Biological Forum – An International Journal **15(2): 1053-1063(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Molecular characterization and Efficacy Study of Different Treatments Against *Cucumber mosaic virus* in Pumpkin (*Cucurbita moschata*)

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(Received: 01 January 2023; Revised: 13 February 2023; Accepted: 18 February 2023; Published: 23 February 2023)
(Published by Research Trend)

ABSTRACT: Pumpkin (*Cucurbita moschata* Duch.), a prominent cucurbitaceous vegetable consumed in India is more vulnerable to viral infections under field conditions. Plant viruses are considered major constraints for cucurbit production. Management of viral diseases is crucial for increasing pumpkin yields and value. Viral diseases of pumpkins are widespread in Assam and considered as a major concern for production and productivity of the crop. Pumpkin plants showing chlorotic spots, mosaic, mottling, green vein banding, leaf deformation and blistering symptoms were collected and analyzed for presence of virus using reverse transcription PCR (RT-PCR). Sequence similarity analysis and phylogenetic studies revealed that the isolated virus was *Cucumber mosaic virus* of Subgroup 1B. Further, the study also constitutes to test the efficacy of different seed treatments for the inactivation of seed-borne *Cucumber mosaic virus* in pumpkin variety Arjuna Pumpkin. In the current study, virus inactivation treatments for seeds were prepared using potassium peroxymonosulfate, trisodium phosphate, triton × 100, sodium hypochlorite, hydrochloric acid (HCl), pseudomonas fluorescence, an untreated control, and farmer's practises as treatments. It was found that seeds treated with potassium peroxymonosulfate showed a lower percentage of viral infection (6.70%), followed by HCl treatment (13.48%) and Trisodium phosphate (26.93%). In addition, all other treatments observed an apparent higher viral symptom severity score index than Potassium peroxymonosulfate. Based on the results of this experiment, potassium peroxymonosulfate was found to be the most effective treatment since it considerably reduced the proportion of infected plants and promoted healthy vegetative growth.

Keywords: Pumpkin, CMV, Coat protein, Seed Treatment, Seed-borne Viruses, AUDPC, PCA-biplot.

INTRODUCTION

Pumpkin is considered one of the important vegetable crops belonging to the family Cucurbitaceae and is extensively grown in sub-tropical regions of India. The *Cucurbitaceae* family includes 118 genera and 825 species (Jeffrey *et al.,* 1990), among which pumpkin (*Cucurbita maxima* Duch. and *Cucurbita moschata* Duch.), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus Thunb.*), zucchini (*C. pepo*), fig leaf gourd (*C. ficifolia),* and fluted pumpkin (*Telfairia occidentalis),* Among these, *C. pepo* L., *C. maxima* Duch., and *C. moschata* Duch. are considered to be economically important crop species (Whitaker and Davis 1962; Taylor and Brant 2002). In 2020, the worldwide production of pumpkin, squash, and gourds was about 28 million tonnes from an area of 5 million ha, and in India, the total production was 5.1 million tonnes from an area of 45,000 ha (FAOSTAT 2021). The average fruit weight ranges between 8 and 10 kg, with some fruits weighing up to 20 kg. This vegetable is a rich source of phytonutrients and is an important source of functional components such as vitamin E, ascorbic acids, linoleic acid, zeaxanthin, carotenoids, phytosterols, selenium, and others, which may also act as antioxidants in human nutrition. (Sirohi *et al.,* 1991).

Cucurbits are often attacked by many biotic and abiotic agents, of which diseases and pests are considered major concerns for the productivity of cucurbits (Loebenstein and Thottappilly 2009). Plant viruses are considered major constraints for cucurbit production, with over 39 genera known to infect cucurbits, causing major losses in both quality and quantity (Ayo-John *et al.,* 2014; Lecoq and Katis 2014; Nicaise, 2014). Among the plant viruses, Begomovirus, Crinivirus, Polerovirus, Cucumovirus, Ipomovirus, Tobamovirus,

Tospovirus, and Potyvirus are considered to be known to infect cucurbits (Knierim *et al.,* 2010; Antignus *et al.,* 2001). In India, cucurbits are being attacked by a wide range of plant viruses, which include *Begomovirus,* followed by *Potyvirus*, *Polerovirus*, *Cucumovirus*, and a few others (Kumari *et al.,* 2021). Among these viruses, cucumber mosaic virus (CMV) is considered the most common virus to attack the crop in the field and cause highly prevalent viral disease in vegetables, making the production cumbersome (Roossinck, 1999; Palukaitis *et al.,* 1992; Miao *et al.,* 2016). Cucumber mosaic virus is the predominant virus affecting the major crops of the north-eastern states of India, with a yield loss of up to 30–50% (Baruah *et al.,* 2016; Borah *et al.,* 2019; Routhu *et al.,* 2022). Virus symptoms in cucumbers vary from mild mosaic or vein banding to severe mottling and malformation of leaves, colour change and deformation of fruit, and plant stunting (Davis and Muzuki 1987). According to studies, CMV is seed-borne in nature, resulting in 80% of annual losses and leading to local epidemics. (Florini and Zitter 1987).

Cucumber mosaic virus (CMV), a type member of the genus *Cucumovirus* within the family *Bromoviridae*, is one of the most widespread and significant viruses affecting important solanaceous, cucurbitaceous, and non-crop plants. CMV's host range includes over 1,000 plant species, including major crops, fruits, and vegetables. The transmission of CMV is primarily through aphids in a non-persistent manner. The genome of CMV contains three positive-sense, single-stranded RNAs enclosed in separate particles (Palukaitis *et al*., 1992). RNA 1 and 2 encode 1a and 2a proteins, which constitute the replication and multiplication of the virus (Peden and Symons 1973). RNA 2 encodes the 2b protein, which has multiple functions including cell-to-cell movement, symptom induction, and acting as a virulence determinant by suppressing host gene silencing (Ding *et al*., 1994; Brigneti *et al.,* 1998). The RNA3 encodes two proteins: 3a, which involves cell-to-cell movement protein, and 3b, which encodes capsid protein (CP). The coat protein gene is involved in the aphid-mediated transmission and assemblage of virions (Canto *et al.,* 1997; Mossop *et al.,* 1977). CMV strains have been classified into two main subgroups, *i.e.,* subgroups I and II, based on serology, nucleic acid hybridization, RT-PCR followed by RFLP, and nucleotide sequence identity (Roossinck, 2002).

Several strategies have been developed for the control of viral diseases in plants, including cultural practises (e.g., removal of weeds, eradication of plants, and use of disease-free seeds) and the use of chemical and biological pesticides, such as synthetic pyrethroids, organic halogen compounds, and carbamates, for the control of insect vectors (Palukaitis *et al.,* 1992). In addition, almost all viral pathogens do not have chemical alternatives in their management (Iriti and Varoni 2015; Gerhardson, 2002). Since chemicals can't be easily used to treat seed material and farmers don't know the best ways to apply chemicals to seeds, viruses can cause huge losses in crop production and value (Paylan, 2011).

