ألمجلة أللیبیة لوقایة النبات
Libyan Journal of Plant Protection
http://www.ljpp.org.ly
ISSN : 2709-0329

Identification of Viruses Causes Mosaic Disease on Cucumber Crop in AL-Lusayta area At AL-Jabal AL-Akhdar – Libya

Bukhairallah, Dareen Mohamed Hamad[1] , Mohamed Ali Mousa Adam [2],Suad Mohammed Sulayman [1] and Omar Mousa El-Sanousi[2]

1) Dept. of botany Faculty of Science University of Omar Al-Mukhtar /ElBeida-Libya
2) 2) Dept. of plant protection Faculty of Agriculture / University of Omar Al-Mukhtar /ElBeida-Libya

Received – August 7, 2022; Revision – August 28, 2022; Accepted –September 10, 2022; Available Online – November 10, 2022.

*** Corresponding author E-mail: E-mail \ omar.elsanousi@omu.edu.ly (Omar Mousa El-Sanousi)**

ABSTRACT \

This study was conducted for the identification of the virus or viruses that caused mosaic symptoms on cucumber plants by using biological characters including symptomology, host range, aphid transmission, and polymerase chain reaction. The result of the transmission test by aphids *Myzus persica* showed the ability of aphids to transmit the virus isolates after feeding on the tobacco leaf of *Nicotiana tabacum* cv. Xanthi the results of host range and symptomology were showed that the virus isolates induced mosaic symptoms on cucumber *Cucumis sativus*, cantaloupe *Cucumis melo*, watermelon *Citriullus lanatus*, tobacco *N. glutinosa*, *N.tabacum* cv. White Burley and Xanthi. No symptoms were observed and no virus was recovered from Chenopodium *Chenopodium amaranticolor*, datura *Datura stramonium* and *D. mete*, squash *Cucurbita pepo* pumpkin *Cucurbita maxima*, pepper *Capsicum annum*, tomato *Lycopersicon esculentum* Broad bean *Vicia faba*. The result of PCR produced by the use of the coat protein (CP) specific Reverse primer to detect Cucumber

mosaic virus after gel electrophoreses were revealed in the presences of the band of **540**bp represented the coat protein of CMV in inoculated and not in healthy tobacco plants this result confirmed that the virus isolates belongs to CMV.

Keywords: Cucumber, *Cucumis sativus*, cucumber mosaic virus, aphid transmission.

INTRODUCTION \

Cucumber plants are infected with a wide range of plant diseases, which include viral diseases and cause considerable losses to the crop. Mosaic symptoms were observed on cucumber plants in some fields in the intermediate area of Alwsyta north of AL-Baida city. Besides some factors such as nutrient deficiency, insect feeding several viruses including *Cucumber green mottle mosaic virus*, *Zucchini yellow mosaic virus*, *Watermelon mosaic virus*, *Cucumber vein yellowing virus*, *Cucumber mosaic virus*, and *Squash mosaic virus* produced mosaic symptoms on cucumber plants [9]. Since we suspected the mosaic symptoms induced on cucumber plants are caused by a viral infection, this study aims to isolate and identify the causes of mosaic symptoms on cucumber plants by using the biological and molecular characters of this agent.

MATERIALS AND METHODS \

Samples collection and preservation: Three visits to several farms in the region of AL-Jabal AL-Akhdar along the road between the cities of AL-Bayda and AL-Hamama were conducted in the summer season from June to September 2018, The samples were taken for infected leaves bearing the symptoms of mosaic Figure (1). In these visits, nine samples were taken from three fields planted with the cucumber crop. These samples were placed in plastic bags and each sample was divided into several samples according to the leaves numbers for use in transmitting the virus. The rest of the samples were kept in the freezer at -0C^{o} for use in further study.

Figure 1: Mosaic symptoms on the infected cucumber leaves.

Biological study: Inoculum preparation: 1 gram of each infected sample is taken and crushed it in a mortar with (1 ml of potassium phosphate KH2PO4 0.1 molar and pH 7). Using the method described by [12].

Plants inoculation: The tested plants were placed in the dark for 24 hours, then sprayed with carborandum abrasive and inoculated with the virus containing sap [10].

The isolates used in the study: Nine samples of infected cucumbers from three fields were brought to the lab in plastic bags for testing for virus infection. Nine samples gave mosaic symptoms on the inoculated cucumber plant. Three isolates represented three fields from those that produced mosaic symptoms on the tested cucumber were chosen for this study.

