Canadian Journal of Pesticides & Pest Management

ETC ETERA PUBLICATIONS

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First Report of a Mixed Infection with *Tomato yellow leaf curl virus* TYLCV and *Tomato spotted wilt virus* TSWV in Some Economic Crops in the Syrian Coastal Region

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Submitted: 21.03.2019 | Accepted: 26.11.2019 | Published: 24.12.2019

Abstract A survey of *Tomato spotted wilt virus* (TSWV, *Tospovirus*, Bunyaviridae) and *Tomato yellow leaf curl virus* (TYLCV, *Begomovirus*, Geminiviridae) infection in some important crops in the Syrian coastal region was made. Samples showing leaf curl and yellowing symptoms were collected from 19 regions during the 2017 and 2018 growing seasons (311 mixed samples and 1244 individual samples). Triple antibody sandwich ELISA (TAS–ELISA) was used to detect TYLCV, whereas tissue blot immunobinding assay (TBIA) was used to detect TSWV. The results of TAS–ELISA and TBIA showed the presence of the two viruses in all visited regions, causing single or mixed infections (26.04% and 32.79% assingle infections by TSWV and TYLCV, respectively, and 14.14% as mixed infection). The highest rate of TSWV infection was 50% in Setkheres region, and that of TYLCV infection was 54.54% in Yahmour region. The infection rate of TSWV in tobacco was 47.45%, and that ofTYLCV in pepper was 51.31%, whereas the rate of mixed infection in tomato was 25.45%. This is the first report of TSWV infection in grapevine (6.66%) and *Ocimumbasilicum* (16.66%) and of TYLCV infection in *Phaseolus vulgaris* (27.02%) in Syria.

Keywords TSWV; TYLCV; TBIA; TAS-ELISA; Syrian coast

How to cite Akel, E.; Qusayi, A.-R.; Nadine, A.; Ismail, I.D. First Report of a Mixed Infection with *Tomato yellow leaf curl virus* TYLCV and *Tomato spotted wilt virus* TSWV in Some Economic Crops in the Syrian Coastal Region. *Can. J. Pestic. Pest Manag.* **2019**, *1*(1), 37–45; doi:10.34195/can.j.ppm.2019.12.003.

Introduction

The main crops of *Solanaceae*, such as tomato, potato, tobacco, pepper, and eggplant, are subject to several important viral diseases that adversely affect the crop and cause significant production losses. The symptoms of infection by a single virus vary according to the plant host, its sensitivity, the viral strain, the surrounding environmental conditions, as well as to the age of the plant at the time of infection [1]. *Tomato yellow leaf curl virus* (TYLCV: *Begomovirus*, Geminiviridae) [2] causes one of the most dangerous viral diseases and the most destructive disease of tomato crop in a short period. It has several strains which damage 50%–100% of the crop [3–5]. TYLCV infection of *Phaseolus vulgaris* has been extensively reported in the tropics and sub-tropics, such as in Lebanon [6], Palestine [7],