The yield of crops depends on the quality and viability of the seeds used to grow them. Viruses have been found to be a major threat to seed health and vigour, which ultimately leads to a reduction in crop yield (Johnson *et al.,* 1983; Chatzivassiliou *et al.,* 2008), germination percentage (Pesic and Huruki 1988; Blattny and Osvald 1954), deterioration of seed colour and shape (Inouye, 1962), and seed chemical composition (Ghosh *et al.,* 2011).

To date, several researchers have focused on the inactivation of viruses in vegetable seeds by using various chemicals and other physical or mechanical means. Cordoba-Selles *et al.* (2007) discovered that immersing Pepino seeds in 10% trisodium phosphate for 3 hours followed by an hour of heat treatment at 80 °C successfully inactivated the *Pepino mosaic potexvirus* (PepMV). Although germination was adversely affected, the *Capsicum mosaic virus* affected pepper seeds, which, when heated in an oven at 76 °C for 72 hours following 3 months post-harvest storage, could eliminate the entire viral population (Rast and Stijger 1987). Treatment of BSMV-infected barley seeds with 10,000 rad of gamma rays reduced symptom expression in grown plants (Halliwell and Langston, 1965). Despite the fact that viruses cause numerous and extremely destructive diseases in Cucurbitaceae, little consideration was paid to the management of the viruses. In light of the elevated incidence of cucumber viruses and their growing economic significance, it is necessary to continue research focusing on the molecular identification and characterization of the cucumber mosaic virus. Management of viral diseases is crucial for increasing pumpkin yields and value (Masika *et al.,* 2017). For the development of effective control strategies, information on pumpkin viruses and their incidence according to pumpkin-sortant criteria is necessary. Utilizing healthy, virus-free seeds is the primary strategy for managing plant viruses (Li *et al.,* 2020). A few disinfectants have been found to be effective against certain viruses in crop plants, although there is still a lot of scope for improvement. (Chanda *et al.,* 2021). Six disinfectants were tested for their efficacy against mixed virus infection of the cucumber mosaic virus in pumpkin crops, which affected crop yield. This work aimed to identify the most effective disinfectant(s) to prevent infection by *cucmber mosaic virus* in cucurbits, specifically in pumpkins.

MATERIALS AND METHODS

Sample collection and processing: Suspected leaf samples of naturally infected pumpkin exhibiting typical mosaic, mottling, green vein banding, leaf deformation, and blistering symptoms were collected from the fields of Jorhat and Gossaigaon in Assam,

India, during mid-December 2020. The leaves were properly labeled and stored at -80°C for further use. The virus sap was maintained through repeated sap inoculations on other pumpkin plants.

Molecular detection and characterization of the coat protein gene of CMV:

From the virus-infected leaves of a pumpkin, total RNA was isolated using a Miniscript Nucleospin RNA Plant Kit (Machery-Nagel) according to the manufacturer's protocol. The integrity and quality of total RNA were checked on 1.2% Agarose gel using a spectrophotometer. From the isolated RNA, cDNA synthesis was made using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The reaction mixture for cDNA synthesis consisted of 2µl of RT buffer, 0.8µl of dNTP mix, 2µl of RT random primer, 1µl of reverse transcriptase enzyme, 1µl of RNase inhibitor, 3.2µl of nuclease-free water. The final volume was adjusted to 20µl by adding 100µl of isolated RNA of 100 ng. The reaction cycle was carried out at 25 °C for 10 min, followed by 37 °C for 120 min, and 85 °C for 5 min. The coat protein (CP) region of CMV was amplified using specific primers (CMV CP forward: 5' TTAAGAAATATACCGCTTTT3' and CMV CP reverse: 5' AGTCCTTCCGAAGAAACCG 3') (Borah et al. 2019). The reaction mixture for PCR amplification of the CP gene consisted of 10µl Emerald PCR mix (EmeraldAmp® MAX PCR Master Mix), 1 µl of cDNA as a template, 1µl each of forward and reverse primers (10µM final concentration), and final volume was adjusted to 15µl by adding 2µl of nuclease-free water. The PCR cycle utilized for amplification of the CP gene was initial denaturation of 4 min at 94 °C followed by 35 cycles of denaturation at 94 °C for 30sec, 55°C for 30sec, 72 °C for 40sec and a final extension temperature of 72 °C for 5 min. The PCR products were visualized on an agarose gel (1.2%) in a 1X TBE buffer containing 200ng of ethidium bromide mL^{-1}.

Phylogenetic Analysis. PCR amplicons were sequenced to compare the genetic diversity of CMV in pumpkins from Jorhat and Gossaigaon with other CMV isolates around the world. A basic local alignment search tool (BLAST) from the NCBI (http://www.ncbi.nlm.nih.gov/) was used to try to figure out what the nucleotide sequences of genomic fragments from CMV isolates were. The evolutionary distances were calculated using the p-distance method (Kumar *et al*., 2018). The phylogenetic inference was made using the conserved CP gene fragment of CMV isolates from Jorhat and Gossaigaon and MEGAX software.

Planting material and description of experiments. The experiment was performed during the Rabi season (the year 2021) at the Horticultural Experimental Field of Assam Agricultural University, Jorhat (latitude: 26°45' N, longitude: 94°12' E, altitude: 87 m, with an elevation of 116 m above mean sea level). Viable pumpkin seeds of a pumpkin cultivar (Arjuna Pumpkin, which is a well-known variety in most Asian countries) previously infected with CMV was used as planting material for the present investigation. In this experiment, a total of 7 treatments were considered, including an untreated control (Table 1). A total of 20 seeds were used in each treatment and were sown individually in polybags filled with soil. The germination percentage of seeds was recorded for each treatment, and once fully germinated, 15 seedlings were transplanted to the main field with a spacing of 1 m for each treatment and the control. Uniform cultural practices like weeding and other intercultural operations were adopted in each plot throughout the crop's growth. Along with the above treatments, 15 Arjuna Pumpkin seedlings were given to a farmer in Charigaon village of Jorhat district in Assam, India, to grow in his own field using an indigenous package of practices. This was done so that we could keep track of how often viral diseases happened.

Table 1: Different seed treatments used in the present study.

Treatment	Details
Treatment I	Seeds of pumpkin were soaked in Potassium peroxy monosulfate solution of 2% concentration for 10 minutes and air-dried before sowing.
Treatment II	Seeds of pumpkin were soaked in Trisodium phosphate solution of 2% concentration for 10 minutes and air-dried before sowing.
Treatment III	Seeds of pumpkin were immersed in10%TritonX100for10minand air-dried before sowing.
Treatment IV	Seeds of pumpkin were immersed in 0.4% sodium hypochlorite (NaClO) for 10 min and air-dried before sowing.
Treatment V	Seeds of pumpkin were immersed in 2% hydrochloric acid (HCl) for 10 min and air-dried before sowing.
Treatment VI	Seeds of pumpkin were steeped in a slurry of *Pseudomonas fluorescens*@10g/lt of water and air-dried at normal room temperature before sowing.
Untreated control	Control (untreated seed)
Farmer's practice	Seeds were given to a farmer to grow in his own field using their indigenous technique.

