

LYCOPENE QUANTIFICATION OF TOMATO BY SPE AND HPLC

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Abstract

THAKUR, N., 2016. Lycopene quantification of tomato by SPE and HPLC. *Bulg. J. Agric. Sci.*, 22: 84–90

Indiscriminate use of chemical fertilizers and pesticides could cause adverse changes in biological balance as well as lead to an increase in incidence of cancer and other diseases, through the toxic residues present in the grains and other edible parts. Keeping in view the above criteria, the present studies were carried out in the farmers field (village Basal), 5 km away from Solan town, under mid hill conditions of Himachal Pradesh during the two consecutive years (2013 and 2014), to study the effect of different organic and inorganic treatments on lycopene content of tomato (cv. Solan Lalima). A Field trial was laid out in a randomized block design (RBD) with seven treatments replicated thrice. The results revealed that the combination of organic manures and biofertilizers proved best to improve the lycopene content over the farmer's system of cultivation (conventional treatment). The lycopene extracted by SPE (Solid Phase Extraction) column and further purified by HPLC analysis of organic sample showed maximum increase of 19.66% and 11.06% in lycopene content over control. It can be concluded from the present studies that by adopting appropriate combination of organic production technologies, tomato with better nutritional quality can be easily cultivated

Key words: tomato; organic; conventional; lycopene; SPE; HPLC

Abbreviations: FYM: Farm Yard Manure; VC: Vermicompost; AZO: Azotobacter; PSB: Phosphate Solubilizing Bacteria; SPE: Solid Phase Extraction; HPLC: High Performance Liquid Chromatography; RT: Retention Time

Introduction

Tomato (*Solanum lycopersicum*) is one of the most important, popular and widely grown vegetable throughout the world. It belongs to family Solanaceae. It is world's largest vegetable crop after Potato, but tops the list of processed vegetables. Tomato has become an important vegetable of the world in view of the increasing demand for the fresh consumption as well as for processing industries. High dry matter and soluble solids are desirable characteristic for the canned tomatoes industry since they improve the quality of the processed product (De Pascale et al., 2001). Total area under tomato cultivation in the world is 4.81 Mha with a total production of 163.02 MT, about 2 million producers and 170 countries with certified organic agriculture (Willer et al., 2015). In India it is grown in an area of 876410 ha with a production of 17 848 160 MT. In Himachal Pradesh tomato is being cultivated over an area of 17848 ha with total production of 40, 0000 MT (Anonymous, 2013; Indian Horticultural Database, 2014),

about 3965.38 ha area under organic certification. Solan district is known for the production of tomato having an area of 4298 ha with a production of 195 900 MT. Tomato produced in Himachal Pradesh during June to November becomes off season vegetable in the markets of North Indian plains fetching very remunerative prices to farmers. It seems that the cost of chemical fertilizers will reach beyond the reach of marginal farmers. Tomatoes are important not only because of the large amount consumed, but also because of their high health and nutritional contributions to human. Food quality and safety are of concern to every individual. Quality can be considered as a complex characteristic of food that determines its value or acceptability to a consumer. Consumer awareness of the relationship between foods and health, together with environmental concerns, has led to an increased demand for organically produced food. The presence of carotenoids in the diet and their role in human health has become a subject of extraordinary interest. Carotenoids, due to their unique