Virus transmission by aphids: The transmission was tested using the green peach aphid, *M. persicae*, cucumber plants were bred in cages that prevented entry of insects, a number of which were separated in a petri dish for starvation for an hour, then separated leaves of *Nicotiana tabacum* cv. White burley tobacco plants were infected from virus each isolates and left for feeding for 1-3 minutes and then transferred to healthy tobacco plants by six insects for each plant, seven plants per isolate were left to feed for one hour and then sprayed with Mosby insecticide to kill aphids [16].

Study of the response of several families to virus isolates: The plant seedling of three leaves ages was used for studying response the host range, are shown in Table (1).

Table 1: Plants were prepared to study the response of several hosts to virus isolates.

English name	Family and scientific name
Nightshade family	**Solanaceae**
Tomato	***Lycopersicon esculentum***
Tobacco	***Nicotiana tabacum***
Tobacco	***N. tabacum* cv. White burley**
Tobacco	***N. tabacum* cv. Xanthi**
Tobacco	***N.glutinosa***
Jimson weed	***Datura stramonium***
Jimson weed	***D. metel***
Pepper	***Capsicum annuum***
Black Night Hade	***Solanium nigrum***
Gourd family	**Cucurbitaceae**
Watermelon	***Citriullus lamatus***
Cantaloupe	***Cucumis melo***
Cucumber	***C. sativus***
Squash	***Cucurbita pepo***
Pumpkin	***C. maxima***
Goosefoot family	**Chenopodiaceae**
Pig weed	***Chenopodium amaranticolor***
Legume	**Fabaceae**
Broad bean	***Vicia faba***

Use of a polymerase chain reaction (PCR) test: The use of Reverse transcription-polymerase Chain Reaction (RT- PCR) for the detection of the virus: The Reverse transcription-polymerase Chain Reaction (RT-PCR) for the detection of the virus isolate was carried out in the laboratory of the faculty of science at the University of Alexandria for science in Egypt. The primers that were used in this test Table (2) as described by [4,15].

Extraction of the virus isolate RNA: Total RNA was extracted from leaf samples using the Thermo scientific Gene JET Viral RNA purification kit (Lot:00135006, GE Healthcare, UK). 50µl of column preparation liquid was added to the center of the spin column membrane so that the membrane is entirely moistened. 200 µl of the virus was loaded to an empty 1.5ml lysis tube, then 200µl of lysis solution (supplemented with Carrier RNA), and 50µl of proteinase K were added to the sample and mixed thoroughly by pipetting. The sample was incubated for 15 min at 56 C^o in a thermomixer, and centrifuged for 3-5 seconds at full speed to collect drops from the inside of the lid. 300 µl of ethanol 96-100% was added to the sample, mixed by pipetting, incubated at room temperature for 3min, and centrifuged for 3-5 s at full speed to collect drops from the inside of the lid. The lysate was transferred to the prepared spin column preassembled within the wash tube and centrifuged for 1min at 14,000 rpm. Flow-through was discarded. The spin column was placed into a new 2ml wash tube.700µl of wash buffer1 supplemented with ethanol to the spin column was added and centrifuged for 1min at 14,000 rpm. Flow-through was discarded. The spin column was placed into a new 2ml wash tube. 500µl of wash buffer 2 supplemented with ethanol to the spin column was added and centrifuged for 1min at 14,000 rpm. Flow-through was discarded. The spin column was placed into a new 2ml wash tube. The spin column was centrifuged for 1min at 14,000 rpm. Flow-through was discarded. The spin-column was placed into a new 1.5ml elution tube. 50µl of eluent preheated to 56 C^o was added to the center of the spin column membrane. The column was incubated for 2 min at room temperature and centrifuged for 1min at 14,000 rpm. Flow-through was discarded. Total RNA extracted was stored at -20 C^o until used for PCR analysis [13].

Dye reached the bottom of the gel, then gel was stained overnight in 0.1% Coomassie blue stain (0.25g of Coomassie Brilliant Blue R-250 in 90 ml of methanol: H_2O (1:1; v/v) and 10 ml glacial acetic acid). To diffuse excess dye from the gel, it was placed in a destaining solution (45ml Methanol, 10 ml glacial acetic acid, and 45 ml H_2O) for several hours as required [13].

