

Full Length Research Paper

Tomato yellow leaf curl virus (TYLCV), identification, virus vector relationship, strains characterization and a suggestion for its control with plant extracts in Iraq

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Tomato yellow leaf curl begomovirus (TYLCV) was identified on the basis of symptoms on test plants, transmission by whiteflies *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) biotype B, and its incubation period in the insect. Symptomatic tomato plants showing leaf curling, leaf blade reduction, distortion and general stunting, indicative of TYLCV in greenhouses, were transplanted into pots (20 × 20 cm) containing soil mix and were maintained in growth room for identification. The virus was found to be easily acquired and transmitted by *B. tabaci* biotype B when given an acquisition access period on tomato infected plants for 30 min with incubation period of 24 h. Leaf curl and distortion associated with severe stunting and yellowing were developed on inoculated *Datura stramonium*, *Nicotiana tabacum* cv. Samsun NN, *Nicotiana glutinosa* and *Phaseolus vulgaris* var. Battle and were found as susceptible to the virus without visible symptoms. The virus caused vein clearing on inoculated *Solanum nigrum* leaves. No infections were observed on *Gomphrena globosa*, *N. tabacum* cv. White burly, *Physalis floridana*, *Solanum melongena*, *Cucumis sativus*, *Chenopodium amaranticolor* and *Chenopodium murale* inoculated by the virus. Two strains for TYLCV were characterized by symptoms on tomato and immun-double diffusion test. A spur was formed between the polyclonal antibodies of TYLCV isolate and the extracts from infected tomato plants showing different symptoms. Extracts from Thuja, Tamarix and Henna plants exhibited inhibitory effects on TYLCV multiplication in tomato treated plants with protection periods of 10 to 12 days.

Key words: *Bemisia tabaci*, control, plant extracts, strains, Tomato yellow leaf curl begomovirus.

INTRODUCTION

Tomato yellow leaf curl begomovirus (TYLCV), Genus: Begomovirus, Family: Geminiviridae, is one of the major factor limiting tomato, *Lycopersicon esculentum* Mill., production in tropical and subtropical regions in the world and in many Mediterranean and Middle Eastern countries (Jones et al., 1993; Markham et al., 1994; Czosnek and Latterote, 1997). The virus was first observed in eastern Mediterranean since 1966 (Lapidot and Friedman, 2002).

The incidence of symptomatic plants was up to 100% with heavy losses in tomato yield up to 80% especially when plants are infected in early stage of growth (Cohen and Antignus, 1994; Lourou et al., 1996; Momol et al., 1999). The family Geminiviridae is composed of plant viruses with isometric geminate particles containing

circular single stranded DNA (Harrison, 1985). Most TYLCV strains have monopartite genomes and are transmitted efficiently by whiteflies *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) in persistent circulating manner (Navot et al., 1991; Cohen and Antignus, 1994; Hajimorad et al., 1996; Hanley-Bowdoin et al., 1999; Martinez-Culibras et al., 2001; Salati et al., 2002). TYLCV is readily acquired by immature and adult whiteflies and is retained along the life of the adult whitefly (Cohen and Antignus, 1994; Mansour and AL-Musa, 1992). The spread of the virus in the field or in glasshouses was found linked to increase in *B. tabaci* population (Polston and Anderson, 1997).

Two strains of TYLCV, mild and severe, have been identified. The mild strain occurred in Israel and Spain, whereas the more widely spread severe one found in Caribbean and southern United states (Polston et al., 2006).

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Symptoms induced by TYLCV on tomato plants consist of reduction in leaf size, leaf curling upward, severe stunting and distortion associated with interveinal chlorosis, observed mainly on the upper portion of plants (Martinez-Culebras et al., 2001; Salati et al., 2002). When infection occurs at early stage of growth, the plant exhibited severe stunting, dropping of flowers and stops producing marketable fruits.

Due to the wide spread of TYLCV on tomato in both protected and open field, the study was aimed to identify TYLCV by symptoms on indicator plants and its relationship with the vectors, determine the virus strains by symptoms on tomato plants and serological characteristics, and manage the disease through using plant extracts.

