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Mixed infections of *Watermelon mosaic* potyvirus and *Cucumber green mottle mosaic* tobamovirus in Cucurbit hosts

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Abstract

Mixed infections of cucurbits by *Cucumber green mottle mosaic virus* (CGMMV) and *Watermelon mosaic virus* (WMV) exhibit a synergistic interaction. Watermelon, cucumber and cantaloupe co-infected by the WMV and CGMMV displayed synergistic pathological responses, finally in some cases, progressing to vascular wilt and plant death. Accumulation of CGMMV RNAs in a mixed infection with WMV in some cucurbits was higher than infection with CGMMV alone. Moreover, the level of capsid protein from CGMMV increased in mixed infection. However, the level of WMV did not show any significantly increase in doubly versus singly infected plants. Single infections of WMV or CGMMV on the same hosts produced only vein clearing, blistering, systemic mosaic or mottling on the upper leaves and similar symptoms developed after double infection. It is concluded that co-infections with WMV and CGMMV displayed synergistic interaction which could have epidemiological consequence.

Key words: CGMMV, WMV, mixed infection, synergism.

Introduction

Virus diseases have been generally caused by single or by mixed infection of two or more viruses. The simultaneous infection in hosts by distinct viruses or different strains of the same virus species occurs commonly in nature (Gil-Salas *et al*., 2011). In plants, simultaneous infection by different viruses can lead to a phenomenon described as neutralism, when the viruses do not interfere with the replication, accumulation and transmission of each other (Smith, 1977). Intermittently, it can lead to antagonism, when one virus reduces the infection or accumulation of another virus (Matthews, 1991), or to synergism, when co-infections lead to enhanced symptom expression and ⁄ or increased virus levels (Savenkov & Valkonen, 2001). Synergistic interactions between plant viruses can lead to increased disease in crops that are susceptible to the various virus

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combinations (Hunter *et al*., 2002; Karyeija *et al*., 2000; Wang *et al*., 2002). Besides, interviral synergy can lead to resistance breakage (Choi *et al*., 2002; Wang *et al*., 2004) or limited spread of another virus (Saenz *et al*., 2002). A remarkable increase in host symptoms during double infection compared with single infection was found in several studies involving combinations of potyviruses and viruses belonging to other genera. Combinations of such viruses leading to synergy include the potyviruses *Potato virus Y* (PVY), *Tobacco vein mottling virus*, or *Tobacco etch virus* (TEV) together with the potexvirus *Potato virus X* (PVX) in tobacco (Rochow and Ross, 1955; Vance *et al*., 1995; Vance., 1991); *Maize dwarf mosaic virus* with *Maize chlorotic mottle virus* in maize (machlovirus) (Goldberg., 1987); *Soybean mosaic virus* with two comoviruses, *Bean pod mottle virus* and *Cowpea mosaic virus* in soybean (Anjos *et al*., 1992; Calvert *et al*., 1983); PVY with the polerovirus *Potato leafroll virus* in *Nicotiana clevelandii* (Barker *et al*., 1987); *Zucchini yellow mosaic virus* (ZYMV) with either the polerovirus *Cucurbit aphid-borne yellow virus* in muskmelon (Bourdin & Lecoq., 1994) or the cucumovirus *Cucumber mosaic virus* (CMV) in cucumber (Poolpol *et al*., 1986); and *Turnip mosaic virus* with CMV in radish (Sano and Kojima., 1989). In most of these interactions, the accumulation of the potyvirus did not increase, but the accumulation of the other virus did increase (Anjos *et al*., 1992; Goldberg and Brakke., 1987; Pruss *et al*., 1997; Vance., 1991). In some cases, mixed infection with a potyvirus did not exhibit synergy, as in the cases of *Peanut mottle virus* with either *Tomato spotted wilt virus* (Hoffmann *et al*., 1998) or *Bean pod mottle virus* (BPMV) (Anjos *et al*., 1992).

