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Impact of Two TYLCV Egyptian Isolates on Metabolic and Antioxidant Activities in Some Tomato Cultivars

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Abstract

Tomato yellow leaf curl disease caused by *Tomato yellow leaf curl virus* (TYLCV) leads to substantial tomato production losses in Egypt and worldwide. So, we study the impact of two TYLCV Egyptian isolates on metabolic and antioxidant activities in some tomato cultivars. The infectivity of two TYLCV Egyptian isolates was confirmed by DAS-ELISA. Four tomato cultivars inoculated mechanically with two TYLCV isolates by syringe inoculation showed variation external symptoms. The metabolic parameters and biochemical components in four infected tomato plants were affected with TYLCV isolates. The electrolyte leakage ratio was increased and the membrane stability index was decreased in four tomato cultivars, as well as a significant decrease in both Mg^{+} and N^{-} content compared with healthy plants. While, an increase in the content of sodium, potassium and phosphorus. On the other hand, photosynthetic pigment concentration was reduced in the leaves of tomato plants. A notable reduction in carbohydrates, protein and lipid content of challenged plants. Both phenol and proline content significantly increased in TYLCV infected plants. Antioxidant enzymes (POX, PPO, SOD and CAT) showed an increase in the activity in TYLCV infected. Also hydrolytic enzymes (amylase and protease) activity was significantly increased compared with healthy ones. This study indicated the TYLCV-EGT was more effective regarding biochemical and metabolic parameters in infected tomato plants throughout different four tomato cultivars.

Keywords: TYLCV, Tomato cultivars, Certain minerals, Metabolic characteristics, antioxidant enzymes, Chemical contents, ROS.

Introduction

Tomato (*Solanum lycopersicum*) is one of the most popularly grown vegetable crops in the world. The worldwide high consumption of tomato is mostly due to its acceptable flavor and high nutritive value. (Alhudiab *et al*., 2014). Tomato (*Solanum lycopersicum*) is an economically important vegetable worldwide. The annual global production of tomato in 2014 was more than 171 million tons (FAO, 2014).

Several biotic stresses, including the diseases caused by viruses are responsible for significant tomato production losses world over. Among the viral diseases, whitefly-transmitted geminiviruses (genus: Begomovirus) are the serious tomato production constraints in tropical and subtropical regions of the world. These viruses cause diseases that show varied symptoms, including a destructive tomato yellow leaf curl disease (TYLCD) and tomato leaf curl disease

(ToLCD) (Diaz-Pendon *et al.*, 2010; Prasanna *et al*., 2015).

Tomato yellow leaf curl is a destructive viral disease of tomato caused by Tomato yellow leaf curl virus (TYLCV). In tropical and subtropical regions, total losses of tomato crops have been reported. TYLCV is widespread and can be found in most places where tomato is grown (Michael *et al*., 2009; Navas-Castillo *et al*. 2011; Chen *et al*., 2016).

A dramatic biochemical changes in virus infected plants result in decrease of both quality and quantity of infected crops. Various reports suggest that virus multiplication inside the plant cell alters different biochemical constituents of plants and disrupt the physiological processes like photosynthesis, transpiration and respiration of the infected plants which affect the growth and yield (Tajul *et al*., 2011; El-Dougdoug *et al.,* 2014b). Also, have reported that the determination of cellular constituents in virus infected plant is very important to understand the activities of the host cell and the nature and extent of damage have caused by the virus.

Viral infection induced increased permeability in cells to cause loss of water. This also explains the cup shape of the infected leaves, especially in the severe symptom stages (Oleinikova, 1969)

Cell membrane stability (CMS) has been used as an efficient criterion to discriminate among crop cultivars with respect to degree of salt tolerance (Meloni *et al.* 2003 in cotton; Sairam *et al.* 2005 in wheat, Jamil *et al*. 2008 in canola). In this respect, Farooq and Azam (2006) used the CMS technique to screen salt tolerant, salt sensitive and two salt/water deficiency tolerant wheat genotypes using 100–250 mM NaCl salinity maintained in pots containing gravel and nutrient solution, they concluded that CMS technique is suitable for screening wheat under high salinity levels and for detecting differences that may arise due to the cumulative effects of salinity and reduced water contents.

Membrane damage could indirectly be evaluated by measuring solute leakage (Electrolyte leakage) from cells (Ekmekei *et al.,* 2007) and membrane stability index (Ali *et al*., 2008; Bassuany, *et al*., 2014).

TLYCV caused an increase in electrolyte leakage and decrease in the membrane stability index of tomato plant as compared with healthy plants (Khalil *et al.,* 2014).

Photosynthesis is one of the main physiological processes important for plant growth (Arfan *et al*., 2007), and it is highly affected by viral infection (Radwan *et al.,* 2010). Chlorophyll a and b increased with age. However, these levels were significantly lower in diseased plants than in healthy plants, from early growth stages. The reduction in total chlorophyll content could be due to increased chlorophyllase activity and/or reduced synthesis of the photosynthetic pigments as observed by Ahmed *et al*. (1986).

A number of biochemical changes in infected tomato tissues were observed at the level of photosynthetic apparatus as indicated through photosynthetic pigments. A reduction in the concentration of total chlorophyll in the infected plants indicated compromised photosynthetic apparatus in diseased plants (Montasser *et al*., 2012).

The reduction in photosynthetic pigments may be attributed to the infected action of a virus on the biosynthesis of pigments, increasing their degradation and/or maintaining damage of the chloroplast thylakoid could be a result from the mineral deficiency. In this respect Sawy (2009) found that the reduction in plant pigments concentrations may be due to decrease in absorption of some ions as Mg and Fe which were involved in chlorophyll biosynthesis under stress conditions.

TYLCV decreased chlorophyll biosynthesis. Moreover, the decrease in chlorophyll contents in infected tomato plants. Carbohydrates, which represent one of the main organic constituents of the dry matter, derived from photosynthesis, were found to be affected by infected stress (Khalil *et al*., 2014). Also TYLCV caused markedly decreases in soluble sugar, insoluble sugar and total carbohydrate contents in stem and leaves. Also, a notable reduction in the total protein was seen as a result of TYLCV infection.
Stressed tomato plants accumulate more phenolic compounds as antioxidants to resist viral stress. This may be due to a renewed synthesis of phenolic phytoalexins to fight the viral stress (Yong-ping *et al*., 2009).

Moshe *et al*. (2012) mentioned that reactive oxygen species (ROS) scavenging mechanisms in plants involve enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and catalase (CAT). SODs act as the first line of defense against ROS, dismutating superoxide to H_2O_2. APX, GPX, and CAT subsequently detoxify H_2O_2. Most anti-oxidative enzymes were detected in

TYLCV infected tomatoes. SODS, APX, thioredoxin peroxidase, ferredoxin-nitrite reductase were more abundant in susceptible then in resistant plants. Plant thioredoxins are the key factors in oxidative stress response. The defense enzymes of tomato such as peroxidase (POD), polyphenoloxidase (PPO) and phenylalanine amonnialyase (PAL) increased by TYLCV inoculation. TYLCV enhance the activities of defense enzymes and decrease disease index significantly so as to increase tomato resistance to TYLCV (Li *et al.*, 2012).

Materials and Methods

Source of tomato cultivars and virus isolate: One hundred seedling of each tomato cultivar (G512, 380, 714 and Lugin) were obtained from Horticulture Research Institute belong to Agriculture Researches Center (ARC), Dokki, Giza, Egypt

Tomato cultivars were checked before cultivation for potential infection with TYLCV, PVY, ToMV, TSWV and CMV tested by DAS-ELISA technique using specific polyclonal antibodies (Clark and Adam, 1977).

Two TYLCV Egyptian isolates was isolated from naturally infected tomato plants at Sinnuris and Tamiya, Faiyoum, Egypt**,** also was identified biologically, serologically and molecularly previously (unpublished)

Experimental design:

Sixty seedlings were selected for uniformity by choosing those of equal size from each cultivar which gave –ve results DAS ELISA with TYLCV, PVY, TSWV, ToMV and CMV. One seedling per bag was planted in each plastic bags (diameter 30 cm and depth 40 cm), each filled with about 9.0 kg sterilized soil (clay soil mixed with sandy soil in a proportion of 3:1 (V:V)) and with practical agriculture recommended. Two TYLCV isolates was syringe inoculated on twenty plants at two weeks from planting for each cultivar. Another twenty plants without inoculated as a control of each cultivar. These plants were maintained under a greenhouse. After 3-7 weeks post inoculation the development external symptoms was recorded and infected plants were confirmed by DAS-ELISA.

Serological detection was determined using TYLCV specific polyclonal antibodies by DAS-ELISA according to (Clark and Adam, 1977).

Physiological parameters were determined in healthy and infected plants as follow:

Electrolyte leakage*:* The total inorganic ions leaked out from the leaves were measured by the method described by (Sullivan and Ross, 1979). The electrolyte leakage was calculated by using the formula: $(EC_b\text{-}EC_a/EC_c) \times 100$

Membrane Stability Index (MSI): MSI was estimated using conductivity meter. MSI calculated using the formula described by (Sairam, 1994).

$MSI= [1-(C_1/C_2) \times 100]$

Certain Minerals: Wet ashing method, plant materials were dried in an oven at 80 °C till constant weight. The dried matter digested according to the method of Chapman and Pratt (1978).

Sodium and potassium were estimated by the flame emission technique as adopted by Ranganna (1977). Phosphorus and calcium were determined simultaneously by ICP spectroscopy according to the method of Saltanapour (1985).

Total nitrogen content: The total nitrogen content of the healthy and infected leaves of tomato was determined by Micro-Kjeldahl method, (A.O.A.C 1960)(*).

Photopigments concentration: The method used for the quantitative determination of chlorophyll was that of (Vernon and Selly, 1966). The optical density of the plant extract was measured using spectrophotometer of two wave lengths (649 and 665 nm). These are positions in the spectrum where maximum absorption by chlorophyll (a) and (b) occurs. The concentrations of chlorophyll (a), (b) and total chlorophyll in plant tissue were calculated using the equations mentioned by (Vernon and Selly, 1966).

Mg chlorophyll (a) / g tissue = 11.63(A665) – 2.39(A649).

