Biological, serological and inhibition activity of *Tomato spotted wilt virus* isolated from tomato plants in Taif region, Saudi Arabia

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ABSTRACT

Tomato spotted wilt virus (TSWV) has been isolated from naturally infected tomato plants collected from Taif governorate, Saudia Arabia for the first time. Observed symptoms circumvented mosaic, curling, bronzing and/or purpling, chlorosis and necrotic spot on the leaves. Disease symptoms of infected fruits produced from inoculated healthy tomato seedling showing discoloration, faint concentric rings, necrotic and chlorotic spot on mature tomato fruits. The virus was biologically purified from a single local lesion formed on *Chenopodium amaranticolor* Caste & Reyn. The isolated virus was identified on the basis of symptomatology, transmissibility, host range and serological tests by indirect –enzyme linked immunosorbent assay (Indirect- ELISA) and dot blotting immunobinding assay (DBIA) using an induced antiserum for TSWV. All the five tested tomato cultivars were found to be susceptible when mechanical inoculated under greenhouse conditions. Wide variations of symptoms were found between cultivars. Super strain and super merman were found to be more susceptible than any other cultivar tested which showing 90% infection. Basil essential oil and ethanolic extract of *Plantago major* leaves was used as antiviral to reduce the infection with TSWV at three concentrations levels (500,1000 and 2000 µg/ml and 50,125,250 µg/ml, respectively).Viral infection was reduced by using the highest concentration of Basil essential oil and ethanolic extract reached to 45.99% and 63.82%, respectively. Different treatments with antiviral compounds decreased the content of total soluble sugars, titrable acidity also increased the lycopene and ascorbic acid in tomato fruits compared with infected plants.

Key words:
Tomato (*Lycopersicum esculentum* Mill), *Tomato spotted wilt virus* (TSWV), Tomato cultivars, Inhibation; Basil (*Ocimum basilicum* L.), Essential oil, *Plantago major* leaves

INTRODUCTION:

Tomato (*Lycopersicum esculentum*, Mill) is economically the most important vegetable crop worldwide . It is a major vegetable crop that has achieve tremendous popularity over the last century. It is grown in practically every country of the world, in outdoor fields and greenhouses. The tomato plant is very versatile and the crop can be divided into two categories; fresh market tomatoes, which we are concerned with and processing tomatoes, which are grown only outdoors for the canning industry and mechanically harvested. In both cases, world production and consumption has grown quite rapidly over the past 25 years (Nehemet and Miray, 2012).

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The "spotted wilt" disease of tomato was first described in Australia in 1919 (Brittlebank, 1919). *Tomato spotted wilt virus* (TSWV), the causl agent of spotted wilt disease is the type member of the genus *Tospovirus* in the family *Bunyaviridae* (Moyer1999 and Sherwood, *et.al* .2000) . TSWV has a very broad host range infecting over 1000 species of plants in more than 82 families including both monocots and dicots(Prins and Kormelink,2000). Symptoms induced by TSWV on tomato plants, appear as black and brown necrotic spots on leaves , vessel browning , stem necrosis , mosaic and leaf mottling , yellow spot , discolouration on ripe fruits and subsequent wilting (German *et al*., 1992 and Mumford *et al.*, 1996). The loss of marketable tomato yield due to TSWV epidemics accounted for millions of dollars and reduced the tomato production by 50-90% (Cho *et al*., 1987).Resistant cultivar is considered as the most important tool for management of TSWV. Continuous deployment of resistant cultivars is necessary as the virus evolves and breakdowns resistance rapidly(Cho *et al.*,1996; Moury *et al*.,1997 and Hoffmann *et al*.2001).

Basil (*Ocimum basilicum* L.) contain bioactive phytochemical constituents like alkaloids ,saponins, essential oil and three constituents are responsible for antimicrobial and antioxidant activites of basil (Hanif *et al*., 2011). *Plantago majo*r contain active compounds such as polysaccarides, lipids, flavonoides, alkaloids and some organic acids that involved in the healing activity , antimicrobial and anti-inflammatory (Jamilah *et al.*, 2012). Thus the aim of this study is to (1) Study the biological and serological aspects of *Tomato spotted wilt virus* (TSWV) (2) Investigate the antiviral activity of plantage leaves ethanolic extract and basil essential oil against *Tomato spotted wilt virus* (TSWV) infection of tomato plants under field conditions.(3) Find out some biochemical compounds changes in tomato fruits during the viral infection and treatment with antiviral compounds to evaluate the effective treatments for inhibiting the virus infection.

MATERIALS AND METHODS

Part 1: Biological studies

1.1. *Virus source and symptoms.*

Samples form naturally infected tomato (*lycopersicum esculentum*, Mill) plants with suspected *Tomato spotted wilt* symptoms showing curling bronzing and /or purpling, chlorosis (yellowing) and necrotic spots on the leaves were collected form Taif Governorate, Saudi Arabia. Infected plants were transferred and grown in 25 plastic pots filled with natural soil under greenhouse conditions. Upon recovery of plants, they were used for isolation, Identification and serologic testing.

1.2. Virus isolation and propagation

Naturally infected tomato plants used as a source of TSWV were mechanically transferred onto tomato plants cv. Castle rock grown in the greenhouse. The virus was purified biologically through three consecutive passage onto the local lesion host *Chenopdium amaranticolor* Coste & Reyn plants using phosphate buffer, pH 7(Kuhn,1964),then transmitted mechanically to *Gomphrena globosa* L. which used as a source for virus propagation. On the other hand, the isolate understudy was serologically identified using antisera specific to some tomato viruses such as *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY) and TSWV which induced previously by Virus and Phytoplasma Res. Dept. for routine diagnostic work using indirect ELSA method (Converse and Martin, 1990). Tomato plants reacted positively only against TSWV antiserum were used as source of virus inoculum throughout this study.

1.3. Virus transmission test:

1.3.1. Mechanical transmission:

Seedling at the 2-3 leaf stage of healthy test plants, tomato (*lycopersicum esculentum*, Mill) varieties and other host plants used for host range, varietal susceptibilitiy studies were tested by mechanical transmission. The Inoculated plants were kept under observation in insect proof greenhouse for 30 days. Plants were periodically sprayed with insecticides to prevent contamination with any viral infection.

1.4. Host range studies.

Thirteen plant species belonging to sex different families, Amaranthaceae, Chenopodiacea, Cucurbitaceae, Compositae, Fabaceae and Solanaceae were mechanically inoculated with TSWV infected sap. These tested plants were as follow: *Gomphrena globosa, Chenopodium amaranticolor, Cucumis stivus, Cucumis pubescens, Cucurbita pepo, Cucumis melo, Lactuca sativa, ,Phaseolus vulgaris. Vicia faba, Vigna unguiculata, Capsicum annum, Solanum tuberosum and Solanum melongena*. Tested plants were maintained for 30 days in the greenhouse for symptoms development.An equal number of healthy seedling of the same age and species were left without inoculation as a control. Some of the inoculated plants were serologically tested using indirect ELISA methods.

