Project ID 601

Competitive Research Grant

Sub-Project Completion Report

on

Molecular characterization and integrated management of *Cucumber mosaic virus* infecting Cucumber (*Cucumissativus*) in Bangladesh

Project Duration

May 2017 to September 2018

Plant Pathology Division
Bangladesh Agricultural Research Institute
Joydebpur, Gazipur-1701

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Acronyms

BARC- Bangladesh Agricultural Research Council

BARI- Bangladesh Agricultural Institute

BLAST- Basic Local Alignment Search Tool

cDNA- Complementary DNA

CMV- *Cucumber mosaic virus*

CRG-Competitive Research Grant

DAS-ELIS- Double Antibody Sandwich Enzyme-Linked Immune–Sorbent Assay

DNA- Deoxyribonucleic Acid

Fig.- Figure

GSP- Gene specific primer

MBCR- Marginal Benefit Cost Ratio

M-MLV – Moloney Murine Leukemia Virus

NATP-2- National Agricultural Technology Program-Phase II Project

NCBI- National Center for Biotechnology Information

nm- Nano meter

Nt.- Nucleotide

OD- Optical Density

Primer F- Forward primer

Primer R- Reverse primer

RND- Ribonucleic Acid

RT-PCR -Reverse Transcription-Polymerase Chain Reaction

Tha^{-1} – Tons Per hectare

T_m- Melting Temperature

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Executive Summary

Cucumber **(***Cucumissativus***)** belongs to Cucurbitaceae is an important commercial vegetables crop having export potential, grown throughout the world. Virus diseases especially *Cucumber mosaic virus*(CMV) is considered as the most important and widespread virus of cucumber and causes severe yield loss. However, the project has been taken with a view to molecular detection and develops integrated management option of CMV. Survey and collection of diseased cucumber plant samples were performed from ten districts from June 2017 to May 2018. Among the ten districts, disease incidences were varied from 10.75 to 28.50 %. The diagnostic study of the pathogen was done in 2017–2018 at the Plant Pathology laboratory of Bangladesh Agricultural Research Institute. The determination of causal agent has been done based on mechanical inoculation, symptom expression in the test plant species, DAS-ELISA and morphological properties of the virus particles using transmission electron microscopy (EM), and using specific oligonucleotide primers in reverse transcription-polymerase chain reaction (RT-PCR). EM investigation revealed the presence of isometric virions about 28–30 nm in diameter characteristic of CMV. Serological detection (DAS-ELISA) of collected samples conclusively revealed the presence of CMV in five symptomatic isolates like mild mosaic, mosaic, mosaic & stunting, mosaic & curling and leaf narrowing. Mechanical inoculation test proved that the virus is mechanical/sap transmitted. The virus was purified biologically by inoculation into *Chenopodium amaranticolor* and maintain in *Nicotianabenthamian* and *Cucumissativus*for molecular study. Total RNA was extracted from infected plant by Trizol reagent and RNA isolation kit. cDNA was synthesized by Reverse Transcription using M-MLV reverse transcriptase and cDNA synthesis kit. Amplicons of different lengths of CMV isolates belonging to subgroups I or II were amplified. The RT-PCR assay of infected cucumber leaf and test plants produced a band of 450 to 700 base pairs. The nucleotides were blasted with NCBI blast program and confirmed the virus as CMV. The phylogenetic analysis proved that the virus is belonging to the cluster of subgroup IB. The field trial was conducted to find out the integrated management option against CMV. Six treatment packages with a control were tested to manage the CMV. Two treatment packages T_2(Netting seedling, sticky yellow trap, polythene mulch and 4 sprays of Imidacloprid 0.1% at 15 days interval) and T_1 (Netting seedling, sticky yellow trap, polythene mulch and 4 sprays of Bio-neem 0.2 % at 15 days interval) were considered as the best management options on the basis of minimum disease incidence (9.67; 10.5), higher yield (13.04 t/ha; 12.96 t/ha) and Marginal benefit cost ratio (1:3.17&1:2.93) respectively. However, the management packages are cost effective.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: Molecular characterization and integrated management of Cucumber mosaic virus infecting Cucumber (Cucumissativus) in Bangladesh.

2. Implementing organization: Bangladesh Agricultural Research Institute (BARI)

3. Name and full address with phone, cell and E-mail of PI/Co-PI (s): **PI**- Dr. Mohammad Siddiqur Rahman, Senior Scientific Officer (Plant Pathology), Bangladesh Agricultural Research Institute. E-mail: mdsiddiqurrahman@yahoo.com. **Co-PI-**Dr. Ashraf Uddin Ahmed, Principal Scientific Officer (Plant Pathology), Bangladesh Agricultural Research Institute. E-mail: kajalashraf@gmail.com.

4. Sub-project budget (Tk):

4.1 Total:2484105.00

4.2 Revised (if any): 2484100.00

5. Duration of the sub-project:

5.1 Start date (based on LoA signed): 11 May 2017

5.2 End date: 30 September 2018

6.Justification of undertaking the sub-project: Cucumber (*Cucumissativus*) is an important commercial vegetables crop having export potential, grown throughout the world. In Bangladesh, the crop is cultivated in an area about 9,118 ha with a total production of 57,128 tons and the average yield is only 6.27 tha^{-1} (BBS 2016) which is very low as compared to other cucumber growing countries in the world where average yield is more than 30 $tons'ha^{-1}$. Virus diseases are the major constrain of commercial cucumber production in Bangladesh. Mosaic disease caused by *Cucumber mosaic virus* (CMV) is considered as the most destructive and widespread virus of cucumber worldwide and causes severe yield loss, up to 100% depending on time and stage of infection (Zitter and Murphy 2009; Rahman *et al*. 2016). effective management package for CMV is scanty in Bangladesh. CMV is the type member of the genus Cucumovirus in the family Bromoviridae has the broadest host range known for any plant virus with approximately 1200 plant species in over 100 plant families (Fauquet *et al*. 2005; Zitter and Murphy 2009). The CMV genome consists of three single-stranded, messenger-sense RNA molecules, designated RNA1, RNA2 and RNA 3 having several strains (Zitter and Murphy 2009). Till now plant virus diagnosis in Bangladesh is confined on less reliable methods like Symtomatology and ELISA. An effective and applicable virus management strategy requires an accurate diagnosis of plant viruses. Recent developments in molecular techniques have revolutionized the field of diagnostics in agriculture. So, molecular characterization of CMV is needed urgently to develop appropriate management strategies. Therefore, the project has been undertaken to detect CMV using molecular tools and develop potential management option against CMV.

7. Sub-project goal: Molecular detection of CMV and development of management package

8.Sub-project objective(s): **i)** Detection of Cucumber mosaic virus strain infecting cucumber in Bangladesh using molecular tools. **ii)** Study the virus-vector relationship in development of viral disease in the cucumber field. **iii)**To develop potential management options for minimizing CMV infection through integrated approach.

9.Implementing location (s): Plant pathology Division (Laboratory and Field), Joydebpur, Gazipur.

10. Methodology in brief:

a. Survey & sample collection: Survey was conducted in ten cucumber growing areas of Bangladesh (Table 1). Samples from naturally infected cucumber leaves as well as weeds of adjacent fields exhibiting characteristic symptoms of CMV were collected from different locations of ten districts and also disease incidence were noted. The districts were selected on the basis of major cucumber growing areas as well as to collect representative data on CMV incidence from the maximum part of Bangladesh. Only symptomatic samples were collected randomly throughout the field in each district. During survey 5 to 10 samples/field were collected according to the size of the field including weed samples. The collected samples were tested by DAS-ELISA methods and were preserved at - 80^0C refrigerator for further study. The virus was mechanically inoculated to the indicator host, *Chenopodiumamaranticolor* for biologically purification of the virus isolate (Fig. 1). The biologically purified virus was maintained in the propagation host *Nicotianabenthmmiana* and host plant *Cucumissativus* as virus source for further study in insect proof net house.

Table 1. Location of Survey and CMV sample collection

Sl. No.	Name of district	Sample collection sites
1	Gazipur	Sreepur,Kaliganj, GazipurSadar
2	Dhaka	Savar, Dhamrai, Keraniganj
3	Narsingdi	Belabo,Monohardi, RaipuraShibpur
4	Jamalpur	JamalpurSadar, Melandaha, Islampur
5	Sylhet	Sylhet Sadar, Jaintiapur,Gowainghat
6	Chittagong	Hathazari, Fatikchhari, ChattogramSadar
7	Comilla	ComillaSadar, Chandina,Burichang, Daudkandi
8	Jessore	JessoreSadar, Manirampur, Jhikargacha, Keshabpur
9	Dinajpur	Dinajpursadar, Biral, Birganj
10	Barishal	Babuganj, Barisal Sadar, Banaripara

Fig. 1. Mechanical inoculation techniques

b. Disease incidence

Diseases incidence were calculated using the following formula.

$$\text{Diseases incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total plants in the plot}} \times 100$$

C. Transmission Electron Microscopy (TEM)

Leaf samples collected from naturally infected cucumber plants and test plants were prepared for transmission electron microscopy (TEM). Carbon coated palladium grids were floated on drops of crude extract of virus infected plants for 5 minutes. After 5 minutes the grids were transferred on 2% Ammonium Molybdate dye for 2 minutes for stained. Finally, the grids were dried for transmission electron microscopy as previously described by Dong et al.2008.

d. RNA extraction, RT-PCR and sequencing

Total RNA was extracted from symptomatic cucumber leaves and inoculated *Nicotianabenthmmiana* leaves using TRIzol reagent (Invitrogen, USA) and RNA extraction kit (Eastep Super RNA kit, Promega China). The first-strand cDNA was synthesized by Reverse Transcription (RT) using M-MLV (MoloneyMurine leukemia virus) reverse transcriptase (Promega, USA) and also by cDNA synthesis kit (Verso cDNA Synthesis Kit, Thermo Scientific, India) following the manufacturer's instructions. The synthesized cDNA fragment was amplified through PCR using CMV primers presented in Table 1.

Table 2. Primers used for the amplification of DNA fragment

Primer	Sequence (5'- 3')	Genomic location	T_m
CMV F1GSP	TATGATAAGAAGCTTGTTTCGCG	295-317 Nt.	52 ^{0}C
CMV R1GSP	GCCGTAAGCTGGATGGACAA	1975-1993 Nt.	52 ^{0}C
CMV F2GSP	GTAATACGACTCACTATAGGTTTTGTTTG	114-132 Nt. at CP gene	55 ^{0}C
CMV R2 GSP	GCGCGAAACAAGCTTCTTATC	633-653 Nt.	55 ^{0}C
CMVF3 Sub-group I	GTAATCTTACCACTGTGTGTG	1-21 Nt.	55 ^{0}C
CMVR3 Sub-group I	TGGTCTCCTTTTGGAGGCCC	3341-3360 Nt.	55 ^{0}C
CMVF4 Sub-group II	GTAATCTTACCACTTTCTTTTC	1-22 Nt.	55 ^{0}C
CMVR4 Sub-group II	CTCCTTATGGAGAACCTGTG	3020-3039 Nt.	55 ^{0}C

The amplified desired fragments (PCR product) were purified using agarose gel DNA purification kit (Aidlab Co, Ltd). Purified PCR products were out sourcing for sequencing.

