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Plant Growth Regulators Influence The Contents Of Bioactive Compounds In Tomato

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Abstract
Tomato is a popular vegetable with important health benefits due to its high level of bioactive compounds, especially lycopene, phenolics, and vitamin C. The influence of varied cultural technique and different concentration of plant growth regulators (PGRs) on the bioactive compounds' concentration was studied. BARI Tomato-4 tomato variety was grown and collected from a greenhouse. The obtained results showed that there were significant differences in the mean values between analyzed parameters according to the culture technique and PGRs. The highest vitamin C (6.60 mg/100g) and vitamin A (0.1036 mg/100g) content was measured in the treatment combination of tomato-tone @ 60 ppm in poly tunnel treatment. On the other hand the treatments' combination of open field condition and NAA@30 ppm confirmed the highest amount of lycopene (mg100g-1). But only the highest total soluble solid (TSS) was attained (10.93%) from open cultivation with tomatotone@60 ppm concentration. Therefore, based on the overall performances it can be confined that tomato plant treated by tomato-tone @ 60 ppm and cultivated under poly tunnel is favorable for higher bio-chemical composition in tomato.

Key words: *Bio-chemical composition, tomato, PGRs and cultivation techniques*

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I. Introduction

A very common one is tomatoes (*Lycopersicon esculentum* Mill.) vegetables of strong nutritional value, world-wide (Vinha *et al.,* 2014; Mara, 2018). They are known as food that promotes health. The fruits of tomatoes are rich in vitamins (A, C, K, B1, B2, B2, B3, PP), a strong mineral source (potassium, sodium, Magnesium, copper, calcium, iron, phosphorous, sulphur and produce organic acids, carbohydrates, dietary fibers, a lot of phenolic compounds and carotenoids such as lycopene and b-carotene (Choi *et al.,* 2010; Bhowmik *et al.,* 2012). Since ancient times the health benefits of tomatoes is well recognize. Tomatoes are one of the most important sources of lycopene and other carotenoids that keeps biologically active and protective compounds like flavonoids and tocopherols against diabetes, cancer and cardiovascular diseases (Burda, 2014).

Synthesis of biological active compounds and their antioxidant activity change the fruit color from earlymature to ripening stage(Brandt *et al.,* 2006; Aina *et al.,* 2019; Hussein *et al.,* 2016) and quantity express the intensity of the colors. The green color of unripe tomato fruits is due to the presence of chlorophyll, at maturity stage green pigment degraded and synthesis yellow pigment due to β-carotene and xanthophylls. At ripening stage fruit color varieties differ from yellow to orange-red depending on the lycopene: β-carotene ratio (De Sousa *et al.,* 2014; Muriel Quinet *et al.,* 2019).

Plant growth regulators (PGRs) are used extensively in tomato to enhance yield and quality (Serrani *et al.,* 2007). Gemici *et al.,* (2006) reported that application of synthetic auxin and gibberellins are effective in increasing both yield and quality of tomato. Fruit set in tomato was successfully improved by application of plant growth regulators (Desai *et al.,* 2014). Sarkar *et al.,* (2014) reported that maleic hydrazide @ 60 ppm increases individual fruit weight and yield of tomato. GA3 significantly increases growth characters, yield and also improved quality of tomato whereas NAA application increased total soluble solid percentage significantly (Pundir and Yadav, 2001). High price of tunnel materials, timely non availability of hormones, insect and diseases attack were reported as major problems for summer tomato production by Karim *et al.* (2009). However, Plant growth regulator can be used for avoiding poly tunnel. The aim of this study was to describe differences in concentration of biologically active compounds in tomato depending on culture technique and application of PGR.

II. Materials and Methods

The experiment was conducted in the field of Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. The location of the site is 24° 09" N latitude and 90° 26" E with an elevation of 8.2 m high from the sea level. The soil of the experimental plots was sandy loam in texture with pH 6.15 with 26.85 field capacity. The climate of this area was characterized by very little precipitation during March to July scarcity of rainfall 82.63-436.69 mm; with high temperature (maximum 34.08^{o}C minimum 18.61^{o}C) in early summer and while heavy rainfall with high humidity(83.11%-87.43%) in later. BARI Tomato-4 a summer type variety was considered for this experiment. Plant sample were collected from randomly selected three plants from the center of each plot to get higher precision. Healthy and uniform 30 days old seedlings were uprooted separately from the seed bed and were transplanted in the experimental plots maintaining a spacing of 60 cm × 40 cm between the rows and plants respectively. This allowed an accommodation of 6 plants in each plot. The seedbed was watered before uprooting the seedlings from the seedbed so as to minimize damage of the roots. The seedlings were watered after transplanting.

