



Effects of Beejamrit Seed Priming on Photosynthetic Pigments, Free Amino Acids and Protein Content in Fenugreek (*Trigonella foenum-graecum* L.) Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: <https://doi.org/10.9734/ijpss/2026/v38i106386>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/167698>

Original Research Article

Received: 31/07/2026

Accepted: 17/10/2026

Published: 24/10/2026

Abstract

Beejamrit is a traditional cattle-derived preparation used for seed treatment in natural farming, but its biochemical effects are incompletely characterised in fenugreek. This study compared unprimed seeds with seeds soaked for 3 h in nominal Beejamrit concentrations of 10%, 20%, 30%, 50%, or 100%. Three hundred seeds were allocated across the six groups (50 per group), and seedlings were grown in pots under ambient conditions in Ahmedabad, India. Reported seedling measurements comprised chlorophyll a and b, total chlorophyll, carotenoids, free amino acids, and protein, reported as means $\pm$ standard errors. Relative to the unprimed control, the 100% treatment showed higher mean total chlorophyll (0.6002 versus 0.5211 mg g⁻¹; +15.2%), free amino acids (5.2717 versus 4.2381 mg g⁻¹; +24.4%), and protein (11.0738 versus 9.6645 mg g⁻¹; +14.6%). However, carotenoids were lower (0.1426 versus 0.2002 mg g⁻¹; -28.8%), and intermediate concentrations produced non-monotonic responses. These comparisons are descriptive because the available summary data do not establish the independent replicate structure or permit defensible inferential testing. The results indicate heterogeneous pigment and nitrogenous-compound responses following Beejamrit soaking; experimental confirmation with a fully specified formulation, sampling and replication is needed before conclusions about physiological performance or practical efficacy can be drawn.

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Keywords: Beejamrit; fenugreek; seed priming; chlorophyll; carotenoids; amino acids; protein; biostimulants

1. Introduction

Seed priming involves pre-sowing treatments intended to initiate early physiological activity while limiting premature germination. Appropriate priming treatments can influence germination synchrony, reserve mobilisation and subsequent establishment, but responses depend on crop species, treatment composition and growing conditions (Ashraf & Foolad, 2005; Farooq *et al.*, 2019; Paparella *et al.*, 2015). Seed germination and seedling establishment are accompanied by substantial changes in storage-protein turnover, amino acid metabolism and cellular organisation (Angermann *et al.*, 2024; Bewley *et al.*, 2013; Nonogaki *et al.*, 2010).

Interest in microbial and organic biostimulants arises from their potential effects on crop nutrition and stress responses, although biological activity must be demonstrated for each formulation and application context (du Jardin, 2015; Rouphael & Colla, 2018). Beejamrit is used in Indian natural farming as a seed-treatment preparation made from cattle-derived materials and other ingredients. A recent metagenomic and metabolomic characterisation identified diverse microorganisms and metabolites in independent Beejamrit samples and emphasised variability across ingredients and preparation conditions (Panchal *et al.*, 2026). Such findings offer background for testable hypotheses but do not identify the composition of the specific formulation used in any other experiment.

Previous investigations have provided useful evidence concerning microbial seed treatments and Beejamrit formulations. Patel *et al.* (2023) examined the growth-promoting potential of a defined *Bacillus subtilis* isolate in fenugreek under abiotic stress conditions, while Panchal *et al.* (2026) characterised the microbial and metabolite composition of Beejamrit. However, findings from these investigations cannot be directly extrapolated to the biochemical responses of fenugreek seedlings exposed to the Beejamrit preparation evaluated in the present study. The specific contribution of this investigation lies in the descriptive comparison of photosynthetic pigments, free amino acids and protein concentrations across different nominal Beejamrit seed-soaking concentrations. This approach provides preliminary information on biochemical response patterns without establishing treatment efficacy or the physiological mechanisms underlying the observed differences.

