Characteristics of *Cucumber Mosaic Virus* Isolates Infecting Cucurbits in Egypt

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ABSTRACT

Eight isolates of the *Cucumber mosaic virus* (CMV) were isolated from cucumber (*Cucumis sativus*) and zucchini (*Cucurbita pepo*) plants showing symptoms matched the CMV infection. The identity of these isolates was confirmed as CMV based on diagnostic hosts reaction, enzyme-linked immunosorbent assay (ELISA), and electron microscopy. The identification was confirmed at the molecular level. The obtained isolates were mechanically transmitted to diagnostic hosts which produced typical symptoms of CMV infection on each plant. Also, its easily transmitted by aphid (*Aphis gossypii*) in non-persistent manner. The virus could be transmitted through seeds of pumpkin, zucchini, and cucumber but not through seeds of melon. A variation in aggressiveness between obtained isolates were observed. Evaluating the disease reaction of some cultivated cucurbit types toward CMV artificially infection reveled that all the tested types were susceptible but differed in their susceptibility rate.

Keywords: Cucumber mosaic virus, ELISA, RT-PCR, seed transmission, reaction of cucurbits types.

INTRODUCTION

Cucurbits are important crops grown in many countries worldwide. Some cucurbits such as cucumber, zucchini and melons are considered as very important food crop in Egypt.

Many plant diseases with varied causals (bacteria, virus and fungi) can attack the cucurbitaceous crops and cause economic losses to these crops through reduction in growth and are responsible for deformation mottling, and oily spots on fruits, making the product unmarketable (Zitter *et al*. 1996; Walter *et al*. 2003; Herrera-Vasquez *et al*. 2013 and Lobin, *et al.* 2015). Among these, viral diseases are of great importance for farmers due to severe loss in quality and yield (Lebeda *et al*. 2007).

Cucumber mosaic virus (CMV) the type member of the genus Cucumovirus. CMV is considered as one of the most primitive viruses (Tomlinson., 1987). Unlike other members of the family *Bromoviridae,* CMV have a very broad, collective host range, infecting more than 1200 plant species in over 100 families, including fruit crops, vegetables and ornamentals, both monocots and eudicots. (Edwardson & Christie, 1991).

Since the development of the shadow-casting technique (Muller, 1942; Williams & Wyckoff, 1944), there has been no difficulty in detecting particles of viruses. Electron microscopy are generally applicable technique which was used to the identification of plant viruses in clarified infective sap preparations. It has so far been mainly used to assess the particle sizes and shapes of purified plant viruses.

CMV has been studied extensively at the molecular level. The CMV genome is a single-stranded, positive-sense RNA (Palukaitis and Garcia-Arenal, 2003; Roossinck, *et al.,* 1999 and Suzuki *et al.,* 1991). This RNA contain five genes encoding proteins designated 1a, 2a, 2b, 3a, and capsid protein (CP) which conserved on three segments (Palukaitis *et al.,* 1992; Ding *et al.,* 1994; Palukaitis and Garcia-Arenal, 2003). Depending on phylogenetic analyses of nucleotide sequence similarity, the numerous strains of CMV have been classified into two major groups. Later, the subgroup 1 has been further divided into two subgroups (Aramburu *et al.,* 2007; Chaumpluk *et al.,* 1996 and Roossinck, 2003).

CMV is intractable disease due to its extremely broad natural host range and the ability to be transmitted by various methods. Together, these factors have contributed to the success of CMV as a pathogen and its effects on crop losses. Foremostly, the virus is transmitted by aphids but also can transmit by seeds, beetles, parasitic plants, and mechanically (Doolittle, 1916; Edwardson and Christie,1991 and Jagger, 1916). Aphids are the most efficient and the main insect vectors in CMV transmission from plant to plant (Zitter

& Banik 1984). *Aphis gossypii* and *Myzus persicae* are the most prominent among more than 60 species of aphids, can transmit CMV in a stylet-borne, nonpersistent manner (Francki *et al*. 1979).

The seed-borne naturally of CMV has been reported in styrian hulless oilseed pumpkin (*Cucurbita pepo* subsp. *pepo* var. *pepo* Styrian Hulless Group) by Tóbiás *et al*. (2008). Ali & Kobayanshi (2010) have reported on transmissibility of the virus through pepper seeds. Seed transmission of CMV also occurs in many weeds with frequencies ranging from <1% to 50% (Palukaitis and García-Arenal, 2003 and Palukaitis *et al*.,1992).

The importance of CMV is due to its of increase in tropical and subtropical regions, especially where mixed cropping is undertaken. Although, controlling the aphid vector is important, it is still not effective enough in the field. Therefore, CMV resistant plants becomes the best hope for durable resistance to this pathogen. However, many of resistance genes are for tolerance and others can be overcome by different strains of CMV, resistance genes have been utilized in several instances (Palukaitis *et al*., 1992 and Palukaitis and García-Arenal, 2003).

The present study was conducted aiming at isolation and characterization of CMV from different location in Egypt. Screen out some available commercial cucurbits against this virus and the possibility of transmission through seeds as epidemiological aspects related to the disease has also been studied.

MATERIALS AND METHODS

Virus isolation and detection:

Sample collection

Samples that are used in virus isolation and detection were collected from cucumber (*Cucumis sativus*) and zucchini (*Cucurbita pepo*) plantations located in Beheira, Monoufia and Gharbia Governorates during April and May 2019 growing season. Symptomatic leaves and fruits of both cucurbits plants were collected from plants showing symptoms matched the CMV infection. The symptoms appeared on targeted plants included mosaic (generic light-green/dark-green), chlorosis, stunting, vein clearing, blisters on leaves, deformation, mottling and wart (oily spot) on fruits (Zitter & Murphy, 2009). A total of 43 samples were collected. The collected samples were carefully packed in plastic pages and labeled then kept in ice box until it was transported to the laboratory which processed within 24 hr.