Mg chlorophyll (b) / g tissue = 20.11(A649) – 5.18(A665).

Mg chlorophyll (a + b) / g tissue = 6.45 (A665) +17.72(A649).

(*) Association of Official Agricultural Chemists.

For carotenoids, the concentration was carried according to (Lichtentahler, 1987) equation: Car_{x+c} = 1000 × OD_{470}- 1.82 C_a – 85.02 C_b/ 198= mg/g fresh weight. A= reading of optical density by spectrophotometer (nm).

Extraction of total carbohydrate according to (Said *et al*., 1964).

Total soluble carbohydrates: were determined using anthrone techniques according to (Umbriet *et al*., 1969). Total soluble carbohydrate (in terms of sucrose equivalents).It was measured using spectrophotometer at 620 nm (Unico 2000).

Phenolic compounds in leaves were carried out according to the method described by (Daniel and George, 1972). Using spectrophotometer (Unico 2000) at the wave length 725 nm.

Free proline content was determined according to the method described by (Bates *et al*., 1973) at 520 nm using UV- spectrophotometer (Unico 2000).

Total proteins: According to the method of (Lowery *et al*., 1951) using casein as a standard protein determination of total protein in leaves by spectrophotometer (Unico2000) at the wave length 750 nm. The protein pattern was determined by sodium dedocyl sulphate polyacrelamyde gel electrophoresis (SDS-PAGE) according to Laemmli (1970), modified by Studier (1973).

Total lipids content: One hundred grams of air–dried plant powder were extracted with petroleum ether (40-60°C): ether (1:1 v/v) for 24 hours using Soxhlet apparatus. The lipids were obtained by distilling off the solvent. The last traces of the solvent were removed by heating the liquid sample in a vacuum oven at 50°C to constant weight according to Christie (1982)

Antioxidant enzymes: The plant materials used for estimation of enzymes were 2 g of the terminal buds were homogenized with 10 ml of phosphate buffer pH 6.8 (0.1 M), then centrifuge at 2^{o}C for 20 min at 20000 rpm in a refrigerated centrifuge. The clear supernatant (containing the enzymes) was taken as the enzymes source (MuKherjee and Choudhuri, 1983).

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described by (**Marklund and Marklund, 1974)** at 325 nm using UV- spectrophotometer (Labomed, inc.23)

Catalase (CAT) activity was determined by measuring the rate change of H_2O_2 absorbance with a UV-spectrophotometer (Labomed, inc.23) at 250 nm according to the method of (Chen *et al*., 2000).

Peroxidase (POX) activity was assayed using the rate of increase in absorbance as pyrogallol was determined by UV- spectrophotometer (Labomed, inc.23) at 470 nm (Bergmeyer, 1974).

Polyphenol oxidase (PPO) activity of polyphenol oxidase enzyme was determined according to the method adopted by Matta and Dimond (1963). The absorbance was measured at 495 nm by UV-spectrophotometer (Labomed, inc.23).

Hydrolytic enzymes

Amylase activity: Activities of amylases were estimated using a method modified from that described by Afifi *et al*. (1986). The amylase activity was estimated by measuring O.D. at 660 nm (Unico 2000).

Protease activity: Proteases were determined using the method of Ong and Gauchier (1973), the optical density (O.D.) at 660 nm was determined using spectrophotometer (Unico 2000).

Statistical analysis: All plant chemical analysis data were statistically analyzed using one-way ANOVA and Fisher's Least Significant Difference (LSD) test (SigmaPlot 12.0) at the 0.05 level of probability. Generally, the values recorded in the values of the biochemical analysis are means of six replicates.

Results

The virus infectivity of two TYLCV isolates were confirmed through syringe injection and via whiteflies to healthy tomato plants cvs. Castle Rock, that gave leaf curling, yellowing due to TYLCV-EGS isolate while TYLCV-EGT isolate showed interveinal yellowing, leaf crinkling. As well as gave (+ve) results with TYLCV polyclonal antibodies by DAS-ELISA

Fig. 1: *Solanum lycopersicum* cv. Castle Rock exhibiting TYLCV symptoms in both I: (TYLCV-EGS) and II: (TYLCV-EGT) isolates

(TYLCV-EGS) and II: (TYLCV-EGT) isolates.

Sixty seedlings of each tomato cultivars, (G512, 380, 714 and Lugin) were tested to the extent of its infection or virus free from TYLCV, PVY, TMV, TSWV and CMV viruses using specific antibodies by applying the DAS-ELISA assay.

Physiological processes.

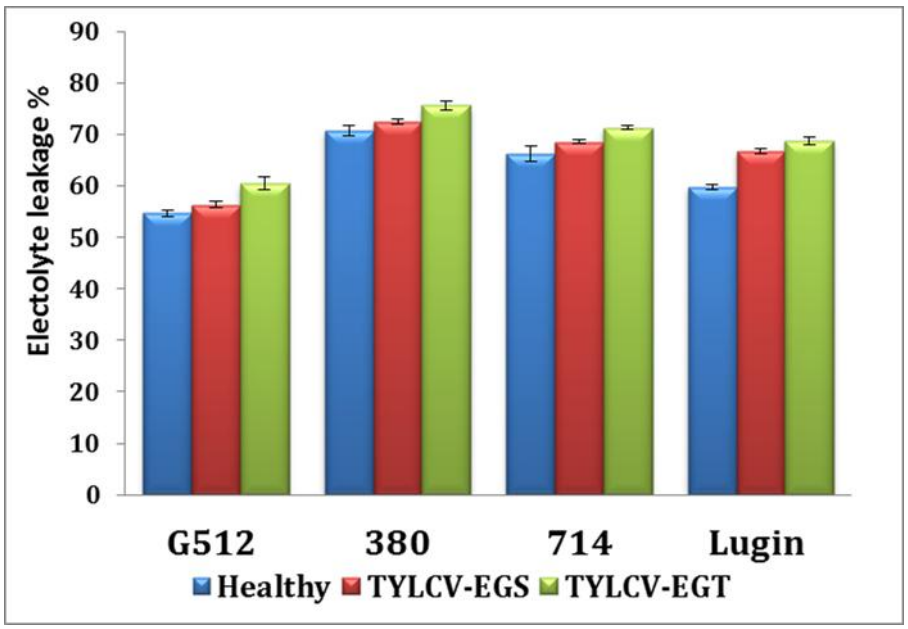
Electrolyte leakage (EL) and membrane stability index (MSI).

Electrical leakage and membrane stability enables for assessing the injury of cell membrane. Figure (1-a & b) revealed that, different cultivars of tomato under the influence of two isolates of TYLCV showed different responses in the rate of electrical leakage and the cell membrane stability comparing with healthy plants of the same cultivars.

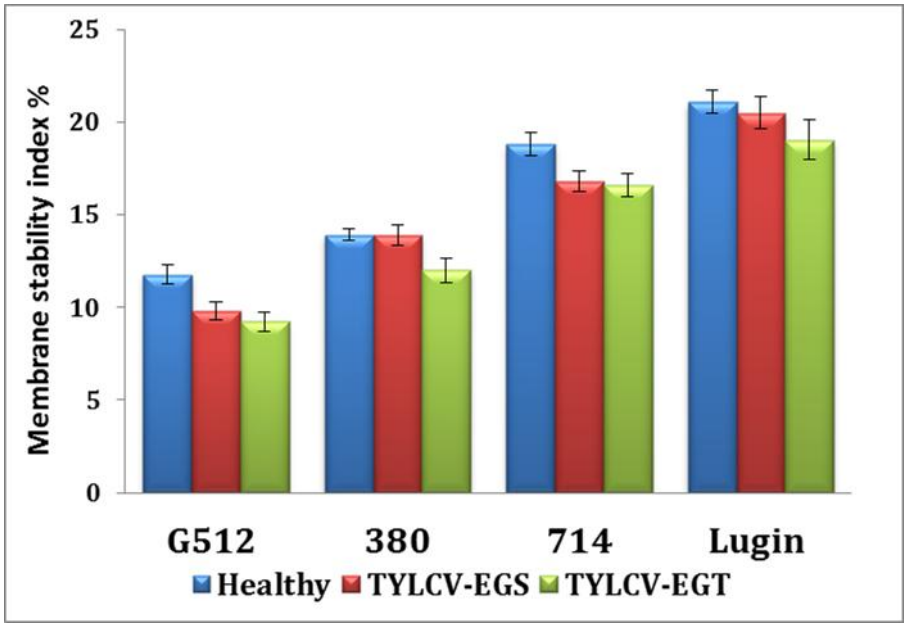
It was found that both of (714) and (Lugin) cultivars (Fig. 1-a) showed a significant increase in the rates of electrical leakage compared to healthy plants of the same cultivars. This was the case throughout the two isolates. While the results also showed a significant increase in values of electrical leakage ratios in cultivars (G512) and (380) at TYLCV-EGT. On the other hand, non-significant increase in ratio of electrical leakage was observed in (G512) and (380) cultivars at TYLCV-EGS.

Concerning the impact of TYLCV isolates on membrane stability of tomato cultivars, the result (Fig. 1-b) also showed that, a significant decrease in values of membrane stability ratios in infected plants of all cultivars compared to healthy plants of the same cultivars throughout two isolates with the exception of (380) and (Lugin) cultivars which showed insignificant decrease throughout at TYLCV-EGS.

The TYLCV-EGT was more effective than TYLCV-EGS regarding the electrolyte leakage and membrane stability index of different tomato cultivars.



(a)



(b)

Fig. 1: Effect of two TYLCV isolates on (a): electrical leakage %, (b): membrane stability index % of four different tomato cultivars. Each value is mean of 6 replicates ± standard error of means, Healthy= Healthy plants, TYLCV-EGS= Infected plants with TYLCV-EGS isolate, TYLCV-EGT= Infected plants with TYLCV-EGT isolate, at P<0.050

Inorganic anion and cations contents

Data illustrated in Fig. (2-a, b, c, d & e) showed that changes in the contents of inorganic anion and cations (N^-, P^{+++}, K^+, Na^+ and Mg^{++}) in all tomato cultivars as a result of TYLCV-EGS and TYLCV-EGT isolates infection compared with healthy plants.

