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Biochemical and physiological traits of spinach (*Spinacia oleracea* L.) by impact of Panchagavya-based botanical formulations

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Abstract
A field experiment was conducted during rabi session of 2019–20 and 2020–21 at district Sultanpur to evaluate the effect of Panchagavya and botanical leaf extracts on physiological and biochemical traits of spinach (*Spinacia oleracea* L.). The experiment was laid out in a randomized block design with eight treatments and three replications. Foliar sprays of 3% Panchagavya and its combinations were applied at 12 and 25 DAS. The results revealed that Panchagavya + botanical neem leaf extract recorded significantly higher plant height (28.03 ± 2.81 cm), leaf area (378 ± 13.15 cm^2), and fresh yield (232.9 ± 15.26 q ha^{-1}) compared to control (16.26 ± 1.05 cm, 260 ± 15.68 cm^2, and 159.6 ± 8.15 q ha^{-1}, respectively). Similarly, biochemical parameters were also enhanced, with maximum dry matter yield (32.74 ± 1.68 q ha^{-1}), ascorbic acid (45.13 ± 3.16 mg 100 g^{-1}), and chlorophyll content (7.31 ± 0.45 mg g^{-1}) recorded under T_3, whereas the lowest values were observed in control (18.51 ± 2.15 q ha^{-1}, 31.72 ± 2.58 mg 100 g^{-1}, and 5.50 ± 0.25 mg g^{-1}, respectively). Thus, foliar application of Panchagavya in combination with neem leaf extract proved most effective in improving growth, yield, and quality of spinach, offering a sustainable and eco-friendly approach for enhancing crop productivity.

Keywords: Panchagavya, botanical extracts, biostimulant, spinach, nutrient uptake, ascorbic acid, sustainable agriculture

Introduction

Vegetables play a crucial role in ensuring nutritional security by supplying essential vitamins, minerals, and dietary fibre, particularly in developing countries (Keatinge *et al*., 2011) [9]. Among leafy vegetables, spinach (*Spinacia oleracea* L.) is widely recognized for its high nutritional value, being rich in ascorbic acid (70 mg 100 g^{-1}), iron (16.2 mg 100 g^{-1}), vitamin A, folate, and natural antioxidants (Ryder, 1979; Nonnicke, 1989; Dicoteau, 2000; Morelock & Correll, 2008; Avsar, 2011; Nivedha *et al*., 2024) [2, 8, 14, 15, 16, 23]. In addition to its dietary importance, spinach possesses medicinal properties and is traditionally used for managing inflammation and liver-related disorders (Roberts & Moreau, 2016) [22].

In India, vegetable cultivation covers approximately 9.575 million hectares with an average productivity of 17.7 t ha^{-1}, contributing significantly to the agricultural economy (Singh *et al*., 2019) [27]. Maharashtra is one of the leading states under vegetable cultivation. However, intensive use of chemical fertilizers to meet the increasing demand for higher yields has resulted in soil degradation, micronutrient imbalances, and environmental pollution (Aadesh *et al*., 2024) [1]. These challenges have necessitated the exploration of sustainable and eco-friendly alternatives for improving crop productivity.

In recent years, organic biostimulants have gained considerable attention due to their ability to enhance plant growth, nutrient uptake, and soil health (De Pascale *et al*., 2017; Sible *et al*., 2021; Rajesaheb *et al*., 2025) [7, 19, 26]. Panchagavya, a traditional cow-based formulation comprising cow dung, urine, milk, curd, ghee, jaggery, and coconut water, is known to contain essential nutrients, plant growth regulators (auxins, gibberellins), vitamins, enzymes, and beneficial microorganisms (Singh *et al*, 2023; Behera *et al*., 2024; Balkrishna *et al*., 2025) [3, 6, 28]. Its application has been reported to improve plant growth, yield, and physiological efficiency across various crops by stimulating microbial activity and enhancing nutrient availability (Mani *et al*., 2025) [12].