Cyprus [8], Iraq, Turkey, Sudan, Tunisia, Nigeria, Senegal, whereas in Thailand [9,10] and Syria, infection has been recordedin tomato and some common wild grasses [11]. This virus was also reported in several cultivated plants such as *P. vulgaris* L., *Petunia hybrid* V., *Capsicum annum* L., *Nicotianatabacum*, *Croton lobatus* L., and *Lens culinaris* M. [12], as well as in wild plants, including *Lycopersicon esculentum* and *Cucurbita pepo*, in Cuba [13]. The virus is transmitted by the white fly *Bemisiatabaci* Gennadius (Homoptera: Aleyrodidae) in a persisting manner [14–16]. The period of virus acquisition by the fly from an infected plant is about 15–30 min [10], and the same period is requested for transmission from the fly to healthy plants. This insect is polyphagous, can infect about 706 plant species belonging to 86 families, and is able to transmit the virus for 20 days since its acquisition, also through its eggs. The virus is also efficiently transmitted by grafting (if not mechanical [17]), but not by seeds [17] or soil [6]. The *L. esculentum* and *Datura stramonium* are used as diagnostic plant species to detect the transmission of TYLCV by whitefly [18,19]. The infected plants show clear dwarfing, wrinkling, and yellowing of the leaves and yellowing between the veins and the edges of young leaves [20–23]. The activity of the virus within the plant increases with the onset of infection, especially in the hot months. Most leaves become wrinkled in sensitive species. The plant appears to be dry, and its leaves seem to shrink. The infection is most common in spring, when symptoms appear early in infected plants in the nursery, about 20–30 days after planting, and cause losses of up to 80% of the cultures. The losses in the case of delayed infection (after flowering and producing fruits) do not exceed 15%–20%. The *Tomato spotted wilt virus* (TSWV *Tospovirus*, Bunyaviridae) is a high-risk virus for many economic crops, such as tomato and pepper, in all their growing regions [24,25]. Tomato is the main host of the virus, among many other hosts belonging to more than 900 species [25–27]. It is also a dangerous virus for ornamental plants in greenhouses [28]. In severe cases, the damage caused by the virus may affect up to 100% of the cultures [24]. The symptoms vary according to the virus strains [29]. Two features characterize TSWV infectionin tomato: (*i*) the bronze coloration of the young leaves, in addition to their wrapping, followed by the appearance of several small dark spots; (*ii*) the folding of the leaves at the bottom of the plant, making the plant look wilted, as if affected by wilt caused by *Fusarium* and *Verticillium* fungi [30]. On green fruits, pale and yellow spots appear and become prominent later. They look like eyes or appear as yellow or dark concentric circles alternating with green ones, which later become purple or red and finally affect the the whole mature fruits, distorting it. The stems and stalks appear dark [31]. Herbs are an important source and reservoir of the virus [32–34]. The virus is transmitted by different species of thrips (at least six species) [35–37]. The virus was recorded in Syria in pepper [38], tomato and pepper [39,40], tobacco and some herbs [39], peanuts [41], and tomatoes in greenhouses [42,43].

The early detection of economic viruses in crops and herbs, especially those of the Solanaceae family, and the determination of their spread are critical for the application of appropriate and integrated methods for their eradication and management. Therefore, this research was carried out to highlight the distribution of TSWV and TYLCV in the Syrian coastal region and to identify the most important host plants for single and mixed infections.

Materials and Methods

Field Survey and Sampling

Field surveys were conducted in the 2017–2018 growing season in different regions of the Syrian coast, in Lattakia and Tartous provinces. In all, 311 mixed samples and 1244 individual samples were collected from Sarsakia, Zagrin, Bloran, Bahlolia, Kesmin, Birneh, Hinnadi, Katria, Rwemia, Set-Kheres, Sheikh Hussamo, Bassa, and Jableh in Lattakia, and from Kadmous, Hraisson, Mhwarti, Gamassa, Amreet, and Yahmour in Tatrous. The samples were selectively and randomly collected from plants showing symptoms of viral-like infection (mottling, mosaic, vein-clearing, curling and deformation of leaves, bronze discoloration between veins, leaves roll, stunting, yellowing of the edges of the leaves and between the veins, vein-banding, etc.).

Serological Tests

Serological tests were carried out in the virology laboratory for Agricultural Scientific Research in Lattakia Centre. Two serological tests were used: the tissue blot immunoassay technique (TBIA) for the detection of TSWV (polyclonal antibodies produced by the Spanish company «Plant Print»), following the protocol according to Whitfield et al. (2003) [44], and the TAS–ELISA technique for the detection of TYLCV (monoclonal antibodies were obtained from the German company «LOWE»), following the protocol according to Clark and Adams (1977) [45].

Results and Discussion

Symptoms Recorded in the Sampled Crops

The results of the field surveys identified various, widely distributed symptoms, similar to those caused by viruses on most crops in the surveyed regions. These symptoms were more pronounced and distinct in some regions than in others, with infection rates ranging from 10% to 80%. Mosaic and yellowing between veins, as well as curling and deformation of the leaves were the most common symptoms in tomato. Other symptoms were also recorded: bronze coloration between veins and dwarfing in some tomato plants, ring spots and vein necrosis in tobacco plants, ringspots and mosaic in peppers. Ring spots were also observed on *O. basilicum* and grapevine in Hraisson (Banias, Tartous). These symptoms were previously reported in several studies as caused by TYLCV [20–23] and TSWV [30,31]. Some tomato samples showed mixed symptoms, consisting in wrinkling and yellowing of the leaves and bronze coloration. The mixed infection in pepper plants showed yellowing, severe leaf deformations, and leaf curling.