1.5. Cultivar susceptibilities.

Five commercial different Tomato CVs.. namely, Radisson, peto86, Castel Rock , Supr Strain, super merman, were sown in20 cm diameter pots in the greenhouse using ten seeds /tested cv. The emerging seedlings were mechanically inoculated with TSWV. An equal number of healthy seedlings of the same cultivars and age were left without inoculation as controls. Symptoms and percentage of transmission were observed and recorded for two weeks then tasted using indirect-ELISA method.

Part 2: Serological studies:

2.1. Indirect enzyme – linked immuno sorbant Assay (ELISA) method.

Antiserum against TSWV (El-shazly *et al*, 2006) was used for detection of the virus isolate, using indirect ELISA method was similar to that described by Converse and Martin(1990). Reading greater than twice the value of healthy control was considered positive.

2.2. Dot blotting immunobinding assays (DBIA) on nitrocellulose membrane:

The dot-blot immunoassay procedures with nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) substrates were used as described by Abdel Salam (1999).

Field experiment

Complete plots design with three replicates was used for two consecutive years during September 2012/2013 and 2013/2014. Seeds of tomato plants (the highest susceptible cv Super strain) were seeded in plastic trays in the greenhouse and transferred when they had four to five leaves under field condition and for simultaneous inoculation. Seedling sown in eight plots, plot/ treatment , each one containing three lines, (Ten plants/ line). Treatments used were three concentrations from each Basil essential oil(500, 1000, 2000 µg/ml) and ethanolic extract of *Plantago majo*(50,125,250 µg/ml). 1 ml of essential oil or extract / concentrations was added to 1ml of the infected sap in a mortar and mixed thoroughly.Plants were inoculated with the mixture by gently rubbing the primary leaves with carborundum. The plants in the seventh plot were inoculated only with TSWV as a positive control, whereas the plants in the eighth plot were inoculated only with distilled water as a negative control. Positive and negative control were used to study the comparison between different treatments. Ten days after inoculation, nine to fifteen samples from inoculated tomato cultivar were taken at random. Two other samples were taken from each positive and negative control. All collected samples were examined with indirect ELISA to calculate the inhibition percentage of virus infectivity.

Part 3: Biochemical studies.

1-Sources of samples

Tomato seeds (*lycopersicon esculentum* Mill) c.v (Radisson, peto86, Castel Rock , SuprStrain,super merman)were obtained from sun seeds company, Cairo, Egypt. Basil (*ocimum basilicum* L.)and (*plantago major)* plants were collected from Taif area, Saudi Arabia. Required plant parts were dusted off and left to dry indoors. Air dried parts were ground in an electrical mill to obtain fine powder.

2-Extraction of essential oil from Basil Leaves.

The essential oil of air dried aerial parts of *ocimum basilicum* was

obtained by hydrodistilation for 3h, according to Guenther (1961).The distillate volatile oil was isolated and dried over anhydrous sodium sulfate. The oil was stored at 4˚C until analysis by GC-MS.

2.1. GC-MS analysis of the essential oil.

Gas-Chromatography-Mass Spectroscopy was used for identification of components of essential oil according to Adams (1995). Analytical GC/MS was carried out on a HP spectroscopy 6890 series with HP selective detector 5973, under the control of a HP chemstation version A02.12 data system. A carbowax capillary column, 50m × 0.53mm I.D., 1.5 m thickness (HP company, U.S.A) was used with helium as carrier gas (flow rate 1.5ml/min). Sample was injected using the split sampling technique, ratio 1: 50 with sample amount 1µl. Injection port temperature 280^{o}C. Column temperature was held at 40^{o}C for 5min and then programmed at 3^{o}C/min to 280^{o}C and held these for 20min. Detector temperature: 300^{o}C. Mass spectroscopy operating parameters were: electron ionization at 70 eV, accelerating voltage 10kV and scan M/z range 30-650. The identification of constituents was carried out by comparing retention time with those of authentic reference compounds, or peak-matching library research using the standard mass library (NIST Standard Mass Library).

3-Preparation of ethanolic extract from *plantago major* leaves:

Ground sample(200g) was extracted with ethanol 80% for 6 hours using soxhlet apprartus,the extract was filtered through whatman NO.1.filter paper-Ethanol was evaporated from the supernatant in a rotary evaporator at 200m par pressure and 35˚c till dryness.The residue was weighted then dissolved in water and made up with distilled water to known volume and stored at -7˚C till usage (Marby *et al*., 1970).

3.1. Determination of active compounds in ethanolic extract of *P.major* leaves.

3.1.1. Determination of flavonoids

The total flavonoids content was determined according to the aluminum chloride colorimetric method of Chang *et al.* (2002)

3.1.2. Determination of total phenolic compounds

Total phenolic contents were determined using the Folin- Ciocalteu method (Meda *et al*.,2005) .

3.1.3 . Determination of tannin content

Quantitative estimation of tannin of the leaves sample was carried out as catechin equivalents using the Vanillin-HCl/methanol(Price *et al*.,1978).

4. Treatments with antiviral compounds

Basil was treated as essential oil emulsion in tween 80 at (500,1000,2000µg/ml)but *plantago*

mojor leaves ethanoil extract was treated as total flavonoids active compounds at (50,125 and 250µg/ml) as Quercetin from the ethanolic extract. The tested concentrations were replicated three times.

5- Chemical analysis of tomato fruit:

5-1. preparation of ethanolic extract.

A Known weight of tomato fruits (30g) were blinded and mixed well with ethanol (80%) then was kept over night. The extract was filtered through Whatman No.1.filter paper. Ethanol was evaporated from the supernatant in a rotary evaporator at 200 m per pressure and 35˚C till dryness. The residue was dissolved in water and made up with distilled water to known volume and stored at -7˚C till usage.

5- 2. Determination of carbohydrates fractions:

5-2-1.Determination of total soluble sugars

Total soluble sugars content was determined in ethanol extract of fruits by the phenol-sulphuric acid method as described by Dubois *et al*. (1956).

5-2-2.Determination of reducing sugars.

Reducing sugars were determined in the ethanol extract,using dinitrosalicylic acid (DNSA) method according to Miller (1959).

5-2-3-Calculation of non-reducing sugars:

Non-reducing sugars were calculated by difference between the total soluble sugars and the reducing sugars.

5-2-4-Determination of lycopene content

Lycopene content was determined in all treatments according to Beerh and Siddappa (1959) .

5-2-5 -Determination of ascorbic acid content:

Ascorbic acid was determined in all treatments according to method of loeffler and Ponting (1942)

5-2-6.Determination of titrable acidity

Acidity was determined in all treatments according to A.O.A.C.(1990)

RESULTS

Part 1: Biological Studies.

1-1. Virus Source and symptom

The virus used in this study was isolated from tomato (*lycopersicum esculentum*,Mill) plants collected from Taif Governorate, Saudi Arabia . Infected leaves with TSWV showed symptoms of curling, bronzing and /or purpling, chlorosis, and necrotic spots on the leaves (Fig.1).

1.2. Virus isolation and propagation

The isolated virus from infected tomato plant produced systemic necrotic spot symptoms on the leaves followed by curling,yellowing,mosaic and discoloration of the veins in brown on tomato plants CV. Castle rock grown in the greenhouse (Fig.2).