Botanical leaf extracts such as neem (*Azadirachta indica*), karanj (*Pongamia pinnata*), gliricidia (*Gliricidia sepium*), and custard apple (*Annona squamosa*) are rich in bioactive compounds that exhibit biostimulant as well as mild pesticidal properties (Sharma *et al*., 2023; Kumari *et al*., 2025) [11, 25]. These extracts can enhance plant vigor, improve resistance against pests and diseases, and contribute to sustainable crop production systems (Sam Ruban *et al*., 2019) [24]. The integration of Panchagavya with such botanical extracts may further improve nutrient assimilation, physiological performance, and yield while reducing dependence on synthetic agrochemicals.

Despite the proven benefits of individual organic inputs, limited studies have evaluated their combined effects on spinach under semi-arid conditions. Therefore, the present investigation was undertaken to assess the effect of foliar application of Panchagavya alone and in combination with selected botanical leaf extracts on growth, yield, nutrient uptake, soil fertility, and quality parameters of spinach under the semi-arid conditions of the Marathwada region.

Materials and Methods

1. Experimental Site and Climate

The present investigation was conducted during the rabi seasons of 2019–20 and 2020–21 at Sultanpur district U.P., India in the supervision of KVK, SMS, Soil Science. The climate of this district is characterized by a hot summer and a pleasant cold season. The climate is semi-arid with very hot summer and equally cold winter season. During the summer months i.e. May-June, the maximum temperature goes beyond 44 degree Celsius and in winter months of December & January, it is around 3-4 degree Celsius. Winds are generally light to moderate with some strengthening during later part of the summer and monsoon season. Winds are mostly from the east or south-east during the period from May to September. The average annual rainfall is about 900 to 1000 mm and confined mainly during July to September. The rainfall in general increases from the south-west towards the north-east and about 80-85 per cent of the annual rainfall is received during the south-west monsoon months June to September, July being the rainiest month. Hail and Dust Storms are observed only during March to June.

Soil: Alluvial soils generally cover the major portion of the district. They have developed an alluvium deposited by the Gomti and its tributaries. The soils are neutral to moderately alkaline and calcareous and have sometimes, well developed clay accumulation in the sub-soils. The large areas of barren waste are striking characteristics of the district. Tahsil Sultanpur has the greatest area of such land. Most of this area is of a very unproductive nature and consists of wide usar plains, which are specially noticeable in the south and the east of district. Nothing grows on such land, not even grass. Most of the land affected by soil erosion in the district lies in the water sheds of the rivers Gomti, Mangar, Majhai and Sai. The mainstay of the population is still agriculture and more than 90 percent of families have excess number of persons engaging themselves in agricultural related activities. Out of the total geographical area, about 67% is devoted to Agriculture. The culturable and non-culturable waste land contributes 14.85 % and 18.21 % respectively. The main occupation of the people is agriculture, the crop pattern was all alike on the usual pattern of harvesting two crops rabi and kharif. But zaid crop are also found in this district.

Agro-Ecological Situations

As per the survey of the district, it has been observed that agro-ecological situations are directly correlated with physiographic situations, land quality and availability of irrigation facilities of the area. Broadly, the farming situations have been divided into four i.e. productive plain, sodic, ravenous and waterlogged conditions. The first three conditions, are further divided into two i.e. irrigated and rain fed. The waterlogged areas are temporarily or permanently submerged in nature. Bore well is the major source of irrigation (48.5%) followed by canal (38.65%) and public tube wells (9.17%).The soil of the experimental field was sandy loam to clay loam soil.

2. Land Preparation

The field was prepared by ploughing by harrow and cultivaters up to a depth of 35 cm using a tractor-drawn plough, followed by harrowing with a tractor-drawn disc harrow. The land was then leveled using a wooden plank to obtain a fine tilth suitable for sowing of crop.

3. Experimental Design and Crop Details

The experiment was laid out in a randomized block design (RBD) with eight treatments and three replications (Table. 1). Each experimental plot measured 1.25 m × 1.55 m. Spinach (*Spinacia oleracea* L.) variety 'All Green' was used as the test crop and was sown at a spacing of 15.55 cm between rows and 5.5 cm between plants. The recommended dose of fertilizers (RDF) at 85:45:42.5 kg, $N:P_2O_5:K_2O$ ha^{-1} was applied uniformly to all treatments except T_8. Nitrogen, phosphorus, and potassium were supplied through urea, D.A.P., and muriate of potash, respectively.

