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# Interaction Between *Cucumber mosaic Virus* (CMV) and *Tomato mosaic Virus* (ToMV) and their Effect on Disease Severity and Growth of Tomato Plants

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**The effect of the interaction between inoculation of *Cucumber mosaic virus* (CMV) and *Tomato mosaic virus* (ToMV) in tomato plants were demonstrated for symptom severity, plant height, leaves number, chlorophyll content and relative virus concentration. Symptoms assessed after 15 and 30 days of inoculation were affected with double inoculation by both viruses. Tomato plants co infected with CMV then ToMV after 7 days caused high increase in the symptoms severity. All inoculations reduced the stem height as well as number of leaves and chlorophyll content but with different percentages. The greatest reduction in stem height, leaves number and total chlorophyll content were 47.54, 37.93 and 53.93%, respectively when tomato seedlings were inoculated first with CMV and 7 days later with ToMV. Relative virus concentration was determined by indirect ELISA. As compared with the corresponding positive controls, simultaneous infection with CMV and ToMV significantly decreased relative concentrations of both viruses. The highest increase in relative concentration of CMV was 33.41% obtained when the plants were inoculated with ToMV first and CMV, 7 days later. While, combined infection with CMV first and ToMV, 7 days later resulted in higher increase of ToMV by 35.12 %.**

**Key words:** Interaction; *Cucumber mosaic virus*; *Tomato mosaic virus*; Symptoms; Relative concentration.

Tomato (*Lycopersicon esculentum* Mill.) is an important economic crop. Many viruses can attack tomato plants including *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Tomato yellow leaf curl virus* (TYLCV), *Tomato spotted wilt virus* (TSWV) (Roselló *et al*., 1996), *potato virus* X (PVX), *potato virus* Y (PVY) (Mansour *et al*., 2008).

CMV is Mediterranean Basin and worldwide in occurrence (Jacquemond, 2012) and among the most important viruses infecting economic crops in Arab region including Egypt. It causes damage for tomatoes production (Mansour *et al*., 2008).

ToMV is widespread wherever tomato is grown (Mansour *et al*., 2008). It causes serious losses in their hosts by damaging the leaves, flowers and fruits and by causing stunting of the plant (Agrios, 2005).

Natural multiple infections with plant viruses are frequent, often leading to unpredictable variations in symptoms, infectivity, accumulation and/or vector transmissibility (Garcia-Cano *et al*., 2006; Martín and Elena, 2009).

ToMV and CMV are considered from the main viruses of tomato plants and co-infection of them resulted in high incidence in tomato. Mixed infection with viruses which occurs in nature may lead to either interference or synergistic effect (Hull,

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2002; Martín and Elena, 2009; Mohamed, 2010). Synergistic interactions resulting from mixed infections with two or more unrelated viruses are common and well documented in plants (Matthews, 1991; Untiveros *et al*., 2007; Rentería-Canett *et al*., 2011; Bondok, 2012; Zaied, 2013; Cuellar *et al*., 2015).

The present study was achieved to investigate the interaction between CMV and ToMV and evaluate their effect on the symptoms severity, plant height, leaves number, chlorophyll contents and relative concentration of both viruses in tomato plants.

## MATERIALS AND METHODS

**Viruses**

CMV and ToMV used in this study were obtained from the collection of Virology Lab., Plant Pathology Dept., Faculty of Agriculture, Alexandria University. ToMV was maintained on *Nicotiana tabacum* cv. Turkish, while CMV was maintained on *N. glutinosa* which served as virus source plants for further studies.

**Interaction between CMV and ToMV in Tomato plants**

Healthy seedlings of tomato cv. Super strain B were transplanted 21 days after sowing, in 15 cm diameter plastic pots filled with sterilized mixture of soil and beat moss (2:1 w/w) in insect proof cages. Tomato plants were mechanically inoculated either with one or two viruses using 0.1M phosphate buffer, pH 7.0. Simultaneous inoculation with one or two viruses was carried out using an inoculum containing equal volumes of infectious sap of each virus. Regarding double inoculation, some additional trials were carried out. Tomato seedlings were inoculated first with one virus and 7 days later with the second one. Ten plants were used for each treatment. Leaves of control plants were dusted with carborandum and inoculated with only one virus each. Negative control plants were inoculated with buffer solution. All plants were maintained under greenhouse conditions.

**Effect of interaction on symptoms severity**

Singly and double inoculated plants were observed for one month after inoculation. External symptoms appeared on inoculated plants (either on inoculated or on non-inoculated leaves) were recorded 15 and 30 days after inoculation.

