

Some ornamental and weed reservoir for Cucumber mosaic virus in Egypt



Moatasem Be Allah A.Y., El-Adly A.M., Wardany A.A.* and El-Shanawny A.A.

Botany & Microbiology Department, Faculty of Science, Al-Azhar University, Egypt

Citation: Moatasem Be Allah A.Y., El-Adly A.M., Wardany A.A., El-shanawny A.A. (2022). Some ornamental and weed reservoir for Cucumber mosaic virus in Egypt. *Journal of Environmental Studies*, Vol. 27(1): 35-41.

Article Information

Received 30 June 2022,

Revised 30 July 2022,

Accepted 3 Aug. 2022.

Published online

30 Sep. 2022

Abstract: An extensive study was conducted during 2020 and 2021 in major zucchini production areas of Assiut, Egypt, where cucumber mosaic virus (CMV) is commonly found, to assess the incidence and prevalence of naturally infected some ornamental and weed species that could serve as CMV reservoirs. Cucumber mosaic virus has rapidly spread to cucurbitaceae farms in most regions of Egypt. More precisely, it has a wide host range causing severe damage in many important agricultural and ornamental crops. CMV was isolated from Zucchini plants (*Cucurbita pepo*) showing virus like symptoms. Cucumber mosaic virus was identified on the basis of symptoms, transmission, serology, transmission, electron microscopy (TEM) and reverse transcription polymerase chain reaction (RT-PCR). Coat protein (CP) gene was amplified using gene specific primer. In this study the CMV was transmitted into propagation host by mechanically and through aphids. The transmission was confirmed through direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA). Isolation was performed depending on *Chenopodium amaranticolor* as a local lesion host. Virus was purified from 200 gm of virus infected *Nicotiana tabacum*. Eighteen plants species belonging to twelve different plant families were studied as the hosts of CMV. Cucumber mosaic virus was detected in seven species only (*Chenopodium amaranticolor*, *Nicotiana tabacum*, *Datura innoxia*, *Ocimum sanctum*, *Vinca rosa*, *Hibiscus rosa* and *Salvia splendens*) by transmission viral isolate through mechanical and insect (*Aphis gossypii*) inoculation from naturally infected *Cucurbita pepo* with CMV.

Keywords: Cucumber mosaic virus, *Aphis gossypii*, ornamental, weed, reservoir, *Cucurbita pepo*

Introduction

Cucumber mosaic virus was first reported in 1916 as the causal agent of plant diseases (Doolittle, 1916). CMV, genus: Cucumovirus, family: Bromoviridae, is one of the most widespread plant viruses with extensive host range infecting about 1300 species from 100 plant families, including cereals, fruits, vegetables and ornamentals crops, often causing serious infection, even death, of plants, reducing yield, and worsening the quality of agricultural products (Roossinck, et al., 1999, Chen, et al., 2006). It causes loss in quantity and quality of produce (Deloko, et al., 2022). The occurrence of CMV has been reported in many hosts such as Egyptian henbane (Samad, et al., 2000), gladiolus (Raj, et al., 2002), Tomato (Sudhakar, et al., 2006), Geranium (Verma, et al., 2006), banana, *Rauwolfia serpentina* and *Jatropha curcas* (Aglave, et al., 2007). CMV played a major role in the deterioration of qualities of many ornamentals as it caused direct damage to the hosts; moreover, it predisposed the plants to secondary invaders (Mahmoud, 2011; Abd El-Aziz & Younes, 2019).

Mokbel, et al., 2020, have reported that CMV can be easily transmitted by mechanical inoculation or aphid. The virus is readily transmitted in a non-persistent manner by more than 80 species of aphids (Palukaitis, et al., 1992).

In certain cases, transmission of the virus also by seeds (most often by those of species of the Leguminosae family) is observed, but the percentage of transmission is low and is no importance for circulation of the virus or for maintaining a permanent reservoir of infection (Kozlovskaya, et al., 2013).