MATERIALS AND METHODS

Virus isolate

Tomato plants *L. esculentum* Mill., showing leaf curling upward or downward, leaf blade reduction, leaf corrugation, leaf marginal and interveinal chlorosis, and severe stunting of entire plant were collected from plastic and glasshouses in different areas of Iraq. The plants were transplanted into 20 × 20 cm pots containing mix soil and maintained in growth room at 21 to 25°C with 16 h photoperiods and used for virus source.

Test plants

The plants, *L. esculentum* Mill., *Nicotiana tabacum* var Xanthi, *N. tabacum* cv. Samsun, *N. tabacum* cv. White Burly *N. glutinosa*, *Datura stramonium*, *Solanum nigrum*, *Physalis floridana*, *Cucumis sativus* Beta alpha, *Chenopodium amaranticolor*, *Ch. murale*, *Phaseolus vulgaris* var Battle (Shafic 1983; Ioannou et al., 1987; Mansour and Al-Musa, 1992), were used. Seeds of these plants were sown in pots (20 × 20 cm) containing sterile soil mix and peatmoss (2:1) in growth room (21 to 25°C). The seedlings were transplanted at 3 to 5 leaves stage to other pots containing the same soil mix. The plants were sprayed with imidacloprid (Confidor) 1 ml/L to prevent insect injuries.

Virus transmission

Adults of whiteflies were collected from cotton plants *Gossypium hirsutum* and reared on the same host in muslin protected cages (90 × 60 × 30 cm) in growth room at 21 to 25°C with photoperiod 16 h light / 8 h dark and relative humidity 70 ± 5%. Numbers of whiteflies were given an acquisition feeding period for 24 h on virus infected tomato plants. Healthy tomato and test plants were exposed to viruliferous whiteflies (10 insect / plant) at 5 leaf stage and maintained in insect proof cages in greenhouse. Non inoculated plants were maintained in isolated cages as control. Symptoms developments were evaluated 3 to 4 weeks of inoculation. Samples of new leaves of inoculated plants were taken for immuno-double diffusion test.

Virus incubation in *B. tabaci* biotype B

Numbers of non-viruliferous whitefly adults were placed on TYLCV-infected tomato plants in cage for 1 h to acquire the virus.

Viruliferous insects were then transferred to tomato healthy plants (10 insects/plant) for periods of 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h in insect proof cages in growth room. The percentage of virus infection was estimated visually by symptoms as well as confirmation serologically by immuno-double diffusion test.

Virus purification

Aerial parts (100 g) of infected tomato plants showing characteristic symptoms of TYLCV (after 4 weeks of inoculation) were homogenized in citrate buffer (100 mM sodium citrate, 60 mM sodium sulfite, 5 mM EDTA, pH = 8, amended with 1% 2-mercaptoethanol) 1:2 (g/ml) by warring blender. The homogenate was passed through 4 layer of muslin and clarified by n-butanol 10% with agitation at 4°C for 1 h. The mixture was centrifuged at 5000 rpm / min. Pellets were resuspended in citrate buffer (10 mM sodium citrate, 1 mM EDTA, and pH = 8) and passed through 0.45 µm Millipore filter. The filtrate was dialyzed against 500 distilled water for overnight at 4°C and the virus concentration was estimated as follows:

mg/ml virus = Absorbance at 260 nm / 7.7 (Czosnek et al., 1988).

Antiserum production

Rabbits were immunized by 4 injections of purified TYLCV at interval of one week. The first injection in the ear marginal vein without Freund's Adjuvant, succeeded by three intramuscular injections emulsified with equal volume of Freund's Incomplete Adjuvant (1 ml at 0.5 mg/ml for each injection) (Sequeira and Harrison, 1982). The blood was collected after one week of the last injection through the marginal ear vein. The antiserum was obtained and purified as reported by Clark and Adams (1977).

