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Synergistic Effects of Mosaic and Leaf Curl Viruses on Growth in Glass-House Tomato Plants

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SN and MAJ designed the study and performed the statistical analysis. Authors MWJ and IH wrote the protocol and managed the analyses of the study. Authors SN and MZI wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Viral diseases are known to be one of the major limiting aspects in tomato production in different countries. Almost 75 viruses are known to infect this crop and adversely affecting the yield annually. Combined attack of more than one viruses become more dangerous and show very drastic effects on the crop. Keeping in view the acuteness of the viral diseases and their effects on tomato yield, the interaction between *Tomato mosaic virus* (ToMV) and *Tomato leaf curl virus* (TLCV), was investigated under controlled conditions. Effects on plant developments and yield was evaluated under viral attack separately as well as in combination and the percent plant infection was recorded. The tested viruses significantly reduced the plant height and fruit yield of infected tomato plants. The greatest effect was obtained in the double infection with TLCV and ToMV with mean fruit yield of plants was 112.28 gm. While singly ToMV and TLCV were slightly reduced fruit yield as 156.08 gm and 141.685 gm respectively. The plant height was also greatly reduced in case of double infection i.e. with mean height of plants was 26.32 cm. While, on other hand it was slightly reduced when ToMV and TLCV were infested separately that was 38.18 cm and 33.22 cm respectively.

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1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the major imperative vegetable crops after potato by virtues of its high nutritive value and it is grown throughout the world. Tomato is a rich source of vitamins A, B and C and is used for different food purposes. It is native to central, south and southern North America extending from Mexico to Peru and it belongs to the Solanaceae family [1]. In Pakistan tomato is cultivated in all provinces but its production is mainly concentrated in Sindh and Punjab. The total area under cultivation is 53.1 thousand hectare with production of 10.4 thousand tonnes [2]. About seven viral diseases have been reported in Pakistan which includes TLCV, ToMV, potato virus X (PVX), potato virus Y (PVY), cucumber mosaic virus (CMV), tomato yellow top virus (TYTV), tomato spotted wilt virus (TSWV) and tomato ring spot virus (TRSV) [3]. Among them TLCV and ToMV are the most important viral diseases and distributed throughout Pakistan. Among these TLCV reported to cause 50% to 75% yield losses in tomato [4].

Particles of ToMV are rigid rods with size of 300 x 18 nm which consist of single-stranded RNA (2000 k Da) and single polypeptide (21 k Da) containing coat protein [5]. Symptoms of tomato mosaic virus can be established throughout developmental stage and all plant components are affected usually, contaminated plant have a light or dark green mottling with twisted of younger leaves, and stunting to unstable amounts. TYLC is one of the major destructive viral infections of cultivated tomato (*Solanum lycopersicum*) in tropical and subtropical areas internationally, and losses of up to 100% are common. In most of the areas, TYLC is the major restrictive issue in tomato production and improvement [6].

Tomato leaf curl virus is characterized by leaf curling, yellowing, chlorosis of leaf margins, leaf distortion, reduction in leaf size, shortening of the internodes or stunting. TYLCV, belonging to Gemini viruses, is a severe viral disease of tomato crops in the Mediterranean basin region. The disease has been reported in several countries. All commercial tomato varieties are susceptible to this disease.

TYLCV is broadcasted through the whitefly (*Bemisia tabaci*) and fails to infect plants when inoculated mechanically [7]. Genome consists of TLCV; single-stranded; circular; of two components i.e. first largest genome part (2.78 kb) and the second largest genome part (2.7 kb) [8].

Thus, the synergistic effect of ToMV and TYLCV on percent plant infection, plant height and plant yield of tomato crop were the aims of the present research work.