Specificity and efficiency of transmission of CMV strains by species of aphids has been mapped to several domains in the amino acid sequence of the CP (Quemada, et al., 1989, Perry, et al., 1998 and El-Borollosy & Waziri, 2013). These domains could be directly involved in the binding of virus particles to aphid receptors or, alternatively, affect the stability of particles in the aphid stylet and foregut (Quemada, et al., 1989). Transmission efficiency varies with the strain of the virus, the aphid species, the species of the plant source of virus and test (recipient) plant, the host

* Corresponding author E-mail: Ahmed_wr2000@yahoo.com

species used to maintain the aphid colony (Chen & Francki, 1990), and the number of CMV transfer to test-plants (Bernal & García-Arenal, 1994). *Aphis gossypii* are the most common species found transmitting CMV and used for experimental transmission of this virus. Most strains of CMV can be transmitted by these two aphids because the specificity of virus transmission is very low. Many species of aphids can transmit multiple strains of CMV (Chen & Francki, 1990).

CMV is an icosahedral virus approximately 28–30 nm in diameter and belongs to the genus Cucumovirus in the family Bromoviridae. CMV is a multicomponent virus with a single stranded positive sense RNA. RNAs 1 and 2 are associated with viral genome replication while RNA 3 encodes for movement protein and coat protein (Palukaitis, *et al.*, 1992; Madhubala, *et al.*, 2005; Oraby, *et al.*, 2008; El-Borollosy & Waziri, 2013). CMV isolates have been divided into two subgroups (named I and II) based on different methods such as: serological data, amino acids of the coat protein, nucleic acid hybridization and nucleotide sequence similarities. Roossinck, *et al.* (1999) suggested further division of subgroup I strains into IA and IB, on the basis of the results of phylogenetic analyses. Ornamental plants, perennial grasses, trees, and shrubs play a major role in the preservation and spread of pathogens of virus diseases, including CMV, in nature. Reservoirs of this virus in natural biocenoses occur regularly and last a long time. The virus on tree and shrub species is found most often in a latent form or its symptoms are expressed weakly (Kozlovskaya, *et al.*, 2013).

The objective of this work was to determine the role of alternative hosts of some ornamental and weed plants that act as reservoir in the survival and epidemiology of cucumber mosaic virus during cucurbitaceae-free periods.

Materials and methods

The present investigations were undertaken to identify the causal virus based on biological, serological and molecular testing. The research work has been carried out at Plant Pathology laboratory, Faculty of Sciences, Al-Azhar University, Assiut and Virus Diagnostic and Identification Laboratory of the Plant Disease Research Institute at the Agricultural Research Center, Giza, Egypt.

Virus isolation and purification

For survey and collection of CMV isolates, the disease incidence was recorded in each field by visual examination. Five samples of Zucchini plants

(*Cucurbita pepo*) leaves showing virus-like symptoms were separately collected from open fields in major Zucchini production areas of Assiut, Egypt, during the growing seasons from 2020-2021. Leaves showing mosaic mottling and malformation were labelled and sealed in polythene bag and kept at 4° C and transported to the Plant Pathology laboratory, Faculty of Sciences, Al-Azhar University, Assiut, Egypt for further analysis. Naturally infected Zucchini leaves that were prevising labeled were used for biological isolation. Virus inoculum was prepared by grinding infected leaf tissues 1:10 (w/v) with a mortar and pestle in 0.1M phosphate buffer (pH 7.0), containing 0.5% of 2-mercaptoethanol. Pure culture was maintained by obtaining single lesion from *Chenopodium amaranticolor* and inoculating it on to *Nicotiana tabacum* at 28° C by dusted leaves of healthy *N. tabacum* plants in seedling stage with carborundum (600 mesh); and then inoculated with a freshly prepared viral inoculum using forefinger method, finally kept in an insect proof greenhouse under observation. The virus was further multiplied on the other hosts that have the ability to infect it Walkey (1991).

Host range studies

Seedlings of seventeen host plants belonging to different taxa were raised under controlled glasshouse condition. The plants selected were ubiquitously present near the Zucchini growing agro-ecosystem.