The table summarizes the experimental treatments comprising foliar application of Panchagavya alone and in combination with different botanical leaf extracts, along with micronutrient spray and organic nutrient management through farmyard manure (FYM), used to evaluate their effects on growth, yield, nutrient dynamics, and quality of spinach.

Table 1: Treatment details of Panchagavya, botanical leaf extracts, and nutrient management practices evaluated in spinach (*Spinacia oleracea L.*)

Sr. No.	Treatment	Treatment details
1	T_1	Control (water spray only)
2	T_2	Panchagavya
3	T_3	Panchagavya + botanical neem leaf extract
4	T_4	Panchagavya + botanical karanj leaf extract
5	T_5	Panchagavya + botanical gliricidia leaf extract
6	T_6	Panchagavya + botanical custard apple leaf extract
7	T_7	Micronutrient Grade II
8	T_8	100% nitrogen through farmyard manure (FYM)

1. Preparation of Panchagavya and Botanical Extracts

Panchagavya was prepared by mixing fresh cow dung (5.25 kg), ghee (0.52 kg), cow urine (3.25 L), milk (2.25 L), curd (2.25 L), jaggery (525 g), and coconut water (2.25 L), followed by fermentation under aerobic conditions according to climatic factors. Botanical leaf extracts were prepared by fermenting freshly collected leaves with cow urine in a 1:1 ratio. The fermented extracts were filtered and mixed with Panchagavya in a 1.15:1 ratio to obtain combined formulations (Pathak & Ram 2013) [18].

2. Application of Treatments and Crop Management

The prepared formulations were diluted to 2.75% concentration and applied as foliar sprays at 12 and 25 days after sowing (DAS) using a hand sprayer. Micronutrient Grade II was applied as a foliar spray at 0.45%

concentration (Table. 1). Irrigation was applied immediately after sowing and subsequently at 7-9 day intervals. One hand weeding was carried out during the crop growth period. The crop was harvested at 50 DAS.

3. Observations Recorded

1. Physiological Properties

Plant height was measured from the base of the plant to the tip of the fully expanded leaf on the main shoot and expressed in centimeters. Leaf area was recorded and expressed in cm². Fresh yield was recorded from each plot and converted into quintals per hectare (q ha^{-1}).

2. Biochemical Properties

Dry matter yield was recorded and expressed in quintals per hectare (q ha^{-1}). Ascorbic acid content was estimated using the method described by Ranganna (1986) [20] and expressed as mg per 100 g of fresh weight. Chlorophyll content was determined according to using the DMSO extraction method and measured spectrophotometrically, and expressed as mg g^{-1} (Barnes *et al*., 1992; Khalyfa *et al*, 1992; Ritchie *et al*., 2021) [5, 10, 21].

4. Statistical Analysis

The experimental data were subjected to analysis of variance (ANOVA) appropriate for a randomized block design (RBD) as described by Panse and Sukhatme (1985) [17]. Treatment means were compared using the critical difference (CD) test at the 5% level of significance (p = 0.05) to determine the significance of differences among treatments.

Results

1. Physiological Properties

The results revealed that different treatments significantly influenced the physiological parameters of spinach, with values expressed as mean ± standard error.

2. Plant Height

Plant height ranged from 16.26 ± 1.05 cm in control (T1) to 28.03 ± 2.81 cm in T3. All treatments recorded higher plant height compared to control, indicating a significant improvement. Among the treatments, T3 recorded the maximum plant height; however, it remained statistically at par Non-Significant (NS) with T5 (26.03 ± 1.95 cm) and T4 (25.34 ± 1.54 cm). Further, T4, T5, and T6 (24.06 ± 1.15 cm) did not differ significantly among themselves, forming a statistically similar group. T7 (22.52 ± 1.55 cm) and T8 (20.19 ± 0.26 cm) recorded comparatively lower values, with T8 being significantly lower than the higher-performing treatments but still superior to control (Table. 2; Figure. 1).

3. Leaf Area

Leaf area varied from 260 ± 15.68 cm² in control (T1) to 378 ± 13.15 cm² in T3. All treatments showed a significant increase over control. T3 recorded the highest leaf area; however, it was statistically at par (NS) with T5 (363 ± 16.42 cm²), T6 (331 ± 13.28 cm²), and T4 (330 ± 12.51 cm²). The differences among T4, T5, and T6 were non-significant, indicating comparable performance of these treatments (Table. 2; Figure. 1).