Serological Tests

The results of the serological tests confirmed the presence of TYLCV and TSWV in 32.79%, 26.04%, and 14.14% of samples in single and mixed infection, respectively (Table 1). These results are consistent with those of previous studies on the presence of TYLCV [11] and TSWV [42,43]. The incidence of TSWV in a previous study in Syria was 11.5% in tomato samples and 41.2% in pepper samples; the highest prevalence of the virus was found in tomato samples collected from Konitracity 41% [40]. These results are in contrast with those of another study that reported the absence of the virus in Tartous. Our results indicated the occurrence of TYLCV in 32.79% of samples, which could be due to the transmission of the virus to several crops by *B. tabaci*. The presence of TSWV was higher in this study than in previous studies in Syria pepper samples, which reported the virus in 3.6% [38], 3.1% [40], and 22.06% of the samples [39]. This virus was also identified in 64% of *P. vulgaris* samples collected from greenhouses in Turkey [46].

Table 2 indicates that the infection by the two viruses spread differently in the sampled regions. The incidence of TSWV infection ranged from 9.09% to 50%. The highest rate of infection was in Set-Kheres (50%), and the lowest rate was in Katria (9.09%). In contrast, TYLCV infection was recorded in all regions except Sheikh Hussamo and Amreet. The infection rate ranged from 14.28% to 54.54%, the highest rate was in Yahmour (54.54%), and the lowest rate was in Zagrin, Bassa, and Rwemia (14.28%). The high distribution of TYLCV in Yahmour could be due to the wide distribution of greenhouses next to the different crops, and consequently, to the presence of its vector in high density. Mixed infection by the two viruses was recorded in all studied regions except in Rwemia, Bassa, Sheikh Hssamo, and Gamassa. The highest rate was in Set-Kheres (37.5%), and the lowest rate was in Zagrin, Katria, and Kadmous (9.09%). This variation could be related to the number of samples collected from each crop and region, as well as to the time of sampling, in addition to the presence of the insect vectors for these two viruses.

Table 1. Rate of infection by *Tomato yellow leaf curl Virus* (TYLCV) and *Tomato spotted wilt virus* (TSWV) in some important crops in the Syrian coastal region. PAbs: polyclonal antibodies.

Region/Site	Host Plant	No. of Tested Samples	Number of Samples That Reacted Positively for the Following Viruses			Total Samples That Reacted Positively with PAbs and Their Percentage (%)		
			TSWV	TYLCV	TSWV + TYLCV	TSWV	TYLCV	TSWV + TYLCV
Sarsakia	Tomato	4	1	1	1	25	25	25
	Pepper	5	2	1	1	40	20	20
	Tobacco	4	2	-	-	50	-	-
	Eggplant	3	-	-	-	-	-	-
	Phaseolus vulgaris	2	-	1	-	-	50	-
Zagrin	Tomato	4	1	1	1	25	25	25
	Pepper	5	2	2	1	40	40	20
	Tobacco	6	4	-		66.66	-	-
	Eggplant	2	-	-	-	-	-	-
	P. vulgaris	1	-	-	-	-	-	-
	Grapevine	3	-	-	-	-	-	-
Bloran	Tomato	2	-	1	-	-	50	-
	Pepper	3	1	1	1	33.33	33.33	33.33
	Tobacco	4	2	1	1	50	25	25
Bahlolia	Tomato	4	-	2	-	-	50	-
	Pepper	3	2	1	1	66.66	33.33	33.33
	Tobacco	6	4	1	1	66.66	16.66	16.66
	Eggplant	4	-	1	-	-	25	-
	P. vulgaris	3	-	-	-	-	-	-
Kesmin	Tobacco	4	2	1	1	50	25	25
	Pepper	3	1	2	1	33.33	66.66	33.33
	P. vulgaris	2	-	1	-	-	50	-
	Potato	4	1	-	-	25	-	-
Birneh	Tomato	3	1	1	1	33.33	33.33	33.33
	Pepper	4	1	2	1	25	50	25
	Tobacco	5	3	1	-	60	20	-
	P. vulgaris	2	-	1	-	-	50	-
	Grapevine	1	-	-	-	-	-	-
Set-Kheres	Pepper	3	3	2	2	100	66.66	66.66
	P. vulgaris	2	-	1	-	-	50	-
	Potato	3	1	1	1	33.33	33.33	33.33
Hinnadi	Tomato	2	1	1	1	50	50	50
	Pepper	4	2	3	1	50	75	25
	Tobacco	2	-	1	-	-	50	-
	Eggplant	5	-	2	-	-	40	-
	P. vulgaris	3	-	1	-	-	33.3	-
	Grapevine	1	-	-	-	-	-	-
	Potato	3	1	1	-	33.3	33.3	-
Katria	Tomato	-	-	-	-	-	-	-
	Pepper	3	1	2	1	33.3	66.66	33.3
	Tobacco	-	-	-	-	-	-	-
	Eggplant	5	-	1	-	-	20	-
	P. vulgaris	3	-	-	-	-	-	-
Rwemia	Tomato	2	-	1	-	-	50	-
	Pepper	3	1	2	1	33.33	66.66	33.33
	Tobacco	-	-	-	-	-	-	-
	Eggplant	2	-	-	-	-	-	-
	P. vulgaris	2	-	-	-	-	-	-
	Potato	2	-	-	-	-	-	-
Bassa	Tomato	-	-	-	-	-	-	-
	Pepper	2	-	1	-	-	50	-
	Tobacco	-				-	-	-
	Eggplant	3	-	-	-	-	-	-
	P. vulgaris	2	-	-	-	-	-	-
Sheikh Hussamo	Pepper	2	-	-	-	-	-	-
	Tobacco	3	1	-	-	33.33	-	-
	Grapevine	1	-	-	-			