Estimation of disease prevalence and severity. The field was observed at regular intervals to check the presence of viral symptoms in the experimental plot during the entire crop season. Plants were randomly evaluated for virus-like symptoms of *cucumovirus, potyvirus* and *begomovirus* such as yellow spots, mosaic/mottling, green vein banding, leaf deformation/distortion and blistering (Nagendran *et al.,* 2017). Viral Disease prevalence (DP) was assessed for all the treatments individually based on the number of

plants infected to the total number of plants present in each treatment.

DP (%) =n/N × 100

Where, n is the number of plants showing symptoms and N is the total number of plants. Disease prevalence in a pumpkin was observed during the 15th, 30th, 60th, 90th and 120th days after transplanting in the main field.

Along with disease prevalence, infection percentage and infection progress rate were also estimated for all the treatments on the 15th, 30th, 60th, 90th and 120th days after transplanting in the main field upon treatment. More specifically, symptoms were classified into five categories, namely: (a) yellow spots, (b) mosaic/mottling, (c) green vein banding, (d) leaf deformation/distortion and (e) blistering. The symptom severity of viral-infected pumpkin plants was estimated by a scoring system (ordinal disease rating scale) using an index ranging from 0 to 5 and defined as follows: 0 :- no infection (none of the above-mentioned symptom categories is observed); 1:- mild infection (any one of the three symptom categories is observed); 2:- Moderate infection (any two of the three symptom categories are observed); 3:- Severe infection (three symptom categories were observed); 4:-highly severe infection(four symptom categories were observed); and 5= Extremely severe infection(all five symptom categories were observed) (Routhu *et al.,* 2022). Furthermore, the impact of each treatment on plant length was estimated at 120 days after transplanting by measuring the average plant length of all 15 plants under each treatment.

Data analysis. For analyzing the data, ANOVA (Analysis of variance), descriptive statistics, and heritability were performed utilising Genstat software (www.biosci.global/softwar-en/genstat) and R v. 3.6.1 software (https://www.R-project.org/.). AUDPC for all the treatments were estimated using Microsoft Excel 2016. Past v.4.03 software was used for the analysis of PCA-Biplot and boxplot (Nayak *et al.,* 2017).

RESULTS

Observation of viral disease symptoms in pumpkin. From the infected plants, symptoms associated with CMV infection were vein banding, downward leaves, mottling, stunting, narrowing, leaf rolling and upward cupping, yellowing and blistering of leaves (Fig. 1).

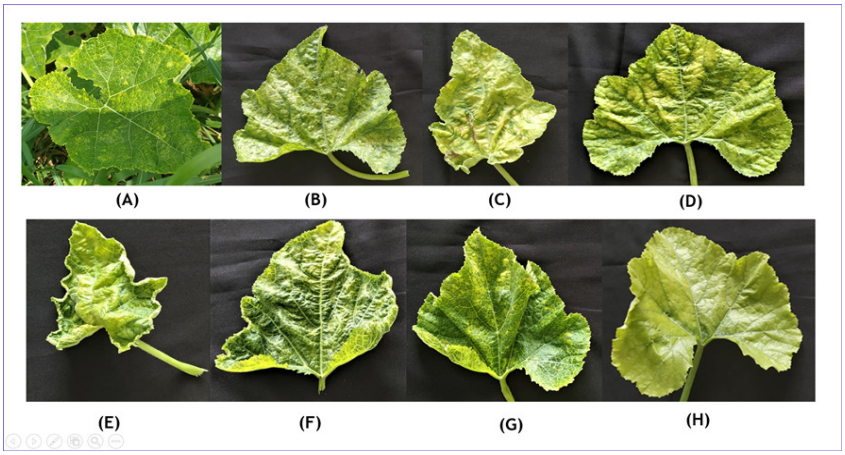

Fig. 1. Disease symptoms observed on pumpkin crop in the experimental field: (A)Yellow spots; (B) Mosaic; (C) Mottling; (D)Vein banding and chlorosis; (E) Leaf rolling and upward cupping, blistering, narrowing; (F) Vein clearing, mosaic, upward curling of leaves; (G)Vein clearing, mosaic, mottling, chlorosis; (H) Leaf chlorosis/ yellowing.

Molecular characterisation and Phylogenetic analysis. Characteristic symptoms of *Cucumber mosaic virus* infection in pumpkin plants were reported in the fields of Jorhat and Gossaigaon places of Assam. The incidence of CMV in pumpkin fields was confirmed based on Semi-Quantitative PCR results. Infected samples generated an amplicon size of 699bp, thus confirming the CMV infection in pumpkin (Fig. 2). The viral origin of the specific amplicon was confirmed by Sanger di-deoxy sequencing and found to the CP gene of the *Cucumber mosaic virus.* The obtained genomic sequence was analysed through multi-alignment (Corpet 1988) and nBLAST (blast.ncbi.nlm.nih.gov/Blast.cgi) tools of NCBI (Enclosed in additional files 1 and 2). It was found that isolated CMV sequence belongs to subgroup IB type of CMV and shares 99% similarity with other CMV isolates around the world. The retrieved sequence was deposited in the NCBI database and assigned with gene accession numbers (MZ219645 and MZ219646). Phylogenetic analysis was conducted with 29 closely related CMV isolates around the world, so as to find the genetic diversity between the isolates (Fig. 3). CMV CP of pumpkin from Assam (MZ219645 and MZ219646) formed maximum similarity with CMV isolate (MW291545.1) infecting chilli in Ecuador, and with other CMV isolates (KM272276.1) infecting chilli in Karnataka, India.

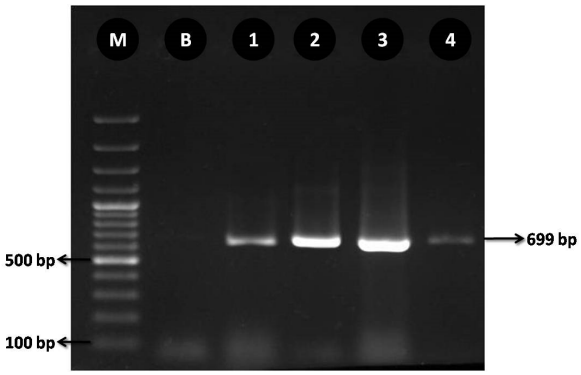

Fig. 2. Semi-Quantitative PCR analysis for the amplification of Coat Protein (CP) gene of CMV infecting Pumpkin; Agarose gel electrophoresis showing amplification of 699bp nt CP gene, with lane M: 100bp ladder (Takara), lane 1-2: Isolates of Jorhat, Lane 3-4: Isolates of Gossaigaon; B-Blank (No DNA).

