

Interaction Between Some Viruses Which Attack Tomato (*Lycopersicon esculentum* Mill.) Plants and Their Effect on Growth and Yield of Tomato Plants.

Mohamed, E.F.

Botany Department, Faculty of Agriculture, Fayoum University, Egypt.

e-mail:emaddwidar@yahoo.com

Abstract: Interaction between tomato mosaic virus (ToMV), tomato yellow leaf curl virus (TYLCV) and cucumber mosaic virus (CMV) and their effect on growth and yield of tomato plants was studied. In symptoms and disease severity experiment, the most pronounced synergistic effects were caused by mixtures of ToMV+TYLCV and ToMV+TYLCV+CMV. Combination of ToMV+CMV caused slight symptoms. Generally, systemic symptoms were of the mosaic or mottling types in addition to different degrees of stunting and malformation. In interaction experiment, all virus combinations tested decreased the effect of ToMV on tomato plants, and few plants showed the characteristic pale-and dark-green mosaic symptoms of ToMV infection. In cross-protection experiment, the effect of double infection is depending on the subsequent of infections. When the plants were first inoculated with TYLCV and later with ToMV, the effect of TYLCV was prominent. On the other hand, first inoculation with ToMV suppressed the effect of the subsequent infection with TYLCV. Infection with ToMV supported the symptoms of the subsequent infection with CMV. This may be due to the weak symptoms of CMV which can be suppressed by the severe symptoms of the ToMV. On the other hand, infection with CMV suppressed the subsequent infection with ToMV. All virus treatments significantly reduced tomato height. TYLCV had the greatest effect (Mean height of plants was 27.75 cm.), while CMV was slightly reduced plant height (Mean height of plants was 34.95 cm). The tested viruses significantly reduced the yield of infected tomato plants. The greatest effect was obtained in the double infection with TYLCV + ToMV and TYLCV+CMV (Mean yield of plants was 130.15 and 139.06 gm. respectively). While CMV was slightly reduced plant yield (Mean yield of plants was 160.08 gm). [Journal of American Science 2010;6(8):311-320]. (ISSN: 1545-1003).

Key words: Tomato mosaic virus (ToMV), tomato yellow leaf curl virus (TYLCV), cucumber mosaic virus (CMV), interference, synergism and antagonism.

1. Introduction

Virus diseases are considered one of the most important problems affecting tomato production in many countries (Daniela *et al.*, 2009; Murad *et al.*, 2009; Salvatore *et al.*, 2009; Sead *et al.*, 2009; Torsten *et al.*, 2009; Weimin *et al.*, 2009; Akos and Ervin, 2010; and Pradeep and Masato, 2010). There are about 75 viruses infect this crop (Thornberry, 1966).

Tomato mosaic virus (ToMV) is widespread wherever tomato is grown. ToMV particles are rigid rods of 300 x 18 nm that contain single-stranded RNA (2000 kDa) and a coat protein of a single polypeptide, 21 kDa (Sutic *et al.*, 1999). ToMV belongs to Tobamoviruses. Tobamoviruses contain more than a dozen rod-shaped viruses that cause serious losses in their hosts by damaging the leaves, flowers and fruits and by causing stunting of the plant (Agrios, 1997).

Tomato yellow leaf curl (TYLC) is one of the most devastating viral diseases of cultivated tomato (*Lycopersicon esculentum* Mill.) in tropical and subtropical regions worldwide, and losses of up

to 100% are frequent. In many regions, TYLC is the main limiting factor in tomato production (Moriones and Navas-Castillo, 2000). TYLCV, belonging to geminiviruses, 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 transmitted by the whitefly *Bemisia tabaci*. and fails to infect plants when inoculated mechanically (Akad *et al.*, 2004; Bosco *et al.*, 2004; Jiang *et al.*, 2004; Noris *et al.*, 2004; and Parrella *et al.*, 2004). Genome consists of DNA; single-stranded; circular; of two parts; largest (or only) genome part 2.787 kb; the 2nd largest 2.7 kb. Virions geminate; 20 nm in diameter; dimers 30 nm in length; angular in profile; without a conspicuous capsomere arrangement (Dalmon *et al.*, 2003; Fekih-Hassan *et al.*, 2003; Cui *et al.*, 2004; and Onuki *et al.*, 2004).

Cucumber mosaic virus (CMV) with its 59 strains causes damage for tomatoes production (Kaper and Waterworth, 1981). CMV infects many crops and cause huge reduction in the yield all over

the world. CMV appears to be one of the most important virus in Eastern China, Croatia, France, Egypt, Greece, Italy, Japan, Poland, Portugal, Sweden, Australia and Northeastern United States (Tomlinson, 1987; Pares and Gunn, 1989; Mavrodieva, 1998; and Stommel et al, 1998). The percentage of crop losses in tomato was 100% in Italy and Spain in 1987 (Jorda et al, 1992); 80% in Australia; 20 % in melons in California (Grafton-Cardwell et al, 1996); and 50% in tobacco and pepper in Florida (Kucharek et al, 1998). Genome consists of RNA; single-stranded; linear; of three parts; largest (or only) genome parts the largest 3.389 kb; the 2nd largest 3.035 kb; the 3rd largest 2.197 kb. Virions isometric; not enveloped; 29 nm in diameter; rounded in profile; without a conspicuous capsomere arrangement. Virions contain 18 % nucleic acid; 82 % protein; 0 % lipid (Brunt, 1996).

