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EFFECT OF ETHEPHON ON RIPENING AND POSTHARVEST QUALITY OF TOMATO (*Solanum lycopersicum*) DURING STORAGE

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Abstract

The experiment was conducted to evaluate the effect of ethephon (2-chloroethyl phosphonic acid) on ripening and postharvest quality of tomato (*Solanum lycopersicum*). Four concentrations of ethephon viz. 250, 500, 750 and 1000 ppm were applied in the experiment for ripening of tomato under ambient conditions (22±2ºC & 80±5% RH) with a control (0 ppm ethephon) in two consecutive years 2012 - 2013. The tomato fruits of 'Udayan' variety at breaker stage were harvested from the farmers'. The treated fruits were assessed for ripening percentage and biochemical properties such as total soluble solids (TSS) (%), titratable acidity (%), ascorbic acid content (mg/100g) and total carotenoids (mg/100g). The observations were recorded at 3, 6, 9 and 12 days after storage. The maximum TSS and total carotenoids were found in tomato treated with ethephon concentration @750 - 1000 ppm after 6 days of storage. From the experiment, it was found that ethephon can be applied @750 – 1000 ppm in breaker stage of tomato for uniform ripening within 6 days of storage at ambient temperature. The applied ethephon was estimated as 0.18 - 0.88 ppm at edible stage (6 days of storage), which was lower than the maximum residue limit (MRL) (2 ppm of ethephon) and safe for human consumption.

Keywords: 2-chloroethyl phosphonic acid, residue, uniform ripening, nutritional quality, shelf life.

Introduction

Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops in Bangladesh. It is popular because of its versatile consumption and nutritional value. The total tomato production in the country is 3.85 lakh metric tons from 69.51 thousand acres of land (BBS, 2019). It is grown all over the country and generally harvested in the mature stage. However, the immature green tomatoes are harvested and some ripening chemicals are used to quicken uniform ripening and to enhance shelf life (Chowdhury *et al.*, 2012). However, the quality of the produce mainly depends on the optimum maturity condition while harvesting.

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Proper maturity is one of the critical factor, which affects the ripening process, eating quality such as flavour and taste development in fruit (Lalel *et al.,* 2003). The immature harvested fruits are not nutritionally rich and will not develop typical flavour and quality (Brackmann *et al.,* 1993). Farmers use ripening chemicals to treat the tomato fruits for ripening without any awareness of the residues and their effects on human health.

Hakim *et al.* (2012) opined that ripening chemicals are considered hazardous to human health and they have to be used within safe recommended level. Recently there have been mixed opinions on the toxicity of ethephon among the consumers all over the country. Ethephon has been registered with EPA (US Environmental Protection Agency) since 1973 as a plant growth regulator used to promote fruit ripening and flower induction (Quoc *et al.,* 2012). It is a chemical which is irritant to the skin or the eyes but it is not skin sensitizer and carcinogen as classified by IARC (International Agency for Research on Cancer) as group D (not carcinogenic to human). According to FAO, the maximum allowable daily intake for ethephon is 0.05 ppm (mg/kg) body weight/day (Bui, 2007) while the recommended maximum residue level of ethephon is 2 ppm (mg/kg) of treated fruit.

Ripening is a process regulated by a gaseous plant hormone called ethylene (Quoc *et al.,* 2012). Ripening chemicals contains 2-chloroethyl phosphonic acid which penetrates into fruit and stimulate endogenous ethylene (Alexander and Grierson, 2002; Tseng *et al.,* 2000). When the mature fruits are dipped into aqueous solution of ethephon, the ethephon enters into the fruit cells and enhance endogenous ethylene, which hastens the ripening process. Considering aforesaid facts, the present study was undertaken to evaluate the effectiveness of ethephon on ripening for marketing of tomato.

Materials and Methods

Site: The experiment was carried out at the Laboratory of the Postharvest Technology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur in an ambient condition (22±2^{o}C & 80±5% RH) at last week of February in two consecutive years 2012 and 2013.

Plant material: The tomato fruits of 'Udayan' variety (commercial hybrid variety) at breaker stage were collected from the farmers' field of Gazipur Sadar Upazilla of Gazipur. Tomato fruits were harvested on 25th February 2012 and 28th February 2013. The harvested fruits were put in plastic crates and carried out at the Laboratory of the Postharvest Technology Division of BARI. About 1200 fruits were used in this study and fruits were selected on the basis of uniformity and size (70 - 90 g). No physical injuries or infections were considered. The selected fruits were divided into two parts, one of which used for examining chemical composition at 3 days interval up to 12 days; and other was directly

placed into plastic boxes at ambient condition to evaluate the physical parameters at the same interval up to 12 days of storage.

