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Research Article

Exogenous application of salicylic acid and NPK promotes tomato growth parameters, yield, and nutraceutical quality under cold stress

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Abstract

The usage of biostimulants such as salicylic acid (SA) and NPK has emerged as an innovative practice for increasing crop production and quality. It plays a crucial role in the control of many physiological and metabolic processes. This experiment aimed to investigate the impact of SA and NPK treatment on the yield, growth, and quality characteristics of tomatoes grown in field conditions. The experiment structure laid out RCBD with three replications. Studied the effect of nine treatments of NPK (7 g/L), SA (0.05 mM, 0.1 mM, 0.5 mM, 1.0 mM), and combination of both (NPK+ 0.05 mM, NPK+ 0.1 mM, NPK+ 0.5 mM and NPK+ 1.0 mM) on two varieties of tomato. The foliar applications of SA, NPK, and their mixtures were administered during the planting phase, the onset of flowering, and the fruiting stage by maintaining intervals of 10 days after 15 days of transplanting. The response variables were yield (Plant height, number of fruits per plant, leaf area) fruit quality parameters (firmness, pericarp thickness, titratable acidity (TA), total soluble solids), nutraceutical quality parameters (Total sugars, ascorbic acid, non-reducing sugars, lycopene, β-carotene, reducing sugars, total phenols, and proline contents). The results indicate that foliar spray with SA and NPK boosts yield and phytochemical component production in tomato fruits compared to control. According to the findings, the treatments (NPK+ 0.5 mM and NPK+ 1.0 mM) showed the best results regarding bioactive compounds, yield, and quality parameters in both varieties of tomato under cold stress conditions.

Keywords: biostimulants; β-carotene; foliar applications; fruit yield; tomato

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Introduction

Tomato (*Solanum lycopersicum* L.) comes from the Solanaceae family and is an annual herbaceous plant (Gonias *et al.*, 2019). Globally, tomatoes are known as a very important vegetable crop and its production was 182 million tons in 2017 (El-Hady *et al.*, 2021). Its fruits contain lycopene, one of the most significant dietary carotenoids for the protection of chronic diseases such as lung, breast, and prostate cancer (Palozza *et al.*, 2011). The lycopene concentration (mg 100^{-1}g) of tomato changes according to the cultivation environment, genotype genetic makeup, disease pressure, and genotype-by-environment interactions (Des Marais *et al.*, 2013). Tomatoes exhibit substantial levels of total lipids (1%), carbohydrates (3%), protein (1.2%), moisture (95%), minerals (calcium [Ca], potassium [K], magnesium [Mg], phosphorus [P], sodium [Na], zinc [Zn], manganese [Mn] and vitamins (A and C, pantothenic acid, riboflavin, thiamin, niacin, and pyridoxine) (Melfi *et al.*, 2018; Perveen *et al.*, 2015). Furthermore, they are high in phenolic compounds (flavonoids and phenolic acids), carotenoids (lycopene, β-carotene), and glycol-alkaloids (tomatine) (Poonam Chaudhary *et al.*, 2018). The role of tomato bioactive compounds is considerable in controlling different diseases and body functions (Ali *et al.*, 2020).

Salicylic acid (SA) is a natural growth regulator of vascular plants that increases production and fruit shelf life (Chen *et al.*, 2016; Kaya *et al.*, 2023; Preciado-Rangel *et al.*, 2019). Furthermore, by modulating the activity of defensive enzymes and minimizing or eliminating plant cell damage induced by oxygen stress, SA can regulate the amounts of reactive oxygen species (ROS) in plants (Jahan *et al.*, 2019). This is attributed to the increased activity of the enzymes phenylalanine ammonium lyase (PAL) and peroxidases (POD) which promote plant antioxidant production (Alali *et al.*, 2018; Hao *et al.*, 2014; Mendoza *et al.*, 2018). Antioxidant substances have a molecular structure that suppresses the generation of free radicals and can thereby prevent oxidative stress-related diseases (Priya Chaudhary *et al.*, 2023). Physiological and metabolic functions such as transpiration, photosynthesis, ion uptake, antioxidant enzyme activities, transportation, and osmoregulation are all influenced by SA (Faried *et al.*, 2017; Jayakannan *et al.*, 2015). The foliar applications of SA may boost vegetable output by lowering stress-induced growth inhibition (Chakma *et al.*, 2021; Sobrinho *et al.*, 2023). Salicylic acid improves flower development, electrolyte mobility in plants, chlorophyll augmentation, and photosynthesis rate (Souri and Tohidloo, 2019). Previously, the advantageous effects of growth hormones on wheat, tomato, chilies, and roses have been reported (El-Gaied *et al.*, 2013; Zahid *et al.*, 2023; Zahid *et al.*, 2021).

Fertilizers comprising the important plant nutrients nitrogen (N), phosphorus (P), and potassium (K) are crucial for productive crops (Mantovani *et al.*, 2017; Yahaya *et al.*, 2023). Nitrogen's role is acceptable because it is required for protein, nucleic acids, chlorophyll, and several essential enzymes (Nunes-Nesi *et al.*, 2010). Nitrogen deficiency in soil, on the other hand, leads to low crop yield and quality. Excessive nitrogen use has a negative impact on agricultural crop quality and yield (Zhao *et al.*, 2022). Phosphorus (P) is a macro-element that is required for plant growth and development. Its scarcity limits plant growth and causes them to remain immature (Ibrahim *et al.*, 2022). Potassium (K) promotes plant development and is useful in a variety of processes such as photosynthesis, food translocation, cell extension, and protein creation (Sardans and Peñuelas, 2021). Foliar-applied NPK fertilizers considerably contribute to increased yield by increasing plant biomass (Naitman *et al.*, 2015). Foliar NPK applications have been shown to improve photosynthetic capacity and maintain healthy leaf nutrition (Ihsan *et al.*, 2013). Fertilization is a significant factor influencing crop output. Foliar application is commonly employed as an alternative or additional soil nutrient supply (Ferrari *et al.*, 2021). Foliar application (SA and NPK) is helpful for improving the growth and fruit quality parameters of tomatoes.

In recent investigations, it has been highlighted that there have been important modifications in the intensity, frequency, and duration of extreme weather events over the past few years, definitely with regard to

temperature extremes (Ummenhofer and Meehl, 2017). The productivity, growth, development and quality of plants are intensely affected by temperature, and each species displays a definite temperature range with an optimal temperature for the numerous processes (Hatfield and Prueger, 2015). In the case of tomatoes, exposure to low chilling temperatures, definitely below 18 °C, can adversely influence several growth aspects such as truss formation, anthesis, and ripening of fruit (Ploeg and Heuvelink, 2005).

In light of this, the objectives of this study are (1) to assess and evaluate the impacts of several treatments, namely NPK alone, SA alone, and combinations of NPK and SA, on two distinct tomato genotypes, (2) to evaluate the influence of SA and NPK treatments on the growth, nutraceutical quality, and yield-related parameters of tomato plants, and (3) to ascertain the ideal SA concentration for foliar application in conjunction with NPK to maximize tomato cultivation's production and quality metrics under the cold stress.

