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INFECTION WITH TOMATO MOSAIC VIRUS REDUCES LYCOPENE ACCUMULATION IN TOMATO FRUITS

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A R T I C L E   I N F O

A B S T R A C T

Lycopene content in tomato germplasm (both local and exotic) was evaluated against isolates of tomato mosaic virus (ToMV), using a locally preferred hybrid *i.e.,* Rio Grande as a control. Promising lines with resistance to ToMV were assessed by total carotenoid and lycopene content in virus-challenged tomato genotypes using spectrophotometry and RP-HPLC. Our data showed that virus infection significantly lessens the total carotenoid and lycopene content in tomato fruit. Lycopene content was significantly reduced in infected tomato compared to healthy, in locally cultivated hybrid Rio Grande. The germplasm GT-47 (CLN-2123-E) showed 60% decrease in lycopene content in infected tomato when in comparison to healthy produce. The virus infection, however, exhibited less deleterious effect on DPPH-based anti-oxidant potential of the ToMV infected tomato genotypes.

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INTRODUCTION

Tomato is of great nutritional value (Helyes *et al.,* 2008), and is one of the main sources of a number of essential dietary-bioactive compounds such as the carotenoid lycopene, polyphenols, ascorbic acid, β-carotene and lutein (Kaur *et al.,* 2013). The carotenoids are of great interest among these compounds, as a deficiency can led to health issues (Krinsky and Johnson, 2005). Consuming sufficient amount of lutein can reduce the risk of cataracts, atherosclerosis and age-related macular degeneration   (Bone *et al.,* 2000). Moreover, the therapeutic value of tomatoes and tomato-based food products also includes a reduced risk of various cancers (Ferreira *et al.,* 2000, Tang *et al.,* 2008, Vaishampayan *et al.,* 2007) and of coronary heart disease (Rissanen *et al.,* 2003, Rao and Agarwal, 2000).

Carotenoid is a major class of phytochemicals in tomato (Khachik *et al.,* 2002), containing around 600 fat-soluble pigments of plants, including nutritional pigments such as anti-oxidants and pro-vitamin A (Krinsky and Johnson, 2005). Amongst the carotenoids, lycopene is the most abundant in tomato, giving it the characteristic red color. The unsaturated hydrocarbon lycopene is the acyclic form of β-carotene and possesses no pro-vitamin A activity. (Bramley, 2002). The quality of tomato is strongly associated with its lycopene content (George *et al.,* 2004) and a dramatic increase in the carotenoids and lycopene content of the fruit, signifies ripening stage of tomato fruit (Laval-Martin *et al.,* 1975). With maturation, the chloroplasts of the green tomato fruit change slowly into chromoplasts, which can store lycopene in membrane-bounded crystals. Lycopene is therefore

important in respect to the final market and nutritional value of tomatoes (Dumas *et al.,* 2003, Harris and Spurr, 1969). As bioactive phytochemical, lycopene is one of the most powerful scavengers of free radicals and singlet oxygen in the body and efficiently eliminates active oxygen species (AOS) from the cells (Rao *et al.,* 1998, Toor and Savage, 2005). Furthermore, it plays a vital role in various biological functions such as the modulation of intercellular gap-junction communication, metabolic pathways, and hormonal and immune systems (Woodall *et al.,* 1997).

Tomato fruit ripening is a regulated process, during which the flavor, color, aroma, and the texture changes in a systematic manner. Ripening is affected by the impact of both biotic and abiotic factors, most of them are attributed to phytohormones (Srivastava and Handa, 2005). Principally carotenoid biosynthesis is regulated during the ripening process by a combination of gene-regulation and post-translation regulation (Bramley, 2002).

Both hybrids and open-pollinated (OP) varieties of tomato are currently incapable to meet domestic demand in Pakistan due to their susceptibility to biotic and abiotic stresses, low genetic potential, water shortages, the limited area under cultivation and competition with key crops (Saleem *et al.,* 2009). Among the yield limiting constraints the key factors are the susceptibility of widely grown tomato varieties to biotic and abiotic stresses and lack of good quality seeds (Akhtar *et al.,* 2010, Akhtar *et al.,* 2012, Saleem *et al.,* 2009, Saleem *et al.,* 2013, Sajjad *et al.,* 2011, Hameed *et al.,* 2010). However, little is known about the impact of biotic stresses on the quality of the produce in agricultural crops of economic importance.

Here we report the impact of ToMV infection on the total carotenoid and lycopene content of tomato under screenhouse conditions. In this study, we investigated the effect of virus infection on glasshouse-grown tomato genotypes, in which their lycopene and the total carotenoids were evaluated and compared by spectrophotometer and reversed-phase high-performance liquid-chromatography (RP-HPLC). Furthermore, the antioxidant activity of the healthy and the virus-infected tomatoes was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method.

