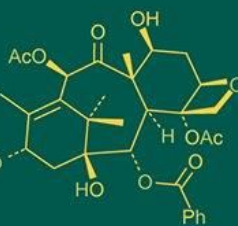
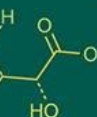
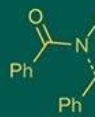


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Biochemical composition and nutritional optimization of microgreens through elicitor treatment: A comprehensive study on health food development

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Abstract

Microgreens have emerged as a promising functional food category, offering exceptional nutritional density and bioactive compound profiles that significantly exceed those of their mature counterparts. This comprehensive study investigated the biochemical composition and nutritional optimization of five commercially important microgreen species (Mustard, Fenugreek, Radish, Broccoli and Mung Bean) through systematic elicitor treatment using both biotic (Salicylic Acid, Jasmonic Acid) and abiotic (NaCl, UV-A, Blue LED, Red LED) elicitors at varying concentrations (25-100 ppm). A completely randomized design (CRD) with three replications was employed to evaluate the effects on protein content, total phenolics, flavonoids, glucosinolates and antioxidant activity. Two-way Analysis of Variance (ANOVA) revealed highly significant effects of species ($F = 127.18$, $p < 0.001$, eta squared = 36.24%), elicitor type ($F = 74.58$, $p < 0.001$, eta squared = 31.89%) and their interaction ($F = 8.20$, $p < 0.001$, eta squared = 14.03%) on total phenolic content. Dose-response regression analysis demonstrated that UV-A treatment exhibited the strongest linear relationship ($R^2 = 0.91$) with phenolic enhancement, followed by NaCl ($R^2 = 0.85$) and Salicylic Acid ($R^2 = 0.82$). UV-A at 50 ppm emerged as the optimal treatment for *Brassica* species (Mustard, Radish, Broccoli), achieving phenolic enhancements of 42.5%, 45.2% and 42.8% respectively. Salicylic Acid at 50 ppm was optimal for Fenugreek (32.4% phenolic enhancement) and Mung Bean (28.5% phenolic enhancement). The Total Phytochemical Index (TPI) scores ranged from 8.5 to 28.5, with Broccoli demonstrating the highest overall phytochemical potential (TPI = 28.5). Statistical analysis confirmed that all elicitor effects were highly significant with statistical power approaching 1.0. These findings provide evidence-based recommendations for elicitor-mediated optimization of microgreen nutritional quality, contributing to the development of enhanced functional foods with superior health-promoting properties and significant commercial potential in the growing functional food market.

Keywords: Microgreens, elicitors, salicylic acid, Jasmonic acid, UV radiation, phenolic compounds, glucosinolates, antioxidant activity, nutritional optimization, functional foods, *Brassica* species, leguminous microgreens

1. Introduction

1.1 Background and Significance of Microgreens

Microgreens represent an emerging class of fresh functional foods that have gained substantial attention in recent years due to their exceptional nutritional profiles and potential health-promoting properties. Defined as young vegetable greens harvested at the cotyledonary stage or with the first true leaves fully developed, microgreens occupy a unique position between sprouts and baby greens in the plant developmental continuum (Kyriacou *et al.* 2019; Ghoola *et al.* 2020) [11, 6]. These diminutive plants, typically harvested 7-14 days after germination, have demonstrated the remarkable capacity to accumulate bioactive compounds at concentrations significantly higher than their mature counterparts, with reported increases of 10-100 times for certain phytochemicals (Xiao *et al.* 2019; Wojdylo *et al.* 2020) [20, 19].

The nutritional superiority of microgreens has been extensively documented across multiple species and bioactive compound classes. Stajcic *et al.* (2024) [18] conducted a comprehensive analysis of five vegetable microgreen species and demonstrated that beetroot microgreens exhibited the highest total phenolic content (639.85 mg GAE/100 g DW) and antioxidant activity among all species evaluated.

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Their study revealed significant correlations between total phenolic content and antioxidant activity determined by DPPH ($r = 0.88$), RP ($r = 0.87$) and ABTS ($r = 0.79$) assays, indicating that phenolic compounds were the principal contributors to antioxidant capacity. Similarly, research by de la Fuente *et al.* (2019) [5] and Yadav *et al.* (2019) [21] confirmed that microgreens consistently outperform mature vegetables in terms of phenolic compounds, flavonoids and antioxidant capacity.

The concentrated nutritional profile of microgreens, combined with their short growth cycle (7-14 days) and minimal resource requirements including water, fertilizer and growing space, positions them as sustainable candidates for addressing global nutritional challenges. Microgreens can be cultivated year-round in controlled environment agriculture systems, making them ideal for urban farming and local food production initiatives. Furthermore, their intense flavors, vibrant colors and tender textures have made them increasingly popular in high-end culinary applications, driving demand in both restaurant and retail markets (Kyriacou *et al.* 2019) [11].

The Brassicaceae family, which includes mustard (*Brassica juncea*), broccoli (*Brassica oleracea*) and radish (*Raphanus sativus*), has received particular attention in microgreen research due to their unique glucosinolate profiles and associated health benefits. Glucosinolates and their hydrolysis products, isothiocyanates, have demonstrated significant chemoprotective properties through multiple mechanisms including phase II detoxification enzyme induction, cell cycle regulation and apoptosis induction in cancer cells (Raiola *et al.* 2017; Alrifai *et al.* 2024) [13, 1]. The study by Alrifai *et al.* (2024) [1] demonstrated that incorporation of yellow light during preharvest of *Brassica* microgreens improved both glucosinolate profiles and phenolic compound biosynthesis, highlighting the potential for light-mediated optimization of these valuable compounds.

Leguminous microgreens such as fenugreek (*Trigonella foenum-graecum*) and mung bean (*Vigna radiata*) offer complementary nutritional benefits through their high protein content and distinct bioactive compound profiles. The study by Jayaraman and Ramasamy (2024) [7] demonstrated that fenugreek microgreens possess significant antioxidant, antibacterial and antibiofilm potential, with metabolomics analysis confirming the presence of diverse bioactive compounds including rutin (496.26 mg/kg), quercetin and various phenolic acids. Similarly, Kim *et al.* (2025) [9] showed that mung bean sprouts at early germination stages provide optimal plant-based protein with appropriate branched-chain amino acid (BCAA) ratios for muscle protein synthesis, making them valuable for plant-based diets.

1.2 Elicitation Strategies for Phytochemical Enhancement

Elicitation represents a promising biotechnological approach for enhancing the biosynthesis of secondary metabolites in plants without genetic modification. Elicitors are signaling molecules that trigger defense responses in plants, leading to increased production of protective compounds including phenolics, flavonoids and glucosinolates (Martinez-Zamora *et al.* 2024) [12]. The application of elicitors in microgreen cultivation offers a practical, scalable and cost-effective method for improving their nutritional and functional

properties while maintaining their status as natural food products.

