ANTIVIRAL ACTION OF HOT PEPPER (*Capsicum annuum*) AGAINST *TOMATO SPOTTED WILT VIRUS* INFECTED TOMATO

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ABSTRACT

Tow field experiments were conducted during April 2006 and September 2006 to study the sensitivity of tomato cultivars against infection with *tomato spotted wilt virus* (*TSWV*) and effect of treatments with ethanolic extract of hot pepper fruits with three concentrations (50, 125 and 250 µg/ml) to induce resistance against infection with *TSWV* in tomato plants (cv. Peto 86). The results showed that all treatments induced resistance to the virus infection when applied to plants as crude extract with *TSWV* in a mixed inoculum. Also all treatments gave a significant increase in photosynthetic pigments, total soluble phenols and flavonoids as well as the activity of catalase, peroxidase and polyphenol oxidase compared with infected plants.
Key words: Tomato (*Lycopersicon esculentum*),*tomato spotted wilt virus* (*TSWV*), infection, hot pepper fruits (*Capsicum annuum*), antiviral activity, chemical composition, leaves.

INTRODUCTION

Tomatoes (*Lycopersicon esculentum* Mill) are important products providing vitamin A and C with a specially appealing flavor. Characteristic tomato flavor is a result of the interaction of taste components and aromatic volatiles. Sugars, organic acids, free amino acids, and salts are the non-volatile constituents of tomato flavor. Glucose and fructose make up about 50% of the dry matter. The main organic acid is citric acid. Glutamic acid is the major free amino acid found in tomato juice. Potassium and phosphate are the most abundant minerals in fresh tomatoes. There are over 400 aroma volatiles in fresh tomatoes but only about 30 contribute significantly to the flavor (Emin, 2001).

However, the presence of several viral diseases greatly limits the use of sustainable growing systems, such as *Tospovirus*, (*Tomato spotted wilt virus*, *TSWV*) and *Begomovirus*, (*Tomato yellow leaf curl virus*, *TYLCV*) are two of the viral agents causing severe economic losses. Although protocols for integrated pest transmit the diseases. Recently, a new virus, (*Potexvirus Pepino mosaic virus*, *PepMV*) has become a threat to tomato production in several areas of Europe, including southeastern Spain (Pico *et al*., 1996; Rosello *et al*., 1996 and Soler *et al*., 2000). TSWV is now classified as the type member of the Tospovirus genus which to date includes 13 species (Van de Wetering, 1999).

TSWV are well-established viruses with increasing economic importance as pathogens. This virus has one of the widest host ranges of any other plant virus, infecting over 900 species of plants in more than 70 families

including both monocots and dicots (Peters, 1998). The loss of marketable tomato yield due to *TSWV* epidemics accounted for millions of dollars and reduced the tomato production by 50 – 90% in the Hawaiian Islands (Cho et al., 1987). The virus is exclusively transmitted by thrips (*Thysanoptera*).

Viruses do not attack the structural integrity of their host tissues, but instead they subvert the synthetic machinery of host cells, acting as molecular pirates. No safe veridical chemical that can eliminate viruses without adversely affecting their hosts has yet been found. This problem has become a serious challenge to plant pathologists, biochemists and molecular biologists to develop a long-term and a sustainable management strategy for *TSWV*.

A large number of substances originating from plants affect the ability of plant viruses to infect and multiply in plants either by inactivation of the virus or through induced systemic resistance (Renuka Devi *et al*., 2004).
Hot pepper, genus *Capsicum*, belongs to the great family of tropical plants *Solanaceae* (Somos, 1984). This besides the large application of *Capsicum* peppers related with cookery, they have been employed in traditional medicine as antimicrobial, insecticide and anticonvulsive (Otero *et al*., 2000). Due to the fact that flavonoids and Phenolic compounds show different kinds of biological activity (Harborne and Williams, 2000 and Hahlbrock and Scheel, 1989).

Flavonoids are low-molecular-mass compounds widely distributed in the plant kingdom (Koes *et al*., 1994). Although their presence in plants has been known for many years, antiviral activity of flavonoids against a plant virus, such as potato virus X (PVX) was first reported several years later, the effects of a range of flavonoids on tobacco mosaic virus (TMV) and potato virus X (PVX) were studied by Verma and Bakt (1973); French *et al*. (1991 and 1992).

