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Enhancing nutrient bioavailability by balancing antioxidant and antinutritional properties in organic tomatoes using *Kappaphycus alvarezii* based biostimulants

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Abstract

Organic farming has gained increasing attention for its sustainability and health benefits, particularly in crops like tomatoes, which are essential due to their high consumption and nutritional value. This study investigates the effects of *Kappaphycus alvarezii* (red seaweed) biostimulants on the nutritional composition, antioxidant content, mineral bioavailability, and antinutritional factors of organically grown tomatoes. This study evaluated the impact of cow urine extracted seaweed (SWC) at 10%, 5% and water extracted seaweed (SWW) at 10% biostimulants on fruit quality. Results revealed significant improvements in the proximate composition of tomatoes, with SWC 10% leading in moisture, protein, fiber, and carbohydrate content. Mineral nutrient analysis showed SWC 10% achieving higher levels of essential minerals like calcium (152.28 mg/100 g), magnesium (41.44 mg/100 g), potassium (563.21 mg/100 g), iron (15.32 mg/100 g), and zinc (0.86 mg/100 g) compared to the control. The biostimulants also boosted antioxidants, particularly vitamin C, carotenoids, and lycopene, with SWC 10% outperforming other treatments. Additionally, SWC reduced antinutritional factors, such as phytates, oxalates, and tannins. Notably, SWC 10% reduced phytates to 98.36 mg/100 g from 129.47 mg/100 g in the control. Also, the mineral bioavailability was enhanced by keeping the phytate : Ca, phytate : Fe, phytate : Zn and phytate*Ca : Zn ratios below critical limits (0.24, 1.0, 18.0, and 200, respectively). Additionally, tomatoes treated with SWC 10% exhibited improved marketable qualities, including total soluble solids (6.76%), titrable acidity (0.59%), taste index (1.16), and fruit firmness (1.98 kgf/cm²). The study highlights the potential of *K. alvarezii* derived biostimulants to enhance the nutritional and marketable qualities of organic tomatoes, contributing to improved human health outcomes besides promoting sustainable agriculture practices.

1. Introduction

In recent years, organic farming has gained prominence as a key approach to sustainable agriculture, recognized for its benefits to both the environment and human health. Among organic crops, tomatoes are particularly important due to their high consumption and associated health benefits. Organic tomato cultivation, which avoids synthetic chemicals, pesticides, and genetically modified organisms (GMOs), is crucial for promoting human health through improved nutritional quality. The nutritional composition, antioxidants, antinutritional qualities, and marketability of tomatoes varied depending on the method of cultivation, growing conditions such as climate, soil type, and inputs used (Bourn and Prescott, 2002; Ali *et al.*, 2021). Research shows that organically grown tomatoes often contain higher levels of vitamins, minerals, and antioxidants such as lycopene compared to conventionally grown varieties (Worthington, 2001; Lairon, 2010). Additionally, organic tomatoes minimize consumer exposure to harmful pesticide residues,

which are linked to risks like cancer, hormonal disruptions and reproductive issues (Aktar *et al.*, 2009). Enhanced levels of carotenoids, essential phytochemicals and a reduction in heavy metals and pesticide residues were noted in organically grown tomatoes (Ilic *et al.*, 2012). A significant increase in the quality of tomato fruits, including titrable acidity, soluble salts, vitamin C and phenolic compounds, was observed with organic farming (Oliveira *et al.*, 2013).

A promising crop growth promoting input in organic farming is red seaweed, particularly species like *K. alvarezii*. Red seaweed is rich in bioactive compounds such as polysaccharides, phytohormones and essential minerals (Raghunandan *et al.*, 2019; Nivetha *et al.*, 2024), making it an ideal candidate for organic tomato cultivation. These compounds contribute to enhanced plant growth, improved nutrient uptake and increased resilience to environmental stressors such as drought and salinity (Hernandez-Herrera *et al.*, 2022; Mughunth *et al.*, 2024). The growth-promoting hormones in *K. alvarezii*, including auxins, gibberellins, and cytokinins, support higher yields and better fruit quality, while its natural disease resistance reduces the need for chemical inputs, aligning with organic farming principles (Shukla *et al.*, 2019; Zodape *et al.*, 2011). Thus, the present study investigated the use of *K. alvarezii* based biostimulants, focusing on their effects on nutritional composition, antioxidants, antinutritional factors of organically grown tomatoes and mineral bioavailability, contributing to improved fruit quality and human health.

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2. Materials and Methods

2.1 Experimental details

A pot culture experiment was conducted with the tomato (*Solanum lycopersicum* L.) hybrid 'Shivam' as a test species (Authentication No. BSI/SRC/5/23/2024-25/Tech./431) from October 2023 to February 2024. The study took place in a green shade net house at the Nammazhvar Organic Farming Research Centre, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. Soil was collected from an organic tomato field in Kuppanur village, processed, and used in a completely randomized design with three replications. Each pot was filled with 5 kg of soil, compacted to a field bulk density of 1.35 Mg/m³, and mixed with 63 g of farmyard manure (FYM), then irrigated to field capacity. Four-week-old organically grown tomato seedlings were transplanted, one per pot, and managed without synthetic inputs. Vermicompost at 12.5 g per

pot was applied on the day of transplanting, following TNAU's recommended organic tomato cultivation practices.

K. alvarezii was collected from Mandapam coastal waters, Ramanathapuram, India. It was cleaned, air-dried for a week, and then oven-dried at 60 ± 2°C. The dried seaweed was ground into powder. Two liquid biostimulants, SWC (Cow urine-extracted seaweed) and SWW (Water-extracted seaweed), were prepared by soaking the powder in a 1:50 mixture of cow urine and de-ionized water for 72 h. After filtering and centrifuging, the supernatant was tested on organic tomatoes at 5% and 10% concentrations, with water spray as a control. Sprays were applied at 30, 45, and 60 days after transplanting. At harvest, 500 g of representative fruits from all treatments, including the control, were selected for analysis. The nutrient composition of soil, water, FYM, vermicompost, RSW, and concentrated biostimulants is shown in Table 1.