Materials and Methods

Plant material

The study was conducted in Pakistan over the year 2021-2022, specifically at the Vegetable Research Institute (VRI) located inside the Ayub Agricultural Research Institute in Faisalabad (longitude 73°74 East, latitude 30°31. 5 North, with an elevation of 184 m (604 ft.)). The seedlings that exhibited robust health and consistent growth were then transferred to the field. In the field, a randomized complete block design with a split plot was followed. The distance between R×R was 150 cm, whereas the distance between P×P was 50 cm. Weeds were removed manually. Each treatment consisted of three repetitions, with five plants in each replication. During the phases of planting, initiation of flowering, and fruit development, foliar sprays of salicylic acid, NPK fertilizer, and a mixture of both were administered 20 days following the transplantation process, with further administrations occurring at 20-day intervals. The experiment included the employment of various concentrations of salicylic acid (SA) and NPK in two varieties of tomatoes, namely 10142 (V1) and LTH-324 (V2). The levels of salicylic acid and NPK were designated as T1 (Control), T2 (NPK 7 g/L), T3 (SA 0.05 mM), T4 (SA 0.1 mM), T5 (SA 0.5 mM), T6 (SA 1.0 mM), T7 (NPK + SA 0.05 mM), T8 (NPK + SA 0.1 mM), T9 (NPK + SA 0.5 mM), and T10 (NPK + 1.0 mM). The tomato crop is sensitive to low temperatures so every year in the month of December and January as temperature is very low and crops are under the cold stress. The metrological data during the stress period was noted (Table 1).

Vegetative growth parameters

Vegetative growth parameters i.e., leaf area, plant height (cm), the number of fruits per plant, and yield (g) were estimated from the plants of each treatment, and their means were determined.

Firmness (kgf) and pericarp thickness (mm)

The firmness of the sample was assessed using a penetrometer. The core of the fruit was utilized for the purpose of measuring the force exerted, expressed in kilograms of force (kgf), and subsequently an average value was computed. The measurement of pericarp thickness is conducted using a Vernier caliper.

Ascorbic acid (AA)

Tareen *et al*. (2012) outlined the methodology used for the quantification of ascorbic acid in tomatoes (Tareen *et al.*, 2012). The experimental protocol consisted of the amalgamation of 5 grams of fruit pulp with 5 milliliters of a 0.1% hydrochloric acid solution (weight/volume). Subsequently, the mixture was homogenized and subjected to centrifugation at a speed of 10,000 rpm for a duration of 10 minutes, with the objective of isolating the supernatant. Subsequently, the absorbance of the supernatant solution at a wavelength of 243 nm was measured using a spectrophotometer (SP 3000 plus).

Table 1. Metrological data during the cold stress period

Days	Temperature (°C)			Relative humidity (%)
	Minimum	Maximum	Average	
12 December 2020	10	13	11.5	90
13	7.5	12.5	10.0	90
14	7.0	15	11.0	85
15	8.0	13.5	10.8	72
16	1.5	17	9.3	70
17	2.5	19.5	11.0	44
18	1.8	21	11.4	49
19	4	22.5	13.3	54
20	4.5	23.5	14.0	51
21	4.5	23	13.8	51
22	4.5	24	14.3	55
23	5	24	14.5	56
24	5.5	23	14.3	57
25	5	21	13.0	70
26	3.5	22.5	13.0	57
27	4.5	13	8.8	74
28	4.5	13.5	9.0	76
29	1	18	9.5	62
30	1.5	19.5	10.5	52
31	1	20	10.5	54
1 January 2021	1	20.5	10.8	57
2	3.5	15.5	9.5	70
3	5.5	20	12.8	66
4	11	19.8	15.4	83
5	13.5	15	14.3	96
6	9.5	14	11.8	87
7	6.5	18	12.3	72
8	6.5	12.5	9.5	92
9	7	16.5	11.8	77
10	6	16	11.0	81
11	5.5	11.5	8.5	89
12	4.5	15	9.8	81
13	4.5	14.3	9.4	86
14	3.5	11	7.3	89
15	5	10	7.5	91
16	5.5	13.5	9.5	84
17	5.5	10.5	8.0	87
18	6.5	12.5	9.5	84
19	4	19.5	11.8	71
20	4.2	21.2	12.7	66
21	4.5	25	14.8	50
22	8	19.6	13.8	62
23	10	18.5	14.3	73
24	3.5	19.7	11.6	53

Total soluble solids (TSS)

TSS was calculated for selected cultivars using an Atago RX 500 digital refractometer. The reading in Brix (percentage) was obtained at 20 °C after a drop of tomato juice was placed on the prism of the refractometer (Ilić *et al.*, 2015).

Titratable acidity

The determination of titratable acidity (TA) in tomato juice was conducted by performing a titration on a 10 g portion of a homogenized sample using 50 mL of distilled water and a 0.1% NaOH solution at a pH of 8.17 (Thakur *et al.*, 1996). The resulting value was reported in grams per liter (g/L).

Total sugar contents

The total sugar contents were calculated by following the previously reported method (Chemists and Chemists, 1920). A 25 mL aliquot was made for reducing sugars in a flask. The non-reducing sugars were mixed with 5 mL of HCl and 20 mL of distilled water to create the reducing sugars. To allow for full hydrolysis, this reaction mixture was kept at room temperature overnight. After that, 1 N NaOH was used to neutralize the reaction mixture while phenolphthalein served as an indicator. By gradually heating Fehling's solution until it turned brick red and adding a few drops of methyl blue, titration was performed against Fehling's solution. Until a brick red hue formed, this titration method was repeated. The sugar contents were taken by the formula:

$$Total\ Sugar\ (\%) = 25 \times (\frac{X}{Y})$$

Where: x = mL of standard sugar solution used against 10 mL Fehling's solution. Y = mL of sample aliquot used against 10 mL Fehling's solution.

Estimation of proline

Bates et al. (1973) explained the step-by-step technique was used to determine the proline concentration (Bates *et al.*, 1973). First, a 0.2 g homogenized sample of leaves was prepared by centrifugation at 12000 rpm for 10-15 min. A freshly made acid ninhydrin solution (1.25 g ninhydrin, dissolved in 20 ml 6 M orthophosphoric acid, and 30 ml glacial acetic acid) was added to the 2 ml leaf sample that was taken in a test tube. The test tube solution was heated for one hour at 100 °C and then cooled to 25 °C. After that, 4 ml of toluene was added to the test tube's contents during the phase separation step, and the test tubes were held vertically for ten minutes. The absorbance reading was observed at 520 nm.

