the highest fresh leaf weight at 3.92 grams and a dry leaf weight of 0.65 grams (Figure 4). Treatments 1, and 3 to 5 showed varying levels, with T<sub>1</sub> and T<sub>3</sub> consistently high. Figure 5 illustrates Kalmegh plant parts: pod (P), flower (F), leaves (L), branches (B), and stem (S).

ANOVA results indicated significant differences in NPK levels among treatment

groups, with low p-values (<0.05) confirming this. Pairwise comparisons showed substantial differences between N vs P, P vs K, and N vs K (p = 0.0001494). Field trials demonstrated that plant-based biofertilizers (PBB) generally outperformed the Negative Control. Specifically, treatment 3 (T<sub>3</sub>) had the highest plant height (88 units), moderate branching (19 branches), and early flowering (70 days), while Treatment 2 (T<sub>2</sub>) also performed well with a plant height of 85 units and flowering in 72 days. Treatments T<sub>2</sub> and T<sub>3</sub> are recommended for optimizing plant growth.

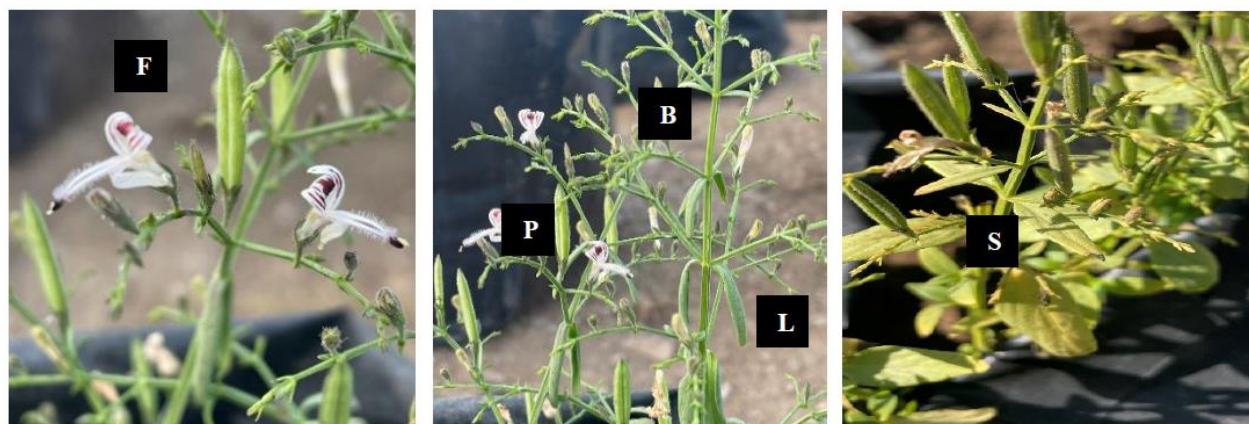


Fig. 5. Kalmegh Plant Parts on Flowering and Maturation

## DISCUSSION

Our study parallels the research conducted by Hussein *et al.*, who utilized a plant-based compost composed of chopped rice and soybean straws in a 1:1 ratio (Hussein *et al.*, 2022). In contrast, our study employed a blend of arrow bamboo, mango tree, and pigeon wood in a 1:1:1 ratio. The compost resulting from the blend made by Hussein *et al.*, demonstrated a significant increase in nutrient content, specifically containing 1.03% N, 0.52% P, and 0.47% K. Our investigation revealed notable enhancements in soil nutrient levels following the application of the plant-based biofertilizer. Initially, the soil contained 175.10: 27: 231 kg/ha N: P: K, respectively. After 15 days of applying the biofertilizer, these levels increased to 208.00:40: 298 kg/ha N: P: K, respectively in treatment 2 (T<sub>2</sub>). These findings underscore the substantial impact of plant-based compost on augmenting nitrogen, phosphorus, and potassium levels in the soil, thereby enhancing their availability for plant uptake through mineralization processes.