Serological detection of virus

The presence of CMV in collected samples was detected using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The test was performed following the standard methods described by Clark and Adams (1977). The commercial kits produced by LOEWE (Germany) were used to perform the test. Samples that positively reacted with DAS-ELISA test has been retained while the others were discarded.

Pathogenicity test

Pathogenicity of CMV isolates was carried out by mechanically inoculating of healthy cucumber plants at two-leaf stage using sap from ELISA positive samples. The symptomatic plant leaves were macerated in 0.1M potassium phosphate buffer (pH 7.5) using a sterile pestle and mortar. The filtrates were inoculated on cucumber plants by leaf rubbing method using carborundum. Plants treated with carborundum and phosphate buffer only were served as control. Treated plants were kept at 25-30ºC in insect proof green house and inspected daily for symptoms development. The inoculated plants were serologically tested by DAS-ELISA test using CMV specific antiserum.

Biological purification and virus propagation

According to Eyvazi *et al.* (2015), the single local lesion technique was used for biological purification of viral isolates. Leaf and fruit sap obtained from CMV-positive samples (based on ELISA test) was mechanically inoculated onto leaves of healthy quinoa seedlings (*Chenopodium quinoa*). For virus propagation, the single local lesions developed on inoculated plants was individually grinded in phosphate buffer solution (pH 7.0) and used for inoculation of cucumber (*Cucumis sativus*) and periwinkle (*Catharanthus roseus)* leaves. These plants were served as a viral inoculum sources for the subsequent experiments.

Virus identification

Diagnostic hosts reaction

The diagnostic responses of the test viral isolates were screened on eight diagnostic plant hosts. These plants include, chilli pepper (*Capsicum annuum)*; periwinkle (*Catharanthus roseus*); Chenopodium (*Chenopodium amaranticolor*); quinoa (*C. quinoa*); datura (*Datura inoxea* and *Datura metel*); tobacco (*Nicotiana glutinosa,* and *N tabaccum* cv

Samsun). The seedlings of these plants were planted in 15 cm plastic pots containing sterilized soil. At the four-leaf stage, the test plants of each host were mechanically inoculated with 20 µl of sap from infected cucumber leaves in 0.01M phosphate buffer (pH 7.0). These experimental plants were kept in net greenhouse (insect proof screen) to excludes the insect vectors. The test plants were monitored daily for symptoms development.

Electron microscopy:

Dip preparation method (Jalender *et al.*, 2017) was used to investigate the ultramicroscopic characteristics of the particles of CMV particles in crude plant sap. Leaf tissues of infected quinoa seedlings were triturated in 2.5% glutaraldehyde and fixed for 10 minutes and centrifuged at 4000 rpm for 5 min. After 5-10 min, excess sap was drained with the help of filter paper. The sap was then mixed with an equal volume of 2% uranyl acetate (pH 7.0) for 1 min and transferred to formvar-coated grids for examination with JEOL-JEM-1010 transmission electron microscope (Egyptian National Research Center).

Molecular detection of CMV by Reverse transcription-polymerase chain reaction (RT-PCR):

Viral RNA was extracted from infected cucumber leaves using TRIZOL procedures, as described by Pecinka lab. The leaves were washed with distilled water and approximately 50 mg of leaves were homogenized in liquid nitrogen. After that, 1 ml of TRIZOL was add to the homogenized tissue and vortexed then incubated at room temperature for 1 minutes. The samples were centrifuged at 13000 rpm for 10 minutes at 4° C. The supernatants were transferred to new tubes and 0.2 ml of chloroform per 1ml TRIZOL and was added. The tubes were shaked vigorously by hand for 15 seconds then incubated at room temperature for 3 minutes. The samples were centrifuged at 13000 rpm for 15 min at 4° C for phase separation. The upper aqueous phase was transferred to new tubes (ca 50-60% of TRIZOL vol.). the RNA was precipitated by mixing with 0.5 ml isopropanol per 1 ml TRIZOL then incubated at room temperature for 10 min. The samples were centrifuged at 13000 rpm for 10 min at 4°C to obtain pellets. The supernatant was removed and the pellets were washed with 1 ml 75% EtOH (diluted with DEPC treated water) then vortex once and centrifuged at 7500x g for 5 min at 4°C. The supernatant was discarded and the pellets were dried for 5 min at room temperature. The pellets were dissolved in DEPC treated water and incubated at 55°C for 10 minutes then stored at -80°C until used. The primer, CMV1F (5′-GCCGTAAGCTGGATGG ACAA-3′) and the primer C M V 2 R (5′-TATGATAAGAAGCTTGTTTCGCG-3′) designated by Wylie *et al,* (1993) were used to amplify a region of about 442 bp in length covering complete CP region of CMV.

The synthesis of cDNA was carried out with Reverase™ M-MuLV reverse transcriptase (BIORON, Germany), following the protocol recommended by the manufacturer.

The amplified DNA was electrophoresed on a 2.0% agarose gel with 1xTAE buffer, stained with 0.5 µg/ml ethidium bromide and photographed using (Gel Doc 2000 Bio-RAD). The molecular weight of the PCR products was determined by comparison with DNA marker, 100-1000 bp ladder (exACTGene, Canada).

Aphid transmission

Melon aphids (*Aphis gossypii*) collected from apparently healthy cucumber plants were used in this experiment. The collected aphids were reared on healthy cucumber plant. New generation of aphids were reared on other healthy cucumber plants in insect proof cages and kept under the optimal conditions, i.e. 20-25°c and 70% climatic humidity for the proper aphid growth. After 3 weeks of rearing, the aphids were collected and starved for one hour then transferred to cucumber plants infected by CMV and allowed an acquisition feeding period for 20 minutes (Francki *et al*. 1979). After that, the aphids were transferred to healthy cucumber plants to feed for 10-15 min. After one hour, the insecticide solution was sprayed to kill all aphid vectors. The plants were observed daily for the symptoms development. After 10-15 days of inoculation, symptoms were noted and ELISA was performed to confirm the presence of CMV in the test plants.

