



Effect of Bioinoculants and Biostimulants on Fodder Yield, Proximate Composition and Fibre Fractions of Berseem (*Trifolium alexandrinum* L.)

Vrushabh Vijay Fiskey¹, Devendra Kumar Dadhich¹, Tejaswini Chandrakar¹, Kamsali Sukeswara Achari¹, Vijendra Kumar Meena¹, Sanjeev Kumar¹

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ABSTRACT

Background: Bioinoculants and biostimulants are gaining importance as sustainable approaches for improving fodder productivity and nutritive quality in forage crops. Their integration with organic nutrient sources may enhance nutrient availability, plant growth and forage quality. Therefore, the present investigation was undertaken to evaluate the effect of bioinoculants and biostimulants on fodder yield, proximate composition and fibre fractions of berseem (*Trifolium alexandrinum* L.).

Methods: A field experiment was conducted during the rabi seasons of 2023-24 and 2024-25 at the Agronomy Research Farm of ICAR-National Dairy Research Institute in a randomized block design with six treatments and six replications.

Result: Among the treatments, PGPR + 75% recommended dose of nitrogen (RDN) through FYM + three foliar sprays of *Panchagavya* recorded the highest green fodder yields of 57.0 and 58.5 t ha⁻¹ and dry fodder yields of 7.6 and 8.1 t ha⁻¹ during 2023-24 and 2024-25, respectively, over the absolute control. The treatment also significantly improved dry matter, crude protein, ether extract and total ash contents while reducing acid detergent fibre, neutral detergent fibre and acid detergent lignin contents. The findings indicated that integrated application of PGPR, FYM and *Panchagavya* is an effective and sustainable nutrient management strategy for improving fodder productivity and nutritive quality of berseem under organic production conditions.

Key words: Beejamrit, Berseem, *Panchagavya*, PGPR, Quality, Seaweed.

INTRODUCTION

Berseem popularly known as the “King of fodder crops,” is one of the most important rabi forage legumes cultivated in India, particularly in the northern and north-western regions. The crop is widely preferred because of its high biomass productivity, excellent palatability, rapid regeneration capacity and superior nutritive value containing nearly 18-22% crude protein. Berseem provides continuous green fodder from November to April through 4-5 successive cuttings, producing approximately 70-75 t ha⁻¹ green fodder under favourable management conditions (Kumar *et al.*, 2024). In addition to its forage value, berseem enriches soil fertility through biological nitrogen fixation, contributing approximately 115-400 kg N ha⁻¹ and benefiting succeeding crops. Its wider adaptability, high digestibility and better economic returns make it an important component of sustainable livestock-based farming systems (Sheoran and Kumar, 2020).

The livestock sector depends heavily on the continuous availability of quality fodder. India supports nearly 11.6% of the world's livestock population, yet faces substantial fodder deficits estimated at 35-36% for green fodder and 10-12% for dry fodder (Islam *et al.*, 2016; Singh *et al.*, 2023). Poor availability of nutritious forage adversely affects animal health, milk quality and livestock productivity, thereby reducing economic returns from dairy farming (Ingvarsten and Moyes, 2013). Therefore, improving fodder productivity and quality through sustainable nutrient

¹ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.

Corresponding Author: Sanjeev Kumar, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.
Email: bhanusanjeev@gmail.com

ORCID: 0009-0007-3020-9744, 0009-0005-3083-0747, 0009-0007-6587-7443, 0000-0003-3357-8098

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management approaches is essential for ensuring feed security and livestock sustainability.

The nutritive value of berseem is determined by its proximate composition and fibre fractions, which directly influence forage digestibility and animal performance. Parameters such as crude protein, ether extract, total ash, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) are important indicators of forage quality (Sharpe and Kenny, 2019; Nurdianti *et al.* 2024). Higher NDF and ADF contents are generally associated with lower digestibility because of increased lignification of plant tissues (Van Soest *et al.*, 1991).