Symptoms appeared three to five days post inoculation. Disease symptoms of infected fruit produced from inoculated healty tomato seedlings with TSWV in experimental farm showing chlorotic and necrotic spot on mature tomato fruits (Fig.2).

1.3. Virus transmission test:

1.3.1. Mechanical transmission:

The virus isolate was easily transmitted mechanically from infected tomato (*lycopersicum esculentum* Mill) plants to healthy greenhouse grown tomato seedlings at the 2-3 leaf stage. Necrotic local lesion appeared three to five days post inoculation. Inoculated plants showed systemic necrotic spot symptoms on the leaves followed by curling, yellowing, dark brown streak on leaf petioles and growing tips (Fig.2).

1.4. Host range studies

Result in Table (1) and (Fig.3) indicate that TSWV had a wide host range. There were thirteen differential hosts belonging to six different families susceptible to TSWV infection. The induced systemic symptoms on the tested hosts ranged between mosaic,chlorotic and necrotic local lesion ,necrotic spot and yellowing (Fig.3). Systemic symptoms appeared after 12-15 days post inoculation (Table1).

1-5 Cultivar susceptibilities

Result in Table (2) indicated that all the five tomato cvs under greenhouse conditions found susceptible to the virus under study.The percentages of infection in different tomato cvs.ranged from 60% for cultivar Peto86 to 90% for Superstrain and Super merman cvs. Observed different levels of resistance and severity of symptoms caused by TSWV on tomato plants varied according to tomato cultivars showed in Table (2). The highest susceptible cv. Super strain was used in this investigation to study the efficiency of basil essential oil and *P.major* ethanolic extract on reducing the infection of TSWV in tomato plants under field conditions and effect of treatments with different concentrations on nutritional components in tomato fruit

Part 2: Serological studies

The virus under study was detected by indirect ELISA; positive reaction was obtained only with TSWV antiserum. Thus, providing further evidence that the virus under study is indeed TSWV . Indirect ELISA was used to confirm the presence of TSWV in the field, greenhouse after mechanical inoculation and to study the effect of various concentration of basil essential oil and P.major ethanolic extract on TSWV infection in experimental tomato plant c.v Super strain Table(4) . On the other hand, the technique of dot –blot on nitrocellulose membrane could be readily applied for detection of TSWV in infected tomato tissues (Fig.4).

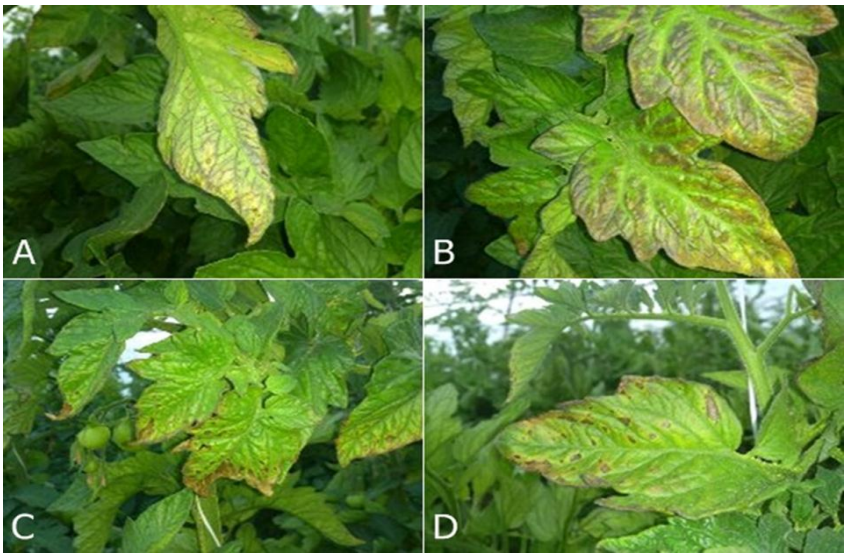

Fig. (1): Symptoms of an infected tomato plant with TSWV in a commercial tomato field in Taif Governorate. 1-A, B): Tomato leaves showing curling, bronzing and /or purpling. (1-C, D): TSWV symptoms showing mosaic and necrotic spot on tomato leaves.

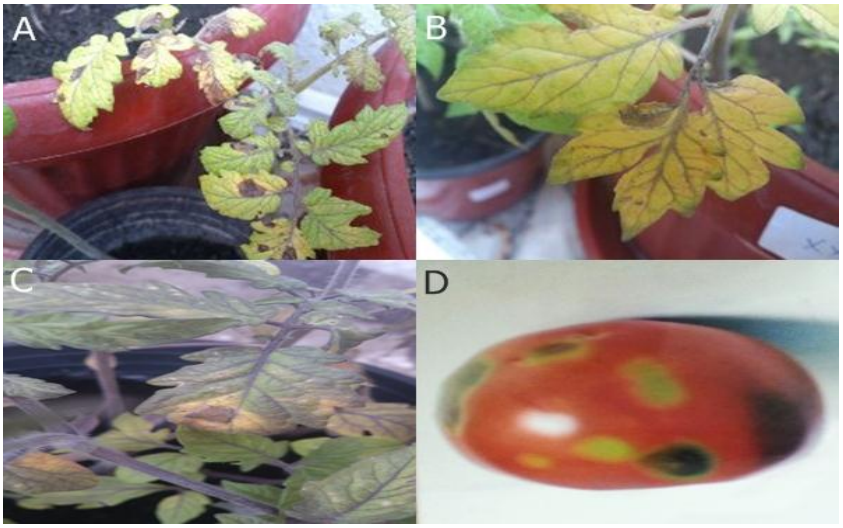

Fig. (2): TSWV developed symptoms after mechanical inoculation of tomato plants. A, systemic necrotic spot symptoms on the leaves followed by curling. B, yellowing and discoloration of the veins in brown. C, symptoms of curling, bronzing and/or purpling and necrotic spots on leaves. D, chlorotic and necrotic spot on mature tomato fruit.

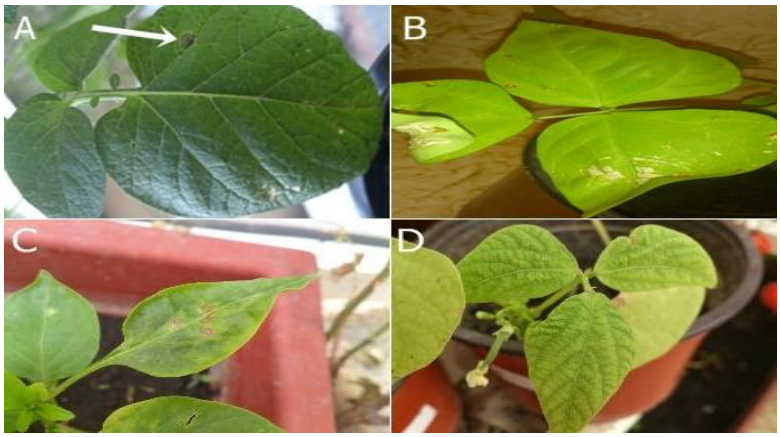

Fig. (3): Symptoms of TSWV infection on some host plants upon mechanical inoculation. A, Potato leaves showing necrotic ring spot. B, Cowpea leaves showing necrotic local lesion. C, Pepper leaves showing mosaic and necrotic spot and D, Green bean showing mosaic and green vein banding symptoms

Table (1): Host range of *Tomato spotted wilt virus* tested by mechanical inoculation.