Table 1. *Cont.*

Region/Site	Host Plant	No. of Tested Samples	Number of Samples That Reacted Positively for the Following Viruses			Total Samples That Reacted Positively with PAbs and Their Percentage (%)		
			TSWV	TYLCV	TSWV + TYLCV	TSWV	TYLCV	TSWV + TYLCV
Jableh	Tomato	9	3	5	2	33.33	55.55	22.22
	Pepper	7	2	4	1	28.57	57.14	14.28
	Tobacco	8	2	3	2	25	37.5	25
	Eggplant	4	-	1	-	-	25	-
	P. vulgaris	3	-	1	-	-	33.33	-
	Basil	2	-	-	-	-	-	-
	Peanut	3	1	1	1	33.33	33.33	-
	Potato	6	1	2	1	16.66	33.33	16.66
Kadmous	Tomato	4	2	2	1	50	50	25
	Pepper	3	-	1	-	-	33.33	-
	Tobacco	2	-	1	-	-	50	-
	Eggplant	1	-	-	-	-	-	-
	P. vulgaris	1	-	-	-	-	-	-
Hraisson	Tomato	5	2	4	2	40	80	40
	Pepper	8	5	4	3	62.5	50	37.5
	Tobacco	9	4	3	2	44.44	33.33	22.22
	Eggplant	3	-	1	-	-	33.33	-
	Peanut	3	1	-	-	33.33	-	-
	Grapevine	3	1	-	-	33.33	-	-
	Basil	4	1	-	-	25	-	
	P. vulgaris	2	-	1	-	-	50	-
Mhwarti	Tomato	4	2	1	1	50	25	25
	Pepper	5	2	3	1	40	60	20
	Tobacco	6	4	2	2	66.66	33.33	33.33
	Eggplant	3	-	1	-	-	33.33	-
	P. vulgaris	2	-	1	-	-	50	-
	Grapevine	4	-	-	-	-	-	-
Amreet	Tomato	3	-	-	-	-	-	-
	Pepper	6	1	3	1	16.66	50	16.66
	Tobacco	-	-	-	-	-	-	-
	Eggplant	-	-	-	-	-	-	-
	Phaseolus vulgaris	2	-	-	-	-	-	-
Gamassa	Tomato	5	2	4	2	40	80	40
	Pepper	4	1	3	1	25	75	25
	Tobacco	-	-	-	-	-	-	-
	Egg plant	2	-	-	-	-	-	-
	P. vulgaris	3	-	1	-	-	33.33	-
	Grapevine	2	-	-	-	-	-	-
Yahmour	Tomato	4	1	3	1	25	75	25
	Pepper	3	2	2	1	66.66	66.66	33.33
	Tobacco	-	-	-	-	-	-	-
	Eggplant	2	-	-	-	-	-	-
	P. vulgaris	2	-	1	-	-	50	-
Total		311	81	102	44	26.04	32.79	14.14

The results in Table 3 show that both viruses were present in most of the sampled crops, as single or mixed infection. The highest rates of infection were 51.31% for TYLCV and 47.45% for TSWV. The incidence of TSWV in tomato, pepper, tobacco, potato, peanut, basil, and grapevine was 29.09%, 38.66%, 47.45%, 22.22%, 33.33%, 16.66%, and 6.66%, respectively. No infection was detected in eggplant. These results are consistent with those of many other studies indicating the distribution of this virus in a large number of *Solanaceae* crops [24,25,39,40,42,43,47]; in contrast, Halabi et al., 2014 [39] reported eggplant infection by TSWV. The incidence of TYLCV infection in tomato, pepper, tobacco, eggplant, potato, peanut, *P. vulgaris* was 50.90%, 51.31%, 23.72%, 20.51%, 22.22%, 16.66%, and 27.07%, respectively.