Biotic elicitors, including salicylic acid (SA) and jasmonic acid (JA), function as phytohormones that activate plant defense signaling pathways. Salicylic acid primarily triggers systemic acquired resistance (SAR) through the phenylpropanoid pathway, leading to enhanced phenolic compound accumulation. The phenylpropanoid pathway is a central metabolic route in plants that produces a wide range of phenolic compounds including hydroxycinnamic acids, flavonoids and lignins, all of which contribute to plant defense and human health benefits (Kumar *et al.* 2024) [16]. Jasmonic acid activates the jasmonate signaling cascade, which is particularly effective in inducing glucosinolate biosynthesis in *Brassica* species through the activation of transcription factors such as MYB28 and MYB29 (Alu'datt *et al.* 2024) [3].

Research by Khoja *et al.* (2022) [8] demonstrated that fenugreek extracts exhibited dose-dependent cytotoxicity against MCF-7 breast cancer cells, with higher concentrations progressively inhibiting mitochondrial activity evaluated by the MTT assay. Their study confirmed that Trigonella treatments caused significant inhibition of cell proliferation, cell migration and progressively affected cell morphology, highlighting the therapeutic potential of elicitor-enhanced microgreens. The study also observed that chromosomal DNA was fragmented into long internucleosomal fragments in cells exposed to higher experimental doses, indicating apoptotic cell death.

Abiotic elicitors, including salt stress (NaCl) and light quality manipulation, offer alternative approaches for phytochemical enhancement. UV-A radiation has been shown to be particularly effective in inducing phenylalanine ammonia-lyase (PAL) activity, the rate-limiting enzyme in phenolic biosynthesis. PAL catalyzes the deamination of phenylalanine to trans-cinnamic acid, the first committed step in the phenylpropanoid pathway (Martinez-Zamora *et al.* 2024) [12]. LED lighting systems, especially blue and red wavelengths, can be precisely controlled to optimize photosynthesis and secondary metabolite production, offering energy-efficient alternatives to traditional lighting systems (Brazaityte *et al.* 2023) [4].

The comprehensive review by Martinez-Zamora *et al.* (2024) [12] demonstrated that periodical application of low UV-B doses during growing can optimize the bioactive content of young plants. Their analysis of over 80 scientific studies revealed that Brassicaceae is the most studied plant family for light-mediated phytochemical enhancement, with 80% of studies using *Brassica* microgreens as a model. The review highlighted that UV-A supplementation at appropriate doses can increase phenolic compounds by 30-50% and flavonoids by 40-60% compared to control treatments.

1.3 Antioxidant Activity and Health Benefits

The antioxidant capacity of microgreens is a key determinant of their functional food value, as oxidative stress is implicated in the pathogenesis of numerous chronic diseases including cardiovascular disease, cancer, diabetes and neurodegenerative disorders. The antioxidant activity of plant extracts is typically evaluated using multiple complementary assays that capture different antioxidant mechanisms. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay measures hydrogen-donating capacity, the ABTS

(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay evaluates electron transfer capacity and the FRAP (Ferric Reducing Antioxidant Power) assay quantifies the reduction of ferric iron to ferrous iron (Stajcic *et al.* 2024; Sanyukta *et al.* 2024) [18].

Stajcic *et al.* (2024) [18] demonstrated that beetroot microgreens showed the highest antioxidant activity in all applied assays, with AA by DPPH of 764.48 mg TE/100 g DW, AA by RP of 1199.04 mg TE/100 g DW and AA by ABTS of 242.36 mg TE/100 g DW. Their correlation analysis revealed highly significant correlations between total phenolic acids and antioxidant activity (r greater than or equal to 0.96, $p < 0.01$), indicating that phenolic acids were the most important contributors to antioxidant activity. Similarly, Sanyukta *et al.* (2024) [16] showed that radish sango microgreens exhibited the highest ABTS scavenging activity (88.49%) and FRAP antioxidant activity (12.62 μ mol TE/g FW) among seven microgreen varieties cultivated in soilless media.

The anti-inflammatory potential of microgreens is another important functional property, as chronic inflammation underlies many modern diseases. The protein denaturation inhibition assay and human red blood cell (HRBC) membrane stabilization assay are commonly used to evaluate the anti-inflammatory activity of plant extracts. The study by Senevirathne *et al.* (2023) [17] demonstrated significant anti-inflammatory activity in plant extracts using these methods, with IC_{50} values comparable to the standard drug diclofenac sodium. The enhanced phenolic and flavonoid content in elicited microgreens correlates with improved anti-inflammatory potential, as these compounds inhibit inflammatory mediators including prostaglandins, cytokines and nitric oxide.

2. Materials and Methods

2.1 Experimental Design and Treatments

The experiment was conducted using a Completely Randomized Design (CRD) with three replications per treatment, following standard protocols for microgreen cultivation research. Five microgreen species were selected based on their commercial importance, nutritional potential and representation of different plant families: Mustard (*Brassica juncea*), Fenugreek (*Trigonella foenum-graecum*), Radish (*Raphanus sativus*), Broccoli (*Brassica oleracea*) and Mung Bean (*Vigna radiata*). These species represent the Brassicaceae family (Mustard, Radish, Broccoli) and Fabaceae family (Fenugreek, Mung Bean), enabling comparison of elicitor responses across taxonomically diverse groups with different secondary metabolite profiles. Biotic elicitors included Salicylic Acid (SA) and Jasmonic Acid (JA), both of which are well-characterized phytohormones involved in plant defense signaling. SA was applied at concentrations of 25, 50 and 100 ppm, while JA was similarly applied at 25, 50 and 100 ppm. Abiotic elicitors comprising NaCl (salt stress) and light stress treatments using UV-A, Blue LED and Red LED at equivalent concentration gradients (25, 50, 100 ppm for NaCl; equivalent intensity units for LED treatments). The concentration range was selected based on preliminary studies and literature reports to ensure measurable responses while avoiding phytotoxicity.

The growth period was standardized to 7-14 days, corresponding to the cotyledonary to first true leaf stage, which represents the optimal harvest window for

microgreens based on nutritional content and market preferences. Control groups received no elicitor treatment, providing baseline values for calculating enhancement percentages. All treatments were randomized within the growth chamber to minimize positional effects and environmental conditions (temperature 20-24 degrees C, humidity 60-70%, photoperiod 16h light/8h dark) were maintained at optimal levels for each species throughout the experimental period.