Table 1: Composition of inputs used for growing tomato organically under pot experiment

Minerals	Soil applied inputs					Concentrated biostimulants	
	Experimental soil	Irrigation water	Farm yard manure	Vermicompost	Cocopeat	SWC	SWW
pH	7.01	6.94	7.36	7.15	6.80	8.47	5.91
EC (dS m ⁻¹)	0.37	0.43	9.04	0.83	0.79	53.20	2.97
Ca (mg/kg)	533.88	ND	380	410	349.8	110.10	30.01
Mg (mg/kg)	110.70	ND	220	280	118.6	960.0	100.12
K (mg/kg)	208 (kg/ha)	80.04	14300	17800	719.1	18300	1240
Fe (mg/kg)	153.7	0.30	5149	876.61	16.20	97.5	5.50
Zn (mg/kg)	21.9	0.48	98.90	85.21	1.49	6.20	0.20

2.2 Proximate composition and mineral nutrients analysis

The proximate composition of organically grown tomatoes *viz.*, ash content, crude fiber, and carbohydrates was analyzed (Abdullahi *et al.*, 2016). Crude protein was derived from nitrogen content. Mineral nutrient content of tomato fruits was quantified by digesting a known weight of sample using a triple acid mixture of nitric, sulfuric and perchloric acids. The digested clear solution was diluted with 1% nitric acid, filtered by Whatman No.42 filter paper and subjected to analysis after volume adjustment (AOAC, 1990). The nutrient elements such as calcium, magnesium, potassium, iron and zinc were analyzed using an ICP-AES (Thermo Fisher, 7000 series).

2.3 Quantification of antioxidants and antinutritional factors in tomato

Representative tomato fruits were sampled from each treatment replication after sub-sampling from the crushed pieces using a pestle and mortar. The sub samples were analyzed for quality attributes such as ascorbic acid content, lycopene content and beta-carotene, following the methods of Ranganna (1986), Negi and Roy (2000), and Sharma and Nautiyal (2009), respectively.

Phytate content was determined adopting method established by Young and Greaves (1940). Oxalate content was measured following the procedure outlined by Adeniyi *et al.* (2009) while tannin content was analyzed according to the method described by Makkar and Goodchild (1996). The method of Bohm and Kocipal-Abyazan

(1994) was used to assess flavonoid content, and phenolic content was estimated according to the standard protocol outlined by Singleton (1999).

2.4 Profiling flavonoids and phenolic acids in organically grown tomatoes

The bioactive compounds in tomatoes were determined by total ion chromatography (TIC) analysis, using LCMS (Shimadzu UFLC-LC-20 AD) with an electrospray ionization detector. A reversed-phase C18 column (4.6 mm × 250 mm, 5 µm) was used for separation, maintaining the column at 35°C, with a 10 µl sample injection. Chromatography was performed for 20 min with an m/z range of 100-1000. Compounds were eluted using a mobile phase of 0.1% formic acid in water (solution A) and methanol (solution B) in gradient mode (5% B for 2 min, increasing to 90% over 10 min, returning to 5% B by 15 min, and holding for 5 min). The flow rate was 0.2 ml/min using a binary pump. Mass spectrometry was conducted in both positive and negative ionization mode with parameters: drying gas flow at 17 l/min, nebulizing gas flow at 3 l/min, and a total flow rate of 0.7 µl/min. Compound identification was achieved by comparing the obtained mass spectra with the Plant Metabolite Database (PmDB).

2.5 Minerals bioavailability assessment

Estimate the relative bioavailability of essential dietary nutrients *viz.*, iron, zinc and calcium, the molar ratios of phytate to each

mineral were calculated by the formula given by FAO/IZiNCG (2018):

Minerals bioavailability =

$$\frac{\text{Phytate content (mg)} / \text{molecular weight of phytate}}{\text{Mineral content (mg)} / \text{atomic weight of mineral}}$$

The phytate to mineral molar ratios calculated can be used to predict the inhibitory effect of the antinutrient on the mineral bioavailability.

2.6 Marketable qualities assessment

To assess the marketable quality of organically grown tomato parameters give the firmness, titrable acidity and taste of fruits were measured. While the tomato fruit firmness was determined with the aid of a fruit penetrometer, the titrable acidity was determined by the method of Sharma and Nautiyal (2009). The total soluble solid was determined with the standard protocol outlined by Mazumdar and Majumder (2003). The taste index was calculated as suggested by Ilic *et al.* (2014) using the titrable acidity and total soluble solids values.

2.6 Statistical analysis

The results were presented as mean values with standard error (SE) to account for variability and provide precision in estimating the population mean. SE was calculated using Microsoft Excel 2010 and was used to assess the reliability of the mean values across different experimental treatments. Data were reported in the format (\pm) and error bars were included in the graphs to represent variability across treatments.

3. Results

3.1 Proximate composition of organically grown tomatoes

The proximate composition analysis of tomatoes treated with different biostimulants showed significant variations (Table 2). Moisture content was consistently high across all treatments, with the highest level of 93.83% at SWC 10%. Crude protein was relatively low and was highest in the SWC 10%. Crude fiber was notably higher in the SWC 10% group, reaching 1.88%, potentially enhancing the health benefits of the tomatoes. The SWC 10% treatments also had the highest carbohydrate percentage at 6.73%, suggesting an increase in energy content by the applied biostimulant. Ash content remained stable across all samples, indicating consistent mineral levels.