Analytical parameters Lycopene & β-carotene

To test for lycopene, 5 grams of tomato fruit tissue were homogenized. Then, 100 mL of the sample was mixed with 8 mL of a 2:1:1 hexane, ethanol, and acetone solution. The solution was immediately vortexed and incubated for an hour without bright light. Each sample with 1.0 ml water, then vortexed once more. The phases were split after ten minutes. Used the top layer of one of the control samples to rinse the cuvette. After discarding the old blank, a new one was used to calibrate the spectrophotometer at 503 nm. Determined the A503 of the upper layers of the lycopene samples. Beta carotene carotenoid standards were commercially purchased with an appropriate solvent and the absorbance was measured at its peak wavelength (λmax). Extinction coefficients were used while considering the dilution factor, the carotenoid sample solutions were assayed by High-performance liquid chromatography (HPLC) (Scott, 2001).

Total phenol contents

Total phenols were assessed using colorimetry and the Folin-Ciocalteau method for the nutraceutical quality assessment (Singleton *et al.*, 1999). To accomplish this, the chemicals were extracted from a 0.5 g

lyophilized sample using 5 mL of 80% methanol. A test tube containing a 750 µL solution of 2% Na_2CO_3 was also filled with 250 µL of 50% Folin-Ciocalteau reagent, 1375 µL of deionized water, and 250 µL of the sample extract. Utilizing spectrophotometry, the absorbance at 725 nm was determined. Gallic acid milligrams per kilogram of dry weight (mg GA g^{-1} DW) were used to express the total phenol values.

Statistical analysis

R Studio (4.2.2) was used to do all the analysis related to the recorded data. ANOVA (Analysis of variance) was used to determine significant differences among the treatments and varieties. Multiple comparison analysis was performed by using Tukey HSD. Significant differences ($p < 0.05$) were shown by different letters. The research used Principal Component Analysis (PCA) to examine the patterns of morphophysiological differences among the ten different treatments and 16 parameters of tomato. The aim was to identify and classify the major factors that contribute to the phenotype. The phenotypic level was used to compute the correlation coefficient (r) between characteristics, with the aim of identifying both positive and negative associations between yield and its associated qualities.

Results

Plant height (cm)

The analysis of variance revealed significant variation between two tomato cultivars treated to varying amounts of salicylic acid and NPK, as well as their combination. However, significant V1 plant height growth changes were noted in response to varied salicylic acid and NPK treatments (Table 2 and Figure 1).

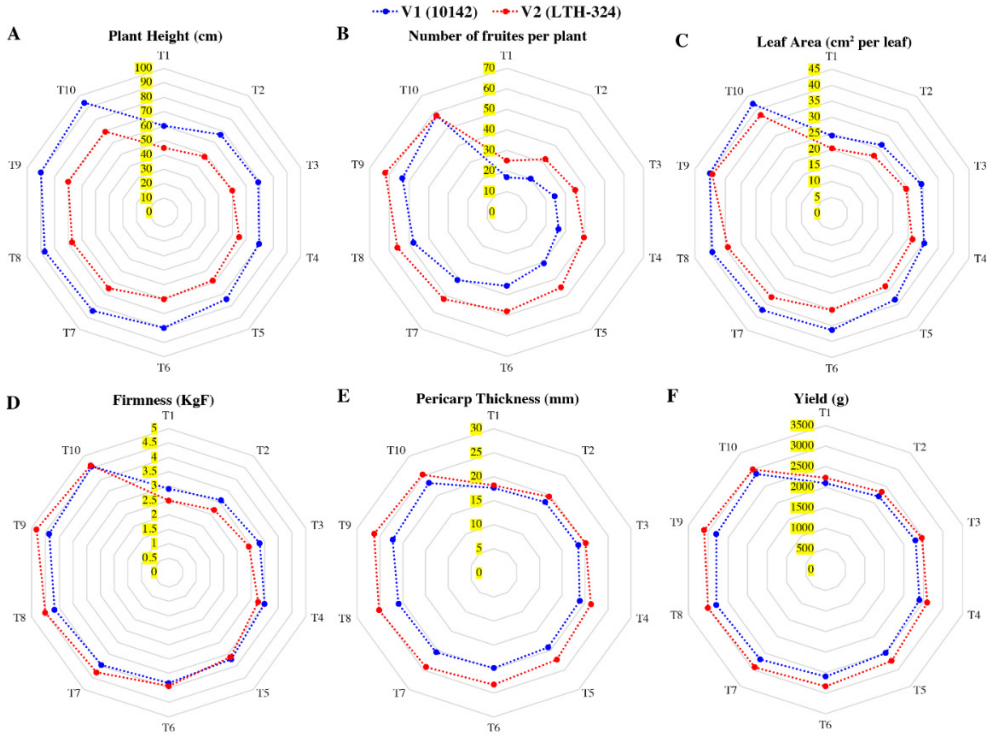

Figure 1. Effect of foliar applications of salicylic acid (SA) and NPK on the growth, yield, and quality-related parameters of tomato

T1 (Control), T2 (NPK 7 g/L), T3 (SA 0.05 mM), T4 (SA 0.1 mM), T5 (SA 0.5 mM), T6 (SA 1.0 mM), T7 (NPK + SA 0.05 mM), T8 (NPK + SA 0.1 mM), T9 (NPK + SA 0.5 mM) and T10 (NPK + SA 1.0 mM).

Table 2. Effect of SA and NPK on growth, yield, and nutraceutical quality-related parameters of tomatoes grown under field conditions at low temperature

