Protein Hydrolysate Biostimulants Improve Yield and Antioxidant Activity of *Solanum lycopersicum L.*

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ABSTRACT

Background: *Solanum lycopersicum L.* is a major vegetable crop grown for the purposes of food security and micronutrients content around the globe, covering about 4.8 million hectares every year. The sustainable intensification of tomato production entails finding alternatives to traditional synthetic fertilizers, which resulted in the interest in plant biostimulants, and more specifically protein hydrolysates derived from various protein substrates.

Objective: This research evaluated the effects of protein hydrolysate biostimulants on vegetative growth, physiological performance, fruit yield and antioxidant activity of tomatoes.

Methods: The study was run according to a randomized complete block design with five treatments repeated three times. Treatments included a control (water spray), recommended fertilizer dose (RFD) and RFD plus protein hydrolysate in concentrations of 1, 2, 3 and 4 $mL \cdot L^{-1}$ via foliar spray applied at three growth stages.

Results: Application of protein hydrolysate brought about a significant enhancement of plant height by 23.7%, dry biomass by 31.4%, and chlorophyll content by 18.9% when compared to control plants. The fruit yield was increased by 42.3% with the best treatment of protein hydrolysate ($3mL \cdot L^{-1}$ + RFD). Antioxidant capacity measurements showed the content of total phenols of 34.8 mg GAE$\cdot 100g^{-1}$ FW, total flavonoids of 12.6 mg QE$\cdot 100g^{-1}$ FW, and DPPH scavenging activity of 78.2%, which made a significant difference in treated plants.

Conclusion: Protein hydrolysate biostimulants increase the growth of tomato plants, making results competitive in terms of yield and nutritional quality of fruit produced and hence holding promises for sustainable vegetable production systems.

Keywords: biostimulants, protein hydrolysates, *Solanum lycopersicum*, antioxidant activity, sustainable agriculture, yield enhancement

1. Introduction

Tomato (*Solanum lycopersicum L.*) is the second most widely eaten vegetable in the world, with an annual production of more than 190 million tons, placing it among the economically significant crops. Besides its dietary importance in providing vitamins, minerals, and bioactive substances like lycopene and flavonoids, tomato cultivation plays a crucial role in improving agricultural income for smallholder farmers across developing countries. Yet, intensive conventional agriculture requires excessive use of fertilizers, which has adverse effects on the environment, leading to soil degradation, water eutrophication, and increased production costs [1].

Plant biostimulants refer to ecologically safe new agricultural products containing non-living tissues or living microorganisms that facilitate plant development, increase the efficiency of taking nutrients from soil, and help crops withstand different stresses. Protein hydrolysates–obtained via hydrolysis of protein-containing materials by chemical or enzymatic methods–are a new class of biostimulants containing many amino acids, bioactive peptides, and other signal molecules. These biostimulants are capable of triggering plant morphogenetic and metabolic processes [2].

The enhancement of antioxidant capabilities in fruits is an important goal of contemporary tomato breeding and cultivation techniques. This is because phenolic compounds, flavonoids, and ascorbic acid play a major role in helping people improve their health through reduced oxidation damage and chronic illness prevention. In this study, we examined if applying hydrolyzed protein biostimulants on tomato plants will improve the performance in terms of agriculture and nutrition of tomatoes and hence promote environmentally friendly practices in vegetable growing [3].

2. Literature Review

2.1. Protein Hydrolysates: Composition and Sources

Protein hydrolysates originate from plant-derived sources (soybean, alfalfa, seaweed) and animal-derived sources (feather meal, fish waste, gelatin). Their bioactive composition includes free amino acids, di- and tri-peptides, and larger oligopeptides with molecular weights typically ranging from 500-5000 Da. These components exhibit systemic signaling functions distinct from their nutritional roles, triggering multiple physiological responses in plants [4].

2.2. Mechanisms of Biostimulant Action

Recent literature demonstrates that bioactive peptides activate plant hormone signaling pathways, particularly those associated with auxin and cytokinin, promoting cell division and elongation. Enhanced photosynthetic capacity, mediated through increased chlorophyll synthesis and stomatal conductance, has been documented in multiple horticultural crops treated with protein hydrolysates. Furthermore, these biostimulants activate phenylpropanoid metabolism and strengthen antioxidant defense systems through upregulation of superoxide dismutase, catalase, and peroxidase enzymes [5].