Test plant	Common name	Days post inoculation	Observed symptoms	ELISA
Amaranthaceae				
Gomphrena globosal.	Gomphrena	4-5 days	N.L.L	+
Chenopodiaceae				
Chenopodium amaranticolor	Goose foot	3-4 days	N.L.L	+
Cucurbitaceae				
Cucumis stivus L.Beta Alfa	Cucnmber	6 days	M.N.L.L.y	+
Cucumis pubescens L.	Hairy Cucumber	4-5 days	N.L.L	+
Cucurbita pepo L.	Squash	5-6 days	M-Y	+
Cucumis meloL.	Melon	4-5 days	CL.L.L-M-N.L.L	+
Compositae				
Lactuca sativa	Lettuce	3 days	M-Y	+
Fabaceae				
Phaseolus vulgaris L. Bronco	Green bean	5-7 days	N.L.L-Vb-M	+
Vicia faba L.	Broad bean	7 days	N.L.L-M-Y	+
Vigna unguiculata	Cowpea	3-4 days	M-N.L.L	+
Solanaceae				
Capsicum annum L.	Pepper	6-7 days	N.L.L-M	+
Solanum tuberosum	Potato	3-4 days	N.RS	+
Solanum melongena	Egg plant	6-7 days	N.L.L.-M-Y	+

N.L.L=Necrotic Local Lesions, M=Mosaic, Y=Yellowing, CL.L.L=Chlorotic Local Lesion, Vb=Vein banding, N.RS= Necrotic ring spots, +=positive reaction

Table (2): Tomato cultivars susceptibilities to infection with tomato spotted wilt virus under greenhouse conditions upon mechanical inoculation.

Cultivars	Symptoms	No. of tested plants	No. of infected plants	Infection%
Radisson	N.L.L,B and/or P	10	7	70%
Peto 86	N.RS-M	10	6	60%
Castel Rock	N.RS-M	10	8	80%
Super strain	NS,B and /or P	10	9	90%
Super merman	N.L.L-M	10	9	90%

N.L.L= necrotic local lesion, B=bronzing ,P=purpling, N.RS=necrotic ring sopt, M=mosaic, NS=necrotic spot

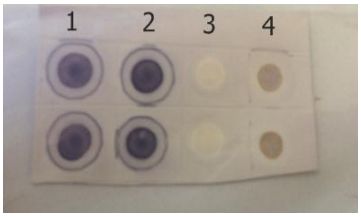

Fig. (4): Detection of TSWV by dot blot immunosorbent assay (DBIA) on nitro cellulose membrane. Lan 1: Infected tomato plant from greenhouse. Lan 2: naturally infected tomato plant from field. Lan3: TBST buffer. Lan4: Healthy tomato plant.

Part 3: Identification and determination of chemical components of the essential oil of basil by GC-MS:

The identified components from *Ocimum basilicum* essential oil and their percentages are summarized in Table (3). The yield of the essential oil (on afresh weight) was 0.19% after steam distillation. Twenty components were identified preliminary, GC/MS examination of the oil indicated that it consists (94.06%) volatile components and (5.94%) components were unknown. The main constituents in the oil were linalool (43.11%)1,8 cineol (12.55%), eugenol (8.33%), methyl cinnamate (3.66%) iso- caryophllene (3.56%), Y-Terpin (1.93%) α-Farnesene(1.81%), Naphthalene (2.44%), trans- β –.ocimene (1.70%), myrtenol (1.44%), -α-cubebene (5.03%), α-caryophyllen (1.65%) ,azulene(1.42%).five compounds were identified as traces constituents (less than 1%) such as camphene (0.92%), germacrene (0.89%) and camphor(0.75%).

3.1. Chemical analysis of *Plantago major* L leaves ethanolic extract:

Data in Table (4) show the highly contents of total flavonoids,phenolic and tannins compounds in ethanolic extract which found to be 1200,667 and 490 mg/100g DW, respectively.

Table (3): chemical constituents of the essential oil from *Ocimum basilicum.*

Compound	Rt	Percentage %
∝ −pinene	5.26	0.79
Camphene	5.61	0.92
β −pinene	6.34	1.24
β − Myrcene	6.78	0.93
1,8-cineol	7.92	12.55
Trans-β −ocimene	8.21	1.70
γ-Terpin	8.56	1.93
Linalool	11.43	43.11
Camphor	12.61	0.75
Myrtenol	12.74	1.44
∝-Cubebene	16.14	5.03
Eugenol	19.19	8.33
Methyl cinnamate	19.60	3.66
iso- caryophyllene	20.00	3.56
∝- caryophyllene	20.11	1.65
Azulene	21.88	1.42
∝-Farnesene	22.99	1.81
Germacrene	23.51	0.89
Naphthalene	24.11	2.44
Total Compound	-	94.06
Unknown compounds	24.66	5.94

Table (4): Total penolic, flavonoids and tannins compounds contents in ethanolic extract of *P.major* leaves (mg/100 g DW).

Compounds	Total phenols	Total flavonoids	Total tannins
	1200	667	490

3.2 Effect of different antiviral treatments against TSWV infection in experimental tomato plants:

Results demonstrated in Table (5),showed that all treatments induced resistance against virus infection when applied to the plants as a mixed inoculums from extract and TSWV. Various Basil essential oil concentrations positively reduced the viral infection and the highest basil concentration (2000 µg/ml) revealed the highest reduction (45.99%) .In addition the highest effective treatment against viral infection in *Plantago. major* extract ,which realized the

highest viral reduction compared with all other treatments, , was(250 µg/ml) realized (63.82%) and the lowest viral infection was (36.18%).

3.3 Chemical analysis of experimental tomato fruits.

3.3 -1 -Effect of infection with TSWV and different treatments with antiviral compounds on lycopene content.

Results in Table(6) showed that infection with virus significantly decreased the lycopene content compared with healthy plants. The highest total lycopene content have been observed with the highest concentrations of basil essential oil and *P.major* extract compared with other concentrations and reached to 6.77 and 8.26 mg/100g respectively, against 4.11mg/100g for the infected plants while the healthy plants had 6.89 mg/100g.

3.3 .2- Effect of infection with TSWV and different treatments with antiviral compounds on ascorbic acid content in experimental tomato fruits.

Results in Table (6) demonstrated that the highest total ascorbic acid content have been observed with highest concentrations of both essential oil and *P.major* extract were reached to 26.44 and 29.11 mg/100g F.W, respectively against 25.01 mg/100g F.W for the infected plants, while the healthy plants was 20.88mg/100g F.W. On the other hand , the gradually increase in the essential oil and extract administrations accompanied with a gradually increase in the fruit ascorbic acid contents

3.3.3- Effect of infection with TSWV and different treatments with antiviral compounds on titrable acidity, total soluble,reducing and nonreducing sugars in experimental tomato fruits in experimental tomato fruits.