Reverse transcription-polymerase Chain Reaction (RT-PCR) for the detection of CMV: First-strand cDNA was synthesized using Moloney Murine Leukemia Virus reverse transcriptase (Lot:00160714, Thermo Scientific) and its buffer (5_X) [50 mM Tris-HCl

(pH 8.3 at 25 C^o), 250 mM KCl, 20 mM $MgCl_2$, and 50 mM DTT] in presence of reverse primer were used. 1µl of RNA was added to (4 µl (5_X) RT-Buffer, 2 µl (10 mM) dNTPs,1 µl of primer, 1 µl (200 u/ µl) of RT-enzyme, 1 µl (20 u/ µl) RiboLockRNase Inhibitor, 12 µl H_2O). The mixture was incubated at 42 C^o for 60 minutes. After cDNA synthesis, the reverse transcriptase was inactivated at 70 C^o for 15 min in the PCR thermocycler (Gene Amp 9700 thermocycler, Applied Biosystem ABI, USA). Viral cDNAs were amplified by PCR in a reaction mixture (25 µl final volume) containing 5_X Mg-free Buffer, 25 mM $MgCl_2$ (Promega), 5 nmol/ml of each primer Table (2), 10 mM dNTP mixes (Promega), 5U/µl Taq DNA polymerase (Promega) and 5 µl of the RT mixture. Table (2) lists specific synthetic oligonucleotides as primers used, region amplified, expected product size. Thermal cycling conditions, such as incubation time, number of cycles, and temperatures during PCR amplification were shown in Table (3) as described by [15].

Table 2: Specific synthetic oligonucleotides primers used for cloning CMV isolate genes.

Primer name	Primer sequence (5'-3')	Region amplified	Expected product size (bp)
CP F	GCGCGAAACAAGCTTCTTATC	coat protein	540
CP R	GTAGACATCTGTGACGCGA		

Table 3: Thermal cycling conditions used for PCR protocol for CMV.

Primer	Initial Denaturing	Cycles	Denaturation	Annealing	Elongation	Final extension
CP	94C/4min	(40)	94°C /1min	(55°C)/2min	72°C /2min	72°C /10 min

Agarose gel electrophoresis of PCR-amplified DNA: According to Sambrook and Russell [13], 1% agarose gel was prepared by dissolving 0.5g agarose powder in 50ml of 1x TBE buffer (10x TBE Buffer:107.80g Tris base, 55g Boric acid, and 7.44g Disodium EDTA.$2H_2O$ pH 8.3 and the final volume were brought to 1liter with distilled water). The running buffer used was 1x TBE .5 µl of the PCR amplification products were mixed with 3 µl 6x gel loading buffer (38% Sucrose, 0.1% Bromophenol blue, and 67mM EDTA), and run in the 1% agarose gel in 1xTBE buffer. Electrophoresis was performed at 80V, the gel was stained with ethidium bromide solution (0.5µg/ml) for 10 min, visualized on an ultraviolet transilluminator (UVP) (M-20, upland, USA), and photographed using a gel documentation system (Alpha-ChemImager, USA). The size of the PCR product was

determined using a 100bp and 1Kb DNA ladder (Promega).

RESULTS \

Response and symptomology: The results of host range and symptomology were summarized in Table (4). The results showed that the virus isolates induced mosaic symptoms on Cucurbitaceae plants included *C. sativus , C. melo , and C. lanatus* Fig (2) and on tobacco plants included *N. glutinosa , N. tabacum* cv. White burley and Xanthi Fig. (3). No symptoms were observed and no virus was recovered from *C. amaranticolor, D. stramonium and D. metel, C. pepo C. maxima, C. annum, L. esculentum V. faba.*

Table 4: Response of tested hosts to virus infection.

English Name	**Scientific name**	**The symptoms on virus isolate**		
		1	2	3
Chenopodium	*C. amaranticolor*	-	-	-
Cucumber	*Cucumis. Sativus*	M	M	M
Cantaloupe	*C. melo*	M	M	M
Squash	*Cucurbita pepo*	-	-	-
Pumpkin	*C. maxima*	-	-	-
Watermelon	*Citriullus lamatus*	M	M	M
Pepper	*Capsicum annum*	-	-	-
Tomato	*Lycopersicon esculentum*	-	-	-
Tobacco	*Nicotiana glutinosa*	M	M	M
Tobacco	*N. tabacum* cv. White burley	M	M	M
Tobacco	*N. tabacum* cv. Xanthi	M	M	M
Datura	*Datura metel*	-	-	-
Datura	*D. stramonium*	-	-	-
Broad bean	*Vicia faba*	-	-	-