Interference, the reduction of infection by one virus when two related viruses are used as inoculum together, has been extensively investigated. Exclusion mechanism may be operating such that when an infection is initiated with a particle of one strain of virus, a particle of a second strain cannot participate in the same infection. Interference by infectious agents occurs after attachment to host cells. Metabolic changes initiated by the interfering strain were the basis of the phenomenon. The proof of competitive exclusion at an infection site requires showing that the interfering strain does not multiply set reduces numbers of lesions produced by another strain. On the other hand, if the interfering strain does infect and multiply, then interference may involve interaction during multiplication (Cohen et al., 1983; Rochow et al., 1983; Sakai et al., 1983; Sherwood and Fulton, 1983; Sackey and Francki 1986; Ammar et al., 1987; and Marchoux et al., 1988).

Thus, the effect of ToMV, TYLCV and CMV on tomato symptoms, disease severity, plant growth and plant yield as well as the interaction of these viruses on tomato plants were the aims of the present investigation.

2. Material and Methods

1. Virus isolate:

1.1. Concerning ToMV, virus inoculum was the crude sap obtained by trituration of frozen leaves of tomato plants (*Lycopersicon esculentum* Mill.) seedlings showing mosaic symptoms. These symptoms developed 14 days after inoculation with a single local lesion obtained from *Nicotiana sylvestris* leaves that were inoculated with sap extracted from naturally infected tomato plants. Inoculation of leaves was carried out by rubbing with finger after their being dusted with carborandum.

1.2. Concerning TYLCV, virus-free colony of specific vector (*Bemisia tabaci*) raised on healthy squash seedlings were used in transmission of TYLCV from diseased tomato to healthy tomato plant. Insects were transferred to the diseased tomato plants to feed and become viruliferous. These viruliferous insects were used for transmission process.

1.3. Concerning CMV, virus inoculum was the crude sap obtained by trituration of frozen leaves of cucumber plants (*Cucumis sativus* L.) seedlings showing mosaic symptoms. These symptoms developed 14 days after inoculation with a single local lesion obtained from *Chenopodium amaranticolor* leaves that were inoculated with sap extracted from naturally infected cucumber plants. Inoculation of leaves was carried out by rubbing with finger after their being dusted with carborandum.

2. Interaction experiment:

The effect of single, double and mixed infection with ToMV, TYLCV and CMV on the symptoms, disease severity, height and yield of tomato (*Lycopersicon esculentum* Mill. cv. Cassel rock) plants was studied. ToMV and CMV were inoculated mechanically, while *Bemisia tabaci* insect was used to transmit TYLCV from diseased to healthy plants. Symptoms were recorded weekly for 3 successive weeks. Plant height was recorded after 4 weeks of inoculation. Four replicates were used in this study, each containing 5 plants. Data obtained were statistically analyzed according to Steel and Torrie, (1960). It was observed that the pale-and dark-green mosaic symptom is characteristic of ToMV infection. Therefore, to determine the interaction between this virus and other viruses of tomato, the tested viruses were inoculated on tomato seedlings, then later (15 days after symptom appearance), ToMV was inoculated to the same infected plants of each treatment. The number of plants which showed pale-and dark-green mosaic symptom of each treatment was recorded.

3. Results and Discussion

1. Effect of ToMV, TYLCV and CMV on symptoms and disease severity of tomato plants:

Tomato seedlings were inoculated with single, double and mixed infection with ToMV, TYLCV and CMV. Measurements on symptoms and disease severity were recorded.

It was found that, symptoms can be used to differentiate between ToMV, TYLCV and CMV 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.

The most damaging symptom is necrosis in leaves, along the stems and on the fruits. This result agrees with that obtained by Singh (1983) and Susic *et al.* (1999).

TYLCV-infected tomato leaves are small, malformed, curled upward, and severely chlorotic. Yield losses can reach 80%. This result agrees with that obtained by Brunt (1996) and Susic *et al.* (1999).

On the other hand, CMV-infected tomato displays pronounced pathological changes. Mild mosaic and mottle first appears in leaves and becomes more evident with development of the disease in the formation of new leaves of abnormally narrowed/elongated thread-like formations Shortened and compressed internodes alter the general plant appearance. Virulent strains cause necrosis along leaf veins, necrotic streaks along the stems, and death of shoot tips. The extent of damage to production is

dictated by the number of infected plants. This result was similar with that obtained by Brunt (1996) and Susic *et al.* (1999). The main symptoms of ToMV, TYLCV and CMV inoculated singly or in combination to tomato plants are shown in Table (1) and Fig. (1). Mixed inoculation with all three viruses caused a more severe disease than either alone. The first symptoms appeared one week after inoculation and the infected plants showed severe symptoms. Mixtures of the two viruses caused less severe symptoms. The most pronounced synergistic effects were caused by mixtures of ToMV+TYLCV and ToMV+TYLCV+CMV. Combination of ToMV+CMV caused slight symptoms. Generally, systemic symptoms were of the mosaic or mottling types in addition to different degrees of stunting and malformation.

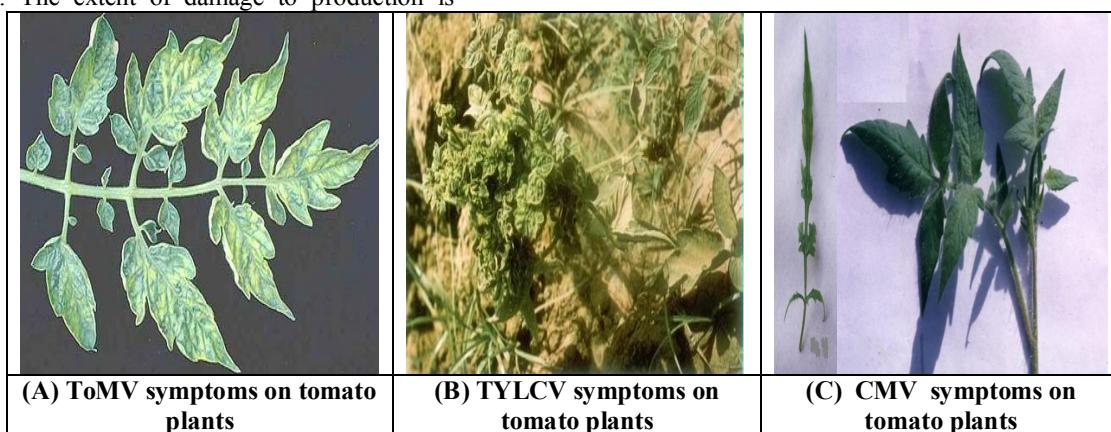


Fig. (1): Symptoms of some viruses affecting tomato (*Lycopersicon esculentum* Mill. cv. Cassel rock) plants. (A) ToMV symptoms, (B) TYLCV symptoms and (C) CMV symptoms.