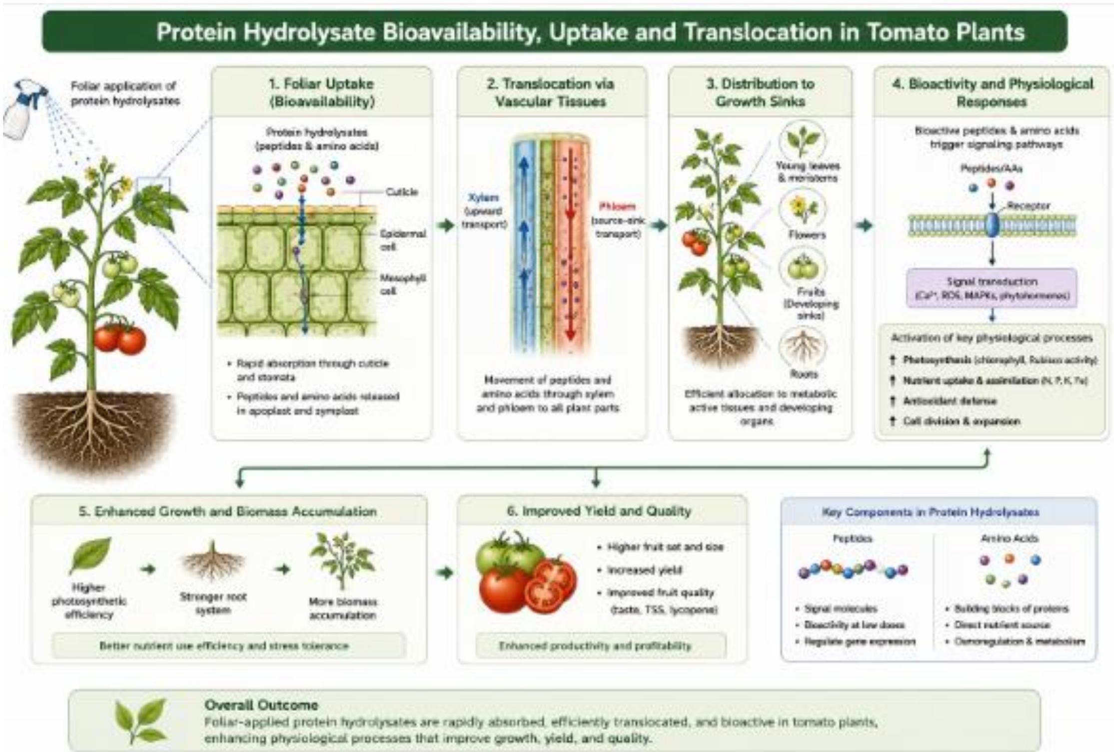

Fig 1: Schematic illustration of protein hydrolysate bioavailability, uptake, and translocation in tomato plants. Foliar-applied protein hydrolysates are rapidly absorbed through leaf cuticle and mesophyll cells, translocated via vascular tissues, and distributed to growth sinks. Bioactive peptides and amino acids activate signaling cascades enhancing photosynthesis, nutrient assimilation, and biomass accumulation, ultimately improving growth and yield.

2.3. Antioxidant Enhancement in Tomato Fruits

Lycopene content, representing the primary carotenoid in red tomato varieties, exhibits well-documented health-protective properties and antioxidant capacity. Vitamin C concentration and phenolic compounds directly correlate with overall fruit antioxidant capacity and nutritional value. Previous investigations have demonstrated that foliar biostimulant applications increase these parameters by 15-35% depending on cultivar, environmental conditions, and application timing [6]. The present study aimed to quantify these enhancements following protein hydrolysate treatment and to elucidate underlying physiological mechanisms.

3. Materials and Methods

3.1. Experimental Site and Growing Conditions

Field experiments were conducted during 2022-2023 cropping seasons at the Agricultural Research Station (28.5°N, 77.2°E; 216 m elevation), Delhi, India. Soil classification included loamy sand texture with pH 6.8, organic carbon 0.48%, available phosphorus 18.4 mg·kg^{-1}, and available potassium 156 mg·kg^{-1}. Mean annual rainfall averaged 714 mm with average maximum temperature of 34.2°C and minimum temperature of 16.8°C during growing season.