Table 2: Proximate composition of organically grown tomatoes treated with SWC and SWW biostimulants

Composition	SWC 5%	SWC 10%	SWW 5%	SWW 10%	Control
Moisture (%)	93.09 \pm 1.60	93.83 \pm 2.32	90.74 \pm 0.76	91.16 \pm 0.48	90.27 \pm 2.02
Crude protein (%)	1.12 \pm 0.03	1.19 \pm 0.03	0.93 \pm 0.02	1.04 \pm 0.01	0.89 \pm 0.02
Crude fibre (%)	1.73 \pm 0.03	1.88 \pm 0.05	1.37 \pm 0.02	1.59 \pm 0.02	1.26 \pm 0.00
Carbohydrates (%)	6.10 \pm 0.07	6.73 \pm 0.16	5.41 \pm 0.04	5.64 \pm 0.11	5.13 \pm 0.06
Ash content (%)	0.90 \pm 0.00	0.87 \pm 0.02	0.98 \pm 0.01	0.96 \pm 0.01	0.98 \pm 0.01

\pm Standard error of replicated values; *SWC: Cowurine extracted seaweed biostimulant, SWW: Water extracted seaweed biostimulant.

The mineral nutrient analysis revealed distinct differences across treatments (Table 3). The SWC 10% treatment recorded the highest levels of all nutrients, with Ca, Mg, K, Fe, and Zn (152.28 \pm 0.32; 41.44 \pm 0.95; 563.21 \pm 2.34; 15.32 \pm 0.38 and 0.86 \pm 0.02 mg/100 g, respectively), significantly surpassing the control as 121.35 \pm 2.27; 20.09 \pm 0.30; 461.99 \pm 4.57; 7.84 \pm 0.06 and 0.72 \pm 0.02 mg/100 g, respectively. The SWC 5% treatment followed closely, offering

competitive levels of calcium (131.41 \pm 1.99 mg/100 g) and magnesium (27.62 \pm 0.89 mg/100 g). SWW treatments provided moderate mineral content, with Ca, Mg, K, Fe, and Zn contents of 130.87 \pm 3.0; 25.63 \pm 0.04; 506.54 \pm 8.70; 10.98 \pm 0.2 and 0.76 \pm 0.0 mg/100 g, respectively in SWW 10% treatment. Overall, the SWC 10% significantly enhanced the nutritional profile of tomato fruit, indicating its potential to surpass average nutrient levels.

Table 3: Composition of minerals in organically grown tomatoes treated with SWC and SWW biostimulants

Minerals	Daily dietary intake recommended (mg/100 g)	Average concentration in tomato fruit	Concentration in tomato fruit (mg/100 g) from experiment				
			SWC 5%	SWC 10%	SWW 5%	SWW 10%	Control
Ca	<1500 mg	50-100	131.41 \pm 1.99	152.28 \pm 0.32	127.55 \pm 1.33	130.87 \pm 3.0	121.35 \pm 2.27
Mg	<400 mg	10.0-15.0	27.62 \pm 0.89	41.44 \pm 0.95	21.81 \pm 0.52	25.63 \pm 0.04	20.09 \pm 0.30
K	<3500 mg	200.0-250.0	521.36 \pm 4.07	563.21 \pm 2.34	489.32 \pm 6.11	506.54 \pm 8.70	461.99 \pm 4.57
Fe	<27 mg	0.5-15	12.63 \pm 0.32	15.32 \pm 0.38	9.01 \pm 0.21	10.98 \pm 0.21	7.84 \pm 0.06
Zn	<13 mg	0.10-0.50	0.78 \pm 0.01	0.86 \pm 0.02	0.74 \pm 0.01	0.76 \pm 0.0	0.72 \pm 0.02

\pm Standard error of replicated values; *SWC: Cowurine extracted seaweed biostimulant, SWW: Water extracted seaweed biostimulant.

3.2 Antioxidants composition of organically grown tomatoes

Tomatoes are one of the most adaptable and commonly eaten vegetables globally, offering a rich supply of antioxidants like vitamin

C, β -carotenoids, lycopene, phenols and flavonoids. The analysis of tomato fruit revealed significant differences in vitamin C by both the biostimulants. The SWC 10% treatment had the highest concentration at 59.17 \pm 1.36 mg/100 g, surpassing the control (44.38 \pm 0.62 mg/

100 g) and other treatments such as SWW 10% (51.49 ± 0.95 mg/100 g) and SWC 5% (53.61 ± 0.30 mg/100 g). In terms of β -carotenoids, the SWC 10% treatment again led with 31.87 ± 0.81 mg/100 g, compared to the control (15.26 ± 0.06 mg/100 g), while SWC 5% had 23.61 ± 0.12 mg/100 g. The SWW biostimulants showed lower values (17.54 ± 0.01 and 20.91 ± 0.24 mg/100 g for SWW 5% and 10%, respectively). For lycopene, SWC 10% reached 10.26 ± 0.22 mg/100 g, significantly higher than the control (5.72 ± 0.01 mg/100 g) and other treatments, including SWC 5% (8.31 ± 0.09 mg/100 g) and SWW 10% (6.97 ± 0.03 mg/100 g). The content of flavonoids and

phenolics also demonstrated significant enhancements in due to organic biostimulants application. The SWC 10% treatment yielded the highest flavonoid concentration at 13.22 mg/100 g while the SWW treatments produced lower levels of 9.49 mg/100 g of SWW 10%. Similarly, the tomatoes received SWW 10% had the highest phenolic (1.89 mg/100 g) and followed by SWW 5% (1.36 mg/100 g). The control group consistently showed the lowest values of both flavonoids and phenols. Overall, these results highlight the effectiveness of SWC biostimulant in enhancing the antioxidant profiles of tomatoes, thereby improving their nutritional quality.

