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Integrated impact of micronutrients and potassium humate applications on growth, yield and fruit quality of tomato

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Abstract

Micronutrients play vital role in improving fruit quality due to their physiological role in metabolism and plant growth. Integrated effect of different rates of micronutrients (0, 50, 75 and 100% of recommended dose) and potassium humate (0, 5, 10 and 15 kg ha^{-1}) on growth, yield, fruit quality and leaf mineral composition of tomatoes was investigated through pot experiments for two years. Results showed significant improvement in growth attributes, leaf mineral contents and finally yield of tomato due to integrated use of potassium humate and micronutrients. Chemical analyses of red-ripe fruits showed improvement in their nutritional characters due to more nutrient accumulation in fruits. Application of Zn, Fe and B mixture along with potassium humate as soil amendment enhanced fruit pulp, total soluble solids, titratable acidity and ascorbic acid of red-ripe fruits and fruit yield over control ones. Our investigation highlights an existing unexploited potential of potassium humate as soil organic amendment in improving tomato nutrition and supports non-conventional programs to improve tomato nutritional value.

Key words: humic acid, leaf mineral contents, *Lycopersicon esculentum*

Introduction

Chemical composition and nutrients content in vegetables and fruits determine their nutritional value (Schnitzler and Gruda, 2002). Now a day, researchers have key focus on quality of products while introducing amendments in food production technologies. Tomato fruit is important part of human diet due to enriched in minerals, lycopene, vitamin C and phenolic compounds (Moigradean *et al.*, 2007). It is a valuable food component because of its ascorbic acid content that acts as antioxidant and has therapeutic properties (Kader, 2002). As quality concerns, have become progressively more important worldwide in the last decade and therefore, most of recently reported investigations have addressed the impact of plant nutrition on the quality of tomatoes (Siddiq *et al.*, 2015).

Six micronutrients i.e. boron, manganese, iron, copper, zinc, and molybdenum play distinct and vital roles in plant physiology and biochemical processes (Yaseen *et al.*, 2013) and ultimately in quality of vegetables and fruits. The deficiency of any micronutrient in the soil can be a barrier to growth, even when all other nutrients are in the soil at optimum levels (Yaseen *et al.*, 2013). In Pakistan, micronutrients deficient soils are very common due to extensive farming of crops, calcareousness nature and low organic matter. The field scale deficiency of three micronutrients i.e. Zn, Fe and B has hampered not only production of tomatoes but is believed a root cause of poor quality tomatoes in Pakistan (Siddiq *et al.*, 2015). This situation is predicted to get worse in the future.

Organic matter affects the growth and yield of crops by modifying soil properties and nutrient uptake. Poor soil aggregation and less moisture containment are characteristics of soils with low amount of organic matter. A lot of studies have reported a significant increase in growth and yield of crops due to the application of organic amendments (Phillips, 2004; Hussain *et al.*, 2005). Although, numerous information is available about the alone use of potassium humate and micronutrients for vegetables particularly tomato, however our main emphasis is to determine the impact of micronutrients on growth, yield and quality of tomato in the presence and absence of potassium humate. As humic substances affect plant micronutrient uptake due to their ability to complex metals under different environmental conditions (Garcia-Mina et al., 2004), therefore, we hypothesized that integrated application of micronutrients and potassium humate is more effective to improve growth, yield and fruit quality of tomato.

Materials and Methods

Pot experiments were carried out at the Institute of Pure and Applied Biology, Bahauddin Zakariya University (BZU), Multan for two consecutive years i.e. during autumn season to investigate integrated effect of potassium humate and micronutrients mixture on growth, yield, fruit quality

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and mineral composition of tomato (*Lycopersicon esculentum* Mill.). A layer of surface soil from 0-15 cm was obtained from University Experimental Farm, air-dried and sieved through 2 mm mesh. A representative sample of soil was analyzed for its physical and chemical characteristics according to methods prescribed by U.S. Salinity Laboratory Staff (1954). The soil was silt loam, alkaline and calcareous with low organic matter and total nitrogen and available phosphorus (Table 1). The air-dried soil was filled in plastic containers having diameter of 24.5 cm with 28.00 cm depth and a drainage hole in the bottom. The tomato nursery of cv. Rio Grande was raised under greenhouse conditions. Four seedlings of one month age, uniform in size and growth, were transplanted in each pot according to experimental treatments on 15th of September each year.

