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RESEARCH ARTICLE

Multilocation evaluation of protein hydrolysates (20 %) foliar application on growth and yield of chickpea under diverse agro-climatic conditions in India

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Abstract

Protein hydrolysates, composed of amino acids and short-chain peptides, have gained prominence as effective bio-stimulants that enhance nutrient uptake, enzymatic activity and overall crop productivity. Chickpea (*Cicer arietinum* L.), a vital pulse crop, often suffers yield losses due to soil fertility constraints and climatic variability. However, multilocation dose-response information on protein hydrolysates in chickpea under contrasting environments remains limited. To address this, a multilocation field study was undertaken during the rabi season of 2024-25 to evaluate the bio-efficacy of a 20 % protein hydrolysate formulation applied as a foliar spray at different concentrations across three agro-climatic regions at Dr PDKV, Akola; DBSKKV, Karjat and KSNUAHS, Shivamogga. The experiment followed a randomised block design (RBD) with five treatments-control, 2.0 mL/L, 3.0 mL/L, 4.0 mL/L and a commercial check and observations were recorded on growth and yield attributes. Improvements in chlorophyll index and nodulation (where measured) suggested enhanced physiological activity contributing to yield improvement. Results revealed that foliar application of protein hydrolysate significantly enhanced plant height, branching, pod number and grain yield across all locations. The 3.0 mL/L concentration showed the most consistent agronomic response, with yield increases of 16.4 % at Akola, 22.5 % at Karjat and 54.69 % at Shivamogga compared to the control. Economic analysis indicated positive returns, supporting practical field applicability. The study indicates that foliar application of 20 % protein hydrolysate at 3.0 mL/L can improve chickpea productivity under diverse agro-climatic conditions and may serve as a sustainable crop management input.

Keywords: amino acids; bio-stimulants; chickpea; multilocation trial; protein hydrolysates

Introduction

Pulses are a vital component of agriculture, serving as a major source of dietary protein, minerals and essential amino acids for millions of people. Among them, chickpea (*Cicer arietinum* L.) is extensively cultivated due to its adaptability to diverse agro-ecological regions and its role in sustaining soil fertility through biological nitrogen fixation (1, 2). However, chickpea productivity remains unstable across environments because crop performance is highly influenced by nutrient availability and climatic variability. Despite their importance, the productivity of pulses remains suboptimal, largely due to abiotic stresses, poor soil fertility and limited adoption of advanced nutrient management technologies. Enhancing crop productivity while ensuring sustainability requires innovative inputs that can complement conventional fertilisers and improve plant resilience under variable field conditions (3).

In recent years, bio-stimulants have emerged as an eco-friendly and sustainable alternative to improve crop performance. Protein hydrolysates, in particular, have gained importance for their ability to enhance nutrient uptake, stimulate enzymatic activity and improve plant growth and yield (4). Derived from enzymatic or chemical hydrolysis of plant (maize) or animal proteins, they contain amino acids, peptides and organic compounds that act as growth promoters and metabolic enhancers (5, 6). Foliar sprays of protein hydrolysates have been reported to enhance vital physiological functions such as photosynthesis, nutrient uptake and stress resilience, which in turn contribute to improved crop yield and productivity (7).

Although several studies have reported the benefits of protein hydrolysates in horticultural and field crops, systematic evaluations under multilocation conditions for pulses remain limited (8). Given the growing demand for cost-effective and sustainable technologies to boost pulse productivity, it is essential to generate region-specific data on their efficacy. Therefore, a multilocation field investigation was conducted to evaluate the agronomic response of chickpea to foliar-applied 20 % protein hydrolysate under contrasting environments. We hypothesised that crop response would be dose dependent, with an optimum concentration providing stable yield improvement across locations. The objective was to identify a practically reliable concentration suitable for field recommendation rather than a location-specific response.

Materials and Methods

Experimental sites and agro-ecological zones

Multilocation field experiments were conducted during the rabi season 2024–25 to evaluate the effect of a 20 % protein hydrolysate bio-stimulant on chickpea. The formulation consisted of amino acids and short peptides derived from enzymatic hydrolysis of plant protein sources (*Zea mays* L.). No additional mineral nutrients were intentionally added. The trials were carried out at three locations representing diverse agro-ecological zones (Fig. 1).