Much less is known about the synergistic interactions occurring in cucurbit species doubly infected by CMV and potyviruses. Infections of cucurbit species by either potyviruses such as ZYMV or *Watermelon mosaic virus* (WMV) or by CMV are very common and cause considerable damage worldwide in severe epidemics in cucurbit fields, either in single or double infections (Luis-Arteaga *et al*., 1998; Grafton-Cardwell., 1996). Economical damage of virus diseases caused by mixed infection was reported on watermelon showing necrosis by mixed infection of *Cucumber green mottle mosaic virus* (CGMMV) and *Watermelon mosaic virus* (WMV) in Korea (Cho, 1998; Kim *et al*., 2000). Nonagon, a specific ultrastructure produced in the complexly infected cells of watermelon and cucumber plants, induced by interaction of the two pathogens for WMV and CGMMV (Cho *et al*., 2000). Nonagon in watermelon necrosis disease had the shape that one particle of watermelon mosaic potyvirus was surrounded by 9 particles of *cucumber green mottle mosaic* tobamovirus (Cho, 1998). The present study investigated the varying symptoms in cucurbit crops by mixed infection of WMV and CGMMV showing different biological characteristics. The isolates were characterized biologically by reaction to inoculation of a variety of range of test plants. Results were also compared symptom expression and levels of WMV and CGMMV viruses in cucurbit plants in single and mixed infections. Results also compared with those obtained for plants infected with the potyvirus WMV in single and mixed infections with CGMMV.

Materials and methods

Virus sources

Samples were collected from different cucurbit plants in 2009 -2011. They were tested by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with commercial antiserum (SEDIAG S.A, Strasbourg, France) to detect to presence of mixed infection with CGMMV and WMV. The WMV-P and WMV-M isolates were collected respectively from diseased pumpkin and melon plants at different areas of Khorasan provices (Moradi *et al*., 2011) and they were used for the associate virus in mixed infection with *cucumber green mottle mosaic* tobamovirus (CGMMV-C) (Moradi and Jafarpour, 2011). The CGMMV-C isolate (isolated from cucumber crops in khorasan, Iran, in 2010) used as a counterpart virus in mixed infection with WMV-P isolate (Cho, 1998). After identification by DAS- ELISA and RT-PCR, to endure the purity of each isolate, all isolates were passed through three successive single-lesion transfers on *Chenopodium amaranticolor* Coste&Reyn. Each isolate was maintained separately in cantaloupe plant, which also served as the source of inoculums.

Plants, Viruses and inoculations

For single- and double-infection studies, the five kinds of Cucurbit plants for watermelon (cv. local), cucumber (cv. Straight 8), cantaloupe (cv. Honeydew, local), pumpkin (Iranian cultivar) and zucchini squash (cvs. Black knight and Local) were grown in the temperature-controlled greenhouse (25˚C) with a photoperiod of 14 h until development of the first true leaf. *Cucumis melo* seedlings were used as source plants to maintain the viral cultures for inoculation of CGMMV-C, and WMV-P, WMV-C. Inoculations of test plants were carried out mechanically by rubbing cotyledons after dusting with Carborundum powder. Inocula were extracted by grinding leaves in 50 mM potassium phosphate buffer (pH 7.0); at least two plants of each of the following species were inoculated.

For symptomatology of WMV-P, WMV-M and CGMMV-C isolates in the indicator plants at least four plants of each species or cultivar belonging to the families *Solanaceae, Chenopodiaceae, Cucurbitacae, Amaranthaceae* and *legominosae* were inoculated (Table. 1). ELISA and PCR were used addition to symptoms observation for checking the presence of the virus particularly in the case of symptomless infections. Plants were maintained in an insect–proof screen house and approximately 4 weeks after inoculation, plants that showed typical symptoms of virus infection were used as the virus source.

Mixed infection

Double inoculation was performed by preparing extracts from equal fresh weights of young leaves of source plants and mixing the extracts prior to inoculation. In mixed infections watermelon, cucumber, cantaloupe and pumpkin plants were inoculated with a combination of CGMMV+WMV (in a 4 vol. of 0.01M potassium phosphate buffer, pH 7.0). Mock inoculations and individual inoculations with CGMMV and WMV were employed as controls.At 14, 28, 42 and 56 days post-inoculation (dpi) 0.2 g tissue from the second or third leaf was removed for analysis by RT-PCR detection of CGMMV and WMV.