This study aimed to describe the association between nominal Beejamrit soaking concentrations and six biochemical measurements in fenugreek seedlings: chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, free amino acids and total protein. The comparisons were intended to identify response patterns rather than to infer specific biochemical pathways, photosynthetic rates or field-scale agronomic efficacy.

2. Materials and Methods

2.1 Seed Material and Cultivation

Fenugreek (*Trigonella foenum-graecum* L.) seeds were obtained from a local source. Healthy, visually uniform and undamaged seeds were selected. In total, 300 seeds were allocated to six treatment groups: an unprimed control and five Beejamrit concentrations, with 50 seeds per group. Three replicate sets were reported for each treatment; the allocation of the 50 seeds among replicates and the number of independently maintained pots per treatment were not specified. Therefore, the biological unit corresponding to the reported standard errors could not be established from the available summary records.

Earthen pots contained a 1:1 (v/v) mixture of garden soil and cocopeat. The experiment was conducted in Ahmedabad, India, during March 2026 under ambient light and ventilation. Reported daytime temperatures ranged from 34.0 to 39.5 °C and nighttime temperatures from 20.0 to 23.0 °C (mean daily maximum 37.2 °C; mean daily minimum 21.9 °C); average relative humidity was 39.4%. Pots were watered to limit waterlogging and desiccation.

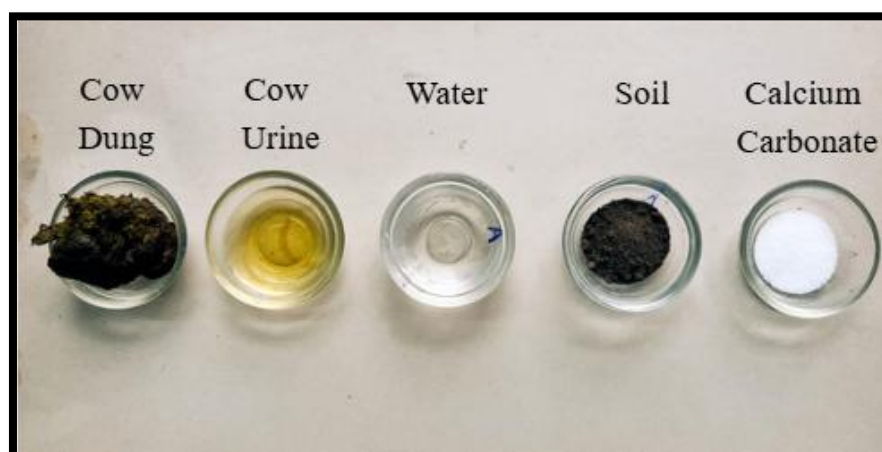


Fig. 1. Ingredients required for the preparation of Beejamrit

2.2 Beejamrit Preparation

The preparation was formulated according to the method described by Bishnoi and Bhati (2017), using fresh cow dung (125 g) and cow urine (125 mL), calcium carbonate (1.25 g), neem-rhizosphere soil (1.25 g) and tap water (500 mL). Cow dung and urine originated from Gir (*Bos indicus*) cattle in Gujarat; the urine had been collected approximately 24 h before formulation. The ingredients were mixed with 500 mL of water in a glass vessel and the suspension was maintained under ambient laboratory conditions (approximately 25–30 °C and 40–70% relative humidity) for 72 h, with stirring twice daily. The reported pH during preparation ranged from approximately 6.5 to 7.5. The amount of added water and final volume of the stock suspension were not specified in a reproducible form; accordingly, the mass-to-volume composition of the parent suspension cannot be determined precisely.