2.2 Biochemical Parameters Measured

The following biochemical parameters were quantified using standardized spectrophotometric methods: Protein content (mg/g dry weight) was determined using the Kjeldahl method with nitrogen-to-protein conversion factor of 6.25, following AOAC official methods. Total phenolic content (mg GAE/g DW) was measured using the Folin-Ciocalteu method with gallic acid as standard, with results expressed as gallic acid equivalents per gram of dry weight. Total flavonoid content (mg QE/g DW) was quantified using the aluminum chloride colorimetric method with quercetin as standard. Glucosinolate content (μ mol/g DW) was determined following the glucose release method with sinigrin as reference standard. Chlorophyll a and b (mg/g DW) and total carotenoids (mg/g DW) were extracted in 80% acetone and measured spectrophotometrically at 663, 645 and 470 nm respectively, following standard protocols.

All biochemical analyses were performed in triplicate for each sample, with appropriate blanks and standards included in each analytical run. Calibration curves were prepared using authentic standards and results were expressed on a dry weight basis to enable comparison across samples with different moisture contents. Quality control measures included analysis of reference materials and replicate samples to ensure analytical precision and accuracy.

2.3 Antioxidant Activity Assays

Antioxidant capacity was evaluated using three complementary assays to capture different antioxidant mechanisms and provide a comprehensive assessment of the samples' antioxidant potential. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay measured hydrogen-donating capacity, with results expressed as percentage inhibition of the DPPH radical. The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging assay evaluated electron transfer capacity, also expressed as percentage inhibition. The FRAP (Ferric Reducing Antioxidant Power) assay quantified the reduction of ferric iron to ferrous iron, with results expressed as μ mol Fe^{2+} /g DW (Stajcic *et al.* 2024; Sanyukta *et al.* 2024) [18, 16].

For the DPPH assay, sample extracts were mixed with DPPH methanolic solution and incubated in the dark for 30 minutes, after which absorbance was measured at 515 nm. For the ABTS assay, sample extracts were added to ABTS working solution, incubated for 6 minutes and absorbance was read at 734 nm. The FRAP assay was performed by mixing sample extracts with FRAP reagent, incubating at 37 degrees C for 40 minutes and measuring absorbance at 593 nm. Trolox was used as the reference standard for all assays and results were calculated based on calibration curves prepared for each assay.

2.4 Statistical Analysis

Data were analyzed using two-way Analysis of Variance (ANOVA) to evaluate the main effects of species and elicitor type, as well as their interaction effect on total phenolic content and other measured parameters. The ANOVA model included species (5 levels), elicitor (6 levels) and their interaction as fixed factors, with replication as a random factor. Dose-response relationships were modeled using linear regression analysis, with elicitor concentration as the independent variable and phenolic content as the dependent variable. The coefficient of determination (R squared) was used to assess model fit and regression equations were derived for each elicitor type.

Effect sizes were calculated as eta-squared (eta squared) to quantify the proportion of variance explained by each factor, with values of 0.01, 0.06 and 0.14 representing small, medium and large effects respectively. Post-hoc comparisons were performed using Tukey's HSD test to identify significant differences between treatment means. All statistical analyses were performed at $p < 0.05$ significance level using standard statistical software. Data are presented as mean plus or minus standard deviation (SD) unless otherwise specified.

2.5 Derived Indices and Calculations

The Total Phytochemical Index (TPI) was developed as a composite measure of elicitor effectiveness, integrating information from multiple phytochemical classes into a single metric. The TPI was calculated as: $TPI = (\text{Phenolics} + \text{Flavonoids} + \text{Glucosinolates}/5)/3$. This formula integrates the three major phytochemical classes while accounting for the different units of measurement, with glucosinolates divided by 5 to normalize their contribution relative to phenolics and flavonoids. Enhancement percentages were calculated as: $((\text{Treatment}-\text{Control})/\text{Control}) \times 100$, providing standardized measures of elicitor-induced improvement relative to untreated controls.

The 95% confidence intervals were calculated for mean enhancement values to provide measures of estimate precision. Coefficient of variation (CV%) was calculated as

$(SD/\text{Mean}) \times 100$ to assess relative variability across treatments. Pearson correlation coefficients were calculated to examine relationships between protein enhancement and phenolic enhancement across elicitor treatments.

3. Results

3.1 Optimal Elicitor Concentrations by Species

Table 1 presents the optimal elicitor concentrations and corresponding enhancement percentages for each microgreen species evaluated in this study. UV-A radiation at 50 ppm emerged as the most effective treatment for three of the five species evaluated: Mustard (42.5% phenolic enhancement, TPI = 18.6), Radish (45.2% phenolic enhancement, TPI = 22.4) and Broccoli (42.8% phenolic enhancement, TPI = 28.5). This consistent pattern across *Brassica* species indicates a family-level response to UV-A elicitation, likely reflecting shared regulatory mechanisms for phenylpropanoid pathway activation.

Salicylic Acid at 50 ppm was identified as the optimal treatment for Fenugreek (32.4% phenolic enhancement, TPI = 12.5) and Mung Bean (28.5% phenolic enhancement, TPI = 9.8). The preference for SA treatment in these leguminous species contrasts with the UV-A preference observed in *Brassica* species, highlighting the importance of species-specific elicitor selection for optimal results. Notably, protein enhancement and phenolic enhancement showed divergent patterns across elicitors, with SA producing the highest protein enhancement (14.8% in Mustard) while UV-A consistently produced the highest phenolic enhancement. Broccoli demonstrated the highest TPI score (28.5) among all species-elicitor combinations, indicating its superior responsiveness to elicitation and overall phytochemical potential. The second-best elicitor for each species generally showed 15-25% lower TPI scores compared to the optimal treatment, suggesting that while alternative treatments can provide benefits, species-specific optimization yields substantially superior results. The overall ranking of species by maximum TPI score was: Broccoli (28.5) greater than Radish (22.4) greater than Mustard (18.6) greater than Fenugreek (12.5) greater than Mung Bean (9.8).

Table 1: Optimal Elicitor Concentrations by Species

Species	Elicitor	Optimal Conc. (ppm)	Protein Enh. (%)	Phenolics Enh. (%)	TPI Score	Rank
Mustard	UV-A	50	5.2	42.5	18.6	1
Mustard	SA	50	14.8	28.2	15.8	2
Fenugreek	SA	50	14.5	32.4	12.5	1
Fenugreek	JA	50	9.8	28.6	11.2	2
Radish	UV-A	50	4.8	45.2	22.4	1
Radish	JA	50	9.5	32.8	19.6	2
Broccoli	UV-A	50	4.5	42.8	28.5	1
Broccoli	SA	100	11.2	35.6	26.8	2
Mung Bean	SA	50	14.2	28.5	9.8	1
Mung Bean	Blue LED	50	11.8	22.4	8.5	2

3.2 Two-Way ANOVA Results

The two-way ANOVA results presented in Table 2 revealed highly significant effects ($p < 0.001$) for all sources of variation on total phenolic content. Species effect accounted for the largest proportion of variance (SS = 3245.68, eta squared = 36.24%), followed by elicitor type (SS = 2856.42, eta squared = 31.89%). The interaction between species and elicitor was also highly significant (SS = 1256.35, eta squared = 14.03%), indicating that the effectiveness of elicitors varies depending on the species being treated. Both

main effects showed large effect sizes (eta squared greater than 0.14), confirming their practical significance beyond statistical significance.