Therefore, the aim of the present study was to investigate the effect of various concentration from hot pepper fruits ethanolic extract treatments in controlling *TSWV* infection for tomato plants under field conditions, as well as , investigate the proposed mechanism of action of these anti-viral natural plant extracts throw the alteration of the chemical composition, secondary metabolites accumulation and activity of antioxidant defense enzymes compared with healthy plants.

MATERIALS AND METHODS

Two field experiments were carried out at The Experimental Station of Faculty of Agricultural, Cairo university, Giza, Egypt. During April 2006 and September 2006.

1- Source of tomato seeds and hot pepper fruits:

Tomato seeds (*Lycopersicon esculentum* Mill. cv. Castle rock, Peto 86, Super merman, UC 97 and Strain B) and Hot pepper fruits (*Capsicum annuum*) were obtained from local market.

2- Virus source and identification:-

Samples of *Gomphrena globosa* plants showing typical systemic mottling, chlorotic ring spot and necrotic local lesions of *tomato spotted wilt virus* were collected and tested for the presence of *TSWV* by indirect ELISA.

TSWV were Obtained from Dr. Manal A. Elshazly (virus and phytoplasma Research Department, plant pathology Research Institute, Giza. Egypt).

3. Mechanical transmission:-

Mechanical transmission were conducted by homogenizing the infected leaves with *TSWV* in distilled water. Some of the sap was used for inoculate a number of healthy tomato plants as a positive control. The other sap was mixed 1:1 (v/v) with the different concentrations of extract then it used to inoculate a number of healthy plants as a treatment which slightly dusted with the carborundum powder. On the other hand a number of healthy tomato plants were leaved as a negative control. Inoculated plants were rinsed thoroughly and kept for four days then the leaves tested with indirect ELISA using polyclonal antiserum supplied from Manal. Elshazly et al. (2006) for the presence of the virus after inoculated mechanically. .

4. Indirect ELISA method:-

Indirect ELISA method was carried out according to Converse and Martin (1990) as follows:-

The extracted samples of treated and control plants were ground with a homogenizer, separately at 1:10 dilution with 50µm carbonate buffer pH 9.6 and incubated overnight at 4°C. Wells were washed with PBS-tween (PBST) buffer 3 times and a blocking agent (1% egg albumin) was applied for 60 min. at room temperature. The plates were dried without washing antiserum was then added at dilutions 1/500 with antigen buffer (PBs pH 7.4) and incubated for 3h at 37 °C. The plates were washed and dried. Secondary antibody, (goat anti-rabbit antibody), conjugated to alkaline phosphatase was added to wells at dilution of 1/1000 in conjugate buffer and incubated in weels for 3h at 37 °C. The plates were washed with (PBST). P- nitrophenyl phosphate 1mg/ml dissolved in substrate buffer pH 9.8 was added. Absorbance reading at 405 nm were taken after incubation with substrate for 2h.

5. Hot pepper fruits extraction and analysis :

5.1. Preparation of ethanolic extract:

200g from the air-dried fruits powder of hot pepper were extracted with ethanol (80%) using neutral aqueous ethanol for 6 hours using soxehl

apparatus The extract was filtered through filter paper whatman No. 1. after filtration, the clear solution was evaporated under vacuum, the residue was weighted then dissolved in water and made up with distilled water to known volume, hot red pepper treated as a crude ethanolic extract (control, 50, 125 and 250 µg/ml) as total flavonoids active compounds.

5.2. Preliminary Phytochemical screening of hot pepper fruits ethanolic extract:

Preliminary phytochemical tests were carried out on the ethanolic extract of hot pepper, carbohydrates and / or glycosides were tested according to Harper(1975) method, flavonoids were tested by Geissman (1962) method, saponins were tested by Shellard (1957) method, tannins were tested by Claus (1967) method, sterols and / or triterpenes were tested by Brieskorn and Klinger-Hand (1961) method and alkaloids and / or nitrogenous bases were tested by Farnseorth *et al*. (1964) method.

6. Chemical analysis:

The ethanol extracts of tomato leaves after four days from the virus infection were used to determine total soluble, reducing and non-reducing sugars, total soluble phenols, and total flavonoids.