The selected plant hosts are *Sorghum halepense*, *Portulaca oleracea*, *Chenopodium amaranticolor*, *Nicotiana tabacum*, *Imperata cylindrical*, *Datura innoxia*, *Convolvulus arvensis*, *Cyperus fuscus*, *Ocimum sanctum*, *Solanum nigrum*, *Malva parviflora*, *Vinca rosa*, *Salvia splendens*, *Sonchus oleraceus*, *Plantago major*, *Hibiscus rosa* and *Rottboellia*. The viral isolate was transmitted through *Aphis gossypii* from infected *Cucurbita pepo* to all these test plants at 3–4 leaf stage, each test plants were replicated thrice. Inoculated plants that did not show any disease symptoms were checked for latent infection by back-inoculation to the indicator host *Chenopodium amaranticolor*. The inoculated plants were kept under controlled conditions and were examined daily for four weeks for symptoms expression. Confirmation for the presence of virus was done by DAC-ELISA using specific antisera (Kaper & Waterworth, 1981; Thottappilly & Rossel, 1987; Hampton, *et al.*, 1997; Abd El-Aziz, 2015; Abd El-Aziz & Younes, 2019).

Aphid transmission

Individuals of the aphids, *Aphis gossypii*, were collected from cotton and broad bean plants. New

generation of aphids were transferred to other healthy plants of cotton and broad bean growing in insect proof cages and the aphids were left for reproduction. Several virus free adults of each of the above-mentioned aphids were starved for two hours.

They were allowed to feed for 20 min (acquisition period) on infected *N. tabacum* plants. After that the aphids were transferred to other selected healthy hosts for allowed to feed for 10-15 min (inoculation period), and then the insects were sprayed by insecticide (Malathion). The plants were maintained in insect proof cage at 25-30° C for 25 days and the plants were inspected daily for symptoms development. The aphid inoculated plants were serologically tested using CMV antiserum. *Nicotiana tabacum* was chosen because, in case of infection with any plant virus, it gives specific symptoms, which is local lesion, each one represent presence of one virus, so that no mix viral infection overlaps in one local spot.

Production of CMV polyclonal antiserum

An antiserum against CMV was prepared according to Chalam, *et al.*, (1986). A male white New Zealand rabbit over two Kg was injected intramuscularly four times at 10 days intervals with one ml containing 3 mg of purified CMV and emulsified with an equal volume of Freund's incomplete adjuvant. Ten days after the last injection; the blood was collected from the marginal ear vein and allowed to clot, and then the antiserum was separated and stored frozen until required. The separated antiserum was clarified by centrifugation at 5000 g/ 35 min.; 0.2% NaN₃ was added, and then antiserum was divided in aliquots, kept frozen until needed for different serological assays. Antiserum titer was determined using indirect ELISA as described by Fegla, *et al.*, (2000). Extracts from infected and healthy *Nicotiana tabacum* plants were diluted with coating buffer to 1: 10. Serial dilutions of double fold up to 1:256000 of antiserum from cross absorption and filtered extracts from healthy tissues diluted 1: 20 in serum buffer, were used according to Younes, (2003). Cucumber mosaic virus antiserum provided by Dr. Rehab Dawood, Agriculture Reaearch Center, Dokki, Giza, Egypt was used for serodiagnosis study.

CMV detection by enzyme-linked immunosorbent assay (ELISA)

Direct antigen coating-ELISA (DAC-ELISA) test was performed as described by Clark & Joseph (1984) to test the presence of CMV in collected or inoculated plants using CMV antiserum. One g. of each leaf sample collected and ground in liquid nitrogen then, one ml of 0.2M potassium phosphate buffer with 0.5%

sodium sulphite and 1% polyvinyl pyrrolidone (PVP) was added to each sample. The sample squeezed gently with absorbent cotton and equal volume of chloroform was added and vortexed thoroughly. The homogenate was centrifuged at 5000 rpm for 5 min and the supernatant was used for coating the ELISA plates.

Plates were coated with 200µl of prepared antigen sample and incubated at 37° C for 1 hr or overnight at 4° C. After incubation, the well's contents were discarded and washed with PBS-T buffer thrice (flooding with PBS-T for 3min every time). The blocking solution was added (200 µl / well) and incubated for 1 hr. at 37° C. After washing the plate (thrice with PBS-T), 200µl of diluted rabbit antiserum (1: 500) was added in the wells and incubated at 37° C for 1½ hrs. The plate was washed and 200 µl of enzyme conjugate (anti- rabbit alkaline phosphate) was added and incubated at 37° C for 1½ hrs. After incubation, the plates were washed with PBS-T. After final washing, 200 µl of substrate (p-Nitro phenyl phosphate- 0.6 mg/ml) was added and allowed for color development at room temperature by avoiding exposure to light. Then the reaction was stopped by adding 3M NaOH and the color intensity was read in ELISA reader at 405 nm.