Table 1: Physico-chemical characteristics of soil used in pot experiments

Parameter	Unit	Value
Textural class	-	Silt loam
Organic matter	%	0.76
ECe	dS m^{-1}	2.11
pH	-	8.20
CEC	Cm_c kg^{-1}	5.42
Total N	%	0.039
Available P	mg kg^{-1} soil	8.60
Extractable K	mg kg^{-1} soil	172.2

The treatments consisted of two factors; four levels of potassium humate (0, 5, 10 and 15 kg ha^{-1}) and four rates of micronutrients mixture (0, 25, 50 and 100% of recommended dose). The recommended rate of micronutrients used per hectare was 5 kg of Zn, 1.5 of B and 5 kg of Fe in the form of $ZnSO_4$, Borax and $FeSO_4$, respectively (NDFC, 1998).

The micronutrient mixture was prepared by mixing $ZnSO_4$, Borax, $FeSO_4$ and surfactant. The treatments were arranged in a Completely Randomized Design (CRD) with factorial layout and having four repeats. The recommended dose of NPK @ 160, 100, 100 kg ha^{-1}, respectively was applied to the crop. The sources of fertilizers were urea, DAP and sulfate of potash. Complete dose of P and K, and 1/3rd dose of nitrogen were mixed in the soil while filling the pots. The remaining dose of nitrogen (2/3rd) was applied in two equal splits i.e. one at flower initiation and the second at peak flowering stage. Similarly, different rates of potassium humate were also mixed in the soil of each pot before transplanting seedlings while micronutrients mixture was top-dressed after 30 days of seedlings transplanting according to the treatment plan. The pots were irrigated at water holding capacity throughout the growing periods. The plants were protected from damage caused by birds and pilferage by humans. Weeds were controlled manually. The crop was protected against the insects, white fly, jassid and army worm by spraying with pyriproxifin (at 5 ml L^{-1} of water); nitenpyram (at 2 ml L^{-1} of water) and lambda cyhalothrin (at 2.5 ml L^{-1} of water), respectively during the growth period. Data regarding plant growth and yield were recorded at 120 days of transplanting.

Tomato fruit analyses

For fruit, mineral composition, sampled fruits of tomatoes were oven dried to a constant weight, ground and analyzed for micronutrients as described by Wolf (1982). Zinc and iron concentrations in di-acid digested solution were identified using an atomic absorption spectrophotometer (Jones *et al.*, 1991). Boron was determined through a UV/Visible spectrophotometer (Gaines and Mitchell, 1979).

For fruit quality attributes, five fully ripe tomato fruits of uniform size and weight from each treatment were selected, washed with distilled water to remove dust and dried with soft tissues and stored till analysis. At the time of analysis, the stored fruits of each treatment were homogenized well by grinding in simple food shaker. The homogenized juice was then centrifuged at 9000 g for 15 minutes to get clear fruit juice for the following estimations.

Total soluble solids

A digital refractrometer (ATAGO) was used to determine total soluble solids (TSS) from clear tomato fruit juice. Results were presented in °Brix after making temperature correction (AOAC, 1999).

Titrateable acidity

Titrateable acidity was measured by titration method which was carried out against 0.1N sodium hydroxide and indicator used was phenolphthlein. The results were presented in percent of anhydrous citric acid (AOAC, 1999).

Ascorbic acid

Ascorbic acid determination was based on reduction of dye, 2, 6-dichlorophenol indophenol by ascorbic acid (AOAC, 1999). Tomato juice (0.5 ml) was mixed with 2.5 ml of extracting solution (3% metaphosphoric acid and 8% acetic acid). Filtration process was carried out through Wattman No. 4 filter paper. The filtrate was titrated against indophenol solution (25% 2, 6-dichlorophenol indophenol and 21% $NaHCO_3$ in water) till a light rose pink colour appeared and persisted for 5 seconds.