Akola, Maharashtra

Nagarjun Medicinal Plants Garden, Dr PDKV, situated in the part of the Eastern Maharashtra Plateau under the hot, moist semi-arid zone. The experiment was conducted on chickpea (*Cicer arietinum* L., var. *PDKV Kanak*) under rainfed conditions.

Karjat, Maharashtra

Regional Agricultural Research Station, DBSKKV, Dapoli, located in the South Konkan Coastal Zone with very high rainfall and lateritic soils. The crop evaluated was Chickpea (*Cicer arietinum* L., var. *Pratap*).

Shivamogga, Karnataka

Zonal Agricultural and Horticultural Research Station, UAHS, located in the Northern Transition Zone with a tropical wet and dry climate. Chickpea was grown under rainfed conditions.

Experimental design and treatments

Across all sites, trials were established following a randomised complete block design (RCBD) based on the respective research protocols. The treatments included foliar application of a 20 % amino acid–based protein hydrolysate at varying concentrations, along with untreated controls and appropriate checks.

Akola (Chickpea)

Four treatments (T_1: Control, T_2: 2.0 mL/L, T_3: 3.0 mL/L, T_4: 4.0 mL/L) with five replications.

Karjat (Chickpea)

Five treatments (T_1: Control, T_2: 2.0 mL/L, T_3: 3.0 mL/L, T_4: 4.0 mL/L, T_5: Vasant Urja) with four replications.

Shivamogga (Chickpea)

Five treatments and three replications (T_1: Control, T_2: 2.0 mL/L, T_3: 3.0 mL/L, T_4: 4.0 mL/L, T_5: University practice).

Differences in replication and check treatments were due to site-specific institutional trial protocols; therefore, combined statistical pooling across locations was interpreted cautiously.

Crop management

Plot size and spacing

Net plot dimensions varied from 3.0 × 4.5 m to 3.0 × 5.4 m, with row spacing of 30 cm and plant-to-plant spacing of 10 cm.

Seed rate

75 kg/ha

Nutrient management

Recommended dose of fertilisers (25:50:30 NPK kg/ha at Akola; RDF at Karjat and Shivamogga) was applied as basal at sowing.

Irrigation

At Akola, irrigation was provided at pre-sowing, pre-flowering and pod-filling stages. At Karjat and Shivamogga, crops were cultivated under rainfed conditions with supplemental irrigation when required.

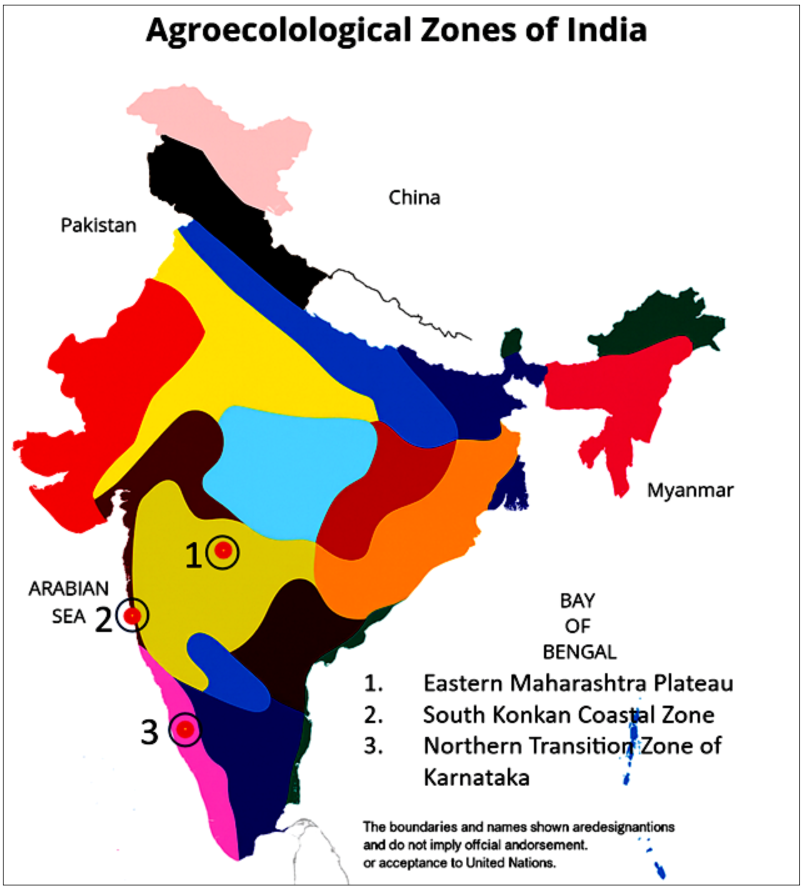

Fig. 1. Experimental sites with their agro-ecological zones.