Ripening chemical used: The source of ethephon was Ethrel (Ethephon 39%) which was available in the market and registered by Department of Agriculture Extension (DAE), Kamarbari, Dhaka.

Treatment setting: The experiment consisted of five levels of ethephon concentration, T_1 = control, T_2 = 250 ppm, T_3 = 500 ppm, T_4 = 750 ppm and T_5 = 1000 ppm. The fruits were immersed into different ethephon solutions for 5 minutes. After that, the fruits were kept at ambient temperature for 10 mins in plastic crates in an attempt to reduce possible chemical injury and being dried up. Twenty-four fruits were used for each treatment group. For control treatment, fruits were dipped into water without using the ethephon solution. The experiment was laid out in Completely Randomized Design (CRD) with three replications.

Parameter studied: The parameters studied were percentage of ripening, firmness, total soluble solids (TSS), titratable acidity, ascorbic acid and total carotenoids.

Percentage of ripening: In order to determine ripening percentage, tomato fruits were daily examined for their colour and when the skin of the fruits were fully yellow in colour, they were considered as ripe. Percent ripening was calculated by using the following formula:

$$\text{Ripening (\%)} = \frac{\text{Number of ripe fruits} \times 100}{\text{Total number of fruits}}$$

Total titratable acidity content: Titration method was used for estimating total titratable acidity content (Ranganna, 2007). Briefly, 5 g tomato fruits were blended with distilled water filtered and transferred to a 100 mL volumetric flask and made the volume up to the mark. Titratable acidity was determined by titration of a known quantity of a sample (5 mL) against 0.1 N NaOH and results were expressed as percentage using the following equation:

$$\text{Total tritratable accidity (\%)} = \frac{T \times N \times E \times V_1 \times 100}{V_2 \times W}$$

Where, T =Titre, N=Normality of NaOH, E=Equivalent of weight of acid, V_1=Volume made up, V_2=Volume of extract taken for estimation, and W=Weight of sample.

Total soluble solids content: The total soluble solids (TSS) content of tomato pulp was determined using a digital hand refractometer by placing a drop of pulp solution on its prism. The percentage of TSS was obtained from the direct reading of the refractometer.

Fruit firmness: Fruit firmness was measured by using a Digital Firmness Tester (DFT 14, Agro Technologie, France). The following method was used (Rahman *et al.*, 2013) to determine fruit firmness. The firmness value was expressed as a resistance force (N) of the surface. Eight millimeter diameter stainless steel flathead probe was applied as penetration force to be reached during the tissue breakage, which penetrates the fruit by 5 mm. Measurements were taken at two places of each tomato fruit which was marked earlier by marker pen and mean value calculated.

Ascorbic acid content: Ascorbic acid content of tomato pulp was estimated by 2, 6-Dichlorophenol-indophenol visual titration method described by Ranganna (2007). Briefly, 10 g of tomato fruit tissue was homogenized in 50 mL of 3% cold metaphosphoric acid (HPO3) using a blender for 2 min and filtered through Whatman filter paper No. 2. The clear supernatant was collected for assaying ascorbic acid. Then 10 mm of aliquot was titrated with 0.1% 2,6-dichlorophenolindophenol solution until the filtrate changed to pink colour persisted for at least 15 seconds and the titration volume of 2,6-dichlorophenolindophenol was recorded. Prior to titration 2,6-dichlorophenolindophenol solution was calibrated by ascorbic acid standard solution. The results were expressed as mg 100 g^{-1} fresh weight. The ascorbic acid was calculated using the following formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{T \times D \times V_1 \times 100}{V_2 \times W}$$

Where, T =Titre, D= Dye factor, V_1=Volume made up, V_2=Volume of extract taken for estimation, and W=Weight of sample

Total carotenoids content: Total carotenoid content was measured by taking 5 g of the sample, grounded with acetone and anhydrous sodium sulphate in a pestle and mortar and filtered (Ranganna, 2007). The absorbance of the filtrate was measured by spectrophotometer (T-80, PG Instrument Ltd., UK) at 451ηm (Alasalvar *et al.*, 2005). The results was expressed as mg/100g.