Table 2: Variations in growth and yield variables of tomato cv. Rio Grande as result of integrated application of potassium humate and micronutrients mixture (data of two years were pooled and means are given)

Treatment		Growth and yield attributes						
Zn+Fe+B (% of rec. dose)	Potassium humate (kg ha^{-1})	Plant height (cm)	Leaves plant^{-1}	Leaf area (cm^2)	Chloro-phyll (SPAD)	Fruits plant^{-1}	Fruit yield (kg plant^{-1})	Harvest index (%)
0%	0	61.0 g	36.5 o	0.31 f	17.54	16.25 l	1.19 k	47.41 l
	5	63.3fg	40.3 n	0.32 h	17.94	16.75 k	1.23 j	47.56 j
	10	65.5 e-g	44.8 m	0.36 gh	18	17.00 j	1.35 i	47.66 h
	15	67.5 e-g	48.5 l	0.38 f-h	18.15	17.25 i	1.40 h	47.73 f
50%	0	73.0 d-g	52.0 k	0.40 e-h	18.27	17.15 ij	1.33 i	47.45 k
	5	81.8 c-e	64.0 h	0.45 d-g	20.61	18.15 g	1.46 fg	47.61 i
	10	84.8 cd	68.5 g	0.47 d-f	21.24	19.11 d	1.57 d	47.68 gh
	15	86.8 cd	72.0 f	0.50 c-e	21.96	19.11 d	1.59 d	47.87 d
75%	0	77.3 c-g	58.0 j	0.43 d-g	19.02	17.50 h	1.42 gh	47.57 j
	5	89.3 cd	76.5 e	0.53 b-d	22.17	18.50 f	1.53 e	47.81 e
	10	92.5 c	80.0 d	0.53 b-d	22.39	19.50 c	1.72 c	47.94 c
	15	93.50 bc	82.0 d	0.58 a-c	22.67	20.20 b	1.79 b	48.04 b
100%	0	79.5 c-f	60.5 i	0.44 d-g	19.23	18.25 g	1.48 f	47.71 fg
	5	110.0 ab	86.3 c	0.62 ab	23.63	18.95 e	1.69 c	47.88 d
	10	113.5 a	89.5 b	0.65 a	24.52	19.25 d	1.72 c	47.96 c
	15	121.0 a	92.0 a	0.66 a	24.87	20.75 a	1.86 a	48.08 a

Values sharing same letter(s) in each column do not differ by each other at $p \leq 0.05$ according to LSD test.

Lycopene content

Lycopene analysis was performed by using the method of Ranganna (1986). Measured sample (10 ml fruit juice) was repeatedly extracted with acetone to get the colourless residues. The extract so obtained was shifted to separating funnel having 10-15 ml petroleum ether. Thereafter, 5% sodium sulfate solution was added. Repeated extraction of acetone phase was carried out with petroleum ether, till it became colourless. The upper petroleum ether layer was pooled and volume was made to 50 ml with petroleum ether. The intensity of colour was measured at 503 nm in a spectrophotometer (UV-VIS 2201 Double Beam). Petroleum ether was used as blank.

Tomato Leaf analyses

Sampled leaves of tomato plants were oven dried to a constant weight, ground and analyzed for macro and micronutrients as described by Wolf (1982). Nitrogen was analyzed according to Jackson (1962), while phosphorus was analyzed using the vanadate-molybdate spectrophotometric procedure (Jones et al., 1991). Potassium was determined by a flame photometer (Chapman and Pratt, 1961).

Data analysis

Statistical procedures were applied to analyze the data (Steel et al., 1997) using completely randomized design with factorial arrangement while means were compared by LSD test at 5% probability level.

Results

Plant Growth and yield attributes

Application of potassium humate and micronutrients significantly affected plant growth (Table 2). Among all tested combinations, application of 15 kg ha^{-1}potassium humate along with full dose of micronutrients (100%) resulted in maximum increase in growth and yield attributes such as plant height, leaves per plant, leaf area, number of fruits per plant and fruit yield per plant (Table 2). A substantial increase in plant growth parameters occurred as result of integrated application of potassium humate and micronutrients compared to alone application of potassium humate or micronutrients. Data on average fruit yield per plant obtained during two seasons revealed that application of 15 kg ha^{-1} potassium humate along with 100% of recommended micronutrients dose caused about 27% increase in fruit yield compared to untreated ones (Table 2).

Fruit quality attributes

Integrated application of micronutrients and potassium humate significantly affected tomato quality attributes such as fruit pulp, TSS, ascorbic acid and lycopene contents (Table 3). Results indicated that fruits of control plants were of inferior quality as compared to those of treated ones. Fruit pulp increased on increasing dose of micronutrients along with rate of potassium humate (Table 3). However, plants supplied with higher rates of potassium humate had more fruit pulp than those treated with lower rates of potassium humate (Table 3).