Foliar application

Treatments were applied using a knapsack sprayer fitted with a hollow cone nozzle. Sprays were imposed at approximately 25–30 DAS (vegetative stage), 45–50 DAS (pre-flowering) and 60–65 DAS (pod initiation).

The stated 500 ml/acre refers to product quantity diluted in water; spray solution volume was adjusted to achieve uniform leaf wetting under field conditions.

Observations recorded

Growth parameters (plant height, branches, LAI, chlorophyll content, nodulation), phenological traits (days to flowering, pod formation) and yield parameters (pods/plant, 100-seed weight, grain yield, stover yield, harvest index).

Statistical analysis

Data from each location were analysed separately using analysis of variance (ANOVA) appropriate to RCBD. ANOVA was performed using statistical software and treatment means were compared using the critical difference (CD) at $P = 0.05$ after checking homogeneity of variance. A formal combined ANOVA across locations was not performed due to differences in varieties, checks and replication structure; hence, results are interpreted site-wise for consistency of response.

Results

Location 1: Chickpea at Akola

The foliar application of protein hydrolysates (20 %) exerted a significant influence on growth and yield parameters of chickpea under rainfed conditions of the Eastern Maharashtra Plateau (Table 1).

Growth attributes

Plant height ranged from 55.04 cm in control (T_1) to 60.76 cm in T_4 (4.0 mL/L). Treatments T_3 (3.0 mL/L) and T4 were statistically at par, both showing 10 % higher plant stature over the untreated control. Branching ability improved considerably, with 8.22–8.24 branches per plant in T_3 and T_4 compared with 6.42 branches in the control, registering an increase of about 28 %.

Phenological traits

The treatments had no significant effect on days to 50 % flowering, which remained within the range of 56 to 57 days. This indicates that the application of protein hydrolysate primarily influenced vegetative and reproductive vigour rather than altering crop phenology.

Yield attributes and yield

Pods per plant improved from 26.6 (control) to 31.8 in T_4, while T_3 recorded a comparable value of 31.7. The 100-seed weight remained stable across treatments (23.78–24.04 g), showing that the formulation did not alter grain size. Seed yield per plant was significantly higher in T_3 (7.94 g) and T_4 (7.98 g) compared with the control (6.62 g). Consequently, grain yield at the field level increased from 1578 kg/ha in T_1 to 1837 and 1856 kg/ha in T_3 and T_4, respectively. Economic observations indicated a marginal yield difference between T_3 and T_4. No phytotoxicity symptoms were observed at any concentration.

Location 2: Chickpea at Karjat

At the high-rainfall lateritic soils of Karjat, protein hydrolysate sprays significantly enhanced plant growth and yield (Table 2).

Growth attributes

Plant height increased from 42.35 cm in the control to 45.85 cm in T_3 (3.0 mL/L), showing a 8.3 % improvement. Branch number rose from 4.90 in T_1 to 6.95 in T_3, denoting enhanced vegetative vigour.

Yield attributes

Pod number per plant increased by 30 % in T_3 (55.7 pods) compared with T_1 (42.7 pods). Pod weight per plant followed a similar trend, reaching 14.59 g in T_3 versus 10.40 g in the control. The 100-grain weight was highest in T_3 (22.98 g), showing improved seed filling.

Table 1. Effect of protein hydrolysate on growth and yield of chickpea at Akola

Tr. No	Treatment	Plant Height (cm)	No. of Branches	Days to 50 % Flowering	Pods/Plant	100-Seed Weight (g)	Seed Yield/ Plant (g)	Seed Yield (kg ha^{-1})
T_1	Control	55.04	6.42	56	26.63	23.78	6.62	1578
T_2	PH 2.0 mL/L	57.54	7.40	56	28.56	23.86	7.18	1714
T_3	PH 3.0 mL/L	60.68	8.22	57	31.74	24.04	7.94	1837
T_4	PH 4.0 mL/L	60.76	8.24	57	31.80	24.00	7.98	1856
	SE(m)+	1.08	0.23	NS	0.52	0.24	0.21	19
	CD (5 %)	3.33	0.71	NS	1.61	NS	0.65	59

Means followed by **CD** (*P=0.05*) differ significantly. **PH** – Protein hydrolysate.