Sequence analysis

The partial sequences of CMV was edited and analyzed with the aid of DNAMAN version 5.0 (LynnonBiosoft, QC, Canada).Phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replications in MEGA 6.0 (Tamura *et al.* 2013). Comparison sequences were obtained from the GenBank database.

e. Field trial:

Integrated management of Cucumber mosaic virus (CMV) infecting cucumber in Bangladesh

The field trial was conducted at the research field of Plant Pathology Division, Bangladesh Agricultural Institute, Gazipur during November 2017 to April-2018. The management packages tested in the present field trial was as follows:

Package-T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval

Package-T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of with Imidacloprid 0.1%at 15 days' interval

Package-T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval

Package-T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval

Package-T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval

Package-T6: Netting Seedling +Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid;

Package-T7: Control

*Maize were sown in line at 10 cm spacing around the plot at 20 days before transplanting of seedling.

i) Disease incidence

Diseases incidence were calculated using the formula described earlier.

ii) Disease severity

Severity of Cucumber mosaic virus (CMV) will be determined according to Monma and Sakata (1997) with some modification based on a 0-4 scale as follows.

Symptom index 0= No Symptom, 1= Mild Mosaic, 2= Mosaic, 3= Mosaic and deformed leaf 4= mosaic and stunted plants

$$\text{Severity Index} = \frac{\sum (\text{Symptom index} \times \text{Number of plants with each symptom index})}{\text{Total number of plants}}$$

iii) No. of aphid/leaf was counted by average of randomly selected 10 leaves/plot

iv) Yield/plot

v) Yield/ha

vi) Economic analysis were performed by partial budget technique as described by Rahman et al. (2011) to find the economically suitable package. Following formulae were used for economic analysis:

Variable Cost = Cost (Taka) that vary in different packages

Gross Return (TR) = Yield in terms of money

Gross margin = Gross Return – Variable cost

Marginal benefit = $\text{Grossmargin(Packages)} - \text{Grossmargin(control)}$

Marginal benefit cost ratio (MBCR) was calculated by the following formula:

MBCR (over control)

g. Design of experiment and data analysis

RCBD with 3 replications were used for field experiments. Data were analyzed statistically for analysis of variance (ANOVA) using MSTAT-C or R software and means were compared according to Duncan's Multiple Range Test (Gomez and Gomez 1984). Data were transformed as and when necessary using Arcsine transformation method.

11. Results and discussion:

Survey, Sample collection, mechanical inoculation and DAS-ELISA test

During survey naturally infected symptomatic cucumber leaves were collected (Fig. 2). Primarily percent disease incidence was recorded from each field on the basis of symptomatology. After confirmation by DAS-ELISA and RT-PCR the average disease incidence was calculated for each districts. During survey 5 to 10 samples/field were collected according to the size of the field including weed samples. The collected samples were tested by DAS-ELISA methods and further confirmed the virus by RT-PCR. The samples were also preserved at - 80^0C refrigerator for further molecular study. Among the ten districts, disease incidences were varied from 10.75 % to 28.50 % presented in Table 1. The highest disease incidence was recorded in

Gazipurdistrict (28.50%) followed by Jamalpur (22.00 %), Dinajpur (21.00%) and Comilla (20.00%). The lowest disease incidence 8.50 % was found in Sylhet. It was also observed that disease was higher in hybrid varieties as compared to op/local varieties. Most of the farmers of Gazipur, Jamalpur, Comilla, Dhaka, Dinajpur use hybrid varieties like Al-Amin, Mallika, ACI green, All-rounder etc. where the disease incidence was higher that ranged from 18.20 to 28.50 %. Disease incidence was found lower (10.75-16.25 %) in other five districts where most of the farmers use OP/local varieties. The lowest disease incidence was recorded in Sylhet districts (10.75 %). It might be due to more use of OP/local varieties that may have some extend natural resistance/tolerant or theweather condition may disfavorthespread of CMV vector population in the field.

A considerable amount of other virus symptoms was also observed in cucumber field during the survey (Data were not shown). Among them some were mechanically transmitted and some were non-transmitted but all of them were not response against the antibody of CMV. So the symptoms were not developed for CMV infection. Furthermore, white fly and thrips vectors were also observed in the cucumber field that transmit Geminivirus and tospovirus respectively. Therefore, due to climatic changes new virus may be immerged or resurgence in the cucumber field and they may become a new thread for cucumber production.

Fig. 2. Survey and collection of different symptomatic isolate of CMV (A-I).

Table 3. Incidence of *Cucumber mosaic virus* in different Cucumber growing areas in Bangladesh

Location	No. of locations surveyed	No. of fields surveyed	CMV incidence	
			Range (%)	Mean (%)
Gazipur	8	15	12-37	28.50
Dhaka	5	9	06-28	18.20
Narsingdi	6	10	10-25	15.50
Jamalpur	6	10	10-30	22.00
Sylhet	5	12	05-21	10.75
Chittagong	6	10	09-25	16.25
Comilla	7	14	08-30	20.50
Jessore	5	12	09-23	14.50
Dinajpur	4	10	10-35	21.00
Barishal	5	8	07-20	13.50

Mechanical inoculation test

Collected samples were mechanically inoculated to *Chenopodiumamaranticolor* (indicator plant), *Nicotianabenthmmiana* (propagation host) and *Cucumissativus*(Host plant).The test plants were showed characteristic symptoms 8-12 days after mechanical inoculation (Fig. 3)and maintained as virus source in insect proof net house for further studies. The mechanical inoculation test proved that the virus is sap or mechanically transmitted.

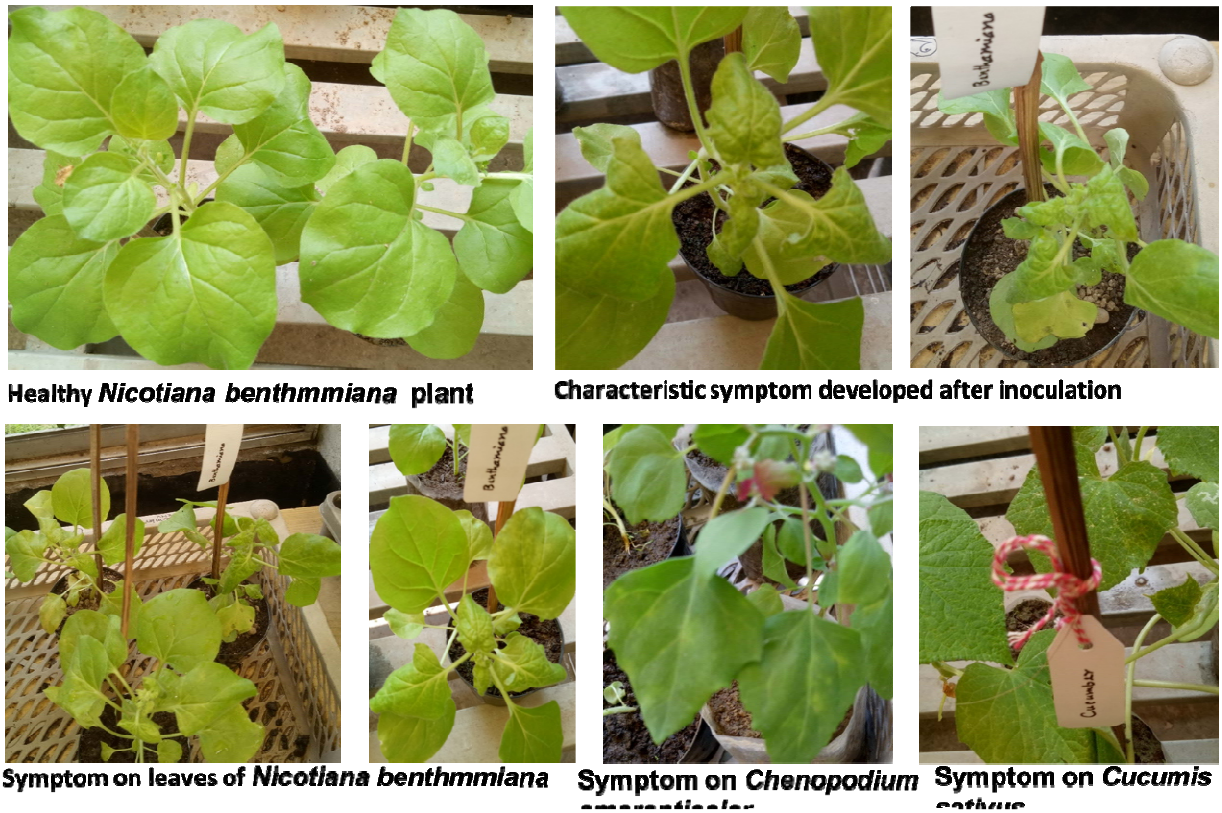

Fig. 3. Symptoms developed after mechanical inoculation.

Serological (DAS-ELISA) detection of CMV symptomatic isolate

Naturally infected characteristics symptoms of cucumber leaf as well as weed samples collected from ten districts were tested by DAS-ELISA against the antisera of Cucumber mosaic virus (CMV). The positive reaction against the antisera indicated that CMV produce five distinct symptoms like Mild mosaic, Mosaic, Mosaic & Stunting, Mosaic & curling and Leaf narrowing. The OD values (Optical density value at 405 nm) of the positively reacted samples in between positive and negative control are presented in Table 3. It was further proved that the samples were infected with CMV. Therefore, the results of ELISA conclusively revealed the presence of CMV with all the five symptoms (Table 3).

Table.4 Serological (DAS-ELISA) detection of CMV symptomatic isolates

Major symptom	Reaction against the CMV antisera	OD value (405 nm) (Average of 5 positive samples)
- Control	-	0.12
+ Control	+	0.40
Mild mosaic	+	0.28
Mosaic	+	0.30
Mosaic & Stunting	+	0.25
Mosaic & curling	+	0.32
Leaf narrowing	+	0.35

"+" positive reaction; "-"Negative reaction

Transmission Electron Microscopy (TEM)

Prepared samples were carefully observed under TEM. Spherical or round shape virus particles about 28-30 nm in diameter was observed under Transmission Electron Microscope (TECNAI G^2 Spirit TWIN 9432 050 18111, Czech Republic). The size and shape of the virus particles was typical of CMV i.e. Cucamovirus (Fig. 4).

