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DECODING *CUCUMBER MOSAIC VIRUS*: UNDERSTANDING THE MOLECULAR CHARACTERIZATION, IMPACTS, AND ITS REMEDIES

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A B S T R A C T

Cucumber mosaic disease (CMD), caused by *Cucumber mosaic virus (CMV)* is the most devastating disease that can cause 100% losses in severe cases. *CMV* transmission is accomplished through mechanical or contact inoculation and sometimes through aphid in stylet borne manner. After entry into the host, *CMV i*nterrupts with normal physiology of cucumber plants resulting in mosaic patterns on leaves, reduction in leaf area leading to reduced yield. This review paper aims at assessment of yield losses caused by *CMV,* efficient disease diagnosis, finding the existing resistance sources and breeding potential for sustainable disease management. Various methods of diagnosis, symptomology, ecology, epidemiology and management tactics of *CMV* have been studied in this review. Usage of insecticides, for *CMV* management is not rational because it is occasionally transmitted through aphid and too in a non-persistent manner. The efforts for vector control even though through botanicals and organic oils is not as much effective due to quick virus transmission. *CMV* impairs the plant photosynthetic activity resulting in yield reduction, however the damages could be managed by using different nutrients. It is focused upon boosting the plant defense by using external application of different resistance inducing chemicals. *CMV* impairs the plant photosynthetic activity resulting in yield reduction, however the damages could be managed by using different nutrients. It is focused upon to boost the plant defense by using external application of different resistance inducing chemicals. Hence this review covers the aspects of ecofriendly management of *CMV* through genetic resistance and innovative cultural practices, which is the major future research area.

Keywords: *CMV*, Pathogen, Host, Transmission, management, aphid, sap transmission.

INTRODUCTION

The *Cucurbitaceae* family contains flowering and herbaceous plants that are mostly annual or perennial and belong to the order *Cucurbitales* (Akbar *et al.,* 2015). The origin of Cucumbers is considered India in Asia and old history points out the origin 3000 years ago. Romans brought cucumbers to different parts of Europe (Waris *et al.,* 2014). Cucumbers are ranked third in importance and vegetable production after potato and tomato (Berke, 2002). Some wild cucumber species show traits like resistance to various nematodes, insects, mites, fungi, bacteria and viruses, which can be used in genetic engineering experiments (Greber, 1978). Cucumber has a major water content of about 95% and low caloric value as well as folic acid, potassium, carotenoids and vitamins A and C. Cucumbers are grown on 28,600 ha of Pakistani lands and produce 261,306 tons of cucumber in quantity annually. Among the diseases affecting cucumbers, major viral disease affecting cucumber is the *Cucumber mosaic virus* (Akbar *et al.,* 2015). *CMV* is the type specie of genus *Cucumovirus CMV* can infects over 1000 host species. *Cyprus rotundus, Datura stramonium*, *D. metal,*

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Trianthema pentandra and *Portulaca olercea* also known as Kulfa are the alternative hosts when a primary host is absent (Kaper and Waterworth, 1977; Iqbal *et al.,* 2011; 2012). *Cucumber mosaic virus* has different modes of transmission. *CMV* is a mechanically transmitted virus but can also be transmitted through aphid spp., (Akbar *et al.,* 2015). About more than 75 different aphids are the vector of *CMV* in non-persistent. The most efficient vector is *Aphis gossypii* (Palukaitis *et al.,* 1992). *CMV i*s also transmissible through dodder plants, 10 Species of Cuscuta are involved efficiently to transmit the virus into host plants (Chen and Francki, 1990; Schmelzer, 1957). Iqbal *et al.* (2012) calculated the incidence level of *CMV* infection in chili crop. They used DAC-ELISA to distinguish 706 samples collected from 4 provinces of Pakistan. The quantity of Sindh samples was 191, while KPK, Punjab and Baluchistan were 51,257 and 207 respectively. The calculated incidence of *CMV* infection was 51.8% in Sindh province, while Baluchistan, KPK and Punjab were at 17.47%, 7.8% and 8.5%, respectively. Not even a single district of Sindh province was free from *CMV* attack. A total of 33 host were found to be the alternative host of *CMV* infection (Ashfaq *et al.,* 2014)**.** Major symptoms of *CMV* are stunting (short stature of plants) , malformation of leaves and Dark and light green Mosaic patterns on the plant (Palukaitis *et al.,* 1992). Different cultural practices, chemical sprays, extracts, natural oils and biological control agents are used for this purpose. Most used strategy from these is the chemical control which is feasible, best and east to apply on the fields but this strategy and other techniques have disadvantages too, that's why integrated pest management or Integrated disease management strategies are used to manage the disease.