Previous studies on nutrient management in berseem have primarily focused on organic manures, biofertilizers applied individually and conventional integrated nutrient management practices for improving fodder yield and quality (Sheoran and Kumar, 2020; Kumar *et al.*, 2024). Recent studies have demonstrated the beneficial effects of plant growth-promoting rhizobacteria (PGPR), *Panchagavya*, *Beejamrit* and seaweed extracts individually on crop growth, nutrient uptake, microbial activity and stress tolerance in different field crops. However, studies evaluating their integrated application, particularly under FYM-based nutrient management systems in forage legumes such as berseem, remain scarce. Furthermore, information on their combined effects on forage quality attributes, especially proximate composition and fibre fractions, is limited. The selected bioinputs were chosen because they represent complementary modes of action: PGPR enhances nutrient acquisition and root growth (Hasan *et al.*, 2024), *Panchagavya* and *Beejamrit* supply diverse beneficial microorganisms and bioactive metabolites (Shafiq *et al.* 2025), while seaweed extract provides natural phytohormones and biostimulatory compounds that improve plant growth and nutrient utilization (Ali *et al.*, 2021). Their integration was therefore hypothesized to produce synergistic improvements in fodder productivity and nutritive quality beyond the effects of individual inputs. Accordingly, the present study was undertaken to evaluate integrated bioinoculant- and biostimulant-based nutrient management strategies for improving fodder yield, proximate composition and fibre fractions of berseem under sustainable production conditions.

MATERIALS AND METHODS

The field experiment was conducted during the *rabi* seasons of 2023-24 and 2024-25 at the Agronomy Section of ICAR-National Dairy Research Institute, Karnal, Haryana, India, located at 29°71' N latitude, 76°97' E longitude and 245 m above mean sea level. At initial experimental soil was clay loam in texture containing 44.08% sand, 21.28% silt and 34.64% clay with pH 7.74, electrical conductivity of 0.238 dS m⁻¹, organic carbon content of 0.65%, available nitrogen of 198.47 kg ha⁻¹, available phosphorus of 27.54 kg ha⁻¹ and available potassium of 184.51 kg ha⁻¹. The experimental period was characterized by semi-arid climatic conditions. During the berseem growing season (October to March), the mean maximum and minimum temperatures were 22.73°C and 10.63°C during 2023-24 and 24.81°C and 11.53°C during 2024-25, respectively, with cumulative rainfall of 61.7 mm and 89.4 mm, indicating favorable conditions for berseem growth.

The experiment was laid out in a randomized block design (RBD) comprising six treatments replicated six times. The treatments included: T₁: absolute control, T₂: 100% RDN through FYM, T₃: PGPR + 75% RDN through FYM +

three foliar sprays of *Panchagavya*, T₄: PGPR + 75% RDN through FYM + three foliar sprays of seaweed extract, T₅: *Beejamrit* + 75% RDN through FYM + three foliar sprays of *Panchagavya* and T₆: *Beejamrit* + 75% RDN through FYM + three foliar sprays of seaweed extract. The integrated treatments received 75% recommended dose of nitrogen through FYM because previous studies have demonstrated that bioinoculants and biostimulants enhance nutrient mineralization, nutrient availability, nutrient use efficiency and rhizosphere biological activity, thereby allowing partial substitution of external nitrogen inputs.

Berseem cv. BL-42 was sown by broadcasting at a seed rate of 25 kg ha⁻¹ under well-prepared field conditions. Farmyard manure obtained from the Livestock Research Centre of ICAR-NDRI, Karnal, was incorporated uniformly into the soil 15 days before sowing according to treatment requirements to satisfy the recommended nitrogen dose. Irrigations were applied uniformly to all treatments as per crop requirement. Weed management was carried out uniformly across all treatments by manual hand weeding whenever required. No major incidence of insect pests or diseases was observed during the experimental period.

The PGPR inoculum used for seed treatment in T₃ and T₄ was procured from the Division of Microbiology, ICAR-Indian Agricultural Research Institute. The liquid biofertilizer consortium consisted of *Azotobacter chroococcum*, *Pseudomonas striata* and *Bacillus decolorationis*, each maintained at a viable population of 10⁸ CFU mL⁻¹. Seeds were inoculated immediately before sowing to ensure uniform microbial coating and effective root colonization. *Beejamrit* was prepared following standard organic farming procedures using fresh cow dung, cow urine, lime solution and rhizospheric soil collected from the root zone of a healthy tree. The mixture was homogenized thoroughly and used immediately for seed treatment in T₅ and T₆.