Seed transmission investigation

The ability and the rate of CMV transmission via cucurbit seeds were investigated under experimental conditions. Seeds of cucumber, zucchini, melon and pumpkin were seeded in plots (10 cm in diameter) and the emerged seedlings were artificially inoculated by the virus preparation at the four-leaf stage.

Mature fruits were harvested from heavily symptomatic plants and seeds were obtained from these fruits and left to dry in shaded place. After the seeds has been completely dried, the seeds of each type were mixed together and 200 seeds were randomly selected from each type and placed in plastic jar then kept under room conditions until used. The selected seeds of each plant type were divided into 4 portions (each with 50 seeds) and each portion were planted in 4 successive planting dates. In each planting date, the regarded seeds were sown individually in plastic pots (10 cm in diameter) containing autoclaved mixture of soil, sand and compost (2:2:1). These pots were kept in net greenhouse (insect proof screen) to excludes whitefly, aphids and leaf miners. The emerged plants were monitored for CMV symptoms development for the duration of the foliar stage. All plants showed any foliar symptoms matched CMV symptoms were tested by DAS-ELISA for the presence of CMV. The rate of seed-transmission for each cucurbit type was estimated from entirety of plants that reacted positively with ELISA test during the four planting dates.

Aggressiveness of cucumber mosaic virus isolates

The aggressiveness of the obtained isolates was evaluated on cucumber and zucchini plants as sensitive hosts in pots experiment.

Prior to the experiment, the tested isolates were individually propagated by mechanically inoculation of healthy cucumber seedlings using the sap of single lesions obtained from infected quinoa plants which previously inoculated by each regarded isolates. These infected cucumber plants were served as inoculum source.

Seeds of cucumber (cv. Barracuda) and zucchini (*cv.* Eskandarani) were seeded in sterilized 25 cm plastic pots (each with 1seeds) containing sterilized soil. At the four-leaf stage, the teste plants of each cultivar were mechanically inoculated with 20 µl of sap from infected cucumber leaves (represented each isolate) in 0.01M phosphate buffer (pH 7.0). Twenty µl of buffer were applied to mock-inoculated controls. Twenty plants were used for each isolate and the experiment was performed twice. These experimental plants were kept in net greenhouse (Anti-Virus insect screen) to excludes the insect vectors. The teste plants were monitored daily for symptoms development.

Infection percentage and disease severity were recorded at 14 and 28-day post inoculation according to the following scale

0 = no symptoms; 2 = mild mosaic symptoms on leaves; 4 = severe mosaic symptoms on leaves; 6 = mosaic and deformation symptoms on leaves; 8 = severe mosaic and deformation of leaves; 10= severe mosaic and deformation of leaves with stunted. (Murphy *et al.,* 2003):

$$\text{Disease severity} = \frac{\sum(\text{disease grade} \times \text{number of plants in each grade})}{\text{total number of plants} \times \text{highest disease grade}} X100$$

Disease reaction of cucurbits type

A pots experiment was conducted to evaluate the pathological response of 15 cultivated cucurbits plants toward CMV. The seeds of the regarded plants were collected from different Egyptian research institutes and local markets. To confirm that these seeds are virus free, thirty seeds from each were randomly selected from the total number of each plant type and seeded in sterilized pots (25 cm in diameter) containing sterilized soil. These pots were kept in net greenhouse (insect proof screen) to exclude the insect vectors. The emerged plants were monitored for symptoms development. Plants showed viral symptoms or abnormality growth during the duration of the growing period were directly eliminated. The healthiest plants within the experimental plant were subjected to DAS-ELISA test to further confirmation of the absence of CMV in these plants. Fruits resulted from these plants were harvested after maturity stage and left to dry in shaded place. The seeds of each type were extracted from the fruits and air dried then mixed together and kept until used. At the appropriate time for planting of each experimental plant, a total of 20 seeds of each plant type were surface-sterilized by 1.0% sodium hypochlorite (20% household bleach) for 5 min and then seeded in sterilized soil in 25-cm plastic pots (4 seeds per pot).

The inoculation of the test plants disease severity assessment and the experiment procedures were performed as described earlier in aggressiveness experiment. Disease severity was evaluated during 2 consecutive planting seasons. Each treatment (virus-inoculated or buffer mock-inoculated) involved five replicated pots and the plants were grown in a completely randomized design.

Results

Virus isolation and detection

Subjection of the collected sample to DAS-ELISA test revealed that, from 43 tested samples only 14 samples positively reacted with the test which represent 32.55% (Table 1). Regarding to the sample's location, the samples collected from al-Gharbia Governorate showed the highest level (53.33%) of infected samples followed by Monoufia (26.66%) and Beheira (15.38%). In regard to the plant type, the positive cucumber samples checked by DAS-ELISA test were more than the positive samples of zucchini.

Pathogenicity test

The CMV in the present investigation was found to be mechanically transmissible by sap inoculation cucumber after 9 to 12 days of incubation time. The inoculated plants manifest yellowing, mosaic, mottling, necrosis, blisters vein banding on leaf plants were stunted. Oily spots on fruits (Fig. 1). All the inoculated plants showed symptoms identical to those observed under field conditions (Fig.2).

Biological purification and virus propagation

The mechanically inoculation of quinoa leaves by leaf and fruit sap obtained from CMV-positive samples in ELISA test lead to formation of single local lesions only on the inoculated leaves. The lesions beginning to appear 8 days after inoculation (Fig 3). All ELISA positive samples produced this symptom on inoculated seedlings.