Pathogen and Disease: *CMV* was first described 1916 as a new infectious entity (Doolittle, 1916). *CMV* is icosahedral particles in shape that have 29 nm diameter. *CMV* particles comprise a single capsid protein comprising 180 subunits and 18% RNA composition (Jacquemond, 2012). *CMV* is single-stranded, positive-sense RNA. This virus contains three particles in its genome that encode for five different proteins designated as 1a, 2a, 2b, 3a, and capsid protein (CP) (Palukaitis *et al.,* 1992). Sequences of RNA 4 particles of *CMV* belonged to subgroups I and II. RNA 4 particles of *CMV* strains Ny for subgroup I and SN for subgroup II were sequenced completely for genetic information (Anderson *et al.* (1995). These results provide the base for classifying *CMV* isolated with the help of a simple method using RNA EcoRI sites. Experiments on genetic studies of *CMV* were conducted in Spain to understand the population genetics of epidemiology. Different isolates were studied at different times of the year in different outbreaks. The results proved that the heterozygous isolates were not able to cause disease efficiently and satRNAs also attacked the solanaceous crops. The genetic structure of satRNAs was different from those helper viruses and the process was named as molecular epidemiology. The isolates were detected to be the Subgroup II of *CMV* while there were also two types present from Subgroup I (Thackray *et al.,* 2000). *CMV* satRNA analyzed molecularly indicated that the isolated have high variations in genetic makeup and showed diversity due to recombination and ad process of point mutation in their genetic makeup. The NT strain of *CMV* belonged to Subgroup I. RNA 2 particle of the NT strain is involved in severe infection and symptoms in tomato plants (Hellwald *et al.,* 2000). Subgroup I strain were selected from the Asian continent along with the any strain isolated from USA and their action and effect on tomato plants were studied. Viral genome has four particles termed RNA 1, 2, 3 and 4. First three particles are packed in individual particles while RNA 4 is packed with RNA 3 particles. Roossinck (2001) described physical and taxonomical properties of *CMV* as well as their host and satellite RNAs. In *Cucumovirus* genus the *CMV* is a type member that belongs to the *Bromoviridae* family. RNA 3 has an exchangeable genome while other exchange within a specific space. The virus particles contain 180 subunits and have the diameter about 29 nm. The genome of RNA particles contains length of first particle as 3.3, The second particle have 3.0 kb length while the third one has 2.2 kb. *CMV* also support some other RNAs that are termed as known as satellite RNAs or sat RNA. these satRNAs can change the symptoms of the infection induced by *CMV*. The *CMV* satRNAs rely on helper Virus (*CMV*) for their replication and do not encode any protein. Roossinck (2002) reported the evolution of *CMV* and performed different phylogenetic tests by using Molecular Evolutionary Genetics Analysis (MEGA) (Fig 1, 2 & 3). The virus was divided into the IA, IB and II subgroups based on detecting the 5'nontranslated region (NTR) of RNA 3 and the sequence of coat proteins. Still, open reading frame analysis doesn't support this classification. Analysis of RNA 3 suggested that the virus is divided into three parts. The subgroup I and II sequencing was done by Chen *et al.*

