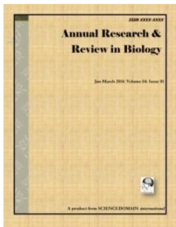

Annual Research & Review in Biology
4(18): 2804-2814, 2014

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Quantitative Effects of Single and Multiple Infections with Four Viruses on Tomato Production

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Author's contribution

This whole work was carried out by the author HS.

Original Research Article

Received 22nd January 2014
Accepted 4th April 2014
Published 7th May 2014

ABSTRACT

Studying the quantitative effect of tomato viruses including tomato yellow leaf curl (TYLCV), cucumber mosaic (CMV), tomato mosaic (TMV) and potato virus Y (PVY) revealed that tomato productivity and fruit weight were severely affected by the number of viruses infected the plant at the same time. Simultaneous occurrence of the four viruses on tomato plants caused drastic reduction in the number of fruit and weight approaching 96% and 93% respectively. Also, mixed infection of tomato with three viruses reduced the number of fruit and weight by72-92% and 52-84% respectively. Plant infection with TYLCV alone reduced the fruit number by 77% and 46% for fruit weight. Single infection by either CMV or TMV caused a reduction in the number of fruit and weight by 63% and 25% or 52% and 12% respectively.

Keywords: Tomato viruses; multiple infection; Palestine.

1. INTRODUCTION

Tomato is one of the most essential and commercially recognizable vegetable crops throughout the world for both the fresh market and food industry. The crop is a significant source of nutrition for substantial portions of the world's population because this crop is

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widely cultivated and consumed extensively. Tomatoes are rich sources of vitamins, especially ascorbic acid and carotene, and antioxidants [1].

Tomato is the most important crop grown in Palestine, occupying 2371 hectares, with an annual production of about 204,000 metric tons. It is the main greenhouse crop and it is also cultivated in low tunnels and in open fields [2]. Tomato production, however, has not yet reached its full potential due to many factors of which diseases are the most important [2]. In this regard, virus diseases affect tomato production causing serious losses every year. The amount of damage they cause varies, depends on the particular virus or combination of viruses present, the virulence of the virus strains, the susceptibility of the variety, the timing of infection, the abundance of insect vectors and environmental conditions [3,4]. Tomato yellow leaf curl virus (TYLCV) has become a worldwide economically recognizable disease of tomato [4]. In the Jordan Valley for example, tomato crop during the fall-growing season is infected with such a virus to the extent that production becomes less profitable [5]. TYLCV was first reported in Israel in the 1960s [4]. Cucumber mosaic virus (CMV) is the second serious tomato virus distributed worldwide causing significant losses in the fields and greenhouses [6]. The virus has an extensive host range and is transmitted by aphids in a nonpersistant manner [7]. In Jordan, the virus is highly encountered on cucumber and has the ability to infect tomato, resulting in low grade fruits [8,9]. Tomato mosaic virus (TMV) is another important disease infecting tomato worldwide [7]. This disease was reported as one of the viruses infecting tomato in the neighboring countries including Jordan [9] and Lebanon [4].Tomato is liable for infection with potato virus Y (PVY) as many reports recorded tomato as a good host for such a virus [10]. In Jordan, Al-Musa and Mansour [11] reported that PVY can infect tomato in the Jordan Valley.

In Palestine, TYLCV was highly encountered on summer grown tomato in the northern districts approaching a rate of infection up to 93% [12]. In such a case production has become less beneficial forcing many farmers to switch their choice of crop production from tomato to other vegetables. Furthermore, TMV, CMV and PVY are another important viruses attacking tomato in the northern regions of Palestine with different frequencies [13].

Since a previous investigation was carried out in Palestine and identified TYLCV, CMV, TMV and PVY as diseases parasitize tomato in the region, the current research aims to study the quantitative effects of such viruses on crop production. The amount of crop loss will be determined for a single virus infection and a combination of viruses present

2. MATERIALS AND METHODS

2.1 Collection of Virus Isolates

Twenty seven leaf samples were collected from tomato plants showing viral symptoms including yellowing, mosaic, mottling, curling, malformation and stunting. The samples were collected during May of 2011 from seven fields in Al-Far'a region. The samples were labelled and transferred to the laboratory for analysis using an ice box.

