



# Development and Evaluation of Palmyra Fruit Pulp Based Bioformulations on Growth and Yield of Spinach

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**Abstract:** This study aimed to develop an organic plant growth promoter using Palmyra fruit pulp combined with organic supplements—fish waste, cow urine, curd, and Bougainvillea leaf extract—to enhance sustainable crop production. Eight formulations were prepared and analyzed for their physico-chemical properties and nutrient content. The formulation containing Palmyra fruit pulp + Cow urine + Fish waste + Curd + Bougainvillea leaf extract (5:4:2:0.2:2) exhibited the highest macro- and micronutrient levels and, in pot culture experiments with spinach, produced the largest plants (28.3 cm height, 8.6 cm width) and highest wet weight (30.31 g/plant). GC–MS analysis identified bioactive compounds, including fatty acids, Glucobrassicin, Phytol, and Squalene, linked to plant defense, antioxidant activity, and chlorophyll biosynthesis. These results highlight the potential of integrating natural organic resources into farming practices to improve plant growth, yield, and overall agricultural sustainability.

**Keywords:** bioformulations, seed coating, seedling growth, paddy, germination, Azospirillum, Phosphobacteria, Palmyra, Fruit Pulp, Growth, Yield of Spinach.

## I. INTRODUCTION

The global shift towards sustainable agriculture has underscored the need for environmentally friendly alternatives to chemical fertilizers and synthetic growth enhancers. The excessive use of synthetic chemicals in agriculture has led to numerous ecological concerns, including soil degradation, water contamination, and a decline in biodiversity (Agudelo-Morales et al., 2021). In this context, the current project focuses on the development and evaluation of Palmyra fruit pulp-based bioformulations for improving the growth and yield of spinach (*Spinaciaoleracea* L.). Palmyra fruit pulp, derived from the *Borassusflabellifer* tree, is rich in essential nutrients, carbohydrates, vitamins, and bioactive compounds such as phenolics and antioxidants (Selvakumar et al., 2020). These components have the potential to act as natural growth stimulants, enhancing plant development and productivity.

The study also incorporates other organic supplements such as cow urine, fish waste, curd, and Bougainvillea leaf extract into the bioformulations. Cow urine is well-known for its high content of nitrogen, potassium, and other essential minerals, making it an effective natural fertilizer (Mohanty et al., 2014). Fish waste contributes additional nutrients, including phosphorus and calcium, which are critical for plant growth (Rishitha&Rao, 2019). Curd, with its lactic acid bacteria, improves soil microbial activity, thereby facilitating nutrient uptake by plants (Li et al., 2016). Lastly, Bougainvillea leaf extract offers bioactive compounds that enhance plant defense mechanisms and promote growth (Gad, 2019).

## II. OBJECTIVES OF THE RESEARCH

The research aims to identify the optimal combination of organic materials that maximizes plant productivity while enhancing soil health. Through systematic pot culture experiments and field trials, various growth parameters such as plant height, leaf number, biomass, and yield—will be evaluated under different treatment conditions. The findings are expected to advance sustainable agricultural practices by providing an effective, eco-friendly alternative to synthetic fertilizers. In light of the current emphasis on environmental safety and sustainability in agriculture, the present study has been formulated with the following objectives:

1. To develop bioformulations using palmyra fruit pulp as a key ingredient for enhancing plant growth.
2. To evaluate the effects of palmyra fruit pulp-based bioformulations on the growth and yield of spinach.



### III. MATERIALS AND METHODS

The methodologies related to the study on “Development and Evaluation of Palmyra Fruit Pulp Based Bioformulations on Growth and Yield of Spinach (*Spinaciaoleracea L*)” are presented in this chapter. In this study, we collected the palmyra fruit and extracted the pulp.

The organic formulations were prepared by adding pulp, cow urine, curd, bougainvillea leaf extract, fish waste under various proportions. They were kept for fermentation and thereafter the digested extract is strained and laboratory analyses were done. Data collected from experiment and laboratory analyses are used for calculating the additional parameters for interpretation. The details of the experiment conducted, methods of analysis of various samples and the efficacy of the treatments obtained are described in this chapter.

#### Components used for preparation of bioformulations:

##### 1) Palmyra fruit pulp (50g)

Palmyra fruit pulp can be used as an organic foliar spray due to its rich content of nutrients, vitamins, and antioxidants, which promote plant growth and enhance resistance to pests and diseases. When applied to foliage, the pulp's natural sugars and bioactive compounds improve leaf health, boost photosynthesis, and increase overall plant vitality.