MATERIAL AND METHODS
Collection of samples for ToMV isolates
Prevalent isolates of ToMV in tomato were collected from farmer's field areas of Malakand. Collection of the samples was done based on the presence of characteristic symptoms on the leaves of the plants under the natural field conditions. The leaf samples were labelled, bagged and transferred to the laboratory at IBGE, The University of Agriculture, Peshawar, for further analysis. Virus identity was confirmed by using DAC-ELISA (Antisera Source: Dr. S.M Mughal, Virology lab, NARC, Islamabad.

Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV) are both closely related tobamoviruses infecting tomatoes and DAC-ELISA cannot differentiate these two viruses. Therefore, the collected virus isolates were further confirmed on the differentiating hosts *Chenopodium amaranticolor* and *Nicotiana tobaccum* cv. White Burly. Local lesions are produced in these hosts by ToMV while TMV results in a systemic infection.

Source of tomato genotypes
Tomato genotypes were obtained from PGRI-NARC Islamabad, AVRDC (Taiwan) and the local market. The detail of tomato genotypes used in this study is given below (Table 1).

Table 1. List of tomato genotypes used in this study.

	Tomato genotypes	Source/origin
1	GT-47 (CLN-2123-E)	PGRI-NARC (Plant Genetic and Research Institute) (Islamabad)
2	GT-29 (AVTO 1002)*	AVRDC (Asian Vegetable Research Development Centre) (Taiwan)
3	GT-34 (AVTO 1007)*	AVRDC (Asian Vegetable Research Development Centre) (Taiwan)
4	Rio Grande	Local market

*resistant against Pakistani isolate of ToMV (data not shown),

Establishment of tomato nursery and transplantation
The potting mixture was prepared in a balanced ratio of

sand, soil and organic matter (1:1:1 v/v) and sterilized in heat resistant plastic bags. All the collected tomato genotypes nurseries were raised in the sterilized potting

mixture, in small clay pots until ready for transplantation. Pots were watered according to the pot capacity with proper drainage was provided to each pot. Seedlings of all the genotypes were transplanted and allowed to establish prior to the virus inoculation.

Mechanical transmission of virus onto tomato genotype
After ToMV confirmation, all plants were inoculated by mechanical inoculation. ToMV infected leaves were ground in a mortar and pestle with phosphate buffer (0.05 M and pH 7.0). The homogenate was filtered through double-layered muslin cloth and applied on carborundum sprinkled leaves of tomato plants using a cotton swab. Negative controls were inoculated with the buffer only. Once inoculated the leaves were washed and left in a glass house. Pots with three replicates and healthy control were arranged in a completely randomized design in the glass house. Normal horticultural practices were followed to maintain the growth and the development of the plants.

Confirmation of ToMV infection
The inoculated tomato plants were observed on a weekly basis for the appearance of mosaic symptoms. The leaves from the virus-infected plants were collected and processed for DAC-ELISA.
Tomato leaf extract was prepared in coating (carbonate) buffer and an aliquot (200µl) was added to each well of a microtiter plate. The plates were incubated at 37ºC for four hours. Afterwards, the plates were washed thrice using PBS-Tween, to remove any unbound virus in the leaf sap extract. ToMV antiserum was added to the wells. The microtiter plates were tightly sealed with parafilm and were incubated as described previously. The washing step was repeated as before to remove any unbound antibodies. DAC-ELISA works on the principle of detecting virus-specific antibodies using goat anti-rabbit antibodies, labelled with alkaline phosphatase (at 1:5000 v/v dilution). The goat anti-rabbit antisera were added to the wells. The plates were incubated as described previously and subsequently washed with PBS-Tween three times in the same manner. The enzyme substrate p-nitrophenol phosphate (Sigma Chemical Co, USA) was added to the wells, tightly sealed and incubated in the dark at room temperature for one hour to allow color development from the interaction of the enzyme conjugated with goat anti-rabbit antisera and the p-nitrophenol phosphate. The reaction was quenched by addition of NaOH (3N, 50µl/well). Microtiter plates were read at 405 nm using an ELISA plate reader (TC-96 Elisa Microplate Reader, Teco Diagnostic U.S.A.).

Total carotenoids and lycopene estimation by spectrophotometer
Total carotenoids and lycopene in healthy and virus-infected tomato genotype were estimated by the procedure previously described (Zakaria *et al.,* 1979). Briefly, the total carotenoids and lycopene were extracted from tomato fruit pulp into petroleum ether. The total carotenoids were assessed in UV/visible spectrophotometer at 450nm. The same extracts were used for assessment of lycopene at 503nm. At 503nm, carotenes have a negligible absorbance while lycopene has a maximum absorbance.
To avoid photolysis of carotenoids and lycopene the procedure was carried out in the dark by wrapping the test tubes in aluminum foil. Saponification of 5g of the tomato paste from each sample was done by using 12% ethanolic potassium hydroxide (2.5ml) in a water bath for 30 minutes at 60ºC. The saponified extracts were transferred into a separating funnel that was packed with glass wool and calcium carbonate, which contained petroleum ether (10-15ml). This was then mixed gently. The upper petroleum ether layer, containing the carotenoid pigment and lycopene, was collected. The aqueous layer was extracted repeatedly until it become colorless. A small quantity of Na_2SO_4 was added to the organic extract to remove excess water. The final volume of the organic extract was recorded.
The absorbance of the yellow color extracts was recorded at 450nm for total carotenoids and at 503nm for lycopene in a spectrophotometer with petroleum ether as a blank.
The amount of total carotenoids and lycopene was calculated using the formula;