Residue level of ethephon: The residue level of ethephon (0-1000 ppm) treated fruits of green mature stage was measured by Gas Chromatography (GC) flame-ionized detector in the Toxicology Laboratory of the Entomology Division, BARI, Gazipur. The tomato samples were grounded thoroughly with an electrical grinder (Handmixer M-22, Bamix, Switzerland). Each sample having 25 g was taken into a wide mouth jar and 100ml of hexane was added to it. Then 1ml of prepared (thrimethylsilyl) diazomethane solution was added for methylation of ethephon. Sodium sulphate (Na_2SO_4) was also added (as per required) with sample until water was removed from the sample. The mixture was then macerated with high-speed homogenizer (Ultraturax, IKA T18 basic, Germany) for 2 mins. The homogenized material was poured into 250 mL conical flask and placed into saker (Orbital Shaking Incubator, Rexmed, Sweden) for 12 hours

continuously shaking. After shaking, the slurry was filtered through a buchner funnel with suction. The flask and filter cakes were rinsed with 25 mL of hexane solution. The filtrated was then transferred into 250 mL round bottom flask and was dried to 3-5 mL by evaporation using a rotary vacuum evaporator (Laborota-4001, Heidolph, Germany). The concentrated filtrated was then transferred into volumetric flask making 10 mL in volume. For colour removal, around 20 mL methanol was added with 10 mL filtrated and shaked vigorously for 3-5 mins. Now, the clear part of the solution from the bottom of the separatory funnel was collected in vial, which was centrifuged at 1200 rpm for 5 mins. (Laboratory Centrifuge, Sigma-3K30, Germany). After centrifuge, supernatant was collected for injection.

The concentrated extracts were subjected to analysis by GC-2010 equipped with FTD. The capillary column used was ATM-1, length 30mm, ID 0.25mm and film thickness 0.25µm. Helium was used as carrier gas, as well as make up gas. Prior to the injection of the sample extract, standard solution of different concentration of ethephon were prepared and injected. The samples were calibrated (retention time, peak area etc.) against 3-4 pointed calibration curve of standard solutions of ethephon. Each peak was characterized by its retention time. Sample results were expressed in ppm automatically by the GC software, which represented the concentration of the final volume injected. From this value, the actual amount of ethephon residue present in the sample was determined by using following formula.

$$\text{Residue in sample (ppm)} = \frac{\text{Cone Obtained in injected volume (ppm)} \times \text{Quantity of final volume (L)}}{\text{Amount of sample taken (kg)}}$$

Experiment design and statistical analysis: The experiment was designed as Completely Randomized Design (CRD) and the data obtained were subjected to an analysis of variance (ANOVA) using statistical software R. Mean comparison was done by Tukey's student t test at 5% level of probability. Percent ripening data were transformed to Arcsine transformation for homogeneity the data. All data were expressed as mean value of triplicate replication.

Results and Discussions

Effect on ripening: It was noticed in Table 1, the colour development of fruits initiated after 3 days of storage in all treatments except control treatment. Fruits in control became turning to tannish yellow after 6 days of storage. Almost 100% fruits turned into uniform red colour with full ripen after 9 days of storage when treated with ethephon @ 750 and @ 1000 ppm. The colour development was better due to rapid degradation of chlorophyll and synthesis of carotenoid pigmentation as well as alteration in pigment due to ethephon treatments might develop attractive pericarp colour of fruit (Chesworth *et al.*, 1998). Fruits treated with 250, 500, 750 and 1000 ppm ethephon over ripened after 12 days as shown

in Table 1. The ethephon (2-chloroethyl phosphonic acid) penetrates into fruit and enhance endogenous ethylene, which regulates the expression of several genes involved in fruits ripening. Ripening which soften the skin of the fruit and convert complex polysaccharides into simple sugars. It was explained by Hall (1977) that the ethylene probably brings about the climacteric, since in many fruits the rise in respiration is directly preceded by an elevation in the ethylene concentration. This respiratory climacteric can be induced by ethylene treatment without a simultaneous change in tissue permeability. It has also been reported that ethylene alters the proportion of individual transfer RNA species. In support of the present study, the color development in tomato was remarkably affected by postharvest application of ethephon. Out of all the concentrations of ethephon tested, 750 ppm and 1000 ppm gave the most attractive and deep colored fruits (Table 1 & Table 2). The findings of Shanmugavelu *et al*. (1976) in mango and papaya support the contention that ethephon treated fruits develop attractive color.