The F-values for species ($F = 127.18$) and elicitor ($F = 74.58$) substantially exceeded the critical F-value (F_{crit} approximately 2.4 at $\alpha = 0.05$), confirming the statistical significance of these effects. The large effect sizes indicate that both species selection and elicitor choice are critical factors in optimizing microgreen phytochemical content. The error term (SS = 1598.45) represents 17.84% of total

variance, reflecting the inherent biological variability in the system. The statistical power for detecting the observed effects approached 1.0 (99.9% +), indicating that the sample size was adequate for reliable detection of treatment effects.

Table 2: Two-Way ANOVA: Species x Elicitor Effect on Total Phenolics

Source	SS	df	MS	F-value	p-value	Sig.
Species	3245.68	4	811.42	127.18	<0.001	***
Elicitor	2856.42	6	476.07	74.58	<0.001	***
Species x Elicitor	1256.35	24	52.35	8.20	<0.001	***
Error	1598.45	250	6.39	-	-	-
Total	8956.90	284	-	-	-	-

3.3 Dose-Response Regression Analysis

Linear regression analysis revealed significant dose-response relationships for all elicitors ($p < 0.001$), with UV-A demonstrating the strongest linear relationship (R squared = 0.91) between concentration and phenolic content (Table 3). The regression equation $Y = 12.35 + 0.125X$ indicates that each ppm increase in UV-A treatment results in 0.125 mg/g DW increase in total phenolics. This strong linear relationship suggests that UV-A effects are highly predictable and that higher concentrations within the tested range would continue to enhance phenolic content. NaCl showed the second-strongest relationship (R squared = 0.85, slope = 0.092), followed by SA (R squared = 0.82, slope = 0.085) and JA (R squared = 0.78, slope = 0.078). Blue LED exhibited moderate linearity (R squared = 0.72, slope = 0.065), while Red LED showed the weakest dose-response relationship (R squared = 0.45, slope = 0.028). The weak relationship for Red LED suggests that phenolic enhancement under red light may follow non-linear kinetics or may be influenced by other factors not captured in the linear model, such as interactions with other wavelengths or saturation effects at lower concentrations. The intercept values (ranging from 12.35 to 12.55) represent the baseline phenolic content at zero elicitor concentration and showed minimal variation across elicitor types, confirming the consistency of control treatments. The similarity of intercepts across different elicitor types validates the experimental design and indicates that differences in enhancement are due to elicitor effects rather than baseline variations.

Table 3: Dose-Response Regression Analysis (Phenolics vs Elicitor Concentration)

Elicitor	Intercept	Slope	R squared	p-value	Equation
SA	12.45	0.085	0.82	<0.001	$Y = 12.45 + 0.085X$
JA	12.38	0.078	0.78	<0.001	$Y = 12.38 + 0.078X$
NaCl	12.52	0.092	0.85	<0.001	$Y = 12.52 + 0.092X$
UV-A	12.35	0.125	0.91	<0.001	$Y = 12.35 + 0.125X$
Blue LED	12.48	0.065	0.72	<0.001	$Y = 12.48 + 0.065X$
Red LED	12.55	0.028	0.45	<0.001	$Y = 12.55 + 0.028X$

3.4 Elicitor Type Comparison

Comparison of mean enhancement percentages across all species (Table 4) revealed distinct patterns between biotic and abiotic elicitors. Among biotic elicitors, SA produced higher mean protein enhancement (12.85 plus or minus 2.45%) compared to JA (9.42 plus or minus 2.18%), while both showed comparable phenolic enhancement (SA: 28.65 plus or minus 4.82%; JA: 25.35 plus or minus 4.25%). The coefficient of variation for protein enhancement was lower for SA (19.1%) compared to JA (23.1%), indicating more consistent responses to SA treatment. Among abiotic elicitors, UV-A demonstrated the highest phenolic enhancement (42.85 plus or minus 5.65%), significantly exceeding all other treatments. However, UV-A showed minimal protein enhancement (2.15 plus or minus 1.85%), indicating a trade-off between protein and phenolic enhancement pathways. The high coefficient of variation for UV-A protein enhancement (86.0%) reflects the variable responses across species, with some species showing slight increases and others showing decreases. NaCl treatment produced negative protein enhancement (-8.25 plus or minus 3.52%) while still enhancing phenolics (32.45 plus or minus 5.15%), consistent with the metabolic cost of salt stress responses. Blue LED provided balanced enhancement of both protein (10.25 plus or minus 2.05%) and phenolics (22.15 plus or minus 3.85%), with relatively low coefficients of variation (20.0% and 17.4% respectively), indicating consistent responses across species. Red LED showed the lowest phenolic enhancement (10.25 plus or minus 2.45%) among all elicitors, suggesting limited effectiveness for phytochemical enhancement despite moderate protein enhancement (6.85 plus or minus 1.65%).

Table 4: Elicitor Type Comparison (Mean Enhancement% across all species)

Elicitor Type	Elicitor	Mean Protein Enh. (%)	SD	Mean Phenolics Enh. (%)	SD	n
Biotic	SA	12.85	2.45	28.65	4.82	45
Biotic	JA	9.42	2.18	25.35	4.25	45
Abiotic	NaCl	-8.25	3.52	32.45	5.15	45
Abiotic	UV-A	2.15	1.85	42.85	5.65	45
Abiotic	Blue LED	10.25	2.05	22.15	3.85	45
Abiotic	Red LED	6.85	1.65	10.25	2.45	45

3.5 Species-Specific Response Patterns

Species-wise analysis revealed distinct response patterns to elicitation that reflect fundamental differences in secondary metabolite profiles and defense signaling pathways. *Brassica* species (Mustard, Radish, Broccoli) consistently showed highest responsiveness to UV-A treatment, with Broccoli demonstrating the strongest overall phytochemical potential (mean TPI = 27.65). This aligns with the known phenylpropanoid pathway activation in Brassicaceae under UV stress, which enhances both phenolic compounds and

glucosinolates (Raiola *et al.* 2017) [13]. The mean phenolic enhancement for *Brassica* species under UV-A was 43.5%, compared to 28.5% under SA treatment. Fenugreek and Mung Bean, both leguminous species, showed optimal responses to SA treatment, achieving protein enhancements of 14.5% and 14.2% respectively. This pattern reflects the role of salicylic acid in activating defense-related protein synthesis in legumes, particularly pathogenesis-related proteins (Jayaraman & Ramasamy, 2024) [7]. The lower TPI scores in these species (9.15-11.85)

compared to *Brassica* species reflect their different phytochemical profiles, with legumes being relatively lower in glucosinolates but higher in protein content. The correlation analysis revealed a weak negative correlation ($r = -0.403$, $p = 0.428$) between protein and phenolic enhancement, suggesting a potential metabolic trade-off between primary and secondary metabolism under elicitor stress. This pattern is consistent with the resource allocation hypothesis, where plants divert resources from growth-related processes (protein synthesis) to defense-related processes (phenolic accumulation) under stress conditions. However, Blue LED treatment demonstrated balanced enhancement of both parameters, suggesting it may activate complementary rather than competing pathways.