Sodium and potassium contents showed significant increased by subject tomato plants to TYLCV virus this observed throughout two isolates and all tested tomato cultivars compared with healthy tomato plants (Fig. 2-a & b).

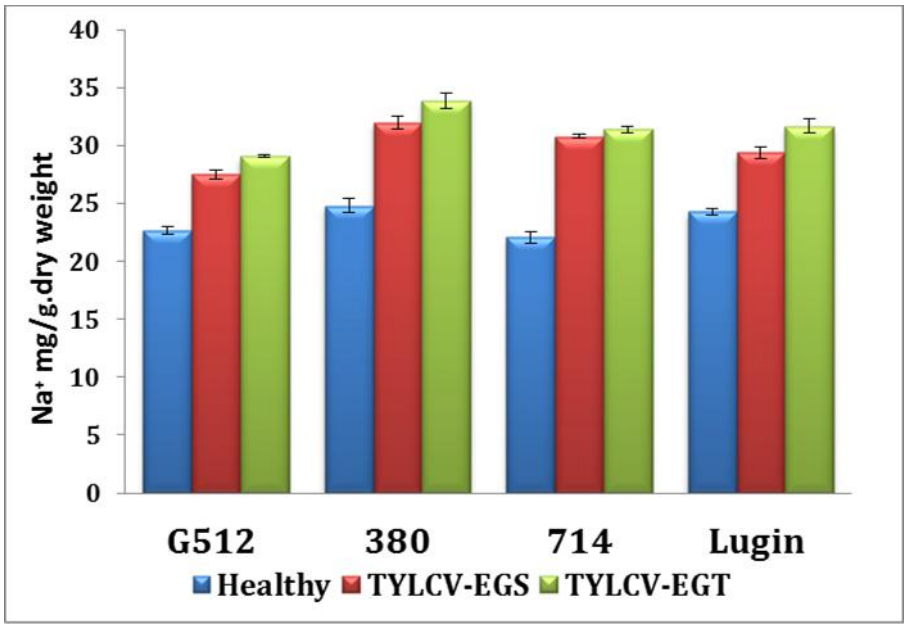
At all tested tomato cultivar plants under the influence of two TYLCV isolates, phosphorus content as shown in Fig. (2-c), increased significantly compared with the same healthy tomato plants, with one exception in (714) cultivar TYLCV-EGS isolate that increased insignificantly.

Among two TYLCV isolates, the most vital impact on sodium, potassium and phosphorus content was TYLCV-EGT isolate.

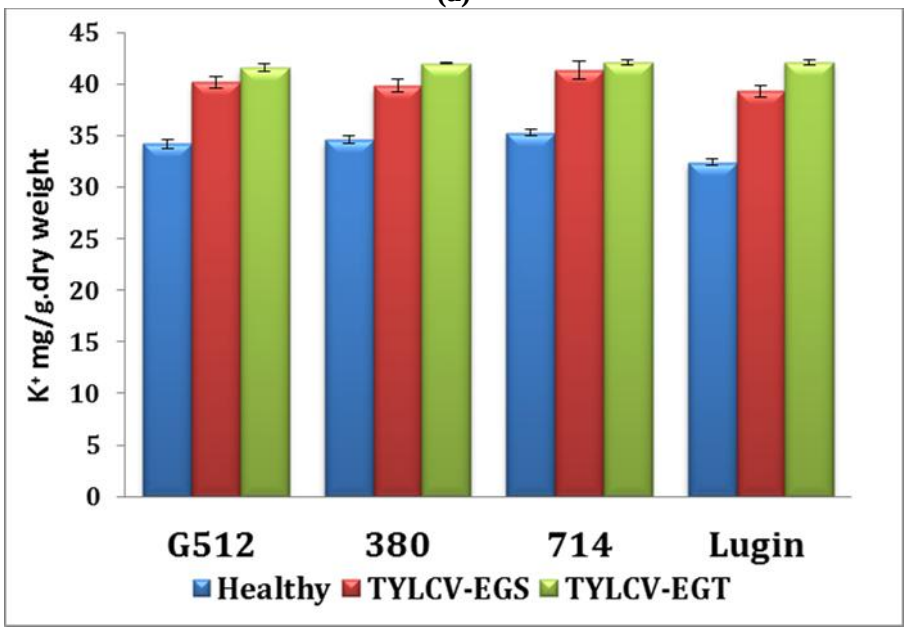
On the other hand, the nitrogen contents significantly decreased with infected tomato plants in all tested tomato cultivars as compared to healthy plants throughout two TYLCV isolates except (380) cultivar that increased significantly at TYLCV-EGS isolate (Fig. 2-d).

As well as magnesium contents also decreased significantly due to TYLCV infection in four tested tomato cultivars throughout two isolates except TYLCV-EGS isolate at (714) cultivar which showed insignificant decreased as in Fig. (2-e).

The TYLCV-EGT isolate was more effective than TYLCV-EGS isolate on both nitrogen and magnesium content in infected tomato plants compared with healthy ones. This case throughout four tomato cultivars.



(a)



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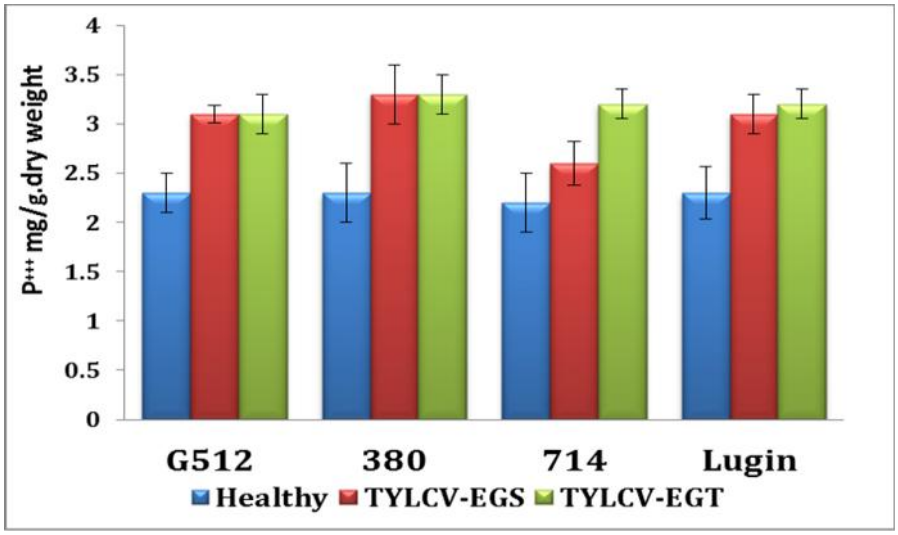

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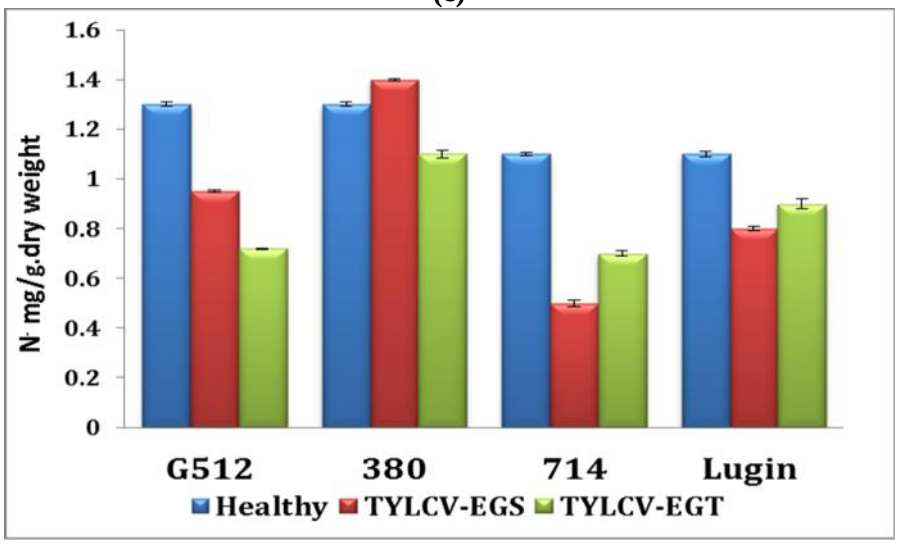

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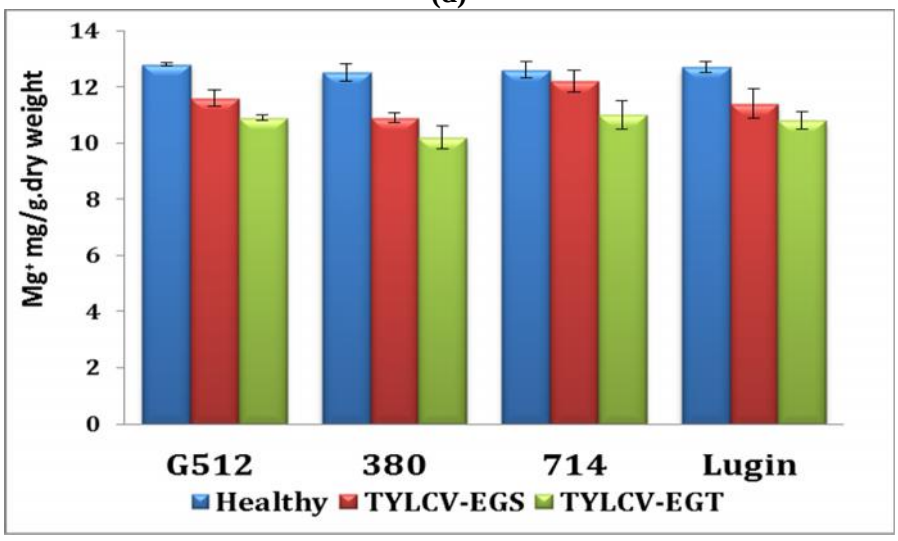


(e)

Fig. 2: Effect of two TYLCV isolates on (a): sodium, (b): potassium, (c): phosphorus, (d): nitrogen, (e): magnesium contents of four different tomato cultivars. Each value is mean of 6 replicates ± standard error of means, Healthy= Healthy plants, TYLCV-EGS= Infected plants with TYLCV-EGS isolate, TYLCV-EGT= Infected plants with TYLCV-EGT isolate, at P<0.050

Phytochemical constituents

Photosynthetic pigments.