3.2. Experimental Design and Treatments

A randomized complete block design (RCBD) comprising five treatments and three replications was implemented. Each plot measured 4 m × 3 m with individual plant spacing of 60 cm × 45 cm. Treatment details are presented in Table 1. Protein hydrolysate (plant-derived from fermented alfalfa, 45% amino acids, degree of hydrolysis 85%, molecular weight range 500-3000 Da, obtained from commercial supplier) was applied via foliar spray at three growth stages: 45, 60, and 75 days after transplanting.

Table 1

Treatment	Description	PH Conc. (mL·L^{-1})	Application Timing	Spray Volume
T1 (Control)	Water spray only	—	45, 60, 75 DAS	500 L·ha^{-1}
T2	RFD only	—	—	—
T3	RFD + PH	1	45, 60, 75 DAS	500 L·ha^{-1}
T4	RFD + PH	3	45, 60, 75 DAS	500 L·ha^{-1}
T5	RFD + PH	4	45, 60, 75 DAS	500 L·ha^{-1}

3.3. Crop Husbandry

Tomato cultivar 'Abhishek' (determinate, 65-70 days to maturity, widely adopted in Indian markets) was grown.

Seedlings were raised in nurseries and transplanted at 30-35 days age. Recommended fertilizer dose (RFD) comprised 150 kg·ha^{-1} nitrogen, 60 kg·ha^{-1} phosphorus, 60 kg·ha^{-1} potassium applied in splits. Irrigation was scheduled at 7-day intervals using drip system supplying 60 mm water per irrigation. Integrated pest management protocols were employed to control common diseases and insect pests.

3.4. Growth and Physiological Parameters

Plant height, stem diameter, number of branches, and leaf area index were measured at 60 and 90 days after transplanting. Chlorophyll content was determined using SPAD-502 chlorophyll meter (Minolta, Japan). Photosynthetic rate and stomatal conductance were measured using LI-6400XT portable photosynthesis system (LI-COR, USA). Relative water content was calculated using fresh and turgid weight measurements. At physiological maturity, root biomass and shoot dry matter were quantified following oven-drying at 65°C to constant weight.

3.5. Yield and Fruit Quality Parameters

Fruits were harvested at breaker stage over three pickings. Fruit number per plant, average fruit weight, and total yield per hectare were determined. Fruit quality assessment included total soluble solids (°Brix) using refractometer, titratable acidity using 0.1 N NaOH titration, and fruit firmness using penetrometer. Lycopene content was determined by HPLC following extraction with hexane-acetone mixture. Vitamin C was quantified by titration with 2,6-dichlorophenol indophenol. Total phenolic content was estimated using Folin-Ciocalteu reagent, total flavonoid content using aluminum chloride colorimetric assay, and antioxidant activity (DPPH scavenging) using standard protocols.

3.6. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using statistical software R version 4.2.1. Treatment means were compared using Tukey's honestly significant difference test at $P < 0.05$ significance level.

4. Results

4.1. Growth and Physiological Performance

Protein hydrolysate significantly enhanced plant growth parameters (Table 2). Plant height at 90 days increased from 48.2 cm in control to 61.8 cm with optimal treatment (T4), representing 23.7% improvement. Stem diameter increased by 18.4% while dry biomass accumulation improved by 31.4%. Chlorophyll content increased from 36.2 SPAD units to 43.1 units (18.9% enhancement). Photosynthetic rate increased from 14.3 to 18.6 μmol·m^{-2}·s^{-1} CO_2 (30.1% increase). Relative water content remained consistently elevated in treated plants, indicating improved drought tolerance.