(M): Mosaic (-): Negative

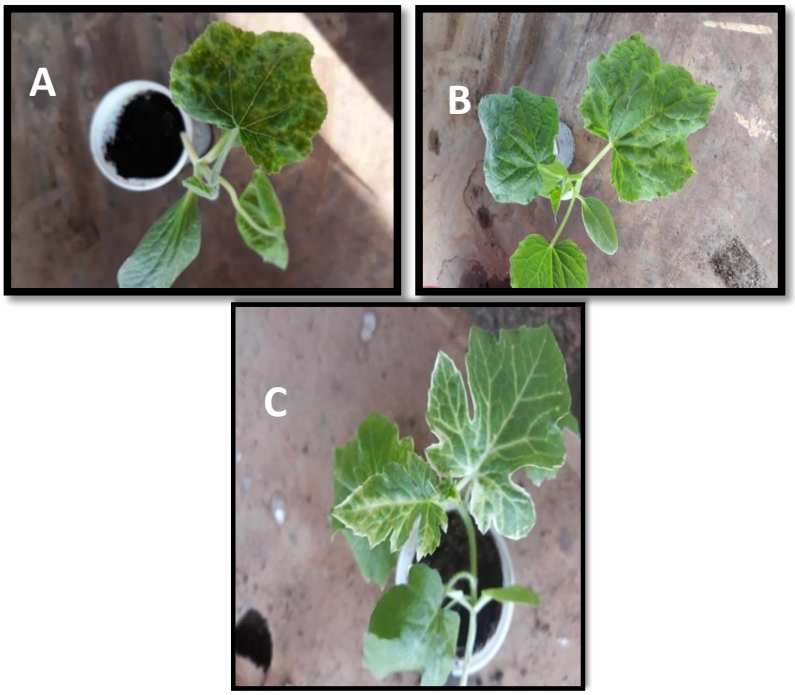


Fig. 2. The mosaic symptoms on cucurbitaceae tested plants.(A) on cucumber *Cucumis.sativus* (B) on cantaloupe *C. melo* (C) on watermelon *Citrullus lamatus*

Fig 3. The virus symptoms on tobacco plants (A) on *N. tabacum* cv. Xanthi (B) on *N. tabacum* cv. White Burly (C) on *N. glutinosa*

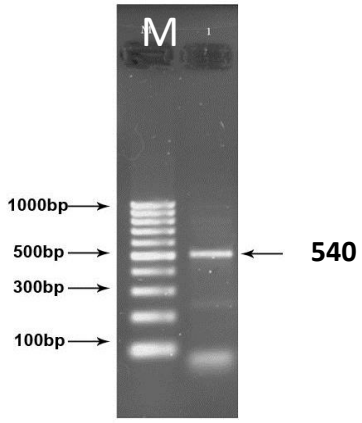
The transmission by aphids: The results showed that *M. persica* could transmit the virus in a non-persistent manner from infected tobacco *N. tabacum* cv. Xanthi to healthy plants after feeding on 1-5 mints Fig. (4). The results of transmission of virus isolates were summarized in Table (5).

Table 5: The transmission of aphids *M. persica* to virus isolates.

The virus isolates	No. of tested plant/ No. of infected
1	6/6
2	6/5
3	6/6

Figure 4: The mosaic symptoms on *N. tabacum* cv. Xanthi induced by aphid Transmission.

Reverse transcription-polymerase Chain Reaction (RT-PCR) for the identification of CMV in inoculated tobacco plant: The result of PCR produced by the use of the CP specific Reverse primer to detect CMV after gel electrophoreses were revealed in the presence of a band of 540 bp represented by the coat protein of CMV in inoculated and not in healthy tobacco plants, the result was shown in Fig. (10).



Number 1: sample 1 positive for CMV

M=1 Kb plus DNA Marker

Figure 5: Electrophoretic mobility of DNA amplicons obtained by Reverse transcription-polymerase Chain Reaction (RT-PCR) from total RNA of host leaves infected by CMV amplified using specific synthetic oligonucleotides primers. Approximately 4 µL aliquots of PCR product were run through 1% agarose gel in 1xTBE buffer, stained with ethidium bromide, and photographed. Lane M: Molecular weight standard 100bp DNA ladder (Jena Bioscience); lane 1: 540bp CMV coat protein.