Panchagavya was prepared using fresh cow dung (10 kg), cow urine (15 L), cow milk (4 L), curd (4 kg) and ghee (1 kg) along with supplementary ingredients such as ripe bananas, jaggery, turmeric powder and coconut water to enhance microbial fermentation and nutrient enrichment. The mixture was fermented under ambient conditions with regular stirring to maintain uniform decomposition. *Panchagavya* was applied as a foliar spray at 30 mL L⁻¹ at 25, 35 and 45 days after sowing (DAS). The seaweed extract used in the study was procured from Dhanuka Agritech and contained *Ascophyllum nodosum* extract enriched with amino acids, vitamins, trace elements, auxins and cytokinins. It was applied as a foliar spray at 3 mL L⁻¹ at 25, 35 and 45 DAS according to the treatment schedule. Foliar sprays were applied uniformly using a knapsack sprayer with an approximate spray volume of 500 L ha⁻¹ to ensure complete foliage coverage across all treatments.

The FYM used in the experiment was analysed before application to ensure uniform nutrient supply. During 2023-24 and 2024-25, FYM recorded pH values of 7.60 and 7.55,

electrical conductivity of 0.85 and 0.90 dS m⁻¹, organic carbon content of 0.95 and 1.00%, total nitrogen content of 0.50 and 0.52%, total phosphorus content of 0.25 and 0.26% and total potassium content of 0.50 and 0.55%, respectively. The quantity of FYM applied under each treatment was calculated based on analysed nitrogen content to fulfil the RDN requirement of berseem. The crop was harvested manually from the net plot area at three successive cuttings during each season. Green fodder yield was recorded on a plot basis and expressed as kg plot⁻¹ and converted to t ha⁻¹. Further, Dry fodder yield was calculated by multiplying dry matter percentage with corresponding green fodder yield. Total green fodder yield and dry fodder yield were obtained by summing the yields of all three cuttings and expressed as t ha⁻¹. Representative fresh fodder samples were chopped into small pieces and oven-dried at 65±5°C until constant weight to determine dry matter content using the following formula:

$$\text{DM (\%)} = \frac{\text{Weight of oven-dried sample}}{\text{Weight of fresh sample}} \times 100$$

The proximate composition of berseem fodder was analysed separately at each cutting and mean values were subjected to statistical analysis. Crude protein content was estimated by the micro-Kjeldahl method using a Kel-Plus N analyser and nitrogen concentration was multiplied by 6.25 (AOAC, 2005). Ether extract was determined through Soxhlet extraction using petroleum ether, while total ash content was estimated by igniting oven-dried samples in a muffle furnace at 550°C (AOAC, 2005). Organic matter was calculated by subtracting ash content from 100, whereas total carbohydrates were estimated as:

$$100 - (\text{CP} + \text{EE} + \text{TA})$$

Fibre fractions including neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to the detergent fibre system of Van Soest *et al.* (1991). Hemicellulose and cellulose

contents were calculated as NDF-ADF and ADF-ADL, respectively. Experimental data were analysed using analysis of variance (ANOVA) appropriate for RBD as described by Gomez and Gomez (1984). Treatment means were compared using the least significant difference (LSD) test at 5% probability level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Green and dry fodder yield