$$Total\ Carotenoids = \frac{(P \times 4 \times V \times 100)}{W}$$

Where,
P = Optical density of the sample
V = Volume of the sample = 2 ml
W = Weight of the sample = 5 gm

$$Lycopene = \frac{(3.12 \times P \times V \times DF \times 100}{1 \times W \times 1000}$$

Where;

DF = Dilution factor

The quantity of total carotenoids and lycopene as expressed as mg/5g tissue.

Total Lycopene estimation by HPLC

For total lycopene identification the HPLC based method of Berra, (2012) with modification was followed. To prevent photodegradation of the lycopene the tomato samples were protected from light and the procedure was performed in the dark. Individual tomatoes were cut into approximately 1.5 cm cubes and the whole fruit was utilized. The chopped tomatoes were frozen in liquid nitrogen, and then ground in a mortar and pestle. The resultant tomato paste (2mg) was dissolved in acetone (20ml) and was shaken for 3 minutes. The acetone layer was removed, and the tomato crude was folded in three layers of filter paper and then oven dried at 48°C for 24 hrs to remove excess water. The dried tomato crude was dissolved in acetonitrile.

Lycopene was analyzed by reversed-phase-HPLC equipped with PDA detector using isocratic elution. . A Phenomenex Luna 5u C18 (II) (250 × 4.6 mm) column was used with MeOH/isopropyl alcohol/THF (25:35:40) containing 250 ppm BHTand 0.05% TEA as mobile phase. The flow rate was 1 ml/min, column temperature was 30°C and the injection volume was 20 µl.

A standard curve was constructed to calibrate HPLC measurements by recording peak areas of the chromatograms of five standard solutions of lycopene covering the concentration range from 0.001 to 0.05 mg/ml. The concentration of lycopene in the tomato samples were then determined by comparing the peak areas of their chromatograms to that of standard curve. The experimentation conditions were exactly the same as those used for the calibration procedure.

Antioxidant activity of tomato lycopene

Six samples of tomato were selected from healthy and virus-infected plants. Each sample (10g) was blended individually for 6 seconds with 50 ml 50% methanol and then homogenized for 30 seconds. The homogenized solution was incubated at 4ºC for 12 hours. After incubation the samples were centrifuged (15,000 g for 15 minutes) and the supernatants were recovered and stored at -20 °C until analysis.

Tomato extracts (0.5ml) were added separately to a DPPH solution (2ml of 0.2 mM in 50% methanol). Reactions were incubated at room temperature for 30 minutes. At this point, the absorbance was measured at 517 nm using a spectrophotometer against a methanol blank. The free radical scavenging activities of solutions of the plant extracts was calculated in terms of percentage inhibition (I%) as follows;

%DPPH scavenging = 100 (A -B)/A

A = absorbance of control, B = absorbance of the sample.

Statistical analysis

Throughout the total carotenoids and lycopene screening experiments of the tomato genotypes, three replicates and a control was used along with a randomized experimental design. Mean and standard deviations were calculated and the statistical analysis of the obtained data was done by using ANOVA and least significant difference (LSD).

RESULTS

Tomato germplasms GT-29, GT-34 (Source: AVRDC), GT-47(Source: PGRI, NARC) and a local variety (Rio Grande) were challenged with ToMV through mechanical inoculation. The presence of virus in inoculated plants was confirmed through DAC- ELISA.

The observed variations among treatments for ELISA absorbance were significantly different (Figure 1). Based on ELISA and infectivity assays all the mechanically inoculated tomato plants, were positive for ToMV. Among the germplasms challenged, Rio Grande was found to be highly susceptible with a higher titer of ToMV compared to the others. Rio Grande is followed by GT-29 and GT34, whilst tomato line GT-47 had the lowest titer of ToMV. To ensure that the plants were free from other common tomato viruses, ELISA was performed for vector-borne viruses such as cucumber mosaic virus (CMV), potato virus X (PVX) and tomato yellow top (TYTV) (data not shown). Tomato plants tested negative for all viruses, except PVX with high titer in all the four germplasm (including Rio Grande as control).