Table 3: Variations in fruit quality attributes of tomato cv. Rio Grande as result of integrated application of potassium humate and micronutrients mixture (data of two years were pooled and means are given)

Treatment		Fruit yield and quality attribute				
Zn+Fe+B mixture (% of rec. dose)	**Potassium humate (kg ha^{-1})**	**Fruit pulp (%)**	**Total soluble solids (°Brix)**	**Total acidity (%)**	**Ascorbic acid (mg 100 g^{-1} FW)**	**Lycopene content (mg kg^{-1} FW)**
0%	0	65.0 j	2.50 h	0.20 d	8.1i	41.4 l
	5	67.0 ij	2.70 g	0.20 d	8.2hi	43.4 l
	10	68.0 i	2.80 fg	0.30 c	8.4gh	44.5 k
	15	70.0 h	2.90 f	0.30 c	8.5fg	45.5 jk
50%	0	71.1 h	3.10 e	0.30 c	8.6fg	47.5 j
	5	76.1 f	4.00 cd	0.40 b	8.9de	67.7 h
	10	77.3 ef	4.08 c	0.40 b	8.9de	70.0 g
	15	78.3 de	4.08 c	0.40 b	9.1d	72.1 f
75%	0	74.1 g	3.88 d	0.30 c	8.7ef	47.5 j
	5	78.4 de	4.10 c	0.40 b	9.1d	81.2 e
	10	80.2 cd	4.10 c	0.40 b	9.5c	82.2 e
	15	81.2 c	4.30 b	0.40 b	9.5c	89.3 d
100%	0	76.1 f	4.00 cd	0.30 c	8.9de	51.5 i
	5	83.2 b	4.40 b	0.40 b	9.9b	92.4 c
	10	83.4 ab	4.60 c	0.40 b	10.0b	101.5 b
	15	89.5 a	4.60 a	0.50 a	11.1a	113.7 a

Values sharing same letter(s) in each column do not differ by each other at $p \leq 0.05$ according to LSD test.

Data on titrateable acidity also revealed significant differences ($p < 0.01$) for rates of micronutrients and potassium humate. Results indicated that potassium humate application improved titrateable acidity of tomato fruits (Table 3) by increasing micronutrients contents in fruits (Figure 1). The concentration of Zn, B and Fe in tomato fruits increased on integrated application of potassium humate and micronutrients at higher rates (Figure 1). Titrateable acidity was found to vary significantly with rates of micronutrients applied. Application of potassium humate and micronutrients significantly affected ascorbic acid (vitamin C) content of tomato fruits (Table 3). However, effect of potassium humate and micronutrients on ascorbic acid contents was rate dependent. The maximum ascorbic acid content (11.1 mg kg^{-1} FW) was recorded in fruits of the plants treated with 100% recommended dose of micronutrients along with 15 kg ha^{-1} potassium humate while minimum ascorbic acid content (8.1 mg kg^{-1} FW) was found in those of control ones (Table 3). Similarly, maximum lycopene content (113.7 mg kg^{-1} FW) was noted in fruits of plants treated with 15 kg ha^{-1} humate and 100% dose of micronutrients whereas least lycopene content (41.4 mg kg^{-1} FW) in fruits of control ones (Table 3).

Leaf mineral composition

Results also indicated that leaves of plants treated with either potassium humate or micronutrients have more macro and micronutrients accumulations compared to those of control ones. Leaf analysis indicated that application of 15 kg ha^{-1} potassium humate along with 100% dose of micronutrients caused maximum accumulation of N (4%), P (0.27%), K (2.62%), Fe (284 mg kg^{-1}), B (64.3 mg kg^{-1}) and Zn (135 mg kg^{-1}) (Table 4). It is also obvious from results that mineral composition of control plants was poor compared to treated ones. Results also revealed that application of potassium humate caused more accumulation of macro and micronutrients than alone application of micronutrients (Table 4).