2.2 Biological Assay

The collected samples showing the dominant symptoms of mosaic and mottling were mechanically-inoculated onto diagnostic assay plants, passed through single lesion transfers and maintained in tomato plants as virus isolates suggestive to be CMV, PVY and TMV [14].

On the rest of samples, whiteflies, *Bemisia tabaci*, were allowed 24hr acquisition-feeding period then transferred to healthy tomato plants [15]. The isolate was tentatively identified as TYLCV.

2.3 Serological Identification of Viruses

Precise identification of the virus isolates was done serologically using the standard virus-specific antibodies. Thus, indirect enzyme-linked immunosorbant assay (I-ELISA) adopted by Clark et al. [16] was used to test the plant samples for CMV, PVY and TMV. With respect to TYLCV, the triple-antibody sandwich (TAS)-ELISA was used to test the virus infection according to Macintosh et al. [17], Sawalha et al. [18] and Sawalha [15]. The virus-specific antibodies of CMV, PVY and TMV and the goat anti-rabbit conjugate were purchased from Bioreba, Inc. The polyclonal and monoclonal antibodies of TYLCV, along with the rabbit anti-mouse conjugate, were purchased from Adgen Ltd (Scotland, UK.). The results of the ELISA tests were recorded one hour after the substrate incubation took place using the automated ELISA-Reader. The light absorbance was measured for ELISA wells at 405 nanometers [19].

2.4 Nursery Experiment

The *In Vitro* experiment was done in a well-conditioned greenhouse established in Al-Far'a valley from June to October of 2011. Healthy tomato seedlings of Izmir cultivar (30 days old) were cultivated in pots filled with a disinfected soil and kept in insect-proof conditions. Uniform healthy tomato seedlings were divided into fourteen groups with 10 seedlings each. Irrigation and fertilization were practiced regularly to maintain the plant hygiene. Izmir cultivar was used as it is widely used by farmers due to its good heat tolerance & setting under hot conditions, intermediate nematode resistance, strong vigor and plant power, high fruit quality, and extremely good shelf life.

2.5 Effect of Viral Infection on Tomato Plants

The pathogenic effect of Palestinian isolates of tomato viruses including TYLCV, CMV, TMV and PVY was studied. The study included the effect of those viruses on both plant productivity and yield. The viruses were used to inoculate the plants either separately or in combination with each other. The experiment was laid out in a Randomized Complete Block Design (RCBD) with fourteen treatments divided into two blocks each with five replicates [20]. The plants were inoculated either mechanically or by graft inoculation depending on the virus transmissibility. Graft inoculation was done only for the TYLCV while the other viruses were inoculated mechanically. For graft inoculation, 1-1.5cm long scions separated from young stems of infected tomato source were grafted on the stems of healthy tomato rootstocks 10 centimetres long. The scions and root stocks were firmly wrapped with Para film tapes and covered with bell jars for 2-3 days. Mechanical inoculation was done using sap extracts prepared in 0.01M neutral phosphate buffer containing 0.01M sodium diethyldithiocarbamate (Na-DIECA) and 0.01M cycteine and then used to inoculate plants dusted with 6000-mesh carborundum. Healthy tomato plants were kept uninoculated as the control part of the experiment.

Twenty days later, the inoculated plants were tested by ELISA to emphasize the virus infection as desired. The diseased plants were kept under observation and the fruit number and weight were determined until the experiment was completed two months after plant inoculation.

2.6 Statistical Analysis

Analysis of the data was made using the Two-Sample Tests of Proportions (TSTP).The results were analyzed using a level of significance when ά = 0.05 [20,21].

3. RESULTS

3.1 Serological Identification of Viruses

ELISA readings recorded for the virus-infected samples were at least two times greater than the readings recorded for the virus-free samples (Figs. 1 and 2).