Table 1. Ingredients and their respective quantities required for the formulation of Beejamrit

Sr. No.	Ingredients of Beejamrit	Quantity
1.	Cow Dung	125g
2.	Cow Urine	125ml
3.	Calcium Carbonate	1.25g
4.	Neem Soil	1.25g
5.	Water	500ml

2.3 Seed Soaking and Sowing

Seeds were soaked for 3 h in Beejamrit treatment solutions prepared by mixing the corresponding volume of undiluted Beejamrit with distilled water to obtain the desired nominal concentrations. The 10% solution was prepared by mixing 10 mL of undiluted Beejamrit with 90 mL of water, the 20% solution by mixing 20 mL of Beejamrit with 80 mL of water, the 30% solution by mixing 30 mL of Beejamrit with 70 mL of water, and the 50% solution by mixing equal volumes (50 mL each) of Beejamrit and water. The 100% treatment consisted of undiluted Beejamrit. Untreated seeds served as the control. After soaking, the seeds were removed, gently blotted and sown immediately in the prepared pots. As no water-soaked control group was included, the experiment cannot distinguish the effects of Beejamrit treatment from those associated with soaking or seed hydration alone.

2.4 Biochemical Measurements

Fresh seedling material was collected after emergence for biochemical assessment. Photosynthetic pigment estimation referred to the chlorophyll estimation approaches evaluated by Ali *et al.* (2021); free amino acids were estimated using a ninhydrin-based colourimetric procedure attributed to Lee and Takahashi (1966); and protein was measured using the Folin phenol reagent method of Lowry *et al.* (1951). The reported outputs included chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, free amino acids and protein in mg g⁻¹ of material on a fresh-weight basis, as indicated by the original graphical axes. Harvest day, sampled tissue fraction, extraction solvents, optical wavelengths, calibration procedures and separation of technical from biological replicates were not documented in the available summary. Such omissions limit independent replication of the analytical procedures.

2.5 Data Treatment

All treatment values are presented as the reported mean $\pm$ standard error (SE). Percentage change from the unprimed control was calculated as $100 \times (\text{treatment mean} - \text{control mean})/\text{control mean}$. No raw replicate values, independent experimental-unit definitions or estimates of within-pot variability were available for a valid treatment-level significance test. Consequently, the results are treated descriptively; no p values, significance letters or confidence intervals were inferred. The total chlorophyll values were reported separately in the summary data; small differences of 0.0001 mg g⁻¹ from the sum of displayed chlorophyll a and b means are consistent with rounding but cannot be resolved without unrounded measurements.

3. Results

3.1 Photosynthetic Pigment Concentrations

Table 2 summarises the pigment values for all six groups. Total chlorophyll declined from 0.5211 mg g⁻¹ in the control to 0.3060 mg g⁻¹ at 50% Beejamrit (a descriptive difference of -41.3%), whereas the 100% group showed 0.6002 mg g⁻¹ (+15.2% relative to the control). Chlorophyll a followed a broadly similar pattern. The 100% chlorophyll b mean was only 0.001 mg g⁻¹ higher than the control. Carotenoids did not show a corresponding increase: the control had the highest mean (0.2002 mg g⁻¹), compared with 0.1426 mg g⁻¹ at 100% Beejamrit.

Table 2. Photosynthetic pigments in fenugreek seedlings after seed soaking (mg g⁻¹, mean $\pm$ reported SE). The independent replication basis of SE was not documented

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
Control	0.3498 $\pm$ 0.0002	0.1713 $\pm$ 0.0017	0.5211 $\pm$ 0.0015	0.2002 $\pm$ 0.0001
10%	0.3521 $\pm$ 0.0013	0.1176 $\pm$ 0.0025	0.4696 $\pm$ 0.0038	0.1624 $\pm$ 0.0004
20%	0.3223 $\pm$ 0.0003	0.1020 $\pm$ 0.0013	0.4243 $\pm$ 0.0009	0.1479 $\pm$ 0.0005
30%	0.2870 $\pm$ 0.0003	0.1095 $\pm$ 0.0015	0.3964 $\pm$ 0.0012	0.1289 $\pm$ 0.0002
50%	0.2261 $\pm$ 0.0351	0.0799 $\pm$ 0.0171	0.3060 $\pm$ 0.0522	0.0988 $\pm$ 0.0174
100%	0.4280 $\pm$ 0.0815	0.1723 $\pm$ 0.0364	0.6002 $\pm$ 0.1177	0.1426 $\pm$ 0.0135