Traits		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	V	T	V × T
DF												1	9	9
PH	V1	60.33g	67f	69ef	69.67ef	74e	80d	84cd	87bc	90ab	94a	***	***	**
	V2	45.00e	48.00e	50.00de	55.00cd	58.00c	60.00bc	65.00ab	67.00a	70.00a	69.33a			
FP	V1	17h	20gh	24.67fg	26.67ef	30.67e	35.67d	40.67c	47.67b	53.33a	58a	***	***	***
	V2	25.00i	32.00h	35.00gh	39.67fg	45.00ef	48.00de	52.00cd	56.00bc	62.00a	58.00ab			
LA	V1	24.26e	26.43de	29.3cde	30.27cd	33.33bc	36.43ab	37.3ab	39.43a	40.2a	42.2a	***	***	NS
	V2	20.27f	22.27ef	24.30def	26.37cdef	28.23bcde	30.23bcd	32.33abc	34.33ab	39.33a	37.90a			
Y	V1	2100d	2200cd	2300bcd	2400abcd	2500abcd	2600abc	2700ab	2800ab	2800a	2866.67a	NS	NS	NS
	V2	2233.33cd	2333.33c	2466.67bc	2600.00b	2733.33ab	2833.33ab	2933.33a	3000a	3100.00a	3000.00a			
ViTC	V1	15.4b	16.4ab	17.2 ab	17.8 ab	18.27 ab	19.77 ab	21.23 ab	22.3 ab	23.7 a	21.4 ab	***	***	*
	V2	15.70e	16.93e	19.20de	21.93cde	24.23bcde	26.40abcd	28.23abc	31ab	33.17a	33.10a			
TSS	V1	2.8e	3.1de	3.5cde	4.1cde	4.3bcde	5.1abcde	5.9abcd	6.3abc	7.1ab	7.9a	NS	***	NS
	V2	2.50d	3.40cd	3.70bcd	4.20bcd	4.50abcd	4.70abcd	5.10abcd	5.9abc	7.10a	6.30ab			
TS	V1	3.56f	3.9ef	4.27def	4.7cdef	5.1bcdef	5.47bcde	5.83abcd	6.33abc	6.9ab	7.47a	NS	***	NS
	V2	3.63d	3.97d	4.30cd	4.83bcd	5.10bcd	5.43bcd	6.20abc	6.8ab	8.40a	7.90a			
RS	V1	1f	1.1ef	1.2e	1.33d	1.57c	1.7b	1.83ab	1.97ab	2.07a	2.17a	NS	***	NS
	V2	1.17g	1.23f	1.33e	1.43de	1.53d	1.70c	1.90bc	2.03b	2.53a	2.50a			
NRS	V1	2.56e	2.87d	3.07cde	3.37cde	3.53bcde	3.8bcde	4abcd	4.37abc	4.83ab	5.3a	NS	***	NS
	V2	2.47d	2.73cd	2.97cd	3.40bcd	3.57bcd	3.77bcd	4.30abcd	4.76abc	5.90a	5.43ab			
F	V1	2.9f	3.1ef	3.33def	3.5cdef	3.7bcde	3.83abcde	3.97abcd	4.17abc	4.37ab	4.57a	NS	***	*
	V2	2.50f	2.70ef	2.93ef	3.27d	3.63cd	3.93bc	4.27abc	4.5ab	4.83a	4.60a			
PT	V1	17.63d	18.2cd	18.5cd	18.8cd	19.2cd	19.8bcd	20.5abc	20.9abc	22.2ab	23.1a	***	***	*
	V2	18.20e	19.53de	20.10de	21.30cd	22.30bcd	23.23bc	24.20abc	25.2ab	26.30a	25.30ab			
P	V1	0.12d	0.18cd	0.22c	0.25bc	0.25bc	0.30b	0.32ab	0.327ab	0.40a	0.479a	***	***	NS
	V2	0.15g	0.22fg	0.29efg	0.33df	0.41cde	0.46bcd	0.53bc	0.58ab	0.72a	0.62ab			
TA	V1	2.12e	2.19de	2.30d	2.39cd	2.47c	2.62bc	2.92b	3.13ab	3.45a	3.64a	NS	NS	NS
	V2	2.46e	2.56de	2.67d	2.78cd	3.12c	3.45bc	3.56b	3.78ab	3.98a	3.65ab			
PHE	V1	18.9f	19.9ef	20.8def	21.8cdef	22.53cde	23.2bcd	24.6abc	25.8ab	26.4a	27.3a	***	***	NS
	V2	17.50f	18.90ef	19.07ef	20.20def	21.33cde	22.47bcd	23.67abc	24.3abc	26.40a	25.30a			
L	V1	15.31b	15.56ab	15.99 ab	16.12 ab	16.45 ab	16.67 ab	16.98 ab	17.19 ab	17.78 ab	18.32a	***	***	NS
	V2	16.12d	16.35cd	16.78bcd	17.12abcd	17.89abcd	18.32abcd	18.91abcd	19.12abc	19.99a	19.45ab			
CAR	V1	4.15e	4.35d	4.62cd	4.98c	5.12bc	5.36b	5.49b	5.98ab	6.23a	6.67a	NS	***	NS
	V2	3.89f	4.14e	4.56de	4.98d	5.15c	5.56bc	5.98b	6.12ab	6.45a	6.17ab			

DF = Degree of freedom; PH = Plant height (cm); FP = Number of fruits per plant; LA = Leaf area (cm^2 per leaf); Y = Yield (g); ViTC = Vitamin C (mg/100 g); TSS = Total soluble solids (Brix %); TS = Total sugars (%); RS = Reducing sugars (%); NRS = Non-reducing sugars (%); F = Firmness (kgf); PT = Pericarp thickness (mm); P = Proline (mg g^{-1}FW); TA = Titratable acidity (meq/day); PHE = Phenols (mg GA g^{-1}DW); L = Lycopene (mg Kg^{-1}FW); CAR = β-carotene (mg Kg^{-1}FW). V = Varieties; T = Treatments. T1 (Control), T2 (NPK 7 g/L), T3 (SA 0.05 mM), T4 (SA 0.1 mM), T5 (SA 0.5 mM), T6 (SA 1.0 mM), T7 (NPK + SA 0.05 mM), T8 (NPK + SA 0.1 mM), T9 (NPK + SA 0.5 mM) and T10 (NPK + SA 1.0 mM). Varieties: 10142 (V1) and LTH-324(V2). According to Duncan's multiple range test, mean (n = 3) with the different letters in a row under the different treatments are statistically comparable at p 0.05 (NS = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$)

T10 (94.0 cm) showed the most plant growth of the nine salicylic acids, NPK, and their combinations, whereas T1 (60.3 cm) provided the least plant growth (Figure 1A). Tomato plant height growth was also observed in variety V2, it showed a maximum at treatment T9 (70.0 cm), whereas T1 (45.0 cm) had the lowest plant height growth. Different doses of salicylic acid, NPK, and their combination strains were shown to have a significant effect on tomato plant height (Table 2 and Figure 1). We also checked the variety and treatment interactions; the results showed a highly significant relation (*** = $p < 0.001$).

Number of fruits per plant

In comparison to the control, both varieties (V1 = 10142 and V2 = LTH-324) increased the number of fruits per plant (FP). V2 showed a maximum of 62 FP in T9, while V1 showed a maximum of 58 FP (Figure 1B). Foliar applications of SA, NPK, and their combinations improved the overall fruits per plant and showed higher significant (*** = $p < 0.001$) levels (Table 2).

Leaf area (cm^2 per leaf)

The leaf area showed the maximum value in T10 (42.2 cm^2 per leaf) in variety V1 as compared to the control. In second variety (V2) showed the highest leaf area (39.3 cm^2 per leaf) in T9 and the lowest leaf area

(20.2 cm^2 per leaf) in T1 (Figure 1C). Both varieties and treatments showed highly significant variations, but their interactions were non-significant (Table 2).

Firmness (kgf) and pericarp thickness (mm)

The SA+NPK (0.5 mM+7g/L, T9) dose was very effective and increased by 4.83 kgf in the firmness and decreased by 2.5 kgf in the weight loss of fruits, in the control treatment, and in variety V2 (Figure 1D). Fruit firmness among all treatments regarding T10 treated plant showed a result (4.5 kgf) while control treatment T1 showed a result (2.9 kgf) of variety V1 (Table 1). Both the varieties behave similarly but they respond significantly differently under applications of SA, NPK, and their combinations (Table 2). V2 showed the highest pericarp thickness values (26.3 mm) for treatment T9 and the lowest (18.2 mm) in the control (Figure 1E). Similarly, the value in variety V1 was measured at T1 (17.6 mm) and T10 (23.1 mm). In comparison to variety V1, variety V2's pericarp thickness was found to be larger (26.3 mm) (Table 1). Both varieties, all treatments, and their interactions differ highly significantly (Table 2).