Table 1. Symptomatology of [a]WMV-P, [b]WMV-M and [c]CGMMV-C in the indicator plants

Test plants	Symptoms of		
	WMV-P	**WMV-M**	**CGMMV-C**
N. glutinosa	-/-	-/-	-/-
Nicotiana benthamiana	-/-	CLL/M	-/M
N. clevelandii	-/-	-/-	-/-
N. tabacum cv. Turkish	L/-	CLL/−	-/-
Nicotiana tabacum cv. samsun	-/-	-/-	CLL/−
Chenopodium amaranticolor	CLL/−	CLL/−	CLL/−
C. quinoa	CLL/−	CLL/−	CLL/−
Datura stramonium	L/-	L/-	-/-
Petunia hybrid	-/-	-/-	-/-
Gomphrena globosa	-/-	NLL/−	-/-
Citrulus vulgaris	-/VC,SM	-/M	-/MO
Cucurbita moschata	-/VB,M	-/VB,M	-/-
Cucurbita pepo cv. zucchini	-/VB,M,B	-/VB,M,B	-/-
Cucumis sativus (cv. Straight 8)	-/SM,DIS	-/CLL,SM	-/M,MO
C. melo (cantaloupe cv. Honeydew)	-/SM,ST	-/CLL,SM	-/M,MO
Pisum sativum cv. Alaska	CLL, N/-	CLL, N/-	-/-

[a]*Watermelon mosaic* potyvirus isolated from pumpkin.
[b]*Watermelon mosaic* potyvirus isolated from melon.
[c]*Cucumer green mottle mosaic* tobamovirus isolated from cucumber.
CLL; Chlorotic local lesion, NLL; Necrotic local lesion, M; Mosaic, SM; Severe mosaic, MO; mottling, DIS; Distortion, ST; Stunt, VB; Vein banding, VC; vein clearing, B; blistering, L; Latent infection, -; Non reaction, *; Not test, Inoculated leaves/Upper leaves.

Figure 1. Symptoms of WMV isolates on a number of indicator plants in greenhouse condition. **Left to right:** chlorotic local lesion on *Chenopodium amaranticolor* and *C. quinoa*, respectively.

Nucleic acid extraction and analyses

Total RNAs were extracted from leaf disks collected from the second true leaves using AccuZol™ Reagent (Bioneer, Alameda, CA) or RNX plus solution (Cinnagen, Iran) according to the manufacturer's instructions. After determination of the RNA concentrations and purity by measuring their optical density (OD260/280) using NanoDrop™ Spectrophotometer (cole parmer-USA), the extracts were equalized for electrophoresis in denaturing gels used for northern blot hybridization analyses, which were performed according to Sambrook *et al.* (1989) The RNAs in the gels, were blotted onto Hybond™-N nylon membranes and hybridized with a ^{32}P-labelled RNA probe containing the CGMMV-CP gene according to the manufacturer's instructions (Amersham Biosciences). CGMMV RNA was detected with a ^{32}P-labelled RNA probe complementary to nt 4322–4681 of CGMMV-SH RNA (Moreno *et al*., 2004), and 30 µg RNA was fractionated on 1.2% agarose gel. Also, RNA samples were tested for the presence of each virus using specific primers designed to amplify a fragment of the coat protein gene. For RT-PCR detection of CGMMV RNA, nucleic acid extracts corresponding to 100mg fresh leaf tissue was used. A 486 nt fragment

representing nt 5763–6248 of (CGMMV-SH. GenBank accession number D12505) was amplified (Figure 3A, lower panel) using CGMMV-For (5'-ATGGCTTACAATCCGATCAC-3', at Positions 5763-5782) and CGMM V-Rev (5'- CTACCACCTCGAAAGCTTAG -3', at Positions 6229-6248) covered a full CP gene. The primers for amplification of WMV were as described by Sharifi *et al*. (2008): primers WMV-For (5'- GAA TCA GTG TCT CTG CAA TCA GG -3'), WMV-Rev (5'- ATT CAC GTC CCT TGC AGT GTG -3'), corresponding to nucleotides (nt) 8926 to 8948 and nt 9727 to 9747 of WMV-Fr (GenBank accession number EU660584), respectively, were used to amplify about 825-bp fragment covering the CP region(Figure 3A, upper panel). RT-PCR using coat protein specific primers produced amplified fragments of the expected size that were subsequently cloned, sequenced. The sequence identity of the viruses was confirmed after comparison with GenBank sequences. PCR products were analyzed by electrophoresis in 1.2% agarose gel and visualized by ethidium bromide staining.