structure, protect tissues against oxidative and photooxidative damage by free radicals or reactive oxygen species produced as a result of the metabolic and pathological processes (Dembinska, 2005). Lycopene has unique structural and chemical features that contribute to specific biological characteristics (Chauhan, 2011). Lycopene is a primary carotenoid in human plasma, present naturally in greater amounts than β -carotene and other dietary carotenoids. This perhaps is an indication of its biological importance in the human defense system (Wilox et al., 2003). A diet containing whole tomato powder and dietary restriction inhibited the development of prostate cancer, however a diet containing pure synthetic lycopene supplement could not (Boileau et al., 2003), provide protecting effects to maintain prostate health (Stacewicz and Bowen, 2005) and lower the risk of lung cancer in human (Wang, 2004). *In vivo* studies have revealed that lycopene inhibits tumor growth in the liver, lung, prostate, breast, and colon. The higher level of antioxidants in the skin effectively reduces skin roughness (Darvin et al., 2008). Diets rich in carotenoids can prevent cell damage, premature skin aging, and skin cancer. Topically applied antioxidants have shown an increase in radical protection after VIS/NIR irradiation (Meinke et al., 2013). Thus, lycopene is an essential nutraceutical compound that provides significant health and medical benefits. Tomato is one of the most important vegetable crops of Solan (HP) with 11 million tons of annual production (FAO, 2008). At present, tomato production is mainly conventional both in open field and in greenhouse in Solan (HP), which has diverse agro-climatic conditions and due to favorable positioning in the Himalayan region, has a great scope for the promotion of organic farming. The state government formulated a policy on organic farming in 2010 and has covered 30 110 farmers with an area of 17 848 ha under organic farming with the future vision of converting 200 villages to complete bio-villages and 20 000 vermicompost units with 50 per cent assistance will be set up. However, government has already initiated the organic cultivation, registration and certification process to use organic fertilizers beside the inorganic fertilizers in tomato production, the farmers are still not aware about the incorporation of organic recommendations. Keeping in view the above facts, the present studies were carried out with an open pollinated and indeterminate tomato variety (cv. Solan Lalima), which has been recently released by University of Horticulture and Forestry (UHF-Nauni) for commercial cultivation of tomato, having superiority over the present tomato hybrids available in the markets in terms of fruit quality and productivity. Being open pollinated variety, it's a suitable option for organic cultivation. Therefore, the farmers can produce its seeds at their own farm. This study was undertaken with the objective to evaluate the contents and quality of lycopene in organically

and conventionally grown tomato, to generate useful information on qualitative and quantitative content of lycopene in tomato fruits. The parameters regarding soil analysis were collected during the study, but here only lycopene analyses is being focused and described.

Material and Methods

Experimental Location

The experimental trial was set up in the farmer's field, located at village Basal, 5 Km away from Solan town, under Solan block of District Solan, Himachal Pradesh at an elevation of 1270 m above mean sea level laying 30-52' North and latitude 77-11' east.

Weather Data of Experimental Site

The experimental area lies under the sub-temperate, sub-humid mid-hill agro-climatic zone of Himachal Pradesh, where summers are moderately hot during May-June, while winters are severe during December-January. The average rainfall in this area ranges from 100 to 300 cm, most of which was received during monsoon months of July and August.

Experimental Layout

The experiment trial consisted of a primary nursery stage and a secondary field trial.

Sources of organic amendments and inputs

Manures used

FYM (Farm yard manure) and VC (Vermicompost) were procured from the farmer's field having compost pits and vermi-bed.

Biofertilizers and Biocontrol agents

AZO (Azotobacter), PSB (phosphate solubilizing bacteria), Neem cakes, *Trichoderma viridae*, *Pseudomonas fluorescens* and Asafetida were procured from Poabs Green Pvt. Limited- Kereala.

Chemicals used

All the chemicals used were of analytic grade. All media component used were from Hi-media chemicals. The SPE column (Octadecyl C18, 1g/ml) with Silica, neutral alumina and basic alumina (500 mg/6 ml; Stepbio, Bologna, Italy) were tested.

Nursery parameters

The Seeds of tomato (cv. Solan Lalima) were sowed in Plastic trays (13 x 9 = 117 seeds). The nursery was set up with three replication and six designed organic treatments. The control was laid according to farmer's practice in field in a seed bed

(1m x 3m). The different combination of media were used which contained both soilless and soil growth media mixed with various organic manures and biofertilizers. The detailed description of various treatments is given in Table 1.

Seed source

Procured from Department of Vegetable crops Dr. Y.S. Parmar -UHF Solan.

Tomato variety used

Solan Lalima (open pollinated). Solan Lalima is an open pollinated and indeterminate variety of tomato having superiority over the present tomato hybrids available in the markets in terms of fruit quality and productivity. Being open pollinated variety, it's a suitable option for organic cultivation.

