Occurrence of Tomato Yellow Leaf Curl Virus on Volunteer Tomato, Jimsonweed, and Tobacco in North West Bank: Distribution of Virus Natural Reservoirs in Summer Season

دراسة وجود فيروس تجعد واصفرار اوراق البندورة على نبات البندورة الطوعي ونباتي الداتورة والدخان في شمال الضفة الغربية: دراسة توزيع عوائل الفيروس الطبيعية خلال فصل الصيف

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Abstract

Volunteer tomato (*Lycopersicon esculentum*), jimsonweed (*Datura stramonium*) and tobacco (*Nicotiana tabacum*) plants were found to be natural reservoirs for Tomato Yellow Leaf Curl Virus (TYLCV) in the northern West Bank regions using the Polymerase Chain Reaction (PCR). The virus can be maintained in these plants during the summer period in the studied regions. Volunteer tomato plants proved to be the most significant reservoirs for the virus because they are so abundant in all of the studied regions, and they demonstrated a high rate of infection, ranging from 52-95%. The maximum infection of volunteer tomato plants was recorded in the Jericho district and Al-Far'a region to be 95% and 93%, respectively. Under field conditions, TYLCV was found to infect tobacco in one of two ways; either as crops planted near the tomato fields or as volunteer plants scattered in tomato growing sites in the Jenin districts. The maximum infection rate of volunteer tobacco plants and tobacco crops was recorded in Qabatyya to be 11% and 6%, respectively.

The research showed that tobacco seedlings in nurseries can be infected with TYLCV by 3.5% in the Jenin district. Jimsonweed was discovered to be a natural host of TYLCV, however with low proportion and only in the Jericho district (one out of every two samples collected).

ملخص

أجريت هذه الدراسة لمعرفة العوائل النباتية الطبيعية لفيروس "تجعد واصفرار أوراق البندورة"، وذلك خلال فترة الصيف (٢٠٠٣-٢٠٠٤)، اذ تبين ان نبات البندورة الطوعي كان أكثر هذه العوائل أهمية، لوفرتة في مناطق الدراسة وارتفاع نسبة اصابته بالفيروس التي تراوحت من ٥٢-٩٥%. وسجلت أعلى هذه الاصابة في منطقة أريحا، اذ بلغت نسبة اصابة نبات البندورة الطوعي ٩٥%، ثم تلتها الفارعة التي وصلت أعلى اصابة بها الى ٩٣%. بينت الدراسة التي أجريت في منطقة جنين أن فيروس تجعد واصفرار أوراق البندورة يصيب نبات الدخان المزروع بجانب حقول البندورة ونبات الدخان الطوعي، فقد سجلت أعلى اصابة لهما في منطقة قباطية اذ بلغت ١١% للدخان الطوعي و٦ % لمحصول الدخان المزروع بجانب حقول البندورة. كذلك بينت الدراسة أن هذا الفيروس يصيب مشاتل الدخان في منطقة جنين بنسبة تصل الى ٣.٥% بالاضافة لذلك فقد أشارت الفحوصات الى أن هذا الفيروس يقضي فترة البيات الصيفي متطفلا على نبات الداتورة النامي في منطقة أريحا وباعداد قليلة، اذ وصلت نسبة اصابته الى ٥٠% (عينة واحدة من أصل عينتين تم جمعهما).

Introduction

Tomato (*Lycopersicon esculentum* L.) is considered to be the most popular vegetable crop planted in Palestine. The crop is planted in an area of 2475.9 hectares, occupying about 18% of the total area planted with vegetables in the West Bank. The major tomato production in Palestine occurs in Jenin, Tobas and Jericho districts which is estimated to be about 26526, 19140, and 13744 metric tons, respectively (Palestinian Central Bureau of Statistics (PCBS), 2006, p. 70-100). The major disease infecting tomato crops in many countries has been determined to be the Tomato Yellow Leaf Curl Virus (TYLCV) (Czosnek *et. al*., 1990, p. 1-6), (Pico *et. al.,* 1996, p. 151-196), (Moriones *et. al*., 2000, p. 123-134), (Morilla *et. al.*, 2004, p. 10715-10723)). TYLCV is very harmful to the crop and can cause serious agricultural and economic tribulations (Czosnek and Laterrot, 1997, p. 1391–1406),