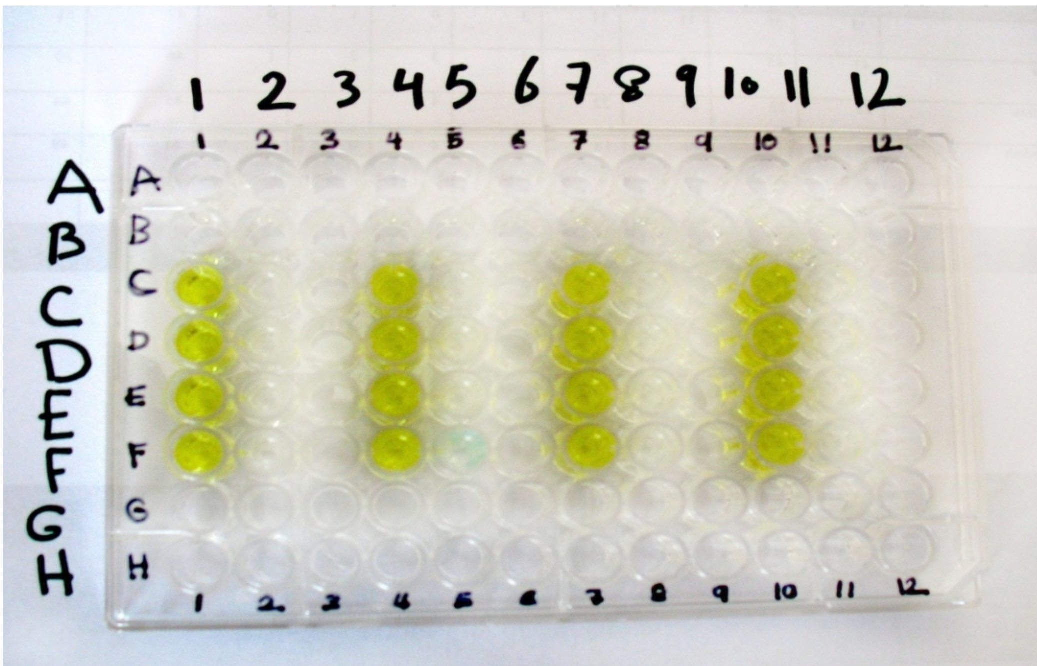

Fig 1. ELISA plate showing the antibody-antigen reaction for the studied viruses. TYLCV (C1-F1), CMV (C4-F4), TMV (C7-F7) and PVY (C10-F10).The negative samples are shown in C2-F2, C5-F5, C8-F8 and C11-F11

3.2 Effect of Viral Infection on Tomato Plants

The results showed tomato plants that were infected with the four viruses produced 0.7 fruit that averaged 47.9grams per plant. Plants that were infected with TMV, CMV and TYLCV produced 1.4 fruit with an average of 109.7grams per plant. Other combination of infection that includes CMV, PVY and TYLCV showed 1.1 fruits that averaged 114grams per plant. Another variation of combination TMV, PVY and TYLCV showed 3.2 fruits with an average 228.4 grams per plant. In addition multiple infection with TMV, CMV and PVY produced 4.8 fruits with an average of 319.2grams per plant. Furthermore, infection with two viruses alone showed an intermediate effect on plant productivity and fruit weight. Thus, infection with TYLCV and CMV was the most effective among this case as the plant infected with those two viruses produced an average of 2.6 fruits with a productivity of 128.5grams per plant. Furthermore, the results showed that infection with single virus had a variable effect on plant production with a maximum effect of TYLCV infection. Plants infected with such a virus produced 3.8 fruits with an average productivity of 360.5grams per plant. Single infection with either CMV or TMV showed an intermediate effect as the plant infected with CMV produced 6.4 fruits with average productivity of 500.4grams per plant compared with plant infected with TMV which produced 8.2 fruits with average productivity of 590.6grams per plant. Infection with PVY alone showed the minimum effect on plant production as the infected plant produced 9.6 fruits in average with a fruit weight of 610grams per plant (Table 1, Figs. 3, 4). Furthermore, the statistical analysis indicates that all treatments were significant when the number of fruit was compared as a variable. In the case of plant productivity variable, all treatments were significant except the plants infected with PVY + TYLCV and TMV + CMV + PVY (Table 1).