PGR's used in this experiment were Gibberelic acid (GA3), Napthalene Acetic Acid (NAA), Maliec Hydrazide (MH) and Tomatotone (4-CPA). The required amount of PGR was taken by using electronic balance. The stock solution of 1000 ppm of PGR with 1ml of ethanol to dilute and then mixed 1 litre of water. 30ml and 60 ml of stock solution were mixed with 1 litre of distilled water. Working solution was prepared just before spraying. The solution was sprayed entire plants three times after transplanting in the main field. All spraying was done by using hand sprayer in the early morning to avoid rapid drying off of the spray solution.

Determination of TSS (%): Total soluble solids (TSS) content of tomato fruit pulp was estimated by using Abbe's refractometer described by Ranganna (1979). A drop of tomato juice was squeezed on the prism of the refractometer. Percent TSS was obtained from direct reading of the instrument.

Determination of Lycopene (mg/100g): Lycopene, a fat soluble carotenoid, is a precursor of β-carotene (Sandmann, 1994). Lycopene (β-carotene) was quantified according to Nagata *et al.,* (1992). At first 1 g of tomato fruit was taken. Then homogenized with twenty millimeters of acetone-hexane (4:6 by V/v) solution. The sample was centrifuged after filtering. Then optical density was measured by using UV-2100 spectrophotometer at 663 ɳm, 645ɳm, 505 ɳm and 453 ɳm. The amount of lycopene in tomato fruit was calculated by using the following formula (mg/100 g on fresh weight basis):

Lycopene (β-carotene) = $0.216\,[OD_{663}] + 0.452\,[OD_{453}] - 1.22\,[OD_{645}] - 0.304\,[OD^{505}]$

Where,

OD_{663} = Optical density at 663 ɳm wave length

OD_{453} = Optical density at453 ɳm wave length

OD_{645} = Optical density at 645 ɳm wave length

OD_{505} = Optical density at 505 ɳm wave length

Determination of Vitamin-A (mg/100g): Weigh about 5 g of well mashed sample was taken and put it into a 250 mL Erlenmeyer flask. Add 1g of α-amylase. About 50 mL of distilled water at 45 - 50°C should be added in to dissolve it. After it is well mixed, use nitrogen gas to exhaust air from the flask. Insert the stopper. Place it in a 60°C±2°C incubator for 30 min.

Vitamin A standard stock solution (based on retinol) (100 g/mL): Weigh 10 mg of standard substance accurately, dissolve it with ethyl alcohol in a 100 mL brown volumetric flask, and dilute to volume.

Draw 0.50 mL, 1.00 mL, 1.50 mL, 2.00 mL, and 2.50 mL of the Vitamin A standard stock solution in 50 mL brown volumetric flasks respectively, and dilute them with ethanol to volume; mix well. The concentrations of this series of standard working solutions are 1.00g/mL, 2.00g/mL, 3.00g/mL, 4.00g/mL, and 5.00 g/mL respectively.

Calculation of Vitamin A: The content of Vitamin A in test sample should be calculated as formula by National Food Safety Standard (2010):

$X = [Cs \times 10/2 \times 5 \times 100]/m$

In which,

X - The content of vitamin A in the test sample, of which the unit µg/100 g

Cs - The concentration of vitamin A test solution resulted from the standard curve (g/mL)

m- The mass of sample, of which the unit is gram (g)

The calculation result of Vitamin A should be expressed as the mean value of two independent tests and accurate to three decimal places.

Note: 1µg retinol= 3.33 IU vitamin A.

Determination of Vitamin-C (mg/100g): At first 20 g of fresh sample was taken and blended with a blender. Then the volume was adjusted to 100 ml with distilled water. Finally, it was centrifuged. After centrifuge 5 ml centrifuged sample was taken for titration. The following formula was used for determining ascorbic acid:

Ascorbic acid (mg/100 g) = [F×V1×V2×100]/[W×V3]

Where,

F= 0.088 mg ascorbic acid for 1 ml of 0.001 N Potassium Iodate (KIO3)

V1= Titrated volume (ml) of 0.001 N KIO3

V2= Total volume of sample (ml)

V3= Volume (ml) of sample titrated with 0.001 N KIO3

W= Weight of the plant sample taken (g)

The data were statistically analyzed by using MSTAT-C software. The recorded data for different characters were subjected to variance analysis. Duncan's Multiple Range Test (DMRT) was used to measure the value of the disparity between the treatment combinations at $p \leq 0.05$ level of significance Gomez and Gomez (1984).