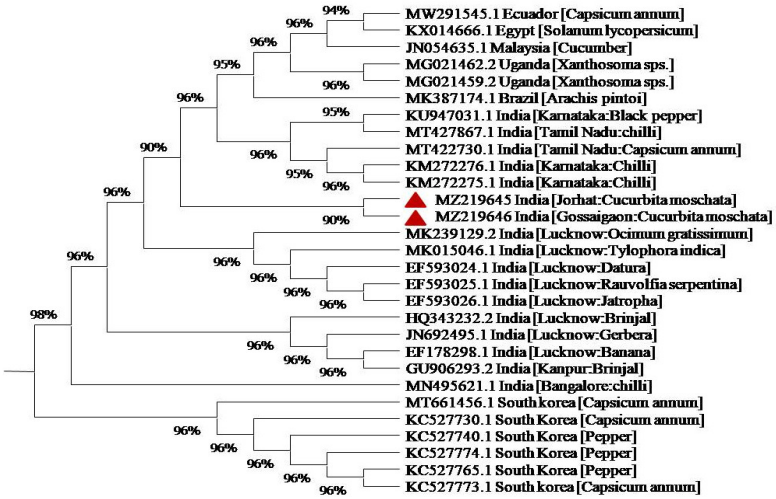

Fig. 3. Phylogenetic relationship of CMV isolates from Assam infecting pumpkin (Red triangle) with other CMV isolates reported worldwide based on coat protein gene. The number at nodes represents the bootstrap percentage score out of 1000 replicates.

Analysis of variance, descriptive statistics and heritability. Analysis of variance showed that a significant difference was observed among the different treatments for the control of viral disease in pumpkins (Table 2). The mean value of disease percentage indicated that expression of viral disease was heavier at 120th days after transplanting in all the treatments (Table 2). None of the seed treatments could completely eliminate the viral infection in natural conditions, but all the treatments are effective over the control and farmer's practice.

Table 2: ANOVA and descriptive statistics based on viral disease incidence percentage of different treatments in pumpkin.

Treatments	Percent Disease incidence at difference days after transplanting (DAT)				
	15th DAT	30th DAT	60th DAT	90th DAT	120th DAT
Potassium peroxymonosulfate (2%, 10min)	0.00	0.00	0.00	6.49	6.70
Trisodium phosphate (2%, 10 min)	0.00	7.11	13.11	13.45	26.93
Triton × 100 (10%, 10 min)	0.00	25.64	41.16	42.12	44.62
Sodium hypochlorite (0.4%, 10 min)	0.00	26.30	27.52	26.30	41.37
HCl (2%, 10 min)	0.00	7.04	6.78	7.13	13.48
Pseudomonas fluorescence (10g/lt, 24 hr)	0.00	13.34	21.40	27.58	27.92
Farmers practice	20.37	32.53	33.45	48.41	59.97
Untreated control	13.42	27.71	27.00	34.67	47.56
Mean	4.22±0.33	17.46±0.46	21.30±0.42	25.77±0.48	33.57±1.34
F-ratio	598.21**	680.83**	1081.06**	1070.58**	180.93**
C.D.(at 5% level)	1.00	1.40	1.28	1.46	4.08
CV(%)	13.48	4.58	3.43	3.23	6.92

"**" Significant at 1%

PCA-Biplot analysis for espial of effective treatment. The PCA analysis based on viral disease incidence percentage on five different days after transplanting of eight treatments, including control (Untreated control and farmers practice), explained that all the treatments have a significant difference in suppressing the viral diseases. The first two PCs in the biplot (PC1 and PC2) explained 95.58 % of the total variation due to the effect of different treatments for viral diseases (Fig. 4).

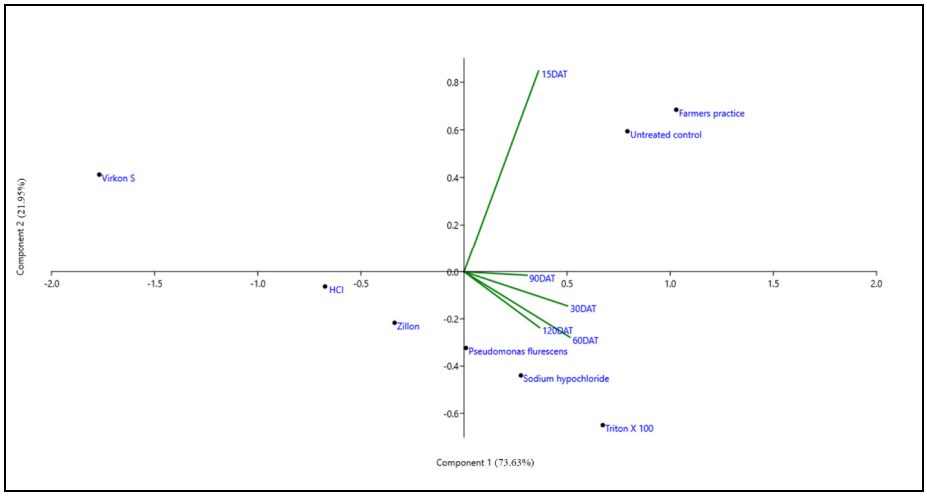

Fig. 4. PCA-Biplot based on viral disease percentage of eight different treatments.

Out of eight treatments, including farmers' practice and un-treated control evaluated for inactivation to seed-borne viral disease, only three treatments (Potassium peroxymonosulfate, HCl and Trisodium phosphate) which had < average 15 % viral disease expression were subjected to efficacy analysis to identify most effective treatments. The first section contains the Farmers/s practice and untreated control are ineffective to viral diseases of pumpkin and also plotted on the same side of 15 DAT. The second section contains the 30 DAT, 60 DAT, 90 DAT and 120 DAT with two treatments like *Pseudomonas fluorescen*ce, Sodium hypochlorite and Triton × 100 as the less effective treatments to control the viral diseases of pumpkin. The third section contains two treatments, HCl and Trisodium phosphate, which are moderately effective in controlling viral diseases. Finally, the fourth section contains only one treatment, Potassium peroxymonosulfate, which is plotted opposite to the disease scoring days, and it is the most effective treatment to control the viral diseases of pumpkin.

Effect of different treatments on seed germination, plant growth and disease pressure. All the treatments showed significant variation in seed germination, plant growth at 20 days after transplanting and disease pressure as expressed by AUDPC value. Germination percentage of treated (including control and farmer's practices) pumpkin seeds ranged from 90% for untreated seeds to 100 % for seeds treated with *Pseudomonas fluorescence,* with an average of 97.21 %. These results indicated that pre-sowing seed treatments could increase the germination percentage in pumpkins. Similarly, all the treatments could enhance the plant length at 20 days after transplanting in comparison to untreated pumpkin seeds and results from further states seed treatment with Potassium peroxymonosulfate resulted in maximum plant length of the crop.

Table 3: ANOVA and descriptive statistics of germination (%), plant length and AUDPC value revealed by different treatments in pumpkin.