Discussion

The aim of these experiments was to investigate potential of potassium humate alone and in combination with micronutrients to improve the quality of tomato fruits due to its regulatory role in soil fertility. Humic substances play the role of organic matter whenever these are used as soil amendment. These substances enhance soil microbial activity and also act as nutrient source (Mackowiak *et al.*, 2001; Charest *et al.*, 2004; Pérez-Piqueres *et al.*, 2005).

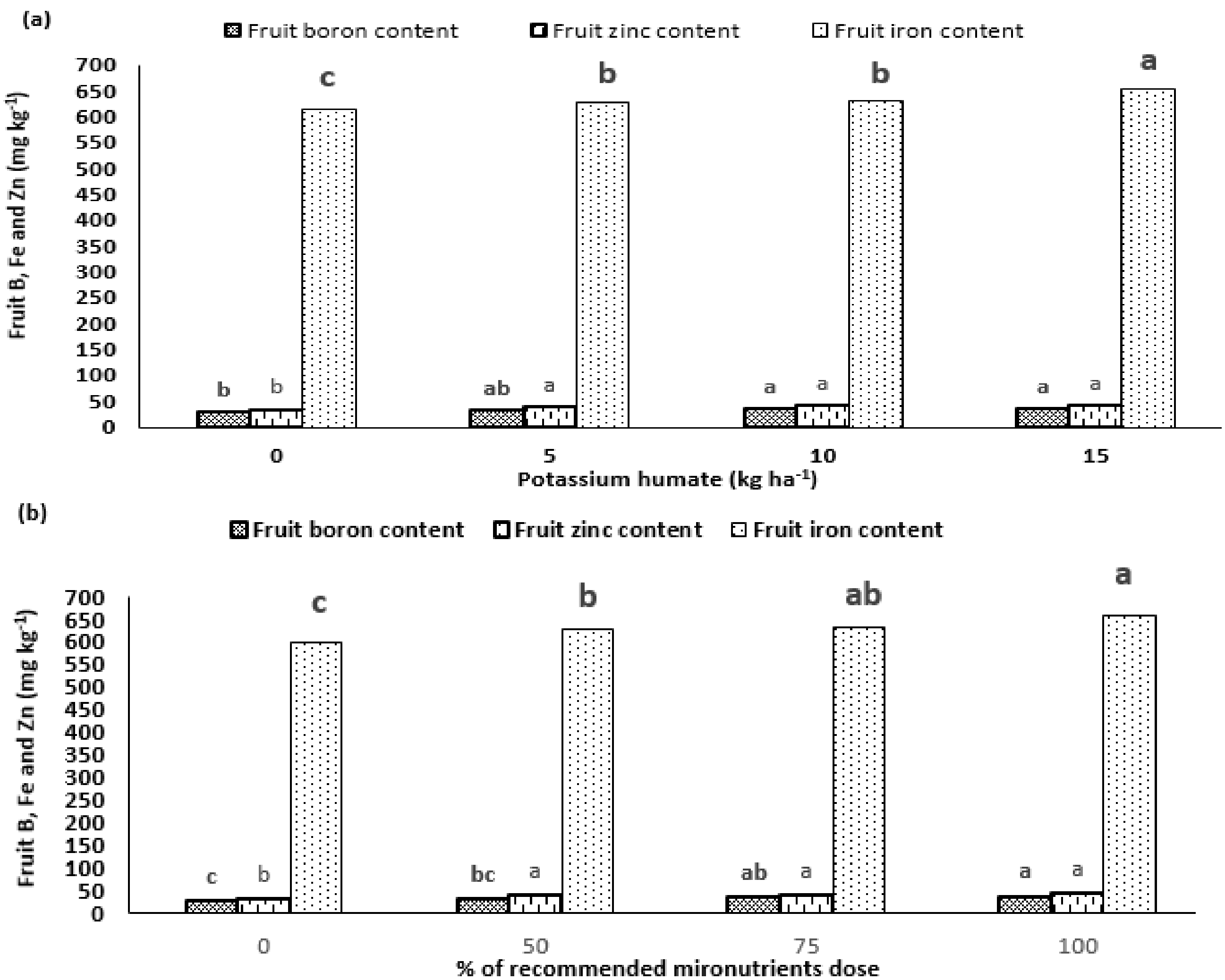

Figure 1: Variations in micronutrient contents in tomato fruits as affected by (a) different rates of potassium humate and (b) reduced and full dose of micronutrients (data of two years were pooled and means are given)