(2007). They identified the molecular evidence for the natural reassortment. *CMV* has three particles in the genome. These three particles were the base for the division of the virus into IA, IB and II. Pratap *et al.,* (2008) studied the shoestring symptom in India and reported for the first time that *CMV* viral isolates are responsible for this symptom in tomatoes. Total RNA of the virus was extracted from the infected tomato samples to detect viral isolates at molecular level. RT-PCR was used for their detection. The primers were designed from the sequence of the strain and used in Rt-PCR to amplify the viral genome. Compared with RNA 3 sequence, virus isolates showed highest identical genome up to 99 percent with Tfn strain and Nt9 strain, Italy and Taiwan isolate of *CMV*. These viruses isolated also showed homology with P1-1 and YN isolates. The similarity index was 97-96 percent with the isolates of China and Spain. Viral genome was identical only 92% with Indian isolates like Amar, Dat and Chry. The viral genomes that showed homology in a 92-99 percent range with the understudy isolates were identified as subgroup 1B. Based on present published data, this was the first report of *CMV* strain's association with shoestring symptom attacks on tomato plants in India, which were detected molecularly. Molecular characterization of *CMV* isolates from India that were similar to the Fny strain of *CMV* (Dubey and Singh, 2010). He analyzed The Gladiolus and identified the *CMV* diseases infecting Gladiolus plants. The primers were designed specifically for coat proteins region of CMC for characterization. The amplified viral sequence showed the expected 657 bp size present in infected samples and further amplified. The comparison of nucleotide and amino acid percentage and study of phylogenetic trees showed that *CMV* isolates infecting Gladiolus plants resemble *CMV* FNY strain that is not present in Asian continent abundantly. Evolutionary history and variation in genome along with diversity were studied by Koundal *et al.* (2011) in New Delhi, India. In the complete nucleotide (nt) sequence analysis of the *CMV*-ND strain *CMV*-ND showed that it is comprise of RNA1 with 3358 nt, have RNA2 of 3042 nt units, and RNA3 comprises of total 2214 nt units. RNA1 of *CMV*-ND have one (ORF) site that encodes for the protein that play a vital part in viral replication and termed as 1a. Sequencing data for ND strain indicated that showed maximum sequence similarity with RNA1 and RNA 3 at nucleotide level with a known strain of *CMV* isolated from Taiwan.

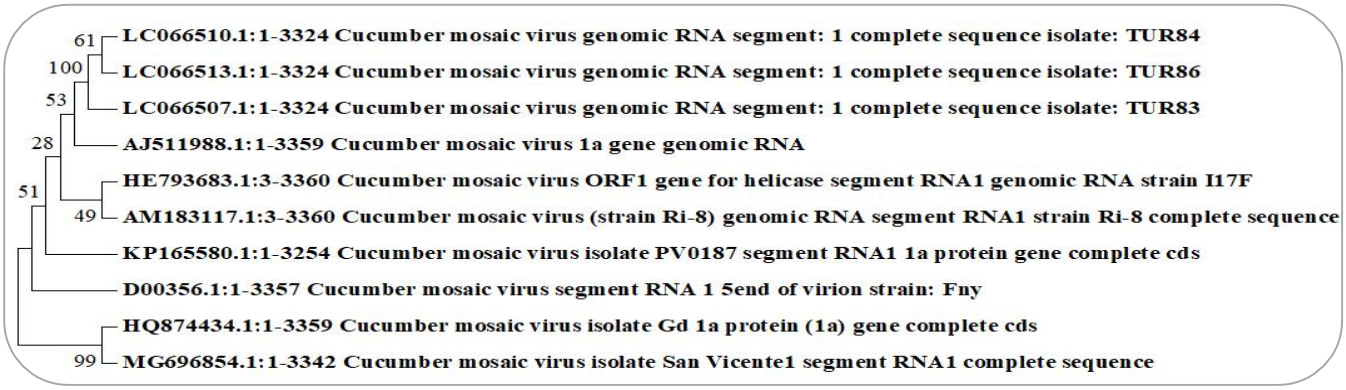

Figure 1. Phylogenetic tree of *CMV* and related Genera based on ITS sequences generated by MEGA.