Treatments	Germination (%)	Plant length at 20 DAT	AUDPC value
Potassium peroxymonosulfate (2%, 10min)	99.67	30.85	295.15
Trisodium phosphate (2%, 10 min)	99.67	22.64	1360.80
Triton × 100 (10%, 10 min)	99.00	22.62	5744.60
Sodium hypochlorite (0.4%, 10 min)	95.00	21.98	2827.05
HCl (2%, 10 min)	99.33	28.74	777.98
Pseudomonas fluorescens (10g/lt, 24 hr)	100.00	24.46	2188.23
Farmers practice	95.00	18.96	4239.85
Untreated control	90.00	18.87	3287.55
Mean	97.21±0.45	23.64±0.31	2340.15±34.71
F-ratio	63.67**	192.54**	1697.59**
C.D.(at 5% level)	1.36	0.94	2231.23
CV (%)	0.80	2.26	49.07

"**" Significant at 1%

Disease pressure caused by mixed virus infection upon different treatments in a pumpkin was expressed by AUDPC value and is represented in Fig. 5. Minimum disease pressure was observed in Potassium peroxymonosulfate treatment, and it was proven to be the most effective treatment resulting total 293.13 AUDPC value against viral diseases. HCL-treated seeds showed medium effectiveness in controlling the viral diseases in pumpkin and emanating 777.98 AUDPC value for the viral diseases. According to the AUDPC analysis, farmer's practice had a poor effect to eliminate viral diseases in pumpkin, and it enhanced the viral disease pressure in comparison to untreated control. The total disease pressure in terms of AUDPC value in the case of farmer's practices was 4239.85. The disease pressure of the remaining treatments was lesser compared to the control (untreated seeds) except for Triton × 100.

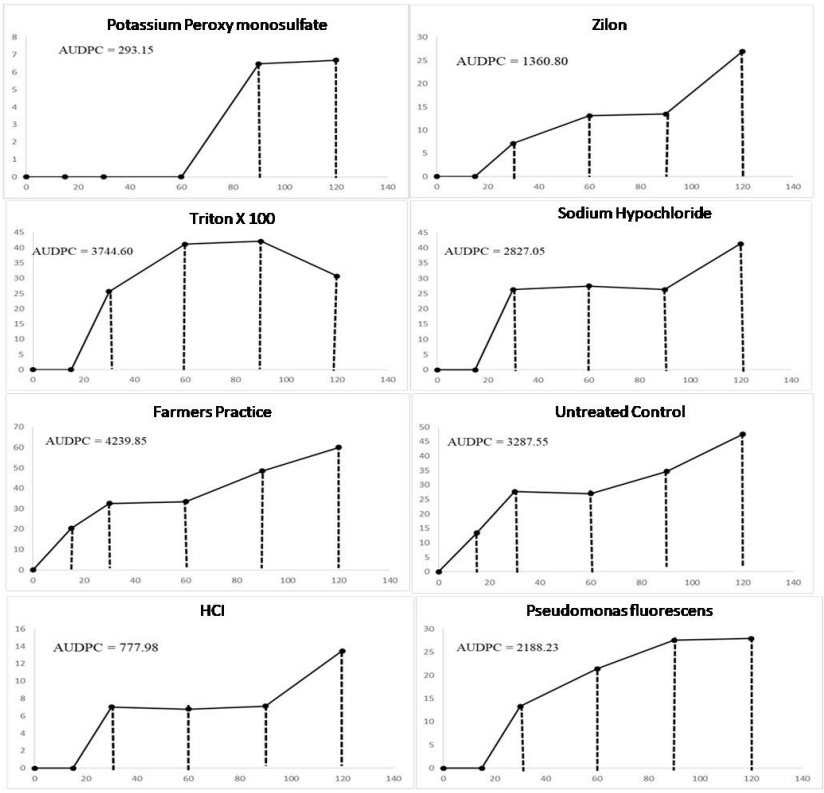

Fig. 5. Effects of different treatments on AUDPC of viral diseases in pumpkin.

The relationship between the germination percentages, plant length and disease pressure with different treatments was explained by the PCA-biplot (Fig. 6). Considering the all parameters, Potassium peroxymonosulfate is the most impressive treatment, and it was plotted on the opposite side of AUDPC in the PCA-biplot.

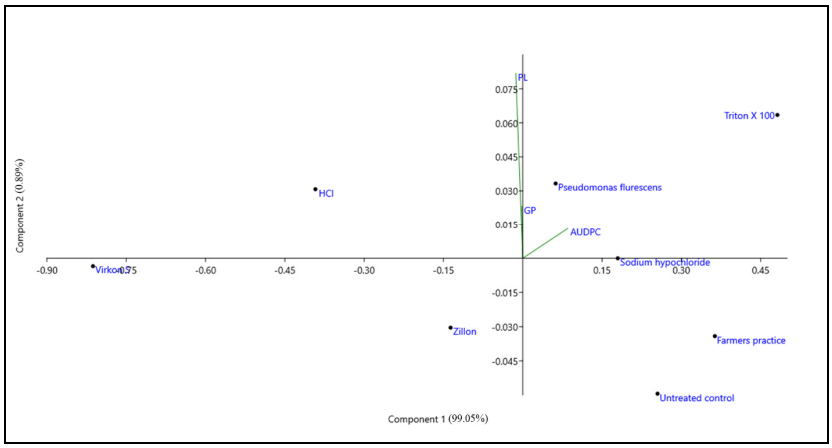

Fig. 6. PCA-biplot shows the relationship among the germination percentage, plant length and AUDPC value with different treatments. [GP: Germination percentage; PL: Plant length at 20 DAT].

DISCUSSION

The *cucumber mosaic virus* (CMV) has become a major threat to pumpkin production in Assam, India. However, it is difficult to visually diagnose CMV in the field because some of the symptoms may be related to other biotic agents such as *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), and *Squash mosaic virus* (SQMV) (Lisa, 1984; Chala *et al*., 1987). As a result, reliable and accurate detection methods for CMV in fields are required. In this study, reverse transcription PCR (RT-PCR) was employed for the detection of CMV infecting pumpkins. CMV was successfully detected through RT-PCR by generating an amplicon size of 699 bp in 1.2% agarose gel electrophoresis.