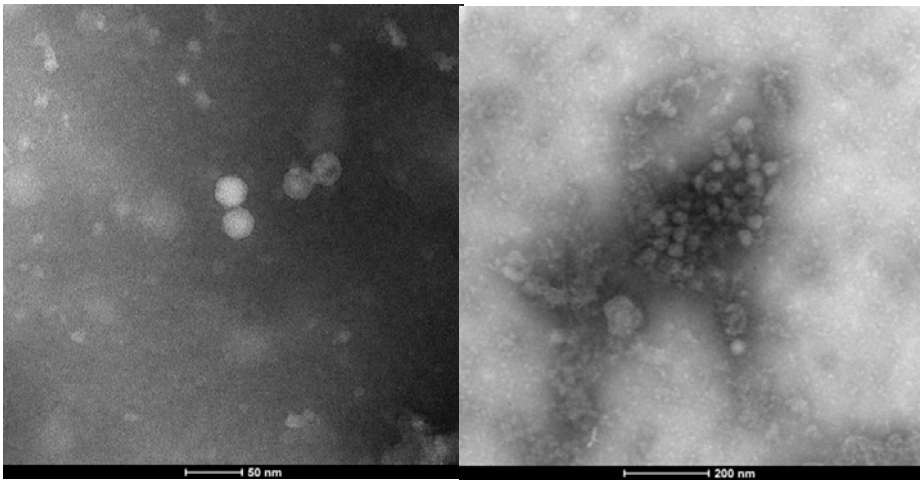

Fig. 4. CMV particles under Electron Microscope (Bar=50 nm left and 200 nm right).

RNA extraction, RT-PCR

Total RNA was extracted from 50 samples including *Nicotianabenthmmiana* leaves. First-strand cDNA was synthesized by Reverse Transcription (RT) using M-MLV (MoloneyMurine Leukemia Virus) reverse transcriptase (Promega, USA) and also by cDNA synthesis kit (Verso cDNA Synthesis Kit, Thermo Scientific, India) following the manufacturer's instructions. The CMV genome about 300-750 bp were amplified by using different primer described earlier. PCR amplified DNA fragments of different samples are presented in Fig. 5. The PCR condition was as the initial denaturing step at 94^0C for 3 min; 35 cycles of 94^0C for 3min, 1 min at 60^0C and 90sec. at 72^0C; with a final extension step at 72ºC for 10 min. The desired DNA samples were purified and were out sourcing for sequencing.

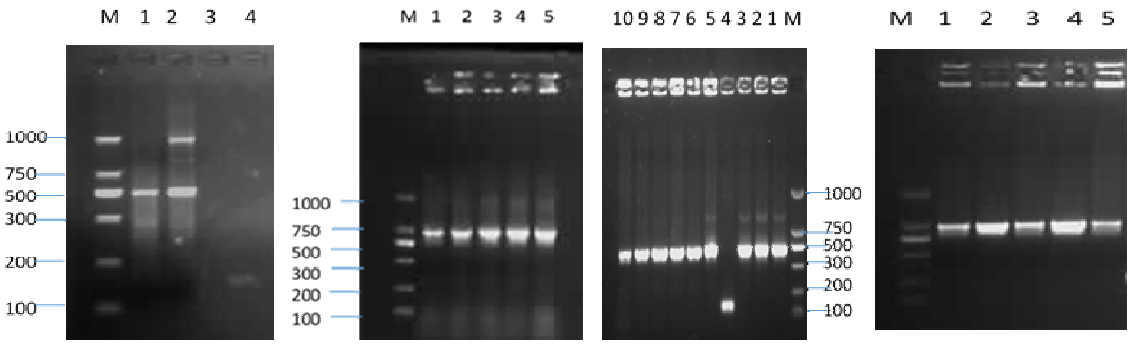

Fig. 5. Amplified different DNA fragments (M- Takara 1000bp DNA Marker; A: 1, 2, 3, 4-Narsingdi; B:1-5 Gazipur C:1-5-Jamalpur, 6-10 Dinajpur; D: 1-5 Comilla)

Sequence analysis

The nucleotide sequences werealigned with BLAST with the NCBI database. The 100% nucleotide identity was found with CMV RNA 1 segment of Genbank accession no. AB179764, 99 % identity with LC339509, 98 % with EU414792, 97% with AF071551, 95% with HQ283392 respectively. Therefore, the identified virus is the partial nucleotide sequence of CMV.

Thirty-nine PCR products were sequenced to detect CMV. All the tested samples were belonging to RNA1 and RNA3 segment of CMV. Therefore, the representative partial sequences of CMV genome (RNA 1 & RNA 3) were edited and analyzed using DNAMAN software (Table 4). The partial nucleotide sequence of RNA 1 and RNA 3 of CMV isolate segment 420 and 412 nucleotides (nt.) respectively were analyzed. The base composition of the 420 nt. segment RNA 1 contained adenine 27.9 %, cytosine 18.8 %, guanine 23.8 %, and uracil 29.5 % are presented in Table 4A. The partial nucleotide sequence of RNA 3 segment of CMV is 412 nucleotides (nt). The base composition of the 412 nt segment RNA 3 contained adenine 19.7 %, cytosine 18.2 %, guanine 24.3%, and uracil 37.9 % are presented in Table 4B.Phylogenetic trees were constructed by the RNA 1 and RNA 3 segment of CMV with the CMV sequences of GenBank database using the neighbor-joining method. Phylogenetic tree based on RNA 1 showed that the CMV isolate of Bangladesh is closely related to the Chinese isolate of AF 071551 (Fig. 6a).The phylogenetic tree based on intergenic region (RNA 3) showed the grouping of CMV isolates and the CMV Bangladesh isolate grouped with the cluster of subgroup IB. The comparison of CMV isolates with three different subgroups showed that the CMV Bangladesh isolate shared 88-91 %, 76-84 % and 62-63 % sequence identity with IB, IA and subgroup II respectively (Fig. 6b). The present investigationrevealed that the CMV Bangladesh isolate belongs to subgroup IB. This is the first molecular identification of CMV occurring in Bangladesh.

Table 5. Analyzed partial sequence of RNA 1 and RNA 3

A. SEQ CMV RNA1: 420 bp; Composition: 117 A; 79 C; 100 G; 124 T; 0 OTHER Percentage: 27.9% A; 18.8% C; 23.8% G; 29.5% T; 0.0%OTHER Molecular Weight (kDa): ssDNA: 130.05 dsDNA: 258.90
ORIGIN 1 GCAAGATCAT CTTGAACGAT CCACAACAGT TCGATGGTCG ACAGCCGGAC TTCTGCACTC 61 ATCCGGCTGC GGATTGCAAA GTACAAGCCC ACTTTGCTAT ATCTATTCAT GGAGGTTATG 121 ATATGGGCTT TAGAGGATTA TGTGAAGCGA TGAATGCTCA CGGAACCACT ATTTTGAAGG 181 GAACGATGAT GTTCGATGGT GCTATGATGT TTGACGACCA AGGTGTAATA CCTGAGCTTA 241 ATTGTCAGTG GAGAAAGATC AGGAGTGCTT TCTCTGAAAC TGAAGACGTC ACACCGCTGA 301 CTGAGAAAAT TAATTCCACG ATATTTTCCC GCGTGCGTAA ATTCAAGACT ATGGTGGCTT 361 TCGATTTTGT CAATGAGTCT ACTATGTCTT ATGTTCATGA TTGGGAGAAT ATAAAATCTT
B. SEQ CMV RNA3: 412 bp; Composition: 81 A; 75 C; 100 G; 156 T; 0 OTHER Percentage: 19.7% A; 18.2% C; 24.3% G; 37.9% T; 0.0%OTHER Molecular Weight (kDa): ssDNA: 127.35 dsDNA: 253.97
ORIGIN 1 GTAATCTTAC CACTGTGTGT GTGTGTGTGT GCGTGTGTCG CGTCGTGTCG AGTCGTGTTG 61 TGTTTCGTTG CGTATTAGTA TATAAGTATG TGTGTGTCTG TACATAATAC TATATCTATA 121 GTGTCCTGTG TGAGTTGATA CAGTAGACAA CTGTGACGCG ATGGTGTAGA GAAGAGAGCA 181 CATCTGGTTT AGTAAAACCC ACAACATTAT CTTTGAGGTT CAATTCCTCT TGATCCCTGT 241 TGGGCCCCTT TTACTTTTCA TGGATGCTTC TCCACAAGAT TGCGTTTCGT CTACTTATCA 301 TTAGTGTATT GTGCTGTGTT TTCTCTTTTG TGTAGTAGAT TTGAGTCGAG TCTCCGCACA 361 TAAGAGTCGT GCTGTCCGCA CATTTTCTTT TCAGTGTGTT AGATTCCCGA GG

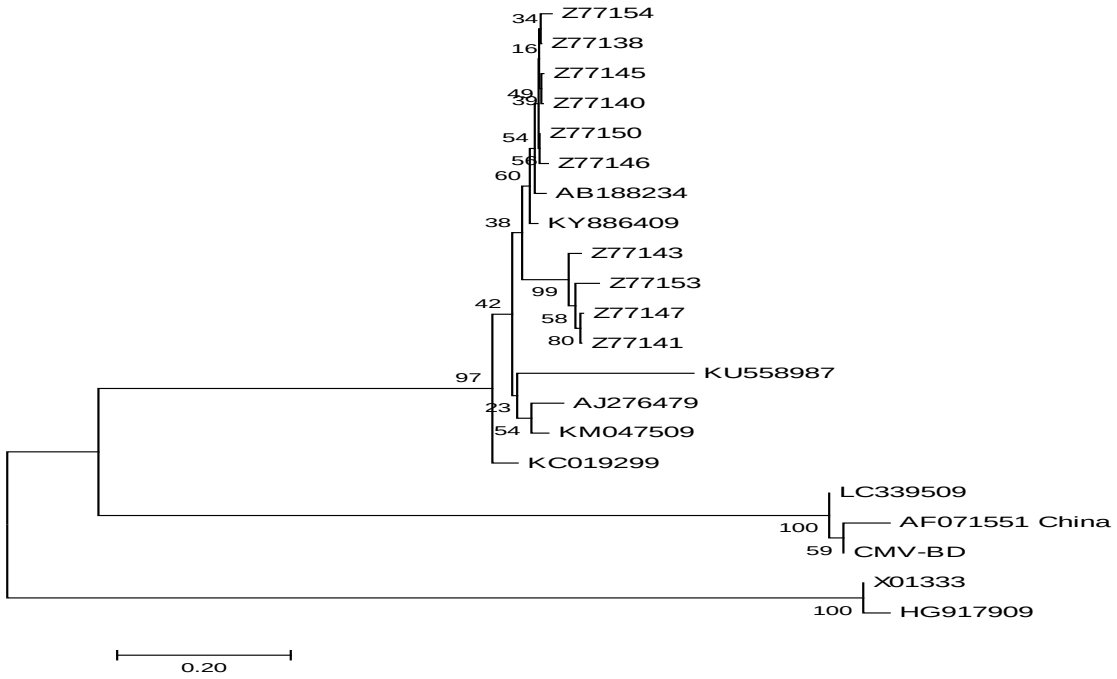

Fig. 6a. Phylogenetic tree based on the partial nucleotide sequences of CMV isolate RNA 1segmentand other 20 related CMV segment. Sequences were aligned through Clustal W. The tree was constructed by the

neighbor-joining algorithm, both which in the MEGA7.0 package. Bar represent exchanged per 100 nucleotides.