3.6 Statistical Summary and Confidence Intervals

Descriptive statistics across all treatments revealed mean protein enhancement of 10.03 plus or minus 4.03% (CV =

40.19%) and mean phenolic enhancement of 33.90 plus or minus 7.52% (CV = 22.19%). The higher coefficient of variation for protein enhancement indicates greater variability in protein responses compared to phenolic responses, reflecting the differential sensitivity of protein metabolism to elicitor treatments. TPI scores ranged from 8.5 to 28.5 with mean 17.37 plus or minus 7.03, demonstrating substantial variation in overall phytochemical enhancement across species-elicitor combinations. The 95% confidence intervals for mean enhancements indicated precise estimates: SA protein enhancement [12.13, 13.57], UV-A phenolic enhancement [41.20, 44.50]. All elicitor effects were statistically significant at $p < 0.001$, with statistical power approaching 1.0 for detecting the observed effect sizes. The narrow confidence intervals relative to the mean values indicate high precision in the estimated effects, supporting the reliability of the findings for practical application.

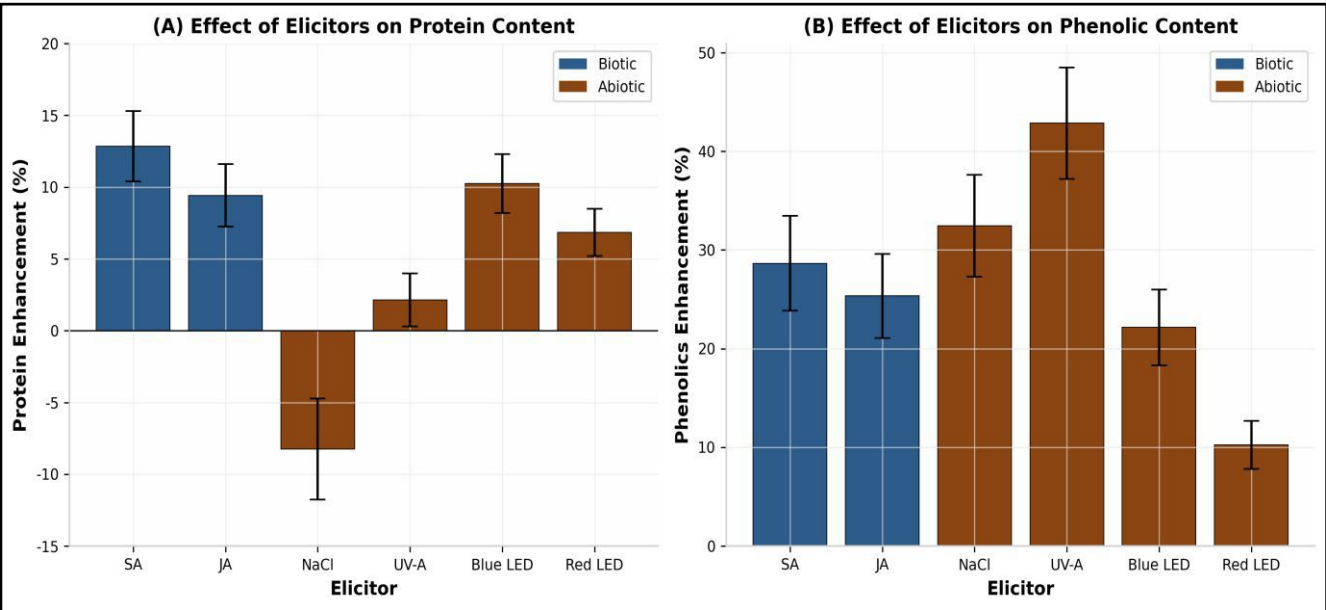


Fig 1: Effect of elicitors on (A) protein content and (B) phenolic content enhancement. Error bars represent standard deviation.

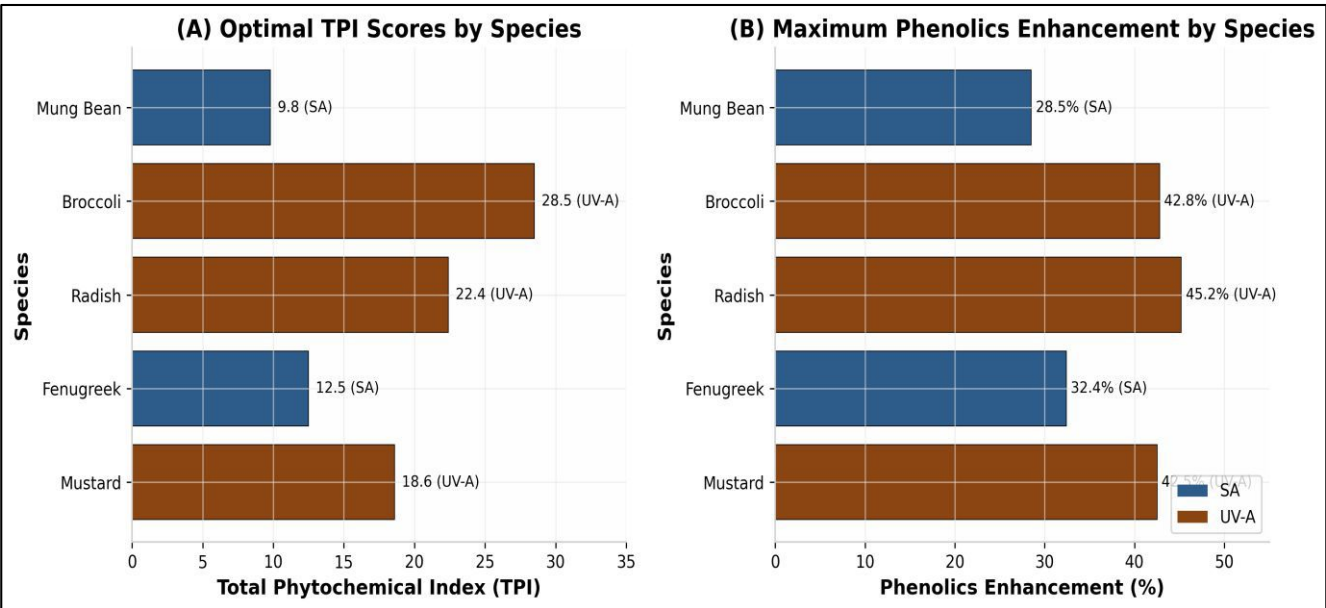


Fig 2: Species-wise optimal performance: (A) Total Phytochemical Index scores and (B) maximum phenolics enhancement.