Analysis of total carotenoids in healthy and virus-infected tomato genotypes

Total carotenoids estimation by spectrophotometer

The total carotenoid content was measured in healthy and virus-infected genotypes of tomato spectrophotometrically (Figure.2). The analysis of ToMV and PVX virus-infected and healthy tomato fruits revealed a significant decrease in the levels of total carotenoids in virus-infected tomato compared to the

healthy control. It was observed that more carotenoids accumulated in the healthy controls in all the four germplasm. The germplasm GT-47 accumulated the greatest amount of carotenoids (78.4mg/5gm) compared to the virus-infected fruit of the same genotype having 14.4mg/5gm. The carotenoid content of GT-29, GT-34 and Rio Grande were comparable amongst healthy and infected plants respectively. Overall, the carotenoid content in healthy genotypes of tomato fruit is significantly greater than in the virus-infected genotypes (Figure. 2).
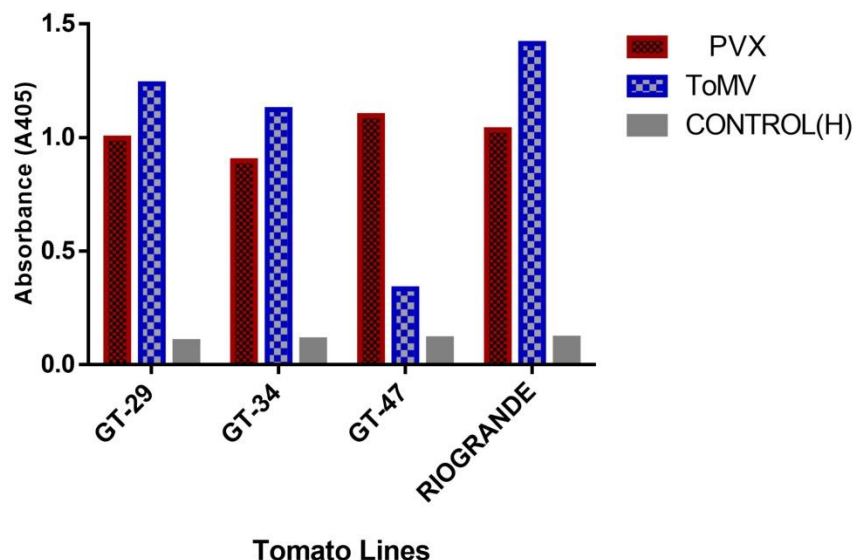
Figure 1. Result of DAC-ELISA for four tomato's genotypes in case of PVX and ToMV. Tomato germplasms GT-29, GT-34 (AVRDC), GT-47(NARC) and Local Check (Rio Grande) were challenged with Tomato mosaic virus through mechanical inoculation. The presence of virus (in inoculated control) and its absence (in healthy control) was confirmed through both DAC-ELISA.
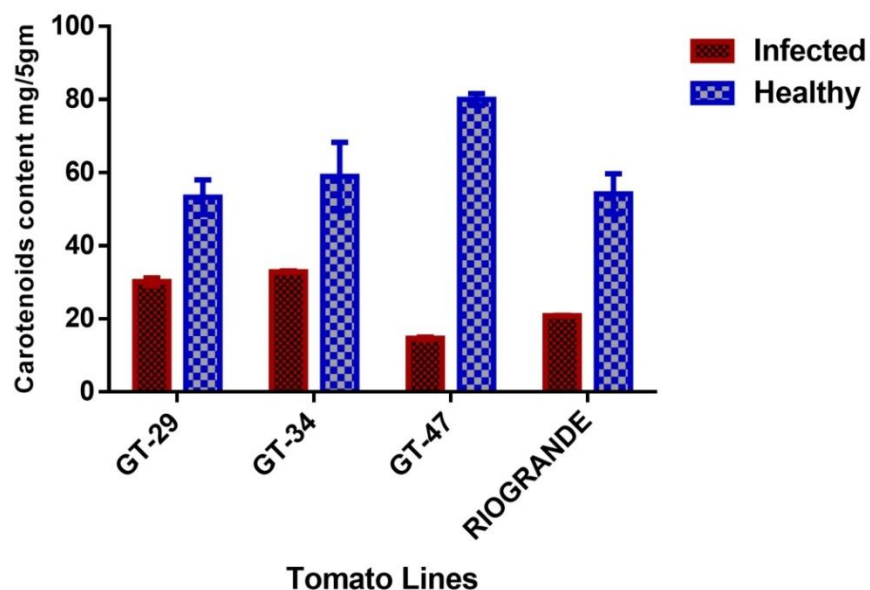

Figure 2. Comparison of total carotenoids content in virus infected and healthy lines of tomato by using spectrophotometer. Data are means ± SD (n = 3).

Total lycopene estimation by spectrophotometer

Total lycopene analysis in healthy and infected tomato genotypes shows that the ToMV and PVX infected tomatoes germplasm have significantly less lycopene than the tomatoes of healthy germplasm (Figure.3). The virus infection leads to a reduction in the lycopene content in infected genotypes. It was found that GT-47 accumulates the greatest amount of lycopene of the four healthy germplasms. It has 0.08mg/5gm lycopene as compared to its virus-infected fruits which have 0.0105mg/5gm. However, AVRDC lines GT-29 and GT-34 (0.027mg/5gm and 0.024mg/5g, respectively) performed significantly better than NARC genotype GT-47 in case of ToMV infection, while the maximum reduction of lycopene was recorded in the local control Rio Grande (0.0012 mg/5gm) in case of virus infection. As expected, each genotype responds differently to virus infection and the degree to lycopene accumulation.