The groupwise pigment patterns in Fig. 2 emphasise the comparatively high chlorophyll mean but substantial reported uncertainty at 100% Beejamrit. The same treatment remained below the unprimed control for carotenoids; therefore, higher chlorophyll concentration did not represent a uniform increase across pigment classes.

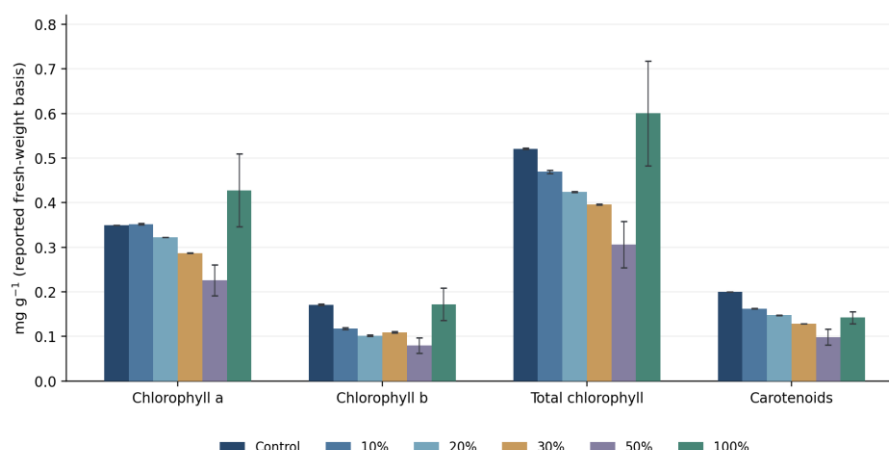


Fig. 2. Photosynthetic pigment means with reported SE across unprimed and Beejamrit-soaked groups. All plotted means and SEs correspond to Table 2

3.2 Free Amino Acid and Protein Concentrations

As shown in Table 3, free amino acid concentration was highest in the 100% group (5.2717 mg g^{-1}), a descriptive increase of 24.4% over the control (4.2381 mg g^{-1}). The 20% treatment also exceeded the control, whereas 10%, 30% and 50% showed lower concentrations. Total protein was highest at 100% ($11.0738 \text{ mg g}^{-1}$; +14.6% compared with the control), but protein remained comparably high at 50% ($10.9933 \text{ mg g}^{-1}$) despite a low free amino acid mean (3.1908 mg g^{-1}).

Table 3. Free amino acid and total protein concentrations in fenugreek seedlings (mean $\pm$ reported SE)

Treatment	Free amino acids (mg g^{-1})	Total protein (mg g^{-1})
Control	4.2381 ± 0.1217	9.6645 ± 0.0296
10%	3.6731 ± 0.0787	6.3425 ± 0.0217
20%	4.8582 ± 0.1012	10.6712 ± 0.0711
30%	3.8660 ± 0.1732	9.6142 ± 0.0142
50%	3.1908 ± 0.0450	10.9933 ± 0.0082
100%	5.2717 ± 0.1405	11.0738 ± 0.0434

Fig. 3 displays the non-monotonic free amino acid response: the mean peaked at 100%, but the 20% treatment also exceeded the control, whereas the 50% treatment was lowest. These comparisons indicate differences in the reported concentrations, not rates of nitrogen uptake or amino acid synthesis.