The integrated application of bioinoculants, biostimulants and FYM significantly enhanced green and dry fodder yields of berseem during both years of experimentation (Table 1). Among the nutrient management strategies, T₃ comprising PGPR + 75% RDN through FYM along with three foliar sprays of *Panchagavya* recorded the highest productivity, producing green fodder yields of 57.0 and 58.5 t ha⁻¹ and dry fodder yields of 7.6 and 8.1 t ha⁻¹ during 2023-24 and 2024-25, respectively. However, the treatment remained statistically comparable with T₄ (PGPR + 75% RDN through FYM + three sprays of seaweed extract), which also produced substantially higher fodder yields than the remaining treatments. Treatments involving *Beejamrit* in combination with FYM and biostimulant sprays (T₅ and T₆) resulted in intermediate productivity but significantly outperformed the sole FYM treatment (T₂). In contrast, the absolute control (T₁) recorded the lowest green and dry fodder yields during both years. Overall, integrated nutrient management treatments improved green fodder yield by 38.4% during 2023-24 and 45.8% during 2024-25 over the T₁. The enhanced productivity under integrated treatments may be attributed to improved soil fertility, enhanced microbial activity and better nutrient availability resulting from FYM application, along with increased root growth and nutrient uptake mediated by PGPR (Dhaliwal *et al.*, 2023). Furthermore, *Panchagavya* and seaweed extract possibly stimulated physiological and metabolic activities through naturally occurring growth-promoting substances

Table 1: Effect of bioinoculants and biostimulants on total green and dry fodder yield of berseem.

Treatment	Total green fodder yield (t ha ⁻¹)		Total dry fodder yield (t ha ⁻¹)	
	2023-24	2024-25	2023-24	2024-25
T ₁ : Absolute control	41.2±0.6 ^e	40.1±0.9 ^e	4.9±0.1 ^d	4.6±0.1 ^e
T ₂ : 100% RDN through FYM	46.8±1.0 ^d	48.3±1.3 ^d	5.9±0.1 ^c	6.1±0.2 ^d
T ₃ : PGPR+ 75% RDN through FYM+3 sprays of <i>Panchagavya</i>	57.0±1.0 ^a	58.5±1.6 ^a	7.6±0.2 ^a	8.1±0.3 ^a
T ₄ : PGPR+ 75% RDN through FYM+3 sprays of Seaweed extract	54.7±1.4 ^{ab}	56.2±0.9 ^{ab}	7.2±0.3 ^{ab}	7.6±0.2 ^{ab}
T ₅ : <i>Beejamrit</i> + 75% RDN through FYM+3 sprays of <i>Panchagavya</i>	52.0±1.0 ^{bc}	53.0±1.1 ^{bc}	6.8±0.2 ^b	7.1±0.2 ^{bc}
T ₆ : <i>Beejamrit</i> + 75% RDN through FYM+3 sprays of Seaweed extract	50.8±1.3 ^c	51.7±1.22 ^d	6.6±0.2 ^b	6.8±0.2 ^c
SEm±	1.12	1.25	0.20	0.21
LSD (P≤0.05)	3.27	3.63	0.57	0.62

*Same letters within each column indicate non-significant difference among the treatments using LSD test ($p < 0.05$).

and beneficial microorganisms (Kaith and Dadhich, 2024; Onte *et al.*, 2025). The numerically higher productivity recorded under T_3 suggests a synergistic interaction among FYM, PGPR and Panchagavya, leading to improved nutrient use efficiency and greater biomass accumulation. This response agrees with Kumar *et al.* (2021a), who also observed that integrating organic nutrient sources with bio-inputs improved forage productivity over the use of organic manure alone. Likewise, Garg *et al.* (2024) observed significant improvements in crop productivity under enhanced organic nutrient management strategies, which is consistent with the positive response obtained under the integrated bioinoculant- and biostimulant-based nutrient management adopted in the present study. However, although T_3 consistently recorded numerically higher green and dry fodder yields than T_4 , the differences were statistically non-significant. This statistical parity indicates that both *Panchagavya* and seaweed extract effectively complemented PGPR and FYM-based nutrient management under the present conditions. Nevertheless, the slight numerical advantage of T_3 may be attributed to the presence of diverse beneficial microorganisms, enzymes, amino acids, vitamins and naturally occurring plant growth regulators in Panchagavya, which may have enhanced nutrient availability, microbial activity and physiological processes more effectively than the seaweed extract used in this study (Behera *et al.*, 2024; Nekar, 2024).