Total lycopene analysis by RP- HPLC

In-depth analysis of both virus-infected and healthy tomato samples was carried out using RP-HPLC (Figure.4). The results confirmed the initial spectrophotometric observations. Overall, the healthy genotypes of tomato contained considerably more lycopene than the virus-infected tomato genotypes. The lycopene content determined by this method was higher compared to the spectrophotometric results indicating that HPLC is more sensitive in detecting lycopene than spectrophotometry. It was observed that the GT-47 contained the maximum (7.9mg/5g) lycopene this was followed by Rio Grande (6.54mg/5g), then GT-34 (5.25mg/5g) and minimum lycopene was found in GT-29(3.54mg/5g) in the case of the four healthy controls.
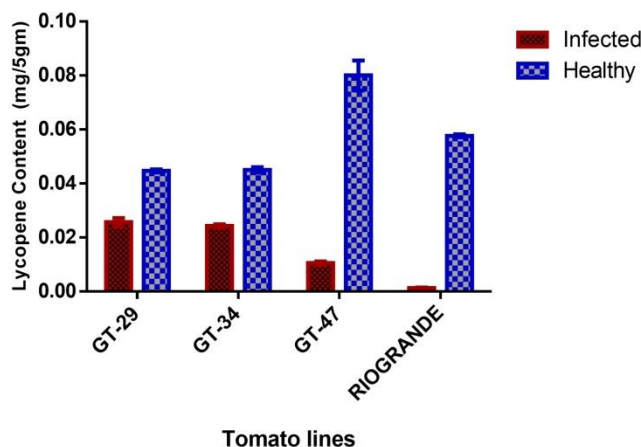

Figure 3. Comparison of total Lycopene content in virus infected and healthy lines of tomato by using spectrophotometer. Data are means ± SD (n = 3).
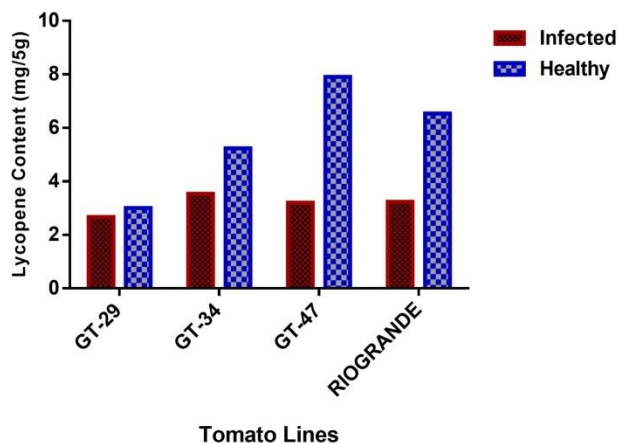

Figure 4. Comparison of total lycopene content in healthy and virus infected tomato genotypes by using RP-HPLC.

Comparison of antioxidant activity of healthy and ToMV infected tomato lines

The antioxidant activity of the healthy and the virus-infected tomato genotypes (Figure 5) was measured by using DPPH free radical scavenging method. A significant difference was found in the antioxidant activity of the genotypes, however. The antioxidant activity of NARC genotype GT-47 in case of healthy tomato fruit was recorded to be the highest as (93 % inhibition) which is followed by AVRD genotype GT-29 (89% inhibition). The minimum antioxidant activity was observed in local control Rio Grande (81% inhibition). The results revealed that ToMV and PVX virus infection does not affect the overall antioxidant activity of the tomato extracts in all the germplasm tested.

Stability of carotenoids and lycopene in virus-infected tomato fruits

The total carotenoids and the lycopene content remain constant at 4ºC storage. When tested in a time-course experiment, the total carotenoids content did not change both in the healthy and in the virus-infected tomato over

the course of five days (Figure 6 and 7). In tomato germplasm GT-29 the lycopene and carotenoids content were higher in the healthy tomatoes than those infected with ToMV and PVX. No change was observed in the lycopene content over the course of the experiment in healthy tomatoes. A slight increase was observed in the virus-infected GT-29 tomato fruits, but the increase was non-significant (p value=0.33). Both the infected and healthy plants had a clear difference in their carotenoid and lycopene accumulation. However, the difference remained identical at all the three data points.