Seed rate

The Seed rate was 400 g/ha (40 gm/bigga).

Seed treatment

The seeds were treated with Beejamrut (6 g/40 g seed) and *Trichoderma viridi* (0.32 g). The seeds were dried in the shade and again treated the seeds with a mixture of *Azotobacter* and PSB (0.8 g each). Finally dried the seeds in shade and sow within 8 h of treatment.

Treatment of trays used for raising nursery

The trays were treated with 1:7 Formalin.

Seedling treatment

Neem spray (7 g/l) was given once for 15 days old seedlings, to protect seedlings from sucking pests like white fly and thrips. Drenching was done with *Pseudomonas fluorescence* (10 g/l) before transplanting to prevent foliar diseases. Dipping

of root portion of seedling in asafetida suspension (100 g in 5 L of water for 20 min) was done to prevent soil-borne pathogens causing wilt diseases, before transplanting. 25 days old tomato seedlings were transplanted to the main experimental field.

Field parameters

Experimental design of the field

The experiment was laid out as RBD (Randomized Block Design) with eight treatments replicated five times. The design consisted of 40 plots (1m x 3m) in which tomato seedlings were planted at a distance 90 cm x 30 cm having 24 plants per plot. The six (T₁-T₆) organic treatments were grown in different consolidate blocks, separated at a distance of 7 m from the farmer's treatment (T₇) which were laid separately (Table 1). Package of practices followed separately for organic, conventional and chemical cultivation during the entire course of studies is summarized in annexure. The dozes of the manures and biofertilizers have been formulated by carrying out the soil and manure analysis and dozes recommendations prescribed in organic package of tomato crop.

Random selection from the field

A random selection of five plants was considered from each bed.

Quantitative analysis of lycopene content:

Sample preparation

Acetone: Hexane extraction method (Shahzad et al., 2014) - The tomato samples of different designed treatments were collected from the field at the last harvest stage for lycopene content estimation. The tomato samples were chopped and dehydrated in a cabinet dryer (70°C) with circulating hot air and ground in a laboratory grinder. The samples (0.6 g) were weighed in diferent beakers to which 5 ml BHT-acetone solu-

Table 1
Description of treatments followed during the nursery and field trials

Treatments	Nursery treatments	Field treatments
T ₁	FYM + Soil (1:1)	FYM @ 312q/ha + <i>Trichoderma viride</i> @ 4kg/ha
T ₂	FYM+ VC+ Soil(1:1:1)	VC @ 78q/ha + <i>Trichoderma viride</i> @ 4kg/ha
T ₃	FYM + coco peat + VC + Vermiculite + <i>Azotobater</i> (1:1:1:1)	VC @ 312q/ha + <i>Azotobacter</i> + PSB + <i>Trichoderma viride</i> (4kg/ha each)
T ₄	FYM + coco peat + Vermiculite + <i>Azotobacter</i> (1:1:1:1)	FYM @ 78q/ha + <i>Azotobacter</i> + PSB + <i>Trichoderma viride</i> @ (4kg/ha)
T ₅	FYM + soil + <i>Azotobacter</i> (1:1:1)	PSB+ <i>Trichoderma viride</i> (4kg/ha)
T ₆	FYM + <i>Azotobacter</i> (1:1)	<i>Azotobacter</i> + <i>Trichoderma viride</i> (4kg/ha)
T ₇ Control (Farmer's practice)	FYM + soil + no seed treatment + Drenching with Dithane and bevisteen (2.5g/L and 0.5g/ L of H ₂ O)	FYM@ 250q/ha + chemical fertilizers (CAN+urea) + pesticides (50-60 no. of sprays) (Farmer's practice).

tion (0.05% w/v) was added. The beaker was placed in a bowl of ice on a magnetic stirring plate for 15 minutes and added 3ml distilled water. It was finally shaken for 5 minutes on ice and incubated for 5 minutes at room temperature to allow the two layers to separate.