RNA extraction and RT-PCR of coat protein (CP) gene

Approximately 100 mg of infected dry leaves of zucchini plant was frozen in liquid nitrogen and ground to powder with a mortar and pestle. Total RNA was extracted with 1 ml TRIzol reagent, according to the instructions of the supplier using the method of Girin, *et al.*, 2007. After ethanol precipitation, the RNA was resuspended in 30 µl RNase free water and treated with RNase free DNase (Ambion), which was then inactivated according to the instructions of the supplier. Approximately 5 µg of total RNA were reverse transcribed using random hexamers (invitrogen) and Revertaid reverse transcriptase without RNaseH activity (Fermentas) in a final volume of 20 µl. For RT-PCR, template was provided as 2 µl of a 50-fold dilution of cDNA in water in a total reaction volume of 20 µl. The CMV specific primers 5'-GCC GTA AGC TGG ATG GAC AA-3' and 5'-TAT GAT AAG AAG CTT GTT TCG CG-3' designed by Wylie, *et al.*, (1993) were used for amplification a part of the CP gene. Amplification was done using Phusion DNA polymerase (Promega), with initial denaturation at 98° C for 2 min followed by 35 thermal cycles of 98° C for 15 s, 60° C for 30 s, and 72° C for 30 s and followed by final extension at 72° C for 10 min.

Results and discussion

During the present investigation, Virus was isolated from zucchini plants showing mosaic, mottling and malformation. The virus was confirmed by specific antiserum and indirect ELISA. Serological detection of infected plants revealed the involvement of a virus namely cucumber mosaic virus (CMV) isolated from zucchini plants (*Cucurbita pepo*) showing mosaic. Results were in harmony with Madhubala, *et al.*, (2005), El-Afifi, *et al.* (2007) and Oraby, *et al.*, (2008).

Host range studies were carried out on fifty-one plants of seventeen species belonging to different taxa. Plant species selected were ubiquitously present near the zucchini growing agro-ecosystem. The species studied are: *Sorghum halepense*, *Portulaca oleracea*, *Chenopodium amaranticolor*, *Nicotiana tabacum*, *Imperata cylindrical*, *Datura innoxia*, *Convolvulus arvensis*, *Cyperus fuscus*, *Ocimum sanctum*, *Solanum nigrum*, *Malva parviflora*, *Vinca rosa*, *Salvia splendens*, *Sonchus oleraceus*, *Plantago major*, *Hibiscus rosa* and *Rottboellia*. These plants were sap inoculated by using naturally infected Zucchini extract and carborundum powder. The results revealed that some species of tested host plants expressed the CMV symptoms after 22 -38 days of inoculation, while other plants have no symptoms expression. Cucumber mosaic virus was detected in seven species only: *Chenopodium amaranticolor*, *Nicotiana tabacum*, *Datura innoxia*, *Ocimum sanctum*, *Vinca rosa*, *Hibiscus rosa* and *Salvia splendens*. Transmission of CMV causes mosaic and leaf deformations in inoculated plants, which was confirmed through DAC-ELISA. Virus isolated from *Cucurbita pepo* (CMV) showing mosaic and severe distortion on *Nicotiana*

tabacum, and green veinbanding mosaic on *Vinca rosa*, *Ocimum sanctum*, chlorotic patches on leaves of *Salvia splendens*; leaf deformation and normal mosaic on leaves *Hibiscus rosa*; whereas, Necrotic local lesions were observed on inoculated leaves of *C. amaranticolor* and *Datura innoxia* (Figure 1).

Transmission experiments by aphids as a vector were designed to analyze the transmissibility of CMV through selected plant species. In this regard, present investigation results successful transmission of CMV to some of test plants by aphids. Vector transmission studies using *Aphis gossypii* that are efficiently transmitted the virus in a non-persistent manner within 20 min of acquisition and inoculation of 10 to 15 min. Inoculated plants by *A. gossypii* showed the symptoms of CMV after 13-25 days of inoculation. These results revealed that the CMV is aphid transmissible and agreed with the data recorded by Rao (1980) who reported. The present investigation results has clearly revealed the occurrence and association of cucumber mosaic virus with zucchini plants was identified based on mechanical transmission, insect vector transmission, host range, serology DAC-ELISA detection. The virus was able to transmit mechanically on some plants used in the study very efficiently and produced the systemic and local symptoms characteristic of CMV. In our present study, aphid species (*A. gossypii*) have the ability to transmit the virus in non-persistent manner. Serology is the most reliable and quick method of detection of CMV infection in *Cucurbita pepo* (Rajasulochana, *et al.*, 2008). Roossinck, (2002) reported that CMV has the broadest host range among the plant viruses, CMV from Zucchini could be transmitted mechanically and by aphid inoculation.