Table 2. Effect of protein hydrolysates on growth and yield parameters of chickpea (Karjat)

Tr. No	Treatment	Plant Height (cm)	Branches / Plant	Pods/Plant	Pod weight $plant^{-1}$ (g)	100 Grain Wt. (g)	Grain Yield (kg ha^{-1})	Stover Yield (kg ha^{-1})
T_1	Control	42.35	4.90	42.70	10.40	20.61	1289.34	1713.72
T_2	PH 2.0 mL/L	44.35	5.95	47.20	12.62	22.08	1455.24	1987.80
T_3	PH 3. mL/L	45.85	6.95	55.70	14.59	22.98	1579.93	2174.21
T_4	PH 4.0 mL/L	43.85	5.00	46.30	11.50	21.17	1429.00	1906.47
T_5	Commercial check	45.10	5.80	47.68	13.16	22.04	1460.48	2139.03
	SE(m)+	0.98	0.13	1.15	0.28	0.68	31.03	26.15
	CD (5 %)	2.73	0.36	3.20	0.77	1.87	86.15	75.58

Means followed by **CD** (*P=0.05*) differ significantly. **PH** – Protein hydrolysate.

Yield performance

Grain yield increased significantly from 1289.3 kg/ha in the control to 1579.9 kg/ha in T_3, recording a 22.5 % yield advantage. Stover yield also improved (1713.7 kg/ha in control vs. 2174.2 kg/ha in T_3), reflecting overall crop vigour. Treatments T_2 (2.0 mL/L) and T_5 (Vasant Urja) performed moderately, while T_4 (4.0 mL/L) showed no consistent superiority, possibly due to excessive dosage. No phytotoxic symptoms were recorded in any treatment.

Location 3: Chickpea at Shivamogga

In the Northern Transition Zone of Karnataka, the treatments of protein hydrolysates significantly influenced both growth (Table 3) and yield parameters of Chickpea (Fig. 2).

Growth attributes

Treated plots recorded higher germination (92.7 % in T_3) than the control (82.5 %). Plant height and branch number were maximised in T_3 (45.5 cm and 5.8 branches). Leaf area index (2.47), root biomass (3.2 g/plant), nodulation (23.9 nodules/plant) and chlorophyll content (44.7 SPAD) were higher in T_3.

Yield attributes

Pod number per plant increased from 31.4 in T_1 to 44.6 in T_3, while seeds per pod improved from 1.6 to 2.2. Pod dry weight nearly doubled in T_3 (6.8 g/plant) compared with the control (3.8 g/plant). The 100-seed weight also increased significantly (24.8 g in T_3 vs. 19.6 g in control), highlighting better grain filling.

Yield performance

Grain yield recorded a sharp increase from 1300 kg/ha in the control to 2011 kg/ha in T_3, representing a 54.69 % yield increase. Biomass and stover yields also improved substantially, while harvest index rose from 35.7 % (control) to 44.0 % (T_3), indicating improved partitioning efficiency. Treatments T_4 and T_5 also recorded high values but remained statistically comparable to T_3. No phytotoxic effects were observed.

Table 3. Effect of protein hydrolysates on growth parameters of Chickpea (Shivamogga)

Tr. No	Treatment	Germination (%)	Plant Height (cm)	No. of Branches/ Plant	Days to 50 % Flowering	Days to First Pod Formation	Leaf Area Index	Root Length (cm)	Root Biomass (g plant^{-1})	Nodulation (No./plant)	Chlorophyll Content (SPAD)
T_1	Control	82.5^d	34.2^d	3.8^d	50.5^a	58.2^a	1.84^d	10.6^d	1.5^d	14.3^d	34.5^d
T_2	PH 2.0 mL/L	88.4^c	40.8^c	4.6^c	48.3^b	55.7^b	2.12^c	12.9^c	2.1^c	18.5^c	38.9^c
T_3	PH 3.0 mL/L	92.7^a	45.5^a	5.8^a	46.1^c	53.6^c	2.47^a	15.8^a	3.2^a	23.9^a	44.7^a
T_4	PH 4.0 mL/L	91.2^a	43.9^a	5.6^a	46.3^c	53.9^c	2.43^a	15.5^a	3.0^a	23.2^a	44.1^a
T_5	Recommended practice	91.0^a	44.1^a	5.4^a	47.0^b	54.1^b	2.39^a	15.0^a	2.9^b	22.5^a	43.7^a
	S.Em	1.21	0.92	0.18	0.52	0.64	0.04	0.32	0.18	0.94	0.85

Values sharing the same superscript within a column are not significantly different at $P = 0.05$ (DMRT). PH – Protein hydrolysate.