Protein analysis: enzyme-linked immunosorbent assay (ELISA)

For ELISA, three leaf disks were collected from each of the second and third true leaves of each test plant and were ground with 1000 µl of either sample buffer. The concentration used (≈200 mg of total tissue per 1000 µl of buffer) was optimal for the analysis of WMV and CGMMV by ELISA. Direct double-antibody sandwich ELISA for WMV (2) and CGMMV were performed with the specific polyclonal antisera according to Clark and Adams (1977). The absorbencies were measured spectrophotometrically at 405 nm using an ELISA plate reader (STAT FAX 2100, USA). A sample was considered virus-positive if the optical density (OD) exceeded the mean plus three standard deviations of the OD of the healthy controls.

Results

Totally of the 690 samples collected from cucumber, watermelon, summer squash, winter squash, cantaloupe and melon, tested by ELISA 180 and 60 were infected with WMV and CGMMV, respectively. Double infections involving different combinations of CGMMV and WMV were noted in 55 samples. The representative isolates were compared with regard to symptoms produced on different test plants (Table 1).

Characteristics of WMV and CGMMV

The reactions on the indicator plants for CGMMV and two different isolates of WMV from Melon and Pumpkin were compared in Table 1. WMV isolates could infect locally *Chenopodium amaranticolor* and *C. quinoa,* and systemically Cucurbits. The symptoms of severe mosaic, blistering and leaf distortion on Cucurbits were produced, but vein banding was produced on the upper leaves of *Cucurbita moschata* and *Cucurbita pepo*. Two isolates infected *N. bentamiana* plants, systemically causing very mild mosaic. WMV-P could not produce any symptoms on *Gomphrena globosa.* However, WMV-M produced necrotic local lesion on *G. globosa*. None of the isolates infected *N. clevelendii* Gray, *N. tabacum* cv. Samsun, *N. glutinosa* and *Phaseolus vulgaris*. CGMMV-C could produce the mosaic symptom on *Citrullus vulgaris, Cucumis sativus* and *C. melo,*

however, it could not produce any symptoms on *Cucurbita moschata* and *Cucurbita pepo* (Table 1).

Symptom appearance by single and mixed infection

The symptoms caused by each virus were distinguishable.

Cucumber: Mild mosaic was produced by CGMMV-C (Figure 2 a) and Vein clearing, chlorotic spot and mosaic were produced by WMV-P at 10 days after mechanical inoculation (Figure 2 b). The doubly infected cucumbers were stunted somewhat with Vein clearing, yellow-green mottle and sever mosaic symptoms, finally had turned completely yellow (Figure 2 c) However, the cucumber plants remained alive.

Watermelon: WMV-P produced blistering, systemic vein clearing and mosaic symptoms on the upper leaves at 10 days after inoculation, followed by severe mosaic symptom (Figure 2 d). CGMMV-C produced somewhat vein clearing and green mild mosaic symptom on the upper leaves (Figure 2 e). The synergistic symptoms of yellowing, vascular wilt, leaf necrosis and plant death were shown at 14 days after double infection with WMV-P and CGMMV-C (Figure 2 f).

Cantaloupe: CGMMV-C could produce yellow-green mottle mosaic on the upper leaves at 10 days after mechanical inoculation (Figure 2 g-i). However, WMV-P produced severe mosaic and malformation on the upper leaves after showing vein clearing at 10 days after inoculation (Figure 2 j). The cantaloupe infected mixedly with WMV-P and CGMMV-C was shown the various symptoms of systemic vein clearing, severe mosaic, malformation, yellowing and vascular wilt, followed by death (Figure 2 k).

Pumpkin: Vein banding was produced on the upper leaves of Pumpkin at 7 days after mechanical inoculation with WMV-P. In the double infection with WMV-P and CGMMV-C, no synergistic symptoms were produced because CGMMV-C could not produce any symptoms. The Pumpkin plants remained alive (data not shown).

Zucchini squash: Mild mosaic, Vein banding and green mild blistering were produced on the upper leaves of Zucchini squash at 10 days after mechanical inoculation with WMV-P. In the double infection with WMV-P and CGMMV-C, no synergistic symptoms were produced because CGMMV-C could not produce any symptoms. Additionally, the squash plants remained alive (data not shown).