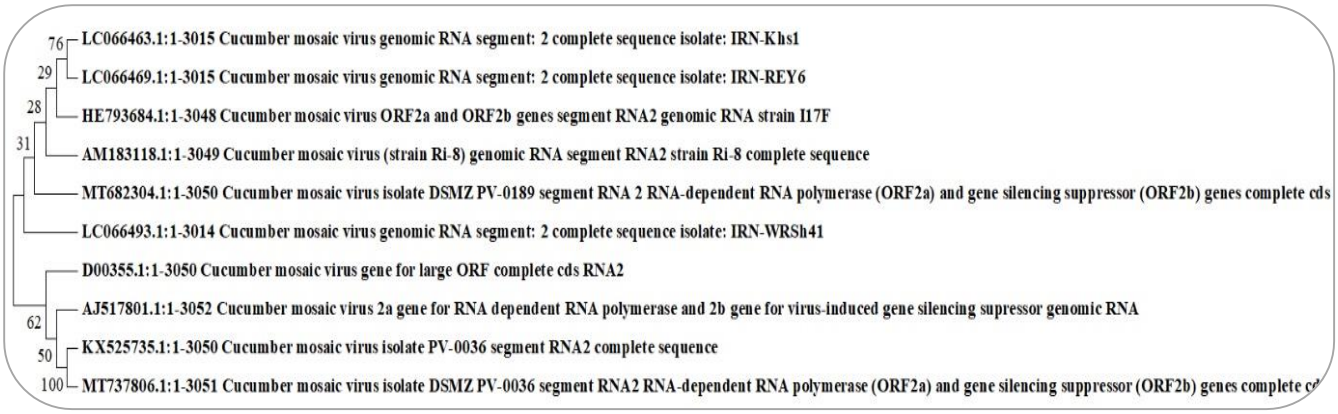

Figure 2. Phylogenetic tree of *CMV* and related Genera based on ITS sequences generated by MEGA.

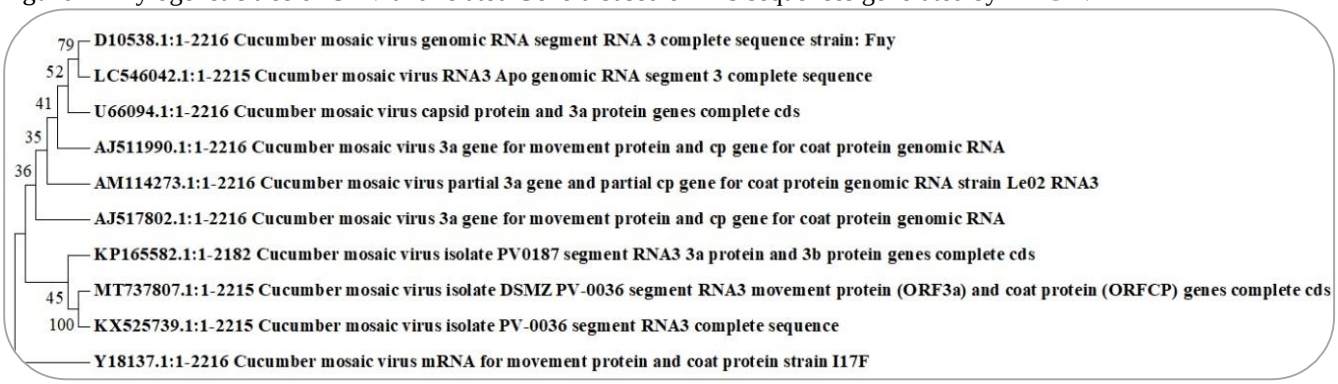

Figure 3. Phylogenetic tree of *CMV* and related Genera based on ITS sequences generated by MEGA.