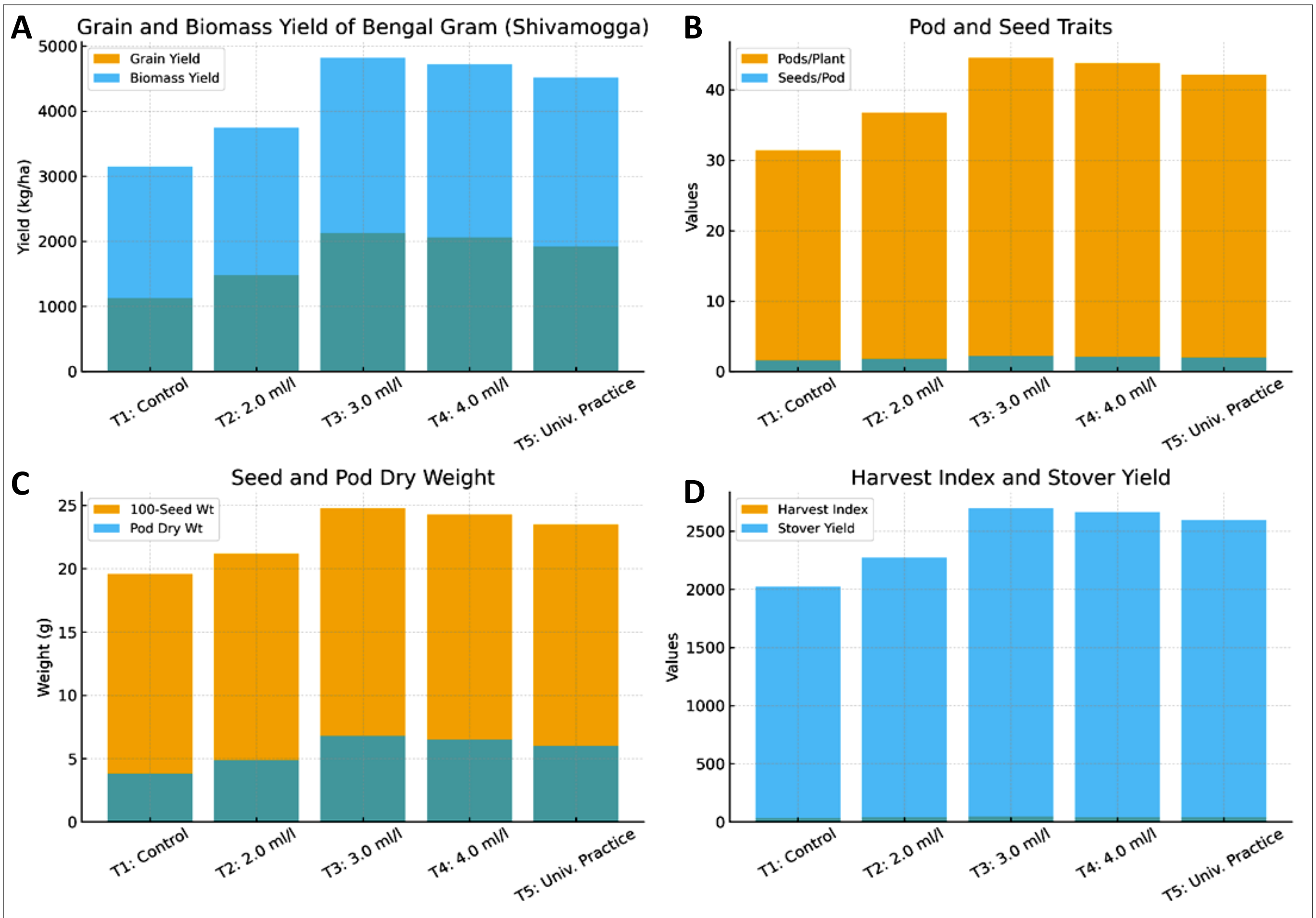

Fig. 2. Effect of protein hydrolysates on yield parameters of chickpea (Shivamogga): **A**. Yield, **B**. Pod and seed traits, **C**. Weight, **D**. Harvest index and stover yield.