(Czosnek *et al.,* 2001, p. 1391–1406). It has been discovered that qualitative and quantitative yield losses in tomato crop often reach 100% in the Middle East, North and Central Africa, and South East Asia (Nakhla *et. al.*, 1994, p. 926). In Jordan, Anfoka *et. al*., (2005, p. 65-70) reported that TYLCV was widely infecting tomatoes planted in several regions of the kingdom including Al-Mafraq, central and north Jordan Valley and north and south Amman. The highest rate of TYLCV infection was recorded in Al-Mafraq (76%), whereas, samples collected from the northern Jordan Valley showed the lowest disease incidence (13%). In Egypt, the virus was considered the most serious disease of autumn-grown tomato in Fayoum, Giza, and Ismailia as the incidence ranged from about 80-99% (Nakhla *et. al.*, 1993, p. 163-173), (Pico *et. al.*, 1996, p. 151-196). In Lebanon, Abou Jawdah *et. al*., (1995, p. 52-57) reported that tomato grown in the coastal plains during December was severely infected with TYLCV, obliging farmers to abandon tomato production in this region. Severe outbreaks of this disease have occurred in Southern Europe and America (Czosnek *et. al.*, 1990, p. 1-6), (Nakhla *et. al.,* 1994, p. 926). TYLCV, much like other Gemini viruses, is not seed-transmissible (Kashina *et al.,* 2003, p. 188–199) and cannot be mechanically inoculated; it is transmitted by the whitefly *Bemisia tabaci* (Brown and Czosnek, 2002, p. 65–100), (Ghanim and Czosnek, 2000, p. 4738-4745). The improvement of diagnostic methods for TYLCV has been more feasible as further information has become available regarding the physical and chemical nature of the virus (Pico *et. al.*, 1996, p. 151-196), and PCR has been used to detect the virus in both plants and vectors (Sinisterra *et. al*., 2005, p. 1525-1532, El-Dougdoug *et. al*., 2006, p. 1151-1155). Since geminiviruses replicate by dsDNA intermediate, PCR techniques are adequate for diagnostic in this group (Rojas *et. al.*, 1993, p. 340-347). This method is more sensitive than hybridization techniques and does not require the use of radioactivity (Pico *et. al.*, 1996, p. 151-196). TYLCV is the most severe viral disease affecting tomato in Palestine; it is an epidemic approaching an occurrence rate of 95% in certain tomato-growing sites (Sawalha unpublished data). Although it has a narrow host range, laboratory studies confirmed that the virus affects numerous botanical families including Solanaceae,

Malvaceae and Leguminosae (Oetting and Yunis, 2004, p. 69), (Sawalha, 2000, p. 104-114).

Therefore, the current research aims to study the possibility of these plants serving as natural hosts that harbor the virus during the summer period and as sources for the virus in the tomato crops in the following growing season. This study was carried out in the summer season because during this period the farmers in the studied regions begin cultivating fall tomato in large areas, including open fields and greenhouses. Moreover, parts of the tomato-growing sites in Jenin districts are used during the summer for tobacco (*Nicotiana tabacum* L.) cultivation. Additionally, farmers in this region used to begin transplanting tomato near tobacco fields before the tobacco removal from the lands ever occurred, after crop maturation and last harvesting. Furthermore, in Jericho and parts of the Tobas district, farmers begin to cultivate fall tomato after leaving the lands for about four months, a direct result of the hot conditions that prevail during this time. This situation therefore emphasizes the necessity for investigation of the summer's natural hosts that act as viral sources for fall-grown tomato. The research also investigates the distribution of these natural hosts and their probability of infection in several locations in Palestine.

Material and Methods

Sample Collection

The research was conducted during the summer months of June through September, 2003 and 2004, in three main Palestinian districts including Jenin, Tobas and Jericho. These locations were selected since they represent the main tomato growing sites in the country. The fields located in Jenin were selected in Al-Zababdeih, Al-Jededeih, Qabatyya and Seris regions. In Tobas, fields were selected in Kashda and in Al-Far'a regions. The fields of Jericho were selected in Lehef Sumeet (Sahel Sumeet) region, which lies on the edges of the Jordan Valley about 10

kilometres west of the Jordan River and 50 kilometres north of the Jericho city.