Yield (g)

The foliar application of SA, NPK, and their combination was used to evaluate the yield of tomato cultivars. Compared to the T1 treatment (control), variety V1 had the highest yield in T10 (2866 g) and in T9 of variety V2, the maximum yield (3100 g) was measured (Figure 1F). It was evident that the different administrations of NPK and SA greatly increased the yield of all kinds as compared to the control (Table 2).

Total soluble solids (TSS, Brix %)

Among all treatments regarding TSS, T10, and T9 treated plants revealed (7.9 brix) while control treatment T1 showed (2.6 brix) and (2.7 brix) in both varieties, respectively (Figure 2A). All the foliar applications of SA, NPK, and their combinations showed highly significant effects (Table 2). It was concluded that T10 and T9 treated plants observed the maximum result as compared to others.

Total sugars (%), reducing, and non-reducing sugars (%)

SA + NPK (T10) significantly improved the total sugar, reducing and non-reducing sugars in variety V1 (Table 1). Among different treatments of SA+NPK, maximum total sugars (8.4%), reducing sugars (2.5%), and non-reducing sugars (5.9%) were noticed in treatment T9 of variety V2 (Figures 2B, 2C, and 2D). The highest sugars were observed in V2 as compared to V1. NPK and SA combinations significantly boosted the sugar contents of tomatoes (Table 2).

Titratable acidity (g/L)

The titratable acidity of the two tomato cultivars was subjected to statistical analysis ($P < 0.05$), revealing substantial variance for SA, NPK, and their combinations. The recorded values varied from 2.12 to 3.64 g/L for V1 and 2.46 to 3.98 g/L for V2 (Figure 2E). It was depicted that treatment T9 calculated the highest value (3.98 g/L) in variety V2 (Table 2). This was because of enhanced photosynthesis activity and carbohydrate accumulation, which resulted in higher TSS, titratable acidity, and fruit firmness (Table 2). The highest value of T10 (3.64 g/L) was measured in V1 while the control treatment showed a minimum value for TA (2.1 g/L).

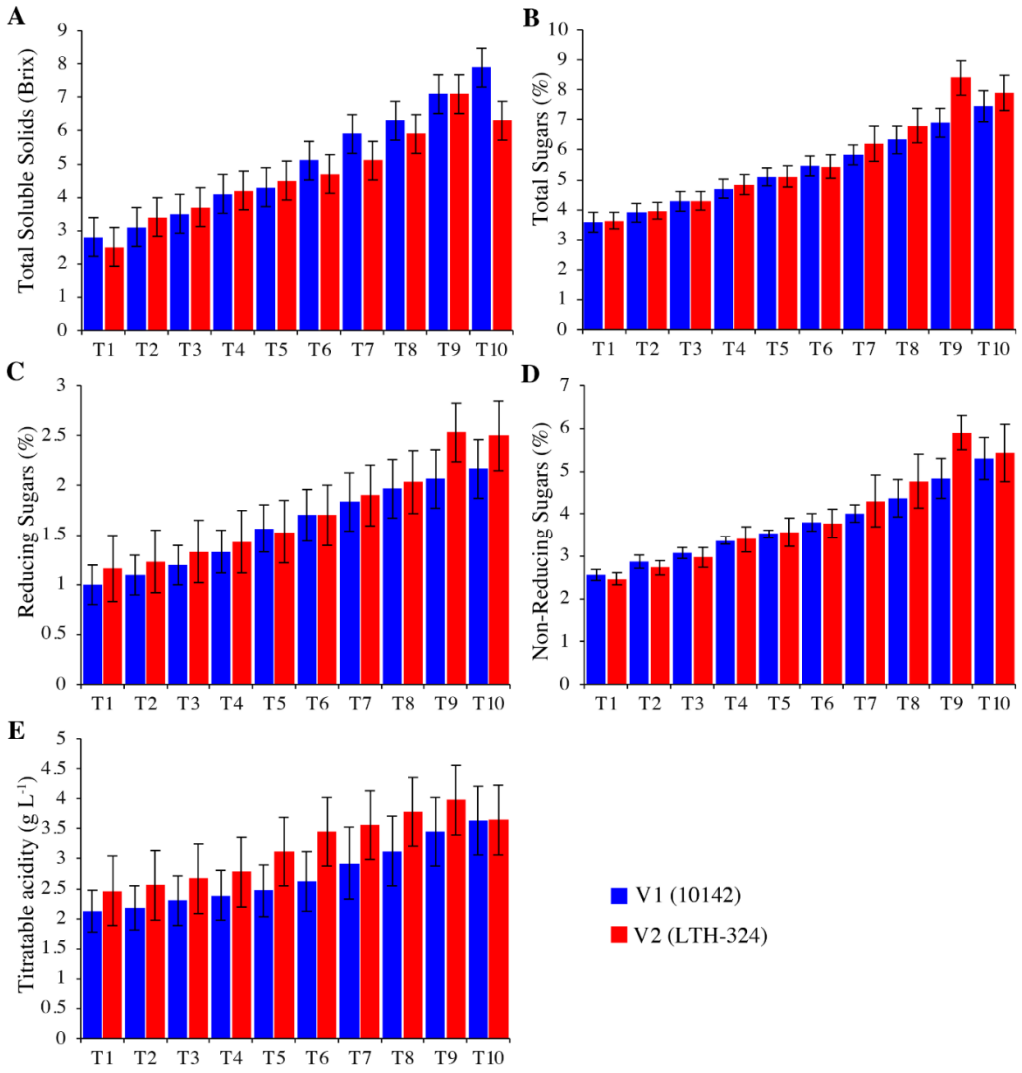

Figure 2. Effect of foliar applications of Salicylic Acid (SA) and NPK on total soluble solids (TSS), total sugars (TS), reducing sugars (RS), non-reducing sugars (NRS), and titratable acidity (TA) contents of tomato

Bars with the standard error was used to denote the data. T1 (Control), T2 (NPK 7 g/L), T3 (SA 0.05 mM), T4 (SA 0.1 mM), T5 (SA 0.5 mM), T6 (SA 1.0 mM), T7 (NPK + SA 0.05 mM), T8 (NPK + SA 0.1 mM), T9 (NPK + SA 0.5 mM) and T10 (NPK + SA 1.0 mM).