2. Interaction between ToMV and other viruses of tomato plants:

A pale-and dark-green mosaic symptom is characteristic of ToMV and was used to differentiate between the effects of ToMV and other viruses in tomato. Table (2) and Fig. (2) show that infection with ToMV alone caused extremely severe pale-and dark-green mosaic symptoms, whereas, pale-and dark-green mosaic symptoms on leaves with dual infection were less severe and few plant showed such symptoms. When all three viruses were inoculated simultaneously on tomato leaves, ToMV produced slight pale-and dark-green mosaic symptoms. It can be concluded that all virus combinations tested decreased the effect of ToMV and few plants showed the characteristic pale-and dark-green mosaic symptoms of infection.

3. Cross protection between ToMV and other viruses of tomato plants:

Data obtained in Table (3) and Fig. (3) show

that, the effect of double infection is depending on the subsequence of infections. When the plants were first inoculated with TYLCV and later with ToMV, the effect of TYLCV was prominent. On the other hand, first inoculation with ToMV suppressed the effect of the subsequent infection with TYLCV. In this respect, partial antagonism may be suggested to be occurred between ToMV and TYLCV. Results obtained on the effect of double infection on disease symptoms indicated that infection with ToMV supported the symptoms of the subsequent infection with CMV. This may be due to the weak symptoms of CMV which can be suppressed by the severe symptoms of the ToMV. On the other hand, infection with CMV suppressed the subsequent infection with ToMV. So, antagonistic effect may be suggested to be occurred between ToMV and CMV. Generally, these results show that the extend to which antagonistic or Synergistic effects occur depends on the timing of the inoculations.

4. Effect of single, double and mixed infection with ToMV, TYLCV and CMV on the height of tomato plants:

According to our knowledge, the effect of these three viruses on plant height have not carried out before. The effect of virus infection on growth of tomato plants was measured (Table 4 and Fig. 4). Under greenhouse condition, it was found that single infection with ToMV, TYLCV or CMV significantly reduced plant height. Inhibitory effect of virus infection on tomato growth is a common phenomenon and it had reported by several investigators. It could be mentioned that TYLCV markedly reduced plant height and significant differences were detected between TYLCV-infected plants and those infected either with ToMV or CMV. When tomato plants were inoculated with ToMV or CMV and 15 days later re-inoculated with ToMV or CMV respectively, no significant differences were detected between double infected plants and those single inoculated either with ToMV or CMV. Concerning TYLCV, the effect of double infection is depending on the subsequence of infections, when the plants were first inoculated with TYLCV and later with ToMV or CMV, the effect of TYLCV was prominent. On the other hand, first inoculation with ToMV or CMV suppressed the effect of the subsequent infection with TYLCV. In this respect, partial antagonism may be suggested to be occurred between ToMV and TYLCV or CMV. Simultaneous infection with the three viruses (ToMV, TYLCV and CMV) at the same time, significantly reduced the plant height as compared by healthy plants or those inoculated with ToMV only. No significant were detected between simultaneously inoculated plants and those singly

inoculated with TYLCV. Results obtained on the effect of double infection on disease symptoms indicated that infection with ToMV supported the symptoms of the subsequent infection with CMV. This may be due to the weak symptoms of CMV which can be suppressed by the severe symptoms of the ToMV. On the other hand, infection with CMV suppressed the subsequent infection with ToMV. So, antagonistic effect may be suggested to be occurred between ToMV and CMV. Generally, these results show that the extend to which antagonistic or Synergistic effects occur depends on the timing of the inoculations. On the other hand, no significant differences were detected between plants inoculated with ToMV and those infected with CMV.

5. Effect of single, double and mixed infection with ToMV, TYLCV and CMV on the yield of tomato plants:

Regarding the yield of tomato plants, it was found that the tested viruses significantly reduced the yield of infected plants (Table 5 and Fig. 5). Previous infection with TYLCV suppressed the symptoms of the subsequent infection either with ToMV or CMV. Previous infections with ToMV elongated the incubation period necessary for TYLCV symptoms for about 2-3 weeks and then, the symptoms of TYLCV started to suppress the symptoms of ToMV. Suppression of ToMV or CMV symptoms by TYLCV infection may be due to severe symptoms and leaf curling caused by TYLCV. In this experiment, the greatest effect was obtained in the double infection with TYLCV + ToMV and TYLCV+CMV (Mean yield of plants was 130.15 and 139.06 gm. respectively).

Table (1): Interaction between three viruses in tomato (*Lycopersicon esculentum* Mill.cv. Cassel rock) plants and its effects on symptoms and disease severity.

Virus combination		Symptom	Disease severity
Control (no virus)	-	-*	-
Virus alone	ToMV	Severe Mosaic, leaf malformation	Severe
	TYLCV	Curling, leaf malformation	Severe
	CMV	Slight mosaic	Slight
Pair of viruses	ToMV+ TYLCV	Mosaic, Curling, leaf malformation	Severe
	ToMV+CMV	Mosaic	Moderate
	TYLCV+CMV	Curling, Mosaic	Moderate
All three viruses	ToMV+ TYLCV+CMV	Mosaic, Curling, leaf malformation	Severe

*(-): no symptoms.