On the other hand, the virus was readily propagated in periwinkle leaves after inoculation by sap of single local lesions formed on quinoa leaves. The inoculated plants showed mosaic symptom due to the systemic infection (Fig. 3).

Virus identification

Diagnostic hosts:

Eight plant species were mechanically inoculated by CMV isolate. Symptoms appeared on host plants could be divided into two groups as follows:

The first group, which is exhibiting systemic symptoms included: periwinkle (*Catharanthus roseus*); and tobacco (*Nicotiana glutinosa*, and *N tabaccum* cv. Samsun) (Fig 4). The second group, showing local lesions only on the inoculated leaves included: Chenopodium (*Chenopodium amaranticolor*); quinoa (*C. quinoa*); and Datura (*Datura inoxea*); (Fig. 4). The third group, showing local lesions on inoculated leaves followed by systemic symptoms, included Chilli pepper (*Capsicum annuum*); and Datura (*Datura metel*); (Fig. 4). Infection was confirmed by back inoculation to periwinkle as an indicator host plant and /or by ELISA technique.

Infection was confirmed by back inoculation to *C. roseus* as an indicator host plant.

Electronic microscopy:

The preparation of extracts from plants infected with CMV, examined under an electron microscope, revealed pleiotropic particles and a diameter of around 28 to 30 nm (Fig. 5).

Reverse transcription-polymerase chain reaction RT-PCR:

The RT-PCR was used for detection of CMV in infected cucumber. PCR fragment of 442 bp size was amplified with the primers used for amplification of the coat protein of CMV. Agarose gel electrophoresis analysis of the amplified PCR products is demonstrated in Fig. (6). However, no product was amplified from healthy cucumber plants using the same procedure.

Aphid transmission:

Aphid transmission experiment using *Aphis gossypii* revealed that CMV was efficiently transmitted by this transmission method. Also, the shortness of the feeding time on diseased plants proved that the virus was transmitted in a non-persistent manner. The symptoms of CMV began to appear on cucumber plants after 12 days of inoculation. The presence of CMV in the test plants was confirmed by ELISA test.

Seeds transmission of the virus:

CMV seed transmission was investigated in 4 cucurbit species under experimental conditions. Generally, the ability of CMV to transmission via seeds were established in cucumber, zucchini and pumpkin but not in melon (Table 2). The rates of transmission were generally low and significantly differed between the three crops. The highest rate of transmission was recorded in pumpkin (2.55 %) followed by zucchini (1.51) and cucumber (1.015). None of the emerged plants from melon seeds showed foliar symptoms matched CMV symptoms in the duration of the experiment.

Isolates aggressiveness:

Variation was observed between the isolates in terms of their virulence on cucumber and zucchini after 14 and 28-day post inoculation.

The results in Table (3) show that all isolates of CMV have different percentages of disease severity (DS) after 14 and 28-day post inoculation. All isolates showed high percentages of diseases severity after 28-days post inoculation comparing to 14-days post inoculation.

On cucumber plants, isolate 6 gave a higher of disease severity (84%) after 28-day post inoculation, followed by isolate 7 (85%) after 28-day post inoculation, On the contrary, isolate 7 gave a lowest disease severity (28%) after 14-day post inoculation.

On zucchini plants, isolate 6 gave a higher of disease severity (88%) after 28-day post inoculation, followed by isolate 3 (74%) after 28-day post inoculation, On the contrary, isolate 2 gave a lowest disease severity 25% after 14-day post inoculation followed by isolate 8 (27%) after 14-day post inoculation.

Reaction of cultivated cucurbits type

The reactions of cucurbit types against cucumber mosaic virus were evaluated under experimental conditions. In general, all the tested types were susceptible but differed in its susceptibility rate (Table 4).

Categorically, the tested cucurbit types divided into three categories depending on the statistical analysis. The first category included cucumber and zucchini (Regardless of its tested cultivars) followed by muskmelon (*Cucumis melo* v. reticulatus which showed the highest level in terms of susceptibility to infection by CMV. The second category included squash (*Cucurbita muschata),* pumpkin (*Cucurbita maxima*), cantaloupe *(Cucumis melo* v. cantalupensis), colocynth (*Citrullus lanatus* v. colocynthis and snake cucumber (*Cucumis melo elongates*) rating scale equivalent to 6.6, 6.4, 5.6, 5.2 and 5.0 respectively. The third category included sponge gourd (*Luffa aegyptiaca)* and watermelon (*Citrullus lanatus*) which showed the lowest rating scale values (4.4 and 3.6, respectively).

Regarding to the tested cultivars of cucumber and zucchini, the rating scales varied between each cucurbit type. In the case of cucumber, the tested cultivars significantly differed in their susceptibility rate. Cultivar Barracuda showed the highest susceptibility rate which the rating scale reached 7.4 (equivalent to 74% disease severity percentage). Beit alpha cultivar comes in second category with 6.4 rating scale (64% disease severity) followed by Faris F1 with 4.8 rating scale (48 % disease severity). Likewise, the tested cultivars of zucchini significantly differed in its susceptibility rate. Whereas, Eskandarani and Sandy cultivars were the most susceptible (with 8.2 and 8.0 rating scales respectively) with a significant difference between it and other tested cultivars. Cultivars, Dahab, and Light showed moderately susceptibility levels with rating scales equivalent to 6.4 (64%), 4.6 (46%) respectively.

Discussion

In this study, isolation of CMV from cucumber and zucchini samples showed virus like symptoms. The Isolation was performed depending on two sequential steps that started with subjection of the collected sample to double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and then inoculating of *Chenopodium quinoa* as a local lesion host.