The studied regions were monitored weekly and leaf samples were collected in the Jenin district from tobacco nurseries, tobacco located near tomato fields, and volunteer tomato and tobacco grew in or near tomato fields and greenhouses. In addition, leaf samples were also collected from jimsonweed that grew in very few locations in Palestine. Samples were collected randomly from the top leaves of these plants because they have the maximum viral content. Leaves were then labelled and kept frozen for further laboratory analysis (Sawalha, 2000, p. 71-72).

PCR Testing

The collected samples were tested for TYLCV infection using the polymerase chain reaction (PCR) as described by Navot *et. al.* (1992, p. 1199-1202) and Tortora *et. al.* (2002, p. 254-255) using TYLCV-specific oligonucleotide primers. Sub-genomic fragments of the virus's genome were amplified. The primers were purchased from the Alltech Company, Paisley, UK. The primer sequences were from 5' to 3', P1V, ATACTTGGACACCTAATGGC, nucleotides (nt) 61-80, and P4C, TGGACATCTAGACCTAAG, nt. 2054-2071. The sequence of the P1V corresponds to the viron positive strand, whereas, the P4C is complementary to the viron strand. The reaction was performed 35 cycles using Perkin Elmer automated thermocycler. The cycling protocol was done in tubes containing 25 µl of PCR cocktail using the cycling program of the table 1.

Table (1): Cycling program of the PCR.

Initial cycle	Temperature in degree Celsius	Time in minutes
Annealing	65	5
Extension	72	5
Denaturation	92	1
Subsequent cycles		
Annealing	55	2
Extension	72	4
Denaturation	92	1

The amplified products were electrophoresed in 1.2% agarose gel, stained with 0.5 µg/ml ethedium bromide and photographed. Fragment size of DNA product was estimated depending on the slandered curves of the typical relationship between the fragment size in base pair (bp) and the mobility of DNA bands *in Lambda Hind III EcoR1*, 125-21226 BP (Sawalha, 2000, p. 55-58).

Population and Distribution of TYLCV Natural Reservoirs

The population density of the virus's natural reservoirs, including jimsonweed and volunteer tomato and tobacco plants, was determined in the tomato growing sites of the studied regions especially in the fields and their edges. The density of plant species (D) was calculated by looking at the number of individual plants of the same species (N) with relation to the total area (A) according to the following equation:

$$D = N/A$$

The average number was estimated by observing the plant species about 100 meters away from the center of the main tomato growing site in each of the studied regions, and then calculated in terms of hectares (Stehlik *et. al.,* 2006, p. 387–394), (Ricklefs and Miller, 2000, p. 271-297).

Occurrence of TYLCV on Collected Samples

The occurrence of the virus was determined by calculating the percentage of viral infection for each of the different samples. The percentage of infected samples was calculated by dividing the number of infected samples by the total number of the collected ones (Sawalha, 2000, p. 24-25).

Development of TYLCV on Volunteer Tomato Plants

The virus's effect on volunteer tomato plants was determined in all regions during the summer months. Therefore, several tomato plants were selected in each region and checked regularly every two weeks for sample collections. The samples were observed for any indication of TYLCV infection and the percentage of infection was calculated.

Statistical Analysis

The field experimentation and the sample collection from the studied regions were done according to the standards of the Completely Randomized Design (CRD). The analysis of the data was conducted using the Two-Sample Tests of Proportions (TSTP) to compare the rate of occurrence of the virus in each of the studied regions. The results were then analysed using a level of significance when $\acute{\alpha} = 0.05$ (Lind *et. al.*, 2005, p. 262-263).

Results

Sample Collection and Field Survey

One hundred plant samples from each volunteer tomato and tobacco were collected from each location in the studied regions. Jimsonweed was collected only from Lehef Sumeet of the Jericho district seeing as this plant was not found in any of the other locations (Table 2).

Table (2): Samples collected from the studied regions and statistical analyses using TSTP when Z table (critical value) = 1.645.