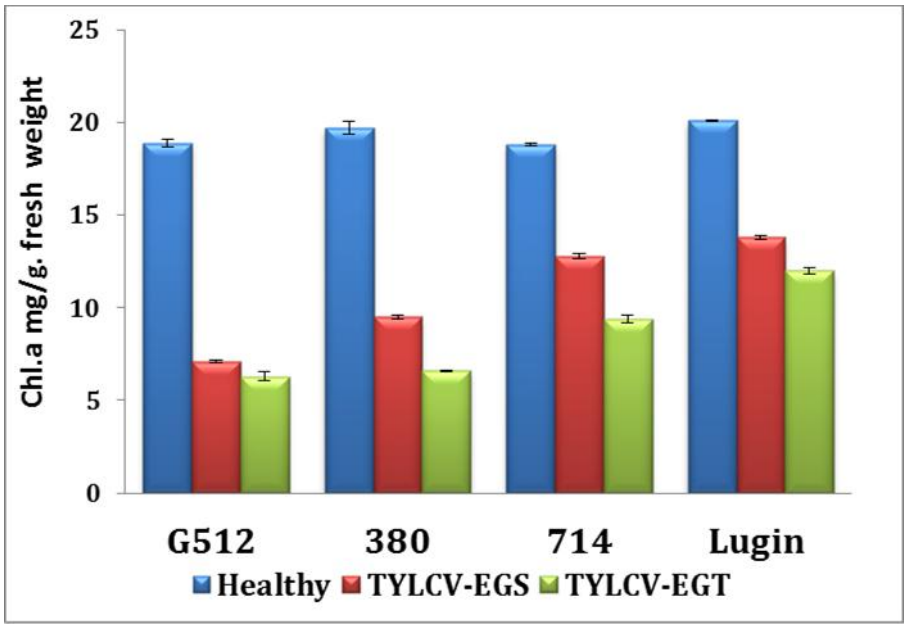
Photosynthesis is one of the main physiological processes important for plant growth.

TYLCV effect on the photosynthesis process in different cultivars of tomato, cultivars also showed different responses as a result of two TYLCV isolates infection by estimating the content of both chlorophyll and carotenoids in the leaves of tomato plants.

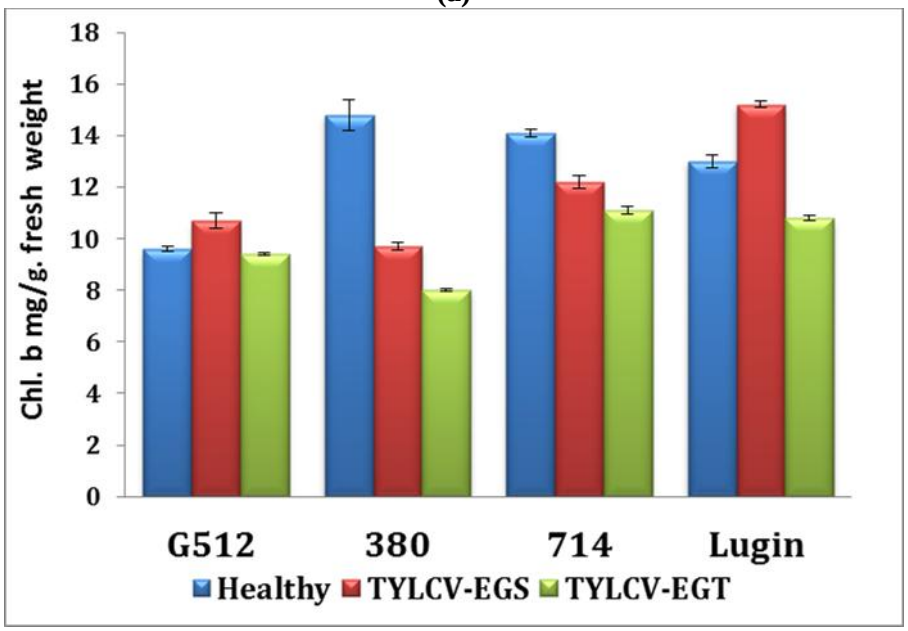
Data illustrated graphically in figure (3-a, b, c & d) clearly revealed that, the contents of chlorophylls (a, b and total a+b) and carotenoids were most significant decreased in different tomato cultivars than of the healthy ones due to TYLCV infection. This was the case throughout the two TYLCV isolates.

While, only in (G512) cultivar not significantly decreased in chlorophyll b was recorded throughout TYLCV-EGT.

The maximum impact in chlorophylls (a, b and total a+b) and carotenoids contents throughout tested tomato cultivars in comparison with the same healthy plants was observed at TYLCV-EGT.



(a)



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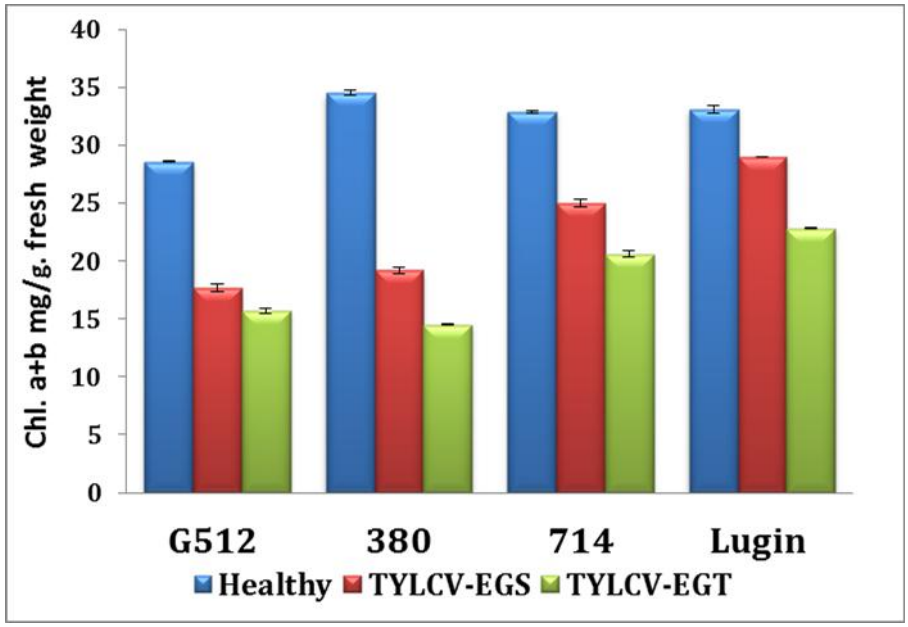

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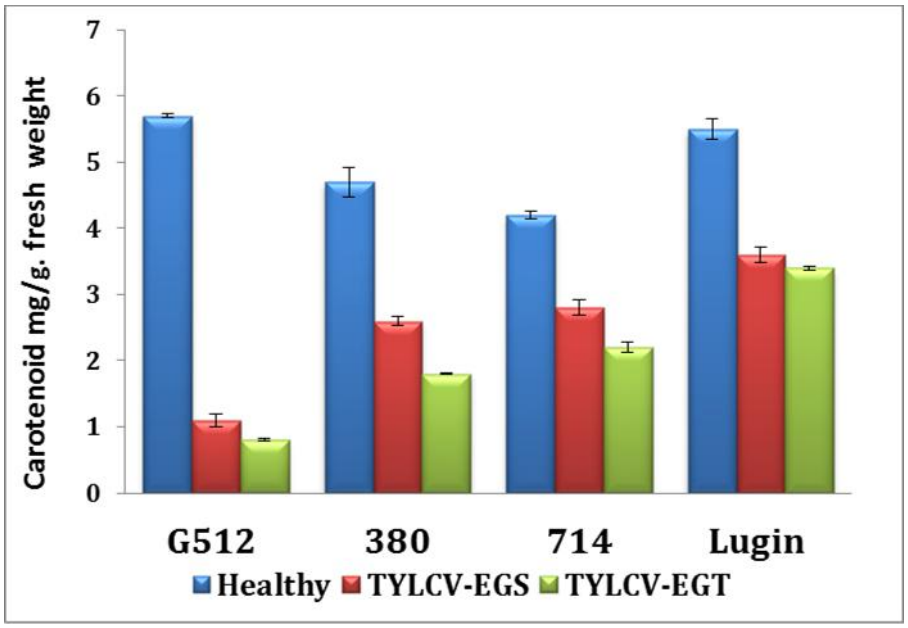


(d)

Fig. 3. Effect of two TYLCV isolates on (a): chlorophyll a, (b): chlorophyll b, (c): chlorophyll a+b, (d): carotenoids level of four different tomato cultivars. Each value is mean of 6 replicates ± standard error of means, Healthy= Healthy plants, TYLCV-EGS= Infected plants with TYLCV-EGS isolate, TYLCV-EGT= Infected plants with TYLCV-EGT isolate, at P<0.050.

Biochemical and bioactive components

Two TYLCV isolates effect on the content of biochemical components (carbohydrates, proteins and lipids) in different cultivars of tomatoes, tested cultivars also showed different responses as a result of TYLCV infection by estimating the content of carbohydrates, proteins and lipids on leaves of both healthy and infected tomato plants.

Data generated (Fig. 4-a) revealed that contents of total soluble carbohydrates in leaves were significantly decreased in infected plants at all tested tomato cultivars as compared to healthy plants throughout TYLCV-EGS and TYLCV-EGT isolates.

Regarding the effect of two TYLCV isolates on total protein content, results in Fig. (4-b) showed significantly decreased in the total protein contents in leaves of TYLCV infected plants compared to the same healthy cultivars at all tested tomato cultivars throughout two TYLCV isolates.

Concerning the impact of TYLCV on total lipids, at all tested tomato cultivar plants under the influence of two TYLCV isolates, data recorded in figure (4-c) illustrated that, lipid content decreased significantly on challenged plants with TYLCV compared with healthy tomato plants, with two exceptions, the first exception was in cultivar (380) at TYLCV-EGS and the second was cultivar (714) in TYLCV-EGT isolate that decreased insignificantly. Among two TYLCV isolates, the most vital effect on carbohydrates, proteins and lipid content was TYLCV-EGT isolate.