Table 4: Antioxidants contents of organically grown tomatoes treated with SWC and SWW biostimulants

Minerals	Concentration in tomato fruit (mg/100 g) from experiment				
	SWC 5%	SWC 10%	SWW 5%	SWW 10%	Control
Vitamin C(ascorbic acid)	53.61 ± 0.30	59.17 ± 1.36	47.26 ± 0.30	51.49 ± 0.95	44.38 ± 0.62
β -carotenoids	23.61 ± 0.12	31.87 ± 0.81	17.54 ± 0.01	20.91 ± 0.24	15.26 ± 0.06
Lycopene	8.31 ± 0.09	10.26 ± 0.22	6.01 ± 0.09	6.97 ± 0.03	5.72 ± 0.01
Flavanoids	11.26 ± 0.05	13.22 ± 0.08	6.38 ± 0.11	9.49 ± 0.05	4.91 ± 0.05
Phenols	2.24 ± 0.02	2.53 ± 0.03	1.36 ± 0.01	1.89 ± 0.01	1.08 ± 0.00

± Standard error of replicated values, *SWC: Cowurine extracted seaweed biostimulant, SWW: Water extracted seaweed biostimulant.

3.3 Antinutritional properties of organically grown tomatoes

In comparing the antinutritional properties of tomato fruit, the SWC 10% treatment emerged as the most effective, showing the lowest levels of phytates (98.36 ± 0.68 mg/100 g), oxalates (0.35 ± 0.01 mg/100 g) and tannins (0.11 ± 0.00 mg/100 g, significantly reducing these factors compared to the control. SWC 5% closely followed, with results similar to SWC 10%. SWW 10% demonstrated moderate effectiveness, with phytates at 121.65 ± 1.6 mg/100 g, oxalates at 0.66 ± 0.01 mg/100 g and tannins at 0.19 ± 0.00 mg/100 g higher than

SWC 10% and 5% but still lower than the control. In contrast, the control group exhibited the highest levels across all components, with phytates at 129.47 ± 1.02 mg/100 g, oxalates at 0.76 ± 0.01 mg/100 g and tannins at 0.24 ± 0.00 mg/100 g. Results showed that the SWC 10% treatment significantly reduced antinutritional factors, particularly phytate. Since all antinutritional factors were present at very low levels except for phytates, only the phytate content was selected for further bioavailability assessment studies.

Table 5: Antinutritional properties of organically grown tomatoes treated with SWC and SWW biostimulants

Antinutritional properties	Concentration in tomato fruit (mg / 100 g) from pot experiment				
	SWC 5%	SWC 10%	SWW 5%	SWW 10%	Control
Phytates	116.21 ± 2.46	98.36 ± 0.68	127.84 ± 0.39	121.65 ± 1.69	129.47 ± 1.02
Oxalates	0.42 ± 0.00	0.35 ± 0.01	0.73 ± 0.01	0.66 ± 0.01	0.76 ± 0.01
Tannins	0.17 ± 0.00	0.11 ± 0.00	0.23 ± 0.00	0.19 ± 0.00	0.24 ± 0.00

± Standard error of replicated values.

* SWC: Cowurine extracted seaweed biostimulant, SWW: Water extracted seaweed biostimulants.

Table 6: Relative ratio of phytate: nutrient of organically grown tomatoes treated with SWC and SWW biostimulants

Minerals	Phytate: Nutrient ratio in tomato fruit (mg/100 g) of pot experiment					Critical limit
	SWC 5%	SWC 10%	SWW 5%	SWW 10%	Control	
Phy : Ca	0.054 ± 0.00	0.039 ± 0.00	0.061 ± 0.00	0.056 ± 0.00	0.065 ± 0.00	< 0.24
Phy : Fe	0.78 ± 0.01	0.54 ± 0.00	1.20 ± 0.01	0.93 ± 0.01	1.40 ± 0.03	< 1.0
Phy : Zn	14.75 ± 0.05	11.32 ± 0.22	17.10 ± 0.31	15.85 ± 0.23	17.80 ± 0.11	< 18.0
Phy*Ca: Zn	48.37 ± 0.06	43.03 ± 0.85	54.44 ± 1.39	51.76 ± 1.16	53.91 ± 0.42	< 200

± Standard error of replicated values.

* SWC: Cowurine extracted seaweed biostimulant, SWW: Water extracted seaweed biostimulants.

3.4 Mineral nutrients bioavailability of organically grown tomatoes

The phytate-to-nutrient ratios in tomato were assessed alongside antinutritional properties, revealing notable trends. All treatments maintained low phytate: calcium ratios (SWC 5%: 0.054 ± 0.00 , SWC 10%: 0.039 ± 0.00 , SWW 5%: 0.061 ± 0.00 , SWW 10%: 0.056 ± 0.00 , control: 0.065 ± 0.00), well below the critical limit of 0.24, indicating minimal impact on calcium availability. For phytate: iron, SWC 10% (0.54 ± 0.00) and SWC 5% (0.78 ± 0.01) showed the lowest ratios, improving iron bioavailability compared to the control (1.40 ± 0.03), which exceeded the critical limit of 1.0. Similarly, phytate: zinc and phytate*calcium: zinc ratios were below the critical limit of 18.0 and 200, respectively with SWC 10% followed by SWC 5% showing the most favourable results, while the control approached the limit. Overall, SWC 10% effectively reduced phytate levels relative

to iron and zinc, highlighting its potential to improve mineral bioavailability in tomatoes.