Symptoms and Host Range: Epidemiological studies on *Cucumber mosaic virus* Showed the Systemic symptoms on naturally infected plants were mosaic and leaf distortion on brinjal, while chilli showed chlorotic spotting, mosaic, leaf puckering and malformation and symptoms recorded on tomato was mild mosaic, leaf narrowing and fern leaf symptoms (Kiranmai *et al.,* 1998). Characteristics symptoms of *CMV* were studied by Kim *et al.,* (2010) which was infecting the Zucchini plants from the Korean fields. *CMV* causes symptoms like stunting of zucchini plants and yellowing of leaves and also causes mosaic patterns in severe form. Malformation of leaves symptoms, uneven ripening and development, and malformation on fruits were observed on zucchini plants. According to survey and sampling *CMV* pas prevalent at Goseong area, Gyeongsangnam-do province of Korea. The scientist analysed *CMV* in bitter gourd and Snake gourd belonging to the family *Cucurbitacae* showing mosaic, mottling, stunted growth puckering and chlorosis and mosaic (Nagendran *et al.,* 2018). In India, shoestring symptom was studied by Pratap *et al.* (2008), and they reported for the first time that *CMV* viral isolated are responsible for this symptom in tomato. Shoestring was also present on infected leaves of tomato plants growing near the bank of Gomti River in Lucknow. The survey was made for gladiolus plants growing in the botanical gardens of NBRI, Lucknow and other adjacent areas. The symptoms of mosaic patterns, colour breaking of flowers, stunting of spikes and size reduction of the flower were caused by *CMV* on gladiolus plants. Molecular and biological characterization detected the *CMV* infection associated with a mosaic symptom (Kumari *et al.,* 2013). *CMV* reflects a large host range and causes severe losses in the yield of plants. *Cucumber mosaic virus* Showed the Systemic symptoms on naturally infected plants were mosaic and leaf distortion on brinjal. In contrast, chilli showed chlorotic spotting, mosaic, leaf puckering and malformation and symptoms recorded on tomato were mild mosaic, leaf narrowing and fern leaf symptoms (Kiranmai *et al.,* 1998).

Transmission: *Cucumber mosaic virus*es have different modes of transmission, so it is very difficult to manage the *CMV* by interfering with the transmission process of the virus. *CMV* is a mechanically transmitted virus but it can also be transmitted through aphid spp., (Akbar *et al.,* 2015). About more than 75 different aphids are the vectors of *CMV*. The virus is nonpersistent. The most efficient vector is *Aphis gossypii* Glover (Palukaitis *et al.,* 1992). This vector works nonpersistent/Stylet-borne manner; its Acquisition

feeding period is 5-10 seconds and its Inoculation Feeding Period is 2 minutes (Hoggan, 1933). *CMV* in more than 19 species of plants Transmitted with the help of but having different levels of efficacy, including some weed species (Neergaard, 1977). *CMV* is also transmissible through dodder plants. 10 species of Cuscuta are involved in efficiently transmitting the virus to host plants (Chen and Francki, 1990; Schmelzer, 1957). Detection of *CMV* in the aphid body by applying ELISA on aphid vector species. ELSIA helped detect *CMV* in aphid body, and the vector confirmed transmission. Infection of *CMV* was proved to be transmittable through the aphid vector (Gera *et al.,* 1979). Effect of infection on *CMV* on lupin (*Lupinus angustifolius*) and infection's seed borne nature, transmission through aphid, infection dispersal and *CMV* effect on yield. When infected seeds (5% and 0.5%) were sown into different plots, they were established into plants having infection percentages of 152.9% and 0.2-0.3% respectively (Jones and Proudlove, 1991). Aphid vectors, the rate of virus transmission is much faster than seed borne and results in severe infection at maturity in plants that were established from the seeds infected 5% than the seeds that were infected at the percentage of 0.5% infected seed. In the experiments. The seeds sown, which were infected 5% yield was reduced, and the yield losses were 34-53%. Seeds harvested showed an infection range of 6-13%. The effect of viral infection caused by the seed sowing having 0.5% infection seed had no significant effect on yield decrease. *CMV* infection's late spread and transmission caused more than 1% seed infection. Viral transmission was done by *Myzus persicae* in the pattern of non-persistent way (Akhtar *et al.,* 2008). *CMV* is vector transmitted through *Aphis gossypii* (Iqbal *et al.,* 2011)**.** *CMV* have mosaic, mottling and other symptoms and it is transmitted through the seeds, mechanically as well as with vector. Additionally, it is transmitted with the help of dodder plants as well (Dragich *et al.,* 2014)**.**