Our study also aligns with the research conducted by Ramadass *et al.*, who conducted a comparative study using enriched and unenriched compost, where the enriched compost was mixed with municipal solid waste whereas the unenriched compost was deprived of municipal solid waste. The enriched compost was supplemented with poultry litter (10%), spent wash (10%), rock phosphate (0.5%), and a microbial consortium (0.5%) comprising *Azotobacter* sp., *Phosphobacteria* sp., and *Pseudomonas* sp. This microbial consortium, formulated from a mixture of microorganisms including *Azotobacter* sp., *Phosphobacteria* sp., and *Pseudomonas fluorescence*, significantly enhanced the nutrient content of compost (Ramadass and Palaniyandi, 2007). The control, which consisted of normal compost,

exhibited N: P: K levels of 0.64: 0.56: 0.70 per cent, respectively, whereas the enriched compost showed higher levels with N: P: K at 1.75: 1.16: 1.83 percent, respectively. This clearly indicates that the addition of waste materials along with a microbial consortium contributes to the enhancement of micronutrients in compost. In our study, we utilized a blend of plant wastes including arrow bamboo, mango tree, and pigeon wood, enriched with a microbial consortium comprising *Bacillus subtilis*, *Lactobacillus* spp., and *Aspergillus niger*. These microorganisms were selected for their effectiveness in organic waste degradation. The resulting compost demonstrated substantial nutrient enhancement, with N; P; K levels reaching 208.0:40:298 kg/ha, respectively, which represented the most favourable concentrations among our treatments. This highlights the efficacy of integrating plant waste with specific microbial consortia in augmenting nutrient availability and enriching compost quality.

Our study also demonstrates effectiveness in enhancing the growth and yield of a potential herb using PBB (a blend of plant waste and microbial consortium). Our findings are consistent with those of Chandana *et al.*, who conducted a study on kalmegh using FYM, Vermicompost, Neem cake, and sheep manure combined with different spacing in a factorial randomized block design (Chandana *et al.*, 2018). Significant differences in leaf weight were observed among treatments. Plants treated with FYM had a fresh weight of 32.39 g and a dry weight of 18.16 g, while vermicompost-treated plants had 31.57 g (fresh) and 16.07 g (dry). Neem cake-treated plants showed 31.95 g (fresh) and 17.16 g (dry), and sheep manure-treated plants had 31.51 g (fresh) and 14.93 g (dry). The control group had lower values at 30.70 g (fresh) and 13.53 g (dry). Our study similarly showed that our

treatment improved leaf weight to 3.92 g (fresh) and 0.65 g (dry) compared to 2.98 g (fresh) and 0.50 g (dry) in the control group, highlighting the effectiveness of our approach in enhancing yield.

Tables 2 and 3 show that increasing fertilizer concentration initially boosts plant growth but may negatively affect it beyond a certain threshold. This aligns with Liebig's principle of nutrient limitation, which states that growth is limited by the least available nutrient. (Rizhinashvili, 2022). Our study corroborates this understanding with supporting evidence from various research endeavours. For instance, Marschner *et al.*, in their work titled "Mineral Nutrition of Higher Plants," discuss how nutrient uptake and utilization by plants are influenced by nutrient availability (Marschner, 2011). They emphasize that while nutrients are essential for growth, excessive concentrations can induce toxicity or imbalance, thereby constraining growth rather than stimulating it. In the study conducted by Hetal *et al.* (Patel *et al.*, 2016), various treatments involving both chemical fertilizers and different biofertilizers were assessed for their effects on the growth parameters of *Plumbago zeylanica*. The treatments included a control with farmyard manure (T<sub>1</sub>), a chemical fertilizer (T<sub>2</sub>), individual biofertilizers such as *Azotobacter* (T<sub>3</sub>), *Azospirillum* (T<sub>4</sub>), and Phosphate Solubilizing Bacteria (PSB) (T<sub>5</sub>), and a combined biofertilizer mix (T<sub>6</sub>). ANOVA of treatments on root length, fresh weight, and dry weight showed a highly significant p-value of 0.000404 ( $p < 0.001$ ), confirming significant differences among treatment groups and rejecting the null hypothesis. Similarly, in our study on Kalmegh plants, ANOVA revealed a p-value of 0.02, below the 0.05 threshold, indicating significant effects of the treatments and validating their positive impact on plant growth. This indicates significant differences in the nutrient levels and growth responses among the treatment groups. Both studies demonstrate the effectiveness of biofertilizers and their combinations in enhancing plant growth compared to traditional chemical fertilizers and control treatments. The significant p-values in both cases underscore the substantial impact of the chosen treatments on the respective plant species, suggesting that carefully selected biofertilizers and their combinations can lead to improved growth parameters. Our findings are consistent with those of Hetal *et al.*, supporting the notion that biofertilizers can offer superior benefits. Future research could further explore the optimal combinations and