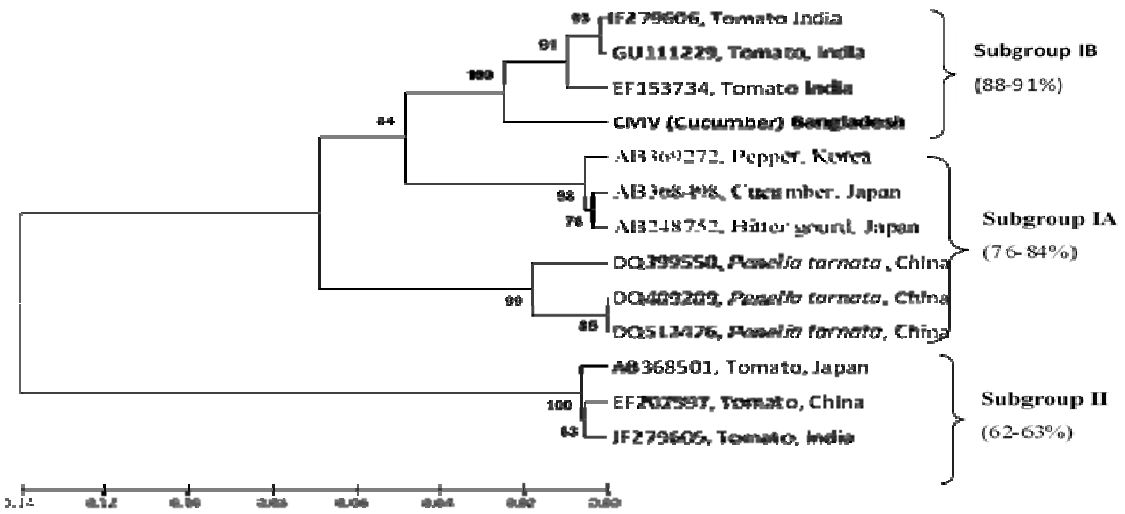

Fig. 6b. Phylogenetic tree based on the partial nucleotide sequences of CMV isolate RNA 3 and other 12 related CMV segment. Sequences were aligned through Clustal W. The tree was constructed by the neighbor-joining algorithm, both which in the MEGA6.0 package. Bar represent exchanged per 100 nucleotides.The tree showing the grouping of the CMV isolates. The CMV Bangladesh isolate grouped with the cluster of subgroup IB.

Field trial: Integrated management of Cucumber mosaic virus infecting cucumber in Bangladesh

Disease incidence and severity of CMV: Disease incidence and severity of Cucumber mosaic virus on different treatments are presented in Fig. 7. Incidence of CMV of all the management packages (T1-T6) was found lower as compared to control. The highest disease incidence (42.00%) was recorded from T7 (control). The lowest incidence (9.67%) was observed in T2 which was statistically similar to T1 (10.5%). The incidence of CMV in T4, T5 and T6, was statistically similar but significantly higher compared to T1, T2 and T3. Similarly, the highest disease severity was found in T7 (control) and each of the management packages (T1-T6) reduced severity of CMV significantly over control. The lowest severity was found in T2, which was statistically similar to T1. Among the treatments T2 and T1 was found very much effective in reducing disease incidence and severity. However, treatments involving sticky yellow trap, polythene mulch with 4 spray of Bio-neem/imidacloprid (T2 and T1) was better than other management packages (Fig. 6). It might be due to better control of CMV vectors (aphids) in the treated plot. CMV is an aphid born non- persistent virus, so only insecticides spray is not enough to control the vector as it required only few seconds to transmit virus from infected to healthy plant. So use of disease free seedling, sticky yellow rap, polythene mulch and then spray insecticide effectively controlled the vectors and reduced the disease incidence and severity in the management packages.

Aphid population

The effect of different management options on aphid population per leaf is shown in Fig. 8. The highest number of aphid per leaf (14.50) was recorded from the plants under control. Every management packages caused significant reduction in number of aphid population per plant over untreated control. Significantly lower number of aphids was recorded from plant treated with management packages T2 and T1 compared to other packages. However, efficacy of two packages was statistically similar and very few aphid was

observed in treatment plot of T2 and T1. It might be due to effectively control of aphids in the treatment i.e. Sticky yellow trap act as continuous barrier against the aphid and again spray with insecticide reduce the colonization of aphid vector on leaf in the treated plot. Therefore, the disease incidence was less in treated plot as compared to control.

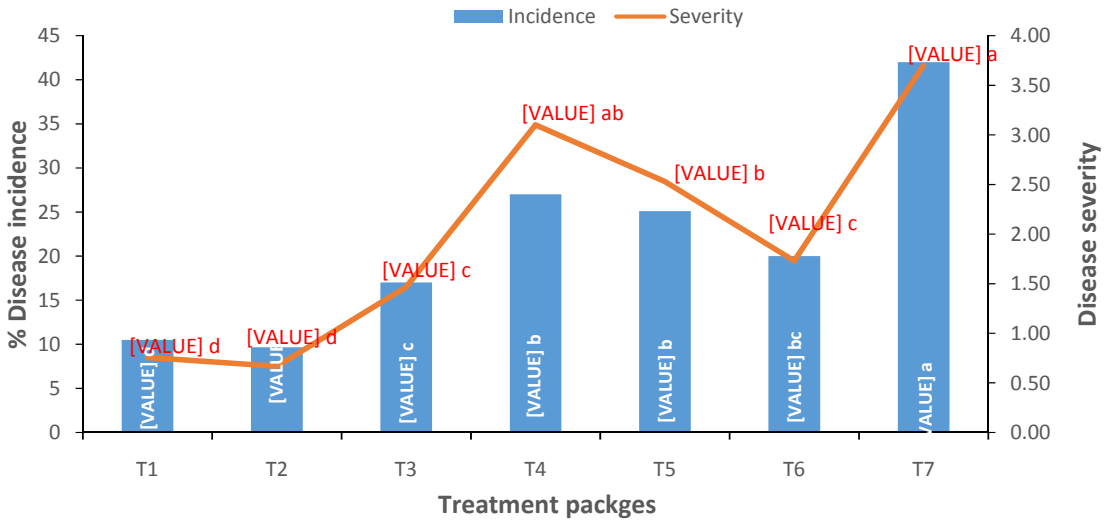

Fig. 7. Effect of different management packages on the incidence and severity of CMV in Cucumber. (T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays ofImidacloprid 0.1% at 15 days' interval Imidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

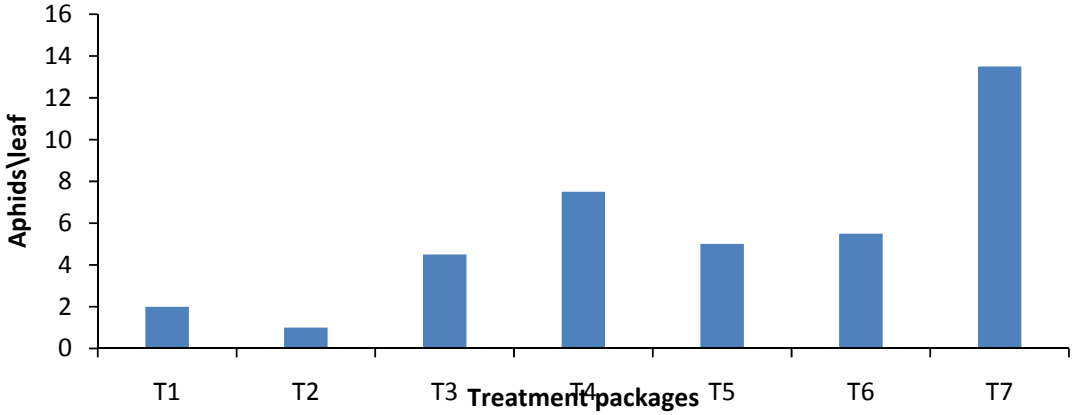

Fig. 8. Effect of different management packages on number of aphids/leaf. (T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays ofImidacloprid 0.1% at 15 days' intervalImidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

Relationship between aphid population and incidence of CMV

In the field trial it was found that the number of CMV infected plants were higher with the increase of aphid number per plant. The relationship was linear, positive and significant (R^2 = 0.7553) and could be expressed

by the regression equation Y= 3.6484x + 4.8546, where Y= incidence of CMV (%) and x =number of aphids per plant (Fig. 9). The R^2 value indicates that the spread of CMV in the field might be attributed by aphid population by 75.53 %.

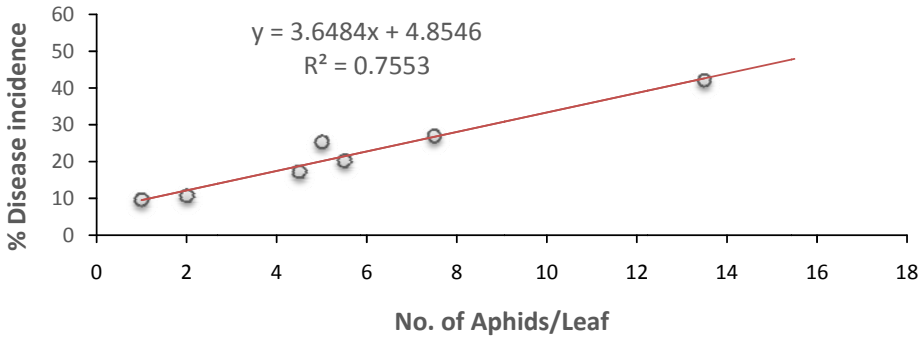

Fig. 9. Relationship between aphid population and percent disease incidence in different management options.

Yield

All the management options reduced disease incidence and gave higher yield as compared to control (Table 5). The highest yield was found 13.07 ton/ha in treatment packages T2 (Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of imidacloprid 0.1% at 15 days' interval) which was statistically similar to T1 (Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem at 15 days' interval) but significantly higher from other management options (Fig. 10). The lowest yield (6.67 t/ha) was found in T7 (untreated control). The yield of other treatments ranged from 7.04 to 10.15 t/ha. The highest reduction of disease incidence was found 76.97% in treatment T2 which was statistically similar by T1 (75 %). Other treatment packages also reduce disease incidence at a considerable level (35.71-59.52 %). However, among the treatment packages, performance of packages T2 and T1 was the best.

Table 6. Effect of management packages on disease reduction and yield of cucumber

Treatments	Incidence	Reduction in disease incidence (%)	Yield T/ha	Yield increase T/ha
T1	10.50 d (18.88)	75.00	12.96 a	6.29
T2	9.67 d (18.05)	76.97	13.07 a	6.40
T3	17.00 c (24.31)	59.52	10.15 b	3.48
T4	27.00 b (31.29)	35.71	7.04 d	0.37
T5	25.10 b (30.06)	40.23	9.30 b	2.63
T6	20.00 bc (26.51)	52.38	8.52 c	1.85
T7	42.00 a (40.36)	-	6.67 d	-
LSD	4.76		0.87	
CV %	9.91		14.50	

* Means followed by same letter are not significantly different at 5% level by DMRT. Value within parenthesis are arcsine transformed value. (T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of

Imidacloprid 0.1% at 15 days' interval Imidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

Fig. Experiment plot

T_1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bioneem at 15 days interval

T2: Same as T_1, except spray with Imidacloprid 0.1%

T_3: Same as T_1, except spray with Imidacloprid 0.1%

T_4:Netting seedlings + 4 sprays of Imidacloprid 0.1% at 15 days' interval

T_5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval

T_6: Netting Seedling + Maize as barrier crop + straw mulch + 2 spray of Imidaclopri

Insect vector trapping by Sticky yellow trap

Fig. 10. Different management packages under field trial

Economic analysis

Results obtained from economic analysis of various treatments are presented in Table 6 and 7. All treatments more or less increase the gross return over control. However, grows return was highest in T2 followed by T1, T3, T5, T6 and T4. The lowest was obtained from Control. Marginal analysis has pointed out that all the management packages increase marginal benefit as well as marginal benefit cost ratio (MBCR) over control (Table 7). The highest MBCR was obtained from T2 and the lowest from T4. The results showed that additional investment of Taka 1 in T2 over control had additional income of Taka 3.17 and similarly Tk. 2.93 in T1, Tk. 1.61 in T3, Tk. 1.47 in T5, Tk. 1.31 in T6 respectively. Considering cost and return and MBCR from the economic analysis indicating that all the management packages except T4 (MBCR 1:0.85) were economically viable and maximum gain could be obtained from T2 (integration with netting seedlings, sticky yellow trap, polythene mulch and 4 spray with imidacloprid 0.1%).