DISCUSSION \

This study was conducted for the identification of the virus or viruses that caused mosaic symptoms on cucumber plants by using biological characters including symptomology, host range, aphid transmission, and polymerase chain reaction. The result of the transmission test by aphids *M. persica* showed the ability of aphids to transmit the virus isolates after feeding on the tobacco leaf of *N. tabacum* cv Xanthi Table [5]. The aphid *M. persica* has been known in several reports with the ability of transmission of CMV [3,10], the transmission of *Zucchini yellow mosaic virus* [8] and the transmission of *Watermelon mosaic virus* [14]. But could not transmit the *Cucumber vein yellowing virus* which was transmitted by Whitefly [11]. *Squash mosaic virus* was transmitted by Beetle (7). *Cucumber green mottle mosaic virus* is only transmitted mechanically without any biological vector (3). The result of the transmission of the virus isolates through *M. persica* restricted these isolates to three viruses, *Cucumber mosaic virus, Watermelon mosaic virus,* and *Zucchini yellow mosaic virus* [5,10].

The result of Reverse transcription-polymerase Chain Reaction (RT-PCR) by using the CP-specific Reverse primer to detect CMV and production bands of 540 bp revealed that the virus isolate was CMV [15].

The inoculation of several hosts showed the ability of the virus to cause mosaic symptoms on cucumber *Cucumis sativus,* cantaloupe *Cucumis melo*, this result is in an agreement with several reports [3,9]. But it is contradicting to several studies, Ali [1] study different CMV isolates in the middle region of AL-Jabal Al-Akhdar including Al-Haniya, AL-Lusayta, AL-Bayda, Shahat, and Soussa from pepper, wild tobacco, and squid plants all of these isolates did not produced mosaic on *Cucumis sativus*

and *C. melo*. Similarly, Ejmal [6] studied several isolates of CMV from wild tobacco from different areas in the west of Libya, all of these isolates did not produce mosaic on the cucumber plant. Also, Mousa [10] studied several isolates of CMV from the tomato crop in the AL-Lusayta area and he reported that these isolates were not produced mosaic on cucumber and cantaloupe plants. The virus isolate produced mosaic symptoms on tobacco *N. glutinosa*, this result was in agreement with the result of CMV isolates from tomato plants in the AL-Lusayta area a, [10]. And with the CMV isolates from wild tobacco in a different area in the middle region of AL-Jabal Al-Akhdar [1]. The virus isolates induced mosaic symptoms on *N. tabacum* cv. White burley and Xanthi these results are in agreement with the result of CMV isolates from tomato plants in the AL-Lusayta area [10]. The virus isolates could not have infected *D. metel* plants. Similar results of non-infection of this plant with CMV were reported in CMV isolates of wild tobacco in AL-Jabal Al-Akhdar [1,2], and on the west coast of Libya [6]. On the other hand, Mousa [10], reported that CMV isolates from tomato in AL-Lusayta produced mosaic symptoms on *D. metel*. These virus isolates did not infect the Chenopodium *C. amaranticolor* and this result is in consistent with tomato isolate in AL-Lusayta [10], and contrary to the result of Ejmal [6] who reported that the *N. glauca* CMV isolates produced local lesions on this host.

REFERENCES \

1. **Ali, S. S. 2008.** Study of cucumber mosaic virus on several families in the central region of Jebel Akhdar. Thesis submitted as part of the requirements for the attic license (Masters) in plant protection. College of Agriculture, Omar Al-Mukhtar University, Libya. [Arabic]
2. **Ali, S. S. Omar, M. E. and Fathi, S. A. 2009.** Study of cucumber mosaic virus on several families in the Jabal Al-Akhdar region. The Tenth Arab Conference on Plant Protection. Beirut, Lebanon. Research summaries book, 61 pp. [Arabic]
3. **Brunt, A., Crabtree, K. and Gibbs, A. 1990.** Viruses of tropical plants. CAB International. Wallingford Oxon, UK, 706 pp.
4. **De Blas, C., Borja, M., Saiz, M. and Romero, J. 1994.** Broad-spectrum detection of *Cucumber mosaic virus* (CMV) using the polymerase chain reaction. Journal of Phytopathology, 141: 323-329 pp.