Table 1. Effect of ethephon on ripening and colour development (fruit colour) of tomato (var. 'Udayan') during storage *(pooled of year 2012 and 2013)*

Ethephon concentration	Storage (days)				
	0 DAS	3 DAS	6 DAS	9 DAS	12 DAS
T_1=Control	Green	Green	Green to tannish yellow (≤10%)	Turning, Green to tannish-yellow (10%-30%)	Pink (30%-60%)
T_2=@250 ppm	Green	Green to tannish-yellow (≤10%)	Turning, Green to tannish-yellow (10%-30%)	Pink (30%-60%)	Red (≥90%)
T_3=@500 ppm	Green	Turning, Green to tannish-yellow (10%-30%)	Pink or red (30%-60%)	Light red, pinkish red 60%-90%)	Over-ripe
T_4=@750 ppm	Green	Pink or red (30%-60%)	Light red, Pinkish red or red (60%-90%)	Red (≥90%)	Over-ripe
T_5=@1000 ppm	Green	Pink or red (30%-60%)	Light red, pinkish red or red (60%-90%)	Red (≥90%)	Over-ripe

Note: DAS=Days after storage

Effect on total soluble solids (TSS) content: In Table 3 it was observed that the highest TSS (5.50%) was found in treatment T_5 (1000 ppm) after 12 days of storage which was followed by treatment T_4 (750 ppm) (5.45%). The TSS increased due to post harvest application of ethephon was also reported by Sabuz *et al.* (2019) in mango, Mahajan *et al.* (2008) in guava and Abbas *et al.* (1984) in orange. Declining trend was noted thereafter irrespective of the treatments. The initial TSS was increased and it might be happened due to water rapid loss of

water from the fruits and the conversion of starch into sugar at a faster rate (Fernandez *et al*., 2006). The decreased TSS content at later stage of storage might be occur due to exhaustion of substrate of conversion i.e. starch (Mahajan *et al.,* 2010).

Table 2. Changes of ripening (%) on the application of ethephon of tomato (var. 'Udayan') during storage *(pooled of year 2012 and 2013)*

Ethephon concentration	Ripening (%)			
	3 DAS	6 DAS	9 DAS	12 DAS
T_1=Control	0.37e)	23.47e	30.42e	40.60d
T_2=@250 ppm	23.00d	30.30d	34.54d	51.49c
T_3=@500 ppm	25.10c	32.16c	38.84c	75.07b
T_4=@750 ppm	26.78b	34.71b	42.67b	89.63a
T_5=@1000 ppm	30.75a	38.37a	46.35a	89.63a
CV (%)	0.42	1.61	1.76	0.81
Level of significance	***	***	***	***

Note: DAS=Days after storage; all values are means of triplicate determinations. Means within columns with different letters are significantly different at 5% level of probability by Tukey's t test.

Table 3. Changes of TSS (%) on the application of ethephon of tomato (var. 'Udayan') during storage *(pooled of year 2012 and 2013)*

Ethephon concentration	TSS (%)			
	3 DAS	6 DAS	9 DAS	12 DAS
T_1=Control	3.52e	4.43d	4.58c	4.88b
T_2=@250 ppm	4.24d	4.70cd	4.83b	4.67c
T_3=@500 ppm	4.46c	4.83bc	4.93b	4.93b
T_4=@750 ppm	4.80b	5.10ab	5.30a	5.45a
T_5=@1000 ppm	5.00a	5.20a	5.20a	5.50a
CV (%)	2.14	3.84	1.88	1.22
Level of significance	***	**	***	***

Note: DAS=Days after storage; all values are means of triplicate determinations. Means within columns with different letters are significantly different at 5% level of probability by Tukey's t test.

Effect on titratable acidity content (TAC): Maximum TAC was found in control treatment T_1 (0.48%) after 12 days of storage which was followed by treatment T_2 (0.45%) (Table 4). Batista-Silva *et al.* (2018) observed that transformation of organic acids into sugars was one of the reasons for deceasing organic acids during fruit ripening. Therefore, another possibility seemed that ethephon might enhance the conversion of organic acids to sugars since present findings revealed

that sugar content was increased and acidity was decreased following ethephon application.

Table 4. Changes of TAC (%) on the application of ethephon of tomato (var. 'Udayan') during storage *(pooled of year 2012 and 2013)*

Ethephon concentration	TAC (%)			
	3 DAS	6 DAS	9 DAS	12 DAS
T_1=Control	0.72b	0.65a	0.50ab	0.48a
T_2=@250 ppm	0.82a	0.66a	0.55a	0.45ab
T_3=@500 ppm	0.52d	0.56b	0.55a	0.43bc
T_4=@750 ppm	0.54d	0.52b	0.46b	0.41c
T_5=@1000 ppm	0.59c	0.53b	0.47 b	0.44bc
CV (%)	4.26	4.90	6.85	4.75
Level of significance	***	***	*	*

Note: DAS=Days after storage; all values are means of triplicate determinations. Means within columns with different letters are significantly different at 5% level of probability by Tukey's t test.