DISCUSSION

Tomato is the world's second largest vegetable crop in terms of production. Sufficient data is available on the impact of viruses on vegetable production that often leads to complete annihilation of a crop with tangible output for the farmers. However, few studies focus on the quality losses that result from a non-catastrophic crop infection. Here we have investigated the quality losses in tomato in response to virus infection.
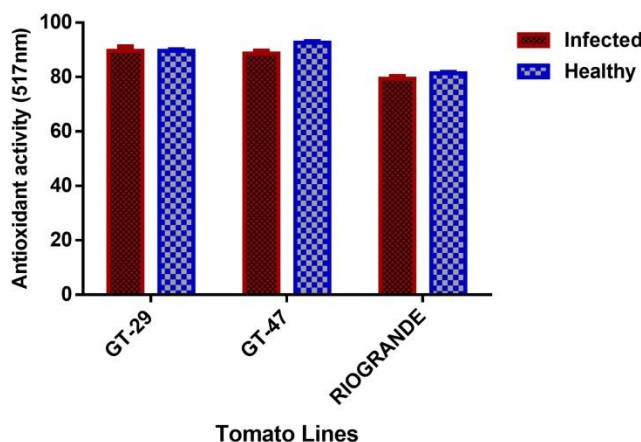

Figure 5. Antioxidant activity of healthy and virus infected tomato lines at wavelength of 517nm by using DPPH method. Data are means ± SD (n = 3).

Tomato contains the major nutritionally important anti-oxidant lycopene that gives the fruit its characteristic red color. Lycopene constitutes 60–74% of the total carotenoids present in tomatoes (Clinton, 1998). Many factors affect the lycopene content, such as maturity, cultivar and heat treatment (Sharma and Le Maguer, 1996, Thompson *et al.,* 2000, Stahl and Sies, 1992). To our knowledge, no studies have been

carried out on the impact of virus infection on tomato quality i.e., lycopene content. Therefore, our initial approach was to test the lycopene content in the virus-infected tomato and compare it with fruits from the healthy plants. For this purpose, the experiment was set out in an insect-proof screen house. Plants were, however, found doubly infected with ToMV and the soil-borne virus PVX.
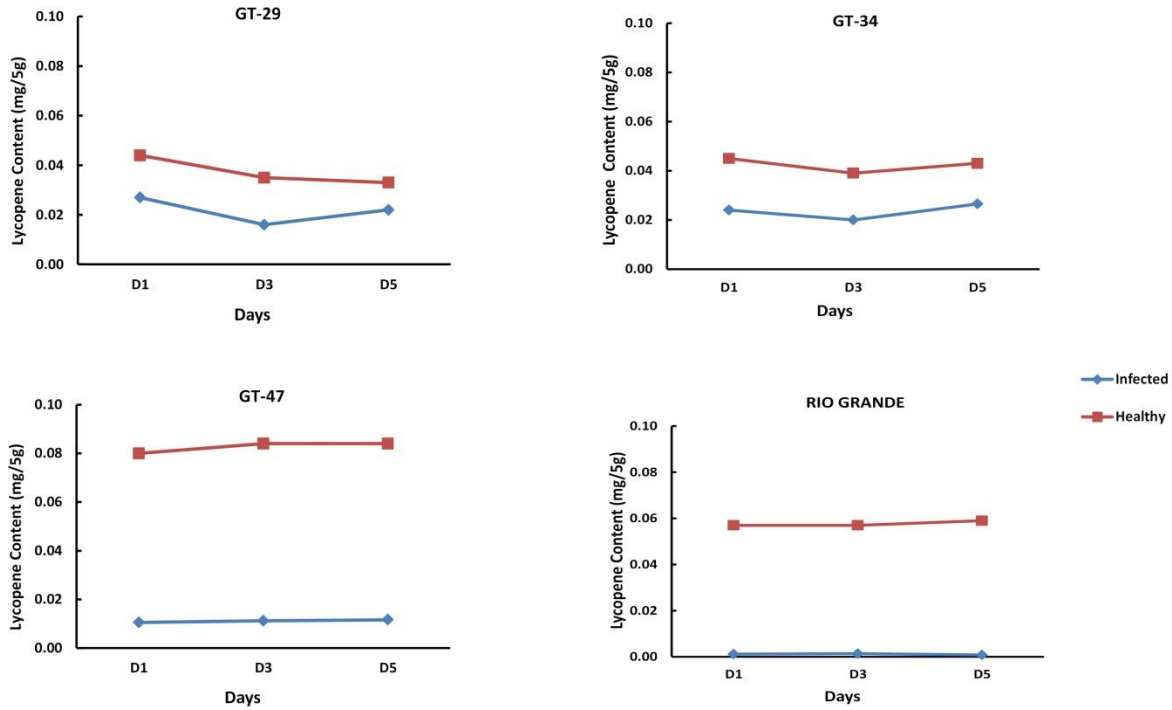
Figure 6. Temporal graphs of total lycopene content in healthy and virus infected tomato lines at 5 days interval calculated by spectrophotometer at wavelength of 503nm in 5gm tomato paste stored at 4°C. Each value are the mean of three replication (n=3r) ± standard deviation (SD).
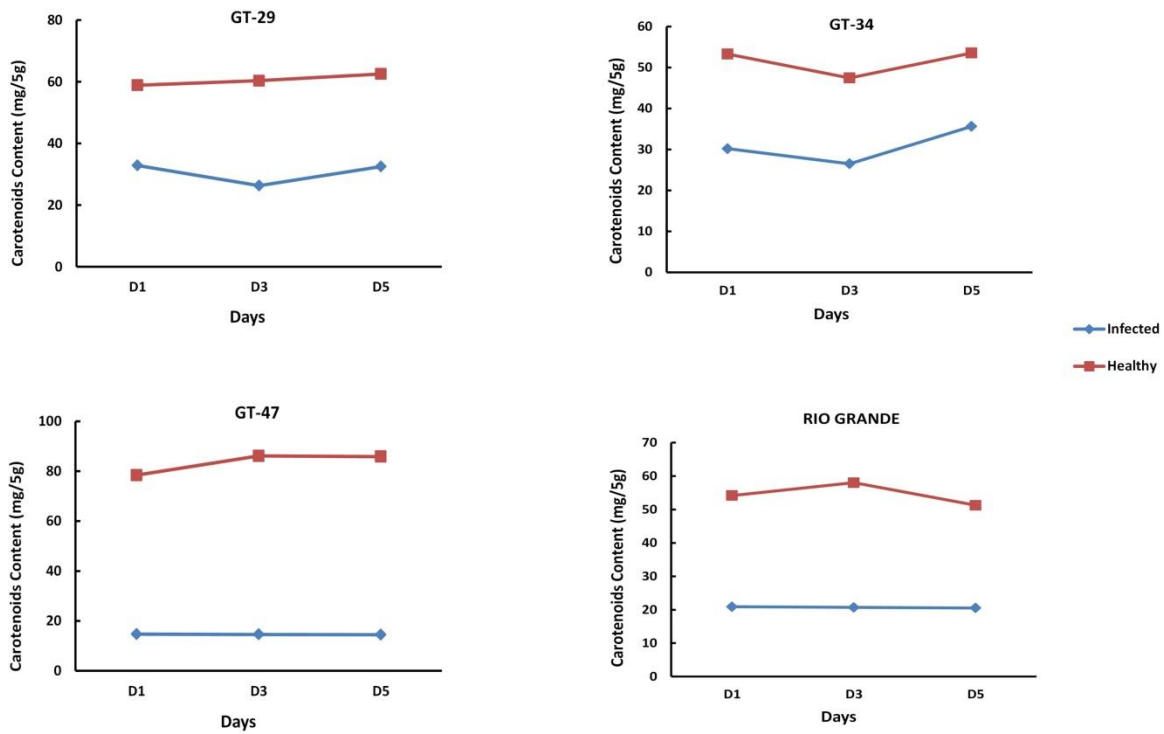