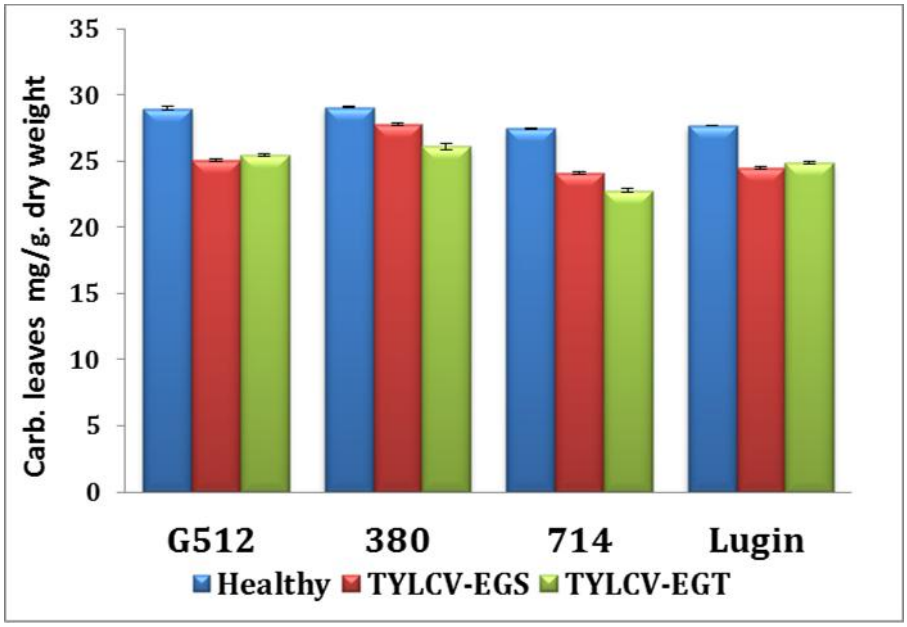
Both of TYLCV-EGS and TYLCV-EGT isolates effect on the content of bioactive components (phenols and proline) in different cultivars of tomatoes, tested cultivars also showed different responses as a result of TYLCV infection by estimating the content of phenols and proline on leaves of both healthy and infected tomato plants.

Data presented in figure (4-d) indicated the effect of two TYLCV isolates on total phenol content, results showed significantly increased in the total phenol contents in leaves of TYLCV infected plants compared to the same healthy cultivars at all tested tomato cultivars, this case generally throughout two TYLCV isolates. While in TYLCV-EGT, cultivar (380) showed insignificant increased and (714) cultivar gave the same value.

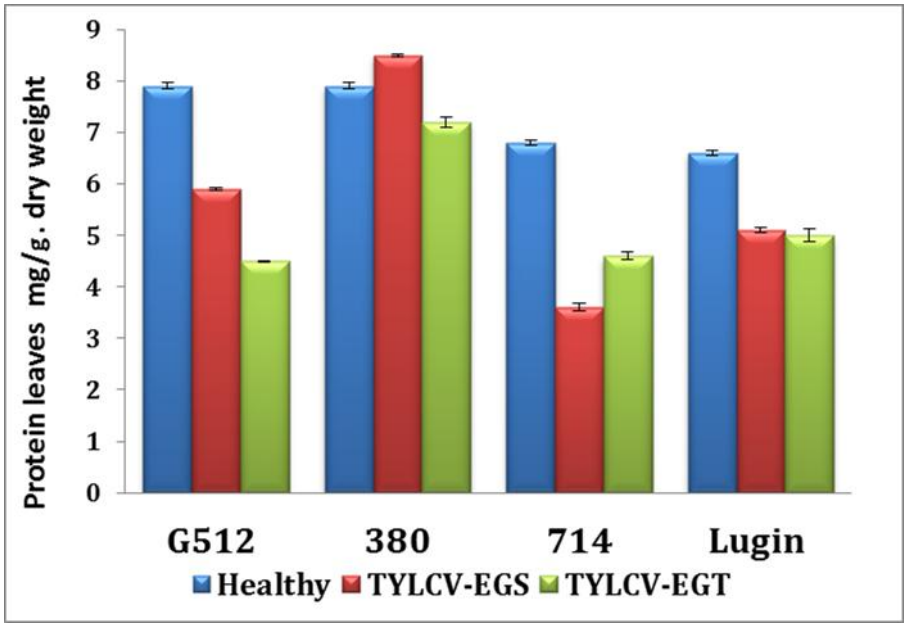
Regarding the impact of two TYLCV isolates on total proline content, results in figure (4-e) showed significantly increased in the total proline contents in leaves of TYLCV infected plants compared to the same healthy cultivars at all tested tomato cultivars throughout two TYLCV isolates.

On the other hand, results showed an insignificant decrease at cultivar (380) only due to TYLCV-EGS infection.

The TYLCV-EGT was more effective than TYLCV-EGS regarding the phenol and proline content of different tomato cultivars that comparing with the same cultivars.



(a)


Protein leaves mg/g. dry weight
9
8
7
6
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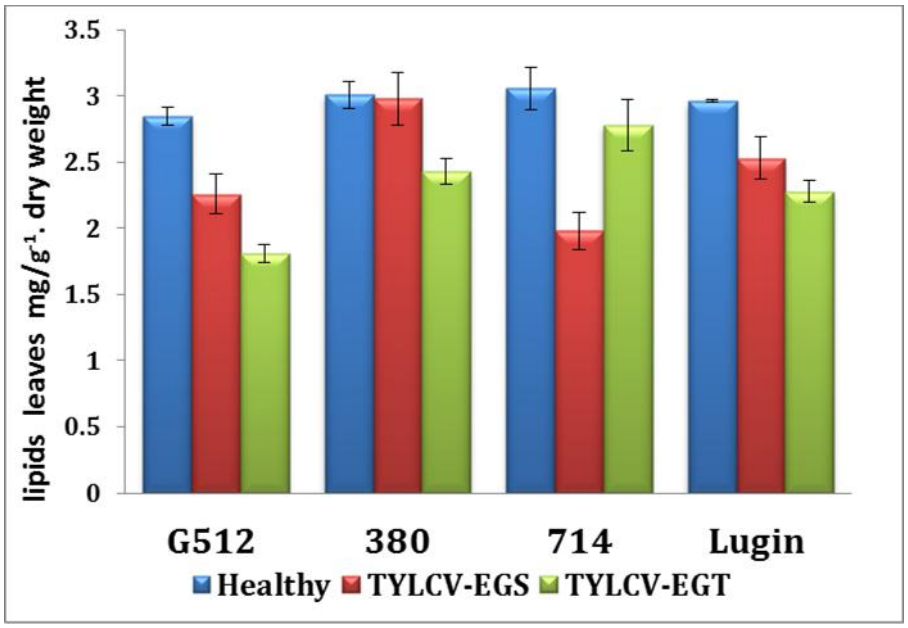
G512
380
714
Lugin
Healthy
TYLCV-EGS
TYLCV-EGT

(b)


lipids leaves mg/g-1. dry weight
3.5
3
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1
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0
G512
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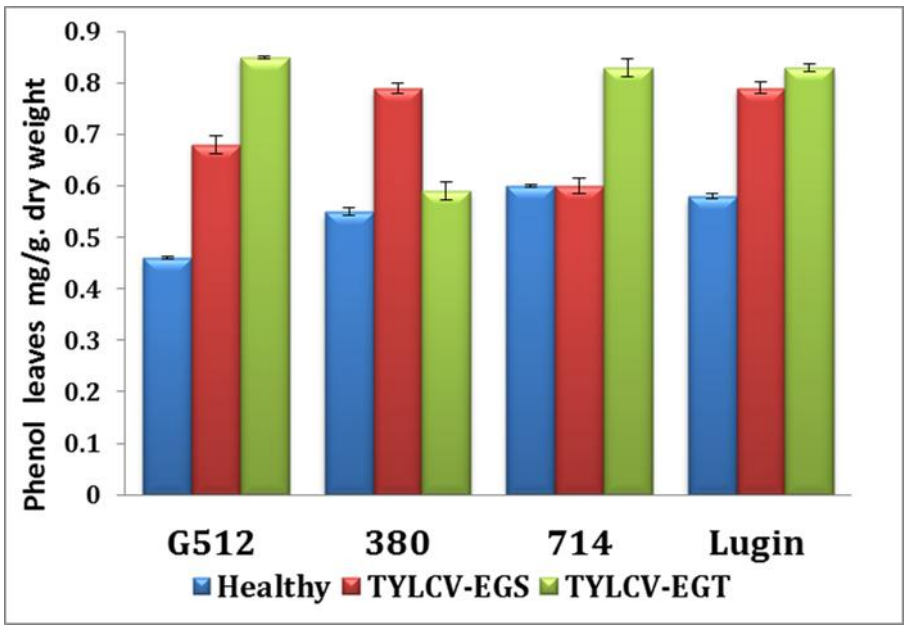
714
Lugin
Healthy
TYLCV-EGS
TYLCV-EGT

(c)



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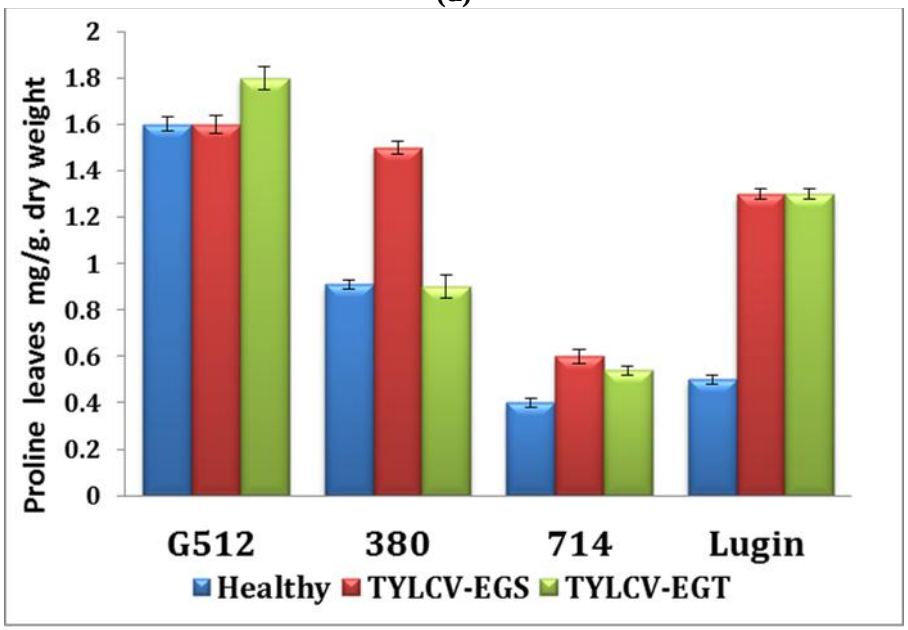