Table 7. Cost and return in different management packages

Packages	*Var. Cost (Tk ha^{-1})	Yield (t ha^{-1})	**Gross return (Tk ha^{-1})
T_1	24000.00	12.96	194400.00
T_2	23000.00	13.07	196050.00
T_3	22000.00	10.15	152250.00
T_4	5000.00	7.04	105600.00
T_5	19000.00	9.30	139500.00
T_6	12000.00	8.52	127800.00
T_7 (Control)	-	6.67	100050.00

* Var. Cost: Cost that vary in different packages

** Whole Sell rate of cucumber @ TK 15.00/Kg

(T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays ofImidacloprid 0.1% at 15 days' interval Imidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

Table 8. Marginal analysis of different treatment packages

Packages	Gross return (Tk ha^{-1})	Var.Cost (Tk ha^{-1})	Gross margin (Tk ha^{-1})	Marginal benefit (Tk ha^{-1})	MBCR
T_1	194400.00	24000.00	170400.00	70350.00	1: 2.93
T_2	196050.00	23000.00	173050.00	73000.00	1: 3.17
T_3	152250.00	20000.00	132250.00	32200.00	1: 1.61
T_4	105600.00	3000.00	100600.00	550.00	1: 0.85
T_5	139500.00	16000.00	123500.00	23450.00	1: 1.47
T_6	127800.00	12000.00	115800.00	15750.00	1: 1.31
T_7 (Control)	100050.00	-	100050.00	-	-

(MBCR: Marginal benefit cost ratio)

(T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays ofImidacloprid 0.1% at 15 days' interval Imidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

Different management packages caused 35.71-76.97 % reduction in disease incidence and increase yield 0.37-6.40 ton/ha (Table 5). In the present investigation, treatment packages comprising with Netting Seedling, sticky yellow trap, Polythene mulch and 4 sprays of Imidacloprid 0.1% /Bio-neem at 15 days interval (T2 & T1) were found better than any other packages in terms of disease suppression and yield improvement (Table 5& Fig. 10). Successful application of integrated management for CMV has also been postulated in the review by Hooks and Fereres (2006). Among the treatment packages, T4 was found less effective. This is obvious, because the non-persistent manner of virus transmission like CMV. Only use of insecticides is not always effective as the aphids become irritated and therefore jump from leaf to leaf or plant to plant in an attempt to avoid the insecticides, subsequently infecting healthy plants because the acquisition and inoculation time is very short. For this, aphids are capable to inoculate healthy plants within few seconds. That is why disease incidence and severity was high as compared to other packages and ultimately reduce the yield (Fig. 10 and Table 5). Because of the very short time needed to transmit a virus, aphids are capable of transmitting NPVs (Non-persistent viruses) prior to being killed by an insecticide. This observation is an agreement with the findings of Hooks *et al*. (2007).

Again treatment T2 gave higher yield than T1 although statistically similar. It might be due to less suppression of aphids by Bio-neem as compared to Imidacloprid 0.1 %. However, in case of diseases incidence and yield both the packages more or less similar. However, the better result was achieved from the treatment packages T2 and

T1. It might be due to sticky yellow trap acted as continuous "spread breakers" by attracting aphids and preventing the colonization on the cucumber leaves and insecticidal sprays further suppressed disease spread. The finding is also the conformity of the previous findings of Anandam and Doraiswamy 2002 in case of non-persistent virus like CMV. The environmental point of view spray with Bio-neem, integration with sticky yellow trap, netting seedling and use polythene mulch during winter season may be a good option to reduce CMV incidence and increase yield of cucumber.

Economic analysis revealed that profit varies depending on the management packages. Results of the present investigation indicate that T2 is the best treatment in terms of economic gain. It has got chemical back up in addition to sticky yellow trap. So that successful control was achieved against aphid vector which reduced incidence and severity of CMV. Furthermore, polythene mulch increases the soil temperature that enhance the growth and development of cucumber as well as suppress weeds in the field. Therefore, higher yield was achieved from that treatment. From environmental point of view T1 may be used. Because it has got botanical insecticide (Bio-neem) instead of chemical which is environmentally safe although marginal benefit cost ratio (MBCR) was little lower than T2. Although the variable cost of T2 and T1 (Tk 24000 and 23000) is higher but the treatments are cost effective considering return for additional cost.

Effect of CMV on yield depends on a number of factors, including plant age and growth stage when infected, viruliferous vector population, environmental conditions etc. (Agrios*et al*. 1988; and Rahman *et al.* 2008). Results of the present investigation demonstrate that CMV of cucumber may be effectively managed through integration of netting seedlings, use sticky yellow trap, polythene mulch (winter season) and spray of imidacloprid 0.1% or Bio-neem. This is the first report of an integrated management of Cucumber mosaic virus (CMV) of cucumber in Bangladesh. Further trial is needed for more confirmation.

12. Research highlight/findings:

- Determined incidence of *Cucumber mosaic virus* (CMV) (10.75-28.5%) in major cucumber growing areas of Bangladesh through survey.
- Identified CMV from like mild mosaic, mosaic, mosaic & stunting, mosaic & curling and leaf narrowing symptoms by DAS-ELISA and molecular tools (RT-PCR).
- Determined *Cucumber mosaic virus* subgroup IB infecting Cucumber in Bangladesh which are similar to accession JF270606 & EF153734 based on phylo genetic grouping.
- Strong positive correlation between vector (aphids) and CMV infection was observed in developing CMV disease in the cucumber field.
- Developed effective management option for CMV of Cucumber-

Integration of netting seedling, sticky yellow trap, polythene mulch and 4 sprays of Imidacloprid / Bioneem at 15 days interval effectively reduced CMV incidence and increase yield of Cucumber.

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B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	8	265000.00	8	265000.00	Achieved
(b) Lab &field equipment	2	17000.00	2	17000.00	Achieved
(c) Other capital items	N/A	N/A	N/A	N/A	

2. Establishment/renovation facilities:

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
N/A	N/A	N/A	N/A	N/A	

3. Training/study tour/ seminar/workshop/conference organized:

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	N/A	N/A	N/A	N/A	
(b) Workshop	N/A	N/A	N/A	N/A	

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)
A. Contractual staff salary	247549.00	247549/-	247549/-	0	100
B. Field research/lab expenses and supplies	1166556.00	1166556/-	1166556/-	0	100
C. Operating expenses	27620.00	276280/-	275700/-	0	100
D. Vehicle hire and fuel, oil & maintenance	200000.00	200000/-	200000/-	0	100
E. Training/workshop/seminar etc.	-	-	-	0	-
F. Publications and printing	49300.00	24300/-	24300/-	0	47.56
G. Miscellaneous	110000.00	110000/-	110000/-	0	100
H. Capital expenses	435000.00	435000/-	435000/-	0	100

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	**Major technical activities performed in respect of the set objectives**	**Output(i.e. product obtained, visible, measurable)**	**Outcome(short term effect of the research)**
Detection of Cucumber mosaic virus strain infecting cucumber in Bangladesh using molecular tools.	i) Survey & virus sample collection ii) Virus detection by TEM, DAS-ELISA, iii) RNA extraction and RT-PCR, amplification of DNA fragments & sequencing.	-Identified five CMV biotypes like mild mosaic, mosaic, mosaic & stunting, mosaic & curling and leaf narrowing.- Find out Cucumber mosaic virus subgroup IB infecting Cucumber in Bangladesh based on phylogenetic grouping	Accurate detection of CMV using moleculartools inBangladesh thatfacilitate furthermolecular study of different plant viruses.
Study the virus-vector relationship in development of	i) Data record on CMV infected plants ii) Observe vectorpopulation on	Strong positive correlation between vector (aphids) and CMV infection was observed in	Help in adopting virus management strategies through vector control

viral disease in the cucumber field.	leaf	developing CMV disease in the cucumber field.	
To develop potential management options for minimizing CMV infection through integrated approach.	i) Six treatment packages with a control were evaluated through field trial. ii) CMV incidence, disease severity and yield were recorded under different treatment packages.	Management option developedfor CMV of Cucumber-"Integration of netting seedling, sticky yellow trap, polythene mulch and 4 sprays of Imidacloprid /Bio-neem at 15 days interval effectively reduced CMV incidence and increase yield of Cucumber"	

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication	1	-	
Information development			
Other publications, if any			

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

"Integration of netting seedling, sticky yellow trap, polythene mulch and 4 sprays of Imidacloprid (0.1%) or Bioneem (0.2%) at 15 days interval effectively reduced CMV incidence and increase yield of Cucumber"

ii. **Generation of new knowledge that help in developing more technology in future**

The knowledge of the present investigation helps in developing more effective management technology regarding the non-persistent viruses in Bangladesh.

iii. Technology transferred that help increased agricultural productivity and farmers' income

The developed technology may help increased cucumber production that enhance farmer's income.

iv. Policy Support

The findings of the present project may assist the policy makers of the agricultural sectors for planning their future research directions regarding plant viruses for sustainable food and nutrition security in Bangladesh.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):N/A

ii) Field Monitoring (time& No. of visit, Team visit and output):

Monitoring team	Date(s) of visit	Total visit till date (No.)	Remarks
Technical Division/ Unit, BARC	14/03/2018	1	
PIU-BARC, NATP-2	14/03/2018	1	

Internal Monitoring (BARI)	06/02/2018, 19/02/2018	2	Satisfactory

H. Lesson Learned/Challenges (if any)

i) Delayed fund release hindered smoothly run the project activities.
ii) Unstable electricity supply hampered the lab work especially molecular work and also storage of molecular chemicals
iii) Timely release of fund is essential for better achievement of the project.

I. Challenges (if any)

The supply of molecular chemicals and backup supports for molecular work still time consuming.

Signature of the Principal Investigator Date ……. Seal	Counter signature of the Head of the organization/authorized representative Date ……. Seal

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: Molecular characterization and integrated management of Cucumber mosaic virus infecting Cucumber (Cucumissativus) in Bangladesh.