Table 2. Symptom progression by WMV-P, CGMMV-W or their mixed inoculums

Plant	[a]Symptoms produced by		
	WMV-P	**CGMMV-C**	**W+ C**
Citrullus vulgaris (local)	-/VC,M	-/MM, VC	-/LN,W,D
Cucumis melo (cantaloupe cv. Honeydew)	VC/VC,M	-/MM	-/VC,W,SM,D
C. sativus (cv. Straight 8)	CLL/VC,M	-/MM	-/VC,SM,W, ST
Cucurbita pepo (cv. Zucchini squash)	-/VC,DIS	-/-	-/ ST,MM
Cucurbita moschata	-/VC,M,B	-/-	-/ST,VB

a
VC; Vein clearing, VB; Vein banding, M; Mosaic, MM; Mild mosaic, SM; severe mosaic, W; Wilt, D; Death, CLL; Chlorotic local lesion, DIS; Distortion, ST; Stunt, B; blistering, LN; Leaf necrosis; -; Non reaction, Inoculated leaves/Upper leaves. Symptoms were investigated on two plants of each treatment at 7-10 days after mechanical inoculation.

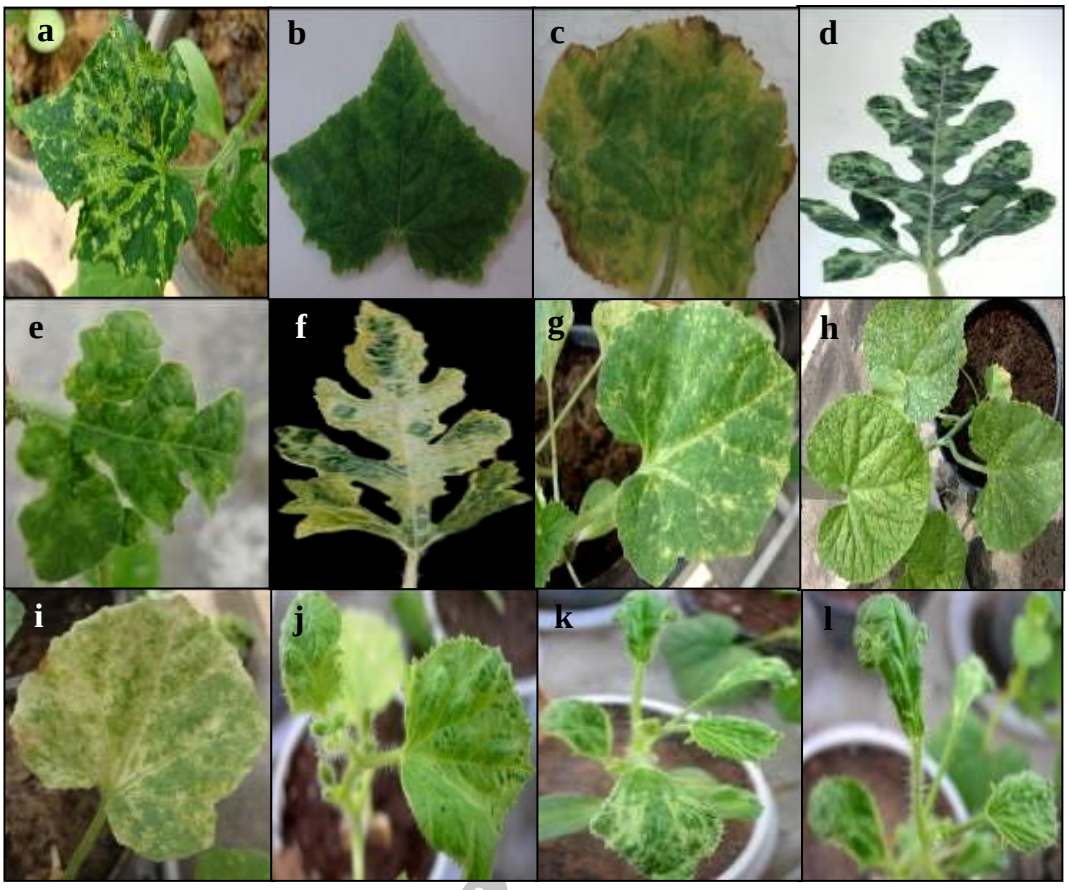

Figure 2. The infection symptotms of *Watermelon mosaic virus* (WMV) and *Cucumber green mottle mosaic virus* (CGMMV) alone or together on cucurbit plants in greenhouse condition. Leaf symptoms on cucumber at 2 weeks post-inoculation with single infections of CGMMV (a), WMV (b), and mixed infections of WMV + CGMMV (c). The infection symptoms of WMV and CGMMV alone or together in Watermelon. In single infection of WMV-P, the virus produced blistering and mosaic symptom on the upper leaves (d), In single infection of CGMMV-C, the virus produced mild mosaic symptom on the upper leaves (e), Yellowing, vascular wilt, leaf necrosis of Watermelon infected mixedly with two different viruses of WMV-P and CGMMV-C finally declined at 14 days after mechanical inoculation (f), CGMMV-C produced mottle mosaic symptom on the upper leaves after producing vein clearing and chlorotic spots and finally sever mottle mosaic and yellowing on cantaloupe (g-i), WMV-P produced malformation and blistering on cantaloupe leaf at 10 days post-inoculation with single infections (j), and finally the mixed infection of WMV-P and CGMMV-C killed the cantaloupe (k).