concentrations of biofertilizers to maximize growth and yield across different plant species. Furthermore, Geisseler and Scow, in their review titled "Long-term effects of mineral fertilizers on soil microorganisms," explore how excessive fertilization can disrupt soil microbial communities, affecting nutrient availability to plants (Geisseler and Scow, 2014). Long-term application of mineral fertilizers can significantly impact soil microorganisms, often leading to shifts in microbial community structure and function. While these fertilizers typically enhance nutrient availability and can boost microbial activity initially, their continuous use may disrupt soil microbial diversity. Over time, excessive mineral fertilizers can lead to imbalances in soil pH, nutrient ratios, and organic matter, which in turn may favour certain microbial species over others and reduce overall microbial diversity. This alteration can affect soil health and ecosystem services, such as nutrient cycling and soil structure, potentially leading to reduced soil fertility and increased vulnerability to diseases and erosion. Thus, while mineral fertilizers are crucial for crop production, their long-term use requires careful management to mitigate adverse effects on soil microbial communities (Yang *et al.*, 2021). This underscores the detrimental impact that overly concentrated fertilizers can have on plant growth by disturbing soil health and nutrient cycling. In analysing the data presented in Table 3, a notable trend is observed in the N:P:K ratio across different treatments, which initially increased from 197.00:34:295 kg/ha to 202.60:36:302 kg/ha, before showing a decrease in treatments 4 and 5, specifically with Soil + PBB (7 kg/45 gm) and Soil + PBB (7 kg/55 gm). This pattern suggests that while increasing fertilizer concentration can initially enhance the nutrient levels available to plants, excessive amounts may not necessarily yield additional benefits and could even be detrimental. Our comparative evaluation of growth characteristics further supports this observation. Treatment 5 (T<sub>5</sub>), which utilized 7 kg/55 gm of PBB, showed lower efficiency in plant growth metrics such as plant height (80 cm), number of branches (22 nos.), leaf length (2.10 cm), leaf breadth (1.10 cm), days to flowering initiation (70 days), fresh leaf weight (3.63 gm), and dry leaf weight (0.65 gm) compared to Treatment 2, which employed a lower concentration of 7 kg/25 gm PBB. The latter treatment displayed more favourable growth characteristics, reinforcing the notion that there is a threshold beyond which additional fertilizer concentration does not



continue to enhance plant growth. This finding is consistent with established principles in agronomy, which suggest that while an appropriate nutrient supply is crucial for optimal plant growth, there exists an optimal concentration beyond which further increases do not correlate with additional benefits (Goulding *et al.*, 2008). Excessive fertilization can lead to nutrient imbalances, potential toxicity, or other detrimental effects that inhibit plant growth and development (Chouhan *et al.*, 2023; Detpiratmongkol *et al.*, 2014).

The results of our study, alongside the observed trend in the N: P: K ratio, underscore the importance of precision in fertilizer application. They advocate for a balanced approach to fertilization, where the concentrations are carefully optimized to achieve the best possible growth outcomes without overloading the plants with excessive nutrients (Bhatt *et al.*, 2024). This approach not only enhances plant health and productivity but also aligns with sustainable agricultural practices by minimizing waste and reducing the environmental impact. Hence, while fertilizers derived from plant waste offer benefits such as organic composition and gradual nutrient release, it is crucial to identify the optimal concentration that promotes plant growth without triggering negative outcomes such as nutrient imbalances or toxicity. Achieving this balance is essential for sustainable agriculture practices aimed at maximizing crop productivity.