6.1. Determination of flavonoids:

The total flavonoids content were determined in ethanolic extract of hot pepper fruits and tomato leaves according to the aluminum chloride colorimetric method described by Chang *et al*. (2002).

6.2. Determination of Phenolic compounds:

Total phenolic contents were determined in ethanolic extract of hot pepper fruits and tomato leaves by the Folin–Ciocalteu method (Meda *et al*., 2005).

6.3. Total soluble, reducing and non-reducing sugars:

Total soluble sugars were determined in ethanolic extract of tomato leaves using the phenol-sulfuric method according to Dubois *et al*. (1956), reducing sugars were determined using dinitrosalicylic acid method (Miller, 1959) and non-reducing sugars were calculated by the difference.

6.4. Total pigments:

Chlorophyll a, chlorophyll b and carotenoids were extracted from tomato leaves after four days of inoculation according to the method of Holden (1965). The concentration of chlorophyll a, b, total chlorophyll and carotenoids were calculated by means of wettstein's formula (Wettstian, 1957).

6.5.Antioxidant enzymes determination:

6.5.1. Enzymes extraction:-

The leaves collected after 1,2,3, and 4 days from infection (3 : 1 buffer volume: fresh weight) was homogenized in a pastel and mortar with 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 3mM DL-dithiothreitol and 5% (W/V) insoluble polyvynilpyrolidone. The homogenate was centrifuged at 10,000 xg for 30 min and supernatant kept stored in separate aliquots at - 40°C, to determinate catalase, peroxidase and

polyphenol oxidase activity according to the method reported by Vitoria et al. (2001).

6.5.2. Soluble Protein Determination:

Soluble protein was estimated by using the Coomassie Brillent Blue G-250 according to Bradford (1976) method with bovine serum albumin as standard.

6.5.3. Determination of Catalase specific activity:

Catalase was assayed in leaves extracts by measuring the decrease in absorbance due to disappearance of H_2O_2at 240 nm according to Chance and Maehly (1955) method.

6.5.4. Determination of peroxidase activity:

Peroxidase activity was assayed in leaves extracts by photochemical method as described by Amako *et al*. (1994).

6.5.5. Determination of polyphenol oxidase activity:

Polyphenol oxidase activity was assayed by using photochemical method as described by Coseteng and Lee (1987).

7. Statistical analysis:

Statistical analyses have done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

RESULTS AND DISCUSSION

1. Effect of elimination of *TSWV* in tomato cultivars:-

The obtained results from table (1) showed that, the most susceptible cultivar to *TSWV* infection compared with the other cultivars (castle rock, super merman, UC97 and strain B) was peto 86 cultivar when tested by indirect ELISA using polyclonal antiserum, which realized the highest O.D.(0.80) compared with the negative control (0.32). so that, the most adequate cultivar for continuing the anti-viral investigation studies was the Peto 86.

Table (1): Effect of elimination of *TSWV* in tomato cultivars.

CULTIVAR	O.D.(405)nm
Negative Control	0.32
Positive Control	0.84
Castle rock	0.51
Peto 86	0.80
Super merman	0.61
UC97	0.68
Strain B	0.53

The obtained data was in accordance with Mumford *et al*. 1996) who found that, various variables affecting symptom expression include the cultivar, age, nutritional and environmental conditions of the plant, and differences between different isolates of *TSWV* on the same hosts**.**

2. Preliminary Photochemical screening and chemical analysis of hot pepper ethanolic extract:

Data shown in table (2) indicate that, the phytochemical analysis of ethanolic extract from hot pepper fruits revealed the presence of carbohydrates and/or glycosides, flavonoids, saponins, tannins, sterols and/or triterpenes and phenolic compounds. Alkaloids was absent in the extract.The highly contents of phenolic compounds and total flavonoids reflect the highly biological activity of this extract containing various anti-viral natural products.

Table (2): Preliminary Photochemical screening of hot pepper ethanolic extract.

Test	Ethanolic extract
Carbohydrate and/or glycosides	+++
Flavonoids	++
Saponins	+
Tannins	++
Sterols and/or triterpens	+
Alkaloids	-
Phenolic compounds	+++

3. The total soluble phenols , and flavonides content of hot pepper extract:

Data in table (3) showed that ethanolic extract contain highly contents of total flavonoids and phenolic compounds which found to be (60 and 394 mg/100g D.W) respectively. These results are relatively similar to those obtained by (Lin and Tang, 2007) they found that, the flavonoids and phenolic compounds were found to be (10.180 mg/100g F.W) respectively in hot pepper fruits.