Table 2: Effect of Panchagavya and Botanical Leaf Extracts on Physiological Properties of Spinach (*Spinacia oleracea* L.)

Treatments	Plant height (cm)	Leaf area (cm²)	Fresh yield (q ha^{-1})
T1: Control (water spray)	16.26 ± 1.05^{c}	260 ± 15.68^{c}	159.6 ± 8.15^{c}
T2: Panchagavya	17.83 ± 1.36^{c}	282 ± 21.15^{c}	163.4 ± 7.76^{c}
T3: Panchagavya + botanical neem leaf extract	28.03 ± 2.81^{a}	378 ± 13.15^{a}	232.9 ± 15.26^{a}
T4: Panchagavya + botanical karanj leaf extract	25.34 ± 1.54^{ab}	330 ± 12.51^{b}	216.7 ± 13.40^{ab}
T5: Panchagavya + botanical gliricidia leaf extract	26.03 ± 1.95^{a}	363 ± 16.42^{ab}	222.6 ± 13.28^{a}
T6: Panchagavya + botanical custard apple leaf extract	24.06 ± 1.15^{ab}	331 ± 13.28^{b}	210.2 ± 10.54^{ab}
T7: Micronutrient Grade II	22.52 ± 1.55^{b}	319 ± 9.64^{b}	203.3 ± 9.07^{b}
T8: 100% N through FYM	20.19 ± 0.26^{bc}	307 ± 8.67^{bc}	170.01 ± 5.89^{c}

Fresh Yield

Fresh yield ranged from 159.6 ± 8.15 q ha^{-1} in control (T1) to 232.9 ± 15.26 q ha^{-1} in T3. All treatments exhibited significantly higher yields compared to control. Although T3 recorded the highest yield, it was statistically at par (NS) with T5 (222.6 ± 13.28 q ha^{-1}), T4 (216.7 ± 13.40 q ha^{-1}), and T6 (210.2 ± 10.54 q ha^{-1}).

These treatments formed a statistically similar group, indicating that their effects on yield enhancement were comparable (Table. 2; Figure. 1).

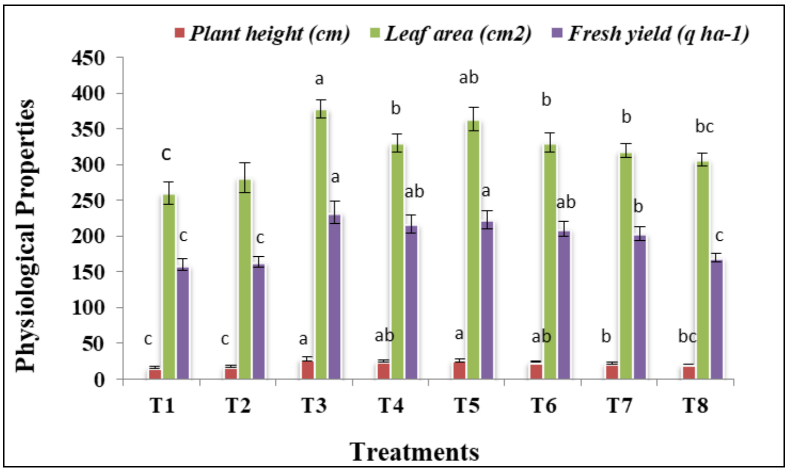

Fig 1. Effect of Different Treatments on Physiological Properties of the Crop. The figure illustrates the impact of various treatments on key physiological parameters, namely plant height, leaf area, and fresh yield. Among all treatments, T3 (Panchagavya + Botanical neem leaf extract) exhibited the highest values for all three parameters, indicating its superior effectiveness. Treatments T5, T4, and T6 also showed notable improvements compared to the control (T1), while the control consistently recorded the lowest values. The error bars represent standard error, indicating the variability within treatments. Overall, the graphical representation clearly demonstrates that the combined application of Panchagavya with botanical extracts significantly enhanced plant growth and yield attributes.