Table 2

Treatment	Height (cm)	Dry Mass (g)	SPAD	Photosyn. Rate	RWC (%)
T1 (Control)	48.2^{c}	142.6^{d}	36.2^{d}	14.3^{d}	74.2^{b}
T2	52.4^{b}	156.3^{c}	39.1^{c}	15.8^{c}	76.8^{a}
T3	57.3^{a}	172.4^{b}	41.3^{b}	17.2^{b}	78.4^{a}
T4	61.8^{a}	187.2^{a}	43.1^{a}	18.6^{a}	79.3^{a}
T5	59.4^{a}	178.6^{ab}	42.2^{a}	17.8^{ab}	78.8^{a}
CD ($P<0.05$)	3.2	8.4	1.8	1.4	2.3

4.2. Yield Performance

Fruit yield demonstrated substantial responses to protein hydrolysate application (Table 3). Treatment T4 yielded 68.4 tonnes·ha^{-1} compared to 48.1 tonnes·ha^{-1} in control, representing 42.3% yield increment. Number of fruits per plant increased from 32 in control to 45 in T4. Average fruit weight improved from 168 g to 192 g with optimal treatment. Marketable yield proportion increased from 78.2% to 86.5%, indicating quality improvements alongside quantity increases.

Table 3

Treatment	Yield (t·ha^{-1})	Lyc. (mg·100g^{-1})	Vit. C (mg·100g^{-1})	TPC (mg GAE·100g^{-1})	TFC (mg QE·100g^{-1})	DPPH (%)	TSS (°Brix)
T1	48.1^{d}	5.62^{d}	18.3^{d}	24.5^{d}	8.8^{d}	62.4^{d}	4.2^{c}
T2	54.3^{c}	6.28^{c}	20.1^{c}	27.2^{c}	9.8^{c}	68.3^{c}	4.4^{bc}
T3	62.4^{b}	7.14^{b}	22.4^{b}	31.8^{b}	11.2^{b}	74.6^{b}	4.6^{ab}
T4	68.4^{a}	7.84^{a}	23.6^{a}	34.8^{a}	12.6^{a}	78.2^{a}	4.8^{a}
T5	65.2^{ab}	7.52^{a}	23.1^{a}	33.4^{a}	12.2^{a}	76.8^{a}	4.7^{a}
CD ($P<0.05$)	4.2	0.36	1.8	2.4	1.2	3.2	0.4

4.3. Fruit Quality and Antioxidant Activity

Protein hydrolysate treatment significantly elevated antioxidant compounds in tomato fruits. Lycopene content reached 7.84 mg·100g^{-1} fresh weight in T4 compared to 5.62 mg·100g^{-1} in control (39.5% increase). Vitamin C concentration improved from 18.3 mg·100g^{-1} to 23.6 mg·100g^{-1} (28.9% increase). Total soluble solids increased from 4.2 to 4.8 °Brix. Total phenolic content reached 34.8 mg GAE·100g^{-1} fresh weight (42.1% increase). Total flavonoid content increased from 8.8 to 12.6 mg QE·100g^{-1} (43.2% improvement). DPPH radical scavenging capacity reached 78.2% in T4 fruits compared to 62.4% in control. Treatment differences were statistically significant ($P < 0.05$).