Region and its number	Volunteer tomato			Volunteer tobacco		Jimsonweed	
	CS	NIS	SR+ZV	CS	NIS	CS	NIS
1- Qabatyya	100	70	4(2.46)	100	10	0	0
2- Al-Zababdeih	100	61	6(4.48) 7(5.80)	100	6	0	0
3- Al-Jededeih	100	65	4(1.72)	100	8	0	0
4- Seris	100	53	6(6.37)	100	5	0	0
5- Kashda	100	70	4(2.47)	0	0	0	0
6- Al-Far'a	100	93	1(4.23) 3(4.86) 5(4.23)	0	0	0	0
7- Jericho	100	95	1(4.70) 3(5.30) 4(5.80) 5(4.70)	0	0	2	1

CS: Collected samples (total), NIS: Number of infected samples, SR+ZV: Region with significant difference and Z-Value between brackets

* Note: there is no significant difference between volunteer tobacco plants in the studied regions as the calculated Z-values are less than critical value

PCR Testing

A subgenomic fragment of TYLCV with a fragment length of about 2000 base pair (bp) was amplified by a combination of P1V (20-mer primer from the intergenic region) with an 18-mer primer (P4C). The PCR was able to detect TYLCV from tomato, tobacco and jimsonweed (Plate 1).

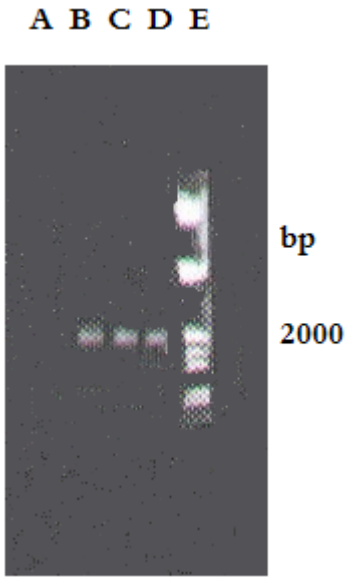

Plate (1): Agar gel electrophoreses of amplified PCR products of TYLCV DNA from infected plants. The primers are P1V and P4C.

Lane A: Healthy tomato plant

Lane B: Infected tomato

Lane C: infected tobacco

Lane D: Infected jimsonweed

Lane E: DNA size marker (*Lambda Hind III Eco* R1, 123-21226 bp).

Population and Distribution of TYLCV Natural Reservoirs

Results demonstrated that the maximum population of volunteer tomato was recorded in the tomato growing site of Al-Far'a, followed by Kashda and Qabatyya. The estimated population of volunteer tomato plants in these regions was 65, 50, 40 plants per hectare of the tomato growing sites, respectively (Fig. 1). The tomato growing sites of Jericho, Al-Jededeih, Al-Zababdeih and Seris showed a low population of volunteer tomato plants per hectare. Observing volunteer tobacco, the maximum population recorded in Seris was 62 plants per hectare, followed by Al-Jededeih and Al-Zababdeih regions with lower distribution of tobacco plants at 60 and 55 plants per hectare (Fig. 1). Qabatyya illustrated the lowest presence of tobacco plants at 13 plants per hectare (Fig. 1).

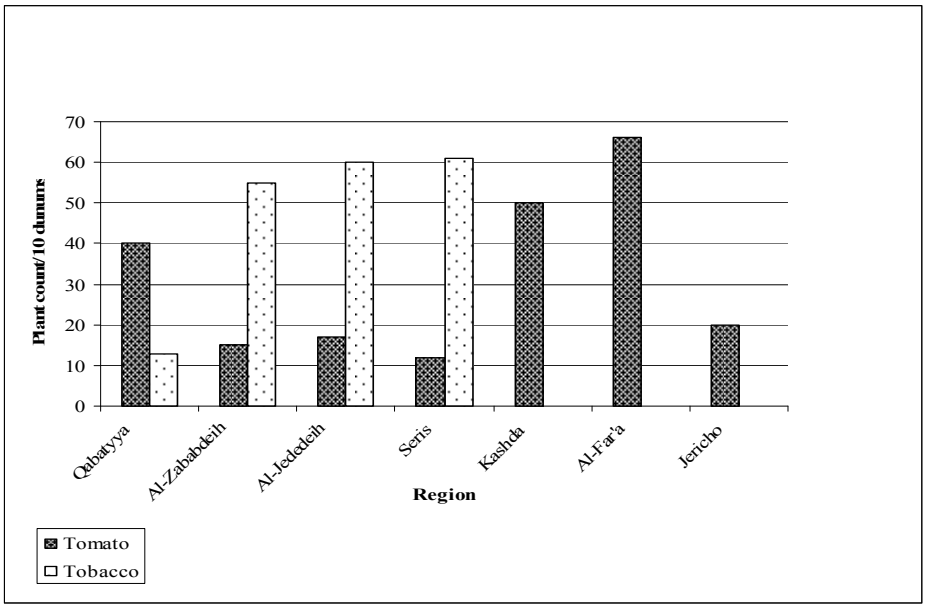

Fig. (1): Population density of volunteer tomato and tobacco plants in Jenin, Tobas and Jericho districts