**Effect of interaction on stem height and number of leaves**

Plant height, number of leaves and shoot fresh weight of infected as well as control plants were recorded after 30 days of inoculation.

**Effect of interaction on chlorophyll content**

Chlorophyll was extracted according to the method of Goodwin (1965). The concentration of chlorophyll a, b and total chlorophyll were calculated as described by Ross (1974).

**Effect of interaction on relative concentration**

Indirect ELISA was used to determine CMV and ToMV levels in sap of young leaves of infected plants 21 days after inoculation with each virus either alone or in different combinations.

**Indirect ELISA**

Indirect ELISA first reported by Koenig (1981) was used. Infected or healthy leaves were ground in extraction buffer tested as mentioned later using a mortar and pestle, The ELISA values, measured by Universal automated microplate reader ELx 800, were expressed as absorbance at 405 nm. In each ELISA plate, six wells containing extracts of samples from singly infected plants with each virus were used to determine the effect of the interaction on their absorbance values. Changes in absorbance value at 405 nm of viruses in combined treatments were considered significant when they were higher than the corresponding positive controls plus three standard deviation or lower than the corresponding positive controls minus three standard deviation (Fegla and Kawanna, 2013; Fegla *et al*., 2009 and Fu *et al*., 2012). The experiments were repeated twice.

**Source of Antisera**

Antisera used in this study were locally produced in Virology Lab., Plant Pathology Dept., Faculty of Agriculture, Alexandria University.

**Statistical analysis**

Analysis of variance was carried out using SAS software (SAS Institute, 1997) and level of significance was determined by L.S.D comparisons at the 5% probability level.

## RESULTS AND DISCUSSION

In addition to natural infection with one virus, hosts are subjected to simultaneous infection with two viruses or more. Interaction of

CMV and ToMV was studied to determine the effects of single and double infection of these viruses on symptom severity and some plant growth parameters such as plant height, leaves number and chlorophyll content as well as relative virus concentration of each virus.

**Effect of interaction on symptoms severity**

Tomato plants, grown under greenhouse conditions, were inoculated with CMV and ToMV either singly or in different combinations and observed for external symptoms. Obtained data were recorded after 15 and 30 days of inoculation. Data in Table (1) showed that, Severity of symptoms was increased in plants infected first with CMV then by ToMV, 7 days later. Simultaneous infection with CMV and ToMV resulted in less symptoms severity when compared with infection with ToMV followed by CMV, 7 days later.

Variation of severity of the external symptom was correlated with the type of infection. Double inoculation showed obvious effect on symptoms severity as compared with single inoculation with any of CMV or ToMV. When plants were simultaneously inoculated with CMV and ToMV showed symptom severity similar to those appeared on plants inoculated singly with ToMV. Severe symptoms were observed when plants were inoculated with CMV first and after 7 days with ToMV. It may reflect a case of synergistic interaction wherever, disease complexes elicit symptoms that are more severe than the ones induced individually by the members of the complex. Such results agreed with those of (Anjos *et al*., 1992; Taiwo *et al*., 2007; Untiveros *et al*., 2007; Rentería-Canett *et al.*, 2011; Aliyu *et al*., 2012; Bondok, 2012; Zaied, 2013; Cuellar *et al*., 2015) with other viruses and other plants.

On the Contrary, Mohamed (2010) found that, combination of ToMV and CMV caused slight symptoms in tomato plants. In this case interaction resulted in interference effect. Martín and Elena (2009) showed that, the infectivity of Cauliflower mosaic caulimovirus (CaMV) and Turnip mosaic potyvirus (TuMV) was not affected by the presence of the other and no symptom synergism was observed.

It may be concluded that the effect of double inoculation depend on the inoculated virus and the sequence of inoculation.

**Effect of interaction on plant height and number of leaves**

All inoculations reduced the stem height as well as number of leaves but with different percentages. The greatest reduction in stem height (47.54%) was due to double inoculation with the two viruses ToMV after 7 days of CMV (Table 2). The reduction percentage of leaves number was 37.93 and 31.89% when tomato plants were inoculated with ToMV after 7 days of CMV and with CMV after 7 days of ToMV, respectively. However, Simultaneous inoculation with ToMV and CMV resulted in decrease in the leaves number by 32.75%.