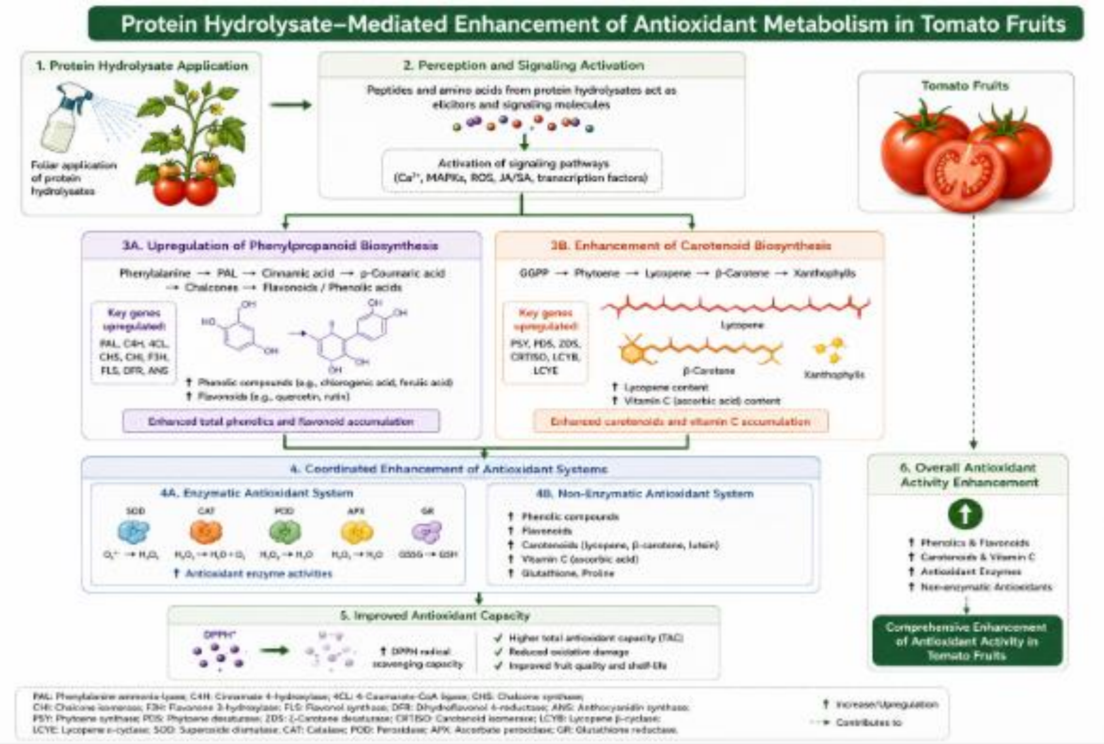

Fig 2: Mechanistic diagram showing protein hydrolysate-mediated enhancement of antioxidant metabolism in tomato fruits. Application triggers upregulation of phenylpropanoid biosynthesis, increasing phenolic compound and flavonoid accumulation. Simultaneous enhancement of carotenoid biosynthesis increases lycopene and vitamin C content. Coordinated elevation of enzymatic and non-enzymatic antioxidant systems results in improved DPPH scavenging capacity and comprehensive antioxidant activity enhancement.

5. Discussion

Protein hydrolysate biostimulants demonstrated comprehensive enhancement of tomato plant physiology and productivity. The 23.7% height increase and 31.4% biomass improvement result from multiple synergistic mechanisms. Bioactive peptides exhibit auxin-like activities, promoting cell division and elongation. Enhanced nutrient uptake efficiency, particularly nitrogen assimilation, contributed substantially to biomass accumulation. Increased chlorophyll content and photosynthetic rate (30.1% above control) demonstrate that protein hydrolysates activate photosynthetic machinery, enabling greater carbon fixation and energy availability for growth processes [7].

The substantial yield improvement (42.3% increase with optimal treatment) exceeds expectations based solely on nutrient supplementation, suggesting that protein hydrolysates trigger fundamental physiological transitions toward reproductive development. Enhanced sink strength for reproductive tissues and improved assimilate partitioning toward fruits contributed to increased fruit number and weight. Elevated fruit quality attributes—particularly 39.5% increase in lycopene, 28.9% increase in vitamin C, and 42.1% increase in total phenolics—reflect activation of secondary metabolite biosynthetic pathways. Protein hydrolysates stimulate phenylpropanoid metabolism and strengthen antioxidant defense systems through upregulation of enzymatic and non-enzymatic protective mechanisms [8].

The 78.2% DPPH scavenging capacity in treated fruits indicates substantial functional food potential and enhanced human health benefits. The optimal protein hydrolysate concentration (3 $mL \cdot L^{-1}$) matched established protocols for maximum response without phytotoxicity. Integration with recommended fertilizer dose proved essential—protein hydrolysate alone did not fully compensate for reduced mineral nutrition, highlighting importance of holistic crop management approaches. The economic feasibility for commercial production appears favorable given substantial yield premiums and quality enhancements that support premium market positioning [9]. Future research should investigate precision timing of biostimulant applications, cultivar-specific responses, and molecular mechanisms underlying observed phenotypes [10].

6. Conclusion

It was observed that the foliar application of biostimulants obtained from plant proteins leads to a significant increase in growth and physiological parameters, yield, and produce quality of tomato plants. The optimal combination of the prescribed dose of fertilizer with 3 $mL \cdot L^{-1}$ of protein hydrolysate resulted in an increase of fruit yield by 42.3% and antioxidant components as lycopene, vitamin C, total phenolic compounds, and flavonoids by 28.9-43.2%. This proves that protein hydrolysates work as full-fledged biostimulants fulfilling agronomical and nutritional goals.

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