Proximate composition

Fodder quality, as reflected by proximate composition parameters, was significantly influenced by different nutrient management treatments (Fig 1). Among all treatments, T_3 (PGPR + 75% RDN through FYM + three foliar sprays of *Panchagavya*) recorded the highest dry matter (13.2% and 13.7%), CP (19.7% and 19.8%), EE (3.62% and 3.7%) and TA contents (17.8% and 18.0%) during 2023-24 and 2024-25, respectively. Treatments T_4 , T_5 and T_6 remained statistically at par with T_3 for certain

quality attributes, particularly dry matter and crude protein content, whereas T_2 exhibited comparatively lower values among nutrient management treatments, although it remained significantly superior to the absolute control. The statistical similarity between T_3 and T_4 for dry matter and crude protein indicates that both Panchagavya and seaweed extract were equally effective in improving these major quality attributes when integrated with PGPR and FYM, although T_3 consistently recorded slightly higher numerical values. Compared with T_1 , treatment T_3 enhanced dry matter, crude protein, ether extract and total ash contents by 12.3–19.8%, 13.3–14.4%, 52.1–65.3% and 21.8–25.5%, respectively, across both years. The improved fodder quality under T_3 may be attributed to the synergistic influence of FYM, PGPR and Panchagavya in enhancing nutrient mineralization, nutrient uptake and physiological activity, thereby promoting protein synthesis and lipid metabolism (Choudhary *et al.*, 2024). These findings are consistent with Ngone *et al.* (2023), who also reported that integrating biofertilizers with organic nutrient sources improved forage quality by enhancing nutrient uptake and protein accumulation in maize. In contrast, the relatively lower values under T_2 may be associated with slower nutrient release from FYM applied alone, as also observed by Nirere *et al.* (2021).

Fibre fractions

The fibre fractions of berseem were significantly affected by different nutrient management strategies, with integrated nutrient treatments markedly reducing fibre concentration compared with the absolute control (Table 2). Among the treatments, T_3 (PGPR + 75% RDN through FYM + three foliar sprays of *Panchagavya*) recorded the lowest NDF, ADF and ADL contents, registering 43.1 and 40.8% NDF, 25.5 and 24.8% ADF and 4.0 and 3.9% ADL during 2023 and 2024, respectively. Treatment T_4 (PGPR + 75% RDN through FYM + three foliar sprays of seaweed extract) remained statistically comparable with T_3 for ADF and ADL

Table 2: Effect of bioinoculants and biostimulants on fibre fraction of berseem.

Treatment	Neutral detergent fibre (%)		Acid detergent fibre (%)		Acid detergent lignin (%)	
	2023-24	2024-25	2023-24	2024-25	2023-24	2024-25
T_1 : Absolute control	48.9±0.2 ^a	48.8±0.3 ^a	29.0±0.1 ^a	29.5±0.3 ^a	5.50±0.04 ^a	5.54±0.06 ^a
T_2 : 100% RDN through FYM	46.8±0.4 ^b	44.7±0.3 ^b	27.3±0.3 ^b	27.0±0.2 ^b	5.02±0.05 ^b	4.95±0.05 ^b
T_3 : PGPR+ 75% RDN through FYM +3 sprays of <i>Panchagavya</i>	43.1±0.2 ^e	40.8±0.3 ^e	25.5±0.2 ^e	24.8±0.2 ^e	4.01±0.03 ^d	3.92±0.04 ^d
T_4 : PGPR+ 75% RDN through FYM +3 sprays of Seaweed extract	44.0±0.3 ^d	41.7±0.2 ^d	26.0±0.2 ^{de}	25.3±0.3 ^{de}	4.10±0.05 ^d	4.00±0.05 ^d
T_5 : <i>Beejamrit</i> + 75% RDN through FYM +3 sprays of <i>Panchagavya</i>	44.9±0.3 ^c	42.9±0.3 ^c	26.4±0.3 ^{cd}	25.9±0.2 ^{cd}	4.21±0.03 ^c	4.16±0.04 ^c
T_6 : <i>Beejamrit</i> + 75% RDN through FYM +3 sprays of Seaweed extract	45.3±0.2 ^c	43.4±0.2 ^c	26.7±0.2 ^c	26.3±0.3 ^{bc}	4.28±0.01 ^c	4.23±0.05 ^c
SEm±	0.24	0.26	0.21	0.24	0.04	0.05
LSD ($P \leq 0.05$)	0.70	0.77	0.60	0.71	0.10	0.15