DAS-ELISA results revealed that 32.55% of the tested samples were found to be positive for infection with CMV. ELISAs is apparently a rapid, sensitive, specific and economic detection method for plant viruses. This serological technique is very useful especially for testing large numbers of plant samples in a relatively short time. This method was first introduced to the study of plant viruses in the 1970s (Voller *et al*., 1976) and has since become the most widely used laboratory technique for screening of viruses in plant samples.

Although, 43 samples were tested, only 14 samples (represent 32.55%) were positive to the test. This low percentage can be attributed to the implementation of the test or to presence of symptoms similar to CMV symptoms but actually these represent other viral infection or other causes such as bacteria, fungi or even some abiotic stress factors. To reinforce that point of view, it's known that the successful implementation of ELISA methods reflects the good performance of these tests. On the other hand, cucurbits are natural host of numerous plant pathogenic viruses.

It is firmly established that *Chenopodium quinoa* is a natural local lesion host of numerous plant viruses, including CMV. The single local lesion technique was used for

biological purification of viral isolates. In this regard, leaves of healthy quinoa seedlings (*Chenopodium quinoa*) was mechanically inoculated using sap from ELISA positive sample. It's been proven that *Chenopodium* spp. (particularly *C. quinoa* and *C. amaranticolor*) are important indicator plants that are used to isolation and purification of plant viruses through single-lesion transfer (Yeh *et al,* 1992). The induction of local lesions on Chenopodium leaves through triggering the programmed cell death, known as the defensive hypersensitive response (HR) leads to localizes and eliminates the viruses (Heath, 2000 and Shirasu and Schulze-Lefert 2000).

Electron microscopic examination allowed easy detection of virus particles. Electron micrographs obtained with leaf dip preparation revealed the presence of isometric spherical particles with a membranous aspect and a diameter of around 28 to 30 nm diameter. This indicating the presence of Cucumber mosaic virus in the examined preparation. Similar particle size, morphology and average diameter of 29-30 nm were reported earlier by Lockhart and Fischer (1976), Bidari and Reddy (1990), Holcomb and Valverde (1991), Kiranmai *et al.* (1997), Doomar Singh *et al.* (1999) and Jagadeeshwar (2004).

CMV isolated from cucumber plants was identified by using of specific oligonucleotide primers in RT-PCR. Analysis of PCR products on agarose gel electrophoresis revealed amplification of specific bands of approximately 440 bp, as similarly recorded by Wylie *et al.* (1993). The RT-PCR was used for detection of CMV in infected cucumber. Previous studies of Hadidi *et al.* (1995); Thompson *et al.,* (1994) illustrated the importance and usefulness of PCR as a molecular diagnostic tool of plant viruses.

One of the main reasons of difficulty to control CMV is that its ability to be transmitted in a non-persistent manner by more than 60 species of aphids (Zitter, 1984; Palukaitis *et al.,* 1992). The transmission of CMV from one infected plant to another through the aphid feeding leads to dramatically spread of viral infection. In this manner, the ability of aphid to cause infection is acquired in just one minute after ingestion and is gone within hours which the hundreds of plants can infected during those few hours (; Palukaitis and García-Arenal 2003; Jck; Falk 2006 and Jacquemond 2012).

Seed transmission of CMV was investigated in cucumber, zucchini, pumpkin and melon under experimental conditions. Interestingly, the ability of CMV to transmission via seeds were established in cucumber, zucchini and pumpkin but not in melon.

Although it is widely proven that this virus can't be transmitted through cucurbit seeds, there are many researchers who have mentioned the possibility of transmission in this way. It was reported by Doolittle and Walker (1925) that 55% of wild cucumber seeds were found to be infected by CMV. Sharma and Chohan (1974) reported that the rate of CMV seed infection in pumpkin was 0.7%. It was reported from India that CMV can be transmitted through melons seeds (Sharma *et al.,* 1984). Tóbiás *et al*. (2008) reported that from 225 seedborne virus infected plants of hulless oilseed pumpkin (*Cucurbita pepo),* a total of 166 had ZYMV (Zucchini yellow mosaic virus) and 59 had mixed infection of ZYMV and CMV as identified by ELISA test indicator plants and RT-PCR.

Regarding results of seed transmission in the present work, the honesty and truthfulness requires it to be noted that the experiment were conducted under conditions that might lack some precautions and tools that make the results highly accurate, so we will subject it in the future to more accurate tests in particular RT-PCR.

All eight isolates used in this study observed different variation of aggressiveness on cucumber and zucchini after 14 and 28-day post inoculation. These results are in agreement with Zhang *et al.,* 1994 who reported that differentiation of CMV isolates based on symptom severity and several researchers on tobacco and capsicum. Diveki *et al*., 2004 and Lee 2009 found that CMV isolates can be classified into different pathotypes based on their virulence in specific plant species and varieties.

Management of CMV by cultural practices alone is so difficult due to its wide host range, rapid spread by vectors and lack of suitable host resistance. The best and the most economical method of viral disease control is the use of resistance cultivars. It is easy to adopt and low in cost, and it is also environmentally friendly. Therefore, the screening of cucumber cultivars for resistance to CMV is important because the resistant cultivars could be used in a cucumber-breeding program in order to facilitate the development of new virus resistant commercial cultivars. So, the reactions of

cucurbit types against CMV were evaluated under experimental conditions.

Unfortunately, all the tested types were susceptible but it differed in its susceptibility rate. Among 10 cucurbit types, sponge gourd (*Luffa aegyptiaca) and* watermelon (*Citrullus lanatus*) showed the lowest rating scale values. Nevertheless, this low values remains insufficient and unreliable evidence for resistance of these cucurbit types.