Table 2. Rate of infection by TYLCV and TSWV in the surveyed region on the Syrian coast.

Region	No. of Tested Compound Samples	Number of Compound Samples That Reacted Positively for the Following Viruses			Total Compound Samples That Reacted Positively with PAbs and Their Percentage (%)		
		TSWV	TYLCV	TSWV + TYLCV	TSWV	TYLCV	TSWV + TYLCV
Sarsakia	18	5	3	2	27.77	16.66	11.11
Zagrin	21	7	3	2	33.33	14.28	9.52
Bloran	9	3	3	2	33.33	33.33	22.22
Bahlolia	20	6	5	2	30	25	10
Kesmin	13	4	4	2	30.76	30.76	15.38
Birneh	15	5	5	2	33.33	33.33	13.33
Set-kheres	8	4	4	3	50	50	37.5
Hinnadi	20	4	8	2	20	40	10
Katria	11	1	3	1	9.09	27.27	9.09
Rwemia	11	1	3	1	-	14.28	-
Bassa	7	-	1	-	-	14.28	-
Sheikh Hussamo	6	1	-	-	16.66	-	-
Jableh	42	9	17	7	21.42	40.47	16.66
Kadmous	11	2	4	1	18.18	45.45	9.09
Hraisson	37	14	13	7	37.83	35.13	18.91
Mhwarti	24	8	8	4	33.33	33.33	16.66
Amreet	11	1	3	1	9.09	27.27	9.09
Gamassa	16	3	8	3	18.75	50	18.75
Yahmour	11	3	5	2	27.27	54.54	18.18
Total	311	81	102	44	26.04	32.79	14.14

Table 3. Rate of infection by TYLCV and TSWV according to the plant host in the Syrian coastal region.

Host Plant	No. of Tested Samples	Number of Samples That Reacted Positively for the Following Viruses			Total Samples That Reacted Positively with PAbs and Their Percentage (%)		
		TSWV	TYLCV	TSWV + TYLCV	TSWV	TYLCV	TSWV + TYLCV
Lycopersicon esculentum	55	16	28	14	29.09	50.90	25.45
Capsicum annum	76	29	39	19	38.66	51.31	25
Nicotiana spp.	59	28	14	9	47.45	23.72	15.25
Solanum melongena	39	-	8	-	-	20.51	-
Solanum tuberosum	18	4	4	2	22.22	22.22	11.11
Vitis spp.	15	1	-	-	6.66	-	-
Phaseolus vulgaris	37	-	10	-	-	27.02	-
Arachis hypogaea	6	2	1	-	33.33	16.66	-
Ocimum basilicum	6	1	-	-	16.66	-	-
Total	311	81	102	44	26.04	32.79	14.14

No infection was reported in grapevine and basil, and this result agrees with others on *Solanaceae* [6–11,13,41]. The virus was recorded in *P. vulgaris* (27.77%) for the first time in Syria [26]. The highest occurrence rate of TSWV was in tobacco (47.45%), and that of TYLCV was in pepper (51.31%). Mixed infection was observed on tomato, pepper, tobacco, and potato (25.54%, 25%, 15.25%, and 11.11%, respectively). The differences in infection rates in the same host plant between regions could be due to differences in the cultivated variety and thus in their sensitivity to the virus, in addition to differences in vector activity in relation to the carried virus in the sampled regions or to different viral strains present in each region. More than one strain of TYLCV was recorded in Syria [48]. The absence of infection by one or both viruses in some plants does not necessarily reflect the absence of the viruses [49].

This study indicates an increased risk of TSWV and TYLCV infection for a large number of economic crops in Syria (tomato, tobacco, pepper, potato), as well as that a mixed infection may be due to more aggressive viral strains. This reflects the need to quickly take actions in order to reduce the viruses' distribution and, consequently, the economic damage they cause [50]. Some strategies can be proposed: control of the diffusion of the insect vectors and the development of resistant plant varieties; prevention of the transmission of the viruses from infected areas to healthy ones; control of the presence in the fields of plant herbs that can host the viruses and control of mixed cultures including more than one host, which ensure the appropriate conditions for the spreading of both viruses and thus their continuous presence throughout the year.

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