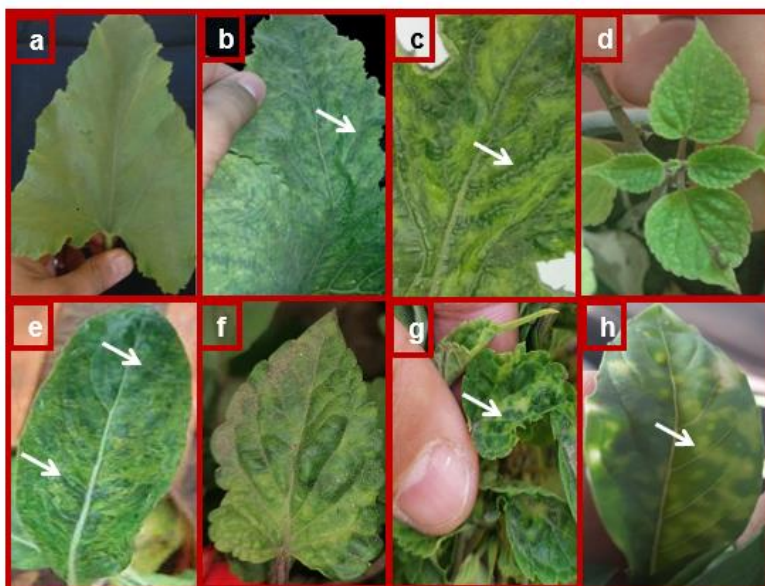


Fig. 1. Systemic symptoms at 22-38 days on different host plants inoculated with CMV, symptoms were inspected daily for development. (a) The non-inoculated *Cucurbita pepo* with CMV, (b & c) Green veinbanding mosaic on leaves of *Cucurbita pepo*, (d) chlorotic patches on leaves of *Salvia splendens* plant, (e) Green veinbanding mosaic on *Vinca rosa* leaves, (f & g) leaf deformation and normal mosaic on leaves of *Hibiscus rosa*, (h) chlorotic local lesions and complete yellowing of the leaves of *Ocimum sanctum* plant.

RT-PCR and cloning of coat protein gene

Previous studies of Thompson, *et al.*, (1994); Hadidi, *et al.*, (1995) illustrated the importance and usefulness of PCR as a molecular diagnostic tool of plant viruses. Analysis of PCR products on agarose gel electrophoresis revealed PCR amplification of the CP gene using gene specific primers resulted in the amplification of 487 bp fragment, similarly, recorded by Abdelsabour, *et al.*, (2015) (Figure 2). Data presented in this study clearly indicate that CP region is

sufficient to provide a simple and reliable method for detection and strain identification of CMV, in agreement with Khan, *et al.*, (2011).

CLL: Chlorotic local lesions; **NLL:** Necrotic local lesions; **LD:** Leaf distortion; **NM:** Normal mosaic; **SM:** Severe mosaic; **GVB:** Green veinbanding mosaic; **YM:** Yellow mosaic; **NS:** Non-symptoms; (+): Symptoms appear on the plant; (-): Symptoms do not appear on the plant.

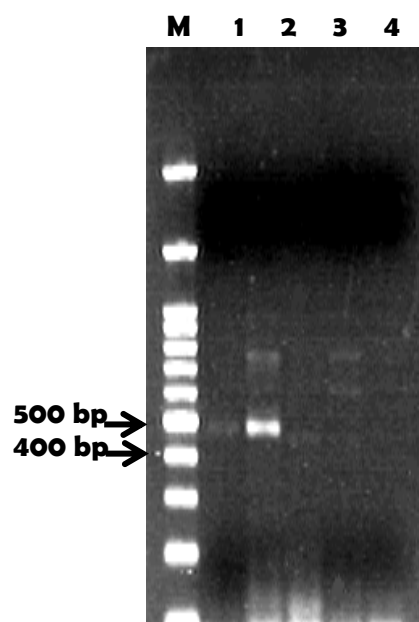


Fig. 2: Agarose gel electrophoresis patterns showing the RT-PCR amplification of CMV isolate coat protein gene. Where; M: DNA Marker (1200 bp), and Lane 1: Sample of the control "non-inoculated" zucchini plant, Lane 2: Sample of mechanically inoculated zucchini leaves, Lane 3-5: Sample of non-infected zucchini leaves.