Table(2): Interaction between tomato mosaic virus and other two viruses of tomato (*Lycopersicon esculentum* Mill.cv. Cassel rock) plants

Virus combination	Severity of pale-and dark-green mosaic symptom	Number of plants showing typical symptom	Percentage of affected plants
Control(no virus)	-	-	-
ToMV	Severe	20/20	100
ToMV+ TYLCV	Moderate	13/20	65

ToMV+CMV	Moderate	9/20	45
ToMV+TYLCV+CMV	Severe	11/20	55

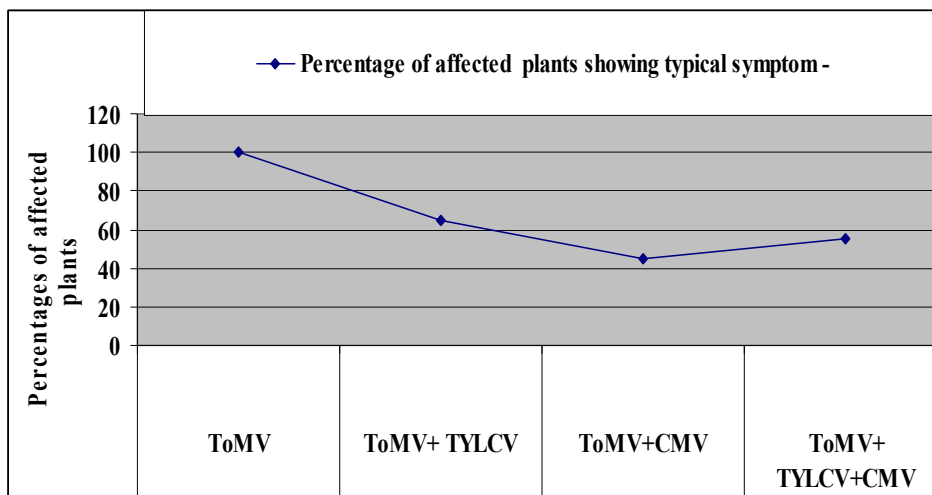


Fig.(2): Percentage of affected plants showing typical symptom(pale-and dark-green mosaic symptom) as a result of interaction between tomato mosaic virus and other two viruses of tomato (*Lycopersicon esculentum* Mill. cv. Cassel rock) plants.

Table(3): Cross protection between tomato mosaic virus and other two viruses of tomato (*Lycopersicon esculentum* Mill.cv. Cassel rock) plants.

Virus combination	Severity of pale-and dark-green mosaic symptom	Number of plants showing typical symptom	Percentage of affected plants
TYLCV	Moderate	10/20	50
CMV	Slight	7/20	35

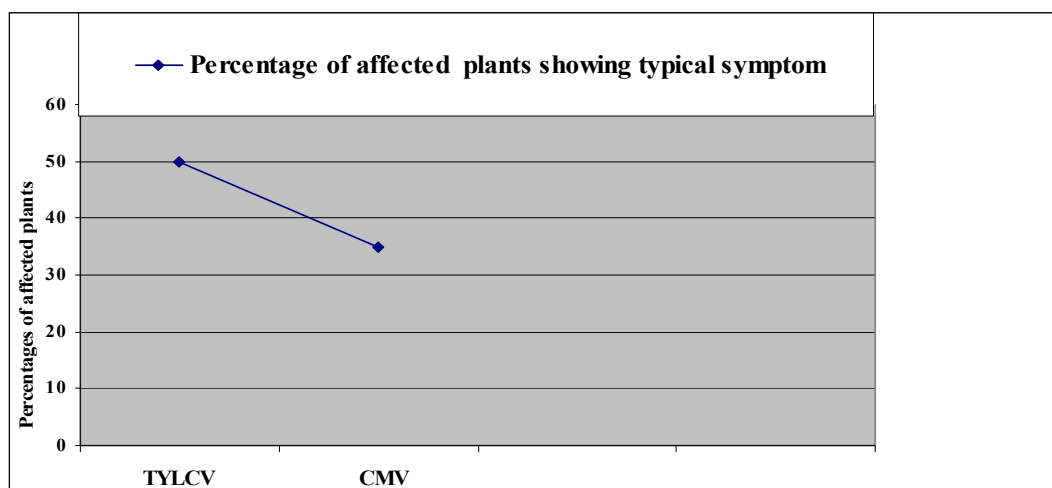


Fig.(3): Percentage of affected plants showing typical symptom(pale-and dark- green mosaic symptom) as a result cross protection between tomato mosaic virus and other two viruses of tomato (*Lycopersicon esculentum* Mill. cv. Cassel rock) plants

Table (4): Effect of single, double and mixed infection with ToMV, TYLCV and CMV on the height of tomato (*Lycopersicon esculentum* Mill. cv. Cassel rock) plants after 4 weeks from the first inoculation.