(e)

Fig. 4. Effect of two TYLCV isolates on (a): carbohydrates, (b): protein, (c): lipids, (d): phenol, (e): proline contents of four different tomato cultivars. Each value is mean of 6 replicates ± standard error of means, Healthy= Healthy plants, TYLCV-EGS= Infected plants with TYLCV-EGS isolate, TYLCV-EGT= Infected plants with TYLCV-EGT isolate, at P<0.050

Enzyme activities

Antioxidant enzymes activities

Plants employ antioxidants detoxifying enzymes activities to combat oxidative stress generated from biotic stress. In order to determine the nature of the antioxidant responses of tomato to TYLCV stress during vegetative stage, we measured the enzymatic activity of PPO, POX, CAT and SOD in shoots of different four tomato cultivars under influence of two TYLCV isolates. Tomato cultivars showed different variation in the enzyme activities under the influence of two TYLCV isolates stress.

Results of the present work (Fig. 5-a) revealed in mostly that, tomato cultivars plants infected with TYLCV-EGS and TYLCV-EGT gave significant increases in PPO activity related to healthy tomato cultivars plants (uninfected) throughout the two isolates.

On the other hand, cultivar (714) showed significant decreasing in PPO activity under effect of TYLCV-EGS.

Data presented in and figure (5-b), indicated the effect of two TYLCV isolates on POX activity, results showed significantly increased in the POX activity in shoots of TYLCV infected plants compared to the same healthy cultivars at all tested tomato cultivars, this case generally throughout two TYLCV isolates.

Data generated in figure (5-c), showed the changes in the activities of CAT enzyme in tomato leaves in response to TYLCV-EGS and TYLCV-EGT isolates.

The high activity of (CAT) was obtained generally, in tomato cultivars plants infected with two TYLCV isolates compared with the same healthy tomato cultivars plants. On the contrary, under the effect of TYLCV-EGS isolate, CAT activity decreased significantly at cultivar (714).

Regarding the impact of TYLCV-EGS and TYLCV-EGT isolates on SOD activity results in figure (5-d), showed generally significant increase in the SOD activity in shoots of TYLCV infected plants compared to the same healthy cultivars at all tested tomato cultivars throughout two TYLCV isolates. On the other hand, results showed cultivar (380) only showed insignificant increase due to TYLCV-EGS isolate infection.

Among two TYLCV isolates, the most vital impact on PPO, POX, CAT and SOD activities was TYLCV-EGT isolate. This case throughout four tomato cultivars.

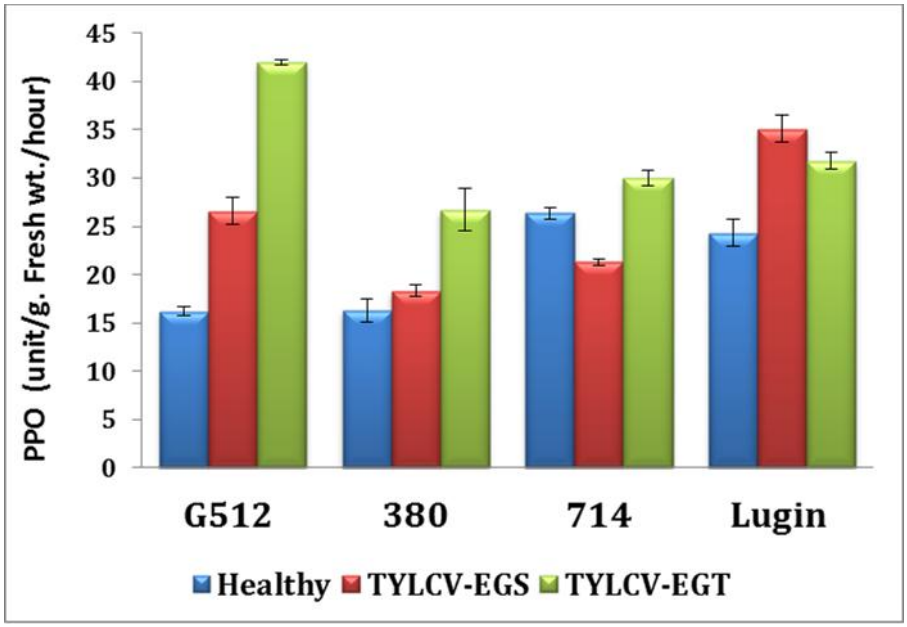
Hydrolytic enzymes activities.

The obtained data (Fig. 6-a) indicated that both TYLCV-EGS and TYLCV-EGT infection, with two exceptions, significantly increased the activities of amylases especially with the impact of TYLCV-EGT. The first exceptional case was represented by significant decreases in amylases at cultivar (Lugin) during two TYLCV isolates and the second was insignificant increased in cultivar (512) at TYLCV-EGS isolate. The highest amylase activity was recorded generally by TYLCV-EGT throughout different four tomato cultivars.

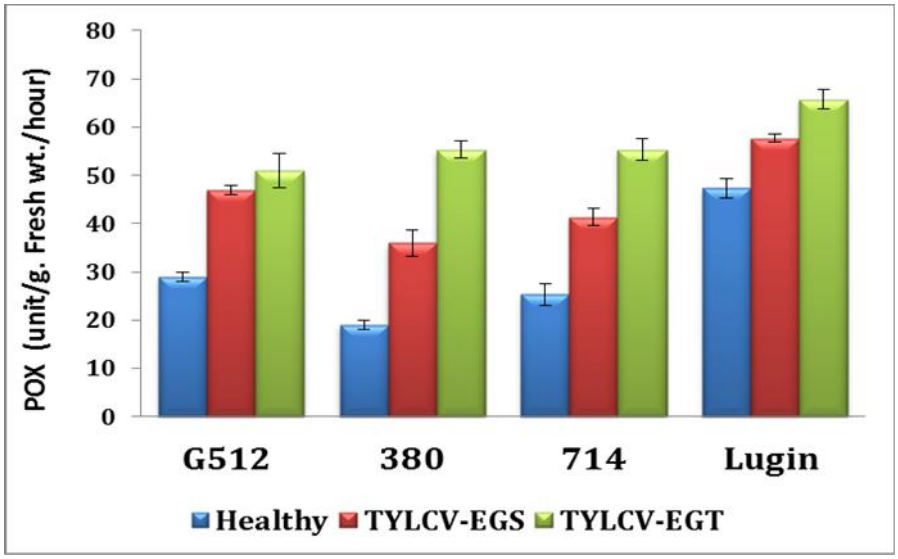
Concerning the activities of protease, results in (Fig. 6-b) indicated that both of two TYLCV isolates resulted, mostly, in either significantly increased at cultivars G512, 380 and 714.

It was also observed that TYLCV-EGS at cultivar (Lugin) caused significantly decreased the protease activities of tomato plants. While TYLCV-EGT hadn't caused any changes in the enzymatic activity of protease. This was valid when being compared with the healthy plants.

The most effective isolate on both amylase and protease activity in infected tomato plants compared with healthy was TYLCV-EGT.



(a)



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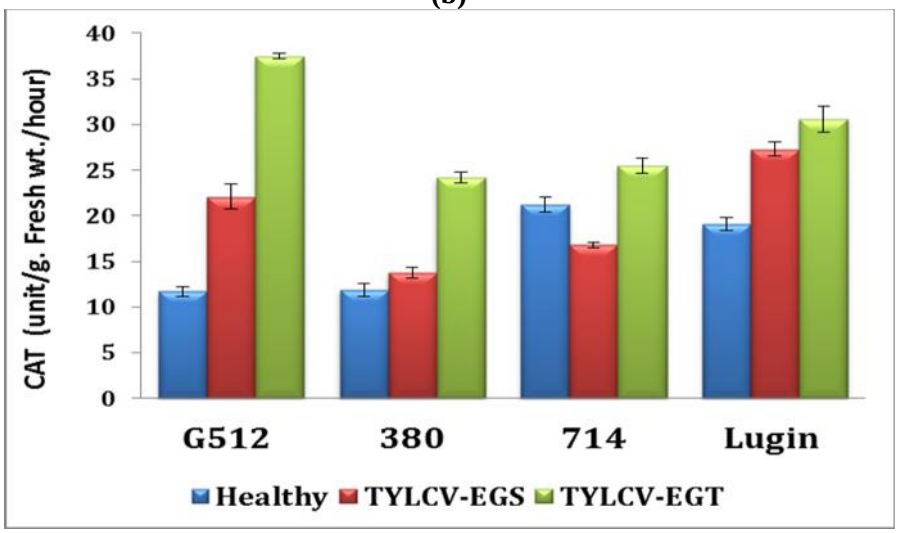