Generally the effect of double inoculation was more pronounced than of single inoculations. It may be concluded that any combination between CMV and ToMV has synergistic reducing effect on height and number of leaves of infected tomato plants. These results are similar to those of (Habib *et al*., 1981; Murphy and Bowen, 2006; Bondok, 2012; Zaied, 2013) with other viruses and other plants. Infection at an early age and by multiple viruses can have devastating effects on the growth, yield and the nutritional quality of cowpea (Taiwo and Akinjogunla, 2006).

**Effect of interaction on chlorophyll content of tomato leaves**

Chlorophyll content of tomato leaves was affected by double inoculation with CMV and ToMV (Table 3). The reduction was much more pronounced especially in chlorophyll a when the plants were inoculated with both viruses in all combinations. The reduction percentage was 61.29 % when the plants were inoculated with ToMV after 7 days of CMV or with CMV after 7 days of ToMV. Inoculation with Both CMV and ToMV in the same time decrease the content of chlorophyll a with 58.06%. The reductions in chlorophyll b and total chlorophyll content were 50.00 , 48.27 and 46.55% and 53.93, 52.80 and 50.56% when the plants were inoculated with ToMV after 7 days of CMV or with CMV after 7 days of ToMV and simultaneous infection with the two viruses, respectively (Table 3).

The reduction in photosynthetic pigments may reflect a disturbance in photosynthetic apparatus. In agreement with our results is the finding of Habib *et al*. (1981) who

recorded that, BCMV and BYMV either alone or together reduced the chlorophyll contents of bean plants.

**Effect of interaction on relative concentration of CMV and ToMV**

Relative concentration of CMV and ToMV determined as absorbance values at 405 nm were significantly affected by interactions (Table 4). Simultaneous infection with both viruses decreased relative concentrations of CMV and ToMV by 12.71 and 23.91 %, respectively as compared with the corresponding positive controls.

**Table 1.** Symptoms severity of tomato plants after 15 and 30 days of inoculation with CMV and ToMV in different combinations

| Treatment | Symptoms description | |
|---|---|---|
| | 15 days after inoculation | 30 days after inoculation |
| $CMV_1$ | Mot | Mot, LM |
| $ToMV_1$ | M M | MM, LM |
| $CMV_1$+ $ToMV_1$ | M M | MM, LM |
| $CMV_2$ | Mot | Mot, LM |
| $ToMV_1$+ $CMV_2$ | SM, LM | SM, Mot, LM |
| $ToMV_2$ | M M | M M |
| $CMV_1$+ $ToMV_2$ | SM, LM | SM, Mot, LM, St |
| Control (Uninoculated) | - | - |

- Inoculation was carried out at 3-4 leaf stage (1) or seven days later (2).
- Ten plants were inoculated in each treatment.
- MM= mild mosaic, SM= severe mosaic, Mot=mottle, LM= leaf malformation, S=stunting.

In plants infected with ToMV first and CMV 7 days later, relative concentration of CMV was increased by 33.41 % whereas relative concentration of ToMV decreased by 20.10 %. Combined infection with CMV first and ToMV, 7 days later resulted in decrease of relative concentration of CMV by 10.67% and higher increase of ToMV by 35.12 %.

The highest values were detected in plants infected with CMV seven days before ToMV followed by inoculation with CMV seven days after ToMV. On the other hand, relative concentration reached to 0.761 in singly inoculated plants with ToMV and 0.590 with CMV. These results was in harmony with those of Garcia-Cano *et al*. (2006) with *Tomato chlorosis virus* (TCV) and TSWV which did not show a consistent tendency when singly and doubly inoculated plants were compared. Neither significant differences were observed slightly higher nor were lower virus titers detected in doubly inoculated plants compared with singly inoculated ones.

It can be concluded that, double inoculations of tomato plants with CMV and ToMV resulted in high susceptibility and enhanced the virus severity when compared with single infection. It has a clear effect on symptoms severity, some growth parameters like plant height and leaf number as well as chlorophyll content and relative concentration of both viruses. The effect of each virus varied according to the viruses, counterpart and sequence of inoculation. Also, there is need for urgent research into the mechanism of the extent to which such interactions impact plants in nature.