1. Biochemical Properties

The biochemical parameters were also significantly influenced by different treatments.

1. Dry Matter Yield

Dry matter yield ranged from 18.51 ± 2.15 q ha⁻¹ in control (T1) to 32.74 ± 1.68 q ha⁻¹ in T3. All treatments showed a significant increase over control. However, T3 was statistically at par (NS) with T5 (31.11 ± 1.08 q ha⁻¹) and T4 (29.94 ± 1.18 q ha⁻¹), indicating non-significant differences among these treatments (Table. 3; Figure. 2).

Table 3: Effect of Panchagavya and Botanical Leaf Extracts on Biochemical Properties of Spinach (*Spinacia oleracea* L.)

Treatments	Dry matter yield (q ha⁻¹)	Ascorbic acid (mg 100 g⁻¹)	Chlorophyll (mg g⁻¹)
T1: Control (water spray)	18.51 ± 2.15^{c}	31.72 ± 2.58^{c}	5.50 ± 0.25^{c}
T2: Panchagavya	19.64 ± 1.34^{c}	34.73 ± 3.05^{bc}	5.85 ± 0.31^{bc}
T3: Panchagavya + botanical neem leaf extract	32.74 ± 1.68^{a}	45.13 ± 3.16^{a}	7.31 ± 0.45^{a}
T4: Panchagavya + botanical karanj leaf extract	29.94 ± 1.18^{ab}	39.07 ± 3.23^{ab}	6.70 ± 0.61^{ab}
T5: Panchagavya + botanical gliricidia leaf extract	31.11 ± 1.08^{a}	41.73 ± 2.06^{a}	7.07 ± 0.31^{a}
T6: Panchagavya + botanical custard apple leaf extract	29.00 ± 1.64^{ab}	39.37 ± 1.26^{ab}	6.61 ± 0.86^{ab}
T7: Micronutrient Grade II	26.59 ± 1.42^{b}	40.20 ± 1.35^{ab}	6.33 ± 0.88^{b}
T8: 100% N through FYM	21.77 ± 1.94^{c}	35.83 ± 1.55^{bc}	6.13 ± 0.46^{bc}

1. Ascorbic Acid Content

Ascorbic acid content ranged from 31.72 ± 2.58 mg 100 g⁻¹ in control (T1) to 45.13 ± 3.16 mg 100 g⁻¹ in T3. All treatments recorded higher values compared to control. However, T3 was statistically at par (NS) with T5 (41.73 ± 2.06 mg 100 g⁻¹), T7 (40.20 ± 1.35 mg 100 g⁻¹), T6 (39.37 ± 1.26 mg 100 g⁻¹), and T4 (39.07 ± 3.23 mg 100 g⁻¹). The differences among these treatments were non-significant, indicating comparable effects on ascorbic acid content (Table. 3; Figure. 2).

2. Chlorophyll Content

Chlorophyll content ranged from 5.50 ± 0.25 mg g⁻¹ in control (T1) to 7.31 ± 0.45 mg g⁻¹ in T3. All treatments were significantly superior to control. Among the treatments, T3 recorded the highest chlorophyll content; however, it remained statistically at par (NS) with T5 (7.07 ± 0.31 mg g⁻¹) and T4 (6.70 ± 0.61 mg g⁻¹). These treatments formed a statistically similar group with non-significant differences among them (Table. 3; Figure. 2).

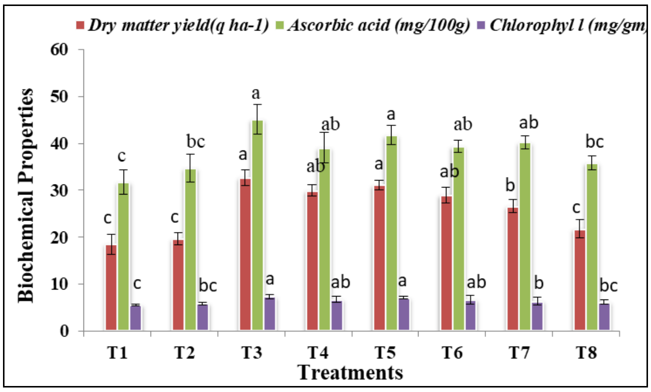

Figure 2. Effect of Different Treatments on Biochemical Properties of the Crop. The figure depicts the influence of various treatments on biochemical parameters, including dry matter yield, ascorbic acid content, and chlorophyll content. Among all treatments, T3 (Panchagavya + botanical neem leaf extract) recorded the highest values for all biochemical traits, indicating its superior performance. Treatments such as T5, T4, and T6 also showed considerable improvement over the control (T1), whereas the control exhibited the lowest values across all parameters. The error bars represent standard error, reflecting the variability within treatments. Overall, the graph highlights that the combined application of Panchagavya with botanical extracts significantly enhanced the biochemical quality of the crop.