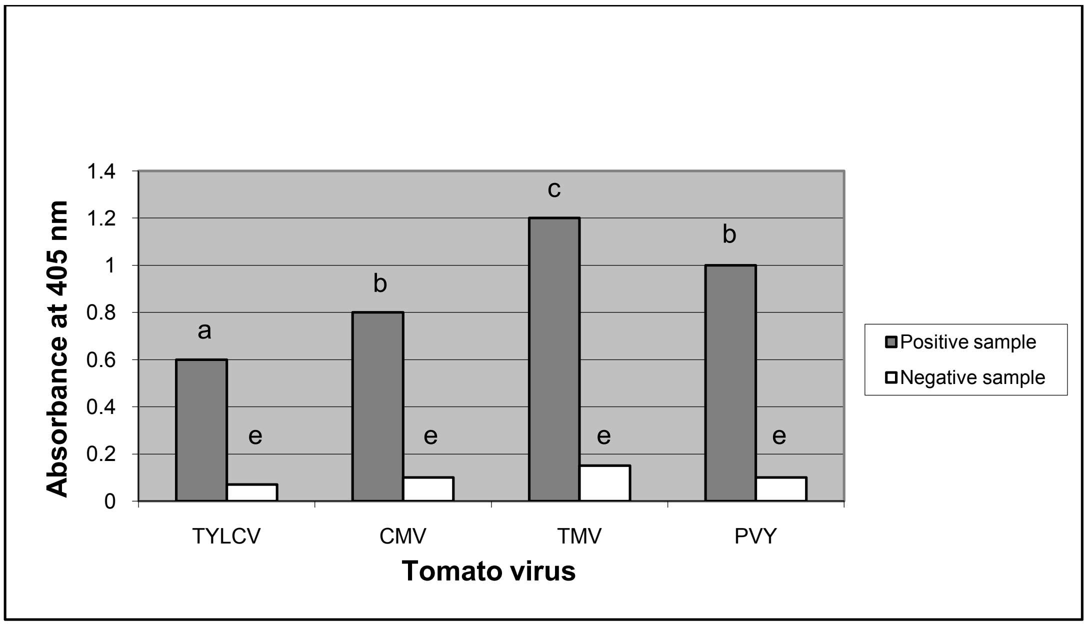

Fig. 2. ELISA detection of tomato viruses showing the difference between positive and negative control samples

****Similar letters above the bins indicate the treatments with no significant difference*

In addition, the mixed infection with four viruses reduced the number and the weight of tomato fruit by 96% and 93% respectively. Also, mixed infection with TMV, CMV and TYLCV or TMV, CMV and PVY reduced the number and the weight of fruit by 92% and 84% or % 72 and 52% respectively. Single infection with TYLCV caused a reduction of 77% for the fruit number and 46% for fruit weight. For CMV and TMV, single infection by either virus caused a reduction in the fruit number and weight by 63% and 25% or 52% and 12% respectively (Table 1, Figs. 3 and 4).