In addition, the cucumber and zucchini cultivars screened in this work were highly susceptible to this virus and when infected showed mosaic, mottling and distortion of leave and fruits. Plapung *et al.* (2014) screened one-hundred varieties of cucumber from different origins for resistance to CMV by mechanical inoculation. They found many resistant lines especially, six accessions from China. This agreed with Heyvey (1997) who reported that, the Chinese long green cucumber varieties were resistant to CMV. Munshi *et al.* (2008) reported that among 31 genotypes of cucumber. sativus var. hardwickii collected from 21 locations in India, 8 genotypes were categorized as resistant, 13 were moderately resistant, 9 were moderately susceptible and one was susceptible.

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Table 1: Detection of CMV in symptomatic sample using DAS-ELISA test.

Governorate	Number of collected samples			DAS-ELISA Positive samples			
	Cucumber	Zucchini	Total	Cucumber	Zucchini	Total	%
Beheira	8	5	13	2	0	2	15.38
Gharbia	8	7	15	5	3	8	53.33
Menofia	9	6	15	2	2	4	26.66
Total	25	18	43	9	5	14	32.55

Table. 2: CMV seed transmission in four cucurbit types under experimental conditions.

Cucurbit type	*Seed number	Emerged plants	Symptomatic plants	ELISA test		**Transmission rate (%)
				Positive	Negative	
Cucumber	200	197	6	2	4	1.015
Zucchini	200	198	9	3	6	1.51
Melon	200	193	4	0	4	0.00
Pumpkin	200	196	13	5	6	2.55
LSD			0.32			

*: divided into 4 portions (each with 50 seeds) and each portion was planted in 4 successive planting dates.

**: calculated based on the numbers of plants that reacted positively with ELISA test.

Table. 3: Aggressiveness of CMV isolates on cucumber and zucchini.

	Disease severity							
	Cucumber				Zucchini			
	14 dpi		28 dpi		14 dpi		28 dpi	
	Rating scale	%	Rating scale	%	Rating scale	%	Rating scale	%
Isolate 1	4.3	43	7.0	70	5.0	50	7.1	71
Isolate 2	2.9	29	5.2	52	2.5	25	4.5	45
Isolate 3	4.1	41	7.4	74	5.1	51	8.1	81
Isolate 5	5.1	51	7.2	72	4.8	48	7.9	79
Isolate 6	5.5	55	8.4	84	5.9	59	8.8	88
Isolate 7	2.8	28	5.4	54	5.7	57	8.5	85
Isolate 8	2.9	29	5.1	51	2.7	27	4.9	49

Table. 4: Reaction of cucurbits toward artificially infection by CMV.

Tested plant		Disease severity			
		14 dpi		28 dpi	
		Rating scale	%	Rating scale	%
cucumber	Barracuda	3.4	34	7.4	74
	Faris F1	1.6	16	4.8	48
	Beit alpha	3.0	30	6.4	64
zucchini	Light	1.4	14	4.6	46
	Eskandarani	5. 2	52	8.2	82
	Dahab	2.2	22	6.4	64
	Sandy	2.0	20	8.0	80
cantalupe		2.4	24	5.6	56
muskmelon		4.8	48	7.8	78
Snake cucumber		2.2	22	5.0	50
pumpkin		3.4	34	6.4	64
squash		3.2	32	6.6	66
watermelon		1.0	10	3.6	36
colocynth		2.4	24	5.2	52
sponge gourd		1.8	18	4.4	44
LSD				1.4	

Figure 1: Symptoms expression on naturally infected cucumber leaves and fruits.

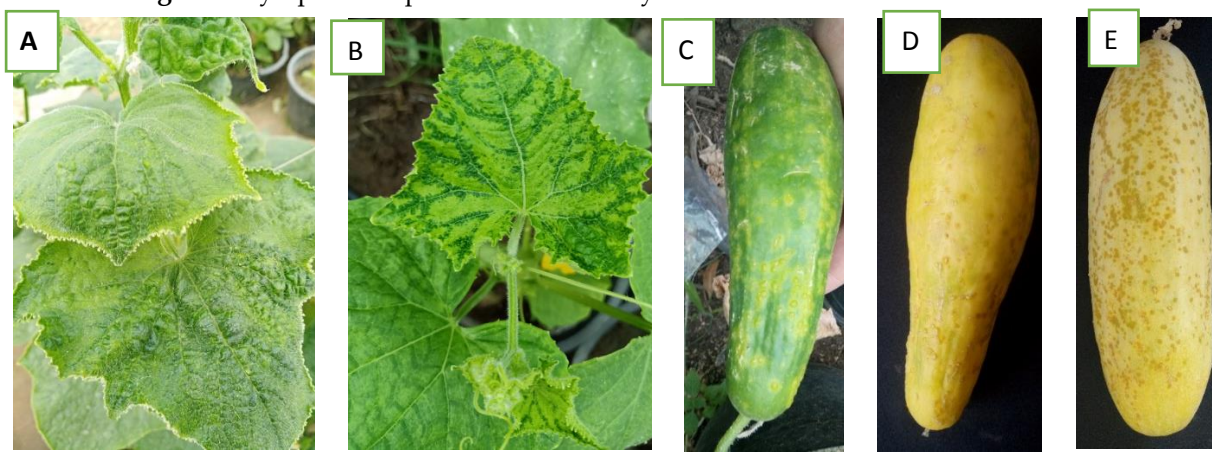

Figure 2: Symptoms observed on cucumber plants after mechanically re-inoculated: A and B vein banding, mosaic, mottling and blisters on leaves. C, D and E: oily spots on fruits.