Changes in CGMMV RNA and capsid protein accumulation associated with synergy in cucurbits

RNA was quantified by scanning the blot and analyzing the sum intensity of each RNA band. At 10 to 12 dpi, before the attack of necrosis, leaves showing mosaic symptoms were analyzed to assess the synergistic effect on the aggregation of viral RNAs in doubly infected plants. CGMMV RNAs increased in doubly infected cucurbit plants (except in pumpkin and zucchini squash) (Figure 3 B). There was a small, but reproducible, increase in the level of CGMMV RNAs accumulating after co-infection with WMV. Also, a similar level of CGMMV capsid protein accumulated in mixed infection with WMV (Table 4). Accumulation of CGMMV-CP is showed in systemically infected cantaloupe in figure 4 .Similar experiments were performed for WMV and the level of WMV

show slight increase in doubly versus singly infected plants (data not shown), which was repeated in many similar experiments in different cucurbit hosts.

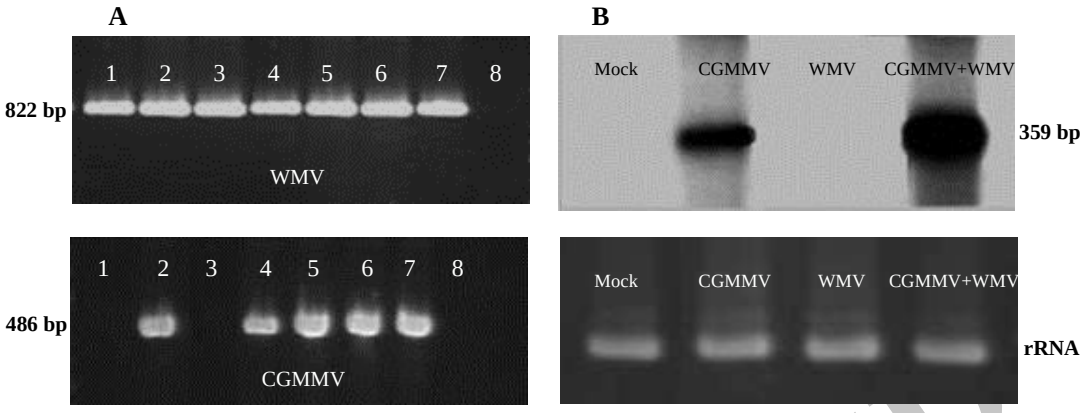

Figure 3. A:*Upper panel*: Electrophoresis pattern of DNA fragments amplified by RT-PCR in 1.7 % agarose gel related to *Watermelon mosaic virus* (WMV) isolates from watermelon, melon, cucumber, pumpkin, zucchini squash, respectively (1-6), **7:** positive control. **8:** healthy pumpkin plant extract as negative control. *Upper panel*: *Cucumber green mottle mosaic virus* (CGMMV) isolates from pumpkin, watermelon, zucchini squash, melon, cantaloupe and cucumber, respectively (1-6), **7:** CV4 isolate as positive control.**8:** healthy cucumber plant extract as negative control.