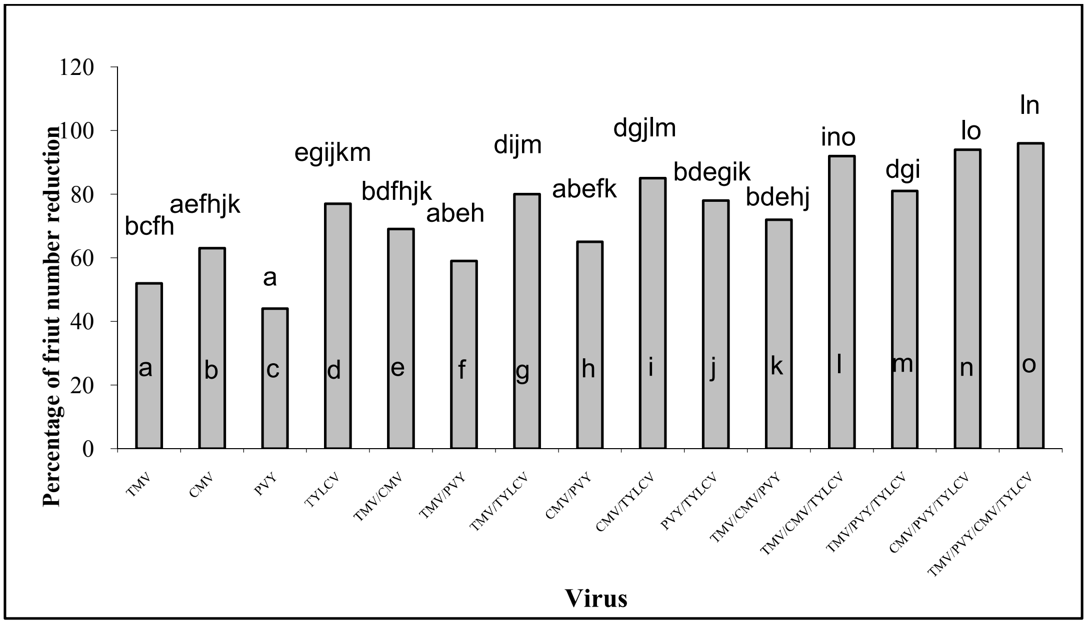

Fig. 3. Effect of viral infection on tomato production

****Letters above the bins indicate the treatments with no significant difference with the treatment inside the bin*

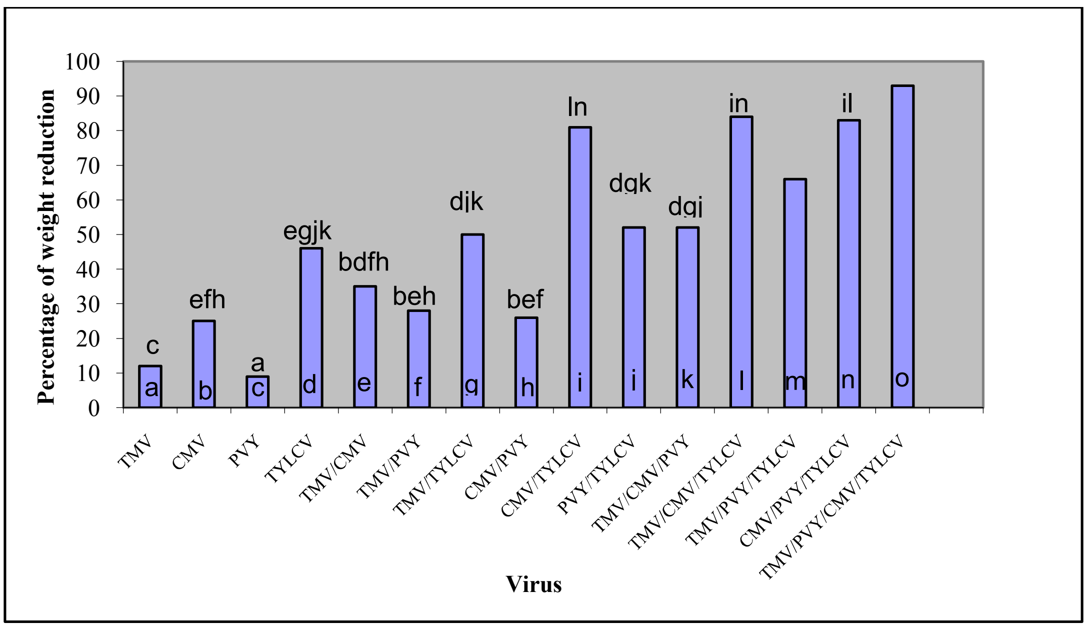

Fig. 4. Effect of viral infection on the wieght of tomato fruits

****Letters above the bins indicate the treatments with no significant difference with the treatment inside the bin*

Table 1. Effect of single and multiple virus infection on number and weight of tomato fruits