Vitamin C (mg/100 g)

The concentrations of vitamin C varied from 15.4 to 23.7 mg 100^{-1} in V1 and 15-7 to 33.10 mg 100^{-1} in V2 (Table 2). Both the varieties showed highly significant (*** = $p < 0.001$) variations in all the treatments, while their interaction showed significant (* = $p < 0.05$) variation. The highest value of Vitamin C was recorded in T9 and T10 sprayed plants (33.1 mg 100^{-1}) while control treatment (T1) showed a result (15.7mg 100^{-1}) in V2. A slight reduction in vitamin C concentration was recorded in T10 compared to T9 (Figure 3A). Treatment T10 (23.7 mg 100^{-1}) gives the best result regarding Vitamin C in variety V1. It was determined that T9 and T10 sprayed plants gave a positively high result as compared to other treatments in both varieties (Figure 3A).

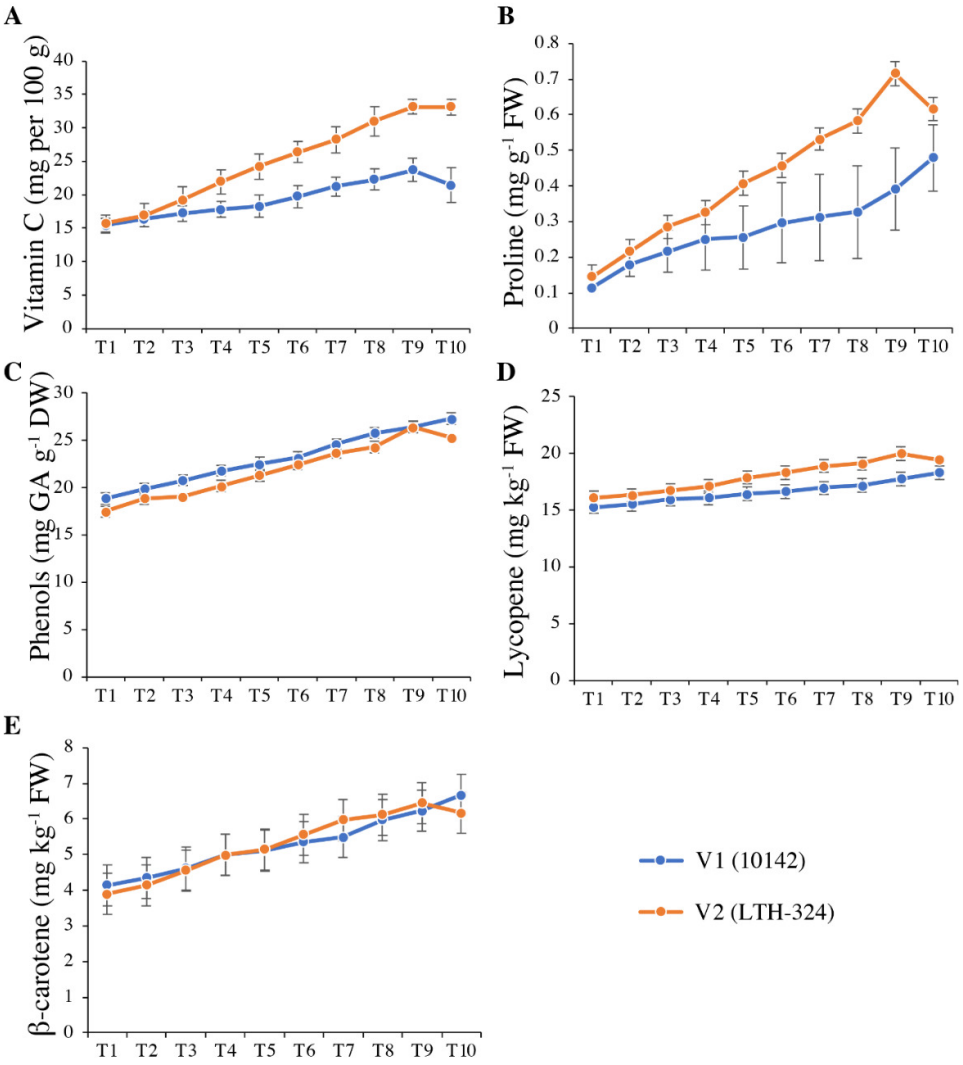

Figure 3. Effect of foliar applications of salicylic acid (SA) and NPK on (A) vitamin C concentration (Vit C, mg per 100 g), (B) proline contents (P, mg g^{-1} FW), (C) Phenols (PHE, mg GA g^{-1} DW), (D) Lycopene (L, mg kg^{-1} FW), and (E) β-carotene (CAR, mg kg^{-1} FW) of tomato
T1 (Control), T2 (NPK 7 g/L), T3 (SA 0.05 mM), T4 (SA 0.1 mM), T5 (SA 0.5 mM), T6 (SA 1.0 mM), T7 (NPK + SA 0.05 mM), T8 (NPK + SA 0.1 mM), T9 (NPK + SA 0.5 mM) and T10 (NPK + SA 1.0 mM).

Proline (mg g^{-1} FW)

The findings on proline content at various SA and NPK foliar spray levels showed the treatments and tomato varieties to differ significantly (Table 2). The range of proline varied from 0.12 to 0.47 mg g^{-1} FW in V1 and 0.15 to 0.72 mg g^{-1} FW in V2. The highest proline content was discovered in the T9 of V2 (0.72 mg g^{-1} FW), while the maximum was observed in T10 of V1 (0.47 mg g^{-1} FW). A slight reduction in proline amount was reported in T10 as compared to T9 in V2 (Figure 3B).

Total phenols contents (mg GA g^{-1} DW)

The obtained results indicate that the phenol contents varied from 18.9 to 27.3 mg GA g^{-1} DW in V1 and 17.5 to 26.4 mg GA g^{-1} DW in V2 under the foliar spray of SA and NPK. Results showed that exogenous applications of SA and NPT promoted significant increases in phenolic compounds as estimated in the controls. T10 and T9 showed the maximum phenols in V1 and V2, respectively (Figure 3C).

Lycopene and β-carotene (mg kg^{-1} FW)

The foliar spray of SA and NPK, and their combinations ameliorated the lycopene contents (19.9 mg kg^{-1} FW), with the highest dose (NPK + SA 0.5mM) in relation to the control of V2 (Figure 3D). The highest value of Lycopene contents (18.3 mg kg^{-1} FW) was found in treatment T10 of variety V1. NPK fertilizer and SA had a major effect on the β-carotene content of both tomato varieties. Open-field grown plants supplied with SA+NPK had the highest β-carotene of V1 (6.6 mg kg^{-1} FW) and V2 (6.4 mg kg^{-1} FW) in T9 and T10 comparison with the control treatments (Figure 3E).