Several studies have been conducted recently to recognize disinfectants to reduce the infectivity of seed-borne plant viruses and viroids in different ornamental crops (Chanda *et al.,* 2021; Lewandowski *et al.,* 2010; Kamenova and Adkins 2004; Hu *et al.,* 1994), including cucurbits (Coutts *et al*., 2013), greenhouse tomatoes (Matsuura *et al.,* 2010; Wintermantel, 2011), etc. Farmers from Assam use seeds from the previous season and usually propagate them without proper seed treatments, thereby increasing the spread of seed-borne viruses in cucurbits. As there are no standard seed treatments for the management of seed-borne viral diseases in pumpkins, we made an attempt to test the efficacy of six different seed disinfectants against virus infection in pumpkins and to suggest a reliable treatment for controlling seed-borne viruses of *begomovirus*, *potyvirus,* and *cucumovirus* in pumpkins. Our results showed that none of the treatments could completely eliminate the viruses in the seeds, but a few of them could manage the viral infection to a greater extent. The efficacy of every seed treatment against viruses completely depends on the location of the virus in the seed. Research by Mathews (1991); Mink (1993); Agarwal and Sinclair (1997) revealed that seed-transmitted viruses are mostly present within the embryo. A suitable disinfectant for any kind of vegetable production should satisfy some criteria, including short contact time, broad efficacy against viruses, safety for workers, not being corrosive to infrastructure, not being phytotoxic to plants, and being economical. According to the results of the present investigation, with its broad-spectrum efficacy against mixed virus infections in pumpkin crops, the most effective disinfectant, 2% potassium peroxymonosulfate, may be recommended to prevent general viral infection in pumpkin crops. Previous research has shown that potassium peroxymonosulfate is the most effective disinfectant and is capable of eliminating various human and animal viral pathogens (Shahid *et al*., 2009; Zou *et al*., 2013). As reported by Li *et al.* (2015), 1% or 2% potassium peroxymonosulfate could successfully deactivate PepMV, ToMV, and TMV (*Pepino mosaic virus*, *Tomato mosaic virus, and Tobacco mosaic virus*) in tomatoes. However, when 2% potassium peroxymonosulfate solution was used, full protection from TMV infection was observed (Li *et al.,* 2015). Li *et al.* (2015) reported that 2% potassium peroxymonosulfate achieved the most consistent effects against four tomato viruses and viroid pathogens: PepMV, PSTVd, TMV, and ToMV. Treatment of contaminated tools with 1% (wt/vol) potassium peroxymonosulfate significantly reduced the contamination during the process of taking cuttings in petunias, as reported by Lewandowski *et al.* (2010). Chemicals such as HCl (hydrochloric acid) were found to be very efficient against seed-borne viruses, as described by Ismail *et al*. (2014); Broadbent (1965); Alexander (1960); McGuire *et al*. (1979). Amrate *et al.* (2020) reported that trisodium phosphate, an antiviral remedy @ 6 ml/L at 15, 30, and 45 DAS, was found to be effective in controlling the yellow mosaic virus of soybean as well as in increasing the soybean yield. Plant growth-promoting microbes (PGPM), which may produce antiviral compounds and endorse the defense potential of plants against different viruses, have become a promising substitute capable of promoting virus tolerance in plants (Yue *et al.,* 2000). Several studies have suggested the possibility of using bacterial as well as plant RNase to protect plants from pathogenic viruses. Thus, identifying the biological properties of plant growth-promoting microbes and their role in the plant microbiome can lead to the development of biological products that may be antiviral, insecticidal, fungicidal, bactericidal, immune, and growth-promoting (Mhlongo *et al.,* 2018). Lewandowski *et al.* (2010) reported that 0.1% Tween 20 or a 1:10 dilution of household bleach (0.6% sodium hypochlorite) plus a 20% (wt/vol) solution of nonfat dry milk (NFDM) completely removed TMV transmission from petunia flowers. Over the past three decades, TritonX-100 (TX-100) has been widely considered the most effective disinfectant in the medical as well as biotechnology industries (Horowitz *et al.,* 1985). Anderson (1987) reported the effectiveness of CH_3COOH, NaClO (Sodium hypochlorite), H_2O_2, and Triton × 100 treatments in eradicating viruses from seeds. The occurrence of viral diseases is observed more in cucurbits and other vegetable crops due to the destitute agronomic management system used by farmers, along with poor weed management in fields, continuous cropping or mono-cropping, and other poor pest management methods (Thresh, 2003; Hull, 2009). Furthermore, the use of uncertified seeds by the farmers, along with the inappropriate use of pesticides due to a lack of knowledge, is found to be the prime cause of the resistance of the aphids and whitefly vectors to the chemical barrier in viral disease management in crop plants (Ayo-John *et al*., 2014; Afouda *et al.,* 2013).

CONCLUSIONS

The present study revealed that the viral diseases of pumpkins are widespread in Assam and considered as a major concern for production and productivity of the crop. Results from reverse transcription PCR and molecular characterization indicates the presence of CMV infection in pumpkin and generated an amplicon size of 699bp in 1.2% agarose gel electrophoresis. It was not possible to establish a correlation between the type of symptom, which might not act as a reliable indicator for a particular virus and for this, molecular characterization is essential. In this study we aimed to test the efficacy of different seed treatments in controlling the virus infection in pumpkin. The results states that 2% Potassium peroxymonosulfate is the most effective treatment against mixed virus infection viruses in pumpkin crops. This information is, therefore, a valuable contribution for the management of viral diseases in cucurbits of Assam, India. The management strategies against viruses in plants also depend on existing viruses, seasons of severe infection by those viruses and the weeds that act as alternate hosts. As mentioned here, the important role that seeds play in virus spread is recognized by researchers and evaluating the optimum dosage and duration of seed treatments for different seed-virus combinations is important without damaging seed vigour and health.

Research involving human participants and/ or animals: This article does not contain any studies with human participants or animals performed by any of the authors.

Acknowledgement. Munmi Borah was supported by the Directorate of Research, Assam Agricultural University, for conducting this research work. Dr. Lohit Ch. Bora, Head, Department of Plant Pathology, AAU, Jorhat, is acknowledged for his assistance in directing this research work.

Conflict of Interest. None.

REFERENCES

Afouda, L. A., Kotchofa, R., Sare, R., Zinsou, V. and Winter, S. (2013). Occurrence and distribution of viruses infecting tomato and pepper in Alibori in northern Benin. *Phytoparasitica*, *41*(3), 271-276.

Agarwal, V. K. and Sinclair, J. B. (1997). *Principles of Seed Pathology*. CRC Press.

Agneroh, T.A., Kouadio, K.T., Soro, K. and Pohé, J. (2012). Identification et distribution des virus du *Lagenaria siceraria* (Molina) Standl et *Citrullus* sp. en Côte d'Ivoire. *Journal of Animal & Plant Sciences*, *13*(2), 1758-1770.

Alexander, L. J. (1960). Inactivation oí TMV from Tomato seed. *Phytopathology*, *50*(9).

Amrate, P. K., Shrivastava, M. K. and Pancheshwar, D. K. (2020). Yield reduction and efficacy of antiviral product against Yellow Mosaic Virus disease in soybean. *Journal of Oilseeds Research, 37* (Special issue, Feb), 229–230.

Andersson, L. (1987). Survey and control of seedborne diseases of tomatoes in Zambia. *Arbetsrapport-Sveriges Lantbruksuniversitet, U-landsavdelningen (Sweden)*.

Andreason, S.A., Olaniyi, O. G., Gilliard, A. C., Wadl, P. A., Williams, L. H., Jackson, D. M., Simmons, A. M. and Ling, K. S. (2021). Large-Scale Seedling Grow-Out Experiments Do Not Support Seed Transmission of Sweet Potato Leaf Curl Virus in Sweet Potato. *Plants 10*, 139.

Antignus, Y., Wang, Y., Pearlsman, M., Lachman, O., Lavi, N. and Gal-On, A. (2001). Biological and molecular characterisation of a new cucurbit-infecting tobamovirus. *Phytopathology*, *91*(6), 565-571.

Ayo-john, E. I., Olorunmaiye, P. M., Odedara, O. O., Abiola, K. O. and Oladokun, J. O. (2014). Assessment of field-grown cucurbit crops and weeds within farms in south-west Nigeria for viral diseases. *Notulae Scientia Biologicae*, *6*(3), 321-325.