## CONCLUSION

In conclusion, our study provides compelling evidence that PBB enhances soil fertility, promotes plant growth, and increases the yield of medicinal plants like Kalmegh. By comparing our findings with established research, we underscore the consistency and reliability of biofertilizers in improving agricultural productivity and sustainability. This knowledge not only contributes to advancing agricultural practices but also supports the cultivation of medicinal plants, thereby addressing global challenges in food security and herbal medicine production effectively.

## AUTHOR CONTRIBUTION STATEMENT

The authors confirm contribution to the paper as follows: study conception and design: Snaa Mistry, R. Krishnamurthy; data collection: Snaa Mistry, Ankit Chaudhary; analysis and interpretation of results: Snaa Mistry, R.

Krishnamurthy, Ankit Chaudhary; draft manuscript preparation: Snaa Mistry, Ankit Chaudhary. All authors reviewed the results and approved the final version of the manuscript.

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## CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

## REFERENCES

- Ahmed, M., Ahmad, S., Qadir, G., Hayat, R., Shaheen, F.A. and Raza, M. A. (2019). Innovative processes and technologies for nutrient recovery from wastes: a comprehensive review. *Sustainability* **11**: 4938.
- Ajila, C., Brar, S. K., Verma, M. and Prasada Rao, U. (2012). Sustainable solutions for agro processing waste management: An overview. *Environ. Prot. Strateg. Sustain. Dev.* 65-109.
- Altomare, C. and Tringovska, I. (2011). Beneficial soil microorganisms, an ecological alternative for soil fertility management. *Genet. Biofuels Local Farming Syst.* 161-214.
- BEFFA, T. (2002). The composting biotechnology: a microbial aerobic solid substrate fermentation complex process. *Compost. Process Manag.* 1-30.
- Bernal, M. P., Albuquerque, J. and Moral, R. (2009). Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresour. Technol.* **100**: 5444-5453.
- Bhatt, R., de Oliveira, M. W. and de Freitas Santos, D. (2024). Improving sugarcane (*Saccharum* spp. hybrid complex) growth, yield and quality through balanced fertilization: an overview. *Rev. De Gestão E Secr.* **15**: e3781-e3781.
- Chandana, M., Joshi, V., Vijaya, D. and Lakshminarayana, D. (2018). Effect of organic treatments and spacing on growth parameters of kalmegh (*Andrographis paniculata*) var. Cim-Megha. *J. Pharmacogn. Phytochem.* **7**: 1695-1699.