**Table 2.** Effect of single and double infection with CMV and ToMV on stem length and the number of tomato leaves after 30 days of inoculation.

| Treatment* | Stem height | | Leaves number | |
|---|---|---|---|---|
| | Height(cm) | Reduction (%) | Number | Reduction (%) |
| $CMV_1$ | 19.22 e | 29.10 | 8.6 c | 25.86 |
| $ToMV_1$ | 22.48 c | 17.07 | 8.2 cd | 29.31 |
| $CMV_1$+ $ToMV_1$ | 21.44 d | 20.91 | 7.8 de | 32.75 |
| $CMV_2$ | 20.97 d | 22.64 | 8.6 c | 25.86 |
| $ToMV_1$+ $CMV_2$ | 19.64 e | 27.55 | 7.9 cde | 31.89 |
| $ToMV_2$ | 24.02 b | 11.39 | 9.9 b | 14.65 |
| $CMV_1$+ $ToMV_2$ | 14.22 f | 47.54 | 7.2 e | 37.93 |
| Control (Uninoculated) | 27.11 a | 0.00 | 11.6 a | 0.00 |
| L.S.D. $_{0.05}$ | 0.842 | | 0.737 | |

* Inoculation was carried out at 3-4 leaf growth stage (1) or seven days later (2).
- Means followed by the same letter(s) within each column are not significantly different at $p$=.05

**Table 3.** Effect of single and double infection with CMV and ToMV on chlorophyll content of tomato leaves after 30 days of inoculation

| Treatment* | Chlorophyll content (mg/g fresh weight) | | | | | |
|---|---|---|---|---|---|---|
| | Chlorophyll (a) | Reduction % | Chlorophyll (b) | Reduction % | Chlorophyll (a + b) | Reduction % |
| $CMV_1$ | 0.14 bcd | 54.83 | 0.34 cd | 41.37 | 0.48 cd | 46.06 |
| $ToMV_1$ | 0.16 bc | 48.38 | 0.37 b | 36.20 | 0.53 b | 40.44 |
| $CMV_1$+ $ToMV_1$ | 0.13 ed | 58.06 | 0.31 fde | 46.55 | 0.44 ef | 50.56 |
| $CMV_2$ | 0.16 b | 48.38 | 0.34 bc | 41.37 | 0.50 bc | 43.82 |
| $ToMV_1$+ $CMV_2$ | 0.12 e | 61.29 | 0.30 fe | 48.27 | 0.42 f | 52.80 |
| $ToMV_2$ | 0.14 cde | 54.83 | 0.32 cde | 44.82 | 0.46 ed | 48.31 |
| $CMV_1$+ $ToMV_2$ | 0.12 e | 61.29 | 0.29 f | 50.00 | 0.41 f | 53.93 |
| Control (Uninoculated) | 0.31 a | 0.00 | 0.58 a | 0.00 | 0.89 a | 0.00 |
| L.S.D. $_{0.05}$ | 0.023 | | 0.028 | | 0.035 | |

- Data average of three replicates.
- Means followed by the same letter (s), in each column, are not significantly different at $p$=.05

**Table 4.** Effect of single and double infection with CMV and ToMV on relative concentration in infected tomato leaves after 21 days of inoculations as determined by ELISA reader at 405nm.

| Treatment* | CMV | | | ToMV | | |
|---|---|---|---|---|---|---|
| | Absorbance values at 405nm | Percent Change | Significance of change** | Absorbance values at 405nm | Percent Change | Significance of change** |
| $CMV_1$ | 0.590 | | | | | |
| $ToMV_1$ | | | | 0.761 | | |
| $CMV_1$+ $ToMV_1$ | 0.515 | -12.71 | + | 0.579 | -23.91 | + |
| $CMV_2$ | 0.422 | | | | | |
| $ToMV_1$+ $CMV_2$ | 0.563 | +33.41 | + | 0.608 | -20.10 | + |
| $ToMV_2$ | | | | 0.464 | | |
| $CMV_1$+ $ToMV_2$ | 0.527 | -10.67 | + | 0.627 | +35.12 | + |
| Control*** (Uninoculated) | 0.098 | | | 0.091 | | |

* Inoculation was carried out at 3-4 leaf stage (1) or seven days later (2).
** Changes in absorbance values of viruses in combined treatment were considered significant when they were higher than the corresponding positive controls plus 3 standard deviation or lower than the corresponding positive control minus 3 standard deviation.
-Mean positive control value plus 3 Standard deviation= 0.830 for ToMV (1), 0.532 for ToMV (2), 0.596 for CMV (1), 0.458 for CMV (2).
- Mean positive control value minus 3 Standard deviation= 0.691 for ToMV (1), 0.395 for ToMV (2), 0.583 for CMV (1), 0.385 for CMV (2).
****Negative threshold values were not included here because absorbance values of virus infected extracted sap were greatly higher than that of healthy sample.

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