##### 2) Fish waste (20g)

Fish waste is an excellent source of macronutrients, particularly nitrogen, which is essential for leaf and stem growth. It also provides phosphorus for root development and potassium for overall plant health. Fish waste contains natural growth hormones like gibberellins and auxins, which can stimulate faster and healthier plant growth.

##### 3) Cow urine (90ml)

Cow urine plays a critical role in organic formulations by providing essential nutrients, enhancing soil health, acting as a natural pesticide, and promoting sustainable agricultural practices.

##### 4) Curd (2g)

Curd is used as a source of *Lactobacillus* which can help by promoting the growth of other beneficial microorganisms and it can also act as a catalyst in the fermentation process.

##### 5) Bougainvillea leaf extracts (1:2)

Bougainvillea species are known to contain a variety of bioactive compounds, including phenolics, flavonoids, alkaloids, anthocyanins, tannins, saponins, phytates, and oxalates (Ikpeme et al., 2015; Singh, 2019). In particular, \**Bougainvillea spectabilis*\* is a rich source of secondary metabolites with diverse pharmacological properties, such as antibacterial, antioxidant, anticancer, antidiabetic, anti-inflammatory, and antihyperlipidemic activities (Alsamadany, 2020; Compaore et al., 2013).

#### Details of Experiment

A replication of the above treatments we're also done. Bougainvillea leaf extract was prepared in 1:2 ratio with 20g of leaves grinded and mixed thoroughly with 40 ml of cow urine and made into an extract.

#### Fermentation process and digestion

The treatments were kept for fermentation for 15 days. Then each formulation is strained and the liquid portion is collected whereas the sludge is discarded. Then 1 ml of strained organic formulations were taken each and digested using diacid and triacid

mixture which was then kept in the sand bath for complete digestion and then analyzed for various chemical properties.

**Table 1: Treatment Details**

TREATMENT DETAILS	
T <sub>1</sub>	Palmyra fruit pulp + Bougainvillea leaf extract (5:2)
T <sub>2</sub>	Palmyra fruit pulp + Cow urine + Bougainvillea leaf extract (5:4:2)
T <sub>3</sub>	Palmyra fruit pulp + Fish waste + Bougainvillea leaf extract (5:2:2)
T <sub>4</sub>	Palmyra fruit pulp + Cow urine + Fish waste + Bougainvillea leaf extract (5:4:2:2)
T <sub>5</sub>	Palmyra fruit pulp + Curd + Bougainvillea leaf extract (5:0.2:2)
T <sub>6</sub>	Palmyra fruit pulp + Cow urine + Curd + Bougainvillea leaf extract (5:4:0.2:2)
T <sub>7</sub>	Palmyra fruit pulp + Fish waste + Curd + Bougainvillea leaf extract (5:2:0.2:2)
T <sub>8</sub>	Palmyra fruit pulp + Cow urine + Fish waste + Curd + Bougainvillea leaf extract (5:4:2:0.2:2)

### Chemical analysis of the organic formulation

After complete digestion, the organic formulation was analyzed for its chemical properties. Parameters such as pH, electrical conductivity (EC), total organic carbon, total nitrogen, total phosphorus, total potassium, calcium, magnesium, sulfur, iron, copper, manganese, zinc, and boron were determined using standard methods, as outlined in Table 2.

## IV. RESULTS & DISCUSSION

The experiment was carried out to assess the effectiveness of Palmyra fruit pulp-based bioformulations in enhancing the growth and yield of spinach. The study included a thorough evaluation of the physico-chemical properties, macronutrient, secondary nutrient, and micronutrient content of the developed formulations. Detailed GC–MS analyses were conducted on two key formulations: T<sub>1</sub>, consisting of Palmyra fruit pulp and Bougainvillea leaf extract (5:2), and T<sub>8</sub>, composed of Palmyra fruit pulp, cow urine, fish waste, curd, and Bougainvillea leaf extract (5:4:2:0.2:2). These analyses provided valuable insights into the nutrient composition and bioactive compounds present in the formulations, demonstrating their potential to improve plant growth and enhance soil health.

### Physico-chemical characteristics of palmyra based bioformulations

All the eight formulations were analyzed for physico- chemical properties and results are presented here under.