Occurrence of TYLCV on Collected Samples

Using PCR to test viral infection, it revealed that the maximum percentage of infection of volunteer tomato plants occurred in Jericho at 95%, and 93% in Al-Far'a regions (Fig. 2). The regions of Kashda and Qabatyya revealed an intermediate infection rate of 70%, followed by Al-Jededeih, Al-Zababdeih and Seris which estimated 65%, 61%, and 53%, respectively (Fig. 2). The utmost infection of volunteer tobacco plants was recorded to be 10% in Qabatyya, and 5-8% in Al-Jededeih, Al-Zababdeih, and Seris regions (Fig. 2). Experimenting with jimsonweed proved that one out every two collected plants was infected (Fig. 2).

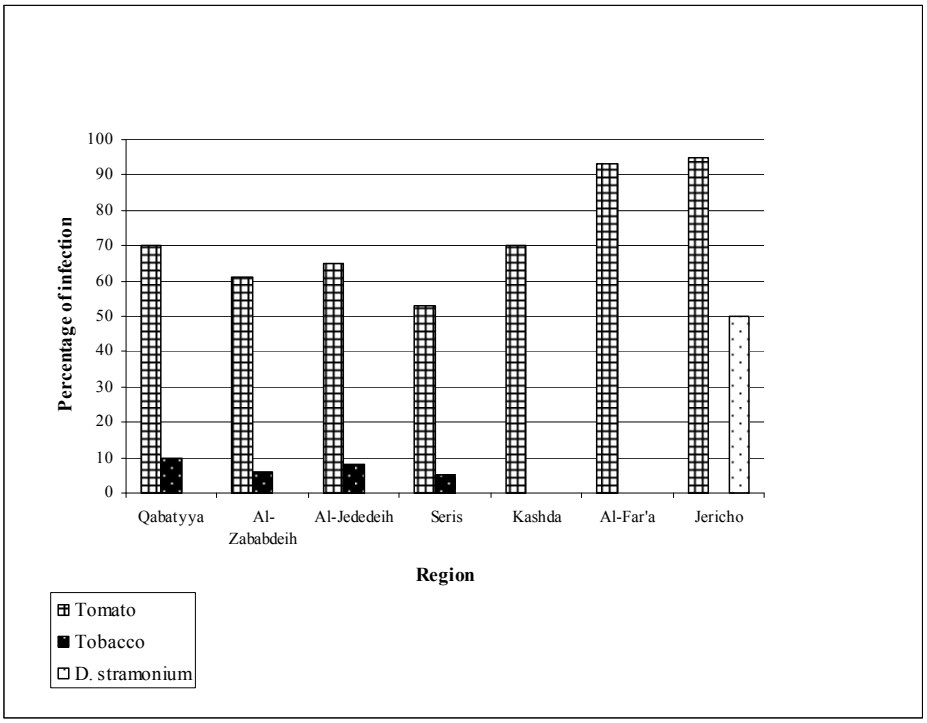

Fig. (2): Percentage of TYLCV infection to volunteer tomato and tobacco plants and jimsonweed in Jenin, Tobas and Jericho districts.

Furthermore, the incidence of the virus in tobacco fields planted near tomato crops revealed that the maximum infection rate occurred in Qabatyya at 6%, followed by Al-Zababdeih and Al-Jededeih regions which showed only 2.5% of infection. The virus was recorded to have a 3.5% infection in the case of the tobacco nursery established in the tomato growing site of Qabatyya (Fig. 3).