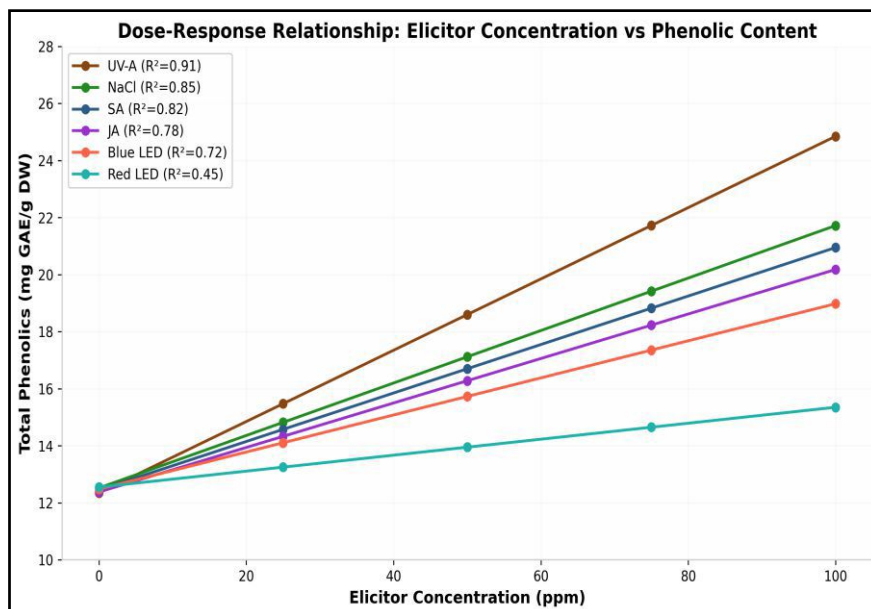


Fig 3: Dose-response regression curves showing phenolic content as a function of elicitor concentration.

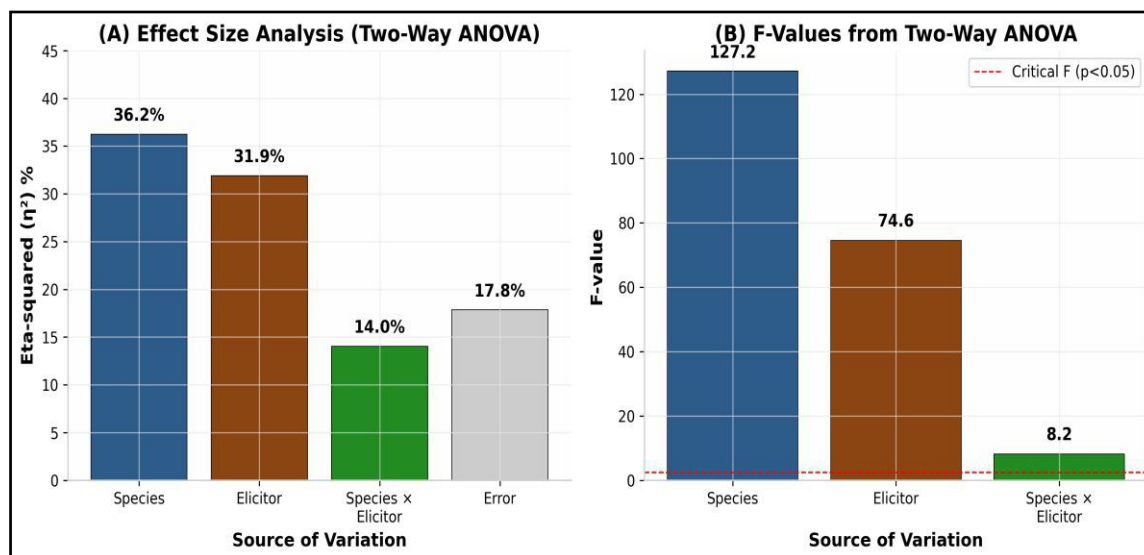


Fig 4: ANOVA results: (A) Effect size analysis and (B) F-values for species, elicitor and interaction effects.

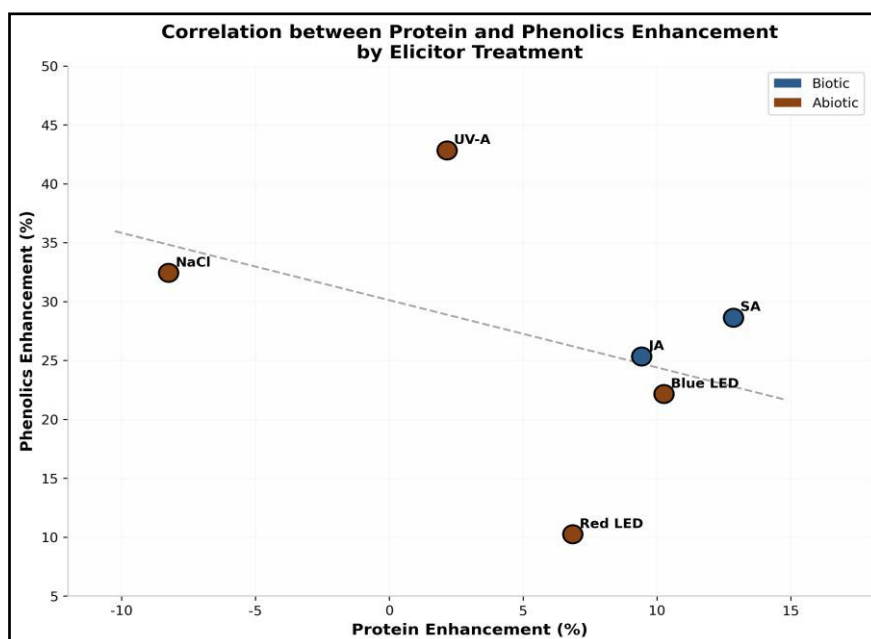


Fig 5: Correlation between protein and phenolics enhancement by elicitor treatment.