Immuno-double diffusion

This test was carried out in 0.85% agarose gel in petriplates. The agarose (0.85 g) was melted in 100 ml of neutral PBS (10 mM sodium phosphate, 0.14 M NaCl containing 0.5% SDS) and poured in the plates before solidification at 50°C (15 ml agarose gel/plate). Wells of 6 mm diameter were made in the gel by cork borer after solidification. TYLCV-polyclonal antibodies (25 µl) was loaded into the central well, and 25 µl of purified TYLCV and extracts from TYLCV-infected foliage were added into Ag wells around Ab well (Ab well was 4 mm from each Ag well). The plants were maintained in a moist chamber at room temperature overnight.

Efficiency of plant extracts on virus multiplication

Air dried of aerial parts from *Thuja orientalis*, *Tamarix brachystachy*, and *Lawsonia inermis* were ground. A volume of 300 ml of ethanol 80% was added to 100 g of aerial parts powder. The mixture was agitated for 24 h and passed through whatman-2 filter paper in Buchner funnel with vacuum. The filtrate was concentrated to a consistent paste in water bath at 40 to 42°C (Al-Ani et al., 2010).

Treatments

Concentrations of 1, 2, 3, 4, and 8 g/L in distilled water, amended with 0.1% Tween-20 were prepared from each extract. TYLCV-inoculated tomato plants (5 plants) were sprayed with each concentration after 48 h of inoculation by *B. tabaci* biotype B, and the virus was detected in the treated plants after 10 days of extract



Figure 1. Severe strain of TYLCV that show leaf reduction, leaf curl upward with yellowing of the new leaves on tomato (A), compared with healthy plant (B).



Figure 2. Mild strain of TYLCV that shown mild leaf curling and blade curvature (A), compared with healthy plant (B).

application. Also, healthy tomato plants were sprayed by the same extract concentrations 1 h prior virus inoculation and the virus was followed in the plants at 3, 5, 10, and 12 days of inoculation. The virus was detected in the plant by immuno-double diffusion test.

RESULTS

Symptoms on test plants

Symptoms of two types were manifested on inoculated tomato plants by *B. tabaci* biotype B previously fed on tomato plants showing leaf reduction, leaf curling,

distortion and general stunting with or without yellowing symptoms. The first type was characterized by leaf reduction, leaf curl upward with yellowing of the new leaves (Figure 1). Similar results were obtained by Shafic (1983), Muniyappa et al. (1991), Mansour and Al-Musa (1992), and Morilla et al. (2003). The second type of symptoms was characterized by mild leaf curling and blade curvature (Figure 2). Similar results were reported by Hajimorad et al. (1996), Morilla et al. (2003), and Lefeuvre et al. (2007) about TYLCV.

Leaf curling, leaf distortion, severe plant stunting associated with yellowing symptoms were developed on



Figure 3. *Datura stramonium* inoculated by viruliferous *B. tabaci* from tomato plants showing characteristic symptoms of TYLCV.

D. stramonium upon inoculation by viruliferous *B. tabaci* from tomato plants showing characteristic symptoms of TYLCV (Figure 3). Similar results were recorded previously (Shafic, 1983; Ioannou, 1985; Mansour and Al-Musa, 1992) about TYLCV on *Datura*, *N. glutinosa*, *N. tabacum* cv. Xanthi, *N. tabacum* cv. Samsun were systemically infected by the virus when inoculated by viruliferous *B. tabaci* but without visible symptoms. It was shown previously that these hosts were susceptible to TYLCV without visible symptoms (Ioannou, 1985; Mansour and Al-Musa, 1992). The virus was detected from these plants by immuno-double diffusion test.

The virus produce symptoms of vein clearing on inoculated *Solanum nigrum* after 23 to 25 days of inoculation by viruliferous *B. tabaci* biotype B, sooner disappeared with the time (Figure 4). These results is in accordance with Shafic (1983), in that this host is susceptible to TYLCV but differ from those reported by Ioannou (1987) and Mansour and AL- Musa (1992), who reported that this host is not susceptible to TYLCV. *P. vulgaris* var. Battle is also systemically infected by the virus without visible symptoms. These results are in agreement with those obtained by Shafic (1983) and Trabulsi (1994).