III. Results

Effect of cultivation practices

Total soluble solid (TSS)

TSS in tomato differed significantly due to different cultivation practices (Table 1). Results showed that the highest TSS (7.60%) was found from open cultivation method (P0) where the lowest (6.74%) TSS was obtained from poly tunnel method (P_1) (Fig. 1[a]).

Lycopene

Non-significant variation was found for lycopene content in tomato influenced by different cultivation practices (Table 1). Cultivation of tomato under poly tunnel method (P_1) expressed the highest lycopene content (0.143 mg/100 g) and the lowest (0.135 mg/100 g) was obtained from open cultivation (P_0) (Fig. 1[b]).

Table 1. Mean square of quality parameters affected by cultivation methods and plant growth regulators

Source	df	Mean Square of quality parameters			
		TSS (%)	Lycopene (mg/100g)	Vitamin A (mg/100g)	Vitamin C (mg/100g)
Replication	2	0.527	0.001	0.001	0.562
Factor A	1	10.05**	NS	NS	6.720**
Error	2	0.534	0.001	0.001	0.515
Factor B	8	8.187**	NS	NS	0.538**
AB	8	3.522**	NS	NS	0.722**
Error	32	0.757	0.001	0.001	0.306

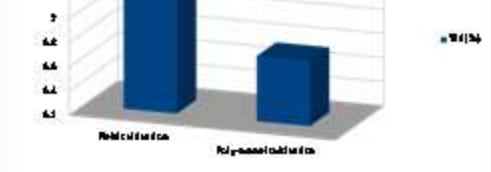
Vitamin A

Vitamin A content of tomato was not significantly influenced by different cultivation practices (Table 1). Cultivation of tomato under poly tunnel method (P1) expressed the highest vitamin A content (0.078 mg/100 g) and the lowest (0.076 mg/100 g) was obtained from open cultivation (P0) (Fig. 1[c]).

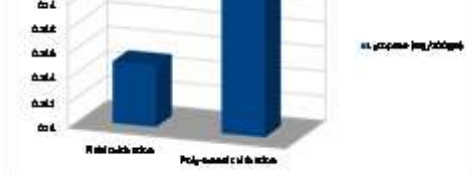
Vitamin C

Vitamin C in tomato differed significantly in response to different cultivation practices (Table 3). Results showed that the highest vitamin C (5.99 mg/100g) was found from poly tunnel method (P1) where the lowest vitamin C (5.29 mg/100g) content was obtained from open cultivation method (P0) (Fig. 1[d]).

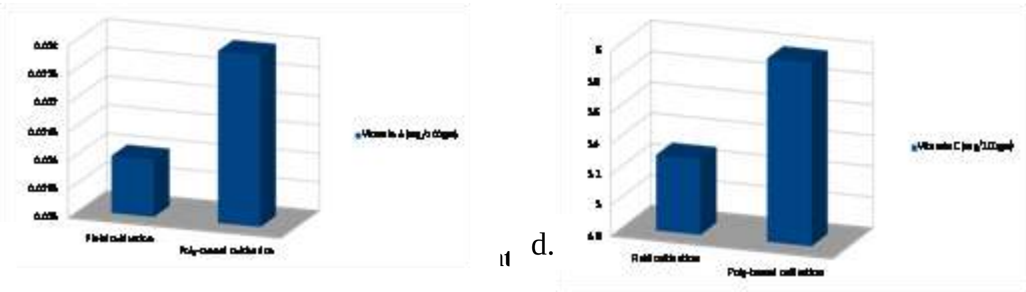
a.



b.



c.



A content (mg/100g) and Vitamin C content (mg/100g) in tomato

Effect of plant growth regulators

Total soluble solid (TSS)

Significant variation was also observed for TSS by different growth regulators (Table 1). Amount of TSS content increased in with hormone application compare to control. But this increased TSS was from higher dose of GA3, NAA and tomato-tone and lower dose MH (Fig. 2.a). However, the highest TSS (9.97%) was from the treatment of T8 (tomatotone@60 ppm) where the lowest TSS (6.08%) was also attained from application of hormone T6 (MH@60 ppm).