2. Implementing organization: Bangladesh Agricultural Research Institute (BARI)

3. Name and full address with phone, cell and E-mail of PI/Co-PI (s): **PI**- Dr. Mohammad Siddiqur Rahman, Senior Scientific Officer (Plant Pathology), Bangladesh Agricultural Research Institute. E-mail: mdsiddiqurrahman@yahoo.com. **Co-PI-**Dr. Ashraf Uddin Ahmed, Principal Scientific Officer (Plant Pathology), Bangladesh Agricultural Research Institute. E-mail: kajalashraf@gmail.com.

4. Sub-project budget (Tk):

4.1 Total:2484105.00

4.2 Revised (if any): 2484100.00

5. Duration of the sub-project:

5.1 Start date (based on LoA signed): 11 May 2017

5.2 End date: 30 September 2018

6.Justification of undertaking the sub-project: Cucumber (*Cucumissativus*) is an important commercial vegetables crop having export potential, grown throughout the world. In Bangladesh, the crop is cultivated in an area about 9,118 ha with a total production of 57,128 tons and the average yield is only 6.27 tha^{-1} (BBS 2016) which is very low as compared to other cucumber growing countries in the world where average yield is more than 30 $tons'ha^{-1}$. Virus diseases are the major constrain of commercial cucumber production in Bangladesh. Mosaic disease caused by *Cucumber mosaic virus* (CMV) is considered as the most destructive and widespread virus of cucumber worldwide and causes severe yield loss, up to 100% depending on time and stage of infection (Zitter and Murphy 2009; Rahman *et al*. 2016). effective management package for CMV is scanty in Bangladesh. CMV is the type member of the genus Cucumovirus in the family Bromoviridae has the broadest host range known for any plant virus with approximately 1200 plant species in over 100 plant families (Fauquet *et al*. 2005; Zitter and Murphy 2009). The CMV genome consists of three single-stranded, messenger-sense RNA molecules, designated RNA1, RNA2 and RNA 3 having several strains (Zitter and Murphy 2009). Till now plant virus diagnosis in Bangladesh is confined on less reliable methods like Symtomatology and ELISA. An effective and applicable virus management strategy requires an accurate diagnosis of plant viruses. Recent developments in molecular techniques have revolutionized the field of diagnostics in agriculture. So, molecular characterization of CMV is needed urgently to develop appropriate management strategies. Therefore, the project has been undertaken to detect CMV using molecular tools and develop potential management option against CMV.

7. Sub-project goal: Molecular detection of CMV and development of management package

8.Sub-project objective(s): **i)** Detection of Cucumber mosaic virus strain infecting cucumber in Bangladesh using molecular tools. **ii)** Study the virus-vector relationship in development of viral disease in the cucumber field. **iii)**To develop potential management options for minimizing CMV infection through integrated approach.

9.Implementing location (s): Plant pathology Division (Laboratory and Field), Joydebpur, Gazipur.

10. Methodology in brief:

a. Survey & sample collection: Survey was conducted in ten cucumber growing areas of Bangladesh (Table 1). Samples from naturally infected cucumber leaves as well as weeds of adjacent fields exhibiting characteristic symptoms of CMV were collected from different locations of ten districts and also disease incidence were noted. The districts were selected on the basis of major cucumber growing areas as well as to collect representative data on CMV incidence from the maximum part of Bangladesh. Only symptomatic samples were collected randomly throughout the field in each district. During survey 5 to 10 samples/field were collected according to the size of the field including weed samples. The collected samples were tested by DAS-ELISA methods and were preserved at - 80^0C refrigerator for further study. The virus was mechanically inoculated to the indicator host, *Chenopodiumamaranticolor* for biologically purification of the virus isolate (Fig. 1). The biologically purified virus was maintained in the propagation host *Nicotianabenthmmiana* and host plant *Cucumissativus* as virus source for further study in insect proof net house.

Table 1. Location of Survey and CMV sample collection

Sl. No.	Name of district	Sample collection sites
1	Gazipur	Sreepur,Kaliganj, GazipurSadar
2	Dhaka	Savar, Dhamrai, Keraniganj
3	Narsingdi	Belabo,Monohardi, RaipuraShibpur
4	Jamalpur	JamalpurSadar, Melandaha, Islampur
5	Sylhet	Sylhet Sadar, Jaintiapur,Gowainghat
6	Chittagong	Hathazari, Fatikchhari, ChattogramSadar
7	Comilla	ComillaSadar, Chandina,Burichang, Daudkandi
8	Jessore	JessoreSadar, Manirampur, Jhikargacha, Keshabpur
9	Dinajpur	Dinajpursadar, Biral, Birganj
10	Barishal	Babuganj, Barisal Sadar, Banaripara

Fig. 1. Mechanical inoculation techniques

b. Disease incidence

Diseases incidence were calculated using the following formula.

$$\text{Diseases incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total plants in the plot}} \times 100$$

C. Transmission Electron Microscopy (TEM)

Leaf samples collected from naturally infected cucumber plants and test plants were prepared for transmission electron microscopy (TEM). Carbon coated palladium grids were floated on drops of crude extract of virus infected plants for 5 minutes. After 5 minutes the grids were transferred on 2% Ammonium Molybdate dye for 2 minutes for stained. Finally, the grids were dried for transmission electron microscopy as previously described by Dong et al.2008.

d. RNA extraction, RT-PCR and sequencing

Total RNA was extracted from symptomatic cucumber leaves and inoculated *Nicotianabenthmmiana* leaves using TRIzol reagent (Invitrogen, USA) and RNA extraction kit (Eastep Super RNA kit, Promega China). The first-strand cDNA was synthesized by Reverse Transcription (RT) using M-MLV (MoloneyMurine leukemia virus) reverse transcriptase (Promega, USA) and also by cDNA synthesis kit (Verso cDNA Synthesis Kit, Thermo Scientific, India) following the manufacturer's instructions. The synthesized cDNA fragment was amplified through PCR using CMV primers presented in Table 1.

Table 2. Primers used for the amplification of DNA fragment

Primer	Sequence (5'- 3')	Genomic location	T_m
CMV F1GSP	TATGATAAGAAGCTTGTTTCGCG	295-317 Nt.	52 ^{0}C
CMV R1GSP	GCCGTAAGCTGGATGGACAA	1975-1993 Nt.	52 ^{0}C
CMV F2GSP	GTAATACGACTCACTATAGGTTTTGTTTG	114-132 Nt. at CP gene	55 ^{0}C
CMV R2 GSP	GCGCGAAACAAGCTTCTTATC	633-653 Nt.	55 ^{0}C
CMVF3 Sub-group I	GTAATCTTACCACTGTGTGTG	1-21 Nt.	55 ^{0}C
CMVR3 Sub-group I	TGGTCTCCTTTTGGAGGCCC	3341-3360 Nt.	55 ^{0}C
CMVF4 Sub-group II	GTAATCTTACCACTTTCTTTTC	1-22 Nt.	55 ^{0}C
CMVR4 Sub-group II	CTCCTTATGGAGAACCTGTG	3020-3039 Nt.	55 ^{0}C

The amplified desired fragments (PCR product) were purified using agarose gel DNA purification kit (Aidlab Co, Ltd). Purified PCR products were out sourcing for sequencing.

Sequence analysis

The partial sequences of CMV was edited and analyzed with the aid of DNAMAN version 5.0 (LynnonBiosoft, QC, Canada).Phylogenetic trees were constructed using the neighbor-joining method with 1000 bootstrap replications in MEGA 6.0 (Tamura *et al.* 2013). Comparison sequences were obtained from the GenBank database.

e. Field trial:

Integrated management of Cucumber mosaic virus (CMV) infecting cucumber in Bangladesh

The field trial was conducted at the research field of Plant Pathology Division, Bangladesh Agricultural Institute, Gazipur during November 2017 to April-2018. The management packages tested in the present field trial was as follows:

Package-T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval

Package-T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of with Imidacloprid 0.1%at 15 days' interval

Package-T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval

Package-T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval

Package-T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval

Package-T6: Netting Seedling +Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid;

Package-T7: Control

*Maize were sown in line at 10 cm spacing around the plot at 20 days before transplanting of seedling.

i) Disease incidence

Diseases incidence were calculated using the formula described earlier.

ii) Disease severity

Severity of Cucumber mosaic virus (CMV) will be determined according to Monma and Sakata (1997) with some modification based on a 0-4 scale as follows.

Symptom index 0= No Symptom, 1= Mild Mosaic, 2= Mosaic, 3= Mosaic and deformed leaf 4= mosaic and stunted plants

$$\text{Severity Index} = \frac{\sum (\text{Symptom index} \times \text{Number of plants with each symptom index})}{\text{Total number of plants}}$$

iii) No. of aphid/leaf was counted by average of randomly selected 10 leaves/plot

iv) Yield/plot

v) Yield/ha

vi) Economic analysis were performed by partial budget technique as described by Rahman et al. (2011) to find the economically suitable package. Following formulae were used for economic analysis:

Variable Cost = Cost (Taka) that vary in different packages

Gross Return (TR) = Yield in terms of money

Gross margin = Gross Return – Variable cost

Marginal benefit = $\text{Grossmargin(Packages)} - \text{Grossmargin(control)}$

Marginal benefit cost ratio (MBCR) was calculated by the following formula:

MBCR (over control)

g. Design of experiment and data analysis

RCBD with 3 replications were used for field experiments. Data were analyzed statistically for analysis of variance (ANOVA) using MSTAT-C or R software and means were compared according to Duncan's Multiple Range Test (Gomez and Gomez 1984). Data were transformed as and when necessary using Arcsine transformation method.

11. Results and discussion:

Survey, Sample collection, mechanical inoculation and DAS-ELISA test

During survey naturally infected symptomatic cucumber leaves were collected (Fig. 2). Primarily percent disease incidence was recorded from each field on the basis of symptomatology. After confirmation by DAS-ELISA and RT-PCR the average disease incidence was calculated for each districts. During survey 5 to 10 samples/field were collected according to the size of the field including weed samples. The collected samples were tested by DAS-ELISA methods and further confirmed the virus by RT-PCR. The samples were also preserved at - 80^0C refrigerator for further molecular study. Among the ten districts, disease incidences were varied from 10.75 % to 28.50 % presented in Table 1. The highest disease incidence was recorded in

Gazipurdistrict (28.50%) followed by Jamalpur (22.00 %), Dinajpur (21.00%) and Comilla (20.00%). The lowest disease incidence 8.50 % was found in Sylhet. It was also observed that disease was higher in hybrid varieties as compared to op/local varieties. Most of the farmers of Gazipur, Jamalpur, Comilla, Dhaka, Dinajpur use hybrid varieties like Al-Amin, Mallika, ACI green, All-rounder etc. where the disease incidence was higher that ranged from 18.20 to 28.50 %. Disease incidence was found lower (10.75-16.25 %) in other five districts where most of the farmers use OP/local varieties. The lowest disease incidence was recorded in Sylhet districts (10.75 %). It might be due to more use of OP/local varieties that may have some extend natural resistance/tolerant or theweather condition may disfavorthespread of CMV vector population in the field.