Transferred the upper layer to clean vial and finally subjected to SPE (Solid phase extraction). The column (Flow rate, 1 g/6 ml; octadecyl C18) used for the separation of pig-

ments was firstly equilibrated with 10 ml hexane. After that loading of sample (2 ml) was done. After loading the first elution process was carried out with 6 ml hexane and remaining fractions were eluted with 6 ml acetone. The elutions gave orange (beta-carotene) and yellow fractions (lycopene). Each sample was eluted with four fractions and these fractions were subjected to spectrophotometric analysis from a range of 360 nm, 443 nm, 476 nm and 503 nm respectively (Table 2).

Table 2
SPE Fractionation for Lycopene

Wavelengths	Fractions (T ₁ sample)				T ₂ sample			
	1	2	3	4	1	2	3	4
360	0.980	0.564	0.980	0.397	0.660	0.502	0.980	0.445
443	1.478	0.568	1.061	0.397	0.680	0.554	0.980	0.440
476	1.482	0.581	1.101	0.405	0.689	0.582	1.123	0.502
503	1.467	0.578	1.098	0.402	0.825	0.673	1.093	0.545
	T ₃ sample				T ₄ sample			
	1	2	3	4	1	2	3	4
360	0.520	0.928	0.914	0.925	0.920	0.824	0.721	0.221
443	2.350	1.268	1.215	1.255	1.441	1.632	1.19	0.451
476	2.377	1.341	1.231	1.268	1.465	1.665	1.242	0.451
503	2.331	1.646	1.231	1.261	1.463	1.654	1.243	0.449
	T ₅ sample				T ₆ sample			
	1	2	3	4	1	2	3	4
360	0.397	0.497	0.670	0.420	0.397	0.497	0.670	0.420
443	0.405	0.564	0.770	0.620	0.405	0.564	0.770	0.620
476	0.409	0.581	0.750	0.652	0.409	0.581	0.750	0.652
503	0.402	0.589	0.780	0.672	0.402	0.589	0.780	
	T ₇ sample							
	1	2	3	4				
360	0.502	0.574	0.981	0.600				
443	0.608	0.770	0.156	0.695				
476	0.614	0.772	0.159	0.700				
503	0.614	0.777	0.162	0.795				

Table 3
HPLC method development for quantitative estimation of Lycopene

HPLC method development for quantitative estimation of Lycopene		
1.	HPLC System:	Agilent 1200 series, Chem-station Software, 6.0 version
2.	HPLC Column :	Intersil ODS-3V; 4.6 × 200 mm, 3.5 μm.
3.	Mobile Phase:	Methanol : 20% THF /Water (v/v) and 80 % Methanol in Gradient Mode
4.	Run time	60 minutes
5.	Flow rate	0.8mL/min
6.	Gradient Mode	RP-HPLC
7.	DAD UV-vis	190-800 nm

The lycopene content was calculated using the following formula:

Formula used - Lycopene content (mg/kg) = Absorbance 503 x 31.2

The sample fractions with maximum lycopene content were selected for further HPLC (Table 3) analysis in comparison to a control.

Results

The Four Light yellow coloured fractions (2 ml each) were obtained for each treatment after SPE extraction. These fractions after analyzing from a range of 360 nm to 503 nm (Table 4), the best samples in comparison to the control were subjected to HPLC analysis (Table 4). The sample (T₁) showed the first fraction with highest (1.467) absorbance with 503nm taken in to consideration as the wavelength maxima for lycopene. Similarly, sample T₂ was recorded with third fraction as preference with 1.093 absorbance at 503 nm. Sample T₃ (first fraction) was recorded with absorbance 2.331, which was marked as highest in all treatments with lycopene content of 72 mg/kg, followed by T₄ (second fraction) with an absorbance of 1.654 and lycopene content of 51.6 mg/kg. The control sample was recorded with an absorbance of 0.795 and lycopene content of 24.80 mg/kg which was higher than T₆ and T₅ treatments, but lower than the other organic treatments. On the whole T₃ and T₄ were recorded with maximum lycopene contents as compared to the control, and were carried forward for HPLC analysis for total quantification of lycopene in tomato fruit sample. The method used for HPLC analysis is given in Table 3. The use of SPE column for separation and purification of carotenoids proved to be an excellent source for easy separation of polar and non-polar compounds. Silica, neutral alumina and basic alumina have been evaluated or their capacity in removing oil phase as required by HPLC. Out of the three columns used the silica columns proved better in extraction efficiency as compared to the alumina columns as surface of silica SPE sorbent is very po-