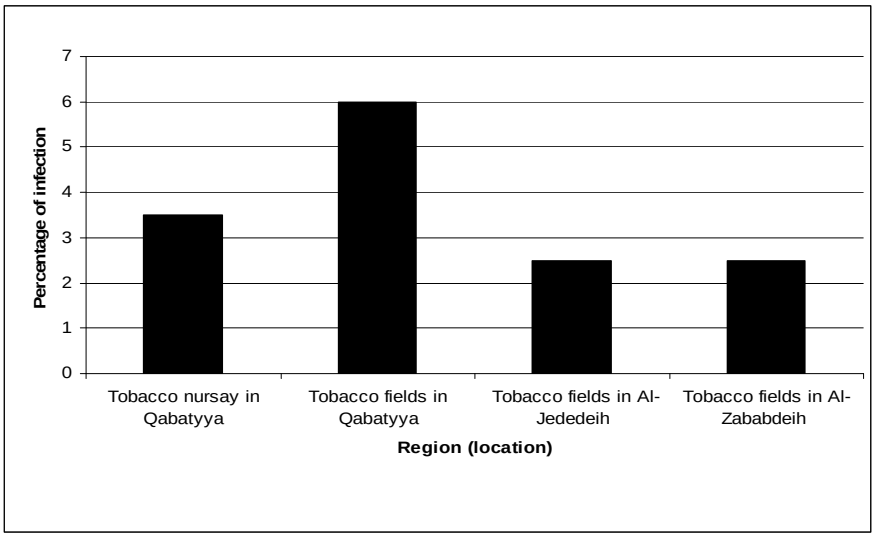

Fig. (3): Incidence of TYLCV in tobacco nursery and in tobacco fields planted near tomato fields in Jenin district.

Development of TYLCV on Volunteer Tomato Plants

PCR tests proved that the virus first appeared during June and increased during September in all regions. The maximum infection percentage was observed in September, except for in the Al-Far'a region which illustrated 93% infection during July. A sudden spike of infection was recorded during July in all regions except Jericho, where it was observed high during the summer months, and peaked at 95% during August and September (Fig. 4).

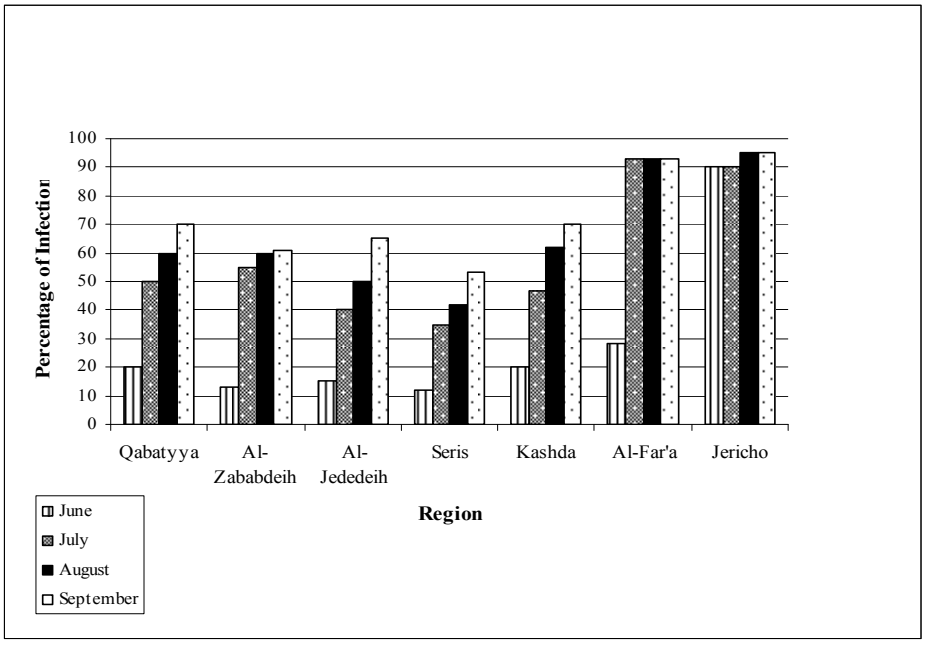

Fig. (4): TYLCV development on volunteer tomato plants in the studied regions during the summer months

Statistical Analysis

Statistical analysis based on the computed Z values revealed that the virus was highly significant on volunteer tomato plants in Jericho, followed by Al-Far'a, Kashda, and Qabatyya. Moreover, statistical comparisons of the effect of the virus on volunteer tomato plants in the studied regions gave evidence to a lot of differences in the significance of the virus. The most significant difference for TYLCV was recorded between the virus's occurrence in Al-Far'a compared with the Seris region, followed by the comparisons between Jericho/Seris, Jericho/Al-Zababdeih and Jericho/Al-Jededieh. In case of volunteer tobacco, the statistical analysis revealed no significant difference in the occurrence of the virus in the volunteer tomato between the regions of study (Table 2).