No infections were obtained on; *Gomphrena globosa*, *N. tabacum* cv. Whiteburly, *P. floridana*, *Solanum melongena*, *C. sativus*, *C. amaranticolor*, and *C. murale*

inoculated by the isolates. Shafic (1983), Ioannou (1987), and Mansour and Al-Musa (1992) reported similar results concerning the response of these plants to TYLCV.

Virus-vector relationship

Whiteflies *B. tabaci* biotype B were found to be able to acquire and transmit TYLCV 22 h after they were caged with infected tomato plants for 30 min (2 over 5 plants infected), while all plants were infected after 24 h. This result indicates that the incubation period of virus in the insect is between 22 to 24 h. Similar results were reported previously (Ioannou, 1987; Mansour and Al-Musa, 1992; Hajimorad et al., 1996; Morilla et al., 2003) concerned the incubation period of TYLCV in *B. tabaci*.

Immuno-double diffusion

Positive reactions were noticed between well containing polyclonal antibodies to TYLCV, severe isolate, and those containing purified virus or extracts from infected tomato plants. Spur was formed between the Abs and extracts from infected tomato plants by two isolates (Figure 5), which indicates that the two isolates represent two different strains of TYLCV.



Figure 4. Vein clearing on inoculated *Solanum nigrum* after 23-25 days of inoculation by viruliferous *B. tabaci*.

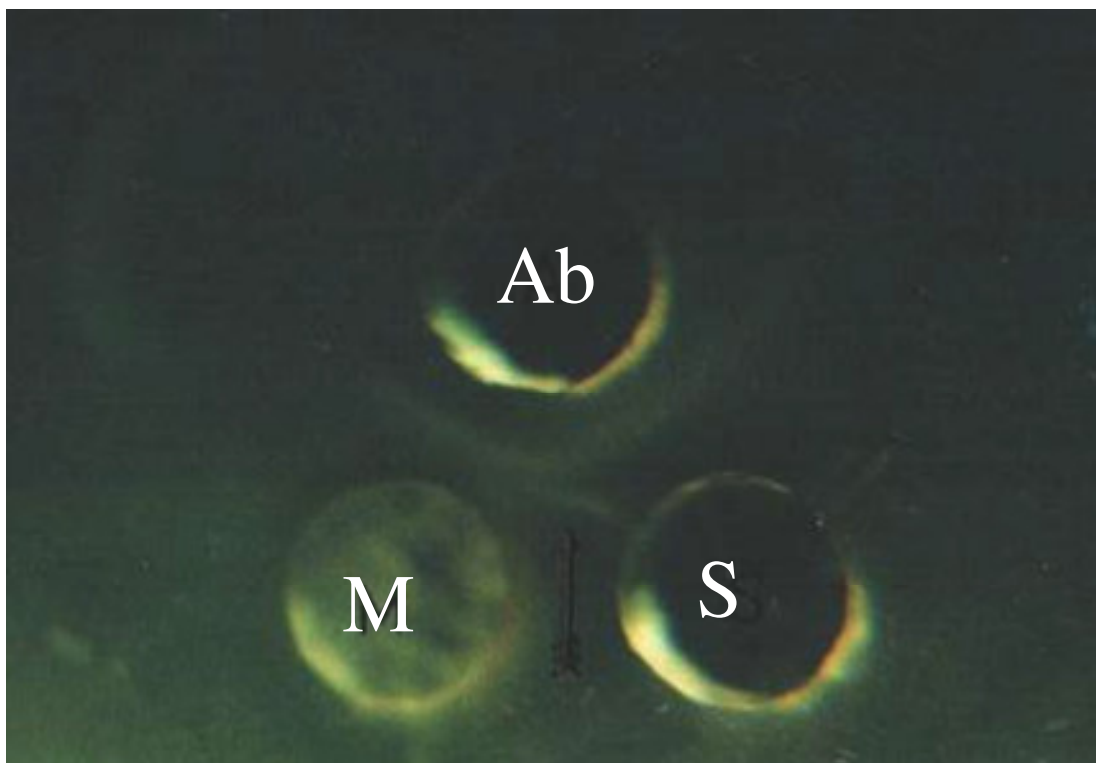


Figure 5. Immunodouble diffusion test shown Spur formed between the Abs and extracts from infected tomato plants by two isolates.