B: Total RNAs extracted at 14 days postinoculation from leaf disks sampled from the second or third true leaves of infected cantaloupe plant inoculated with CGMMV, in the presence or absence of WMV, were analyzed by denaturing agarose gel electrophoresis and northern blot hybridization, Membranes were hybridized with a CGMMV-CP probe labeled with ^{32}P. Lane **1** contains total nucleic acid extracted from mock-inoculated plants. Lanes: **2:** CGMMV RNA accumulation in cantaloupe plants after infection with CGMMV alone, **3:** WMV alone, **4:** or CGMMV plus WMV. The *Lower panel* shows Ethidium bromide-stained gel to verify an indication of total RNA loading.

Table 3. Accumulation of *Cucumber Green Mottle mosaic Virus* (CGMMV) in cucurbit plants infected with CGMMV alone or with *Watermelon mosaic virus* (WMV)

Inoculum[a]				
Host	**none**	**CGMMV-C**	**WMV-P**	**WMV+CGMMV**
Citrullus vulgaris	0	0.983 ± 0.096	0	1.584 ± 0.180
Cucumis melo	0	1.400 ± 0.381	0	1.885 ± 0.116
C. sativus	0	1.993 ± 0.381	0	2.275 ± 0.198
Cucurbita moschata	0	0.0323 ± 0.009	0	0.055 ± 0.012

[a]Plants were inoculated or mock-inoculated with WMV-P, CGMMV-C and WMV-P+ CGMMV-C. Samples were collected at 10 days postinoculation using four leaf disks collected from the second leaves (one disk per leaf) of four plants. The levels of CGMMV were measured by enzyme-linked immunosorbent assay (optical density = 405 nm) with an antiserum specific to CGMMV capsid protein (1: 200). Values are mean ± standard deviation (n = 4).

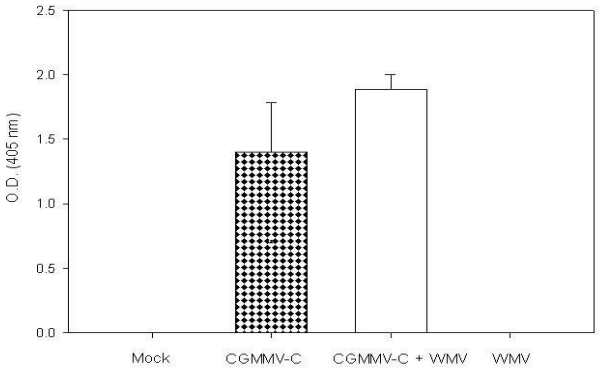

Figure 4. Accumulation of *Cucumber Green Mottle mosaic Virus* (CGMMV) in systemically infected cantaloupe at 14 days post-inoculation. The level of accumulation of CGMMV and WMV isolates were determined by quantitative double-antibody sandwich enzyme-linked immunosorbent assay using antiserum specific to CGMMV (1:200). The value for each histogram represents the average of sample duplicates prepared from four leaf disks of the second leaves (one per leaf) of four plants. Bars stand for standard deviation.