Discussion

The present study demonstrated that Panchagavya application significantly enhanced growth, yield, and biochemical attributes of spinach (*Spinacia oleracea* L.). The improvement in plant height, leaf number, biomass, and yield may be attributed to the presence of essential nutrients, plant growth regulators, and beneficial microorganisms in Panchagavya, which collectively enhance nutrient availability, root development, and overall metabolic activity. The observed increase in growth parameters is in close agreement with earlier studies on biologically active inputs. Mani *et al.* (2025) [11] reported enhanced root length, shoot length, and biomass in spinach due to microbial inoculation, indicating improved nutrient mobilization and phytohormone production. Similarly, Aadesh *et al.* (2024) [1] observed significant increases in plant height, leaf area, and nutrient use efficiency with biofertilizer application, supporting the role of organic inputs in improving crop performance.

The enhancement in biochemical parameters, particularly chlorophyll content and plant vigor, suggests improved photosynthetic efficiency under Panchagavya treatment. These findings are supported by Nivedha *et al.* (2024) [15], who reported increased pigment content (0.58–1.29-fold) and enhanced enzymatic activities such as nitrate reductase and glutamine synthetase in spinach. This indicates that biologically enriched formulations improve physiological processes and nutrient assimilation, thereby contributing to higher yield and better crop quality. The positive effects observed in the present study are also consistent with findings on organic fertilizers in spinach. Balkrishna *et al*. (2025) [3] reported improvements in plant height, chlorophyll content, biomass, and yield, along with enhanced growth indices such as relative growth rate and net assimilation rate. Likewise, Balkrishna *et al*. (2026) [4] demonstrated that sludge-based organic amendments significantly increased growth, yield, and iron content in spinach, highlighting the role of organic inputs in improving micronutrient availability and soil health. In addition to spinach, similar beneficial effects of organic biostimulants have been reported in other crops. Sam Ruban *et al*. (2019) [24] observed that foliar application of humic acid significantly improved growth and yield attributes in brinjal. This suggests that the growth-promoting effects of Panchagavya are not limited to a single crop but are mediated through general mechanisms such as enhanced nutrient uptake, hormonal activity, and microbial interactions.

Beyond growth and yield enhancement, Panchagavya is also known to contribute to improved plant health. Previous studies have indicated that organic formulations containing beneficial microbes and bioactive compounds can help in reducing pest and disease incidence, thereby minimizing reliance on chemical pesticides. This multifunctional role makes Panchagavya an important component of sustainable and eco-friendly agricultural systems. Overall, the findings of the present study, supported by earlier research, confirm that Panchagavya enhances plant growth, yield, and biochemical attributes through improved nutrient dynamics, microbial activity, and physiological efficiency. The consistency of results across different studies and crops further highlights its potential as a sustainable alternative to chemical fertilizers.

Conclusion

The findings of the present investigation clearly demonstrate that the application of Panchagavya in combination with neem leaf extract proved to be the most effective treatment in enhancing both physiological and biochemical characteristics of the crop. This treatment not only resulted in significant improvements in plant growth parameters such as plant height, leaf area, and yield, but also enhanced important quality attributes including dry matter production, ascorbic acid content, and chlorophyll concentration. The consistent superiority of this treatment over control and other treatments indicates a strong synergistic effect of Panchagavya and neem leaf extract in promoting plant growth and metabolic activities. The results further suggest that the use of such organic and botanical inputs can play a crucial role in improving crop productivity and quality in a sustainable manner, reducing reliance on chemical inputs while maintaining soil health and environmental safety.

Competing Interests Disclaimer: Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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