Results in Table (7) showed that, the lowest acidity content have been observed with highest concentrations of both basil essential oil and *P.major* extract which were reached to 0.45 and 0.46% against 0.62% for the infected plants, while the healthy plants had 0.37%. On the other hand, also results in Table(7) indicated that there was an increase in total soluble sugars under viral infection which reached to (10 mg/g F.W) compared with healthy plants (9.41 mg/g F.W). While the non-reducing sugar percentage was decreased in infected plant and reached to(3.66mg/gF.W) compared with healthy plants (4.19mg/gF.W). Moreover values in Table (7) indicated that all previous treatments with essential oil and *P.major* extract decreased total soluble and reducing sugars percentage.The highest decrease found at the concentration of 2000 µg/ ml of essential oil and 50µg/ml of *P.major*, respectively which was 9.62 and9.82mg/g FW and 5.00 and 5.91mg/g FW, respectively.

Table (5): Effect of various concentrations of Basil essential oil and plantago major extract on TSWV Infection in experimental tomato plants CV.Castle rock

Treatments (µg/ml)	Reduction%	Infection%
Basil (500 µg/ml)	24.76±1.92	75.24±4.22
Basil(1000 µg/ml)	37.12±1.85	62.88±2.16
Basil (2000 µg/ml)	45.99±1.89	54.01±1.89
p.major extract (50µg/ml)	30.16±1.35	69.84±3.22
p.major extract (125µg/ml)	43.20±2.24	56.80±2.46
p.major extract (250µg/ml)	63.82±2.90	36.18±1.01
Infected (untredted)	0	100

Each value represents the mean ± SE

Table (6): Effect of various concentrations of basil essential oil and ethanolic extract on lycopene and ascorbic acid contents in experimental tomato fruits.

Treatments (µg/ml)	Lycopene (mg/100g)	Ascorbic acid(mg/100g)
Basil (500 µg/ml)	5.26±0.10	19.55±0.73
Basil (1000 µg/ml)	5.55±0.12	25.78±1.05
Basil (2000 µg/ml)	6.77±0.10	26.44±0.58
p.major (50 µg/ml)	5.40±0.13	18.99±0.74
p.major (125 µg/ml)	7.98±0.16	26.33±0.67
p.major (250 µg/ml)	8.26±0.13	29.11±0.99
Healthy plants	6.89±0.11	20.88±0.52
Infected (untreated)	4.11±0.10	25.01±0.94

Each value represents the mean ± SE

Table (7): Effect of various concentrations of Basil essential oil and *p.major* ethanolic extract on titrable acidity , reducing, non-reducing and total soluble sugar contents (mg/g F.W) in experimental tomato fruits

Treatments (µg/ml)	Acidity as citric acid (%)	Reducing sugars	Non-reducing sugars	Total soluble sugars
Basil(500 µg/ml)	0.54±0.014	6.11±0.10	3.86±0.06	9.97±0.69
Basil(1000 µg/ml)	0.56±0.007	5.09±0.18	4.99±0.16	10.08±0.31
Basil(2000 µg/ml)	0.45±0.008	5.00±0.11	4.62±0.10	9.62±0.05
p.major extract (50µg/ml)	0.52±0.009	5.91±0.19	3.91±0.12	9.82±0.44
p.major extract (125 µg/ml)	0.49±0.008	6.11±0.13	4.03±0.13	10.14±0.59
p.major extract (250µg/ml)	0.46±0.004	6.23±0.14	3.96±0.09	10.19±0.32
Healthy plants	0.37±0.002	5.22±0.10	4.19±0.83	9.41±0.33
Infected (untreated)	0.62±0.004	6.34±0.20	3.66±0.16	10.00±0.70

Each value represents the mean ± SE

DISCUSSION

The virus used in this study was isolated from tomato (*lycopersicum esculentum*, Mill) plants collected from Taif Governorate, Saudi Arabia. Infected leaves with TSWV showed symptoms of curling, bronzing and /or purpling, chlorosis and necrotic spots on the leaves. These symptoms were similar to those that were described previously for the infection of tomato by TSWV (Francki and Hatta, 1981; Anon, 1991;Gera *et al*., 2000;Sharman and Persley, 2006;El-Shazly *et al*., 2008 and Shima *et al*.,2013). The isolated virus from infected tomato plant produced systemic necrotic spot, symptoms on the leaves followed by curling,yellowing,mosaic and discoloration of the veins in brown on tomato plants cv.Castle rock grown in the greenhouse . Necrotic local lesion appeared three to five days post inoculation as described by Wang and Gonsalves(1990); Bezerra *et al*. (1999);El.Shazly *et al*.(2006,2008) and kobeasy *et al*.(2012). On the other hand, disease symptoms of infected fruit produced from inoculated healty tomato seedlings with TSWV in experimental farm showing chlorotic and necrotic spot on mature tomato fruits. Such collective symptoms have previously been described for TSWV infection on TSWV(Best,1968;Peterson *et al*.,1989;Peters *etal*.,1991;Hill and Moran,1996; Sharman and Presley, 2006;El-Shazly *et al*.,2008; Abdel-wahab and El-Shazly,2009;Nehemet and Miray,2012; and Shima *et al*.,2013). The virus isolate was easily transmitted mechanically from infected tomato *(lycopersicum esculentum* Mill) plants to healthy greenhouse grown tomato seedlings at the 2-3 leaf stage. Necrotic local lesion appeared three to five days post inculation.Inoculated plants showed systemic necrotic spot symptoms(12-15days) on the leaves followed by curling, yellowing, dark brown streak on leaf petioles and growing tips. This result agrees with similar results on a disease of tomato caused by TSWV (Peterson *et al.*, 1989; Marchoux and Gebre-Selassie, 1991; Nagata *et al*, 2002; Ullman *et al*., 2002; Medeiros *et al.*, 2004; Sharman and Parsley, 2006; EL-Shazly *et al.*, 2008, 2009 and Kobeasy *et al*., 2012). Results indicate that TSWV had a wide host range. There were thirteen hosts belonging to sex different families susceptible to TSWV. Such results with those obtained by Cho *et al*.(1987);German *et al.*(1992);Canady *et al*.(2001); Groves *et al*. (2002); Momol *et al*.(2002); Parralla *et al*. (2003); Whitfield *et al*.(2005) and Abdel- wahab and El-Shazly (2009)

All the five tomato cvs under greenhouse conditions found susceptible to the virus under study. The percentages of infection in different tomato cvs.ranged from 60% for cultvar Peto86 to 90% for cultvar Superstrain and Super merman. Observed different levels of resistance to the disease caused by this virus are in agree with previously studies by (Boiteux *et al.*(1993) and Moury *et al*. (1997) . The virus under study was identified by indirect ELISA to confirm the presence of TSWV in the field and greenhouse

after mechanical inoculation , to study the host range and the effect of various concentration of basil essential oil and *P.major* ethanolic extract on TSWV infection.On the other hand, the technique of dot –blot on nitrocellulose membrane could be readily applied for detection of TSWV in infected tomato tissues, positive reaction was obtained between infected tissues and TSWV antiserum as strong purple coulor appeared. Such results are confirmed by several authors appling serological tests for TSWV identification(Peters *et al.*, 1990;Marchoux and Gebre-Salassie,1991; Hill and Moran,1996; Mc Michael *et al., 2*002; EL Shazly *et al.*, 2006, 2008, 2009).