Figure 7. Temporal graphs of total carotenoids content in healthy and virus infected tomato genotypes at 5 days interval calculated by spectrophotometer at wavelength of 450 nm in 5g tomato paste stored at 4°C. Each value are the mean of three replication (n=3r) ± standard deviation (SD).

The second virus infection (PVX) was unintended and unexpected. Though the soil was sterilized, the presence of PVX in some experimental pots indicated that the viruses were either not eliminated from the soil or that the sap used for inoculation of ToMV was infected with PVX. Therefore, we conclude that using a differential host for isolating a virus may not be a suitable method. Such differential hosts should be confirmed with molecular diagnostic techniques to ensure that only the desired virus is present in the plant. Neither of the two viruses *i.e.*, ToMV and PVX was detected in the healthy control (uninoculated control).

We then extracted and measured the total carotenoids and lycopene in tomato fruits from the healthy and the virus-infected (ToMV PVX) tomato genotypes using spectrophotometer and RP-HPLC. The results indicated that the virus-infected tomato genotypes contain significantly less carotenoids and lycopene than the healthy control. The carotenoid levels of the analyzed the healthy tomato samples were 11000mg kg^{-1} - 13000mg kg^{-1} in GT-34 and GT-47 respectively, while in infected case carotenoids range from 2944mg/ kg^{-1} to 6624mg/ kg^{-1} in GT-47 and GT-34, respectively. These measured values are much higher than 185-600mg/ kg^{-1} reported by Abushita *et al.* (1997) and 250-350mg/ kg^{-1} reported by Zoran *et al.* (2014). A huge difference in the carotenoids quantity of the varieties may be attributed to factors such as genotype and the environment. (George *et al.,* 2004).

As tomato fruits mature, a rise in the quantity of carotenoids is correlated to the rise in the lycopene's concentration within the plastids (Fraser *et al.,* 1994, Thompson *et al.,* 2000). Concentrations were reported to rise from 0.25 mg kg^{-1} in green tomatoes to values more than 40 mg kg^{-1} in completely ripe fruits.(Brandt *et al.,* 2003). In the healthy tomato fruits, lycopene contents ranged from 9mg kg^{-1} to 17.16mg kg^{-1} in tomato paste of GT-29 and GT-47, respectively. These values are in line with those reported by Kuti et al *et al.* (2005), who also measured the whole lycopene quantity in fresh tomato genotypes grown in greenhouses as ranging from 5.7 mg kg^{-1} to 47.8 mg kg^{-1}. These obtained values of lycopene quantity are also in line with those stated by Clinton (1998) (8.8–42.0 mg kg^{-1}), Although the maximum lycopene content in our experiment is lower are than to those found by Nguyen and Schwartz, (1999) (31–77 mg kg^{-1}) and Thompson *et al.* (2000) (26.22–

57.86 mg kg^{-1}). While in the virus-infected tomato genotypes the lycopene content was 0.012mg kg^{-1} and 0.027 mg kg^{-1} in local control Rio Grande and GT-29, respectively. These values are greatly reduced compared to their healthy tomato fruits which contain 0.057 mg kg^{-1} in Rio Grande and 0.044mg kg^{-1} in GT-29.