First inoculation	Second inoculation	Mean length of plants(cm)
Control(no virus)	-	38.70
ToMV	-	32.25
TYLCV	-	27.75
CMV	-	34.95
ToMV+ TYLCV+CMV	-	30.75
ToMV	TYLCV	31.95
ToMV	CMV	34.50
TYLCV	ToMV	28.50
TYLCV	CMV	30.45
CMV	ToMV	33.30
CMV	TYLCV	31.50
L.S.D at 5%	3.15	

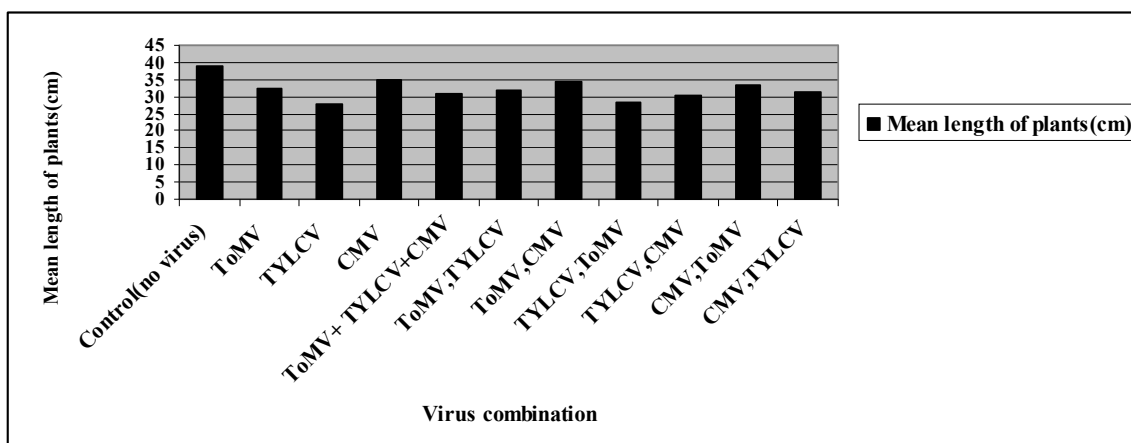


Fig.(4). Effect of single, double and mixed infection with ToMV, TYLCV and CMV on the height of tomato (*Lycopersicon esculentum* Mill. cv. Cassel rock) plants after 4 weeks from the first inoculation.

Table(5): Effect of single, double and mixed infection with ToMV, TYLCV and CMV on the yield of tomato (*Lycopersicon esculentum* Mill. cv. Cassel rock) plants.

First inoculation	Second inoculation	Mean yield of plants(gm)
Control(no virus)	-	219.00
ToMV	-	152.25
TYLCV	-	147.75
CMV	-	160.08
ToMV+ TYLCV+CMV	-	141.34
ToMV	TYLCV	145.91
ToMV	CMV	157.56
TYLCV	ToMV	130.15
TYLCV	CMV	139.06
CMV	ToMV	152.08
CMV	TYLCV	142.86
L.S.D at 5%	17.15	

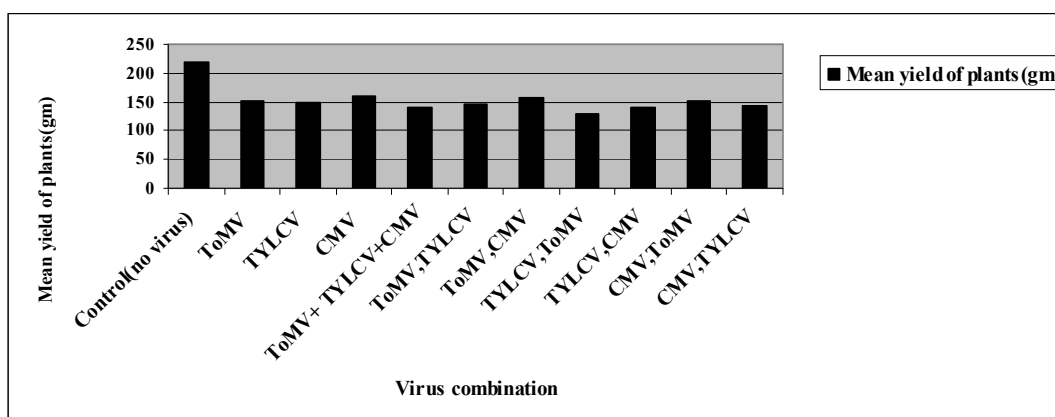


Fig.(5). Effect of single, double and mixed infection with ToMV, TYLCV and CMV on the yield of tomato (*Lycopersicon esculentum* Mill. cv. Cassel rock) plants.

Nitzany and Sela (1962) described interference between tobacco mosaic virus (TMV) and a strain of cucumber mosaic virus, causing local lesions on *Zinnia elegans*, (CMV-LL). Interference was observed on *Nicotiana repanda* and *N. glutinosa* when CMV-LL functioned as protecting virus, as well as on *Z. elegans* when the protecting virus was TMV. Simultaneous inoculations of *N. repanda* or *N. glutinosa* with the two viruses prevented the establishment or the multiplication of CMV-LL. Increasing concentrations of TMV in the challenge inocula resulted in an increased percentage of protection. This supports the hypothesis that the protecting virus occupies some cellular structures or exhausts some precursors needed for the establishment or multiplication of the challenge virus. Eastwell and Kalmar (1997) reported that, in certain cultivars of cowpea (*Vigna unguiculata*) that are operationally immune to cowpea mosaic virus strain SB (CPMV), coinoculation of CPMV with cowpea severe mosaic virus strain DG (CPSMV) reduces severity and delays expression of symptoms normally induced by CPSMV alone. In cultivars susceptible to both viruses, coinoculation delays development of symptoms in response to CPSMV. Using monoclonal antibodies for serological assays and virus-specific RNA probes for hybridization, it is demonstrated that the presence of CPMV in the inoculum yields a concomitant delay in the synthesis of CPSMV coat protein and replication of CPSMV RNA and restricts the transport of CPSMV out of infection centers. Only bottom component of CPMV containing RNA1 is required to offer protection against CPSMV. Destroying the integrity of CPMV RNA eliminates its protective capability. In cowpea cultivars that are operationally immune to CPMV, the presence of CPSMV in the inoculum is unable to compensate for events of CPMV replication that are inhibited. The lack of complementation suggests a high degree of