Table 5. Changes of fruit firmness (N) on the application of ethephon of tomato (var. 'Udayan') during storage *(pooled of year 2012 and 2013)*

Ethephon concentration	Fruit firmness (N)			
	3 DAS	6 DAS	9 DAS	12 DAS
T_1=Control	2.26a	2.06a	1.86a	1.77a
T_2=@250 ppm	1.96ab	1.86ab	1.77ab	1.28b
T_3=@500 ppm	1.86b	1.67bc	1.67ab	0.98b
T_4=@750 ppm	1.86b	1.47c	1.47bc	0.98b
T_5=@1000 ppm	1.67b	1.37c	1.28c	0.88b
CV (%)	11.18	11.33	12.79	11.23
Level of significance	*	**	*	**

Note: DAS=Days after storage; all values are means of triplicate determinations. Means within columns with different letters are significantly different at 5% level of probability by Tukey's t test.

Effect on fruit firmness: The firmness of the tomato fruit declined during storage with the increase of ethephon doses, which was found maximum in case of control (2.26 N) followed by in fruits (1.96 N) treated with 250 ppm (T_2) and minimum in fruits treated with 1000 ppm ethephon (1.67 N) after 3 days of storage (Table 5). After that, firmness was decreased in tomatoes with the advancement of storage period (Table 5). Firmness depends on the fruit maturity and ripening which become increased at the immature stage and then decreased.

Effect on ascorbic acid content (ASC): ASC decreased significantly up to 12 days of storage for all the treatments, but the maximum ASC was noticed in control fruit (26.25 mg/100g) after 3 days of storage (Figure 1). The fruits during storage, in general showed a declining trend in ASC significantly irrespective of the treatments applied (Figure 1). A reduction in ASC with the subsequent prolongation of storage might be happened due to rapid oxidation phenomenon of organic acid in later storage (Gurjar *et al.,* 2017). In addition, high temperature, lack of oxygen, during storage might have a significant contribution to the loss of ascorbic acid (Kamal *et al*., 2019).

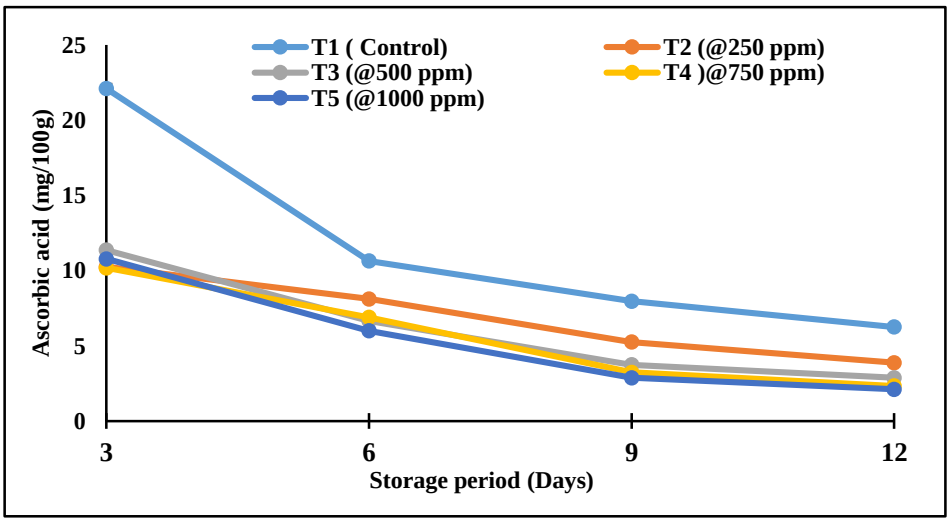

Figure 1. Changes of Ascorbic acid (mg/100g) during 12 days of storage.