HOST RANGE

Virus	Host	Country	Reference
CMV	*Cucurbita pepo1*	Szarvas (Hungary),	Tobias *et al. (*2008)
CMV Subgroup II	Cucumber	Spain	Thackray *et al.* (2000)
NT strain of *CMV*	Tomato	Germany	Hellwald *et al.* (2000)
CMV	Tomato	India	Pratap *et al.* (2008)
CMV	Gladiolus	India	Dubey and Singh, (2010)
CMV Subgroup II	basil plants	India	Khan *et al.*(2011)
ND strain of *CMV*	Tomato	India	Koundal *et al.* (2011)
Tfr and Tss strains of *CMV*	Tomato	India	Geetanjali *et al.*(2011)
CMV Subgroup II	*Nicotiana* (Tobacco)	India	Kumari *et al.* (2013)
CMV	Chili	Pakistan	Ashfaq *et al.* (2014)
CMV subgroup I	Tomato	China	Luo *et al.* (2017)
CMV	Tomato	India	Jalender *et al.* (2017)
CMV	Zucchini	Korea.	Kim *et al.* (2010)
CMV subgroup IA	tomato	Pakistan	Akhtar *et al.* (2008)
CMV	Cucumber	Mauritius	Lobin *et al.* (2015)
CMV	cucumber	Pakistan	Akbar *et al.* (2015)
CMV	chili	Pakistan	Iqbal *et al.* (2011)
CMV	*Portulaca olercea*	Pakistan	Iqbal *et al.* (2011)
CMV	*Trianthema pentandra*	Pakistan	Iqbal *et al.* (2011)
CMV	*Datura metal*	Pakistan	Iqbal *et al.* (2011)
CMV	Musa spp	Costa Rica	Hord *et al.* (2001)
CMV	Alstroemeria	Netherlands	Chen *et al,* (2002)
Ly2 and Ly8 stains	lily plants	Korea	Lee *et al.* (2007)
CMV	watermelon	Iran	Hosseinzadeh *et al.* (2012)
CMV subgroup II	pea	Pakistan	Ahsan ans Ashfaq, (2018)

Detection: In Costa Rica, total RNA was extracted and total nucleic acid with high RNA levels was observed on nylon membranes. RNA hybridization was done against the specific probes of *CMV* related to the subgroups I or II. The *CMV* confirmation was done in 23 samples of 13 crops from 28 sites. *CMV* subgroup I was found to be more abundant in the areas of Costa Rica and subgroup II isolate of *CMV* was found to be present in the Atlantic region, only (Hord *et al.,* 2001). Reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assays (ELISA) are common methods used to detect *CMV*. A more efficient portable RT-exo-RPA assay was developed based upon visual fluorescence for the detection of viruses which needs a specific primer targeting coat protein gene of *CMV* and a probe (Nishant *et al.,* 2019).