Lycopene

Non-significant effect was also found for lycopene content due to different growth regulators (Table 1). Lycopene content was increased with increased concentration of hormone except T_3. Here, lower concentration (30 ppm) of NAA performed best result (0.1799mg/100 g) compare to control and all other treatments. The lowest lycopene content (0.1029mg/100 g) was observed from T5 (MH 30 ppm) (Fig. 2.b).

Vitamin A

Non-significant effect was also found for vitamin A content due to different growth regulators (Table 1). Vitamin A content increased with increased in hormone concentration, but only from Treatment T6 and T8 it was found higher than control (Fig. 2.c). The highest vitamin A content (0.096 mg/100 g) was found in T8 (Tomato-tone 60 ppm) where the lowest vitamin A content (0.059 mg/100 g) was observed from T1 (30 ppm GA3).

Vitamin C

Significant variation was also observed for vitamin C influenced by different growth regulators (Table 1). Vitamin C content increased in hormone application compare to control (Fig. 2.d). This trend of increasing was observed with higher concentration of hormone except T2 (Fig. 8). Results signified that the vitamin C content was the highest (5.98 mg/100g) from the treatment, (Tomato-tone 60 ppm). The lowest vitamin C content (5.16 mg/100g) was attained from control treatment (T0).

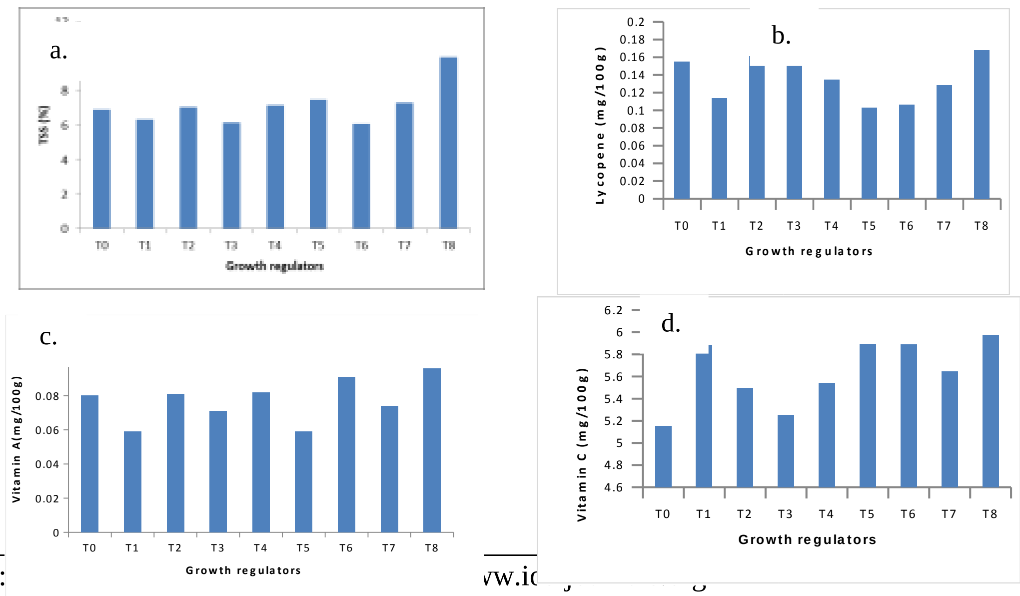


Figure 2. Effect of PGRs on a) TSS content (%), b) Lycopene content(mg/100g), c) Vitamin A content (mg/100g) and d) Vitamin C content (mg/100g) in tomato

[T_0=Control, T_1=GA3 @30 ppm, T_2 =GA3 @ 60 ppm, T_3= NAA @ 30 ppm, T4 = NAA@60 ppm,T_5=MH@30 ppm, T_6=MH@60 ppm, T_7 = Tomato-tone @ 30 ppm and T_8 = Tomato-tone @ 60 ppm]

Combined effect of cultivation practices and plant growth regulators

Treatment combination of cultivation practices and different growth regulators had significant on TSS content in tomato (Table 1). Similarly, TSS was higher in open field than poly tunnel. Only in the open field, amount of TSS content increased with hormone application compare to control except P_0T_1, P0T6 treatment combination (Table 2). However, the highest TSS content (10.93% and 9.00%) was measured from the treatment GA3 (60 ppm) respectively in the open and poly tunnel. The lowest TSS content (5.33%) was attained from P0T6 (Table 2).