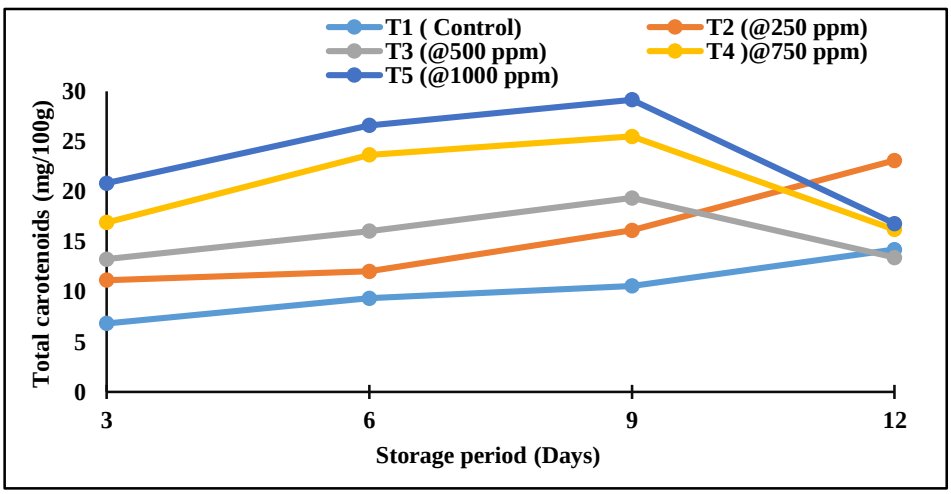

Figure 2. Changes of total carotenoids (mg/100g) during 12 days of storage.

Table 6. Estimated residue level (ppm) ethephon in treated tomato (var. 'Udayan') ***(pooled of year 2012 and 2013)***

Ethephon concentration	Days after application			
	0 DAS	3 DAS	6 DAS	9 DAS
T_1=@Control	-	-	-	-
T_2=@250 ppm	1.28c	0.41c	Not detect	Not detect
T_3=@500 ppm	1.41b	0.56c	0.18c	Not detect
T_4=@750 ppm	1.49b	0.89b	0.65b	Not detect
T_5=@1000 ppm	1.60a	1.13a	0.88a	0.16
CV (%)	3.55	2.82	2.11	-
Level of significance	**	***	**	-

Note: Existing CXL (Codex residue level)-2 mg/kg (ethephon); DAS=Days after storage, all values are means of triplicate determinations. Means within columns with different letters are significantly different at 5% level of probability by Tukey's t test.

Effect on total carotenoids content (TCC): TCC (mg/100g) was increased up to 9 days of storage in all the treatments except control and T_2 (250 ppm) treatment (Figure 2). The maximum TCC was observed in fruits treated with 1000 ppm ethephon (T_5) as early as 9 days after storage (29.15 mg/100g) which was followed by fruits treated with 750 ppm ethephon (T_4) (25.50 mg/100g) and minimum was noted in treatment T_3 (13.40 mg/100g) after 12 days of storage (Figure 2) which was supported by Moniruzzaman *et al.* (2015). Decline in ascorbic acid of the fruits might be due to utilization of ascorbic acid in respiration process during ripening at ambient condition (Olympio and Norman, 2000). Quoc *et al.* (2012) observed a decline trend in ascorbic acid in acerola.

Residue analysis: Initially the maximum residue (ethephon) was observed in treatment T_5 (1000 ppm) (1.60 ppm) followed by treatment T_4 (750 ppm) (1.49 ppm) and the minimum was found in treatment T_2 (1.28 ppm) (250 ppm) (Table 6). After 6 days, it was rapidly decreased and no residue was present after 9 days of storage except treatment T_5 (1000 ppm) (0.16 ppm). The residue of the treated tomatoes was decreased with the duration of storage period (Table 6). It was might be due to volatile compounds produce in tomato fruits which was completely agreed with Beitz *et al.* (1977) findings.

Conclusion

Experimental results showed that there was a little bit difference in nutrient content between treated and non-treated tomatoes. However, the outlook appearance of chemical treated tomatoes were attractive than the non-treated tomatoes. Hence, ethephon @ 750-1000 ppm may be applied in breaker stage of tomato for uniform ripening within 6 days at ambient temperature (22±2˚C) for

marketing purpose. The residual level of the applied ethephon was estimated as 0.18-0.88 ppm at edible stage at 6 days of storage, which was lower than maximum residue limit (MRL) of 2 ppm. The present investigation conclude that the use of ethephon has a significant effect on the ripening and postharvest quality of tomato fruits and 750 to 1000 ppm was best for retaining the various physical and biochemical parameters until the 6 days of storage. The estimated residual level of ethephon in tomato fruits treated with 750-1000 ppm ethephon at 6 days of storage remains lower than the recommended MRL of ethephon (2 mg/kg).

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