Table 2 represents the pH, EC and Organic C of various treatments. The pH values ranged from 5.37 to 5.98, indicating that the formulations create a mildly acidic to near-neutral environment, which is suitable for spinach growth. Treatments such as T<sub>4</sub> (Palmyra pulp + Cow urine + Fish waste + Bougainvillea leaf extract) and T<sub>8</sub> (Palmyra pulp + Cow urine + Fish waste + Curd + Bougainvillea leaf extract) recorded the highest organic carbon content, at 29.00% and 29.18%, respectively. These high organic carbon levels suggest improved soil fertility, promoting better plant growth. Electrical conductivity (EC), an indicator of nutrient availability, varied across treatments, with T<sub>8</sub> having the highest EC at 4.93 dS/m, implying a higher concentration of dissolved salts, which may enhance nutrient uptake by plants. High organic carbon content is crucial for enhancing soil structure, water retention, and microbial diversity. Soils with a good balance of OC show improved microbial resilience and better nutrient cycling, which supports plant growth and yield QiuxiangTian et al.,(2021).

**Table 2: Physico-chemical characteristics of palmyra based bioformulations**

Treatments	pH	EC(dS/m)	OC (%)
T1 Palmyra fruit pulp + Bougainvillea leaf extract (5:2)	5.37	4.48	28.25
T2 Palmyra fruit pulp + Cow urine + Bougainvillea leaf extract (5:4:2)	5.76	4.38	27.75
T3 Palmyra fruit pulp + Fish waste + Bougainvillea leaf extract (5:2:2)	5.55	4.40	28.17
T4 Palmyra fruit pulp + Cow urine + Fish waste + Bougainvillea leaf extract (5:4:2:2)	5.89	4.00	29.00
T5 Palmyra fruit pulp + Curd + Bougainvillea leaf extract (5:0.2:2)	5.93	4.27	28.25
T6 Palmyra fruit pulp + Cow urine + Curd + Bougainvillea leaf extract (5:4:0.2:2)	5.39	4.11	28.08
T7 Palmyra fruit pulp + Fish waste + Curd + Bougainvillea leaf extract (5:2:0.2:2)	5.60	3.73	28.58
T8 Palmyra fruit pulp + Cow urine + Fish waste + Curd + Bougainvillea leaf extract (5:4:2:0.2:2)	5.98	4.93	29.18

**Table 3: Macro and Secondary Nutrient Content of Palmyra Based Bioformulations**

Treatments	N (%)	P (%)	K (%)	Ca (mg/L)	Mg (mg/L)
T1 Palmyra fruit pulp + Bougainvillea leaf extract (5:2)	0.78	0.23	0.57	120	911
T2 Palmyra fruit pulp + Cow urine + Bougainvillea leaf extract (5:4:2)	0.84	0.27	0.86	127	922
T3 Palmyra fruit pulp + Fish waste + Bougainvillea leaf extract (5:2:2)	0.98	0.20	0.56	137	945
T4 Palmyra fruit pulp + Cow urine + Fish waste + Bougainvillea leaf extract (5:4:2:2)	1.68	0.28	0.89	140	968
T5 Palmyra fruit pulp + Curd + Bougainvillea leaf extract (5:0.2:2)	0.77	0.29	0.59	123	920
T6 Palmyra fruit pulp + Cow urine + Curd + Bougainvillea leaf extract (5:4:0.2:2)	0.84	0.27	0.85	128	942
T7 Palmyra fruit pulp + Fish waste + Curd + Bougainvillea leaf extract (5:2:0.2:2)	1.12	0.18	0.64	138	957
T8 Palmyra fruit pulp + Cow urine + Fish waste + Curd + Bougainvillea leaf extract (5:4:2:0.2:2)	1.75	0.29	0.89	143	987

Table 3 presents the nutrient composition (N, P, K, Ca, Mg) of different bioformulation treatments. Nitrogen (N) content ranges from 0.77% to 1.75%, with T<sub>8</sub> showing the highest nitrogen content (1.75%). This suggests that the combination of these ingredients improves nitrogen availability. Phosphorus (P) levels vary between 0.18% and 0.29%. T<sub>5</sub> and T<sub>8</sub> have the highest phosphorus content, indicating a likely enhancement of phosphorus through organic additives. Potassium (K) concentration fluctuates between 0.56% and 0.89%, with T<sub>4</sub> and T<sub>8</sub> exhibiting the highest potassium levels (0.89%), showcasing the efficiency of fish waste and cow urine in potassium enrichment. Calcium (Ca) content ranges from 120 mg/L in T<sub>1</sub> to 143 mg/L in T<sub>8</sub>, indicating the treatment with cow urine, fish waste, curd, and Bougainvillea leaf extract results in higher calcium levels. Magnesium (Mg) levels span from 911 mg/L to 987 mg/L, with T<sub>8</sub> (987 mg/L) having the highest magnesium content, highlighting the potential of the bioformulation to increase magnesium availability.