Table (3): Total flavonides and phenolic compounds contents (mg /100g D.W) in hot pepper ethanolic extract.

component	Total flavonoids	Total phenolic compounds
Hot pepper ethanolic extract	60	394

4. Effect of treatments with hot pepper fruits extract on elimination of *TSWV* in tomato plants:-

Hot pepper fruits extract at three concentrations (50, 125 and 250 µg/ml) were tested for their ability to inhibit *TSWV* multiplication and spread of virus infection in systemically infected tomato plants. Results demonstrated in Table (4) reveal that all these concentration induced resistance to virus infection when applied to the plants as a mixed inoculum from extract and *TSWV*. 250 µg/ml was the most effective from all the three concentrations as the percentage of *TSWV* infection which was decreased by 59.74%.

Table (4): Effect of various concentrations of hot pepper extract on *TSWV* elimination in tomato plants Peto 86 cultivar.

Treatments with ethanolic extract of hot pepper	% Reduction	% Infection
50 µg/ml	28.14±0.62	71.86±0.62
125 µg/ml	38.2±0.57	61.80±0.57
250 µg/ml	59.74±0.89	40.26±0.89
Infected plants	0	100

* Each value represents the mean ± SE

The reduction of infection with *TSWV* may be due to the treatments with hot pepper extract contain several flavonoids and related compounds which have anti viral activity by weakening interactions between coat protein sub-units of the virus leading to increased susceptibility to host RNAses (French *et al*.,1991).

In this respect Malhotra *et al*. (1996) showed that several flavonoids and related compounds have antiviral activity against *TomRSV* such as quercetin, quercetin 3,7,4'-trimethyl ether, quercetin 7,4'-dimethyl ether and fisetin 4'-methyl ether. These compounds showed strong anti-viral activity causing 67 to 76 % inhibition of *TomRSV* infection. Also quercetin does not inhibit viral replication from viral RNA but may be inhibition of virus movement.

5. Effect of treatments with hot pepper fruits extract on photosynthetic pigments of tomato leaves:-

Effect of hot pepper extract with different concentrations on the photosynthetic pigments of tomato leaves when extract was applied together with *TSWV* in mixed inoculums. Table (5) showed that healthy plants (negative control) recorded the highest content of chlorophyll a, b and carotenoids (0.748, 0.531and 0.396 mg/g F.W, respectively) while the lowest values were found in plants infected with virus (positive control) (0.535, 0.331and 0.252 mg/g F.W, respectively).

Table (5): Effect of various concentrations of hot pepper ethanolic extract on chlorophylls and carotenoids contents as mg/g F.W in tomato treated leaves.

Treatments with hot pepper ethanolic extract	Chl (a)	Chl (b)	Total Chl	Carotenoids
50 µg/ml	0.581±0.035ab	0.371±0.016b	0.952±0.062e	0.352±0.026a
125 µg/ml	0.599±0.010ab	0.379±0.024b	0.978±0.048d	0.381±0.017a
250 µg/ml	0.610±0.047ab	0.392±0.016b	1.002±0.069c	0.383±0.013a
Healthy	0.748 ± 0.054a	0.531±0.023a	1.279±0.069a	0.396±0.018a
infected	0.535 ± 0.012b	0.331±0.019b	0.866± 0.036g	0.252± 0.017c
LSD	0.1229	0.0869	0.0165	0.033

* Each value represents the mean ± SE

A significant increasing was noticed in chlorophylls and carotenoids contents with different treatments from hot pepper extract. The levels of chlorophyll a, b and carotenoids at the concentration 250 µg/ml reached (0.610, 0.392 and 0.383 mg/g F.W) respectively compared to positive control. These data showed that chlorophylls and carotenoids gradually increased according to the increase of antiviral compound concentration. These changes in chlorophylls and carotenoids content may be due to virus infection frequently involves yellow mosaic mottling or generalized yellowing of the leaves. Such changes are obviously due to loss of the chlorophylls giving the yellowish coloration due to carotene and xanthophylls, but the latter pigments are also decreased in some diseases (Naidu *et al*. 1986). These changes are accur because many viruses appear to multiply and accumulate in other parts of the cell, generally, the increase of chlorophylls and carotenoids content after treatments with hot pepper extract may be due to the high phenolic and flavonoids compounds may be delayed systemic symptoms development by *TSWV* and suppressed virus multiplication or induced resistance to infection by interfere with an early event in the virus life cycle (Kual *et al*. 1985).