Discussion

Mixed infections of viruses from the family *Potyviridae* and those from other genera typically produce synergism in plants (Gil-Salas *et al.*, 2011). Some of cucurbit plants co-infected by CGMMV and either WMV-P or WMV-C showed more severe symptoms than such plants infected by any of these viruses alone or by CGMMV alone. Eventually, in some plant such as cantaloupe or watermelon developed to wilt and plant death. Cho *et al*. reported that Cucumbers produced only mixed symptoms of the two isolates of WMV-P (isolated from pumpkin) or CGMMV-W (isolated from watermelon), but no synergistic symptoms (Cho *et al*., 2000). However, in this study Cucumbers produced slightly synergistic symptoms in mixed infection of WMV-P and CGMMV-C. Symptoms on the Cucurbits in cantaloupe and cucumber were the only host species displaying symptom expression of the each virus. However, watermelon produced synergistic symptoms of leaf necrosis. From this study, two unrelated viruses of WMV-P and CGMMV-C were influenced each other and the CGMMV-C changed in protein and/or nucleic acid aggregation. The CGMMV RNAs in mixed infection with WMV, accumulated to a higher level than in plants infected by CGMMV alone. CGMMV co-infected by WMV isolate plants displayed synergistic responses in symptom expression (except pumpkin and zucchini squash) compared with plants infected by those viruses alone. Co-infection by CGMMV and WMV isolate led to necrosis of the newly emerging leaves, progressing to vascular wilt and plant death at 15 to 20 dpi in watermelon and cantaloupe plants (Figure 2). The proteins of potyvirus inclusions were involved in the replication of coat proteins and nucleic acid of the potyvirus particles (Ammar *et al*., 1994). Therefore, from the close relationship of two unrelated viruses, the potyvirus inclusions might be a role of helper component in the replication of tobamovirus, or conversely (Cho *et al*., 2000). Potyviruses have been reported to be the major factor in the synergistic interactions in serious virus diseases, and using molecular biology, it has been determined that the genome of potyvirus is the origin of the synergisms (Pruss *et al*., 1997; Vance *et al*., 1995).

Cho *et al*., in 2000 studied on Nonagon, a specific ultrastructure that is produced in the mixedly infected cells of watermelon and cucumber plants, by interaction of the two pathogens for WMV and CGMMV. Potyviruses change its characteristics on its coat protein or somewhere, such as in the case of angled-layer aggregates by CGMMV infected mixedly with WMV-2 on cucurbit (Cho, 1998; Cho *et al*., 2000). WMV coat protein accumulated to similar levels in singly and mixed infected cucurbits. Also, the progression of WMV as quantified by northern blot hybridization analyses was approximately similar in plants with single infections and in mixed infection with CGMMV between 15 and 60 days post-inoculation (dpi). It is concluded that co-infections with WMV enhance the level of CGMMV, which could have epidemiological significance. The progression of CGMMV was clearly different during mixed infections with WMV. Under these circumstances, the accumulation of both viruses was enhanced when compared with single infections (Table 2), suggesting a synergistic interaction between the two viruses. The pumpkin isolate of WMV causes stronger disease symptoms on cucurbits than the cucumber isolate. According to this study interaction between WMV and CGMMV displayed synergistic that could have epidemiological consequence. The data obtained in this study will be beneficial to improve control strategies for these viruses.

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آلودگی مخلوط ویروس موزائیک هندوانه و ویروس موزاییک پیسک سبز خیار در گیاهان کدوییان

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چکیده

آلودگی مخلوط ویروس موزائیک هندوانه (WMV) و ویروس موزاییک پیسک سبز خیار (CGMMV) اثر تشدید کننده‌ای را نشان داد. آلودگی همزمان این دو ویروس در برخی گیاهان کدوییان از جمله هندوانه، خیار و طالبی سبب ایجاد واکنش‌های شدید بیماری‌زایی به همراه زردی، پژمردگی آوندی و در نهایت مرگ گیاهان شد. تجمع RNA ویروس CGMMV در آلودگی مخلوط با WMV تا حدی بیشتر از زمان آلودگی این ویروس به تنهایی بود. به علاوه سطح پروتئین پوششی ویروس CGMMV نیز در زمان آلودگی همزمان افزایش یافت. با این حال سطح RNA و پروتئین پوششی ویروسWMV افزایش قابل توجهی در آلودگی مخلوط نسبت به آلودگی انفرادی نشان نداد. آلودگی انفرادی هر یک از ویروسهای WMV و CGMMV سبب ایجاد علایمی از قبیل رگبرگ روشنی، تاولی، موزاییک سیستمیک یا پیسکی روی برگهای بالایی گیاهان کدوییان شد، ولی در زمان آلودگی مخلوط این دو ویروس همین علایم افزایش پیدا کرد و به صورت سینرژیک (تشدید کننده) نمایان گردید. نتایج حاصل از این بررسی نشان می‌دهد که آلودگی همزمان دو ویروس WMV و CGMMV سبب ایجاد اثرات تشدید کننده می‌شود که این به نوبه خود می‌تواند سبب عواقب همه‌گیر شدن شود.

واژه های کلیدی : ویروس موزائیک هندوانه، ویروس موزاییک پیسک سبز خیار، آلودگی همزمان، اثر تشدید کننده

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