Improvements in plant growth and yield of tomato might be due to application of potassium humate as application of humic substances increase cation exchange capacity, buffering capacity, water holding capacity of soil and nutrient uptake by plants (Vallini *et al.*, 1993; Ayuso *et al.*, 1996; Pertusatti and Prado, 2007). Improved fruit quality in plants treated with potassium humate and micronutrients might be due to more uptake of micronutrients by the plants because humic substances improved micronutrient uptake due to their ability to complex metals (Garcia-Mina et al., 2004). So, difference between quality of fruits from treated and untreated plants could possibly be because of physiological role of micronutrients which was enhanced due to application of humic substance. Hence, application of potassium humate and micronutrients is responsible for improving fruit pulp, TSS, total acidity, lycopene contents etc. (Table 3).

Significant improvement in growth and fruit yield might be as a result of optimum growth of the plants which were under the influence of micronutrients. It may also be due to efficient use of fertilizers particularly N as potassium humate is reported to improve N pool in rhizosphere by preventing N losses.

Soluble solids content (SSC), also known as total soluble solids (TSS) is one of the most unswerving parameters to judge the quality of fruit. The soluble solid content is an important fruit quality attribute that acts as

Table 4: Variations in leaf mineral composition of tomato due to integrated application of different rates of potassium humate and micronutrient mixtures (data of two years were pooled and means are given)

Treatment		Leaf mineral composition					
Zn+Fe+B mixture (% of rec. dose)	Potassium humate (kg ha^{-1})	Macronutrients (%)			Micronutrients (mg kg^{-1})		
		N	P	K	Fe	B	Zn
0%	0	3.33 i	0.17 j	1.63 l	150l	30.1 m	55.2 o
	5	3.38 hi	0.18 i	1.66 kl	181 k	31.9 l	60.2 n
	10	3.43 gh	0.19 h	1.68 jl	193 j	33.8 k	63.7 m
	15	3.49 fg	0.19 h	1.71 ij	198 j	35.8 j	67.7 l
50%	0	3.54 ef	0.21 g	1.73 ij	211i	36.5 j	71.2 k
	5	3.73 c	0.22 f	1.91 g	224 g	43.4 h	85.2 h
	10	3.74 c	0.23 e	1.95 fg	227 g	45.7 g	86.0 h
	15	3.85 b	0.24 d	1.99 ef	234 f	45.7 g	93.1 g
75%	0	3.60 de	0.22 f	1.76 i	218h	40.0 i	74.8 j
	5	3.86 b	0.24 d	2.03 de	236 ef	49.0 f	97.1 f
	10	3.91 ab	0.25 c	2.07 d	240 de	51.4 e	101.6 e
	15	3.97 a	0.26 b	2.15 c	243 d	53.8 d	104.8 d
100%	0	3.66 cd	0.22 f	1.82 h	223 gh	41.0 i	80.8 i
	5	3.99 a	0.26 b	2.55 b	251 c	57.3 c	117.6 c
	10	4.00 a	0.26 b	2.59 ab	267 b	60.0 b	126.2 b
	15	4.00 a	0.27 a	2.62 a	284 a	64.3 a	135.1 a

Values sharing same letter(s) in each column do not differ by each other at $p \leq 0.05$ according to LSD test.

criterion for selection of tomato for processing and canning purposes (Dumas *et al.*, 2003). It is a combination of sugars, nonvolatile organic acids, and soluble cell wall components. Reducing sugars contribute 75 to 80% of the soluble solids content in tomatoes (Siddiq i, 2015). In the present study, fruits of treated plants were comparatively enriched with higher TSS (Table 3). Tomato is the major contributor of vitamin C (ascorbic acid) in food. It has the highest antioxidant activity among all dietary antioxidants. Tomato cultivars and genotypes vary widely in their solids content (Chaves and De-Mello-Farias, 2006) due to variations in ascorbic acid content. The augmentation of ascorbic acid content might be due to either increased ascorbic acid biosynthesis or protection of synthesized ascorbic acid from oxidation due to physiological role of micronutrients.

Conclusion

In comprehensive words, application of micronutrients along with potassium humate not only improved growth attributes and yield of tomato but also resulted in increased fruit quality. Moreover, application of micronutrients at different rates has more impact on plant growth and fruit quality in combination with potassium humate than its alone application.

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