The reduced amount of lycopene in the virus-infected tomato genotypes compared to healthy tomato plants indicates that virus infection affects the expression of genes in the lycopene biosynthesis pathway. As reported by Smith *et al.* (2011) who discovered a small RNA in CMV Y-Sat associated with silenced chlorophyll biosynthetic pathway gene (CHLI) in tobacco. Furthermore, Ibdah *et al,* (2014) provided an evidence that a decrease in production of β-carotene and strigolactone in the roots of host plant by blocking the biosynthesis pathway of carotenoids, using cucumber mosaic virus (CMV). They found that the enzyme phytoene desaturase (PDS) is down regulated by CMV, decreasing the production of carotenoids and infection of *Phelipanche* in tobacco host roots which were infected by both *P. aegyptiaca* and CMV.

Variation in the quantity of carotenoids amongst tomato varieties had been reported previously by different authors like Abushita *et al.* (2000). The huge difference in the total lycopene quantity of glass-house and field-grown tomato cultivars may be due to the variations in the genotypes, and environmental factors such as the amount of sun exposure, and the agriculture procedures such as the water and the fertilizer used (Dumas *et al.,* 2003) which together may affect the carotenoid biosynthesis (Abushita *et al.,* 2000). Total lycopene quantity on average establishes about 80–90% of the total carotenoid quantity of red ripe fruit of tomatoes (Shi and Maguer, 2000). The quantity of carotenoids in the tomatoes depends on genetic factors and thus the variety selected for cultivation affects the quantity at harvest (George *et al.,* 2004).

The lycopene content was further evaluated through HPLC and a slight increase in the values indicate that HPLC is more sensitive and selective in detecting lycopene. Lycopene content in the virus-infected tomato was found to be 7.6% and 10.1% in the AVRDC genotypes GT-29 and GT-34 respectively, whilst healthy tomato genotypes contains 15.03 % to 22.6 % lycopene in GT-34 and GT-47 genotypes respectively. With an increased understanding of the health benefits of lycopene, it is important to know how to

preserve and store lycopene during food processing. Lycopene is a carotenoid and typically exists in nature in the all-trans form. Light, heat, oxygen, and different food conditions can induce lycopene auto-oxidation and isomerization. Lycopene may isomerize to mono- or poly-cis forms in the presence of oil or heat during dehydration. Re-isomerization of lycopene can take place during storage. The lycopene molecule splits during oxidation, which causes loss of flavor and color. The effects of oxygen, heat, oil and the presence of light on the stability of lycopene are constant in many literature studies. Debate still exists though on some details, such as the conditions which cause isomerization and the optimal temperature and humidity for storage (Xianquan *et al.,* 2005). The stability of lycopene analysis in this work shows that it is stable under storage condition of 4°C for five days.Negligible differences in the antioxidant activity of healthy and infected tomatoes is observed. This suggests that the presence of some other antioxidant compounds in tomatoes, such as phenolic compounds, vitamin E and vitamin C, which can also contribute to the total antioxidant capacity of the fruit, may compensate somewhat for the decrease in the carotenoids. In general, the antioxidant activity of vegetables and fruit extracts depends on several different factors, including the plant variety, analysis method, and geographic origin.

CONCLUSIONS
In conclusion, we find that ToMV and PVX infection have a significant effect on the total carotenoid and lycopene content of tomato fruits of infected plants. Tomato genotypes GT-29, GT-34 and GT-47 are more resistant to ToMV and PVX infection than the Rio Grande based on the reduction in carotenoids and lycopene content. The virus has a negligible effect on the antioxidant activity of different tomato genotypes tested. Tomato's genotypes of AVRDC and NARC tested in this study have more carotenoids, lycopene and have more antioxidant activity value compared to the locally sourced Rio Grande. They should, therefore, be incorporated in breeding programs to provide farmers with quality output, despite virus infections. The present study suggests that further work should be done to investigate the virus effect on other compounds of different tomato genotypes such as flavonoids, phenolic acids, ascorbic acid and vitamins.

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CONFLICT OF INTEREST
The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS
Zobia Zafar performed the experiments; Saad H. Shah and Muhammad Numan assisted in analysis of lycopene; Ijaz Ahmad and Asad Ali assisted in virus assay; Hussain Shah, Zafar Iqbal and Muhammad Fahim conceived the study; Saad H. Shah and Muhammad Fahim wrote the manuscript.