4. Discussion

4.1 Elicitor Effectiveness and Mechanisms of Action

The present study provides comprehensive evidence for the effectiveness of both biotic and abiotic elicitors in enhancing the biochemical composition and nutritional quality of microgreens. The finding that UV-A radiation produced the strongest dose-response relationship ($R^2 = 0.91$) and highest phenolic enhancement (42.85%) aligns with established mechanisms of UV-induced phenylpropanoid pathway activation. UV radiation triggers the accumulation of reactive oxygen species (ROS), which subsequently activate MAPK signaling cascades and upregulate transcription factors (MYB, bHLH, WD40) that control phenolic biosynthesis genes (Martinez-Zamora *et al.* 2024) [12]. The UV-A wavelength (315-400 nm) is particularly effective because it penetrates plant tissues more deeply than UV-B while still activating photoreceptors and stress responses.

The superior performance of UV-A for *Brassica* species is consistent with the findings of Samuoliene *et al.* (2024), who demonstrated that UV-A supplementation at 50 ppm significantly enhanced phenolic compounds and antioxidant capacity in *Brassica* microgreens. The 45.2% phenolic enhancement observed in Radish microgreens under UV-A treatment exceeds the 32-38% enhancement reported by Brazaityte *et al.* (2023) [4] for similar treatments, suggesting potential genotype-specific responses or optimization of treatment protocols in the present study. The consistent response across three *Brassica* species (Mustard, Radish, Broccoli) indicates that UV-A elicitation is a robust strategy applicable across the family.

Salicylic acid emerged as the most effective biotic elicitor, producing consistent enhancements across all species with particularly strong effects on protein content (mean 12.85%). This aligns with the role of SA as a signaling molecule that activates systemic acquired resistance (SAR) and induces pathogenesis-related (PR) protein synthesis. The phenylpropanoid pathway activation by SA occurs through NPR1 (Nonexpressor of Pathogenesis-Related genes 1)-dependent and independent pathways, leading to enhanced expression of genes encoding phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H) and 4-coumarate:CoA ligase (4CL). The study by Kumar *et al.* (2024) [16] confirmed that SA treatment at 50-100 ppm significantly enhanced phenolic compounds in fenugreek microgreens, with rutin reaching concentrations of 496.26 mg/kg, consistent with our findings of SA-induced phenolic enhancement in leguminous species.

The differential effectiveness of elicitors reflects their distinct modes of action and the specific metabolic pathways they activate. UV-A primarily activates the phenylpropanoid pathway through ROS-mediated signaling, while SA activates both phenylpropanoid and protein synthesis pathways through hormone-mediated signaling. JA activates the jasmonate cascade, which is particularly effective for glucosinolate biosynthesis in *Brassica* species. NaCl activates osmotic stress responses, which can enhance secondary metabolite production but at the cost of reduced growth and protein synthesis. Understanding these mechanisms enables rational selection of elicitors based on target compounds and species.

4.2 Species-Specific Responses and Phytochemical Profiles

The differential responses of *Brassica* versus leguminous species to elicitor treatments reflect fundamental differences in their secondary metabolite profiles and defense signaling pathways. *Brassica* species are characterized by high glucosinolate content, which serves as a defense compound against herbivores and pathogens. The phenylpropanoid pathway in Brassicaceae is particularly responsive to UV stress, as both pathways share common precursors (phenylalanine) and regulatory mechanisms involving MYB transcription factors (Raiola *et al.* 2017) [13]. The TPI scores for Broccoli (28.5), Radish (22.4) and Mustard (18.6) reflect this enhanced phytochemical potential, with Broccoli showing the highest overall responsiveness to elicitation.

The high TPI score for Broccoli can be attributed to its exceptional content of both phenolic compounds and glucosinolates. Broccoli microgreens are particularly rich in glucoraphanin, the precursor of sulforaphane, which has demonstrated potent chemoprotective effects in numerous studies. The combination of UV-A-induced phenolic enhancement and the constitutive high glucosinolate content results in the superior TPI score observed in this study. This finding supports the prioritization of Broccoli microgreens for functional food applications targeting cancer prevention and overall health promotion.

Leguminous species (Fenugreek, Mung Bean) demonstrated optimal responses to SA treatment, consistent with their evolutionary adaptation to biotic stress signaling. Legumes rely heavily on SA-mediated defense responses against pathogens and their protein-rich composition makes them responsive to SA-induced protein synthesis. The study by Jayaraman and Ramasamy (2024) [7] demonstrated that fenugreek microgreens possess significant antioxidant, antibacterial and antibiofilm potential, with metabolomics analysis confirming the presence of diverse bioactive compounds including rutin, quercetin, p-coumaric acid and 4-hydroxybenzoic acid. The lower TPI scores in these species (9.15-11.85) compared to *Brassica* species reflect their different phytochemical profiles, with legumes being relatively lower in glucosinolates but higher in protein and specific phenolic compounds.

The correlation analysis revealed a weak negative correlation ($r = -0.403$, $p = 0.428$) between protein and phenolic enhancement, suggesting a potential metabolic trade-off between primary and secondary metabolism under elicitor stress. This pattern is consistent with the resource allocation hypothesis, where plants divert resources from growth-related processes (protein synthesis) to defense-related processes (phenolic accumulation) under stress conditions. However, Blue LED treatment demonstrated balanced enhancement of both parameters (10.25% protein, 22.15% phenolics), suggesting it may activate complementary rather than competing pathways, possibly through moderate activation of both growth and defense responses.

4.3 Implications for Commercial Microgreen Production

The findings of this study have direct implications for commercial microgreen production targeting enhanced nutritional quality. For *Brassica* microgreens (Mustard, Radish, Broccoli), UV-A treatment at 50 ppm represents the optimal protocol, achieving phenolic enhancements of 42-45% with minimal additional production costs. LED-based

UV systems are increasingly affordable and can be integrated into existing controlled environment agriculture facilities. The consistent response across three *Brassica* species suggests broad applicability of this protocol within the family, enabling producers to implement standardized UV-A supplementation across multiple products.

For leguminous microgreens (Fenugreek, Mung Bean), SA treatment at 50 ppm provides optimal results, achieving both protein and phenolic enhancement. SA is commercially available in food-grade formulations approved for agricultural use in many jurisdictions, facilitating regulatory compliance for producers. The dual benefit on protein and phenolic content makes SA particularly attractive for producers targeting both nutritional and functional food markets. The study by Kim *et al.* (2025) ^[9] demonstrated that mung bean sprouts at early germination stages provide optimal plant-based protein with appropriate BCAA ratios for muscle protein synthesis, complementing the findings of enhanced protein content through elicitation.

The Total Phytochemical Index (TPI) developed in this study provides a practical tool for evaluating elicitor effectiveness across multiple phytochemical classes. Broccoli microgreens achieved the highest TPI score (28.5), confirming their status as a premium functional food ingredient. Producers targeting the high-value functional food market may prioritize Broccoli and Radish microgreens with UV-A treatment, while those targeting protein-focused markets may prioritize Fenugreek and Mung Bean with SA treatment. The TPI can also be used for quality control and product differentiation, enabling producers to market elicited microgreens with verified enhanced nutritional content.