Table 1. Effect of different concentrations of alcoholic extracts to inhibit TYLCV multiplication.

Alcoholic extracts	Concentration				
	1 g/L	2 g/L	3 g/L	4 g/L	8 g/L
Henna	+	+	+	-	-
Thuja	+	+	-	-	-
Tamarix	+	+	+	-	-

(+)= The virus was found, (-) = the virus not found (used double diffusion test). Results obtained 10 days after application by extracts.

Table 2. Protection period to plant against TYLCV by used alcoholic extracts.

Alcoholic extracts	Periods				
	3 days	5 days	10 days	12 days	16 days
Henna (4 g/L)	-	-	-	+	+
Thuja (3 g/L)	-	-	-	-	+
Tamarix (4 g/L)	-	-	-	+	+
Control	+	+	+	+	+

(+)= The virus was found, (-) = the virus not found (used double diffusion test). Each value in the table represents the mean of 5 replicates.

Efficiency of the plant extracts on TYLCV multiplication

Immuno-double diffusion results revealed that the concentrations 1, 2, and 3 g/L of Henna and Tamarix extracts had no effect on TYLCV multiplication, since precipitation lines were formed between TYLCV-Abs and extracts from TYLCV-inoculated tomato plants treated with these concentrations, whereas the concentrations 4, and 8 g/L exhibited an inhibition effect on TYLCV-multiplication, since no precipitation lines between TYLCV-Abs and extracts from TYLCV-inoculated tomato plants treated with these concentrations were observed.

Results also showed that the concentration 1 and 2 g/L of Thuja extract had no effect on TYLCV-multiplication, while the concentrations 3, 4, and 8 g/L inhibited TYLCV-multiplication for the same indications mentioned earlier (Table 1). The efficient concentration 3 g/L of Thuja extract, 4 g/L of Henna and Tamarix extract confers protection periods to the plants against virus infection 12 and 10 days respectively (Table 2).

DISCUSSION

The symptoms manifested on tomato and test plants inoculated by viruliferous *B. tabaci* biotype B fed on tomato plants; showing leaf reduction leaf curling, stunting and yellowing, provide evidence that the virus infecting tomato is an isolate of tomato yellow leaf curl virus (TYLCV). Many hosts were found to be susceptible to TYLCV without visible symptoms (Symptomless

hosts). These hosts may serve as source of TYLCV transmitted to tomato plants by *B. tabaci* especially those prevailing in the vicinity of plastic or glasshouses cultivated with tomato such as *S. nigrum*. The wide spread of TYLCV is found to be linked with the high density of *B. tabaci* population in the tomato houses cultivation in the presence of virus source. Polston et al. (2006) reported that some genotypes of capsicum species are symptomless hosts and acts as reservoir of TYLCV. The discrepancies between some results in the present study and, those reported by others may be due to difference in cultivar used, virus isolate and biological differences in whitefly biotype which reflect differences in ability to acquire and transmit TYLCV.

Symptoms on inoculated tomato and test plants, and immuno-double diffusion results revealed that the current TYLCV-isolates represent two different groups of strains. The severe one causing severe leaf reduction, upward leaf roll, general stunting and distortion associated with leaf margin and interveinal chlorosis, and the mild one causing moderate leaf rolling with blade curvature. These results are in agreement with others shown previously about the existence of more than one strain for TYLCV (Accotto et al., 2003; Polston et al., 2006; Lefeuvre et al., 2007).

Although TYLCV is transmitted efficiently by *B. tabaci* in persistent manner with an incubation period 24 h in the insect, the use of insecticides to manage TYLCV-disease through vector control was found to be of little effect (Ioannou, 1987). So, research was oriented toward searching of substances safe and effective to control TYLCV. Based on indications that many plants contains

substances that exhibited inhibitory effects on viruses multiplication (Noronha et al., 1995; AL-Jerisi, 1998), the present study was undertaken in attempt to evaluate the efficacy of some plants extracts to control TYLCV on tomato. Results obtained showed that the alcoholic extracts of Henna, Tamarix, at 4 g/L, Thuja at 3 g/L were efficacious to inhibit TYLCV multiplication.