3.5 Marketable qualities of organically grown tomatoes

The marketable quality of organically cultivated tomatoes showed significant variation in all factors, including TA, TSS, TI, and firmness. The application of *K. alvarezii* derived biostimulants, SWC 5% and SWC 10%, resulted in significantly higher TSS content ($5.91 \pm 0.10\%$ and $6.76 \pm 0.13\%$) and TA (0.49 ± 0.01 and $0.59 \pm 0.00\%$), ultimately increasing the taste index (TI) to 1.09 ± 0.03 and 1.16 ± 0.02 , respectively, compared to the control. The SWW biostimulants at both levels (5% and 10%) showed moderate TA and TSS content, with a taste index higher than the control. The highest fruit firmness value was recorded at 1.98 ± 0.05 kgf/cm² with the SWC 10% treatment, indicating improved shelf-life.

Table 7: Enhancement of marketable qualities of organically grown tomatoes treated with SWC and SWW biostimulants

Consumer driven quality attributes	SWC 5%	SWC 10%	SWW 5%	SWW 10%	Control
Titration acidity (%)	0.49 ± 0.01	0.59 ± 0.00	0.36 ± 0.00	0.45 ± 0.01	0.31 ± 0.01
Total soluble solids (%)	5.91 ± 0.10	6.76 ± 0.13	4.78 ± 0.07	5.61 ± 0.04	4.23 ± 0.02
Taste index	1.09 ± 0.03	1.16 ± 0.02	1.02 ± 0.01	1.07 ± 0.02	0.99 ± 0.01
Firmness (kgf/cm ²)	1.27 ± 0.00	1.98 ± 0.05	1.13 ± 0.01	1.21 ± 0.02	1.04 ± 0.00

± Standard error of replicated values.

* SWC: Cowurine extracted seaweed biostimulant, SWW: Water extracted seaweed biostimulants.

4. Discussion

Tomatoes, the second most valuable vegetable globally, are crucial for human nutrition due to their health promoting properties. Heightened consumer concerns over pesticide residues and heavy metals have increased the demand for organic tomatoes. This study examined the effects of *K. alvarezii* organic biostimulants on enhancing the mineral nutrient profile, antioxidant capacity, reducing antinutritional factors and improves the marketable qualities of tomato for better human health outcomes.

Significant variations were observed in the proximate compounds, viz., ash, protein, carbohydrate, and fiber content following the application of organic biostimulants, with SWC 10% leading in all compositions. Similar results with organic tomato over conventionally grown tomato were documented by Ilic *et al.* (2014). The ash content indicates the mineral composition, which is crucial for assessing nutritional value, while high moisture levels affect the shelf life and freshness of tomatoes, influencing their storage stability (Ramdath *et al.*, 2020; Maestri *et al.*, 2019). Although, tomatoes are low in protein, this analysis is important for nutritional labelling, as proteins support cellular structure and function, while lipids are key for quality assessment (Phizicky *et al.*, 2003). Carbohydrate analysis highlights the energy potential of tomatoes, contributing to their desirable textures and flavours. Additionally, fiber provides significant health benefits, including protection against heart disease, colon cancer and diabetes, while improving digestive health and satiety (Nielsen and BeMiller, 2010; Buttriss and Stokes, 2008).

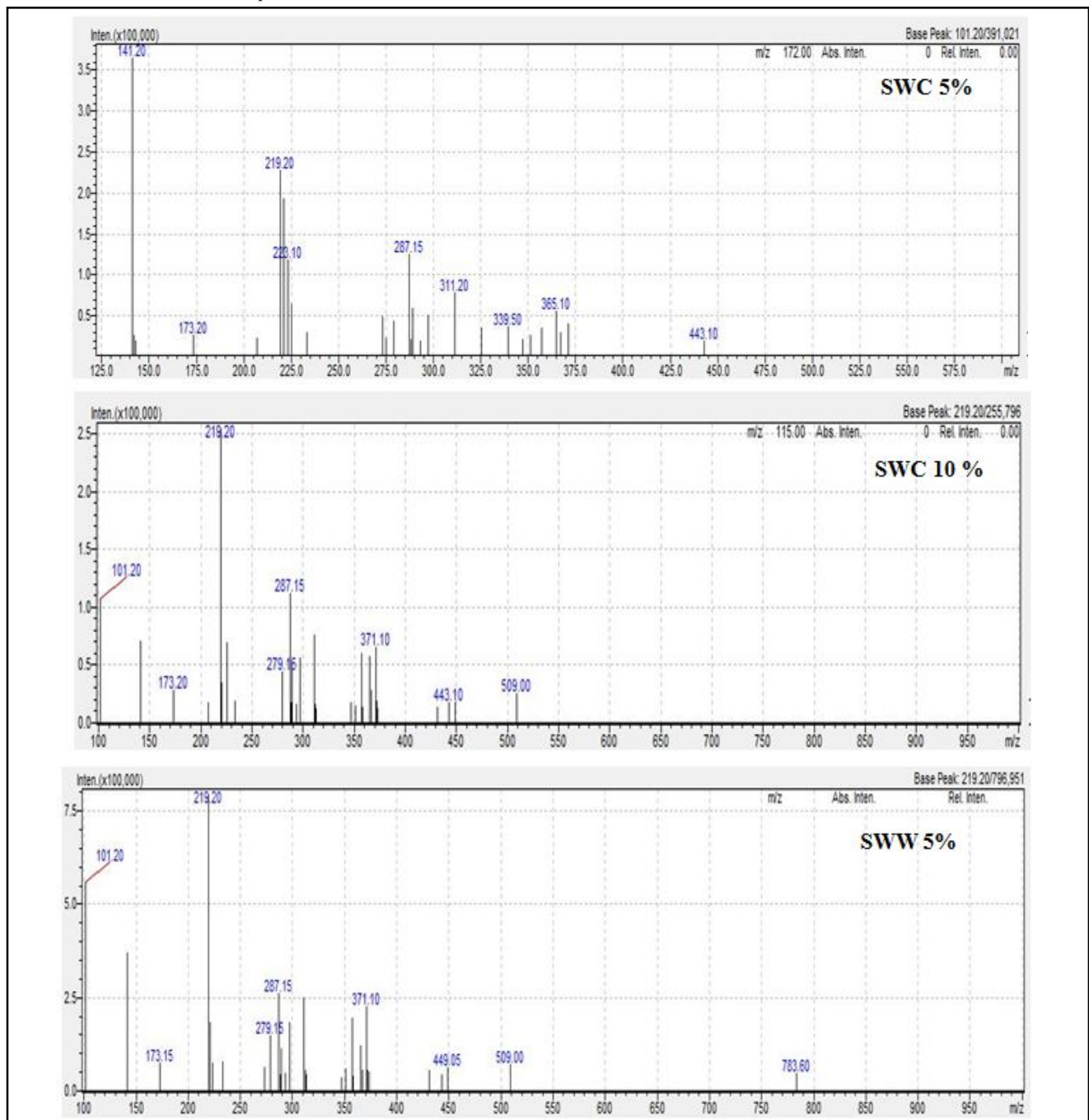
Minerals are essential for numerous physiological functions and their increase was observed in tomatoes grown by applying SWC 10% and 5% as foliar input and could be attributed to the combined effects of soil inputs and biostimulants. These results are consistent