Discussion

The occurrence of high populations of volunteer tomato plants in the studied regions especially in Al-Far'a, Kashda and Qabatyya may be attributed to the poor agricultural practices used by traditional farmers and to the extensive tomato cultivation in these regions.

The higher rate of infection of volunteer tomatoes in Jericho and Al-Far'a regions may be directly related to the warm climate which activates the whitefly vectors for longer periods during the year and increases the possibility of viral transmission to volunteer plants (Jetter *et. al.*, 2001, p. 1-60), (Gerling, 1990, p. 57-112). Therefore, these plants can serve as a good source of primary infection in the following season. Al-Musa (1986, p. 199-208) reported that volunteer tomatoes growing near hedges surrounding citrus groves and along irrigation canals are reservoirs of TYLCV in the Jordan Valley.

Although very few jimsonweeds were found only in Jericho, these plants can serve as natural reservoirs of TYLCV and may enhance the virus' survival as the summer progresses in the region. To this extent, Makkouk and Laterro (1983, p. 1-7) reported that jimsonweed is a potentially important reservoir of TYLCV in the Mediterranean region. Mansour and Al-Musa (1992, p. 122-125) and Sawalha (2000, p. 68) found that, in Jordan, this plant is a good symptomatic host for the Jordanian isolate of TYLCV under laboratory conditions.

Either tobacco crops or volunteer plants were present in all studied regions of the Jenin district. Therefore, the ability of this plant to harbor the virus and provide a primary source of inoculum to newly established tomato crops is feasible. This possibility increases when tobacco is growing in the same area with tomato crops. Such a case increases the number of infected plants that act as a potential source of inoculum to newly established tomato. This finding is supported by other researches which underlined that tobacco reacts with TYLCV in a complete symptomless manner under lab condition (Mansour and Al-Musa, 1992,

p. 122-125). Furthermore, Ioannou and Hadjinicolis (1991, p. 3-6) recovered TYLCV from the complete symptomless tobacco planted near tomato fields in Cyprus. Thus, the crop was considered a secondary host plant that could potentially act as a natural reservoir for TYLCV. Al-Musa (1986, p. 199-208) reported that tobacco serves as an important reservoir for TYLCV in the Jordan Valley.

Additionally, the infection of tobacco nurseries in the Jenin district may facilitate the transmission of the virus through infected seedlings to the regions where they grow. Also, the infected seedlings may act as a primary source of infection for tomato fields established in the region. Al-Musa and Takrouri (1996, p. 243-247) reported that the occurrence of disease inside the nurseries is an important factor in disease epidemiology and effect, since it can spread to the fields and participate in crop infection. The rapid increase of the virus's occurrence rate during July in both the Jenin and Tobas districts may be attributed to the large populations of whiteflies which appear during this period. However, the situation is much different in the Jericho district; the virus infection of volunteer tomato began at a high rate and continued in a steady fashion during the summer months (Fig. 4). Such cases may be attributed to the fact that this area has moderately high temperatures throughout the year which render these plants to be vulnerable to invasion at an early stage by whiteflies, which appear very active during Spring (Jetter *et. al.*, 2001, p. 1-60).

Conclusion

Based on previous research and experimentation, it can be concluded that to be aware of the naturally infected hosts of TYLCV is of dire importance in order to be able to take control of and manage the virus. Ioannou (1987, p. 367-373) observed a significant decrease in disease incidence when the sources of inoculum were eliminated from tomato growing sites. Therefore, removal of volunteer tomato and tobacco plants, as well as solanaceous weeds including jimsonweeds from tomato growing sites is of prime necessity in controlling the disease. In addition,

avoiding tobacco near tomato fields may reduce the sources of the virus and provide a satisfactory level of disease control and prevention. Furthermore, indexing and certification schemes in tobacco nurseries in Jenin are very important factors in minimizing the sources of the virus source in the region.

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