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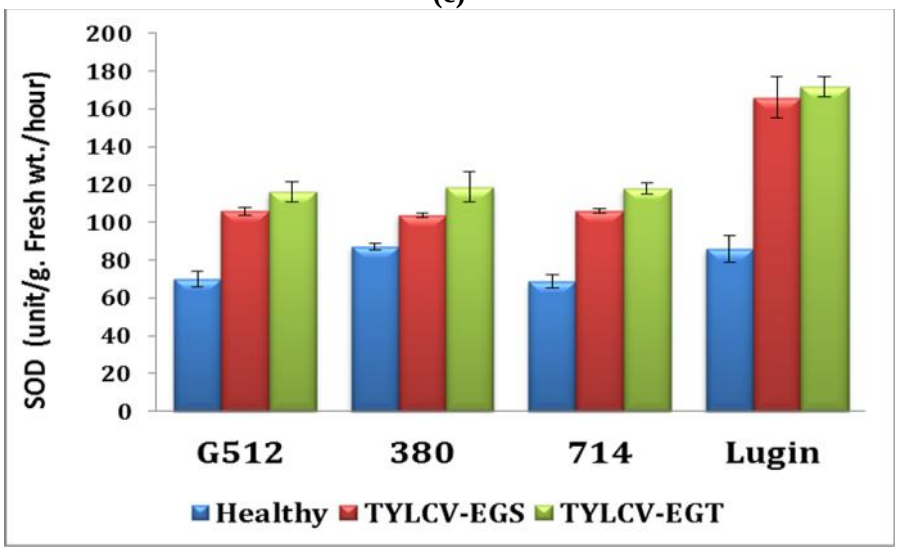
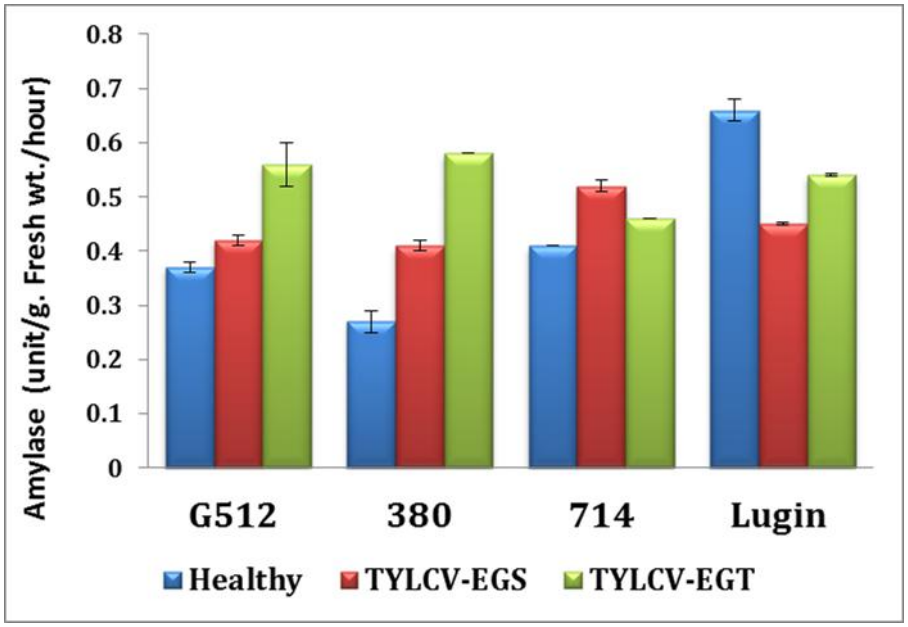
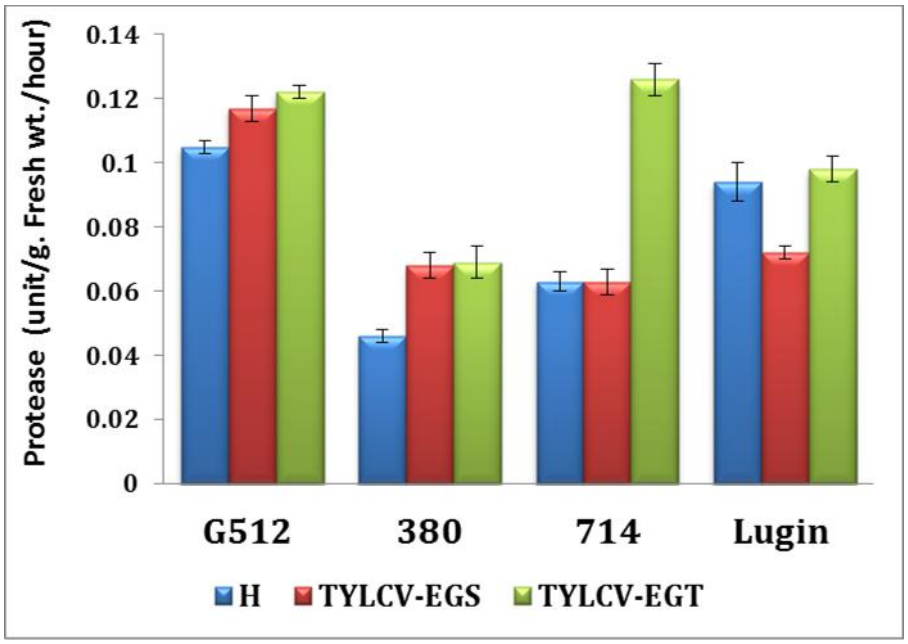


(d)

Fig. 5: Effect of two TYLCV isolates on (a): PPO, (b): POX, (c): CAT, (d): SOD activities of four different tomato cultivars. Each value is mean of 6 replicates ± standard error of means, Healthy= Healthy plants, TYLCV-EGS = Infected plants with TYLCV-EGS isolate, TYLCV-EGT = Infected plants with TYLCV-EGT isolate, at P<0.050



(a)



(b)

Fig. 6: Effect of two TYLCV isolates on (a): Amylase, (b): Protease activities of four different tomato cultivars. Each value is mean of 6 replicates ± standard error of means, H= Healthy plants, TYLCV-EGS= Infected plants with TYLCV-EGS isolate, TYLCV-EGT = Infected plants with TYLCV-EGT isolate, at P<0.050.

Discussion

Tomato (*Solanum lycopersicum*) is one of the most popularly grown vegetable crops in the world. The worldwide high consumption of tomato is mostly due to its acceptable flavor and high nutritive value. **(**Alhudiab *et al*., 2014; Chen *et al.*, 2016).

Tomato yellow leaf curl is a destructive viral disease of tomato caused by Tomato yellow leaf curl virus (TYLCV) (Michael *et al*., 2009; EL-Dougdoug *et al.*, 2013). It is one of the major devastating diseases throughout the world (Lefeuvre *et al*., 2010; Deng *et al*., 2015).

In the present study, both TYLCV-EGS and TYLCV-EGT isolates were able to induce symptoms when transmitted mechanically through syringe injection and via whiteflies to healthy plants. At the same time, the two TYLCV isolates reacted positively with specific antibodies by applying the DAS-ELISA assay. These findings are supported by EL-Dougdoug *et al.* (2014a).

Inoculated four tomato cultivars with TYLCV-EGS or TYLCV-EGT isolates by syringe and by whitefly after 25 to 35 days under a greenhouse condition, showed variation external symptoms. These results were confirmed by DAS-ELISA using specific polyclonal antibodies.

Various reports suggest that virus multiplication inside the plant cell alters different biochemical constituents of plants and disrupt the physiological processes like photosynthesis, transpiration and respiration of the infected plants which affect the growth and yield (Tajul *et al*., 2011; Refaey, 2014). Also have reported that the determination of cellular constituents in virus infected plant is very important to understand the activities of the host cell and the nature and extent of damage have caused by the virus. The current study indicated that both of TYLCV-EGS and TYLCV-EGT affected biochemical and metabolic parameters in infected tomato plants compared with healthy controls throughout different four tomato cultivars.

Membrane damage could indirectly be evaluated by measuring solute leakage (Electrolyte leakage) from cells (Ekmekei *et al.,* 2007) and membrane stability index (Ali *et al.,* 2008; Bassiouny, *et al*., 2014). That marked in obtained results which showed that, infection with two TLYCV isolates mostly increased electrolyte leakage ratio and caused decrease in membrane stability index of tomato plant as compared with healthy plants, that through different four tomato cultivars. These results were in an agreement with Khalil *et al.,* (2014) and Refaey, (2014). Results indicating that viral infection induced increased permeability in cells to cause loss of water (Oleinikova, 1969). This also explains the cup shape of the infected leaves especially in the severe symptom stages. Also results indicated that the TYLCV-EGT was the most effective regarding the electrolyte leakage and membrane stability index of different tomato cultivars.

In the present investigation, results clearly revealed biochemical changes in infected tomato tissues at the level of photosynthetic apparatus as indicated through photosynthetic pigments. A reduction in photosynthetic pigment levels (chlorophyll a, chlorophyll b and carotenoids) in the TYLCV infected tomato leaves. The maximum impact in chlorophylls (a, b and total a+b) and carotenoids contents throughout tested tomato cultivars in comparison with the same healthy plants was observed at TYLCV-EGT. These findings are supported by Montasser *et al*., 2012 and Sofy *et al.* (2014). Research on virus infected tomato plant revealed that photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) decline and this may be due to severity of viral infection These results were also in agreement with Radwan *et al.* (2007), Palanisamy *et al,* (2009), Afreen *et al.* (2011), Raithak and Gachande (2012), Pazarlar *et al.* (2013) and Mushtaq *et al.* (2014). Greater differences in the levels of these pigments were observed with the progression of the disease, which reflected the reduced photosynthetic rate due to loss of photopigments with the progression of the disease (Scholes *et al*., 1989).The decrease in chlorophyll is considered to be a symptom of oxidative stress condition this decrease after virus infection might be due to the generation of reactive oxygen species (ROS) causing damage to chlorophyll *a* that is mean the plant failed to capture the light and so photosynthesis will decrease or stopped (Ali *et al.*, 2006). The current study is in harmony with the study carried by Khalil *et al*., (2014) who reported that the reduction in photosynthetic pigments may be attributed to the infected action of TYLCV on biosynthesis of pigments, increasing their degradation and/or maintaining damage of the chloroplast thylakoid. The observed reduction in Mg^{+}, which is needed for chlorophyll synthesis in infected tomato plants, in the present work, reinforced the view that TYLCV decreased chlorophyll biosynthesis. Moreover, the decrease in chlorophyll contents in infected tomato plants.

In the present investigation, concurrently with the increases in protein and proline level led to the suggestion that nitrogen may be shifted to synthesis of proline as osmoregulating substances instead of chlorophyll. Both of TYLCV isolates induced significant decrease in Mg^{+} and N^{-} content in TYLCV infected tomato cultivars plants when compared with healthy ones. The results also revealed an increase in the content of sodium and potassium ions another increase in phosphorus ion content throughout two TYLCV isolates infection at four tomato cultivars. The results obtained in agreement with Muqit *et al*. (2007), Afreen *et al.* (2011) and Ashfaq *et al*. (2014).