*Same letters within each column indicate non-significant difference among the treatments using LSD test ($p < 0.05$).

contents during both years. This statistical parity suggests that both biostimulants were similarly effective in reducing structural carbohydrate accumulation, although Panchagavya consistently produced marginally lower fibre values. In contrast, T₂ (100% RDN through FYM) exhibited relatively higher fibre fractions among nutrient-managed

treatments, although values remained significantly lower than the control. The reduction in fibre components under integrated treatments may be attributed to enhanced nutrient availability and improved nitrogen uptake resulting from the synergistic action of bioinoculants, biostimulants and FYM. Improved nitrogen nutrition regulates carbohydrate

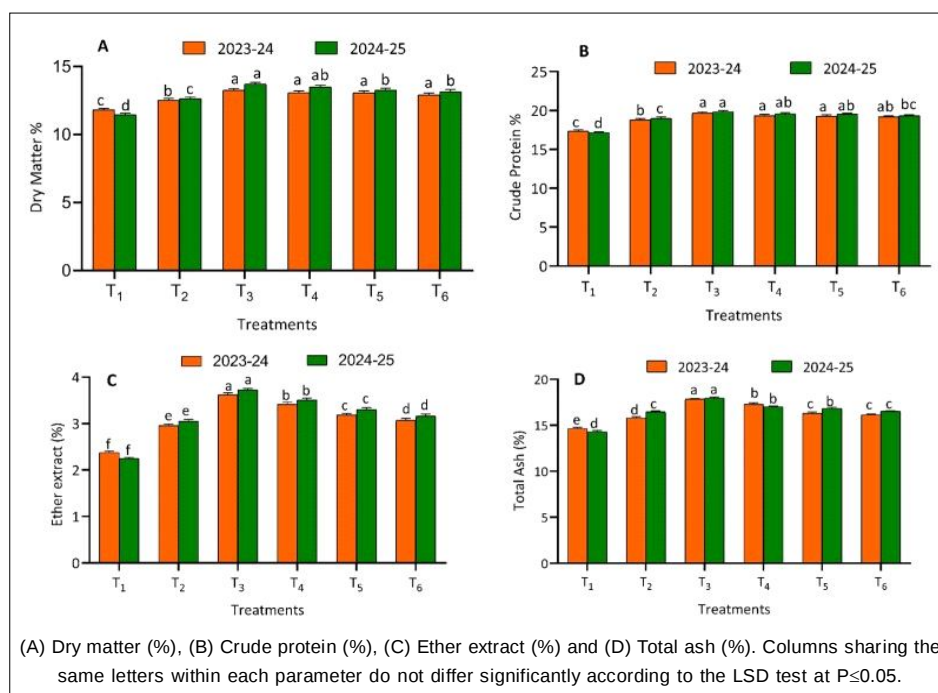


Fig 1: Effects of bioinoculants and biostimulants on proximate composition of berseem.