Replicate	TMV		CMV		PVY		TYLCV	
	No. of fruits	Productivity per plant	No. of fruits	Productivity per plant	No. of fruits	Productivity per plant	No. of fruits	Productivity per plant
Block 1	9	637	7	522	10	691	4	344
	8	601	6	501	10	697	4	339
	8	591	7	531	9	583	4	354
	7	530	6	469	10	657	5	402
	8	595	6	514	11	709	3	374
Sum	Σ40	Σ2954	Σ32	Σ2537	Σ50	Σ3337	Σ20	Σ1813
Block 2	9	598	7	529	10	589	3	339
	8	560	7	501	9	512	4	341
	9	606	6	499	9	523	4	350
	8	579	7	542	10	643	3	364
	8	609	5	396	8	496	4	398
Sum	Σ42	Σ2952	Σ32	Σ2467	Σ46	Σ2763	Σ18	Σ1792
Total Sum	Σ82	Σ5906	Σ64	Σ5004	Σ96	Σ6100	Σ38	Σ3605
Mean	8.2a	590.6(a)	6.4b	500.4 (b)	9.6c	610.0 (c)	3.8d	360.5 (d)
Replicate	**TMV + CMV**		**TMV + PVY**		**TMV + TYLCV**		**CMV + PVY**	
	No. of fruits	**Productivity per plant**	**No. of fruits**	**Productivity per plant**	**No. of fruits**	**Productivity per plant**	**No. of fruits**	**Productivity per plant**
Block 1	6	431	8	522	3	298	7	574
	6	389	7	500	4	305	6	481
	6	422	7	473	3	330	5	469
	5	402	6	412	5	442	6	499
	4	369	8	530	3	313	7	538
Sum	Σ27	Σ2013	Σ36	Σ2437	Σ 18	Σ1688	Σ31	Σ 2561
Block 2	5	428	7	497	3	301	7	603
	5	399	7	464	3	319	6	532
	6	604	8	501	4	386	6	536
	6	513	6	420	4	369	6	401
	4	412	7	479	3	318	5	334
Sum	Σ26	Σ2356	Σ35	Σ2361	Σ17	Σ1693	Σ30	Σ2406
Total Sum	Σ53	Σ4369	Σ71	Σ4798	Σ35	Σ3381	Σ61	Σ4967
Mean	5.3e	436.9(e)	7.1f	479.8 (f)	3.5g	338.1(g)	6.1h	496.7(h)

Table 1 Continued …..

Replicate	CMV + TYLCV		PVY + TYLCV		TMV + CMV + PVY		TMV + CMV + TYLCV	
	No. of fruits	**Productivity per plant**	**No. of fruits**	**Productivity per plant**	**No. of fruits**	**Productivity per plant**	**No. of fruits**	**Productivity per plant**
Block 1	2	208	4	290	5	302	1	121
	2	198	4	299	5	310	1	101
	2	221	4	331	5	331	1	99
	3	297	3	311	4	300	2	163
	4	331	5	389	3	251	0	0
Sum	Σ 13	Σ 1255	Σ20	Σ1620	Σ22	Σ1494	Σ5	Σ484
Block 2	3	247	3	288	5	335	2	132
	2	209	4	341	4	269	1	85
	2	230	3	291	5	325	1	80
	3	278	3	285	6	379	3	187
	3	289	5	369	6	389	2	128
Sum	Σ13	Σ1253	Σ18	Σ1574	Σ26	Σ1698	Σ9	Σ612
Total Sum	Σ26	Σ1285	Σ38	Σ3194	Σ48	Σ3192	Σ14	Σ1096
Mean	2.6i	128.5 (i)	3.8j	319.4 (j)	4.8k	319.2 (j)	1.4l	109.6 (k)