5. Debiez C. and Lecoq 2021. Watermelon mosaic virus and Zucchini yellow mosaic virus potyviridae. Encyclopedia of virology (Fourth edition vol. 3 , P 862-870
6. **Ejmal, M. A. 2007.** Identification of viruses causing mosaic on wild tobacco *(Nicotiana glauca* Graham*)* in western Costal-belt of Libya. MSc, Faculty of Agriculture, University of Al-Tahadi, Sirt, Libya, 91 pp.
7. **Freitag, J. 1956.** Beetle Transmission, Host Range, and Properties of *Squash mosaic virus*. Phytopathology, 46: 73-81 pp.
8. **Lisa, V., Boccardo, G., D'agostino, G., Dellavalle, G. and D'aquilio, M. 1981.** Characterization of a potyvirus that causes *Zucchini yellow mosaic*. Phytopathology, 71: 667-672 pp.
9. **Makkouk, K. M. Gaber, I. F. and Safa, G. Q. 2008.** Viral diseases of important agricultural crops in the Arab region. Arab Renaissance House 45-70 pp. [Arabic]
10. **Mousa, A. O. 2018.** Isolation and identification of the virus that causes mosaic disease on tomato crop in AL-Lusayta area, Al-Jabal Al-Akhdar - Libya. 16 pp. [Arabic]
11. **Nitzany, F. and Cohen, S. 1960.** A case of interference between *Alfalfa mosaic virus* and *Cucumber mosaic virus*. Virology, 11 pp.
12. **Noordam, D. 1973.** Identification of plant viruses. Methods and experiments. Identification of plant viruses. Methods and experiments,207
13. **Sambrook, J. and Russell, D. W. 2001.** Molecular Cloning-Sambrook and Russel-Vol. 1, 2, 3. Cold Springs Harbour Laboratory Press.
14. **Webb, R. and Scott, H. 1965.** Isolation and identification of *Watermelon mosaic viruses* 1 and 2. Phytopathology, 55: 895-900 pp.
15. **Zitikaitė, I. and Samuitienė, M. 2009.** Detection and characterization of *Cucumber mosaic virus* isolated from sweet peppers. Sodininkystė ir Daržininkystė, 28: 281-288 pp.
16. **Zitter, T. and Gonsalves, D. 1991.** Differentiation of pseudorecombinants of two *Cucumbe mosaic virus* strains by biological properties and aphid transmission. Phytopathology (USA), 81: 139-143 pp.

تعريف فيروسات مسببة لمرض الموازيك على محصول الخيار في منطقة الوسيطه بالجبل الأخضر _ ليبيا

دارين محمد حمد بوخيرالله[1] ، محمد على موسي[2]، سعاد محمد سليمان[1] و عمر موسى السنوسى[2].

1. قسم النبات كلية العلوم- جامعة عمر المختار البيضاء-ليبيا

2. قسم وقاية النبات كلية الزراعة- جامعة عمر المختار البيضاء ليبيا

الملخص /

أجريت هذه الدراسة على الفيروس أو الفيروسات المسببة لأعراض الموزاييك على نباتات الخيار باستخدام الخصائص البيولوجية التي تشمل (الأعراض ،المدى العوائلي، إلنقل بحشرة المن)، تفاعل سلسلة البوليمير. أظهرت نتيجة أختبار النقل بحشرة المن*Myzus persicae*قدرة الحشرة على نقل عزلات الفيروس بعد تغذيتها على أوراق التبغ من *Nicotiana tabacum* .نوع *Xanthi* وأظهرت نتائج المدى العوائلي أن الفيروس يحفز أعراض الموزاييك على نبات الخيار *Cucumis sativus* والبطيخ *Cucumis melo*والدلاع*Citriullus lanatus* وكذلك اعراض الموزاييك على نباتات التبغ *Nicotiana glutinosa, N. tabacum cv. White Burley, Xanthi.* ولم يحدث إصابة على كل من نبات الزربيح Chenopodium، الداتورا Datura *D. mete* و *D. stramonium*، القرع *Cucurbita maxima*، الكوسه Cucurbita Pepo ، الفلفل *Capsicum annum* والطماطم *Lycopersicon esculentum* والفول*Vicia faba* . نتيجة استخدام اختبار PCR عن طريق استخدام النسخ العكسي لبروتين الغالف ((CPالخاص بالكشف عن الفيروس CMV بعد الكشف عن الترحيل الكهربائي في وجود الحزمة بطول 540 من القواعد المزدوجة وهي أساسا تمثل بروتين الغلاف لفيروس موازييك الخيار CMV في النبات المعدى بالفيروس وليس في نباتات التبغ السليمة هذه النتيجة تؤكد أن عزلات الفيروس تنتمي إلى فيروس موزاييك الخيار.

Keywords: Cucumber, *Cucumis sativus*, cucumber mosaic virus, aphid transmission.