with previous findings by Layek *et al.* (2018) and Murtic *et al.* (2018), who observed enhanced nutrient content in crops treated with seaweed saps. In addition, the presence of amino acids and growth hormones in *K. alvarezii* contributes to its stimulatory potential, with methionine playing a key role in fruit ripening and lycopene production, and alanine, serine, tyrosine, and proline enhancing nutrient uptake and stress resistance (Alfósea-Simon *et al.*, 2020; Watanabe *et al.*, 2016) in tomato. The significant enhancement of mineral content in organic tomato by SWC 10% treatment correlates closely with its antioxidant properties, particularly about reactive oxygen species (ROS). The elevated levels of essential minerals such as calcium, magnesium, and potassium are crucial for various physiological functions, including the activation of antioxidant enzymes that combat oxidative stress (Garcia-Sanchez *et al.*, 2020). These minerals play a vital role in the defense against ROS, which can cause cellular damage and contribute to chronic diseases. Furthermore, the presence of antioxidants like lycopene, vitamin C and carotenoids, alongside increased mineral content, amplifies the protective effects against oxidative damage (Martinez-Vaverde *et al.*, 2002; Saiharini and Padmaja, 2022). This synergy not only enhances the nutritional quality of tomatoes but also fortifies their capacity to mitigate the detrimental impacts of ROS, thereby promoting better health outcomes. The results underscore the importance of *K. alvarezii* biostimulant applications in optimizing both nutrient and antioxidant profiles in tomatoes, highlighting their potential to improve overall health benefits.

The antioxidant content in tomatoes also showed a significant and favorable increase with the SWC biostimulant. The SWC 10% produced the highest levels of lycopene and vitamin C, surpassing both the control and other treatments. This is crucial, as vitamin C is an essential antioxidant supporting immune function and skin health,

and its elevated levels could contribute to the overall nutritional quality of the tomatoes (Kotikova *et al.*, 2011). Similarly, the SWC 10% significantly boosted β -carotenoids, essential for vision and immune health, surpassing the control and other treatments. This increase underscores the role of SWC biostimulants in enhancing carotenoid synthesis (Leonardi *et al.*, 2000). Moreover, the enhanced lycopene concentration in SWC 10% received tomatoes reveals the potential of biostimulants to boost the antioxidant capacity of tomatoes and its health benefits to consumers. Lycopene's protective effects against various diseases, including certain cancers and cardiovascular conditions (Ali *et al.*, 2021), enhance the health benefits associated with tomato consumption.

Tomatoes are rich in antioxidants like carotene, ascorbic acid, lycopene, tocopherol, phenolic acids, flavonoids, and anthocyanins, which help prevent free radical oxidation. These compounds play a crucial role in reducing oxidative stress, aiding in the prevention of diseases such as CVDs, diabetes, cancer, neurological disorders, arthritis, and aging (Ali *et al.*, 2021; Malik and Madan, 2020; Bamne *et al.*, 2023). Hence, the flavonoid and phenol concentrations were studied with *K. alvarezii* biostimulants, revealing a significant increase in antioxidant content in tomatoes, particularly with 10% SWC, which showed high levels of immune-boosting flavonoids like quercetin, kaempferol, and naringenin (Das *et al.*, 2021).