specificity in the replication of these 2 comoviruses. Hristova and Maneva(1999) studied the effect of cucumber mosaic virus (CMV) and broad bean wilt virus (BBWV) as single and mixed infections on the quantity and quality of *Capsicum annuum* yield. It was found that the susceptibility of *C. annuum* depends on both virus type and plant cultivar, the determining factor being the timing of viral infection. CMV infection had the strongest effect on *C. annuum* followed by BBWV and mixed infection. Mixed infection shows an effect of interference. The total yield and number of marketable fruits as a percentage of total fruits produced by an infected plant was one of the most representative parameters. The proportion number of unmarketable to marketable fruits was a very important characteristic for estimating the reduction caused by a viral infection on the *C. annuum* yield quality. Aguilar *et al.*, (2000). Oilseed rape mosaic virus (ORMV) and tobacco mild green mosaic virus (TMGMV) were mechanically inoculated onto *Arabidopsis thaliana* and *Nicotiana 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 same for both hosts: there was strong interference, with ORMV being the more successful of the two viruses. In tobacco, whichever virus acted as the protecting virus, it interfered significantly with the multiplication of the challenging virus, providing cross-protection. Regardless of the time elapsed between the inoculation of both viruses, the protecting virus always inhibited the

accumulation of the challenging virus. In *A. thaliana*, when TMGMV was the protecting virus, it protected the plants from ORMV infection. When ORMV was used as the protecting virus, TMGMV was not detected, but as TMGMV infection is symptomless and slow to develop in *A. thaliana*, the existence of cross-protection could not be determined. Miguel *et al.*, (2009) illustrated that, tomato rugose mosaic virus (ToRMV) and tomato yellow spot virus (ToYSV) infect tomatoes. ToYSV symptoms in tomato and *Nicotiana benthamiana* appear earlier and are more severe compared to those of ToRMV. Results indicate that ToYSV establishes a systemic infection and reaches a higher concentration earlier than ToRMV in both hosts. ToRMV negatively interferes with ToYSV during the initial stages of infection, but once systemic infection is established this interference ceases. In *Nicotiana benthamiana*, ToYSV (Tomato yellow spot virus) invades the mesophyll, while ToRMV (Tomato rugose mosaic virus) is phloem-restricted. During dual infection in this host, ToYSV releases ToRMV from the phloem.

There are many opinions for explanation of interference phenomenon; Sherwood and Fulton (1983) suggested that the specificity of interference lies at the virus-replication stage. It was concluded that both competition for infection sites and multiplication of the interfering strain are involved in the interference phenomenon Horikoshi *et al.*, (1987) suggest that the inhibitory effect is due to the interference with the binding site of replicase (necessary for RNA synthesis) by partial reassembly of nucleoprotein and that this phenomenon may be a cause of cross protection. Results obtained by Rao- and Hall (1991); Romero *et al.*, (1994); Hsu-YauHeiu *et al.*, (1998) and Teycheney and Tepfer (2001) show that, viral infection can interfere with post-transcriptional gene silencing (PTGS) of a native plant gene, and that this can have profound effects on symptom expression. Khan *et al.*, (1994) suggested that, suppression of bean common mosaic potyvirus (BCMV) str. NL3 symptoms by mosaic-inducing str. NY15 is not caused by impeding its multiplication, but by delaying its transport to the xylem of petiole and stem. Huntley and Hall (1996) concluded that, the observed interference, with bromo mosaic virus replication in transgenic rice, appeared to be mediated through viral RNAs rather than protein products, but was not proportional to detectable levels of messenger expression, suggesting the induction of a host-defense mechanism. Ranjith-Kumar *et al.*, (1998) suggested that, interference with physalis mottle tymovirus replication could be due to the formation of RNA-RNA hybrids at the 3' end of the genomic RNA. DaPalma *et al.*, (2010) concluded that, virus-virus interactions can be organized into three main categories: (1) direct interactions of viral genes or gene

products [such as, Helper-dependent viruses, Pseudotype viruses, Superinfection exclusion, Genomic recombination, Embedded viruses, Heterologous transactivation], (2) Environmental interactions or indirect interactions that result from alterations in the host environment [such as, Indirect transactivation of genes, Breakdown of physical barriers, Altered receptor expression, Heterologous activation of pro-drugs, Modification of the interferon-induced antiviral state] and (3) immunological interactions [such as, Altered immune cell activation, Induction of autoimmunity, Antibody-dependent enhancement of infection, Heterologous immunity].

4. References:

1. Agrios, G.N.(1997). Plant pathology. 4th edition. Academic Press. 508-510p.
2. Aguilar-I; Sanchez-F; and Ponz-F(2000). Different forms of interference between two tobamoviruses in two different hosts. Plant-Pathology. 2000, 49: 6, 659-665.
3. Akad,-F; Dotan,-N; and Czosnek,-H(2004). Trapping of Tomato yellow leaf curl virus (TYLCV) and other plant viruses with a GroEL homologue from the whitefly *Bemisia tabaci*. Archives-of-Virology. 2004; 149(8): 1481-1497.
4. Akos Gellért and Ervin Balázs(2010). The solution structures of the Cucumber mosaic virus and Tomato aspermy virus coat proteins explored with molecular dynamics simulations. Journal of Molecular Graphics and Modelling, 28, 6,2010, 569-576
5. Ammar-ED; Gingery-RE; and Nault-LR(1987). Interactions between maize mosaic and maize stripe viruses in their insect vector, *Peregrinus maidis*, and in maize. Phytopathology. 1987, 77: 7, 1051-1056.
6. Bosco,-D; Mason,-G; Accotto,-G-P(2004). TYLCSV DNA, but not infectivity, can be transovarially inherited by the progeny of the whitefly vector *Bemisia tabaci* (Gennadius). Virology-. 2004; 323(2): 276-283.
7. Brunt, A.A.(1996). Viruses of plants. CAB International Wallingford, UK.
8. Cohen-S; Duffus-JE; and Liu-HY(1983). Acquisition, interference, and retention of cucurbit leaf curl viruses in whiteflies. Phytopathology. 1989, 79: 1, 109-113.
9. Cui,-X-F; Xie,-Y; and Zhou,-X-P(2004). Molecular characterization of DNA beta molecules associated with Tobacco leaf curl Yunnan virus. Journal-of-Phytopathology. 2004; 152(11/12): 647-650
10. Dalmon,-A; Cailly,-M; Bouyer,-S; Arnold-Gaulhiac,-M; Cailly,-A; and
11. Goarant,-G (2003). Emergence of whitefly transmitted viruses on French tomato crops. Colloque-international-tomate-sous-abri,-protection-integree-agriculture-biologique,-Avignon,-France,-17-18-et-19-septembre-2003. 2003; 24-29.
12. Daniela Ribeiro, Jan Willem Borst, Rob Goldbach and Richard Kormelink (2009). Tomato spotted wilt virus nucleocapsid protein interacts with both viral glycoproteins Gn and Gc in planta. Virology, 383, 1, 5 2009, 121-130.