2. MATERIALS AND METHODS

Tomato nursery of 40 days of variety Money Maker was transplant in earthen pots containing mixture of sand, soil and farm yard manures in ratio 1:1:1 in glass house of Agricultural Biotechnology Research Institute, Faisalabad during October-November 2016-17. The environmental conditions were controlled using air conditioners and humidifiers. The temperature of glass house was adjusted at 25-30°C and humidity was maintained around 80. Nursery was collected from Vegetable Research Institute of Ayub Agriculture Research Institute, Faisalabad. The effect of synergism of mosaic and leaf curl viruses was studies on growth parameters of tomato like plant height, fruit yield and percent plant infection.

2.1 TYLCV Symptoms Severity Ranking

Following range was used to assess the infection range visually:

0 = No detectable disease, immunized plants demonstrate comparable growth and expansion as non inoculated plants
1 = Minor yellowing of leaf boundaries on apical leaf
2 = Some yellowing and slight curling of leaflet ends
3 = Broad range of leaf yellowing, twisting, and cupping, with some decreased in size, but the growth was continued
4 = Severe plant stunting and yellowing, prominent leaf cupping and twisting; growth stop. Data was recorded on weekly basis for three consecutive weeks at interval of 7, 14 and 21 days.

There were 4 treatments each was replicated three times.

T1 = Singly infected with ToMV
T2 = Singly infected with TLCV
T3 = Mixed infected with both ToMV and TLCV
T4 = Healthy tomato plants

Mean values were statistically analyzed using LSD test at 5% level of significance [5].

3. RESULTS AND DISCUSSION

The effect of TLCV and ToMV on symptoms and disease severity and growth was observed under glasshouse conditions. Tomato seedlings were infected with single and double or mixed with ToMV and TLCV. Percent plant infection, plant height (cm) and yield of tomato fruit was recorded.

It was found that, symptoms can be used to differentiate between ToMV and TLCV during the early stages of inoculation. ToMV causes a pale- and dark-green mosaic on the young leaves which became malformed, and stunting of the plants.

TLCV-infected tomato leaves are small, malformed, curled upward, and severely chlorotic. Yield losses can reach 80%. This result agrees with that obtained by [9-10]. The synergistic effect of ToMV and TLCV infection on plant height, yield and percent plant infection of tomato plants was measured under glass-house condition shown in following tables.

Regarding the height of tomato plants, it was found that the tested viruses significantly reduced the height of infected plants (Table 1). In this experiment, the greatest reduction in mean plant height was observed in the double infection with TLCV + ToMV as compare to single infection with ToMV and TLCV and healthy plant. Mean plant height of mixed infected plants was 26.321cm as compare to singly infected plants with ToMV and TLCV 38.184, 33.225 cm respectively as compare to healthy plant (43.275 cm).