6. Influence of treatments with hot pepper extract on total soluble, reducing and non-reducing sugars percentage of tomato leaves:-

Regarding to the data of treatments with hot pepper extract in Table (6), it could be noticed that total soluble, reducing sugars percentage were increased after infection with virus and reached 5.56 mg/g F.W compared to healthy plants (5.36 mg/g FW). While the non-reducing sugar percentage was decreased in infected plants and reached 3.08 mg/g F.W compared to control (3.10 mg/g F.W).

Table (6): Effect of various concentrations of hot pepper ethanolic extract on reducing, non-reducing and total soluble sugars contents(mg/g FW) of tomato leaves.

Treatments with hot pepper ethanolic extract	Reducing sugars	Non reducing sugars	Total soluble sugars
50 µg/ml	2.15±0.179b	2.69±0.183b	4.84± 0.197d
125 µg/ml	2.16±0.194b	3.10±0.201a	5.26± 0.312c
250 µg/ml	2.18±0.179b	3.23±0.189a	5.41±0.213bc
Healthy	2.26±0.102b	3.10±0.132a	5.36±0.127bc
Infected	2.48±0.098a	3.08±0.167a	5.56±0.142ab
LSD	0.173	0.173	0.173

* Each value represents the mean ± SE

Values in Table (6) also indicated that the treatments with hot pepper extract decreased total soluble and reducing sugars percentage by increasing antiviral compound concentration. The lowest decrease of total soluble and reducing sugars found at the concentration 250 µg/ml from extract. These may be due to virus infection which can decrease the rate of accumulation of starch in leaves and increase the total soluble sugars content. These results are in harmony with those obtained by Tecsi *et al*. (1994) they reported that antiviral compounds inhibit virus infection and activate the photosynthesis processes while in virus infected leaves a rise in glucose, fructose and sucrose were noticed.

7. Effect of treatments with hot pepper extract on total soluble phenols and flavonides content in tomato leaves:-

From the results in Table (7) it could be noticed that infection with virus and treatments with antiviral compounds increased the content of phenols and flavonides compared with healthy plants. Thes resukts were agreed with Shirasu *et al*. (1997)they showed that hot peppr has reported to potentiat the expression of (PAL) and other defense – related genes allowing higher levels of expression in response to infection with virus.

At the same aim plants can produce antimicrobial compounds to protect themselves from biotic attack that could be essential for microbial infection resistance (Wojtaszek, 1997).

Table (7): Effect of various concentrations of hot pepper ethanolic extract on total soluble phenols , and flavonides content (mg /g F. W) of tomato leaves.

Treatments with ethanolic extract of hot pepper	Total flavonoids	total soluble phenols
50 µg/ml	0.233±0.019b	0.76± 0.038c
125 µg/ml	0.333±0.028b	1.13± 0.097b
250 µg/ml	0.552±0.025a	1.60± 0.089a
Healthy	0.081±0.006b	0.25± 0.016d
Infected	0.162±0.012b	o.74± 0.025c
LSD	0.162	0.150

* Each value represents the mean ± SE

8. Induced systemic resistance of tomato (cv. Peto 86)and specific enzymes activity:

The showed results in Figure 1, 2 and 3 indicated that, *TSWV* infection significantly stimulated the activity of catalase, peroxidase (POD) and polyphenol oxidase (PPO) enzymes compared with healthy plants. On the other hand, all treatments with hot pepper extract realized the highest stimulation of their activity compared with the infected plants. These may be due to increased specific defense gene expression or one possibility is that the initial signal from the inoculated leaf leads to the activation of an enzyme or group of defense enzymes downstream (Smith – Backer *et al*., 1998).

8.1. Specific activities of catalase in tomato leaves which treated with hot pepper fruits extract:

The obtained data (illustrated in fig.1) revealed that the catalase activity significantly was stimulated under viral infection stress. On the other hand, all treatments positively affected catalase activity against viral infection.