13. DaPalma, T.; B.P. Doonan; N.M. Trager; and L.M. Kasman(2010). A systematic approach to virus–virus interactions. *Virus Research*, 149, 1, 2010, 1-9.
14. Eastwell-KC; and Kalmar-GB(1997). Characterizing the interference between two comoviruses in cowpea. *Journal-of-the-American-Society-for-Horticultural Science*. 1997, 122: 2, 163-168.
15. Fekih-Hassan-I; Gorsane-F; Djilani-F; Fakhfakh-H; Nakhla-M; Maxwell-D; and Marrakchi-M(2003). Detection of Tomato yellow leaf curl Sardinia virus in Tunisia. *Bulletin-OEPP*. 2003, 33: 2, 347-350.
16. Grafton – Cardwell , E. ; T. Perring ; R. smith ; J. Valencia ; and C. Farrar (1996). Occurrence of mosaic viruses in melons in the Central Valley of California. *Plant Dis.*, 80 : 1092 – 1097.
17. Horikoshi-M; Nakayama-M; Yamaoka-N; Furusawa-I; and Shishiyama-J (1987). Brome mosaic virus coat protein inhibits viral RNA synthesis in vitro. *Virology*. 1987, 158: 1, 15-19.
18. Hristova-D; and Maneva-S (1999). Effect of cucumber mosaic virus and broad bean wilt virus on pepper yield. *Archives-of-Phytopathology-and-Plant-Protection*. 1999, 32: 6, 453-469.
19. Huntley-CC; and Hall-TC (1996). Interference with brome mosaic virus replication in transgenic rice. *Molecular-Plant-Microbe-Interactions*. 1996, 9: 3, 164-170.
20. Hsu-YauHeiu; Lee-YunShien; Liu-JihShiou; Lin-NaSheng; Hsu-YH; Lee-YS; Liu-JS; and Lin-NS(1998). Differential interactions of bamboo mosaic potexvirus satellite RNAs, helper virus, and host plants. *Molecular-Plant Microbe-Interactions*. 1998, 11: 12, 1207-1213.
21. Jiang,-Y-X; Blas,-C-de; Bedford,-I-D; Nombela,-G; Muniz,-M(2004). Effects of Bemisia tabaci biotype on the transmission of tomato yellow leaf curl Sardinia virus (TYLCSV-ES) between tomato common weeds. *Spanish-Journal-of-Agricultural-Research*. 2004; 2(1): 115-119
22. Jorda, C.; A. Alfaro; M. Aranda; E. Moriones and F. Garcia-Arenal (1992). An epidemic of cucumber mosaic virus plus satellite RNA in tomatoes in Eastern Spain. *Plant Dis*. 76 : 363 – 366.
23. Kaper, J. M and H. E. Waterworth (1981). Cucumoviruses. 257 – 332 in :
24. Handbook of plant virus infections and comparative diagnosis. E. Kurstake, ed. Elsevier / North Holland Biomedical press, New York.
25. Khan-JA; Lohuis-D; Bakardjieva-N; Peters-D; Goldbach-R; and Dijkstra J(1994). Interference between two strains of bean common mosaic virus is accompanied by suppression of symptoms without affecting replication of the challenging virus. *Journal-of-Phytopathology*. 1994, 140: 3, 260-268
26. Kucharek, T. ; D. Purcifull and R. christie (1998). The association of severe epidemics of cucumber mosaic in commercial fields of pepper and tobacco in North Florida with inoculum in *Commelina benghalensis* and *C. communis*. *Plant Dis.*, 82: 1172.
27. Marchoux-G(1988). Interactions between viruses or between viruses and their satellites in a common host. II. Positive interference: synergy, complementation, assistance. *Agronomie*. 1988, 8: 6, 471-490.
28. Mavrodieva, V. A. (1998). Subgroup determination of Bulgarian isolates of cucumber mosaic virus and the presence of satellite RNAs. *Plant Dis.*, 82: 960.
29. Miguel Alves-Júnior, Poliane Alfenas-Zerbini, Eduardo C. Andrade, Débora A. Esposito, Fábio N. Silva, Ana Cláudia F. da Cruz, Marília C. Ventrella, Wagner C. Otoni and F. Murilo Zerbini (2009). Synergism and negative interference during co-infection of tomato and *Nicotiana benthamiana* with two bipartite begomoviruses. *Virology*, 387, 2, 2009, 257-266.
30. Moriones, E. and J. Navas-Castillo(2000). Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Research*, 71, 1-2, 2000, 123-134.
31. Murad Ghanim, Marina Brumin and Smadar Popovski(2009). A simple, rapid and inexpensive method for localization of Tomato yellow leaf curl virus and Potato leaf roll virus in plant and insect vectors. *Journal of Virological Methods*, 159, 2, 311-314.
32. Nitzany, F. E. and I. Sela (1962). Interference between cucumber mosaic virus and tobacco mosaic virus on different hosts. *Virology*, 17, 4, 1962, 549-553.
33. Noris,-E; Luciola,-A; Tavazza,-R; Caciagli,-P; Accotto,-G-P; Tavazza,-M(2004). Tomato yellow leaf curl Sardinia virus can overcome transgene-mediated RNA silencing of two essential viral genes. *Journal-of-General-Virology*. 2004; 85(6): 1745-1749.
34. Onuki,-M; Ogawa,-T; Uchikawa,-K; Kato,-K; and Hanada,-K(2004). Molecular characterization and strain-specific detection of the Tomato yellow leaf curl virus occurring in Kyushu, Japan. *Bulletin-of-the-National-Agricultural-Research-Center-for-Kyushu-Okinawa-Region*. 2004; (44): 55-77
35. Parrella,-G; Alioto,-D; Ragazzino,-A(2004). Yellow leaf curl on tomatoes in Campania. *Informatore-Agrario*. 2004; 60(41): 58-60
36. Pares, R. and L. Gunn (1989). The rule of non – vectored soil transmission as a primary source of infection by pepper mild mottle and cucurbit mosaic viruses in glasshouse – grown capsicum in Australia. *J. Phytopathology*, 126: 353 – 360.
37. Pradeep Sharma and Masato Ikegami(2010). Tomato leaf curl Java virus V2 protein is a determinant of virulence, hypersensitive response and suppression of posttranscriptional gene silencing. *Virology*, 396,1, 2010, 85-93
38. Ranjith-Kumar-CT; Haenni-AL; and Savithri-HS(1998). Interference with *Physalis mottle tymovirus* replication and coat protein synthesis by transcripts corresponding to the 3'-terminal region of the genomic RNA - role of the pseudoknot structure. *Journal-of-General-Virology*. 1998, 79: 1, 185-189.
39. Rao-ALN; and Hall-TC(1991). Interference in trans with brome mosaic virus replication by RNA-2 bearing aminoacylation-deficient mutants. *Virology-New-York*. 1991, 180: 1, 16-22.
40. Rochow-WF; Muller-I; and Gildow-FE (1983). Interference between two luteoviruses in an aphid: lack of reciprocal competition. *Phytopathology*. 1983, 73: 6, 919-922
41. Romero-J; Huang-Q; Pogany-J; and Bujarski-JJ(1993). Characterization of defective interfering RNA