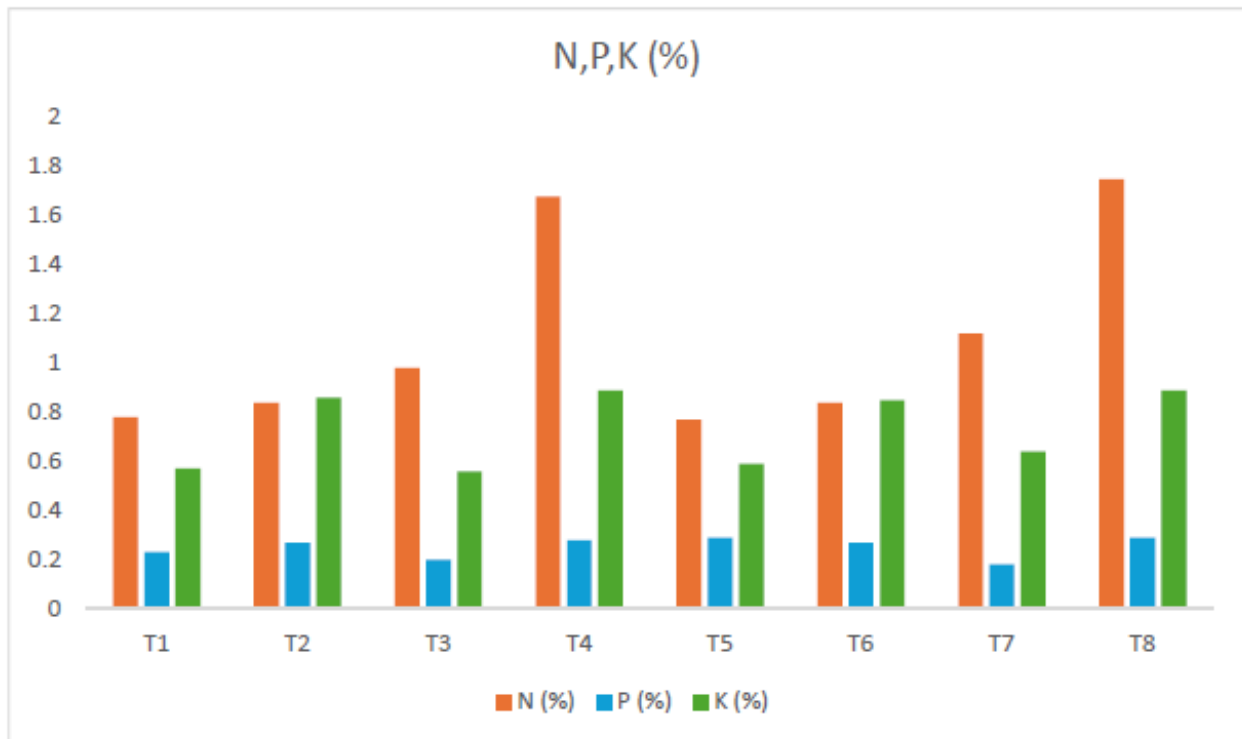


Figure 1: Macro Nutrient Content of Palmyra Based Bioformulations

Table 4: Micro-nutrient Content (mg/L) Of Palmyra Based Bioformulations

Treatments	Cu	Fe	Mn	Zn	B
T <sub>1</sub> Palmyra fruit pulp + Bougainvillea leaf extract (5:2)	0.15	82	0.86	4.02	0.89
T <sub>2</sub> Palmyra fruit pulp + Cow urine + Bougainvillea leaf extract (5:4:2)	0.22	89	0.98	4.184	0.96
T <sub>3</sub> Palmyra fruit pulp + Fish waste + Bougainvillea leaf extract (5:2:2)	0.31	112	1.05	5.10	1.01
T <sub>4</sub> Palmyra fruit pulp + Cow urine + Fish waste + Bougainvillea leaf extract (5:4:2:2)	0.018	116	1.13	5.01	1.07
T <sub>5</sub> Palmyra fruit pulp + Curd + Bougainvillea leaf extract (5:0.2:2)	0.17	85	0.94	4.03	0.91
T <sub>6</sub> Palmyra fruit pulp + Cow urine + Curd + Bougainvillea leaf extract (5:4:0.2:2)	0.27	96	1.01	4.05	1.01
T <sub>7</sub> Palmyra fruit pulp + Fish waste + Curd + Bougainvillea leaf extract (5:2:0.2:2)	0.30	110	1.11	5.11	1.04
T <sub>8</sub> Palmyra fruit pulp + Cow urine + Fish waste + Curd + Bougainvillea leaf extract (5:4:2:0.2:2)	0.32	115	1.12	5.13	1.09

Table 4 represents the Cu concentration varies from 0.018 to 0.32 mg/kg, with treatment T<sub>4</sub> having the lowest and T<sub>8</sub> the highest levels. Fe concentrations range from 82 to 116 mg/kg, with T<sub>4</sub> showing the highest value. Mn concentrations are between 0.86 and 1.13 mg/kg, with T<sub>4</sub> again exhibiting the highest level. Zn concentrations increase with more complex treatments, peaking at 5.13 mg/kg in T<sub>8</sub>, while the lowest is 4.02 mg/kg in T<sub>1</sub>. Finally, B concentrations are fairly consistent across treatments, with slight variation, the highest being 1.12 mg/kg in T<sub>8</sub> and the lowest 0.89 mg/kg in T<sub>1</sub>. Overall, T<sub>8</sub>, which includes all components, shows the highest levels for most nutrients, indicating that it might be the most effective treatment for micronutrient enrichment.