Table 1. External symptoms of the tested- host plants by inoculation of infected sap with Cucumber mosaic virus.

Family	Diagnostic Plant Species	Symptoms	ELISA Detection
Cucurbitaceae	<i>Cucurbita pepo</i>	SM,	+
Poaceae	<i>Sorghum halepense</i>	NS	-
	<i>Rottboellia</i>	NS	-
	<i>Imperata cylindrical</i>	NS	-
Solanaceae	<i>Nicotiana tabacum</i>	GVB	+
	<i>Solanum nigrum</i>	NS	-
	<i>Datura innoxia</i>	NLL	+
Lamiaceae	<i>Ocimum sanctum</i>	YM	+
	<i>Salvia splendens</i>	CLL, LD	+
Malvaceae	<i>Hibiscus rosa</i>	NM	+
	<i>Malva parviflora</i>	NS	-
Apocynaceae	<i>Vinca rosa</i>	GVB	+
Asteraceae	<i>Sonchus oleraceus</i>	NS	-
Plantaginaceae	<i>Plantago major</i>	NS	-
Portulacaceae	<i>Portulaca oleracea</i>	NS	-
Amaranthaceae	<i>Chenopodium amaranticolor</i>	NLL	+
Convolvulaceae	<i>Convolvulus arvensis</i>	NS	-
Cyperaceae	<i>Cyperus fuscus</i>	NS	-

Acknowledgment

The authors briefly acknowledge those who helped them in conducting this research mainly; Prof. Omar ahmed Abd El-Rahem, Prof. of Plant Pathology at Plant Diseases Research Institute, Agricultural Research Center, Egypt.