The efficiency of these extracts may be due to its contents of phenolic compounds especially of tannins. It was previously reported that Thuja contains 70% of tannins (AL-Shammaa, 1989). The effect of these substances may be direct on the virus or indirectly through inducing systemic resistance in the plant against the virus, which may persist for long periods depending on both of plant cultivar and the virus strain (AL-Jerisi, 1998; Kessman et al., 1994; Mahdy et al., 2007). The plant extracts may play an essential role in integrated management of plant viruses in the future.

REFERENCES

- Accotto GP, Bragaloni M, Luison D, Davino S, Davino M (2003). First report of *Tomato yellow leaf curl virus* (TYLCV) in Italy. *Plant Pathol.*, 52: 799.
- Al-Ani RA, Diwan SNH, Adhab MA (2010). Efficiency of *Thuja orientalis* and *Artimisia campestris* extracts to control of *Potato leaf roll virus* (PLRV) in potato plants. *Agric. Biol. J. North Am.*, 1(4): 579-583.
- Al-Jerisi YHZ (1998). Use the *Chenopodium murale* extract for inhibit *Tomato mosaic virus* and induce the tomato plant resistance against it. M.Sc. thesis, Al-Mustansareiah University, College of Sciences, Iraq.
- Al-Shammaa AA (1989). *Drugs and Medical Plants Chemistry*. Ministry of Higher Education and Scientific Research, Iraq, Baitul-Hikma Press, P: 101-111.
- Clark MF, Adams AN (1977). Characteristics of the Microplate method of enzyme- linked immunosorbent assay for the detection of Plant viruses. *J. Gen. Virol.*, 34: 475-483.
- Cohen S, Antignus Y (1994). *Tomato yellow leaf curl virus*, a whitefly-borne geminivirus of tomatoes. In: *Advances in Disease Vector Research*. 10. Springer-Verlag. N.Y., pp. 259-288
- Czosnek H, Laterot H (1997). A world-wide survey of *Tomato yellow leaf curl viruses*. *Arch. Virol.*, 142: 1391-1406.
- Czosnek H, Ber R, Antignus Y, Cohen S, Navot N, Zamir D (1988). Isolation of *Tomato yellow leaf curl virus*, a geminivirus. *Phytopathology*, 78: 508-512.
- Hajimorad MR, Kheyr Pour A, Alavi V, Ahoonmanesh A, Bahar M, Rezaian MA, Gronenborn B (1996). Identification of Whitefly transmitted *Tomato yellow leaf curl geminivirus* from Iran and a survey of its distribution with molecular probes. *Plant Pathology*, 45(3): 418-425.
- Hanley-Bowdoin L, Settlege SB, Orozco BM, Nagar S, Robertson D (1999). Geminiviruses: Models for plant DNA replication, transcriptions, and cell cycle regulation. *Crit. Rev. Plant Sci.*, 18: 71-106.
- Harrison BD (1985). Advances in geminivirus research. *Ann. Rev. Phytopathol.*, 23: 55-82.
- Ioannou N (1985). Yellow leaf curl and other virus disease of tomato in Cyprus. *Plant Pathol.*, 34: 428-434.
- Ioannou N (1987). Cultural management of Tomato yellow leaf curl disease in Cyprus. *Plant Pathol.*, 36: 367-373.
- Ioannou N, Kyriakou A, Hadjinicolis A (1987). Host range and natural reservoirs of *Tomato yellow leaf curl virus*. *Techn. Bull.*, p. 85. Agricultural Research Institute, Nicosia, Cyprus, p. 8.
- Jones JB, Stall RE, Zitter TA (1993). *Compendium of Tomato disease*. The American Phytopathological Society Press. St. Paul, MN, pp. 40-41.