Baruah, B. R., Kashyap, A. and Nath, P. D. (2016). Incidence, detection and integrated management of viral disease complex in Bhut Jolokia, a Chilli cultivar in Assam. *Annals of Plant Protection Sciences, 24*, 136-141.

Blattny, C. and Osvald, V. (1954). Pfenosviroschmele (*Humulus lupulus* L.) napotomstosemenem. *Preslia, (Prague)*, *26*, 1-26.

Borah, M., Kumar, R. G. and Siddappa, S. (2019). Molecular Detection and Phylogenetic Analysis of Cucumber Mosaic Virus Infecting Bhut Jolokia (*Capsicum chinense* Jacq.) of Assam. *Int. J. Econ. Plants*, *6*(3), 126–129.

Brigneti, G., Voinnet, O., Li, W. X., Ji, L. H. and Ding, S. W. (1998). Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. *EMBO J, 17,* 6739–6746.

Broadbent, L. (1965). The epidemiology of tomato mosaic: XI. Seed-transmission of TMV. *Annals of Applied Biology*, *56*(2), 177-205.

Canto, T., Prior, D. A. M, Hellwald, K. H., Oparka, K. J. and Palukaitis, P. (1997). Characterisation of cucumber mosaic virus. IV. Movement protein and coat protein are both essential for cell-to-cell movement of cucumber mosaic virus. *Virology, 237*, 237–248.

Chala, V. P., Harrison, C. W. and Halliwell, R. S. (1987). Identification of two distinct strains of watermelon mosaic virus affecting cucurbits in Texas. *Plant Dis*., *71,* 750-752.

Chanda, B., Shamimuzzaman, M. and Gilliard, A. (2021). Effectiveness of disinfectants against the spread of tobamo viruses: *Tomato brown rugose fruit virus* and *Cucumber green mottle mosaic virus*. *Virol J, 18*, 7.

Chatzivassiliou, E. K., Moschos, E., Gazi, S., Koutretsis, P. and Tsoukaki, M. (2008). Infection of potato crops and seeds with Potato virus Y and Potato leafroll virus in Greece. *Journal of Plant Pathology*, 253-261.

Córdoba-Sellés, M., García-Rández, A., Alfaro-Fernández, A., and Jordá-Gutiérrez, C. (2007). Seed Transmission of Pepino mosaic virus and Efficacy of Tomato Seed Disinfection Treatments. *Plant disease*, *91*(10), 1250–1254.

Corpet, F. (1988). Multiple sequence alignment with hierarchical clustering. *Nucl. Acids Res*., *16*(22), 10881-10890

Coutts, B. A., Kehoe, M. A. and Jones, R. A. C. (2013). Zucchini yellow mosaic virus: Contact transmission,

stability on surfaces, and inactivation with disinfectants. *Plant disease*, *97*(6), 765-771.

Davis, R. F. and Mizuki, M. K. (1987). Detection of cucurbit viruses in New Jersey. *Plant Disease*, *71*(1), 40-44.

Ding, S. W., Anderson, B. J., Haase, H. R. and Symons, R. H. (1994). New overlapping gene encoded by the cucumber mosaic virus genome. *Virology, 198*, 593–601.

FAOSTAT 2021 https://www.fao.org/statistics/en, UN Food and Agricultural Organization, Corporate Statistical Database (FAOSTAT), 2021. Retrieved October 9, 2021.

Fauquet, C. and Thouvenel, J. C. (1987). *Plant viral diseases in the Ivory Coast* (No. BOOK). Institut Francais de Recherche Scientifique Pour le Developpementen Cooperation.

Florini, D. A., and Zitter, T. A. (1987). Cucumber mosaic virus (CMV) in pepper (*Capsicum annuum* L.) in New York and associated yield losses. *PHYTAJ, 77,* 652.

Gerhardson, B. (2002). Biological substitutes for pesticides. *Trends Biotechnol.*, *20,* 338–343

Ghosh, R., Palit, P., Ghosh, S. K. and Roy, A. (2011). A New World virus alters biochemical profiling of jute plants (*Corchorus capsularis*) upon infection. *International Journal of Science and Nature*, *2*(4), 883-885.

Halliwell, R. S. and Langston, R. (1965). Effects of gamma irradiation on symptom expression of barley stripe mosaic virus disease and on two viruses in vivo. *Phytopathology (US)*, *55*.

Horowitz, B., Wiebe, M. E., Lippin, A. and Stryker, M. H. (1985). Inactivation of viruses in labile blood derivatives. I. Disruption of lipid-enveloped viruses by tri (n-butyl) phosphate detergent combinations. *Transfusion*, *25*(6), 516-522.

Hu, J. S., Ferreira, S., Xu, M. Q., Lu, M., Iha, M., Pflum, E. and Wang, M. (1994). Transmission, movement and inactivation of cymbidium mosaic and odontoglossum ringspot viruses. *Plant Disease*, *78*(6), 633-636.

Hull, R. (2009). Comparative Plant Virology, Academic Press.

Inoue, T. (1962). Studies on barley stripe mosaic in Japan. *Berichte des Ohara Instituts furl and wirtschaftliche Biologie, Okayama Universität*, *11*(4), 413-496.

Iriti, M. and Varoni, E. M. (2015). Chitosan-induced antiviral activity and innate immunity in plants. *Environmental Science and Pollution Research*, *22*(4), 2935-2944.

İsmail Can Paylan, Semih Erkan, Nedim Cetinkaya, Muge Ergun and Sercan Pazarlar (2014). Effects of Different Treatments on the Inactivation of Various Seedborne Viruses in Some Vegetables, Ozone: *Science & Engineering,* 422-426.

Jeffrey, C. (1990). An outline classification of the Cucurbitaceae. *Biology and utilisation of the Cucurbitaceae*, 449-463.

Johnson, C. S., Main, C. E. and Gooding, Jr. G. V. (1983). Crop loss assessment for flue-cured tobacco cultivars infected with tobacco mosaic virus. *Plant Disease*, *67*(8), 881-885.

Kamenova, I. and Adkins, S. (2004). Transmission, in planta distribution, and management of Hibiscus latent Fort Pierce virus, a novel tobamovirus isolated from Florida hibiscus. *Plant Disease*, *88*(6), 674-679.

Knierim, D., Deng, T. C., Tsai, W. S., Green, S. K. and Kenyon, L. (2010). Molecular identification of three distinct Polerovirus species and a recombinant Cucurbit aphid-borne yellows virus strain infecting cucurbit crops in Taiwan. *Plant Pathology*, *59*(5), 991-1002.

Koné, D., Aké, S., Abo, K., Soro, S., N'Guessan, C. A., Wipf-Scheibel, C. and Lecoq, H. (2010). First Report of Zucchini yellow mosaic virus in Cucurbits in Ivory Coast. *Plant Disease*, *94*(11), 1378-1378.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution., 35,* 1547-1549.

Kumari, S., Krishnan, N., Dubey, V., Das, B., Pandey, K. K. and Singh, J. (2021). Investigations on annual spreading of viruses infecting cucurbit crops in Uttar Pradesh State, India. *Sci Rep., 11,* 17883.