The yield of the essential oil(on afresh weight) was 0.19% after steam distillation, these results are agree with previously study of Hanif *et al*.(2011), they found that essential oil produced from the original plant of omani basil was 0.171%. The constituents of twenty components were identified preliminary, GC/MS examination of the oil indicated that it consists of (94.06%) volatile components and (5.94%) components were unknown. The main constituents in the oil were linalool (43.11%), 1.8 cineol (12.55%), eugenol (8.33%), methyl cinnamate (3.66%) iso-caryophllene (3.56%), Y-Terpin (1.93%), αFarnesene (1.81%), Naphthalen (2.44%), trans β-.ocimene (1.70%) myrtenol (1.44%), αcubebene (5.03%),α-caryophyllene (1.65%), azulene (1.42%). five compounds were identified as traces constituents (less than 1%) such as camphene (0.92%), germacrene (0.89%) and camphor (0.75%). Several investigations of the essential oils of various basil species showed that linalool as the most component (56.7-60.6%),followed by epiα-cadinol (8.6-11.4%) α bergamotent (7.4-9.2%) and Y –cadinene (3.2-5.4%). Hussain *et al.* (2008) showed that the major components of basil essential oil were eugenol (63.7%),**β** −ocimene (19.6%) and germacrene D(7.3%).

The variability in essential oil composition is present even in several basil species and these variations are sufficient to allow the distinction of different chemotypes or the result of an adaptative process to particular ecologic conditions. These differences may be due to the climatic and storage conditions of basil which could widely influence quantitative composition of the oil. On the other hand, the highly contents of total flavonoids, phenolic and tannins compounds in ethanolic extract which found to be 1200,667 and 490 mg/100g DW, respectively. These results were relatively similar to those obtained by Galvez *et al*.(2005) they found that flavonoids content ranged between 0.69-3.09% in plantago species. Also Grubesic *et al*. (2005) found that tannins content in plantago species leaves was (0.56-2.26%).

Basil essential oil at the three concentrations (500,1000, 2000 µg/ml) and plantago leaves extract at three concentrations(50,125,250 µg/ml) were tested for their ability to inhibit TSWV multiplication and spread of virus

infection in systemically infected tomato plants. Results showed that all treatments induced resistance against virus infection when applied to the plants as a mixed inoculums from essential oil or extract and TSWV. Various Basil essential oil concentrations positively reduced the viral infection and the highest basil concentration (2000 µg/ml) revealed the highest reduction (45.99%) .In addition the highest effective treatment against viral infection in *Plantago major* extract, which realized the highest viral reduction compared with all other treatments, was 250 µg/ml realized 63.82% and the lowest viral infection was 36.18%. The results revealed the antiviral activity of these extracts against TSWV infection. The increased basil essential oil and *P.major* extract administration respectively, companied with highly viral reduction against the infected plants, that is may be due to the natural compounds which already exist in these extracts which separately or together act as antiviral against viral infection with TSWV throw its inhibition effect against reverse transcriptase or interfering with virus coat protein attachment with target cells. In this respect Min *et al*.(2013) found that the essential oils isolated from Artemisia and lemongrass directly inactive TMV activity by interfere with coat proteins or inhibit the formation of capsid proteins which are necessary for adsorption or entry into the host. Also small molecular compounds, which found in essential oil may penetrate into plant cells and exert direct inactivating effects on virus particles. Also Iftikhar *et a*l. (2013) found that high content from bioactive compounds such as eugenol and terpens from clove buds essential oil inhibition of viral activity by preventing viral replication or preventing adsorption of virion to host cells. The reduction of infection with TSWV using *Plantago major* extract may be dut to its contents of several flavonoids and related compounds which have antiviral activity by weakening interactions between coat protein sub- units of the virus leading to increased susceptibility to host RNA ases (French *et al*., 1991).In this respect Malhotra *et al*. (1996)showed that several flavonoids and related compounds have antiviral activity against ToRSV such as quercetin, quercetin 3,7,4 trimethyl ether, quercetin 7,4 dimethyl ether and fisetin 4-methly ether. These compounds showed strong antiviral activity causing 67 to 76% inhibition of ToRSV infection. Also quercetin does not inhibit viral replication from viral RNA but may be inhibit virus movement.

Lycopene, the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and tomato products, and has received much attention in recent years because of its beneficial effect in the treatment of diseases (Shi and Le Maguer 2000). From the results in Table(6) it could be noticed that infection with virus significantly decreased the lycopene content compared with healthy plants. The highest total lycopene content have been observed with the highest concentrations of basil essential oil and

p.major extract compared with other concentrations and reached to 6.77 and 8.26 mg/100g respectively, against 4.11mg/100g for the infected plants while the healthy plants had 6.89 mg/100g.

From other view, the gradually increase in the essential oil and*P. major* extracts administrations accompanied with a gradually recovered in the fruit lycopene contents. The increase in lycopene content may be due to the antiviral compounds effects with increase the secondary metabolites such as carotenoids involved lycopene. These results are in agreement with those of Kunihiko *et al*. (1989), they found that natural products are endowed with the ability to inhibit virus infection. An emphasis is placed on virus reverse transcriptase inhibitors. It was found that a spectacular diversity of chemical structures encompassing proteins, terpenoids, carotenoids, xanthones, alkaloids, flavonoids, polyphenols act as virus reverse transcriptase inhibitors.

The highest total ascorbic acid content have been observed with highest concentrations of both essential oil and *P.major* extract were reached to 26.44 and 29.11 mg/100g F.W against 25.01 mg/100g F.W for the infected plants, while the healthy plants was 20.88mg/100g F.W. On the other hand , the gradually increase in the essential oil and extract administrations accompanied with a gradually increase in the fruit ascorbic acid content it may due to the antiviral action of bioactive compounds in essential oil or extract .

Infection by the virus significantly increased the titrable acidity compared with healthy plants. the lowest acidity content have been observed with highest concentrations of both basil essential oil and *P.major* extract which were reached to 0.45 and 0.46% against 0.62% for the infected plants, while the healthy plants had 0.37%. The obtained data revealed a significant increase in the titratable acidity under various viral treatments when compare with healthy plants, that it may be dut to the plant immune response which proposed to possess a highly resistance to the virus infection, on the other hand, the antiviral treatment recovered the titratable acidity and the fruits parameters closed to the normal quality levels. These results was in accordance with the evidence from a majority of studies around the world suggests that virus GLRaV-3 infected Vines (of both white and red varieties have reduced yield and higher titratable acidity than healthy vines (Charles *et al*., 2006).

All previous treatments with essential oil and extract decreased total soluble and reducing sugars percentage as an antiviral compounds the highest decrease in total soluble and reducing sugars found at the concentration of 2000 µg/ml of essential oil and 50µg/ml of *P.major* extract, respectively which was 9.62 and 9.82mg/g FW and 5.00 and 5.91mg/g FW, respectively. These results are in harmony with those obtained by (Tecsi *et al*,. 1994) they reported that antiviral compounds inhibit virus infection and activated the photosynthesis processes while in virus

infected leaves arise in glucose, fructose and sucrose were noticed.

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REFERENCES

A.O.A.C. (1990). Official methods of analysis of the associate of official agricultural.chemicals.WashingtonD.C .,U.S.A.