- components that increase symptom severity of broad bean mottle virus infections. *Virology-New-York*. 194: 2, 576-584.
42. Sakai-F; Dawson-JRO; and Watts-JW(1983). Interference in infections of tobacco protoplasts with two bromoviruses. *Journal-of-General-Virology*. 1983, 64: 1347-1354.
 43. Sackey-S; and Francki-RIB(1986). Interactions between viruses of the cucumovirus group in common hosts. *Biennial-Report-of-the-Waite-Agricultural-Research-Institute*, 1986, 150.
 44. Salvatore Davino, Chiara Napoli, Chiara Dellacroce, Laura Miozzi, Emanuela Noris, Mario Davino and Gian Paolo Accotto(2009). Two new natural begomovirus recombinants associated with the tomato yellow leaf curl disease co-exist with parental viruses in tomato epidemics in Italy. *Virus Research*, 143, 1, 2009, 15-23.
 45. Sead Sabanadzovic, Rodrigo A. Valverde, Judith K. Brown, Robert R. Martin and Ioannis E. Tzanetakis(2009). Southern tomato virus: The link between the families Totiviridae and Partitiviridae. *Virus Research*, 140, 1-2, 2009, 130-137
 46. Sherwood, J.L. and Fulton, R.W. (1983). Competition for infection sites and multiplication of the competing strain in plant viral interference. *Phytopathology*, 73:1363-1365.
 47. Singh, R.S. (1983). *Plant diseases*. 5th edition. Oxford & IBH Publishing Co. PVT.LTD.508-511p.
 48. Steel, P.G. and Torrie, J.H.(1960). *Principals and procedures of statistic*. McGrain Hill Book Company, INC, New York, 481pp.
 49. Stommel, J.; M. Tousignant; T. Wai; R. Pasini and J. Kaper. (1998). Viral satellite RNA expression in transgenic tomato confers field tolerance to cucumber mosaic virus. *Plant Dis*. 82 : 391 – 396.
 50. Susic, D.D.; Ford, R.E. and Tosic, M.T.(1999). *Handbook of plant virus diseases*. CRC Press LLC. 139-141p.
 51. Teycheney-PY; and Tepfer-M(2001). Virus-specific spatial differences in the interference with silencing of the chs-A gene in non-transgenic petunia. *Journal-of-General-Virology*. 2001, 82: 5, 1239-1243.
 52. Thornberry, H. H. (1966). *Index of plant virus diseases Agric. Handbook, No. 307. Agric. Res, U.S. Dept. Agric., Washington, D.C.*
 53. Tomlinson, J. A. (1987). *Epidemiology and control of virus deseases of vegetables. Ann. Appl . Biol.* 110 : 661 – 681 .
 54. Torsten Gursinsky, Beate Schulz and Sven-Erik Behrens (2009). Replication of Tomato bushy stunt virus RNA in a plant in vitro system. *Virology* 390,2, 1, 250-260
 55. Weimin Li, Dennis J. Lewandowski, Mark E. Hilf and Scott Adkins (2009). Identification of domains of the Tomato spotted wilt virus NSm protein involved in tubule formation, movement and symptomatology. *Virology* 390,1, 2009, 110-121.

5/5/2010