From Table 2 it was indicated that the virus combination of ToMV and TLCV significantly reduced plant yield (112.285gm) as compare to singly infected plant with ToMV and TLCV 156.08gm and 141.685gm respectively and healthy tomato plant (233.085). Percent plant infection was recorded maximum in case of mixed infected plant (4.7) as compare to singly infected plants with ToMV (2.8), TLCV (3.5) and healthy plant (0.43). Another study described intrusion between TMV and a strain of CMV, reasoning local lesions (CMV-LL) on *Zinnia elegans* [11]. Intrusion was detected on tobacco species i.e. *Nicotiana repanda* and *N. glutinosa* when CMV-LL functioned as defensive virus, and on *Z. elegans* when the defending virus was TMV. Concurrent inoculations of *N. repanda* or *N. glutinosa* with the two viruses disallowed the establishment and multiplication of CMV-LL. Growing levels of TMV is the confront immunization resulted in an increased level of defense. This supports the hypothesis that the defensive virus occupies some cellular arrangements or weakens some precursors required for the development and multiplication of the confront virus [12]. *Oilseed rape mosaic virus* (ORMV) and *Tobacco mild green mosaic virus* (TMGMV) were mechanically inoculated onto *Arabidopsis thaliana* and *N. tabacum* 15 days after transplanting and at the 4-leaf stage, respectively. The interactions between the 2 viruses were studied in 2 types of experiments. In the first experiment, ORMV and TMGMV were co-inoculated by mixing and inoculating on one leaf. In the second experiment, ORMV and TMGMV were inoculated onto different leaves at different times. One virus was inoculated as the protecting virus and the second virus was inoculated as the challenging virus 7, 14, 21 or 28 days after inoculation of the protecting virus. The viruses were detected by dot-blot hybridization. The result of co-inoculation was the identical for both hosts: there was strong intrusion with ORMV being the more successful of the two viruses. In tobacco, whichever virus acted as the protecting virus, it interfered significantly with the reproduction of the testing virus, providing cross-protection. In spite of the time beyond between the inoculations of both viruses, the protecting virus always inhibited the accumulation of the testing virus. In *A. thaliana*, when TMGMV was the defensive virus, it protected the plants from ORMV disease. When ORMV was used as the defensive virus, TMGMV was not present, but as TMGMV disease is symptomless and slow to expand in A. thaliana, the survival of cross-protection could not be determined. Further study demonstrated that, *Tomato rugose mosaic virus* (ToRMV) and *Tomato yellow spot virus* (ToYSV) contaminates tomatoes crop [13]. ToYSV symptoms in tomato and *N. benthamiana* emerge before and are more severe compared to those of ToRMV. Results showing that ToYSV create a systemic infection and accomplishes a higher concentration earlier than ToRMV in both hosts. ToRMV adversely interfere with ToYSV during the early stages of disease/infection, but once complete infection/disease is established this interference stops. In *N. benthamiana*, ToYSV attacks the mesophyll, while ToRMV is phloem-restricted. During double infection in this host, ToYSV releases ToRMV from the phloem.

Table 1. Effect of single and mixed infection with ToMV and TLCV on plant height (cm) of tomato (*Solanum lycopersicum*)

Treatments	Weeks			Means plant height (cm)
	1st	2nd	3rd	
ToMV	34.52	37.29	42.74	38.18
TLCV	29.56	32.82	37.29	33.22
ToMV+TLCV	23.14	26.06	29.75	26.32
Control (Healthy)	38.59	42.18	49.05	43.27

LSD values for Treatment = 1.2427; Week = 1.0762; Treatment x week = 2.1524

Table 2. Effect of single and mixed infection with ToMV and TLCV on fruit yield (gm) of tomato (*Solanum lycopersicum*)

Treatments	Dates		Mean tomato yield (gm)
	1st	2nd	
ToMV	152.79	159.37	156.08
TLCV	138.93	144.44	141.685
ToMV+TLCV	106.85	117.72	112.285
Control (Healthy)	250.05	216.12	233.085

LSD values for Treatment = 2.3567; Date = 1.6664; Treatment x Dates = 3.3328

Table 3. Effect of single and mixed infection with ToMV and TLCV on percent plant infection of tomato (*Solanum lycopersicum*)

Treatments	Weeks			Means percent plant infection
	1st	2nd	3rd	
ToMV	2.16	2.83	3.40	2.8
TLCV	2.83	3.80	4.13	3.5
ToMV+TLCV	4.33	4.66	4.95	4.7
Control (Healthy)	0.56	0.90	1.15	0.43

LSD values for Treatment = 0.1701; Week = 0.1473; Treatment x week = 0.2946

4. CONCLUSION

ToMV and TLCV were allowed to infect the tomato plant either singly and in combination. Interaction between these viruses and their effects on tomato yield was investigated and percent infection was also recorded. It was observed that plant height was severely affected by the virus attack. Maximum yield loss was found in the double infection while singly they were slightly reduced fruit yield as well. Percent plant infection were maximum in case of double infection of tomato mosaic virus and tomato leaf curl virus where more than 75% of leaf area was found to be infected. Hence, resistant genotypes should be selected and used in future tomato breeding program to save the yield against viruses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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