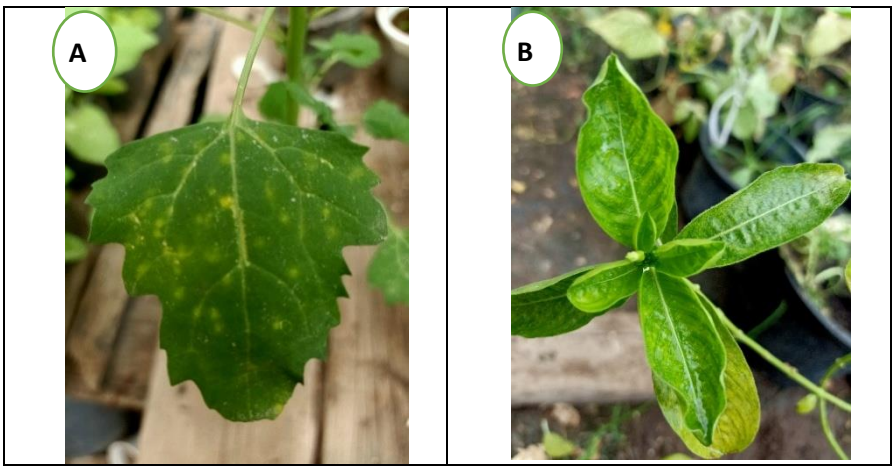

Figure 3: A: Single local lesion formed on quinoa leaves after mechanical inoculation by CMV. B: systemic mosaic symptom formed on periwinkle leaves after mechanical inoculation by CMV.

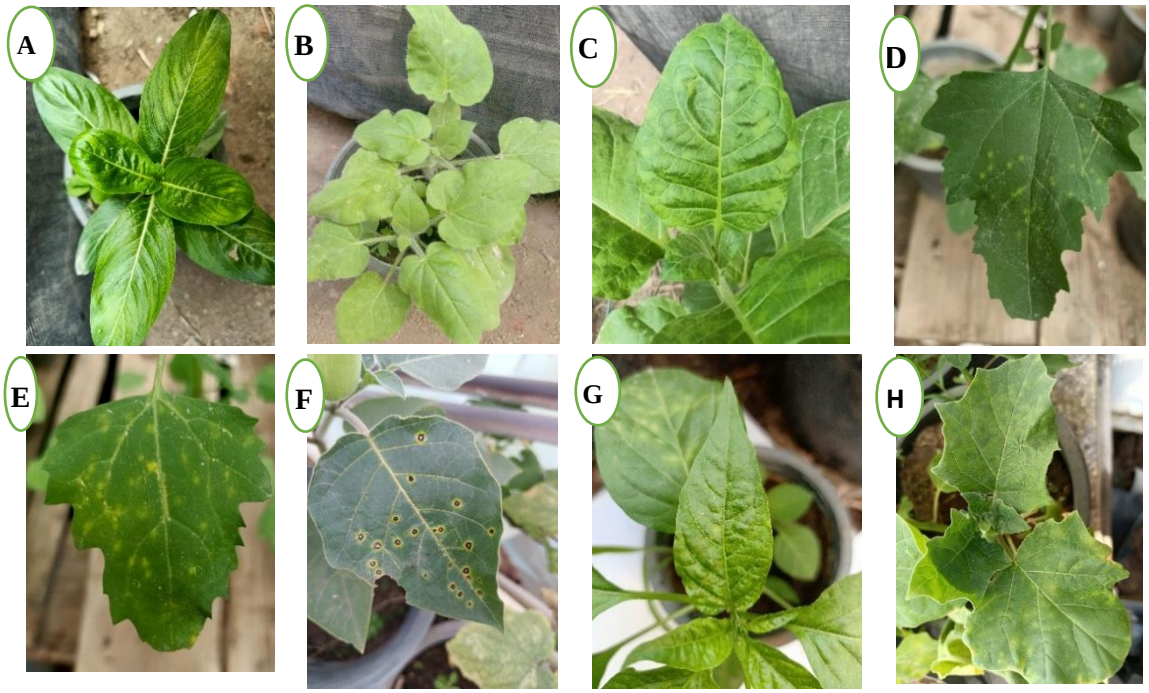

Figure 4: Reaction of different plant species to mechanical inoculation with CMV isolate showing systemic mosaic symptom A: *C. roseus*, B: *Nicotiana glutinosa*, C: *N. tabacum* cv. Samsun, showing systemic symptoms while D: *Chenopodium amaranticolor*, E: *C. quinoa* F: and *D. inoxea,* showing local chlorotic spots.while G *Capsicum annuum* H: *Datura metel*, showing showing local lesions on inoculated leaves followed by systemic symptoms.

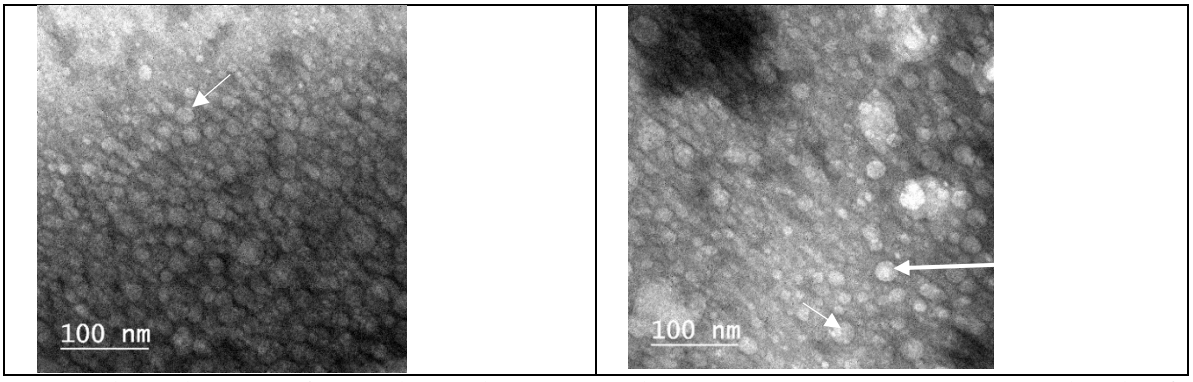

Figure 5: Spherical virions of CMV detected in *C. quinoa* leaves (28 to 30 nm) negative stained with 2% acetate of uranil preparation obtained by sap-dip techniques.