Lecoq, H. and Katis, N. (2014). Control of cucurbit viruses. In *Advances in virus research* (Vol. *90*, pp. 255-296). Academic Press.

Lewandowski, D. J., Hayes, A. J. and Adkins, S. (2010). Surprising results from a search for effective disinfectants for tobacco mosaic virus-contaminated tools. *Plant Disease*, *94*(5), 542-550.

Li, N., Yu, C., Yin, Y., Gao, S., Wang, F., Jiao, C. and Yao, M. (2020). Pepper Crop Improvement Against Cucumber Mosaic Virus (CMV): A Review. *Front. Plant Sci., 11,* 598798.

Li, R., Baysal-Gurel, F., Abdo, Z., Miller, S. A. and Ling, K. S. (2015). Evaluation of disinfectants to prevent mechanical transmission of viruses and a viroid in greenhouse tomato production. *Virology journal*, *12*(1), 5.

Lisa, V. (1984). Zucchini yellow mosaic virus CMI Descriptions of plant viruses, 282.

Loebenstein, G. and Thottappilly, G. (2009). The sweet potato: Springer Science+ Business Media B.

Masika, Fred, Kisekka, Ronald, Alicai, Titus and Tugume, Arthur (2017). Incidence of viruses and virus-like diseases of watermelons and pumpkins in Uganda, a hitherto none-investigated pathosystem. *African Journal of Agricultural Research*, *12*, 177-191.

Mathews, R. E. F. (1991). Plant Virology, 3rd ed. New York: Academic Press.

Matsuura, S., Matsushita, Y., Usugi, T. and Tsuda, S. (2010). Disinfection of Tomato chlorotic dwarf viroid by chemical and biological agents. *Crop Protection*, *29*(10), 1157-1161

McGuire, J. M., Wickizer, S. L. and Goode, M. J. (1979). Association of tomato mosaic virus with tomato seed. *Arkansas Farm Research*.

Mhlongo, M. I., Piater, L.A., Madala, N. E., Labuschagne, N. and Dubery, I. A. (2018). The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. *Frontiers in Plant Science*, *9*, 112.

Miao, B. A. I., Chen, W. T., Xie, B. Y. and Yang, G. S. (2016). A novel strategy to enhance resistance to Cucumber mosaic virus in tomato by grafting to transgenic rootstocks. *Journal of Integrative Agriculture*, *15*(9), 2040-2048.

Mink, G. I. (1993). Pollen and seed-transmitted viruses and viroids. *Annual Review of Phytopathology*, *31*(1), 375-402.

Mossop, D. W. and Francki, R. I. B. (1977). Association of RNA 3 with aphid transmission of cucumber mosaic virus. *Virology*, *81*, 177–181.

Nagendran, S., Mohankumar, S., Aravintharaj, R., Balaji, C. G., Manoranjitham, S. K., Singh, A. K., Rai, A. B., Singh, B. and Karthikeyan, G. (2017). The occurrence and distribution of major viruses infecting cucurbits in Tamil Nadu state, India. *Crop Protection*, *99*, Pages 10-16.

Nayak, Parsuram, Mukherjee, Arup, K., Pandit, Elssa and Pradhan, Sharat (2017). Application of Statistical Tools for Data Analysis and Interpretation in Rice Plant Pathology. *Rice Science, 25,* 1-18.

Nicaise, V. (2014). Crop immunity against viruses: outcomes and future challenges. *Frontiers in Plant Science*, *5*, 660.

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G. and Francki, R. I. B. (1992). Cucumber mosaic virus. Adv. Virus Res., *41*, 281–348.

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G. and Francki, R. I. (1992). Cucumber mosaic virus. In *Advances in virus research* (Vol. 41, pp. 281-348). Academic Press.

Paylan, I. C. (2011). The Investigations on the Detection of the Agents of Viral Diseases in Seeds of Certain Vegetables and Their Inactivation Ways. Ph.D. Thesis, Ege University Graduate School of Natural and Applied Sciences, ˙Izmir, Turkey.

Peden, K. W. C. and Symons, R. H. (1973). Cucumber mosaic virus contains a functionally divided genome. *Virology, 53*, 487–492

Pesic, Z. and Hiruki, C. (1988). Effect of alfalfa mosaic virus on germination and tube growth of alfalfa pollen. *Canadian Journal of Plant Pathology*, *10*(1), 6-10.

Rast, A. T. B. and Stijger, C. C. M. M. (1987). Disinfection of pepper seed infected with different strains of capsicum mosaic virus by trisodium phosphate and dry heat treatment. *Plant Pathology*, *36*(4), 583-588.

Roossinck, M. J. (2002). Evolutionary history of Cucumber mosaic virus deduced by phylogenetic analyses. *J. Virol*, *76,* 3382–3387.

Roossinck, M. J. (1999). Cucumoviruses (*Bromo viridae*)–general features. In 'Encyclopedia of Virology'. 2nd edn (Eds L Granoof, RG Webster) pp. 315–320.

Routhu, G. K., Borah, M., Siddappa, S. and Nath, P. D. (2022). Exogenous application of coat protein-specific dsRNA inhibits cognate *cucumber mosaic virus* (CMV) of ghost pepper. *J Plant Dis Prot., 129*, 293–300.

Shahid, M. A., Abubakar, M., Hameed, S. and Hassan, S. (2009). Avian influenza virus (H 5 N 1); effects of physicochemical factors on its survival. *Virology Journal*, *6*(1), 38.

Sirohi, P. S., Choudhury, B. and Kalda, T. S. (1991). Pumpkin 'Pusaviswas' for tropical and subtropical region, *Indian Horticulture,* 24-26.

Taylor, M. J. and Brant, J. (2002). Trends in world cucurbit production, 1991 to 2001. *DN Maynard (ed.)*, 373-379.

Thresh, J. M. (2003). Control of plant virus diseases in sub-Saharan Africa: the possibility and feasibility of an integrated approach. *African Crop Science Journal*, *11*(3), 199-223.

Whitaker, T. W. and Davis, G. N. (1962). Cucurbits. Interscience Pub. *Inc. New York.*

Wintermantel, W. M. (2011). A comparison of disinfectants to prevent spread of potyviruses in greenhouse tomato production. *Plant Health Progress*, *12*(1), 19.

Yue, Q., Miller, C. J., White, J. F. and Richardson, M. D. (2000). Isolation and characterisation of fungal inhibitors from Epichloëfestucae. *J. Agric. Food Chem, 48,* 4687–4692.

Zou, S., Guo, J., Gao, R., Dong, L., Zhou, J., Zhang, Y. and Shu, Y. (2013). Inactivation of the novel avian influenza A (H7N9) virus under physical conditions or chemical agents treatment. *Virology Journal*, *10*(1), 289.

How to cite this article: Munmi Borah, Gowtham Kumar Routhu, Rupsanatan Mandal and Palash Deb Nath (2023). Molecular characterization and Efficacy Study of Different Treatments Against *Cucumber mosaic virus* in Pumpkin (*Cucurbita moschata*). *Biological Forum – An International Journal, 15*(2): 1053-1063.