lar and because of the –OH groups can form hydrogen bonds with suitable compounds.

HPLC analysis for quantification of lycopene

The samples (T₃, T₄ and T₇) were subjected to HPLC analysis (Table 4) for total quantification of lycopene. The retention time recorded for the analysis of lycopene was 30.1 minutes (Figure 1a). The analysis of the samples revealed that the lycopene percentage recorded in organic samples were higher than the control sample. Organic sample T₃ (Figure 1b) was recorded with 22.8% per cent lycopene purity, followed by T₄ (14.2%) (Figure 1c). Minimum percentage purity (Figure 1d) was recorded in the conventional treatment (3.14%).

The results clearly indicated a higher percentage of lycopene in organic treatments, whereas a very low percentage of lycopene was observed in farmer's practice (control). It was observed that the given loading time for binding does not have significant effects on elution process.

Discussion

India has made spectacular breakthrough in production and consumption of fertilizers during the last four decades, but consumption of renewable form of energy (chemical fertilizers) will be quite a limiting factor for increasing agriculture production in future. Because of escalating energy cost, chemical fertilizers are not available at affordable prices to the farmers. Moreover, the imbalanced and continuous use of chemical fertilizers is leading to a reduction in the crop yields and results in imbalance of nutrients in the soil which has adverse effects on soil health. These organic materials with varying C:N ratios and biochemical composition release nutrients at different pace. Under dynamic multiple cropping systems, the choice of crops in sequence should be based on crop value as its quality and productivity as well as restoration of soil fertility and economics. In sub-tropical climate of eastern India, the high value crops like tomato can be successfully grown as dry and wet season crop, respectively, in a cropping sequence. The crop seems to be promising and gaining popularity with multiple advantages of meeting increasing demand of vegetables. Tomato requires nutrient elements such as N, P, K, Mg, Ca, Na and S for improved production. These nutrients are specific in function and must be supplied to the plant at the right time and in the right quantity (Shukla and Naik, 1993). With intensification of cropping and heavy use of chemical fertilizers, the supplementary and complementary roles of organic materials are being strongly felt for retaining soil productivity (Laudicina et al., 2011). Use of organic farming techniques to grow crops has gained popularity in recent years as a result of both an increase in consumer

Table 4
HPLC method development for quantitative estimation of Lycopene

S.No.	Analyte	Retention time	% Area
	Std. Lycopene	30.1	100%
	Organic sample (T ₃)	30.1	22.8%
	Organic sample (T ₄)	30.1	14.2%
	Farmer's practice (C)	30.1	3.14%

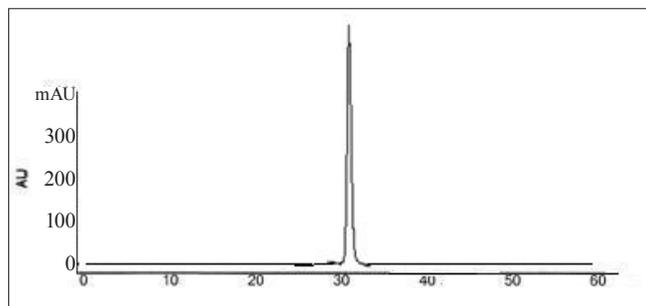


Fig 1a: HPLC chromatogram (standard) of Lycopene (RT 30.1 min)