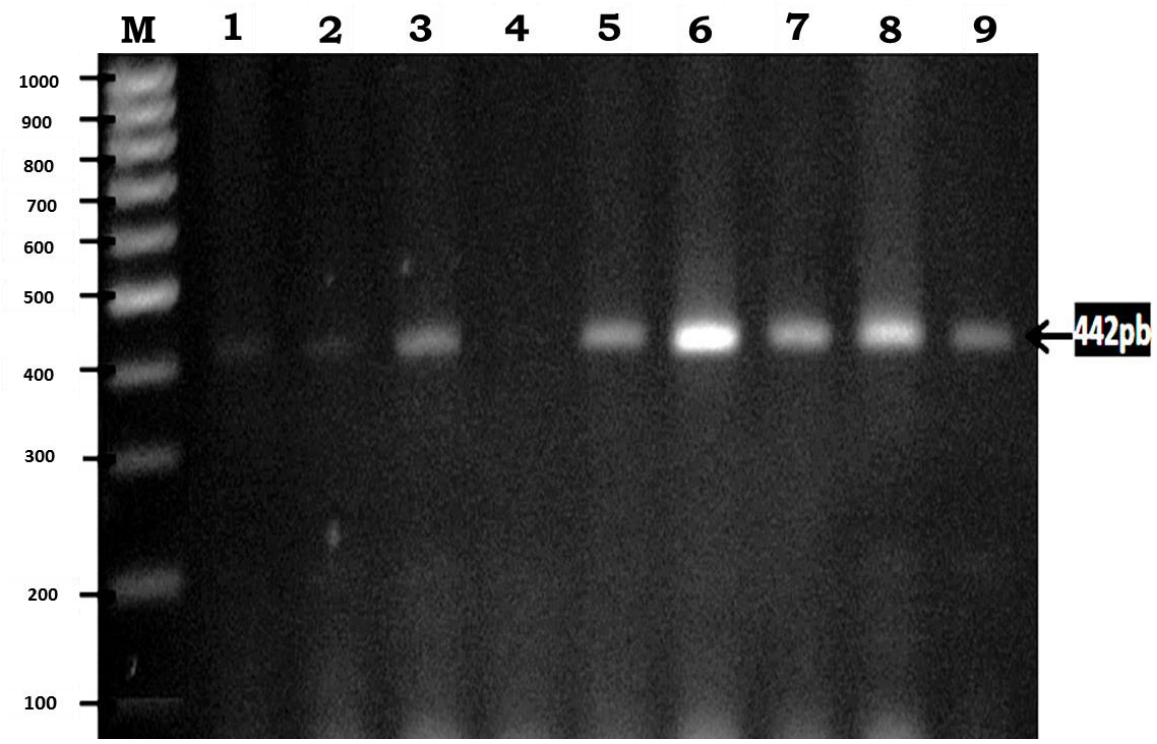

Figure 6: Agarose gel electrophoresis analysis of amplified CMV-cp gene fragment Lanes (1 to 9). RT-PCR products of nine CMV samples showing amplified CMV-cp, Gene fragment of the correct size 442 bp (arrow) in lanes (1,2,3,5,6,7,8 and 9). Lane (4) healthy cucumber. M: 1000 bp ladder.

صفات عزلات من فيروس موزايك الخيار التى تصيب القرعيات فى مصر

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الملخص العربي

تم الكشف عن فيروس موزايك الخيار على نباتات الخيار والكوسة المنزرعة فى بعض محافظات جمهورية مصر العربية اعتمادا على الأعراض الظاهرية على الأوراق فى صورة تجعد واصفرار و مناطق مرتفعة وعلى الثمار الخضراء فى صورة تشوه وتبرقش وبقع زيتية. تم الكشف عن الفيروس باستخدام طريقة الإليزا المباشرة والعينات التى أعطت نتيجة ايجابية تم إجراء اختبار القدرة المرضية للفيروس بها وعمل تنقية بيولوجية باستخدام البقعة الموضعية المفردة على نبات الكينوا وحفظ الفيروس على نباتات الخيار والوينكا. تم تعريف الفيروس باستخدام العوائل المشخصة والميكروسكوب الإلكترونى وتفاعل البلمرة المتسلسل PCR . كذلك تم اختبار طرق انتقال الفيروس حيث ثبتت إمكانية انتقال الفيروس عن طريق حشرة من القطن *Aphis gossypii* بصورة غير باقية، كذلك تم اجراء دراسة احتمالية انتقال الفيروس عن طريق البذور حيث ثبت انتقال الفيروس بالبذور في نباتات الخيار والكوسة والقرع العسلي بنسب مختلفة بينما لم يثبت انتقال الفيروس بواسطة بذور الشمام ، لوحظ تباين في العزلات من حيث الشراسة المرضية على نباتات الخيار والكوسة بعد 14 يوماً و28 يوماً من العدوى. تم إجراء اختبار مدى قابلية أجناس وأنواع وأصناف القرعيات المتاحة للإصابة بالمرض، أظهرت النتائج أن هناك تبايناً فى شدة الإصابة بين أجناس وأنواع وأصناف القرعيات المستخدمة فى الدراسة.

الكلمات الاسترشادية: فيروس موزايك الخيار، طريقة الإليزا، وتفاعل البلمرة المتسلسل، انتقال الفيروس عن طريق البذور، قابلية أجناس وأنواع وأصناف القرعيات المتاحة للإصابة بالمرض.