The catalase activity was stimulated to reach the highest activity by the third day of all treatments then inhibited to get its lowest activity levels by the forth day .The highest catalase activity (4.79 unite) have been recorded with 250 µg/ml of hot pepper ethanolic extract concentration compared with healthy plants (1.49 unite).

The stimulated catalase activity in response to anti viral natural products reflect the induction effect of these extracts on the transcription gene levels of anti oxidant defense catalase enzymes against induced highly levels of hydrogen peroxide signaling molecules during viral infection. These data are in accordance with Jeffrey (2002) who noted that H_2O_2 have been

implicated in plant responses to stress. Catalases and peroxidases are the primary enzymatic detoxifiers of H_2O_2 in most plant tissues under stress conditions

Fig. (1): Effect of various concentrations of hot pepper ethanolic extract (50,125 and 250 µg/ml) on the Specific catalase activities in tomato leaves:

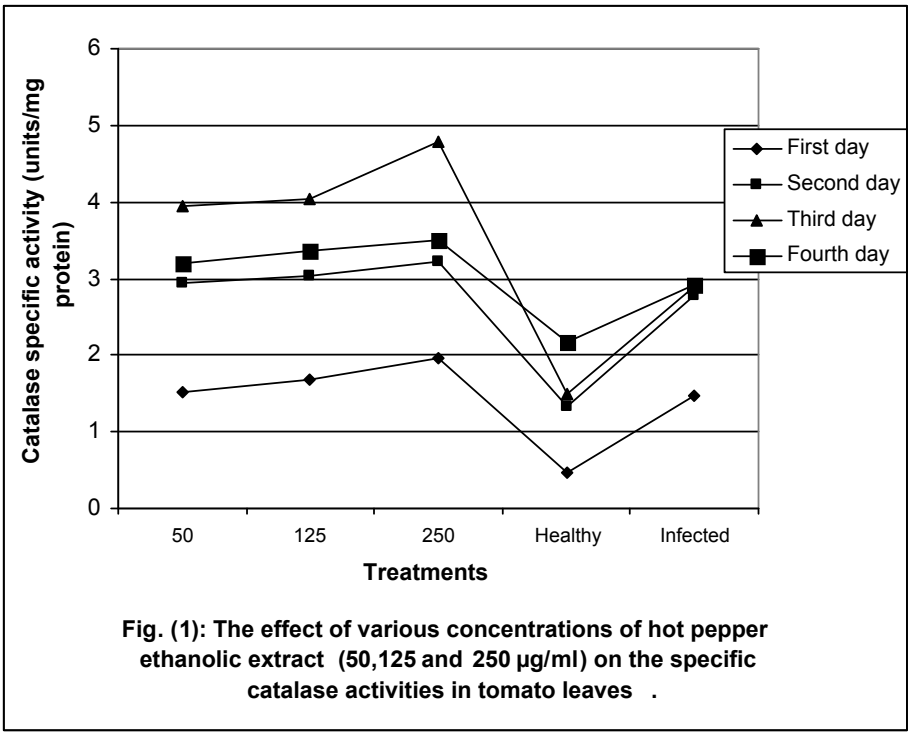

Fig. (1): The effect of various concentrations of hot pepper ethanolic extract (50,125 and 250 µg/ml) on the specific catalase activities in tomato leaves .

8.2. Specific activities of peroxidase in tomato leaves which treated with hot pepper extract:

The obtained data illustrated in figure (2) revealed that, the peroxidase activity significantly was stimulated under viral infection stress compared with healthy plants, on the other hand, all treatments significantly increased the peroxidase activity against viral infection of this study. The peroxidase activity stimulated to reach the highest activity by the third day of all treatments, then inhibited by the fourth day. the highest peroxidase activity have been realized with hot pepper extract (250 µg/ml) to realize 3.85 unite activity compared with healthy plants (1.29).

The induced peroxidase activity in response to various hot pepper extract concentrations reflect the induction effect of these extracts on the transcription gene levels of anti oxidant defense peroxidase enzymes against induced highly levels of peroxides molecules accumulated during highly oxidative burst under viral infection.