References

- Abd El-Aziz, M.H. (2015). Studies on Some Viruses Infecting Cowpea Plants. *Ph.D. Thesis. Faculty of Agric. (Saba - Basha) Alexandria University*. pp. 136.
- Abd El-Aziz H.M.; Younes A.H. (2019). Detection of Cucumber mosaic cucumovirus in infected cowpea plants (*Vigna unguiculata* L.) from northern Egypt. *Novel Research in Microbiology Journal*, 3(2): 326-340.
- Abdelsabour, G.A.; Wardany, A.A. and Sabry, Y.M. (2015). Coat protein gene of new isolate of cucumber mosaic virus infecting banana in Egypt, *Journal of Microbiol. Biotech. Food Sci.*, 5(2): 177-181.
- Aglave, B.A.; Krishnareddy M.P. and Andhale M.S. (2007). Molecular identification of a virus causing banana chlorosis disease from Marathwada region. *International Journal of Biotechnology & Biochemistry*, 3: 13-23.
- Bernal, J.J.; García-Arenal, F. (1994). Analysis of the determinants of the satellite RNA of cucumber mosaic cucumovirus for high accumulation in squash. *Journal of Virology*, 205: 262-268.
- Chalam, T.V.; Reddy, M.V.; Nene, Y.L.; Beniwal, S.P.S.; Subbayya, J. (1986). Some properties of a strain of Cucumber mosaic virus isolated from chickpea in India. *Journal of Plant Disease*, 70: 128-130.
- Chen, C.C.; Hsu, H.T.; Chiang, F.L.; Chang, C.A. (2006). Serological and Molecular Properties of Five Potyviruses Infecting Calla Lily. *Journal of Virus Diseases in Ornamentals. Acta Hort.*, 722: 259-264.
- Chen, B.; Francki, R.I.B. (1990). Cucumovirus transmission by the aphid *Myzus persicae* is determined solely by the viral coat protein. *Journal of Gen. Virol.*, 71: 939-944.
- Clark, M.F.; Joseph, B. (1984). Enzyme Immuno Sorbent Assay in Plant Virology In: *Methods in Virology*, K. Maramorsch and Koprowski (Eds.), *Academic Press, New York* 7: 51-85.
- Deloko, T. C.; Chofong, G. N.; Ali, M. I.; Ibrahim, K., G.; Songolo, O.F.; Manock, N. R.; Kamgaing, M.; Fonkou, T.; Njukeng P.A. (2022). Detection of Cucumber mosaic virus on *Solanum lycopersicum* L. and *Capsicum annuum* L. in the Western region of Cameroon. *Journal of Agriculture and Food Research*, (8): 100294.
- Doolittle, S.P. (1916). A new infectious mosaic disease of cucumber. *Journal of Phytopathology*, 6: 145-147.
- El-Afifi, S.I.; El-Borollosy, A.M.; Mahmoud, S.Y.M. (2007). Tobacco callus culture as a propagating medium for Cucumber mosaic cucumovirus. *International Journal of Virol.*, 3, 73–79.
- El-Borollosy, M.A.; Waziri, A.M.H. (2013). Molecular characterization of a cucumber mosaic cucumovirus isolated from lettuce in Egypt, *Journal of Annals of Agricultural Science*, 58(1): 105–109.
- Fegla, G.I.; El-Samra, I.A.; Younes, H.A.; Abd El-Aziz, M.H. (2000). Optimization of Dot immunobinding assay (DIA) for detection of Tomato mosaic virus (ToMV). *Journal of the Advances in Agricultural Research.*, 5: 1495-1506.
- Girin, T.; Lejay, L.; Wirth, J.; Widiez, T.; Palenchar, P.M.; Nazoa, F.; Touraine, B.; Gojon, A.; Lepetit, M. (2007). Identification of a 150 bp cis-acting element of the AtNRT2.1 promoter involved in the regulation of gene expression by the N and C status of the plant. *Journal of Plant Cell Environment*, 30(11): 1366-1380.
- Hadidi, A.; Levy, L.; Podieckis, V. (1995). Polymerase chain reaction technology in plant pathology. In: *Molecular methods in Plant Pathology* R.P. Singh and U.S. Singh (eds.) CRC press. *Journal of Boca Raton, FL.*, pp. 167-187.
- Hampton, R.O.; Thottappilly, G.; Rossel, H.W. (1997). Viral diseases of cowpea and their control by resistance-conferring genes. In: Singh BB, Mohan DR, Raj Dashiell KE and Jackai LEN (eds), *Journal of Advances in Cowpea research.*, pp.159-175.
- Kaper, J.M.; Waterworth, H.E. (1981). Cucumoviruses. In: *Handbook of plant virus infection and comparative diagnosis*, E. Kurstak, *Journal of Biochemical press.*, pp. 257-332.
- Khan, S.; Jan, A.T.; AQUIL, B.; Mohd, Q.; Haq R. (2011). Coat protein gene based on characterization of cucumber mosaic virus isolates infecting banana in India. *Journal of Phytoogy*, 3: 94-101.
- Kozlovskaya, N.Z.; Volkov, G.Yu.; Kakareka N.N., (2013). Natural Cucumber Mosaic Virus Reservoirs in Seed Farms in Primorskii Krai, ISSN 1068_3674, *Journal of Russian Agricultural Sciences*, 39(4): 330–332.
- Madhubala, R.; Bhadramurthy, V.; Bhat, A.I.; Hareesh, P.S.; Rethesh, S.T.; Bhai, R.S. (2005).