Abdel-Salam,A.M.(1999). Effect of tritonX100 inclusion buffers in reducing non-specific backgrounds in dot blot immune-binding assay (DBIA) on plant viruses .Arab.J.Biotech.2(1):89-96.

Abdel-wahab and EL-Shazly A.M.(2009). Natural incidence of the Thrips- Borne tomato Spotted wilt tospovirus at the Giza region,Egypt.Egyptian.J.virol.6:287-301.

Adams, R. P. (1995). Identification of essential oil components by Gas Chromatography/Mass Spectroscopy. Allured puplishing crop., Carol stream,IL. 71:80

Anon. (1991). *Tomato spotted wilt virus*. Report on Plant Disease No. 665, pp. 1–8.

Beerh, O. P. and Siddappa, G. S. (1959). A rapid spectrophotometric method for the detection and estimation of adulterants in tomatoketchup. FoodTechnol.13:414-418.

Best,R.J.(1968). *Tomato spotted wilt virus*. Adv. Virus. Res.13:65-146.

Bezerra, I.C.; Resende, R.D.; Kormelink, R. and De Avila, A.C.(1999). Increase of Tospovirus viral diversity in Brazil with the identification of two new Tospovirus species one from chrysanthemum and one from zucchini. Phytopathology 89:823-83.

Boiteux, L.S.; de Giordano, L.B.; de Avila, A.C. and Santos ,J.R.M.(1993). linhagem de tomate para mesa resistente a trˆesesp´ecies de tospovirus causadoras do 'vira-cabec¸a'. Hort Bras11: 163–164.

Brittlebank, C.C. (1919). Tomato diseases. J. Agric. Victoria. 17: 231-235.

Chang, C. C.; Yang, M. H.; Wen, H. M. and Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J.of Food and Drug Anal. 10:178-182.

Charles, J. G.; Cohen, D.; Walker, J. T. S.; Forgie, S. A.; Bell, V. A.andBreen, K. C. (2006). Leafroll associated virus type 3 (GLRaV-3). New Zealand, Plant Protec. 59:330-337

Cho, J.J; Custer, D.M.; Brommonschenkel, S.H. and Tanksley, S. D. (1996). Conventional bereeding:host plant resistance and the use of molecular markers to develop resistance to tomato spotted wilt virus.Acta Hortic. 431:367-378.

Cho, J.J.; Mitchell ,W.C.; Mau, L.R.F.and Sakimura, K.(1987). Epidemiology of *tomato spotted wilt virus* on crisphead lettuce in Hawaii. Plant Disease 71, 505–8.

Converse, R.H. and Martin, R.P. (1990). ELISA methods for plant viruses In: Serological methods for detection and identification of viral

and bacterial plant pathgens. (Hamilton, R.O. and Ball, E.M. and De-Bore, S. H (eds), PP. 170-196..

Dubois, N.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. and Smith, F. (1956). Calorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

El- Shazly, Manal. A.; Abdel- Wahab, A.S. and Zein, Salwa N. (2006). Comparative study on two thrips transmitted viruses tomato spotted wilt virus (TSWV) and Iris yellow spot virus (IYSV) as a tospovirus. Egyptian J. Virology 3 (1). 49-70.

EL-Shazly; Manal A.; Ahmed,O.K. and Sabh,A.Z. (2008). Inhibition of *tomato spotted wilt virus* infection by Guava Leaf extract, Clove essemtial oil and neem seed oil in tomato plants . Egyptain. J.virol.5(2).123-148.

EL-Shazly,Manal A.; Dawood , Rehab A. and Soliman, A.M. (2009). Biological, Biochemical Serological, Molecular and tissue cultural studies on an Egyptian chrysanthemum plants. Egypt. J. phylopathology 37 (2):79-94.

Francki, R.I.B.; Hatta, T. (1981). Tomato spotted wilt virus. In: Kurstak E,ed.Handbook of Plant Virus Infections and Comparative Diagnosis Amster:Elsvier/North Holland Biomedical press,492-511.

French, C. J.; Elder, M.; Leggett, F.; Ibrahim, R. K. and Towers, G. H. N. (1991). Flavonoids inhibit infectivity of tobacco mosaic virus. Can. J. Plant Path. 13:1-6.

Galvez, M.; Martin-Cordero, C.; Houghton, P.J. and Ayuso, M. J. (2005). Antioxdant activity af methanol extracts obtained from Plantago species. J Agric. Food Chem. 53:1927-1933.

Gera,A.; Kritzman, A.; Cohen, J.; Raccah, B. and Antignus,Y.(2000). Tospoviruses infecting vegetables crops in Israel. Bulletin. OEPP Bulletin 30:289-292.

German, T. L.; Ullman, D. E. and Moyer, J. W. (1992). Tospoviruses: Diagnosis, molecular biology, phylogeny and vector relationships. Phytopathology 30: 315-348.

Groves, R.L.; Walgenbach, J.F.; Moyer, J.W ., and Kennedy, G.G. (2002). The role of weed hosts and tobacco thrips Frankliniella fusca in the epidemiology of tomato spotted wilt virus. Plant Disease 86:573-582.

Grubesic, R.J.; Vukovic, J.; Kremer, D. and Vladimir-Knezevic, S. (2005). Spectrophotometric method for polyphenols analysis: Prevalidation and application on Plantago L. species. J. Phamaceut. Biomed. Anal. 39:837-842.

Guenther, E. (1961). The essential oils.vol.III. 4th Ed. D.Van Nostrand Company Inc., Princton, New Jersy, Toronto, New Yourk, London.pp.1-150.

Hanif, M.A.; ALMaskari, A. and AL Sabahi, J. (2011). Essential oil composition, antimicrobial and antioxidant activities of unexplored omani basil . J. of medicinal plants research 5:751-757.

Hill, M. F. and Moran, J.R. (1996). The incidence of tomato spotted wilt tospovirus (TSWV) in Australian nursery plants. Australasian Plant Pathology 25: 114-119.

Hoffmann, K.; Qiu, W.P.; Mover; J.W. (2001). Overcominghost-and pathogen-mediated resistance in tomato and tobacoo maps to the mRNA of tomato spotted wilt virus.Mol Plant-Microbe Interact.14:242-249.

Hussain, A.; Anwar, F.; Sherazi, S.T. and Przbylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil(ocimum basilicum)essential oil depends on seasonal Variations . Food chemistry 108:986-995.

Iftikhar, S; Shaid, A.A.; Javed, S; Nasir, I.; Tabassum, B. and Haider, M.N. (2013). Essential oils and Latices as noval antiviral agent against Potato leaf roll virus and analysis of their phytochemical constituents responsible for antiviral activity. J.of Agric.Sci(5):7.167.

Jamilah, J.; Sharifa, A.A and Sharifah. N.R. (2012). GC- MS analysis of various extracts from leaf of plantago major used as traditional medicine . world applied science Journal 17:67-70.

Kobeasy, M.I.; ELshazly, A.M.; Rashed, M.M .and Yousef, S. Rania (2012). Antiviral action of lavender(Lavendular vera) Essential oil against tomato spotted wilt virus infected tomato plants. Journl of chemical acta.(1):6-14.