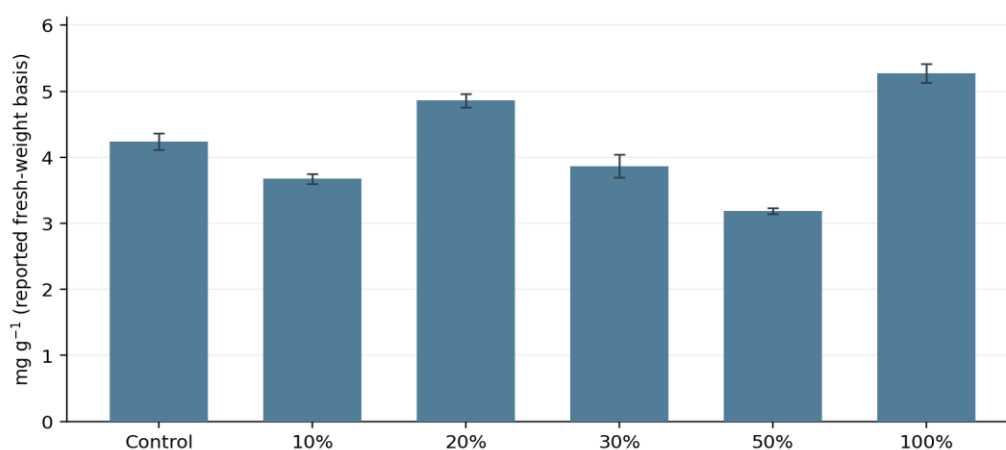


Fig. 3. Free amino acid means with reported SE for the six treatment groups (data from Table 3)

Fig. 4 shows that protein concentration was lowest at 10% Beejamrit and relatively high at 20%, 50% and 100%. Divergence between free amino acid and protein concentrations at 50% Beejamrit demonstrates that the two measured pools did not change in parallel at every treatment level.

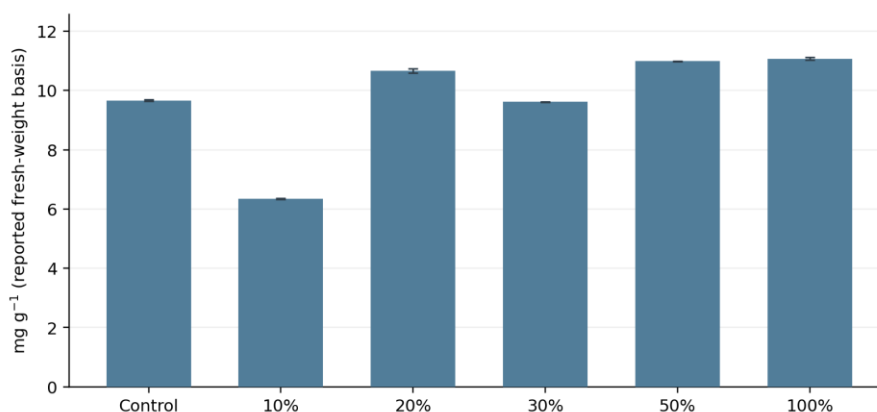


Fig. 4. Total protein means with reported SE for the six treatment groups (data from Table 3)

4. Discussion

The reported measurements show heterogeneous rather than uniformly stimulatory responses to Beejamrit exposure. At the undiluted concentration, average total chlorophyll, free amino acid and protein values exceeded those of the untreated control, whereas carotenoids were lower. At the intermediate concentrations, the response was non-monotonic; for example, the minimum total chlorophyll mean occurred at 50% even though the protein mean at that concentration was close to the maximum. These patterns suggest that conclusions about Beejamrit cannot be generalised across biochemical endpoints solely from treatment strength.

Chlorophyll concentration describes pigment abundance, not photosynthetic carbon assimilation or electron-transport efficiency. Measurements of net photosynthetic rate, chlorophyll fluorescence, nutrient uptake or seedling biomass would be required to infer functional photosynthetic improvement. Similarly, free amino acid and protein concentrations are biochemical pools that can reflect synthesis, mobilisation, degradation, redistribution and growth-related dilution. Their differences cannot establish the direction or rate of nitrogen assimilation without complementary enzyme, isotopic or metabolic-flux evidence. Studies of seed biology demonstrate extensive metabolic reorganisation during establishment, underscoring the limitations of interpreting isolated concentration measurements mechanistically (Angermann *et al.*, 2024; Bewley *et al.*, 2013).