Increased phosphorus content in the diseased leaves might be due to phosphorus containing polypeptide of the virus particles (Haider and Hossain, 1994).

Increment of sodium level synchronized with increase of drought level was reported by Hu *et al.* (2007) that in this research on Purslane (*Portulaca oleracea* L.) leaves, sodium content was increased synchronized with drought stress increment, also, Niakan and Ghorbanli (2007) in studied effect of drought stress on ionic contents in Soybean shoot was reported that sodium level in shoot increment by stress than blank level, but potassium concentration was decreased. Their expression that the reason of decrease potassium content in the shoot is decrement of water potential that was caused decrement of potassium transport of root to shoot in plant. In researches on Sorghum and Maize, increment of sodium and potassium level in root and shoot plants by imposing of drought stress was reported by Erdei and Taleisnik (1993). In another study on wheat (*Triticum aestivum*) was reported that with the increase of the drought stress level and period, sodium and iron concentration was increased in the shoot, but potassium, calcium and magnesium contents were decreased (Abdalla and El-Khoshiban, 2007). Increase of sodium level in plants at stress condition is a defensive mechanism that plants in stress condition by it could controlled of osmotic pressure in cells, and water and nutrient solute absorption of soil.

Carbohydrates in leaves were significantly decreased in TYLCV isolates infected of tested plant cultivars as compared to healthy ones; this may be due to the defense effect of the plant against the virus infection where the strategy altered from defensive to survival. These results are in harmony with the study carried by Radwan *et al*. (2007), Gupta *et al*. (2010), Montasser *et al*. (2012) and Khalil *et al*. (2014). Conflicting reports about the accumulation of sugars in virus-infected plants were recorded. The virus induces changes in the synthesis of fructose-1,6-diphosphate, affecting carbohydrate metabolism and leading to reduced energy reserves of the plant. This reduction in metabolic activity would enhance fructose-1,6-diphosphate accumulation, thereby impeding sucrose synthesis in the infected leaves (Cseke *et al*., 1984). While other reports revealed that increasing in reducing sugars in infected plants could be due to phloem necrosis that may prevent translocation of sugars (Ahmed *et al*., 1986). Reducing of sugars translocation due to damaged phloem in virus-infected plants was also observed by Lastra and Gil (1981).

Data revealed also a notable reduction in the total protein of leaves TYLCV infected plants compared to the same healthy cultivars at all tested tomato cultivars throughout TYLCV-EGS and TYLCV-EGT isolates. This observation also was reported for tomato plants infected with *Tomato yellow leaf curl virus* (Montasser *et al*., 2012; Khalil *et al*., 2014). Similar results also were reported by Taiwo and Akinjogunla (2006) working with a *yellow mosaic virus* infection of mungbean (*Phaseolus aureus* L.). The total protein decrease was observed in infected carrot *(Daucus carota* L.) plant (Afreen 2011). On contrary, Sarma *et al.* (1995) reported that virus infection caused increased crude protein levels in the leaves. Changes in the total amount of protein during virus multiplication show a complex pattern, and depend upon the developmental stage of the leaf during infection. Other reports have shown that during development of the healthy leaf, the total protein content increases during leaf expansion, and declines as the leaf ages (Fraser, 1987). The infection of mature leaves prevents the net loss of total protein during ageing. This may be explained as a result of accumulation of TMV coat protein, with an overall loss of host proteins (Fraser, 1987).

Concerning the impact of TYLCV isolates on lipid content, at all tested tomato cultivars plants under the influence of two TYLCV isolates, lipid content, generally decreased significantly on challenged plants with TYLCV compared with healthy tomato plants. The significant decrease in total lipids may reflect the severe damage to the cell membranes since lipids are an integral part of the cell membrane. Loss of lipids along with the chlorophyll content may be as a result in severe damage to chloroplast membranes (Montasser *et al*., 2012).

Total phenols play a significant role in the regulation of plant metabolic process and overall plant growth as well as lignin synthesis (Kumar *et al*., 2010). In addition, phenols act as free radical scavengers as well as substrates for many antioxidant enzymes.

A number of phenols are regarded as pre-infection inhibitors, providing the plant with a certain degree of basic resistance against pathogenic micro-organisms. Phenol metabolism and cell wall lignification are thus involved in, and have consequences for, a number of cellular, whole plant and ecological processes, that might even provide plants, the immunity against destructive agents (Sudhakar *et al.*, 2007).

Present study indicated that the phenol content significantly increased due to two TYLCV isolates infection. These results were supported by studies of Rai *et al*. (2011); Jaiswal *et al*. (2012); El-Dougdoug *et al.* (2014b) and Khalil *et al*. (2014). Previous studies suggest that stressed tomato plants accumulate more phenolic compounds as antioxidants to resist viral stress. This may be due to a renewed synthesis of phenolic phytoalexins to fight the viral stress (Hutson and Smith, 1980).

The current study was in close agreement with earlier studies where it has been shown that a resistant variety had a higher level of phenolics than the susceptible one (Singh *et al*., 2002). These are in accordance with Sudhakar *et al.* (2007) who stated that phenolic acids are involved in phytoalexin accumulation, biosynthesis of lignin and formation of structural barriers, which play a major role in resistance against the pathogen.

Pathogen infection may cause activation in numerous compounds in the cell or the proline production (Fabro *et al.,* 2004). The obtained results showed significantly increased in the total proline contents in leaves of TYLCV infected plants compared to the healthy ones. These results are in agreement with Shahnaz *et al*. (2013) they reported that infected tomato plants showed a pronounced increase in the levels of proline as compared with the non-infected control plant. Proline accumulation is a common metabolic response to both abiotic and biotic stress and when higher plants are exposed to stress; many plants accumulate high amounts of proline in tissues (Mazid *et al.*, 2011).

The pepper (*Capsicum annuum* L.) infected with *Tobacco mosaic virus* (TMV) showed an increase of proline content in diseased plants as compared to the respective controls (Sercan, 2013; Pazarlar *et al*., 2013). When plants are exposed to microbial pathogens, they produce reactive oxygen species (ROS) that induce programmed cell death in the plant cells surrounding the infection site to effectively wall off the pathogen and terminate the disease process (Apel and Hirt, 2004; Moshe *et al.*, 2012). The amino acid proline may act as a potent scavenger of ROS and this property of proline might prevent the induction of programmed cell death by ROS (Chen and Dickman, 2005).

The accumulation of proline with the rise of salinity levels in flax and faba bean plants were in agreement with the results obtained by Khalil (2011), Kavi *et al*. (2005) they reported that, proline accumulated in response to several environment types of stress to protect the cell by balancing the osmotic strength of cytosol with that vacuole and external environment. Also the accumulation of proline was reported to serve as nitrogen storage compound and/or scavenging hydroxyl radicals (Sharma and Dietz, 2006).

One of the most promising influences of various stresses is the generation of oxidative stress that results from increased levels of reactive oxygen species (ROS) in cell exposed to stress (Kumar *et al*., 2009). In order to repair the damage, generate by ROS, plant evolve complex antioxidant metabolism. Many plant oxidative enzymes such as PPO and POD can catalyze the formation of lignin and other oxidative phenols that contribute to reinforcing the cell structure during the pathogens invasion (Deng *et al*., 2015).

Regarding the impact of TYLCV isolates on antioxidant enzymes activity in tomato leaves, present work showed generally significant increase in the POX, PPO, SOD and CAT activity in TYLCV infected plants compared to the healthy ones cultivars at all tested tomato cultivars throughout two TYLCV isolates. These results are in harmony with the study carried by Sudhakar *et al.* (2006), Rai *et al.* (2011), Jaiswal *et al.* (2012), Huseynova and Aliyev (2012) and Sofy *et al.* (2013). They observed that biotic stress has increased the activities of leaf antioxidant enzymes. Concerning the activity hydrolytic enzymes (amylase and protease), results indicated that both of two TYLCV isolates, mostly, in either significantly increased at all tested cultivars.

Among two TYLCV isolates, the most vital impact on both antioxidant and hydrolytic enzymes activity was TYLCV-EGT isolate. The infected stress in tomato plants revealed the activation of antioxidant enzymes in leaves such as (POX), (CAT) and (SOD) enzymes. Plants have evolved complex antioxidant systems in order to protect cellular membranes and organelles from the damaging effects of ROS (Šubr *et al.,* 2006; Sofy *et al.,* 2014). Increase in peroxidase activity is also a response to viral infection, and has been reported in peaches, apricots (Dıaz-Vivancos *et al.*, 2006; Radwan *et al.*, 2010).

The antioxidant enzymes and redox metabolites act in synergy to carry out ROS detoxification. SOD catalyses the dismutation of O_2^- to H_2O_2, CAT dismutates H_2O_2 to oxygen and water, and APO reduces H_2O_2 to water by utilizing ASC (ascorbate) as a specific electron donor. These are considered the main enzymatic systems for protecting cells against

oxidative damage. The balance between SOD and APO or CAT activities in cells is crucial for determining the steady-state level of O_2^- and H_2O_2 (Gill and Tuteja, 2010).

Almagro *et al.* (2009) and Sofy *et al.* (2014) revealed that peroxides are important pathogenesis-related proteins (PR-proteins). They have an important role in plant defense mechanisms, due to their involvement in the removal of hydrogen peroxide from the cells. Therefore, timing and localization of increased guaiacol peroxidase (GPX) and ascorbate peroxidase (APX) activity and their involvement in cell wall lignification, clearly suggested that peroxidases are involved in the formation of barrier substances confined to the site of pathogen penetration. Kumar *et al.* (2009) reported that a significant enhancement of SOD, CAT, GPX and APX activities was observed in the infected plant parts, accompanied with increased H_2O_2 formation during viral infection. SOD, CAT, GPX and APX were over-expressed due to viral infection, indicating their role in detoxification of ROS. POX was considered to be one of the antioxidant enzymes involved in the plant defense response to pathogen attack; it was reported to be the first enzyme to show changes in its activity under stress (Radwan *et al*., 2007).

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