Principal components analysis

Principal component analysis (PCA) is a straightforward nonparametric technique. The primary objective of PCA is to identify a limited set of components that explain the highest amount of variability within a given dataset. In this research, we further conducted Principal Component Analysis (PCA) to assess the variability and associations across agronomic, biochemical, and yield-related characteristics within the tomato genotypes. The PCA was conducted using 16 characteristics that accounted for 100% of the diversity. The tomato genotypes were subjected to several treatments, including SA, NPK, and their combinations. These treatments were then analyzed and visualized using biplots, where the genotypes were grouped together, and the characteristics were represented by different vectors. The total variance of the first eight principal components (PCs) amounted to 99.9%, whereas the range of variability observed among these PCs spanned from 0.1% to 85.6%. The first three principal components (PC1 = 85.6%, PC2 = 11.9%, and PC3 = 1.1%) exhibited statistical significance (Figure 4A). Agronomic traits (PH, FP, F, PT, Y, and LA), sugars-related traits (TSS, TS, RS, NRS, and TA) were the major contributing factors in PC1 (Figure 4B). FP showed a high association with Y (Figure 4B). Treatments T9 and T10 exhibited contrasting effects compared to treatments T1 and T2. According to the PCA findings, the treatments under consideration may be classified into two distinct groups: those that exhibit superior performance and those that demonstrate subpar performance. These findings have implications for their potential use in future efforts aimed at enhancing tomato cultivation.

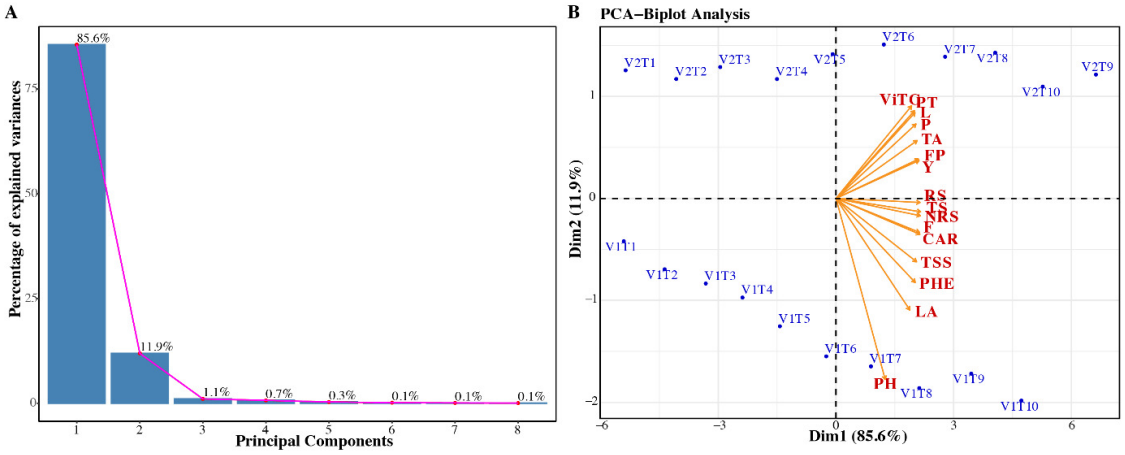

Figure 4. PCA analysis among 16 parameters of tomato grown under field conditions and effect of SA, NPK, and their combination on it.

PH = Plant height (cm); FP = Number of fruits per plant; LA = Leaf area (cm^2 per leaf); Y = Yield (g); ViTC = Vitamin C (mg/100 g); TSS = Total soluble solids (Brix %); TS = Total sugars (%); RS = Reducing sugars (%); NRS = Non-reducing sugars (%); F = Firmness (kgf); PT = Pericarp thickness (mm); P = Proline (mg g^{-1}FW); TA = Titratable acidity (meq/day); PHE = Phenols (mg GA g^{-1}DW); L = Lycopene (mg Kg^{-1}FW); CAR = β-carotene (mg Kg^{-1}FW). V1 = 10142 and V2 = LTH324. T1 (Control), T2 (NPK 7 g/L), T3 (SA 0.05 mM), T4 (SA 0.1 mM), T5 (SA 0.5 mM), T6 (SA 1.0 mM), T7 (NPK + SA 0.05 mM), T8 (NPK + SA 0.1 mM), T9 (NPK + SA 0.5 mM) and T10 (NPK + SA 1.0 mM).

Association among growth parameters, yield, and nutraceutical quality

Correlation is often understood as the quantification of the relationship between two variables that are independent of each other. The relationship between two qualities might exhibit either positive or negative attributes. PH was positively and highly significantly associated with LA, TSS, PHE, and CAR (Figure 5). Y and CAR were positively associated with all the studied parameters. PT was associated with all the parameters except PH and LA.

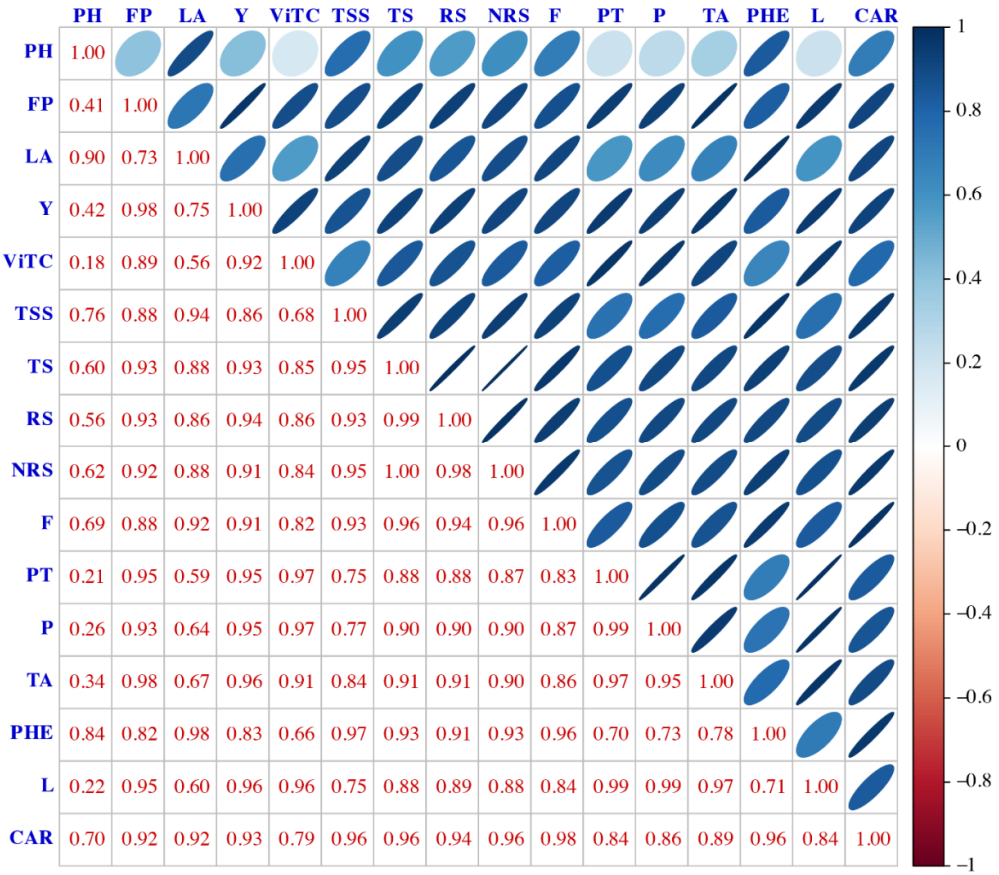

Figure 5. Association between 16 parameters of tomato grown under field conditions and effect of SA, NPK, and their combination on it

Shapes from round to oblong showed poor to strong association. PH = Plant height (cm); FP = Number of fruits per plant; LA = Leaf area (cm^2 per leaf); Y = Yield (g); ViTC = Vitamin C (mg/100 g); TSS = Total soluble solids (Brix %); TS = Total sugars (%); RS = Reducing sugars (%); NRS = Non-reducing sugars (%); F = Firmness (kgf); PT = Pericarp thickness (mm); P = Proline (mg g^{-1}FW); TA = Titratable acidity (meq/day); PHE = Phenols (mg GA g^{-1}DW); L = Lycopene (mg Kg^{-1}FW); CAR = β-carotene (mg Kg^{-1}FW).