- Chouhan, S., Meena, K., Soni, N., Patidar, D., Kachouli, B. and Haldar, B. P. A. (2023). Response of recommended dose of fertilizers with organic manures on growth, yield and economics of kalmegh (*Andrographis paniculata* Nees.): A way to reduced use of chemical Fertilizers. *Pharma Innov. J.* **12**: 119-124.
- Detpiratmongkol, S., Ubolkerd, T. and Yoosukyingstaporn, S. (2014). Effects of chicken, pig and cow manures on growth and yield of Kalmegh (*Andrographis paniculata* Nees). *J. Agric. Technol.* **10**: 475-482.
- Diacono, M., Persiani, A., Testani, E., Montemurro, F. and Ciaccia, C. (2019). Recycling agricultural wastes and by-products in organic farming: Biofertilizer production, yield performance and carbon footprint analysis. *Sustainability* **11**: 3824.
- Gajalakshmi, S., Ramasamy, E. and Abbasi, S. (2001). Potential of two epigeic and two anecic earthworm species in vermicomposting of water hyacinth. *Bioresour. Technol.* **76**: 177-181.
- Garcia-Gil, J., Plaza, C., Soler-Rovira, P. and Polo, A. (2000). Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. *Soil Biol. Biochem.* **32**: 1907-1913.
- Gautam, K., Sirohi, C., Singh, N. R., Thakur, Y., Jatav, S. S., Rana, K., Chitara, M., Meena, R.P., Singh, A. K. and Parihar, M. (2021). Microbial biofertilizer: Types, applications, and current challenges for sustainable agricultural production. In *Biofertilizers*. Amsterdam: Elsevier; pp. 3-19.
- Geisseler, D. and Scow, K. M. (2014). Long-term effects of mineral fertilizers on soil microorganisms—A review. *Soil Biol. Biochem.* **75**:54-63.
- Goulding, K., Jarvis, S. and Whitmore, A. (2008). Optimizing nutrient management for farm systems. *Philos. Trans. R. Soc. B: Biol. Sci.* **363**: 667-680.
- Gupta, C. and Prakash, D. (2020). Medicinal Plants in Natural Health Care as Phytopharmaceuticals. In *Herbal Product Development*. Cambridge: Academic Press; pp. 1-36.
- Hossain, M.Z., von Fragstein, P., Heß, J. and Heß, J. (2016). Plant origin wastes as soil conditioner and organic fertilizer: a review. *J. Agric. & Environ. Sci* **16**: 1362-1371.
- Hussein, M., Ali, M., Abbas, M. H. and Bassouny, M.A. (2022). Composting animal and plant residues for improving the characteristics of a clayey soil and enhancing the productivity of wheat plant grown thereon. *Egypt. J. Soil Sci.* **62**: 195-208.
- Jacoby, R., Peukert, M., Succurro, A., Koprivova, A. and Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Front. Plant Sci.* **8**: 1617.
- Jat, R. and Gajbhiye, N. (2019). Productivity, andrographolide, and NPK content influenced with organics and inorganics in kalmegh. *Agron. J.* **111**: 851-858.
- Khan, K., Pankaj, U., Verma, S. K., Gupta, A. K., Singh, R. P. and Verma, R. K. (2015). Bio-inoculants and vermicompost influence on yield, quality of *Andrographis paniculata*, and soil properties. *Ind. Crops Prod.* **70**: 404-409.
- Koul, B., Yakoob, M. and Shah, M. P. (2022). Agricultural waste management strategies for environmental sustainability. *Environ. Res.* **206**:112285.
- Kumar, R., Kumawat, N. and Sahu, Y. K. (2017). Role of biofertilizers in agriculture. *Pop. Kheti* **5**: 63-66.
- Li, P., Zhu, F., Weiping, W., Zhou, Y., Yao, Y., Hong, L., Zhu, W., Hong, C., Liu, X. and Chen, H. (2023). Physicochemical properties and risk assessment of perishable waste primary products. *J. Environ. Manag.* **337**: 117549.
- Marschner, H. (2011). *Marschner's mineral nutrition of higher plants*. Cambridge: Academic press.
- Matu, S. U. A. (2010). *Utilization of Agro-waste to Produce Biofertilizer*. Kota Samarahan: Universiti Malaysia Sarawak.
- Ministry of Agriculture and Farmers Welfare, G.o.I. (2024). DUS Characteristics Guideline. Available online: <https://plantaauthority.gov.in/crop-dus-guidelines> (accessed on 2 June 2024).
- Mishra, S., Kesharwani, S., Chivate, M., Chauhan, A. K. and Mishra, V. (2023). Development of Novel Biofertilizer by Combining the Banana Waste and Cyanobacteria in Sustainable Agriculture. *Int. J. Food Ferment. Technol.* **13**: 69-86.
- Patel, H.D., Krishnamurthy, R. and Azeez, M. A. (2016). Effect of biofertilizer on growth, yield and bioactive component of *Plumbago zeylanica* (Lead Wort). *J. Agric. Sci.* **8**: 142-155.
- Prakash, O., Sharma, R., Rahi, P. and Karthikeyan, N. (2015). Role of microorganisms in plant nutrition and health. *Nutr. Use Effic. : Basics Adv.* 125-161.
- Ramadass, K. and Palaniyandi, S. (2007). Effect of enriched municipal solid waste compost application on soil available macronutrients in the rice field. *Arch. Agron. Soil Sci.* **53**: 497-506.
- Raza, S.T., Wu, J., Rene, E.R., Ali, Z. and Chen, Z. (2022). Reuse of agricultural wastes, manure, and biochar as an organic amendment: A review on its implications for vermicomposting technology. *J. Clean. Prod.* **360**:132200.
- Reay, D.S., Smith, P., Christensen, T.R., James, R.H. and Clark, H. (2018). Methane and global environmental change. *Annu. Rev. Environ. Resour.* **43**: 165-192.
- Reddy, P. P. (2010). *Fungal diseases and their management in horticultural crops*. Glendale: Scientific Publishers.
- Rizhinashvili, A. (2022). An outline of the theory of the functioning of aquatic ecosystems: nutrient limitation. *Biol. Bull. Rev.* **12**: 596-608.
- Saleem, I., Riaz, M., Mahmood, R., Rasul, F., Arif, M., Batool, A., Akmal, M. H., Azeem, F. and Sajjad, S. (2022). Biochar and microbes for sustainable soil quality management. In *Microbiome under Changing Climate*. Amsterdam: Elsevier; pp. 289-311.
- Sánchez, Ó. J., Ospina, D. A. and Montoya, S. (2017). Compost supplementation with nutrients and microorganisms in composting process. *Waste Manag.* **69**: 136-153.
- Sarker, A., Ahmmmed, R., Ahsan, S., Rana, J., Ghosh, M.K. and Nandi, R. (2024). A comprehensive review of food waste valorization for the sustainable management of global food waste. *Sustain. Food Technol.* **2**: 48-69.
- Sharma, P., Gaur, V.K., Kim, S.-H. and Pandey, A. (2020). Microbial strategies for bio-transforming food waste into resources. *Bioresour. Technol.* **299**:122580.
- Sharma, S., Kumar, A., Singh, A. P. and Vasudevan, P. (2009). Earthworms and vermitechnology—A review. *Dyn. Soil Dyn. Plant* **3**: 1-12.
- Shruthi, R. and Shivashankara, G. (2022). Effect of HRT and seasons on the performance of pilot-scale horizontal subsurface flow constructed wetland to treat rural wastewater. *Water Pract. Technol.* **17**: 445-455.
- Singh, S. and Sinha, R.K. (2022). Vermicomposting of organic wastes by earthworms: Making wealth from waste by converting 'garbage into gold' for farmers. In *Advanced organic Waste Management*. Amsterdam: Elsevier; pp. 93-120.