A considerable amount of other virus symptoms was also observed in cucumber field during the survey (Data were not shown). Among them some were mechanically transmitted and some were non-transmitted but all of them were not response against the antibody of CMV. So the symptoms were not developed for CMV infection. Furthermore, white fly and thrips vectors were also observed in the cucumber field that transmit Geminivirus and tospovirus respectively. Therefore, due to climatic changes new virus may be immerged or resurgence in the cucumber field and they may become a new thread for cucumber production.

Fig. 2. Survey and collection of different symptomatic isolate of CMV (A-I).

Table 3. Incidence of *Cucumber mosaic virus* in different Cucumber growing areas in Bangladesh

Location	No. of locations surveyed	No. of fields surveyed	CMV incidence	
			Range (%)	Mean (%)
Gazipur	8	15	12-37	28.50
Dhaka	5	9	06-28	18.20
Narsingdi	6	10	10-25	15.50
Jamalpur	6	10	10-30	22.00
Sylhet	5	12	05-21	10.75
Chittagong	6	10	09-25	16.25
Comilla	7	14	08-30	20.50
Jessore	5	12	09-23	14.50
Dinajpur	4	10	10-35	21.00
Barishal	5	8	07-20	13.50

Mechanical inoculation test

Collected samples were mechanically inoculated to *Chenopodiumamaranticolor* (indicator plant), *Nicotianabenthmmiana* (propagation host) and *Cucumissativus*(Host plant).The test plants were showed characteristic symptoms 8-12 days after mechanical inoculation (Fig. 3)and maintained as virus source in insect proof net house for further studies. The mechanical inoculation test proved that the virus is sap or mechanically transmitted.

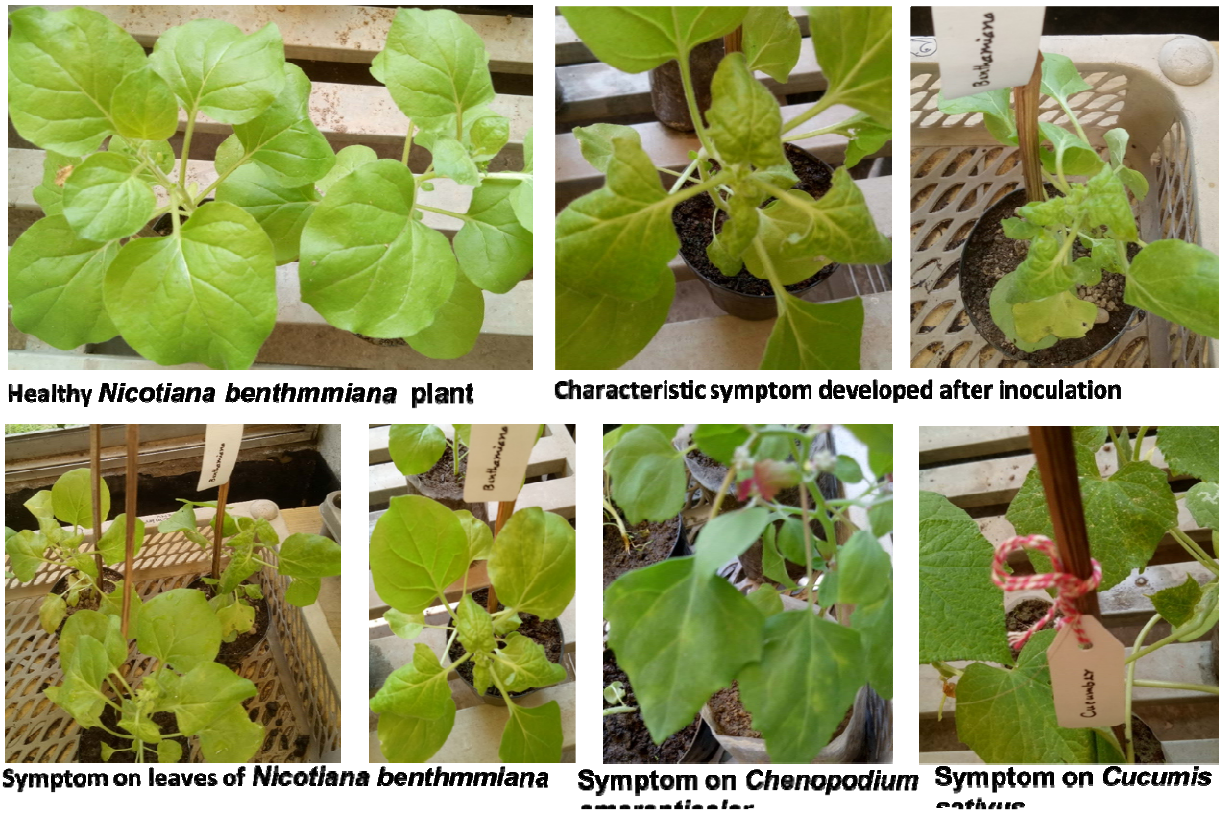

Fig. 3. Symptoms developed after mechanical inoculation.

Serological (DAS-ELISA) detection of CMV symptomatic isolate

Naturally infected characteristics symptoms of cucumber leaf as well as weed samples collected from ten districts were tested by DAS-ELISA against the antisera of Cucumber mosaic virus (CMV). The positive reaction against the antisera indicated that CMV produce five distinct symptoms like Mild mosaic, Mosaic, Mosaic & Stunting, Mosaic & curling and Leaf narrowing. The OD values (Optical density value at 405 nm) of the positively reacted samples in between positive and negative control are presented in Table 3. It was further proved that the samples were infected with CMV. Therefore, the results of ELISA conclusively revealed the presence of CMV with all the five symptoms (Table 3).

Table.4 Serological (DAS-ELISA) detection of CMV symptomatic isolates

Major symptom	Reaction against the CMV antisera	OD value (405 nm) (Average of 5 positive samples)
- Control	-	0.12
+ Control	+	0.40
Mild mosaic	+	0.28
Mosaic	+	0.30
Mosaic & Stunting	+	0.25
Mosaic & curling	+	0.32
Leaf narrowing	+	0.35

"+" positive reaction; "-"Negative reaction

Transmission Electron Microscopy (TEM)

Prepared samples were carefully observed under TEM. Spherical or round shape virus particles about 28-30 nm in diameter was observed under Transmission Electron Microscope (TECNAI G^2 Spirit TWIN 9432 050 18111, Czech Republic). The size and shape of the virus particles was typical of CMV i.e. Cucamovirus (Fig. 4).

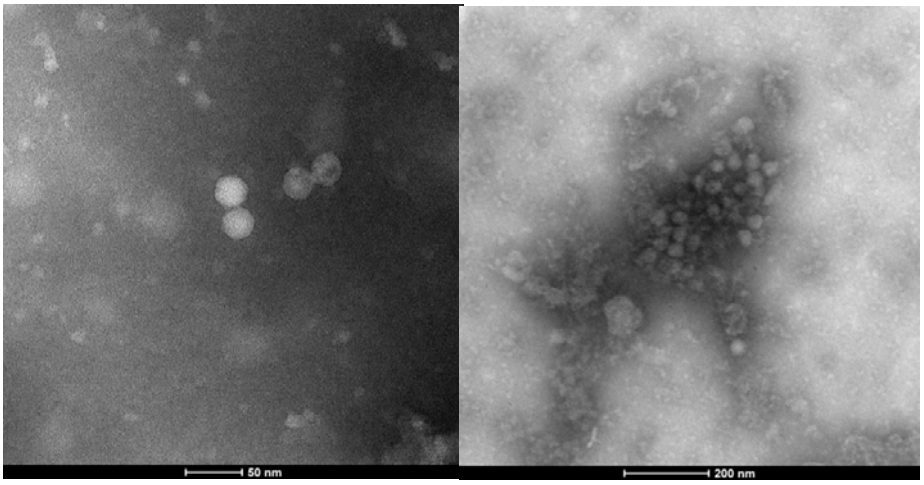

Fig. 4. CMV particles under Electron Microscope (Bar=50 nm left and 200 nm right).

RNA extraction, RT-PCR

Total RNA was extracted from 50 samples including *Nicotianabenthmmiana* leaves. First-strand cDNA was synthesized by Reverse Transcription (RT) using M-MLV (MoloneyMurine Leukemia Virus) reverse transcriptase (Promega, USA) and also by cDNA synthesis kit (Verso cDNA Synthesis Kit, Thermo Scientific, India) following the manufacturer's instructions. The CMV genome about 300-750 bp were amplified by using different primer described earlier. PCR amplified DNA fragments of different samples are presented in Fig. 5. The PCR condition was as the initial denaturing step at 94^0C for 3 min; 35 cycles of 94^0C for 3min, 1 min at 60^0C and 90sec. at 72^0C; with a final extension step at 72ºC for 10 min. The desired DNA samples were purified and were out sourcing for sequencing.

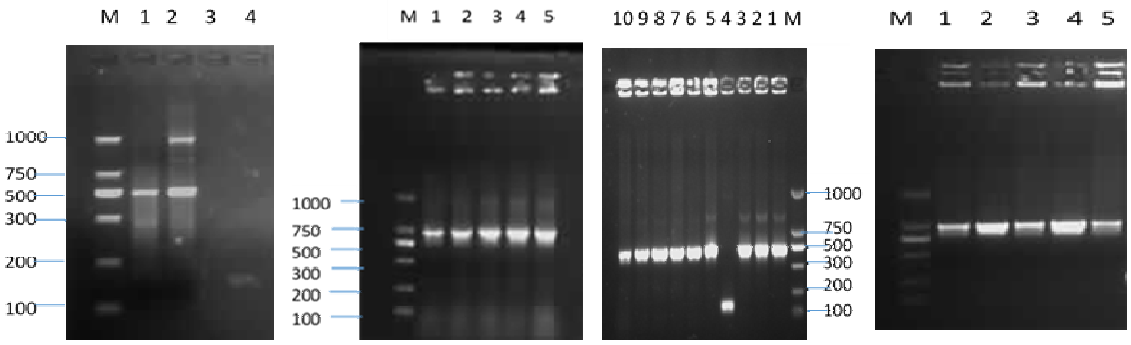

Fig. 5. Amplified different DNA fragments (M- Takara 1000bp DNA Marker; A: 1, 2, 3, 4-Narsingdi; B:1-5 Gazipur C:1-5-Jamalpur, 6-10 Dinajpur; D: 1-5 Comilla)

Sequence analysis

The nucleotide sequences werealigned with BLAST with the NCBI database. The 100% nucleotide identity was found with CMV RNA 1 segment of Genbank accession no. AB179764, 99 % identity with LC339509, 98 % with EU414792, 97% with AF071551, 95% with HQ283392 respectively. Therefore, the identified virus is the partial nucleotide sequence of CMV.