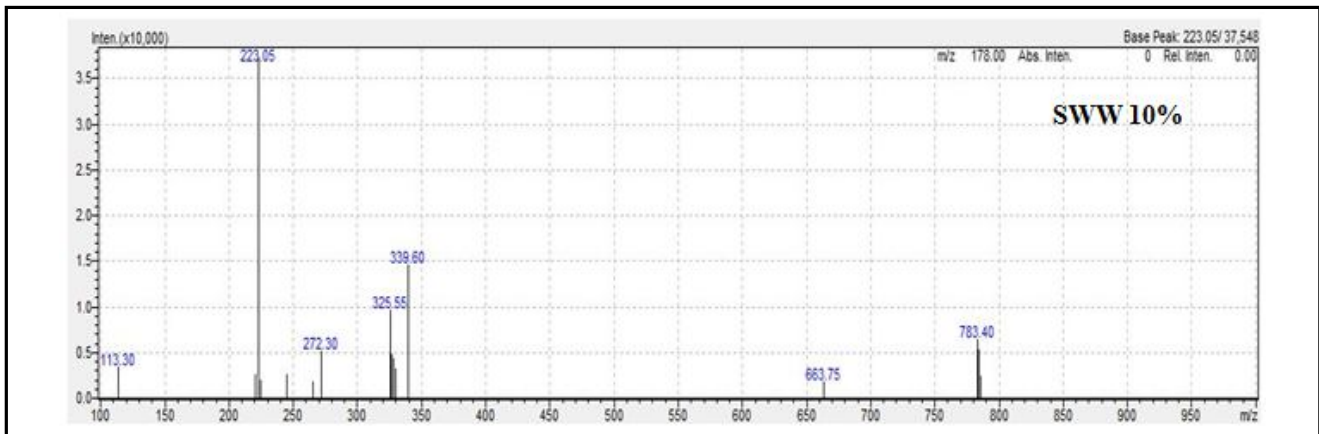


Figure 1: Total ion chromatograms (TIC) of organically grown tomatoes showing various bioactive compounds determined in negative mode by LC-MS.

The health benefits of antioxidants of tomatoes were also documented by Kotikova *et al.* (2011) and Jesus Periago *et al.* (2009). Conversely, the SWW 10% demonstrated a notable intensity of phenolic compounds *viz.*, sinapic and cinnamic acids (Figures 1-2), illustrating varying effects of different biostimulants on antioxidant profiles (Jesus periago *et al.*, 2009; Erge and Karadenz, 2011). The protective effects of tomato phenolic compounds include the anti-inflammatory and antioxidant roles of cinnamic acid, free radical scavenging and

membrane stabilization by kaempferol and sinapic acid, cardiovascular improvements by naringenin and ferulic acid, anticancer properties of quercetin, and prevention of cardiac hypertrophy by p-coumaric acid (Ali *et al.*, 2021; Yeligar *et al.*, 2021). Our findings indicate that both SWC and SWW treatments not only enhance the quantity of these beneficial compounds but also amplify their potential health effects, such as anti-inflammatory and antidiabetic properties (Chao *et al.*, 2010).

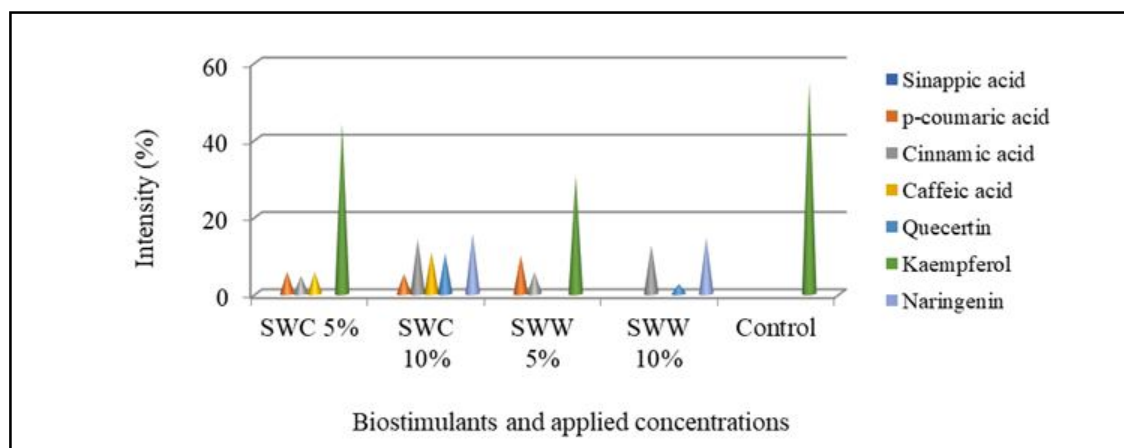


Figure 2: Major flavonoids and phenolic compounds detected in organically grown tomatoes treated with SWC and SWW biostimulants by LC-MS.

In this study, SWC treatments significantly reduced phytate levels, improving phytate-to-nutrient ratios for calcium, iron, and zinc. This reduction is linked to the production of antioxidants like vitamin C, carotenoids, and lycopene, regardless of treatment type. Additionally, SWC treatments lowered other antinutritional factors (phytates, oxalates, and tannins) and enhanced the bioavailability of essential minerals. Conversely, the highest antinutritional properties were found in SWW biostimulants across all levels, including the control, with the lowest levels of vitamin C, carotenoids, and lycopene (Oyetayo and Ibitoye, 2012; Aliyu *et al.*, 2018). This was further supported by a significant negative correlation ($r^2 \leq 0.933$) between phytates and analysed mineral elements in tomato fruits (Figure 3).

The observed ratios for phytate : calcium, phytate : iron and phytate : zinc and phytate*calcium : zinc, indicated that all treatments

remained below critical limits (< 0.24 , < 1.0 , < 15.0 , and < 200 , respectively) (Tura *et al.*, 2023), suggesting favourable nutrient absorption. Notably, the SWC treatments at 10% and 5% demonstrated significantly lower phytate ratios compared to the control (Castro-Alba *et al.*, 2019). This reduction indicates that these treatments may enhance the bioavailability of essential minerals, which is crucial for alleviating nutritional deficiencies in populations that rely on plant based diets. These findings suggest that *K. alvarezii* derived biostimulants enhance the bioavailability of essential minerals, which is critical for addressing nutritional deficiencies, especially in populations reliant on plant based diets. Miller *et al.* (2015) further explained that while dietary calcium can affect zinc absorption in high phytate diets, the phytate*calcium molar ratios observed in this study remained below critical thresholds, indicating a lower risk of zinc malabsorption.