## GC-MS Analysis

Gas Chromatography–Mass Spectrometry (GC–MS) is a powerful analytical technique for identifying and quantifying volatile and semi-volatile organic compounds in complex mixtures. In this study, GC–MS was employed to examine the chemical composition of Palmyra fruit pulp-based bioformulations, with a focus on two key formulations: T1 (Palmyra fruit pulp + Bougainvillea leaf extract) and T8 (Palmyra fruit pulp + Cow urine + Fish waste + Curd + Bougainvillea leaf extract). The analysis provided a comprehensive profile of bioactive compounds, including fatty acids, phenolic compounds, amino acids, and other organic molecules. These compounds are known to contribute to plant growth promotion, stress tolerance, and soil health enhancement. By comparing the GC–MS profiles of T1 and T8, the study assessed how the incorporation of multiple organic supplements in T8 enhanced the bioactivity and overall efficacy of the formulation.

The insights gained from the GC-MS analysis were crucial for understanding the underlying biochemical mechanisms that contribute to the observed differences in plant growth and yield between the various treatments.

## GC-MS results

**Instrument and GC–MS Conditions:** The analysis was performed using a PerkinElmer Clarus SQ 8C GC–MS system equipped with a DB-5 MS capillary column, a standard non-polar column. A 1  $\mu$ L sample was injected. The column dimensions were 30 m length, 0.25 mm internal diameter (ID), and 0.25  $\mu$ m film thickness. Helium (He) was used as the carrier gas.