The obtained data was in accordance with Renuka Devi *et al*. (2004) they noted that, antiviral principals against *TSWV* infection revealed that they induced defense mechanisms in plants challenged with *TSWV* and accumulation of peroxidase was observed from frist day after challenge inoculation with *TSWV* on cowpea.

Also Jeffrey (2002) found that H_2O_2 have been implicated in plant responses to stress. Catalases (CAT) and peroxidases are the primary enzymatic detoxifiers of H_2O_2 in most plant tissues under stress conditions.

Fig. (2): Effect of various concentrations of hot pepper ethanolic extract (50,125 and 250 µg/ml)on the specific activities of peroxidase in tomato leaves:

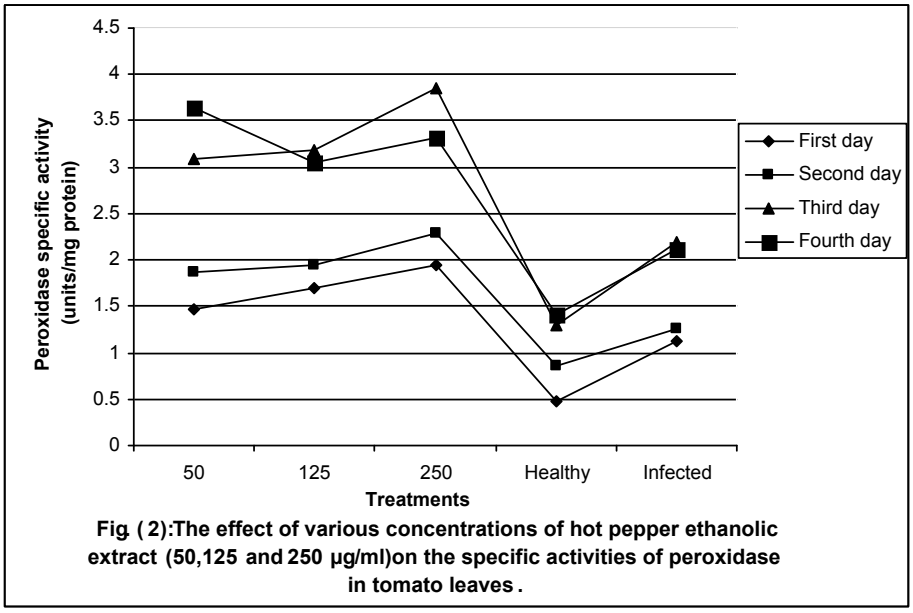

Fig. (2):The effect of various concentrations of hot pepper ethanolic extract (50,125 and 250 µg/ml)on the specific activities of peroxidase in tomato leaves .

8.3. Specific activities of polyphenol oxidase in tomato leaves which treated with hot pepper extract:

Data illustrated in figure (3) revealed that, the polyphenol oxidase activity significantly was stimulated under viral infection stress compared with healthy plants , on the other hand, the polyphenol oxidase activity was stimulated to reach the highest activity by the third day of all treatments then inhibited. The highest peroxidase activity (294.6 unite) have been reported with hot pepper ethanolic extract 250 µg/ml concentration.

Fig. (3): Effect of various concentrations of hot pepper ethanolic extract (50,125 and 250 µg/ml)on the specific activites of polyphenol oxidase in tomato leaves which treated with hot pepper extract:

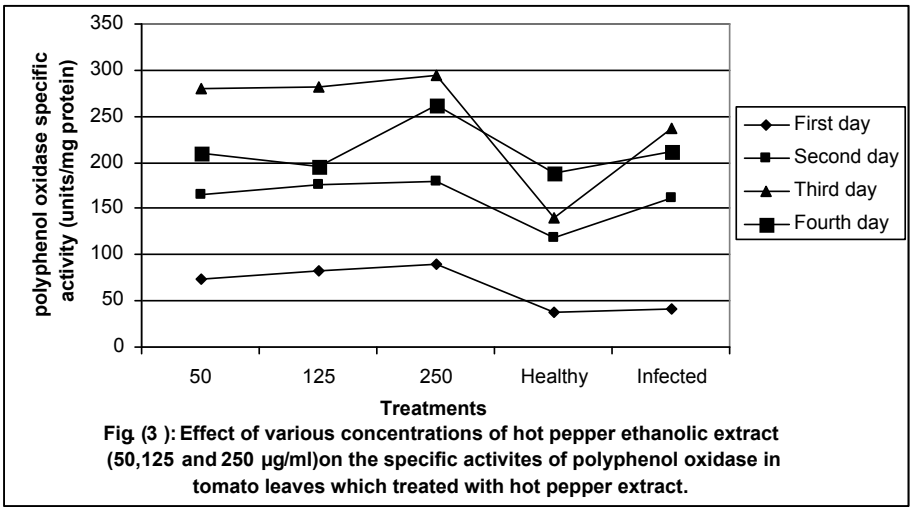

Fig. (3): Effect of various concentrations of hot pepper ethanolic extract (50,125 and 250 µg/ml)on the specific activites of polyphenol oxidase in tomato leaves which treated with hot pepper extract.

When plants are attacked by pathogens they respond by activating a variety of defence mechanisms, including the rapid production and accumulation of reactive oxygen species (ROS) is thought to be an early event that can fundamentally influence the balance of the intraction between the plant and the pathogen (Bolwell, 2004 and Gayoso *et al*., 2004). In sequence ROS are predominantly represented by superoxide anion, hydrogen peroxide(H_2O_2), hydroxyl radical and single oxygen. Plants possess a complex array of enzymatic and non-enzymatic antioxidants that can protect cells from oxidative damage by scavenging ROS. Plant enzymatic defenses include antioxidant enzymes such as peroxidase, superoxide dismutase and catalase which together with the other enzymes of the ascorbate-glutathione cycle, promote the scavenging of ROS (Hernandez *et al*., 2001).

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و ب ذ ل ي ر و ر ي ف ب ى و د ع ل د ا ض م ك ر ا ح ل ف ل ف ل ا ر ا م ث ل ا و ن ا ث ي إ ل ا ط ل خ ت س م ل ا ي ا ث أ ت
م ط ا م ط ل ى ف ط ق ن م ل ا

ف س و ي ب ا ص ا ي ن ا ر ، د م ح ت ؤ ص ن ق ق م ا م ى ل ص ي ب ق د م ح م ي ه ا ر ب إ د م ح م ، د ش ا ر ى د ج م د م ح م
ة ر ه ا ق ل ا ة ع م ا ج – ة ع ا ر ز ل ة ا ي ل ك ق م ي و و ي ح ل و ا ي م ي ك ل م س ق

ع ت ي ن ا ث ا ق ب ت ر م ل ا ل ا ث م ي ث ي ع ل ا ع ل ا ف ى ل ا ع ل ا ص ا ت ق إ ل ا ي د ا ص ت ق ق م ا ط ل ا ل و ص ح د ع ي
ن ذ ل ط ق ن م ل ا ل و ب ذ ل ي ر و ر ي ف ا م م أ ن ي ه ت ا ل ا س ر و ر ي ف ل ا ن ي د ع ل ا ب ب ا ص ي ر ض خ ل ا ي ص ا ح ي ب س ط ا ط ب ل ا
ن ا ك ل ذ ا ق ب ا ص م ل ا م ث ي ا م ق ل ي و س ت ل ا ي ذ ا غ ق ل ط ا ف خ ن إ ع م 50% ى ل ل ا ص ي ل و ص ح م ل ى ف ص ق ن ب س ي
ي م ي ك ل ا و ل ا ب ق م ا ع م ل ا ا م م أ ن ي ر و ر ي ف ل ا ا ذ ه ب ا ص إ ل ا ن ي ج ت ا ن ل ا ر ا ر ض ل ل ل ق ت ل ي س و د ا ج ي إ ن م ب ا ل
ا ه ب ق م ا ع م ل ا ت ا ب ن ل ى ا ل ع ء ي س ي ث أ ت ا م ل ن أ ل ق م ا ع م ل ا ل ي ن ل ق ل ا ر و ر ي ف ل ا ش ت ن أ ل ن ي ف ض ف ت ع ا ض ت ل ا ق د ا ض م ل ا
ا م ن ث ا ي ت خ إ ت ق ل ا ر و ر ي ف ل ا ا ذ ه ق ا و ق ه م ا د خ ت س ا ت ا ب ر ق ي ع ف ه م ا ط ل ا ض ل خ ت س م ل ض ع ب ا ي ت خ إ ت ا ذ ل