Further studies on the genome proposed that these recombination are the reason for the biological fitness increase in *CMV* virus (Chen *et al.,* 2002). The help of real time PCR and TAS-ELISA did detection and subgrouping of *CMV*. Eight mouse hybridoma cell lines were used which produced anti *CMV* monoclonal antibodies. One hundred thirty samples proved to be infected by *CMV* infection. Out of 130, 121 samples founded to be infected with Subgroup-I viruses and showed a percentage of 93.1% while 9 remaining samples were found as infected with Subgroup-II viruses with percentage of 6.9%. MAbs and specific primers specific for coat proteins region used in PCR process (Yu *et al.,* 2005). In the *CMV* genome detection, 2b protein encoded by *CMV* genome particles are the suppressors because these can inhibit gene silencing at post-transcriptional levels (PTGS). RNA silencing to inhibit the RNA or gene expression. These proteins inhibit plant host defense genes and help the viral infection to prevail. To encounter the host defense system, viruses encode chemicals that act as suppressors and block RNA silencing by hindering their pathways. 2b proteins encoded by *CMV* are known as and considered first identified gene suppressors that can inhibit defensive actions through chemical war. Argonaute1 (AGO1) is the chemical used in plant host defense mechanism and 2b suppressant interacts with Argonaute1 (AGO1) and suppresses its gene regulatory functions to save the virus from the chemical arms of a host (Zhang *et al.,* 2006). Two different isolated *CMV* strains were detected from lily plants and the name of the isolated strains was kept as *CMV* strain Ly2 and Ly8. These isolates' comparison was done with *CMV* in different hosts to know *CMV*'s pathogenicity in lily plants. Ly2 and Ly8 stains produced symptoms of mosaic patterns that were symptomatic *Nicotiana benthamiana* plants. Still, the Ly2 strain could not cause systematic infection in the tomato host plants and the cucumber plants which were the basic (Lee *et al.* 2007). It was identified subgroups I and II of *CMV*-infecting pea plants by using TAS-ELISA. Monoclonal antibodies were used in this assay. This report was the first time published about subgroups I and II (Ahsan and Ashfaq, 2018). The scientist analyzed *CMV* in bitter gourd and Snake gourds belonging to the family *Cucurbitacae,* showing mosaic, mottling, stunted growth puckering and chlorosis. Phylogenetic analysis showed a close relationship of *CMV* to Japan and isolates of Italy than the isolates of the Asian continent. Sequencing showed more than 92 percent similarity with *CMV* subgroup IB (Nagendran *et al.,* 2018).

Management: The effect of PGPRs against *CMV* was studied by treating cucumber seeds with PGPRs that significantly reduced the mean number of symptomatic plants when cotyledons were inoculated with *CMV* and no viral antigen was detected in ELISA (Raupach *et al.,* 1996). The effectiveness of benzothiadiazole (BTH) chemical responsible for SAR induction was studied against *CMV* in tomato leaves (Anfoka, 2000). There are no viricides present in the market, some microbial toxic compounds are also produced by some plants (Kähkönen *et al.,* 1999). These chemicals are termed as botanicals and are extracted from *Eucalyptus globulus*, *Nicotiana tabacum, Curcuma longa, Azadirachta indica, Zingiber officinale* and *Allium sativum* provide excellent results in boosting plant defense against biotic stresses and repelling insect vectors of the viruses (Gurjar *et al.,* 2012). There was significant reduction in *CMV* disease after application of Menno Florades 2%, Virocid 3% and Green Up D 25% (Darzi *et al.,* 2020). Menno Florades contains benzoic acid that is accumulated in plant as a defense component and act as phytoanticipin (Oros and Kállai, 2019). Benzoic acid (BA) is converted into salicylic acid by the action of enzyme benzoic acid-2- hydroxylase that increases tolerance in plants against stresses (Kumar *et al.,* 2015). Apart from boosting defense mechanism, foliar application of BA also enhances growth and yield of the plants which further helps in repairing the damages caused by viral attack (Anjum *et al.,* 2013).

Conclusions and Future Aspects: *CMV* poses deteriorating threats for agricultural and horticultural

production round the globe. Due to wider host range, mechanical and stylet borne aphid transmission, and high infectivity followed by huge yield losses, *CMV* is positioned as significant pathogen both for researchers and farmers. Current article has focused on *CMV* etiology, taxonomy, biology, symptomology, epidemiology and management. Regardless of wide research works to know and control *CMV*, its flexibility and the deficiency of resistance in genotypes is a noteworthy problem. The continuous evolution of this virus underlines the need for a multidisciplinary method, comprising genetic resistance, cultural practices, and eco-friendly management to alleviate the menace of *CMV* and protect worldwide crop production. The global spread of *CMV* has been viewed in terms of integrated and sustainable management. The advancements in precise detection, identification and diagnosis of the virus have also been emphasized.

Future aspects in the study of the ubiquitous *Cucumber mosaic virus (CMV)* promise intriguing pathways for both research and practical applications. Understanding and addressing the challenges posed by *CMV* offer several potential avenues for exploration and development. More research on the genetic basis of resistance in plants against *CMV* is vital through CRISPR/Cas9 and gene editing. Climate change has a significant effect on the transmission rate and pattern of pathogen spread so the climate adaptive cultural practices are the dire need of the time.

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