- Thakur, A., Kumar, A., Kumar, C. V., Kiran, B. S., Kumar, S. and Athokpam, V. (2021). A review on vermicomposting: By-products and its importance. *Plant. Cell Biotechnol. Mol. Biol* **22**: 156-164.
- Tiwari, J., Ramanathan, A., Baudh, K. and Korstad, J. (2023). Humic substances: Structure, function and benefits for agroecosystems—A review. *Pedosphere* **33**: 237-249.
- Vijaya, S. and Nanavati, D. (1978). *A. paniculata* (Burm. f.) Wall. ex Nees (kalmegh). *Indian Drugs* **15**:187-190.
- Vurukonda, S. S. K. P., Fotopoulos, V. and Saeid, A. (2024). Production of a Rich Fertilizer Base for Plants from Waste Organic Residues by Microbial Formulation Technology. *Microorganisms* **12**: 541.
- Yahaya, I., Shuwa, M., EL-Jumma, A.M. and Bulama, A.M. Design of Composting Unit for Bio-fertilizer Production using Organic Matter: An Assessment of Municipal Solid Waste Generation in Maiduguri-Nigeria. *Int. J. Inf. Eng. Technol.* **12**: 41-49
- Yang, T., Lupwayi, N., Marc, S.-A., Siddique, K. H. and Bainard, L. D. (2021). Anthropogenic drivers of soil microbial communities and impacts on soil biological functions in agroecosystems. *Glob. Ecol. Conserv.* **27**: e01521.