Replicate	TMV + PVY + TYLCV		CMV + PVY + TYLCV		TMV + PVY + CMV + TYLCV		Control	
	No. of fruits	**Productivity per plant**	**No. of fruits**	**Productivity per plant**	**No. of fruits**	**Productivity per plant**	**No. of fruits**	**Productivity per plant**
Block 1	3	222	0	0	1	90	18	700
	4	270	1	101	0	0	19	850
	3	210	1	109	1	77	16	590
	3	212	2	208	0	0	14	509
	3	236	1	99	0	0	17	587
Sum	Σ16	Σ1150	Σ5	Σ517	Σ2	Σ167	Σ84	Σ3236
Block 2	4	272	1	98	1	68	20	789
	3	211	1	99	0	0	17	692
	3	220	2	209	1	75	17	681
	3	232	2	217	1	68	16	602
	3	199	0	0	2	101	18	698
Sum	Σ16	Σ1134	Σ6	Σ623	Σ5	Σ312	Σ88	Σ3462
Total Sum	Σ32	Σ2284	Σ11	Σ1140	Σ7	Σ479	Σ172	Σ6698
Mean	3.2m	228.4(l)	1.1n	114.0(m)	0.7o	47.9(n)	17.2p	669.8(o)

****Means with similar letters indicate no significant difference, Productivity is measured in grams*

4. DISCUSSION

It has become clear that viral diseases are the most common diseases of tomato plants in Palestine [12,13,22,23]. Almost all tomato plantings have at least one virus infected plants before harvest is complete [12,13]. These diseases are often overlooked, perhaps, because the symptoms may be inconspicuous [7,24]]. Furthermore, TYLCV was reported as the most prevalent virus threatening tomato fields in the northern districts of the West Bank followed by CMV, TMV and PVY respectively [13]. For this reason, researchers of agronomy from public and private sectors have been working hard to rescue tomato fields from the problems caused by the spread of such viruses throughout tomato growing sites especially TYLCV.

Studying the pathogenic effect of tomato viruses on tomato yield showed, in one hand, how much serious those viruses are, especially TYLCV. In the other hand, the current research proved that the cumulative reaction of those viruses occurs when tomato plants are infected with more than one of them. Tomato mixed infection with such viruses has more effect on plants compared with single infection with either tomato viruses as the reduction in yield is much greater in mixed infections than either virus alone. Also, the infection with the four or three viruses is much destructive than the infection with two or a single virus. Such finding may be attributed to the accumulative effect of these viruses on the plant. The present results also demonstrated that TYLCV is the key virus affecting tomato plants and causes significant yield reduction. When TYLCV is present in the virus complex infection or even alone, a serious damage results. Mixed infection with TYLCV and other tomato virus reduced the fruit number up to 77%. Low productivity of TYLCV-infected plants is expected because the virus infection caused significant reduction of fruit number on plants due to the tremendous flower drop. This may be attributed to the great pathogenicity and destructive ability of such virus compared with other viruses. The impact of TYLCV on yield depends upon the time of virus infection. Early infection results in the highest yield reduction. In this regard, other researches have obtained similar results in other countries. El-Dougdoug et al. [25] reported that the height and yield of tomato plants are affected by the interference between TYLCV and TMV in which this affect depended on which virus was inoculated first. In general, the effect of two viruses on decreasing tomato yield is more than when the two viruses are found individually. In regard to other viruses, Giesler *et. al.* [26] reported that a mixed infection with bean pod mottle virus (BPMV) and soybean mosaic virus (SMV) reduced the yield of soybean up to 85%. Also, García-Cano et al. [27] observed synergistic reactions in mixed infections in tomato plants doubly infected with the positive-sense and phloem-limited single-stranded RNA (ssRNA) crinivirus tomato chlorosis virus (ToCV) and the negative-sense ssRNA tospovirus tomato spotted wilt virus (TSWV). Synergism of those viruses in a susceptible tomato cultivar resulted in a rapid death of plants.

5. CONCLUSION

As the results above indicate, it can be concluded that TYLCV was the most destructive virus affecting tomato production in the West Bank of Palestine. Therefore, efforts must be directed towards the control of such a virus. Furthermore, because tomato infection with any of CMV, TMV and PVY together with TYLCV causes drastic reductions of tomato yield, it is important that efforts towards the prevention of those viruses must take place. Although infection with TMV and/or PVY causes low effect on tomato production, efforts should be directed towards the control of those viruses to prevent the possible development of new strains with possibly severe effect that can adapt to ecosystems in the region.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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