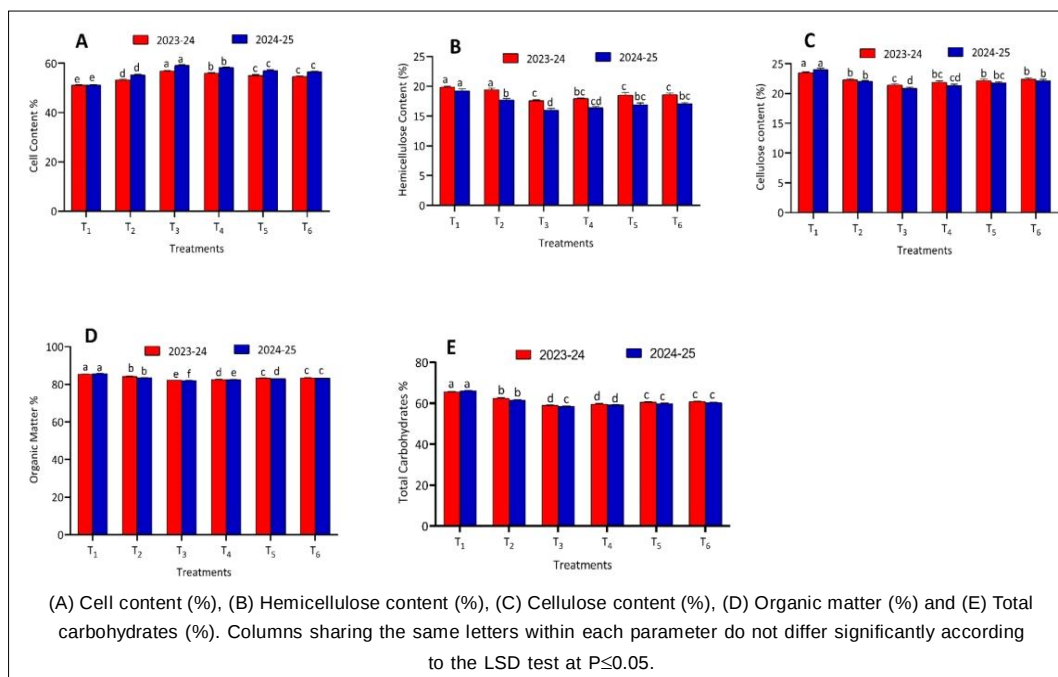


Fig 2: Effects of bioinoculants and biostimulants on cell wall components, organic matter and total carbohydrates of berseem.

metabolism and limits the accumulation of lignified structural carbohydrates, thereby reducing fibre concentration in forage crops (Kumar *et al.* 2021b). Similar findings were also reported by Moore *et al.* (2020).

Quality attributes

The quality attributes of berseem fodder were significantly influenced by different nutrient management practices, particularly with respect to cell wall constituents, organic matter and total carbohydrate content (Fig 2). Among the treatments, T₃ (PGPR + 75% RDN through FYM + three foliar sprays of *Panchagavya*) recorded the highest cell content during both years (56.9% and 59.2%, respectively), remaining statistically comparable with T₄ (PGPR + 75% RDN through FYM + three foliar sprays of seaweed extract). In contrast, T₃ exhibited the lowest cellulose, hemicellulose, organic matter and total carbohydrate contents during both experimental years. Compared with the T₁, T₃ increased cell content by 11.4% and 15.6%, while reducing cellulose, hemicellulose, organic matter and total carbohydrate concentrations during 2023 and 2024, respectively. Treatments T₅ and T₆ showed intermediate responses but performed significantly better than the control. The reduction in organic matter and carbohydrate content under T₃ may be attributed to enhanced nitrogen availability and greater nitrogen accumulation in plant tissues, which likely promoted carbohydrate utilization and metabolic activity within the forage. Similar observations were also reported by Iqbal *et al.* (2017) and Sumon *et al.* (2018).

CONCLUSION

The present investigation demonstrated that the integrated application of bioinoculants and biostimulants with FYM significantly improved fodder yield, nutritive quality and fibre characteristics of berseem under organic production conditions. Among the treatments, PGPR + 75% RDN through FYM combined with three foliar sprays of *Panchagavya* recorded the highest green and dry fodder yields, along with increased dry matter, crude protein, ether extract and total ash contents, while significantly reducing NDF, ADF and ADL concentrations, thereby improving forage digestibility and overall feed quality. These improvements were primarily associated with enhanced nutrient availability, microbial activity and nutrient uptake under integrated nutrient management. Therefore, the combined use of PGPR, FYM and *Panchagavya* represents a promising nutrient management strategy for enhancing berseem productivity and fodder quality in organic production systems. However, further studies on economic analysis, long-term effects on soil health and multi-location validation are required before recommending this practice for wider adoption.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed Consent

Not applicable. This study involved only laboratory analysis of plant/fodder samples and did not involve human participants or animal experimentation.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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