The biological properties of Beejamrit depend on how the suspension is formulated and handled. Panchal *et al.* (2026) demonstrated that different Beejamrit preparations can contain diverse bacterial taxa and metabolites, but these were determined by direct compositional analysis. The present preparation was not characterised by microbial counts, molecular sequencing, metabolomics or hormone assays; therefore, its observed concentration patterns cannot be attributed specifically to beneficial bacteria, bioactive chemicals or hormone-mediated signalling. The work of Bonini *et al.* (2020) illustrates how biostimulant-associated metabolic shifts can be investigated mechanistically in another crop, but its species, inocula and assays differed from those used here.

The biochemical differences observed among treatment groups should be interpreted cautiously because the experimental design does not permit definitive attribution of these differences to Beejamrit-specific activity. In particular, the absence of a water-soaked control prevents separation of the potential influence of seed hydration and soaking from that of exposure to the Beejamrit suspension. Furthermore, the incomplete documentation of independent biological replication, formulation composition and analytical procedures limits the strength and reproducibility of the observed associations. Although certain Beejamrit concentrations were associated with higher mean concentrations of selected biochemical constituents, these observations do not establish enhanced photosynthetic performance, improved nitrogen assimilation or beneficial effects on seedling growth. Further investigations incorporating appropriate controls, independently replicated experimental units and standardised analytical procedures are required to determine whether these biochemical differences are reproducible and attributable to the Beejamrit treatment.

The interpretation of the findings is further constrained by limitations in formulation standardisation, analytical documentation and experimental replication. Although the principal ingredients and general preparation conditions of Beejamrit were described, the final stock volume and precise dilution procedures were not sufficiently documented to ensure reproducibility or establish consistency between independently prepared batches. Furthermore, the microbial composition and microbiological safety of the formulation used in this experiment were not evaluated through documented microbial enumeration or screening for potentially harmful microorganisms. Consequently, the present biochemical measurements cannot establish the microbiological safety of the preparation, although the absence of such assessments does not itself indicate contamination or adverse health effects. Seed allocation across the reported replicates, harvest age and assay calibration also require fuller documentation. As raw experimental-unit measurements were unavailable, statistical significance and between-treatment uncertainty could not be independently verified. In particular, the reported standard error for total chlorophyll at 100% Beejamrit (0.1177 mg g⁻¹) exceeds the absolute difference

between its mean and that of the control (0.0791 mg g⁻¹), indicating that claims of a clear treatment advantage are unwarranted without appropriate inferential analysis. Future investigations should incorporate standardised preparation protocols, independently replicated formulation batches, appropriate microbiological quality-control assessments and validated analytical procedures before practical recommendations concerning agronomic efficacy or microbiological safety are advanced.

5. Conclusion

In this pot experiment, undiluted Beejamrit seed soaking was associated with higher reported mean total chlorophyll, free amino acid and protein concentrations than the untreated control, while carotenoid concentration was lower. Intermediate soaking concentrations produced mixed, non-monotonic biochemical patterns. These results are descriptive and do not demonstrate improved photosynthetic performance, nitrogen assimilation, germination success, yield or environmental benefit. The findings provide preliminary biochemical observations that require confirmation using a fully specified formulation, independent replication, appropriate controls and validated analytical procedures.

Acknowledgements

The authors acknowledge St. Xavier's College (Autonomous), Ahmedabad, for research facilities and academic support; Dr Rashmi Yadav for mentorship; Ms Manundra Disha for technical assistance with laboratory procedures and formulation preparation; and Mr Mahil Shah for assistance in procuring materials.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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