Table 2. Effect on quality parameters as influenced by treatment combination of cultivation methods and plant growth regulators

Treatment	Quality parameters			
	TSS (%)	Lycopene (mg/100g)	Vitamin A (mg/100g)	Vitamin C (mg/100g)
P_0T_0	6.83ef	0.1634	0.08987	4.69 j
P_0T_1	5.67 h	0.1112	0.05707	5.95cd
P_0T_2	10.93 a	0.1682	0.07950	4.92hi
P_0T_3	7.00 e	0.1900	0.07243	4.79ij
P_0T_4	7.67 d	0.1406	0.07657	5.07gh
P_0T_5	8.83 bc	0.0539	0.05870	5.34f
P_0T_6	5.33 h	0.0991	0.08533	5.95cd
P_0T_7	7.67d	0.1133	0.07043	5.17g
P_0T_8	8.50 c	0.1719	0.08980	5.69e
P_1T_0	7.00 e	0.1459	0.07043	5.14g
P_1T_1	7.00 e	0.1154	0.06017	5.82de
P_1T_2	9.00 b	0.1536	0.08257	6.07c
P_1T_3	5.50 h	0.1699	0.07000	5.72e
P_1T_4	6.67 ef	0.1282	0.08753	6.02c
P_1T_5	6.17 g	0.1519	0.05997	6.45a
P_1T_6	6.83 ef	0.1134	0.09780	5.83de
P_1T_7	6.17 g	0.1435	0.07163	6.27b
P_1T_8	6.50 fg	0.1642	0.10360	6.60a
$LSD_{0.05}$	0.3971	NS	NS	0.1488
CV(%)	8.13	4.39	5.49	6.81

The data represent the mean values. Different letters denote significant differences at $p \leq 0.05$ by Duncan's multiple range tests. P_0 = Open air cultivation, P_1= Poly tunnel cultivation, T_0=Control, T_1=GA3 @30 ppm, T_2 =GA3 @ 60 ppm, T_3= NAA @ 30 ppm, T4 = NAA@60 ppm,T_5=MH@30 ppm, T_6=MH@60 ppm, T_7 = Tomato-tone @ 30 ppm,T_8 = Tomato-tone @ 60 ppm, LSD = Least significant difference and CV = Coefficient variance.

Combination of cultivation practices and different growth regulators had non-significant influence on lycopene content in tomato (Table 1).

There was no significant effect on vitamin A content in tomato affected by different growth regulators and cultivation practices (Table 1). Only combination of P_1T_6 and P_1T_8 treatment vitamin A content increased compared to control (Table 2). However, the highest vitamin A content (0.104 mg/100 g) was in the treatment combination of P_1T_8, while the lowest vitamin A content (0.057mg/100 g) was observed in P_0T_1 (Table 2).

Treatment combination of cultivation practices and different growth regulators had significant variation on vitamin C content in tomato (Table 1). Similarly, Vitamin C content was increased with application of hormone compared to control (Table 2). This increasing trend of vitamin C content was observed with increased concentration of hormone except P0T3 and P_1T_6 (Table 2). Significantly the highest vitamin C content (6.60 mg/100g) was produced in the treatment combination of P_1T_8 where the lowest vitamin C content (4.69

mg/100g) was attained from P_0T_0 (Table 2).

IV. Discussion

Total Soluble Solid is an important quality parameter for suitability containing mainly sugars(65%) in tomato(Brandt *et al.,* 2006). Sucrose the component of sugar synthesis by photosynthesis. Full time sunlight in open field might increase the photosynthetic activity as well as accumulation of TSS compare to poly tunnel. Fruits stored at 15°C had higher total soluble solids and soluble sugars but shows red color formation (Islam *et al.,* 2006).

After sugar content, acids (13%) are major components of TSS. GA3 plant hormone increases the synthesis of major two components of TSS. Application of GA3 at 50 ppm increases ascorbic acid (Chaudhary *et al.,* 2006 and Ouzounidou *et al.,* 2010) and TSS in tomato (Gelmesa *et al.,* 2012; karim *et al.,* 2009; Kavipriya & Boominathan 2018). Graham and Ballesteros (2006) reported that GA3 increased proteins, soluble carbohydrates, ascorbic acid, starch and β-carotene in the tomato. Kumar et al., 2014 observed the highest ascorbic acid and total soluble solid (TSS) treated with GA3 50 ppm. Kumar *et al.* (2014) also found the highest TSS from higher dose of GA3 (50 ppm), while Singh and Singh (2011) reported similar result from NAA 50 ppm. Meena (2008) observed that higher total soluble solid (TSS) with application of GA3 at 50 ppm compared to 25 and 75 ppm GA3 and NAA. Saha *et al.,* (2009) and Khaled *et al.,* (2015) shown that combined application of NAA (25 ppm) and GA3 (40 ppm) was more effective than their individual application in terms of TSS, respectively.