Kuhn, C.W. (1964). Seprartion of Cowpea virus mixture. Phytopathology 54:739-740

Kunihiko, F. , Hiroshi, S.; Takuo, O.; Tsutomu, H.; Sei-ichi, T.; Ken, K.; Yasuo, I.; Sadako, I.; Shinya, I.; Meihan, N. and Kunio, K. (1989). Inhibition of herpes simplex virus infection by tannins and related compounds. Elsevier Scien. B., 11:285-297.

Loeffler, H.J. and Ponting, J. D. (1942). Ascorbic acid. Rapid determination in fresh, frozen, or dehydrated fruits and vegetables. Ind. Eng. Chem.Anal. 14:846.

Malhotra, B.; Onyilagha, J. C.; Bohm, B. A.; Towers, G. H. N.; James, D.; Harborne, J. B. and French, C. J. (1996). Inhibition of tomato ringspot virus by flavonoids. Phytochem 43(6):1271-1276.

Marby, T. J.; Markham, K. R. and Thomas, M. B. (1970). The systematic identification of flavonoids. Springer-Verlag. Berlin 354.

Marchoux, G. and Gebre-Selassie, K. (1991). Detection of tomato spotted Wilt virus and transmission by Frankliniella occidentalis in France. Plant Pathotogy 40: 347 -351.

McMichael, L.A.; Persley, D.M. and Thomas, J.E. (2002). A new tospovirus serogroup IV species infecting capsicum and tomato in Queensland, Australia. Australasian Plant Pathology 31: 231–239.

Meda, A.; Lamien, C. E.; Romito, M.; Millogo, J. and Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoide and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91:571-577.

Medeiros, R.B.; Resende, R.O. and de Avila, A.C. (2004). The plant virus tomato spotted wilt tospovirus activates the immune system of its

main insect vector. Frankliniella occidentalis, J. Virol. 78:1-38

Merfort, I.; Wray ,V.; Barakat, H.H.; Hussen, S.A.M; Nawwar, M.A.M.and Willuhan, G. (1997). Flavonol triglycerdes from seeds of Nigella sativa Phytochem 46(2):359-363.

Miller, G. L. (1959). Use of dinitro salicylic acid reagent for determination of reducing sugar . Anal. Chem. 31(3):426-428.

Min,L.; Han, Z.; Xu, Y. and Yao,L. (2013). In vitro and In Vivo anti-tobacco Mosaic virus activities of essential oils and Individual Compounds.J-Microbiol Biotechnol 23(6):771-778.

Momol, M.T.; Funderburk, J.E.; Olson, S., and Stavisky, J. (2002). Management of Tomato spotted wilt tospovirus (TSWV) on tomatoes with UV-reflective mulch and Acibenzolar-S-methyl. Thrips and Tospoviruses. Proceedings of the 7th International Symposium on Thysanoptera, pp. 111–116. Australian National Insect Collection, Canberra (AU).

Moury,B.; Palloix,A.; Gebre Selassie,K. & Marchoux,G. (1997). Hypersensitive resistance to tomato spotted wilt virus in three Capsicum chinense accessions is controlled by a single gene and is overcome by virulent strains. Euphytica 94: 45–52.

Moyer, J.W. (1999). Tospoviruses (Bunyaviridae). In Encyclopedia of Virology, pp. 1803–1807. Eds A. Granoff and R.G. Webster. San Diego, California: Academic Press.

Mumford, R.A.; Barker, I. and wood , K.R. (1996). The biology of the tospoviruses. Ann. Apple. Biol. 128: 159- 183.

Nagata, T.; Inoue-Nagata A.K., van Lent, J.; Goldbach, R. and Peters, D. (2002). Factors determining vector competence and specificity for transmission of Tomato spotted wilt virus. J.Gen. Virol. 83: 663–671.

Nehmet,A.S. and Miray, A.S. (2012). Estimation of the effect of tomato spotted wilt virus(TSWV) infection on some yield compounents of tomato. Phyto parasitica 40:87-93.

Parrella, G.; Gognalons, P.; Gebre-Selassie, K.; Vovlas, C., and Marchoux, G. (2003). An update of the host range of Tomato spotted wılt vırus. Journal of Plant Pathology 85:227-264.

Peterson, R.G.; Scott, S.J. and Gergerich R.C. (1989). Resistance in two Lycopersicon species to an Arkansas isolate of tomato spotted wilt virus. Euphytica 43 : 173-178.

Peters, D.; de Avila, A. C.; Kitajima, E. W.; de OResende, R.; de Hann, P. and Goldbach, R.W. (1990). Anoverview of *tomato spotted wilt virus*. In VirusThrips-Plant Interactions of *Tomato Spotted Wilt*. Australasian Plant Pathology Vol. 25 (2).pp.1-14.

Peters, D; De Avila, A.C.; Kitajima, E.W.; Resende, R.O.; De Haan ,P. and Goldbach ,R.W. (1991). An overview of *tomato spotted wilt virus.* In: Hsu TH, Lawson HR, eds. Virus–Thrips–Plant Interaction of *Tomato Spotted Wilt Virus*. Proceedings USDAWorkshop, United States Department Agriculture Research and

Service ARS-87; Beltsville, Maryland.pp120-150.

Price, M.L.; Socoyoc, S.V. and Butter, L.G. (1978).A critical evaluation of the vanillin reacation as an assay for tannin in sorghum grain .Agric. Food Chem. 26:1214-1218.

Prins M. and Kormelink, R. (2000). Classification of Tospoviruses. http://www.spg.wau.nl/viro/research/html

Sharman,M. and Persley, D. M.(2006). Field isolates of *Tomato spotted wilt virus* overcoming resistancein capsicum in Australia. Australasian Plant Pathology 35: 123–128.

Sherwood ,L.J.; German, T.L.; Moyer, J.W.;Ullman, D.E.and Whitfield A.E. (2000). Tomato spotted wilt. In Encyclopedia of Plant Pathology. pp. 1030–1031. Eds O.C. Maloy and T.D. Murray. New York: John Wiley & Sons.

Shi, J. and Le Maguer, M. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. Crit Rev Food Scien. Nutr. 40(1):1-42.

Shima, M.N.; Mojdeh, M. and Ghotbi, T. (2013) .Biological and serological detection of TSWV on three commercial cultivars chrysanthemum molifor lium in Markazi province of Iran. Annals of Biological Research 4(4):112-119.

Tecsi, L. I.; Maule, A. J.; Smith, A. M. and Leegood, R. C. (1994). Complex, localized changes in CO2 assimilation and starch content associated cucumber mosaic virus and a cucurbit host. Plant J. 5:837- 847.

Ullman, D.E.; Medeiros, R.B.; Campbell, L.R. ; Whitfield, A.E.; Sherwood, J.L. and German, T.L. (2002). Thrips as vectors of tospoviruses. Adv. Bot. Res. 36: 113–140.

Wang, M. and Gonsalves, D. (1990). ELISA detection of various *tomato spotted wilt virus* isolates using specific antisera to structural proteins of the virus.Plant Disease 47:145-158.

Whitfield, A.E.; Ullman, D.E., & German, T.L. (2005). Tospovirus–thrips interaction. Annual Review of Phytopathology 43: 459–489.