Consolidated results across locations

Across all agro-climatic zones, foliar application of 20 % protein hydrolysate improved growth and grain yield of chickpea. Although the higher dose (4.0 mL/L) occasionally recorded numerically greater values, the 3.0 mL/L concentration showed the most consistent response across sites. Yield increases were 16.4 % at Akola, 22.5 % at Karjat and 54.69 % at Shivamogga compared to the control. Variability in the magnitude of response was observed among locations. No phytotoxicity symptoms were recorded at any site.

Discussions

The present multilocation study established a positive agronomic response of chickpea to foliar application of protein hydrolysate across contrasting environments. Rather than uniformly increasing all parameters, the treatment mainly improved reproductive attributes, which translated into yield enhancement.

At Akola, protein hydrolysate improved branching and pod number with a 16.4 % yield increase over the control. Research indicates that amino acid–based bio-stimulants improved the yield of chickpea under stress conditions (9). Similarly, research has demonstrated that improved nodulation and yield follow foliar nutrition at reproductive stages. Because flowering time remained unchanged, the response appears associated with improved resource utilisation rather than altered crop duration (10).

At Karjat, the increment in plant height (8.3 %), pod number (30 %) and grain yield (22.5 %) corroborates earlier evidence that bio-stimulants improve nutrient assimilation and photosynthetic efficiency, resulting in enhanced reproductive vigour (11, 12). The increase in 100-seed weight in T3 (22.98 g) indicates improved grain filling, which can be attributed to enhanced translocation efficiency (13). Bio-stimulant applications improved germination, nutrient cycling and symbiotic associations with N- and P-mobilising bacteria in chickpea (14). However, the higher rainfall environment likely reduced stress intensity, resulting in a moderate response compared with other locations.

At Shivamogga, grain yield increased by 54.69 % compared to the control, along with improvements in nodulation, chlorophyll index and leaf area index. Similar physiological responses were reported in chickpea (13). The larger response magnitude may be related to initial lower crop vigour and stronger responsiveness to external bio-stimulant inputs rather than a fundamentally different mechanism. The increase in harvest index indicates improved partitioning efficiency, aligning with the mode of action of protein hydrolysates, which are known to influence enzymatic activities, hormonal balance and assimilate distribution (12).

An important observation across all locations was that the 3.0 mL/L concentration consistently produced optimal results, whereas the higher concentration provided little additional benefit. This suggests a dose–response plateau where metabolic stimulation reaches saturation and higher concentrations may not proportionally increase physiological activity (11). The variability in response among locations indicates that environmental conditions and baseline soil fertility influenced treatment effectiveness (15). The study is based on a single season and primarily morphological observations; therefore, mechanistic interpretations should be considered indicative rather than conclusive. Future work, including tissue nutrient analysis or physiological measurements, would strengthen causal understanding. Overall, the multilocation performance indicates that protein hydrolysates function as bio-stimulatory inputs improving crop performance under field conditions rather than acting as conventional nutrient fertilisers.

Conclusion

The multilocation evaluation indicated that foliar application of protein hydrolysate improved chickpea performance across contrasting environments, with responses mainly expressed through reproductive growth rather than changes in crop duration. Among the tested concentrations, 3.0 mL/L provided the most consistent agronomic response across sites. Higher concentration did not proportionally enhance yield, indicating a practical optimum dose under field conditions. The variation in response among locations suggests that environmental conditions influence the magnitude but not the direction of the treatment effect. No phytotoxic effects were observed during the study. Overall, the results support the hypothesis that protein hydrolysate can function as a field-level bio-stimulant, though multi-season validation is required before broad regional recommendations.

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Authors' contributions

JU conceived the study, designed the experiment and prepared the manuscript. SBDS carried out supervision and provided technical guidance throughout the research. APP, AN, NP, SB and GD conducted the field experiments and data collection. RKS provided overall project guidance, critical inputs and final manuscript approval. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (GPT-5, OpenAI) to assist in language refinement and formatting. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the final version of the manuscript.

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