Lycopene is strongly influenced by environmental factors, especially daily air temperature (optimum between 22 and 25°C) and exposure to sunlight (Lumpkin, 2005; Dumas *et al.* 2003). The production of lycopene is inhibited by excessive sunlight (Brandt *et al.,* 2006; Brandt *et al.,* 2006b). In field cultivation above factors are beyond control and lycopene content is greatly inhibited.

From PGRs aspect GA3 significantly increases growth characters, yield of tomato (Pundir and Yadav, 2001). Khan et al. (2006) indicated the significant role of GA3 in tomato plant to increase fruit set that leads to larger number of fruits per plant and increased fruit size and final yield. Khan et al. (2006) observed GA3 increase in leaf phosphorous, nitrogen, and potassium content in addition to increased lycopene content of tomato fruit when treated with GA3. Masroor *et al.,* 2006 also reported that foliar application of gibberelic acid significantly increased lycopene content of tomato fruits (Pramanik *et al.,* 2017).

Like other bioactive compound vitamin A is present in tomato, but it is minute (5%) amount (Hussein *et al.,* 2016;Johns *et al.,* 1992). Vitamin A is so sensitive to light, temperature and wind that is well prevailed in field cultivation. Eze J.I. (2012) found that the concentrations of vitamin A in the open-air system were generally low due to exposure to both light and wind.

The study revealed significant differences in the vitamin C content observed in tomato harvested from poly tunnel cultivation. Temperature and relative humidity may have an effect on the vitamin C content of the tomato fruit (Brandt 2006; Seung *et al.,* 2020). High temperature in the open field compare to poly tunnel may affect in vitamin C content. Environmental factors have a marked influence on the level of ascorbic acid (AA) in tomato fruit (Hamner *et al.,* 1945).

PGRs have shown a significant effect on vitamin C content. Haque MA (2017) showed ascorbic acid, total sugar and reducing sugar increased with increased levels of gibberellic acid and were maximum in treatment GA3 @ 60 ppm. Interestingly, Saha *et al.* (2009) found that Combined application of NAA (25 ppm) and GA3 (40 ppm) was more effective than their individual application in terms of vitamin C content, respectively.

V. Conclusion

The study exerted that the highest vitamin C (6.60 mg/100g) and vitamin A (0.1036 mg/100g) content was measured in the treatment combination of tomato-tone @ 60 ppm in poly tunnel treatment. The highest total soluble solid (TSS) was attained (10.93%) from higher concentration of tomatotone under open field. On the other hand the treatments' combination of open field condition and NAA at 30 ppm concentration confirmed the higher amount of lycopene (mg100g-1) in BARI Tomato-4 grown at the farming areas of Gazipur (24° 09" N latitude and 90° 26" E), Bangladesh.

Abbreviations

PGRs: Plant Growth Regulators NAA: α-Naphthalene acetic acid BAP 6-Benzylaminopurine 2,4-D: 2,4-Dichlorophenoxyacetic acid GC-MS: Gas Chromatography Mass Spectrometry

Authors' contributions

Dr. Md. Mijanur Rahman Rajib was involved in conceptualization, Ruby Akter conducted experiment, literature search and evaluation and writing of manuscript drafts. All authors review the manuscript drafts, literature review and approval of the final version of the manuscript. Both authors read and approved the final manuscript.

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Supplementary Information

Table S1. Temperature, relative humidity and rainfall during the growing period of tomato

Month	Air temperature (°C)		Relative Humidity (%)	Rainfall (mm)
	Maximum	Minimum		
March	29.85	18.61	83.11	82.63
April	33.10	23.38	83.00	207.31
May	34.08	24.94	83.00	225.32
June	33.23	26.25	85.38	408.12
July	32.19	27.10	87.43	436.69

Table S2. Physical and chemical characteristics of top soil of experimental field, BSMRAU, Gazipur

Physical		Chemical	
Parameter	Value	Parameter	Value
Field capacity (%)	26.80	pH	6.15
Bulk density (g/cc)[1]	1.59	Organic matter (%)	1.64
Texture		Total N (%)	0.08
Sand (%)	16.90	Available P (ppm)	12.00
Silt (%)	47.50	Exchangeble K. (m.e./l00 g)	0.34
Clay (%)	35.60	Available S (μg/g)	17.60
Textural class	Silty clay loam		

Source: Department of Soil Science, BSMRAU, Gazipur

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