- Occurrence of Cucumber mosaic virus on vanilla (*Vanilla planifolia* Andrews) in India. *Journal of Biosci.*, 30: 339–350.
- Mahmoud, S.Y.M. (2011). Detection of Cucumber mosaic virus in some ornamental plants and elimination of nonspecific ELISA reactions. *Journal of Phytopathology and Plant Protection.*, 44(14): 1410-1424.
- Mokbel, A.S.; Ahmed, A.E.; El-Kammar, F.H.; Kheder A.A. (2020). Molecular Characterization of Cucumber Mosaic Virus and Structural Changes of Infected Sugar beet Plants. *Journal of Virol. Sci.*, 8: 12- 27.
- Oraby, M.M.; El-Borollosy, A.M.; Abdel Ghaffar, M.H. (2008). Molecular characterization of a “Sugar beet” Cucumber mosaic cucumovirus isolate and its control via some plant growthpromoting rhizobacteria. *Journal of Biol. Chem. Environ., Sci.* 3: 147–167.
- Palukaitis, P.; Roossinck, M.J.; Dietzgen, R.G.; Francki, R.I.B. (1992). Cucumber mosaic virus. *Journal of Adv. Virus Res.*, 41: 281-348.
- Perry, K.L.; Zhang, L.; Palukaitis, P. (1998). Amino acid changes in the coat protein of cucumber mosaic virus differentially affect transmission by the aphids *Myzus persicae* and *Aphis gossypii*. *Journal of Virology*, 242: 204-210.
- Quemada, H.; Kearney, C.; Gonsalves, D.; Slightom, J.L. (1989). Nucleotide sequences of the coat protein genes and flanking regions of cucumber mosaic virus strain C and WL RNA3. *Journal of Gen. Virol.*, 70: 1065-1073.
- Raj, S.K.; Srivastava, A.; Chandra, G.; Singh, B.P. (2002). Characterisation of Cucumber mosaic virus isolate infecting *Gladiolus* cultivars and comparative analysis of serological and molecular methods for sensitive diagnosis. *Journal of Current Science*, 83: 1132-1136.
- Rajasulochana, P.; Dhamotharan, R.; Srinivasulu, P. (2008). Comparison of DAC-ELISA and DOT-BLOT-ELISA for the detection of cucumber mosaic and banana streak viruses infecting banana. *Journal of American Science*, 4: 18-27.
- Rao, D.G. (1980). Studies on a new strain of banana mosaic virus in South India. In: Proc. National Seminar on Banana Production Technology. Muthukrishnan C.R. & Abdul Khader J.B.M. (Eds.). *Tamilnadu Agricultural University, Coimbatore, India*, 155-159.
- Roossinck, M.J. (2002). Evolutionary history of cucumber mosaic virus deduced by phylogenetic analyses. *Journal of Virology*, 76(7): 3382-3387.
- Roossinck, M.J.; Zhang, L. and Hellwald, K.H. (1999). Rearrangements in the 5' nontranslated region and phylogenetic analyses of Cucumber mosaic virus RNA 3 indicate radial evolution of three subgroups. *Journal of Virology*, 73: 6752-6758.
- Samad, A.; Raj, S. K.; Srivastava, A.; Chandra, G.; Ajayakumar, P.V.; Zaim, M.; Singh, B.P. (2000). Characterization of a cucumber mosaic virus isolates infecting Egyptian henbane (*Hyoscyamus muticus* L.) in India. *Journal of Acta Virologica*, 44: 131-136.
- Sudhakar, N.; Nagendra-Prasad, D.; Mohan, N.; Murugesan, K. (2006). First report of Cucumber mosaic virus subgroup II infecting *Lycopersicon esculentum* in India. *Journal of Plant Diseases*, 90(11): 1457.
- Thompson, J.D.; Higgins, D.G.; Gibson, T.J. (1994). Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Journal of Nucleic Acids Research*. 22: 4673-4680.
- Thottappilly, G.; Rossel, H.W. (1987). Seed transmission of cowpea (yellow) mosaic virus unlikely in cowpea. *Journal of Tropical Grain Legume Bulletin.*, 34: 27-28.
- Verma, N.; Mahinghara, B.K.; Ram, R.; Zaidi, A.A. (2006). Coat protein sequence shows that Cucumber mosaic virus isolate from geraniums (*Pelargonium* Sp.) belongs to subgroup II. *Journal of Biosciences*, 31(1): 47-54.
- Walkey, D.G.A. (1991). Applied Plant Virology- 2nd ed. *British Library Cataloguing in Publication Data*. pp. 338.
- Wylie, S.; Wilson, C.R.; Jones, R.A.C.; Jones, M.G.K. (1993). A polymerase chain reaction assay for Cucumber mosaic virus in lupin seeds. *Australian Journal of Agricultural Research*, 44(1): 41.
- Younes, H.A. (2003). Natural infection of luffa (*Luffa aegyptiaca*. Mill) with Zucchini Yellow mosaic virus in Egypt. *Journal of Advances in Agricultural Research.*, 8: 227-239.