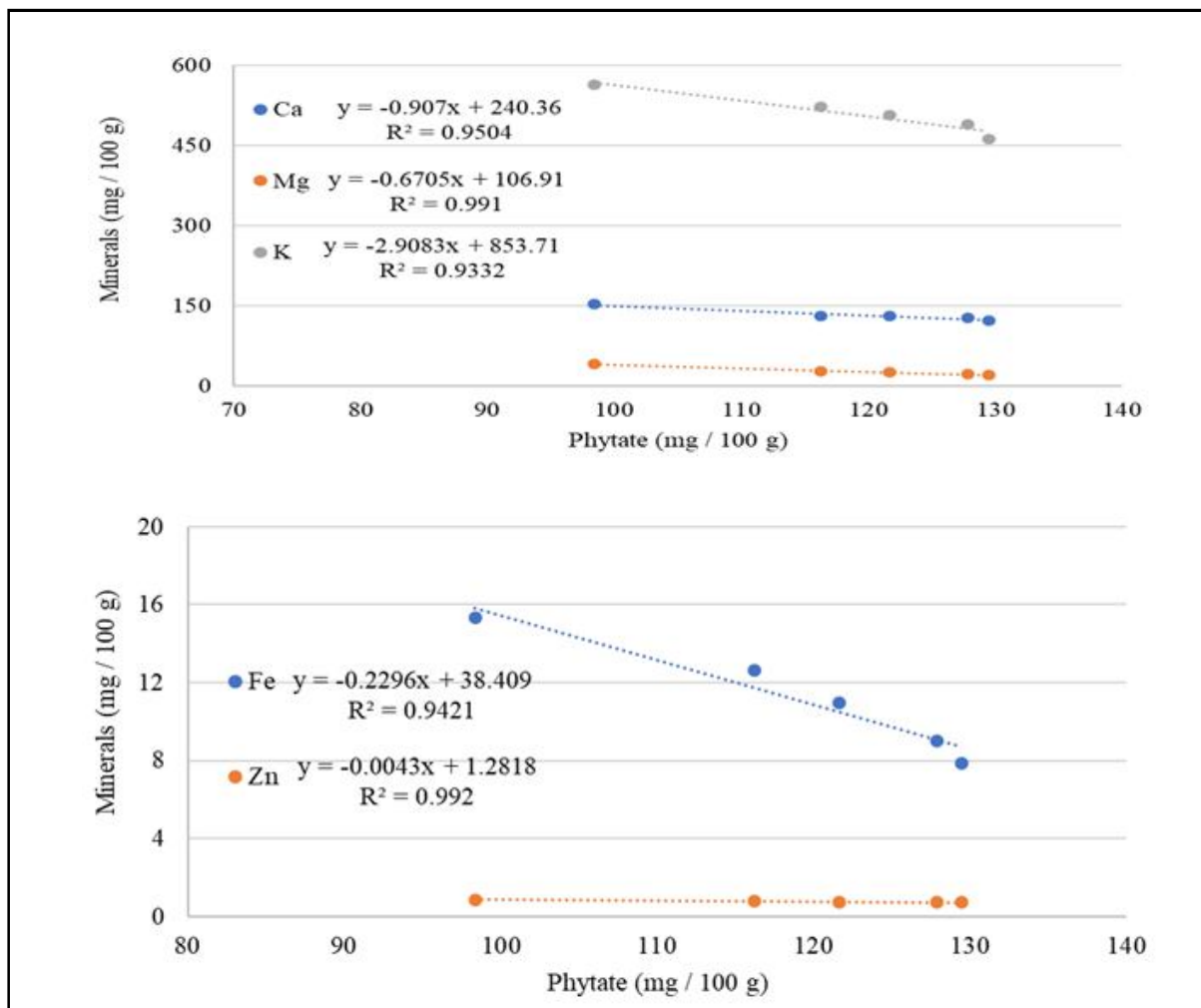


Figure 3: Linear regression correlation of phytate with the minerals concentration in organically grown tomatoes

Lastly, the marketable quality of tomatoes, including total soluble solids (TSS), titrable acidity (TA), taste index (TI), and firmness, was significantly improved by SWC treatments. The increased TSS and TA contributed to a higher taste index, making the tomatoes more appealing to consumers. Enhanced firmness, particularly in SWC 10%-treated tomatoes, suggests prolonged shelf life, a key factor for both farmers and retailers. Taste and flavor, largely influenced by the sugar/acid ratio, are critical for consumer acceptability (Tigist *et al.*, 2011). The improved taste index, coupled with the presence of umami compounds like glutamate, further highlights the ability of *K. alvarezii* biostimulants to enhance the sensory qualities of organically cultivated tomatoes (Behrens *et al.*, 2011; Ghirri and Bignetti, 2012).

5. Conclusion

The study on *K. alvarezii* based biostimulants in organically grown tomatoes showed significant improvements in nutritional quality. These biostimulants increased essential minerals like calcium, magnesium, potassium, iron, and zinc while reducing antinutritional factors like phytates. They also boosted antioxidants such as vitamin C, vitamin A, lycopene, and phenolic compounds, highlighting the

health benefits of *K. alvarezii* treated tomatoes. Enhanced mineral bioavailability, improved taste, firmness, and sensory appeal suggest that *K. alvarezii* biostimulants can address nutritional deficiencies and increase consumer acceptance of organic tomatoes. This research also emphasizes the effectiveness of *K. alvarezii* biostimulants in promoting sustainable agriculture by improving the crop resilience and productivity. Additionally, the findings suggest potential for using *K. alvarezii* biostimulants in cultivating mineral- and antioxidant-rich vegetables, which could help combat oxidative stress and degenerative diseases, contributing to advancements in health and pharmaceutical sciences.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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