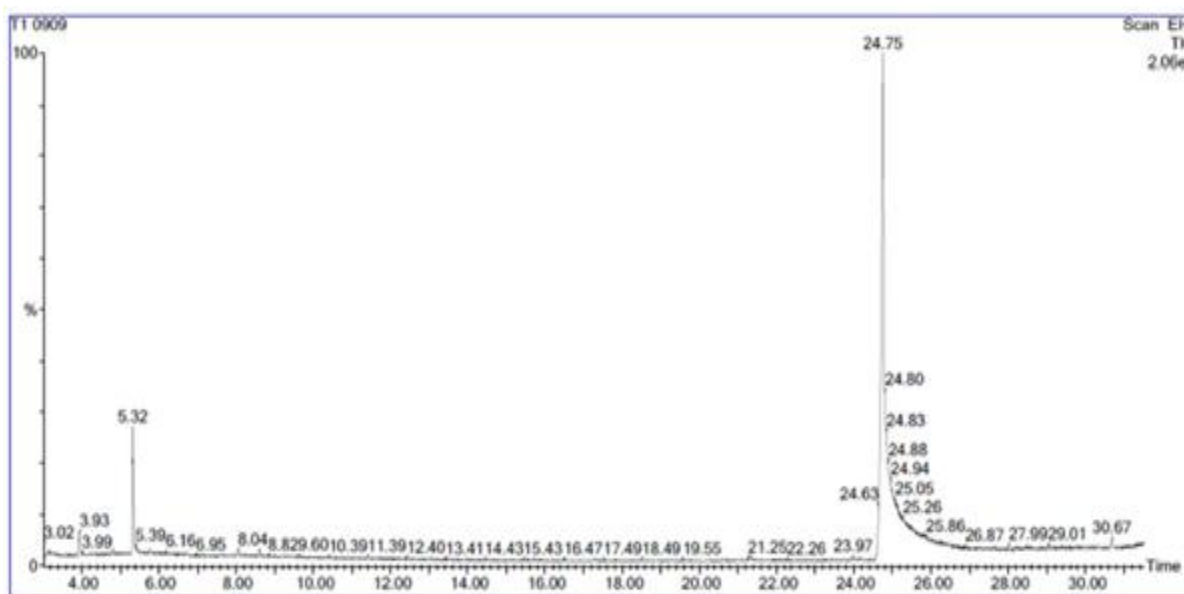


Figure 2: GC-MS result: T1 Palmyra fruit pulp + Bougainvillea leaf extract (5:2)

## Cost Economics of Bioformulation (1L)

The Table 6 represents the cost economics analysis for preparing 1 liter of the T8 bioformulation reveals that the majority of the components, including Palmyra fruit pulp, cow urine, fish waste, and Bougainvillea leaf extract, are available at no cost, resulting in a minimal production cost. The only direct expense is for curd, which amounts to 0.8 for the required 16 grams. However, the labor charge, which is Rs.350 for half a day's work, significantly contributes to the overall cost of Rs.351 per liter. It's important to note that this labor cost is calculated based on small-scale production. If the bioformulation is produced commercially on a larger scale, the labor cost per liter would be substantially reduced due to economies of scale. This reduction in labor costs would make the production of the bioformulation more cost-effective and commercially viable, allowing for broader application in agricultural practices.

**Table 5: Cost Economics of Bioformulation (1L)**

Component	Quantity Required	Unit Cost (₹)	Total Cost (₹) per litre
Palmyra Fruit Pulp	400g	Free	0
Cow Urine	720 ml + 320 ml (1.04 L)	Free	0
Fish Waste	160g	Free	0
Curd	16g	10 for 200g	0.8
Bougainvillea Leaf Extract	160g leaves in 320 ml cow urine	Free	0
Labour	1 day (Half a day work)	350 per half day	350
Total Cost	-	-	₹351

### Pot Culture Experiment

Crop: Spinach

Treatments: 8

Replications: 3

Date of sowing: 29.07.2024

In this project, a pot culture experiment was conducted to evaluate the effectiveness of organic plant growth regulators formulated from Palmyra fruit pulp, cow urine, fish waste, curd, and Bougainvillea leaf extract. Eight different treatment combinations were prepared and applied to plants grown in pots under controlled conditions. Each treatment was replicated twice to ensure reliability. Key growth parameters, including plant height, root length, leaf number, and overall biomass, were measured over a defined period to assess the impact of the treatments.

The results from the pot culture experiment provided crucial insights into the impact of the organic formulations on plant growth, nutrient uptake, and soil health.