- Kessman H, Staub T, Hofmann C, Maetzke T, Herzog J (1994). Induction of systemic acquired disease resistance in plants by chemicals. *Annu. Rev. Phytopathol.*, 32: 439-459.
- Lapidot M, Friedman M (2002). Breeding for resistance to whitefly transmitted geminiviruses. *Ann. Appl. Biol.*, 140: 109-127.
- Lefeuvre P, Hoareau M, Delatte H, Reynaud B, Lett JM (2007). A multiplex PCR method discriminating between the TYLCV and TYLCV-Mld clades of *Tomato yellow leaf curl virus*. *J. Virol. Methods*, 144: 165-168.
- Lourou D, Noris E, Veratti F, Accotto GP (1996). First report of *Tomato yellow leaf curl virus* in Portugal. *Plant Dis.*, pp. 80-1079.
- Mahdy AMM, Fawzy RN, Hafez MA, Hanan AN, Eman SM (2007). Inducing systemic resistance against *Bean yellow mosaic potyvirus* using botanical extracts. *Egyptian J. Virol.*, 4: 129-145.
- Mansour A, Al-Musa A (1992). *Tomato yellow leaf curl virus*: Host range and virus-vector relationship. *Plant Pathol.*, 41: 122-125.
- Markham PG, Bedford ID, Liu S, Pinner MS (1994). The transmission of geminiviruses by *Bemisia tabaci*. *Pestic. Sci.*, 42: 123-128.
- Martinez-Culebras PV, Font I, Jorda C (2001). A rapid PCR method to discriminate between *Tomato yellow leaf curl virus* isolates. *Ann. Appl. Biol.*, 139: 251-257.
- Momol MT, Simon W, Sprengel K, Olson SM, Momol EA, Polston JE, Hiebert E (1999). First report of *Tomato yellow leaf curl virus* in tomato in South Georgia. *Plant Dis.*, 83(5): 487.
- Morilla G, Antunez C, Bejarano ER, Janssen D, Cuadrado IM (2003). A new *Tomato Yellow leaf curl virus* strain in Southern Spain. *Plant Dis.*, 87: 8-1004.
- Muniyappa V, Swanson MM, Duncan GH, Harrison BD (1991). Particle purification, properties and epitope variability of Indian *Tomato leaf curl geminivirus*. *Ann. Appl. Biol.*, 118: 595-604.
- Navot N, Picherski E, Zeidan M, Zamir D, Czosnek H (1991). *Tomato yellow leaf curl virus*. A whitefly-transmitted geminivirus with a single genomic molecule. *Virology*, 185: 151-161.
- Noronha AB, Amelia M, Alexandre V, De-Gaetan R, Vicente M (1993). Protection against *Tobacco mosaic virus* induced by some caryophyllales plant extracts. *Microbios*, 73: 75-80.
- Polston JE, Anderson PK (1997). The emergence of whitefly-transmitted geminiviruses in tomato in the western hemisphere. *Plant Dis.*, 81: 1358-1369.
- Polston JE, Cohen L, Sherwood TA, Ben-Joseph R, Lapidot M (2006). Capsicum species: Symptomless hosts and reservoirs of *Tomato yellow leaf curl virus*. *Phytopathology*, 96(5): 447-452.
- Salati R, Nahkla MK, Rojas MR, Guzman P, Jaquez J, Maxwell DP, Gilbertson RL (2002). *Tomato yellow leaf curl virus* in the Dominican Republic: Characterization of an infectious clone, virus monitoring in whiteflies, and identification of reservoir hosts. *Phytopathology*, 92: 487-496.
- Sequeira JC, Harrison BD (1982). Serological studies on *Cassava latent virus*. *Ann. Appl. Biol.*, 101: 33-42.
- Shafic HL (1983). Studies on identification and control of *Tomato yellow leaf curl virus* in the plastic houses. M.Sc. Thesis, College of Agriculture, University of Baghdad, p. 65.
- Trabulsi R (1994). *Bemisia tabaci*: A report on its pest status with particular reference to the near east. *FAO Plant Prot. Bull.*, 42: 33-58.