Discussion

Salicylic acid (SA) and NPK are reported to help increase the plant height in wheat (Azimi *et al.*, 2013; Naitman *et al.,* 2015; Qasim *et al.*, 2019; Ahsan *et al.*, 2022). In tomatoes, SA application resulted in a moderate improvement in growth parameters and an increase in the number of fruits per plant and height (Agamy *et al.*, 2013; Aires *et al.*, 2022). According to previous research, SA increases the number of flowers and fruits per plant, resulting in a better yield (Basit *et al.*, 2018). The number of fruits in strawberry plants is boosted by

foliar NPK treatment because of the direct availability of potassium. Foliar application of NPK makes the fruit healthier (Shahzad *et al.*, 2017). Foliar SA treatments significantly increase leaf area in tomatoes and strawberries (Jamali *et al.*, 2011; Mady, 2009). This increase in leaf area might be attributable to increased cell division and cell expansion. Foliar NPK fertilizer treatment can help to enhance leaf area and plant height in the dahlia (Kashif *et al.*, 2014). SA foliar spray significantly increases the yield. Yield improvements may be related to changes in vegetative growth factors, such as plant length, leaf area and number of leaves per plant SA could potentially enhance tomato yield (Javaheri *et al.*, 2012; Jo and Shin, 2020; Loc and Thinh, 2020) and chili yield (Zahid *et al.,*, 2023). NPK are an essential nutrient for plant growth, and foliar application of NPK boosts maize production (Kakar *et al.*, 2014). In our study, all these parameters showed similar trends.

SA may assist in enhancing the thickness of the pericarp in pepper (Elwan and El-Hamahmy, 2009). This outcome was comparable to a study that found that NPK foliar spray enhanced pericarp thickness, which improved tomato fruit firmness (Ashraf *et al.*, 2021). The firmness of fruits is one of the quality factors most valued by customers, as probable mechanical defects drastically reduce the shelf life of fruits after harvest (Moggia *et al.*, 2017). The results are consistent with those of an earlier investigation that discovered SA boosted fruit firmness by lowering the softening (Islam *et al.*, 2018) and delayed ripening by blocking the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) into ethylene, which is responsible for cell senescence (Shafiee *et al.*, 2010). The present outcome was comparable to a study that found that NPK foliar spray enhanced pericarp thickness, which improved tomato fruit firmness (Ashraf *et al.,*, 2021). Sugars are one of the most abundant fruit components, accounting for the majority of TSS (Wang *et al.*, 2021). Our findings suggest that SA raised TSS in fruits, which is consistent with the findings of (Baninaiem *et al.*, 2016), who observed large increases in TSS in tomato fruits caused by SA. According to Chandra *et al.* (2007), SA boosted total soluble sugar and soluble protein in cowpea plants (Chandra *et al.*, 2007). This present study finding was consistent with the findings of (Anoop and Indiresh, 2015), those who discovered that NPK foliar spray produced a greater level of TSS, good pericarp thickness, minimal physiological loss, fruit weight, and the highest fruit yield/plant.

These findings show that SA foliar produced considerable increases in the phenolic compounds measured. It is an efficient biostimulant for the preservation and enhancement of phenolic compounds and antioxidants (De la Rosa *et al.*, 2019). Foliar NPK treatment increased yield, total phenols, total antioxidant, and nutrient content in olive and fig fruit (Fawy and El-Shazly, 2016). SA has a substantial influence on retaining a greater vitamin C content in peach fruits (Kazemi *et al.*, 2011). NPK fertilizer application improved the vitamin C content of the okra (Adekiya *et al.*, 2019). According to Shaaban *et al.* (2011), applying SA enhanced non-reducing sugars in apples (Shaaban *et al.*, 2011). SA increases the TSS, ascorbic acid, titratable acidity, total sugars, and reducing sugars in grapes (Hazarika and Marak, 2022). Spraying NPK before the spathe opening and at an early stage of date palm Khidrawi cultivar increased total soluble solids, total sugars, and reducing sugars (Shareef, 2011). The application of NPK is beneficial in increasing the ascorbic acid content, total sugars, reducing sugars, and non-reducing sugars in guava (Binepal *et al.*, 2013).

Lycopene is the primary carotenoid found in tomato fruits, and it is thought that consuming it might help prevent a variety of ailments (Ghadage *et al.*, 2019). SA boosts the tomato lycopene concentration (Kant *et al.*, 2016). SA treatment improved the number of fruits, average fruit weight, fruit output, vitamin C, carotenoid content, and sugar translocation from leaves to fruits in pepper (Elwan and El-Hamahmy, 2009). Foliar use of NPK fertilizer is beneficial in increasing strawberry lycopene content (Abdullah *et al.*, 2021). Increasing NPK fertilizer treatment increased the beta-carotene contents of pepino melon fruits (Mutua *et al.*, 2021). Salicylic acid foliar spray can help improve the proline content of linseed (Bakry *et al.*, 2012). SA foliar treatment is also beneficial in increasing the proline content of chilies (Zahid *et al.,*, 2023). Foliar NPK increased the content of proline and total soluble sugars, which increased wheat production (Shabbir *et al.*,

2016). Plants treated with SA exhibited greater TA in the Washington navel orange (El-Khayat, 2020). Foliar NPK fertilizer spray boosted TA in the Roselle plant (Abbas and Ali, 2011).

Conclusions

In conclusion, foliar spray of SA and NPK improves tomato fruit output, growth, and nutritional quality as compared to the control group that received no treatment under cold stress. Finally, to increase the number of bioactive components while maintaining the production and commercial quality of tomato fruits. For optimum tomato growth and quality, a foliar spray of NPK and SA (NPK+ 0.5 mM and NPK+ 1.0 mM) is recommended. Finally, it should be mentioned that the application of SA and NPK is a practical and sustainable solution for increasing horticulture crop output and nutraceutical quality at low temperatures.

Authors' Contributions

F and GY designed the study. AZ, SAN and SASC helped F for data collection, data analysis, results and their presentation in first draft. GY, KAA, AA, and MU provided with technical expertise, streamlined the basic idea, and funding acquisition. GY supervised the research.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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