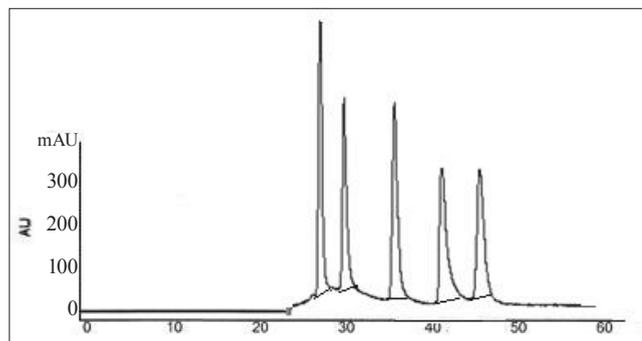


Fig 1b: HPLC chromatogram of purified organic tomato (T₃) extract showing peak of Lycopene (RT 30.1 min) with 22.8 per cent purity

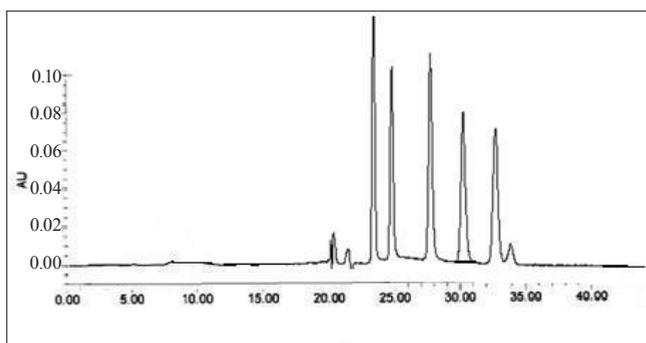


Fig 1c: HPLC chromatogram of purified organic tomato (T₄) extract showing peak of Lycopene (RT 30.1 min) with 22.8 per cent purity

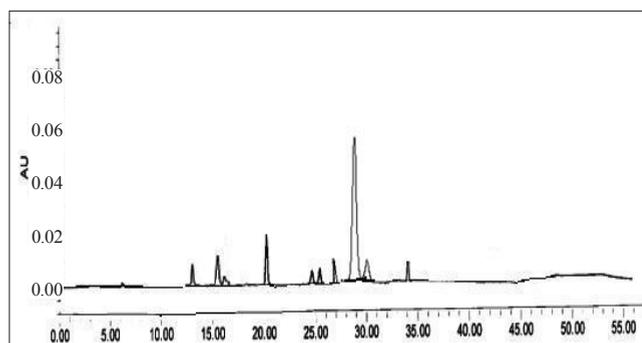


Fig 1d: HPLC chromatogram of purified conventional tomato extract (T₇) showing peak of Lycopene (RT 30.1 min) with 3.41 per cent purity

demand for organically grown produce and a genuine desire on the part of many growers to sustain or improve the soil health (Dimitri and Greene, 2002). Moreover, higher price of organically produce food than conventional produce (Oberholtzer et al, 2005) prompting producers to grow crops organically. The increased consumer demand appears to be driven primarily by the perception that organically grown produce is safer and more nutritious to eat than produce grown conventionally (Lester, 2006). Significantly higher lycopene content was recorded under vermicompost and farm yard manure integrated with various biofertilizers treatments compared to the control. HPLC analysis of organic sample showed maximum increase of 19.66 per cent and 11.06 per cent in lycopene content (T₃ and T₄) over control. Similar increase in lycopene content in organically grown tomatoes and carrots was also reported by Lumpkin (2005) and Evers (1989) in comparison to plants that have been raised with inorganic fertilizers. Furthermore, VC and FYM are also known to contain vitamins (Vit B₁₂ and other vitamins) with higher hormonal and enzymatic

activity which has been reported to affect the vitamin synthesis (Maronik and Vasilchenko, 1964).

Conclusion

The studies indicate that the integration of different manures with biofertilizers resulted in higher lycopene content than the traditional method of cultivation. The farmers should therefore adopt organic strategies for obtaining a produce that gives them better health security and attractive returns.

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Received August, 19, 2015; accepted for printing December, 23, 2015