**Plate 1: Pot Culture of Spinach**



**Plate 2: Replications of Pot Culture**



**Plate 3: Plant growth after 15 days of spraying**



**Plate 4: Foliar spray of 5% of the bioformulations**

**Table 6: Height, Width & Wet Weight of Spinach**

Treatments	Height (cm)	Width (cm)	WET WT (g/Plant)
T <sub>1</sub> Palmyra fruit pulp + Bougainvillea leaf extract (5:2)	25	6.9	24.56
T <sub>2</sub> Palmyra fruit pulp + Cow urine + Bougainvillea leaf extract (5:4:2)	26.4	7.2	25.36
T <sub>3</sub> Palmyra fruit pulp + Fish waste + Bougainvillea leaf extract (5:2:2)	27	8.1	28.36
T <sub>4</sub> Palmyra fruit pulp + Cow urine + Fish waste + Bougainvillea leaf extract (5:4:2:2)	28.2	8.5	29.35
T <sub>5</sub> Palmyra fruit pulp + Curd + Bougainvillea leaf extract (5:0.2:2)	25.3	6.9	25.00
T <sub>6</sub> Palmyra fruit pulp + Cow urine + Curd + Bougainvillea leaf extract (5:4:0.2:2)	26.5	7.6	27.36
T <sub>7</sub> Palmyra fruit pulp + Fish waste + Curd + Bougainvillea leaf extract (5:2:0.2:2)	27.5	8.2	29.00
T <sub>8</sub> Palmyra fruit pulp + Cow urine + Fish waste + Curd + Bougainvillea leaf extract (5:4:2:0.2:2)	28.3	8.6	30.31
Mean	26.78	7.75	27.41

The Table 6 presents the effects of various treatments on the height, width, and wet weight (g/plant) of plants. The height varies from 25 cm (T<sub>1</sub>) to 28.3 cm (T<sub>8</sub>), with T<sub>4</sub> and T<sub>8</sub> yielding the tallest plants (28.2 cm and 28.3 cm, respectively). The lowest plant height is observed in T<sub>5</sub> (25.3 cm). The average height across treatments is 26.78cm. Plant width ranges from 6.9 cm (T<sub>1</sub> and T<sub>5</sub>) to 8.6 cm (T<sub>8</sub>). The broader plant widths are seen in more complex treatments (T<sub>4</sub>, T<sub>7</sub>, and T<sub>8</sub>), where multiple ingredients are used. The average width is 7.75 cm. Wet weight (g/plant) increases from 24.56 g (T<sub>1</sub>) to a maximum of 30.31 g (T<sub>8</sub>). T<sub>3</sub>, T<sub>4</sub>, and T<sub>8</sub>, which involve fish waste, yield the highest wet weights, indicating more substantial plant biomass. The mean wet weight is 27.41 g/plant.

Overall, treatment T<sub>8</sub> produced the largest plants in terms of both height and width and the greatest wet weight, suggesting that this combination is the most effective for promoting plant growth.

## V. RESULTS SUMMARY

Eight distinct bioformulations were developed using Palmyra fruit pulp as the base, combined with various organic supplements, including cow urine, fish waste, curd, and Bougainvillea leaf extract. These formulations were designed to evaluate their effectiveness in enhancing the growth and yield of spinach (*Spinaciaoleracea* L.).

The macro- and micronutrient analysis of the bioformulations revealed significant differences among the treatments. Treatment T<sub>8</sub> was particularly enriched with essential macronutrients—nitrogen (1.75%), phosphorus (0.20%), and potassium (0.64%)—as well as key micronutrients, including manganese (1.12 mg/L), zinc (5.03 mg/L), copper (0.32 mg/L), boron (1.09 mg/L), and iron (115 mg/L). The balanced availability of these nutrients in T<sub>8</sub> contributed to enhanced chlorophyll production, robust plant growth, and overall improved plant health. In comparison, T<sub>1</sub>, while still beneficial, contained lower levels of these critical nutrients, highlighting

the added advantage of the supplementary organic inputs in T8.

GC–MS analysis of the formulations demonstrated a diverse array of bioactive compounds. T1 predominantly contained fatty acids, phenolic compounds, and organic acids, which are known to support plant growth and stress tolerance. In contrast, T8 displayed a more complex chemical profile, encompassing amino acids, bioactive peptides, glucobrassicin, and flavonoids, in addition to the compounds found in T1. The synergistic effects of these bioactive compounds in T8 indicate a higher potential for promoting plant growth and improving soil health compared to T1.

## VI. CONCLUSION

The pot culture experiment was conducted to evaluate the effectiveness of different bioformulations in promoting spinach growth, with key parameters such as plant height, width, wet weight, and overall biomass being measured. Among all treatments, T8 consistently showed the highest growth and yield. The combination of cow urine, fish waste, curd, and Bougainvillea leaf extract in T8 created a nutrient-rich and balanced environment that significantly enhanced the development of spinach plants.

Results from both the GC–MS analysis and the pot culture experiment clearly identified T8 as the most effective formulation among the eight tested. Its comprehensive nutrient profile and the presence of a diverse range of bioactive compounds contributed to superior plant growth and yield. This formulation demonstrates strong potential as an effective and sustainable plant growth regulator, offering a viable alternative to conventional chemical fertilizers in organic farming systems.

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