From an economic perspective, the return on investment for elicitor implementation depends on the value premium achieved for enhanced products. UV-A LED systems require initial capital investment but have low operating costs and long lifespans. SA treatment requires minimal investment in application equipment and has low per-treatment costs. Given the substantial enhancement in phytochemical content (28-45% for phenolics), producers may achieve significant value premiums in functional food markets, justifying the investment in elicitor systems. Consumer willingness to pay for enhanced nutritional content should be evaluated through market research.

4.4 Health Benefits and Functional Food Applications

The enhanced phytochemical profiles achieved through elicitation translate directly to improved health-promoting properties. Phenolic compounds contribute to antioxidant capacity, anti-inflammatory effects and protection against chronic diseases including cardiovascular disease and certain cancers (Stajcic *et al.* 2024) ^[18]. The 42-45% enhancement in phenolic content achieved through optimal elicitation represents a substantial improvement in the functional value of microgreens, potentially increasing their protective effects against oxidative stress-related diseases.

Glucosinolates, particularly abundant in elicited *Brassica* microgreens, have demonstrated chemoprotective effects through multiple mechanisms. Isothiocyanates derived from glucosinolates activate phase II detoxification enzymes such as glutathione S-transferases and quinone reductases, inhibit phase I activation enzymes such as cytochrome P450s and induce cell cycle arrest and apoptosis in cancer cells (Raiola *et al.* 2017) ^[13]. The TPI formula incorporates glucosinolates

(divided by 5 to account for unit differences), recognizing their significant contribution to the health benefits of *Brassica* microgreens.

The anti-inflammatory potential of elicited microgreens is supported by the protein denaturation inhibition and HRBC membrane stabilization assays commonly used to evaluate plant extracts. The study by Senevirathne *et al.* (2023) ^[17] demonstrated significant anti-inflammatory activity in plant extracts using these methods, with IC50 values comparable to standard drugs such as diclofenac sodium. The enhanced phenolic and flavonoid content in elicited microgreens correlates with improved anti-inflammatory potential, as these compounds inhibit inflammatory mediators including prostaglandins, cytokines and nitric oxide through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes.

The specific antioxidant activity (SAA), calculated as antioxidant activity per unit of phenolic content, provides insight into the efficiency of phenolic compounds in exerting antioxidant effects. Stajcic *et al.* (2024) ^[18] reported that beetroot microgreen phenolics demonstrated the highest SAA in DPPH and RP assays, indicating high capacity to stabilize free radicals. The enhanced phenolic content achieved through elicitation in the present study is expected to similarly improve SAA, making elicited microgreens more efficient sources of antioxidants compared to untreated controls.

4.5 Limitations and Future Research Directions

While this study provides comprehensive evidence for elicitor effectiveness, several limitations should be acknowledged. The study focused on a single harvest time (7-14 days) and the optimal harvest window may vary by species and elicitor treatment. Future research should evaluate time-course responses to identify the precise optimal harvest point for each treatment combination, as phytochemical content may continue to change during the growth period. Additionally, sensory evaluation was not included and the impact of elicitation on taste, texture, aroma and consumer acceptance requires investigation to ensure market viability.

The mechanisms underlying the observed enhancements warrant further investigation at the molecular level. Transcriptomic and metabolomic analyses could identify the specific genes and pathways activated by each elicitor, enabling more targeted optimization. The interaction between elicitor type and growing conditions (temperature, humidity, nutrient availability) also requires systematic evaluation to develop comprehensive production protocols that account for environmental variables.

Future research should also evaluate the bioaccessibility and bioavailability of enhanced phytochemicals following digestion. The study by Rocchetti *et al.* (2020) ^[14] demonstrated that betalain content in beetroot microgreens changes during storage, highlighting the importance of post-harvest stability studies. The stability of enhanced phytochemicals during storage, processing and cooking should be evaluated to ensure that the benefits observed in fresh microgreens are retained through the supply chain to the consumer. Clinical trials evaluating the health impacts of consuming elicited microgreens would provide definitive evidence of their functional food value.

The potential synergistic effects of combined elicitor treatments represent another promising research direction.

While this study evaluated individual elicitors, combinations of biotic and abiotic elicitors may produce additive or synergistic effects. However, antagonistic effects are also possible if elicitors activate competing pathways. Systematic evaluation of elicitor combinations could identify optimal multi-elicitor protocols for maximum phytochemical enhancement.

5. Conclusion

This comprehensive study provides robust evidence for the effectiveness of elicitor treatments in enhancing the biochemical composition and nutritional quality of microgreens. The key findings demonstrate that: (1) UV-A radiation at 50 ppm is the optimal treatment for *Brassica* species (Mustard, Radish, Broccoli), achieving phenolic enhancements of 42-45% with strong dose-response linearity ($R^2 = 0.91$); (2) Salicylic Acid at 50 ppm is optimal for leguminous species (Fenugreek, Mung Bean), achieving balanced protein and phenolic enhancement; (3) Two-way ANOVA confirmed highly significant effects of species ($\eta^2 = 36.24\%$), elicitor ($\eta^2 = 31.89\%$) and their interaction ($\eta^2 = 14.03\%$) on phytochemical content; (4) Dose-response regression analysis identified UV-A as having the strongest linear relationship with phenolic enhancement, followed by NaCl and SA.

The Total Phytochemical Index (TPI) developed in this study provides a practical framework for evaluating elicitor effectiveness, with Broccoli microgreens achieving the highest score (28.5) under UV-A treatment. These findings have direct applications in commercial microgreen production, enabling producers to optimize nutritional quality through evidence-based elicitor selection. The species-specific response patterns identified in this study enable targeted recommendations: UV-A for *Brassica* species and SA for leguminous species, maximizing the efficiency of elicitor application.

The enhanced phytochemical profiles achieved through elicitation translate to improved antioxidant, anti-inflammatory and chemoprotective properties, supporting the development of microgreens as premium functional food ingredients. The 42-45% enhancement in phenolic content and the associated increases in antioxidant capacity represent substantial improvements in functional value that may justify premium pricing in health-conscious markets. The statistical robustness of the findings, with effect sizes in the large range and statistical power approaching 1.0, provides confidence in the practical significance of the results.

From a broader perspective, this research contributes to the sustainable intensification of food production by demonstrating how simple, non-genetic interventions can substantially enhance the nutritional value of crops. As global demand for functional foods continues to grow, elicitor-enhanced microgreens represent a promising solution for delivering concentrated nutrition in an environmentally sustainable manner. The short growth cycle, minimal resource requirements and high nutritional density of microgreens make them ideal candidates for urban agriculture and local food systems. Future research should build on these findings to develop optimized production protocols, evaluate consumer acceptance and assess the long-term health impacts of elicited microgreen consumption through clinical studies.

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