Thirty-nine PCR products were sequenced to detect CMV. All the tested samples were belonging to RNA1 and RNA3 segment of CMV. Therefore, the representative partial sequences of CMV genome (RNA 1 & RNA 3) were edited and analyzed using DNAMAN software (Table 4). The partial nucleotide sequence of RNA 1 and RNA 3 of CMV isolate segment 420 and 412 nucleotides (nt.) respectively were analyzed. The base composition of the 420 nt. segment RNA 1 contained adenine 27.9 %, cytosine 18.8 %, guanine 23.8 %, and uracil 29.5 % are presented in Table 4A. The partial nucleotide sequence of RNA 3 segment of CMV is 412 nucleotides (nt). The base composition of the 412 nt segment RNA 3 contained adenine 19.7 %, cytosine 18.2 %, guanine 24.3%, and uracil 37.9 % are presented in Table 4B.Phylogenetic trees were constructed by the RNA 1 and RNA 3 segment of CMV with the CMV sequences of GenBank database using the neighbor-joining method. Phylogenetic tree based on RNA 1 showed that the CMV isolate of Bangladesh is closely related to the Chinese isolate of AF 071551 (Fig. 6a).The phylogenetic tree based on intergenic region (RNA 3) showed the grouping of CMV isolates and the CMV Bangladesh isolate grouped with the cluster of subgroup IB. The comparison of CMV isolates with three different subgroups showed that the CMV Bangladesh isolate shared 88-91 %, 76-84 % and 62-63 % sequence identity with IB, IA and subgroup II respectively (Fig. 6b). The present investigationrevealed that the CMV Bangladesh isolate belongs to subgroup IB. This is the first molecular identification of CMV occurring in Bangladesh.

Table 5. Analyzed partial sequence of RNA 1 and RNA 3

A. SEQ CMV RNA1: 420 bp; Composition: 117 A; 79 C; 100 G; 124 T; 0 OTHER Percentage: 27.9% A; 18.8% C; 23.8% G; 29.5% T; 0.0%OTHER Molecular Weight (kDa): ssDNA: 130.05 dsDNA: 258.90
ORIGIN 1 GCAAGATCAT CTTGAACGAT CCACAACAGT TCGATGGTCG ACAGCCGGAC TTCTGCACTC 61 ATCCGGCTGC GGATTGCAAA GTACAAGCCC ACTTTGCTAT ATCTATTCAT GGAGGTTATG 121 ATATGGGCTT TAGAGGATTA TGTGAAGCGA TGAATGCTCA CGGAACCACT ATTTTGAAGG 181 GAACGATGAT GTTCGATGGT GCTATGATGT TTGACGACCA AGGTGTAATA CCTGAGCTTA 241 ATTGTCAGTG GAGAAAGATC AGGAGTGCTT TCTCTGAAAC TGAAGACGTC ACACCGCTGA 301 CTGAGAAAAT TAATTCCACG ATATTTTCCC GCGTGCGTAA ATTCAAGACT ATGGTGGCTT 361 TCGATTTTGT CAATGAGTCT ACTATGTCTT ATGTTCATGA TTGGGAGAAT ATAAAATCTT
B. SEQ CMV RNA3: 412 bp; Composition: 81 A; 75 C; 100 G; 156 T; 0 OTHER Percentage: 19.7% A; 18.2% C; 24.3% G; 37.9% T; 0.0%OTHER Molecular Weight (kDa): ssDNA: 127.35 dsDNA: 253.97
ORIGIN 1 GTAATCTTAC CACTGTGTGT GTGTGTGTGT GCGTGTGTCG CGTCGTGTCG AGTCGTGTTG 61 TGTTTCGTTG CGTATTAGTA TATAAGTATG TGTGTGTCTG TACATAATAC TATATCTATA 121 GTGTCCTGTG TGAGTTGATA CAGTAGACAA CTGTGACGCG ATGGTGTAGA GAAGAGAGCA 181 CATCTGGTTT AGTAAAACCC ACAACATTAT CTTTGAGGTT CAATTCCTCT TGATCCCTGT 241 TGGGCCCCTT TTACTTTTCA TGGATGCTTC TCCACAAGAT TGCGTTTCGT CTACTTATCA 301 TTAGTGTATT GTGCTGTGTT TTCTCTTTTG TGTAGTAGAT TTGAGTCGAG TCTCCGCACA 361 TAAGAGTCGT GCTGTCCGCA CATTTTCTTT TCAGTGTGTT AGATTCCCGA GG

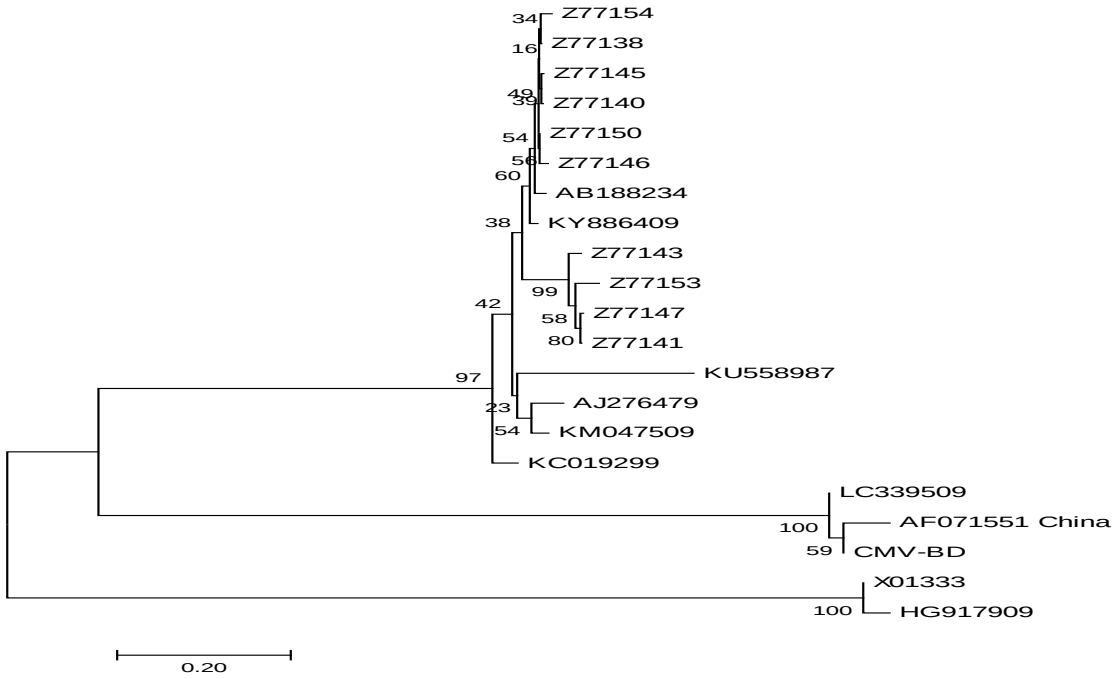

Fig. 6a. Phylogenetic tree based on the partial nucleotide sequences of CMV isolate RNA 1segmentand other 20 related CMV segment. Sequences were aligned through Clustal W. The tree was constructed by the

neighbor-joining algorithm, both which in the MEGA7.0 package. Bar represent exchanged per 100 nucleotides.

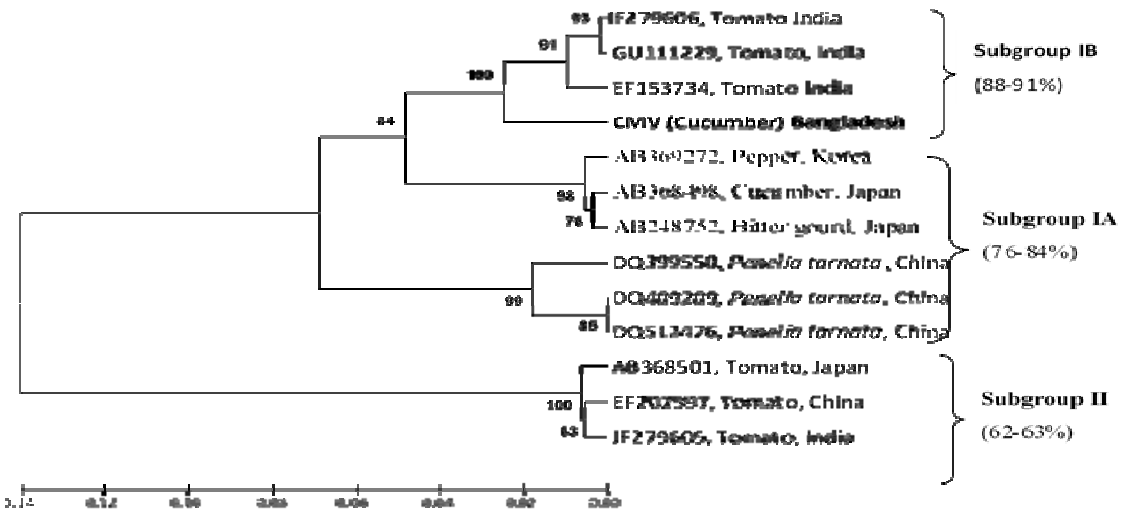

Fig. 6b. Phylogenetic tree based on the partial nucleotide sequences of CMV isolate RNA 3 and other 12 related CMV segment. Sequences were aligned through Clustal W. The tree was constructed by the neighbor-joining algorithm, both which in the MEGA6.0 package. Bar represent exchanged per 100 nucleotides.The tree showing the grouping of the CMV isolates. The CMV Bangladesh isolate grouped with the cluster of subgroup IB.

Field trial: Integrated management of Cucumber mosaic virus infecting cucumber in Bangladesh

Disease incidence and severity of CMV: Disease incidence and severity of Cucumber mosaic virus on different treatments are presented in Fig. 7. Incidence of CMV of all the management packages (T1-T6) was found lower as compared to control. The highest disease incidence (42.00%) was recorded from T7 (control). The lowest incidence (9.67%) was observed in T2 which was statistically similar to T1 (10.5%). The incidence of CMV in T4, T5 and T6, was statistically similar but significantly higher compared to T1, T2 and T3. Similarly, the highest disease severity was found in T7 (control) and each of the management packages (T1-T6) reduced severity of CMV significantly over control. The lowest severity was found in T2, which was statistically similar to T1. Among the treatments T2 and T1 was found very much effective in reducing disease incidence and severity. However, treatments involving sticky yellow trap, polythene mulch with 4 spray of Bio-neem/imidacloprid (T2 and T1) was better than other management packages (Fig. 6). It might be due to better control of CMV vectors (aphids) in the treated plot. CMV is an aphid born non- persistent virus, so only insecticides spray is not enough to control the vector as it required only few seconds to transmit virus from infected to healthy plant. So use of disease free seedling, sticky yellow rap, polythene mulch and then spray insecticide effectively controlled the vectors and reduced the disease incidence and severity in the management packages.

Aphid population

The effect of different management options on aphid population per leaf is shown in Fig. 8. The highest number of aphid per leaf (14.50) was recorded from the plants under control. Every management packages caused significant reduction in number of aphid population per plant over untreated control. Significantly lower number of aphids was recorded from plant treated with management packages T2 and T1 compared to other packages. However, efficacy of two packages was statistically similar and very few aphid was

observed in treatment plot of T2 and T1. It might be due to effectively control of aphids in the treatment i.e. Sticky yellow trap act as continuous barrier against the aphid and again spray with insecticide reduce the colonization of aphid vector on leaf in the treated plot. Therefore, the disease incidence was less in treated plot as compared to control.

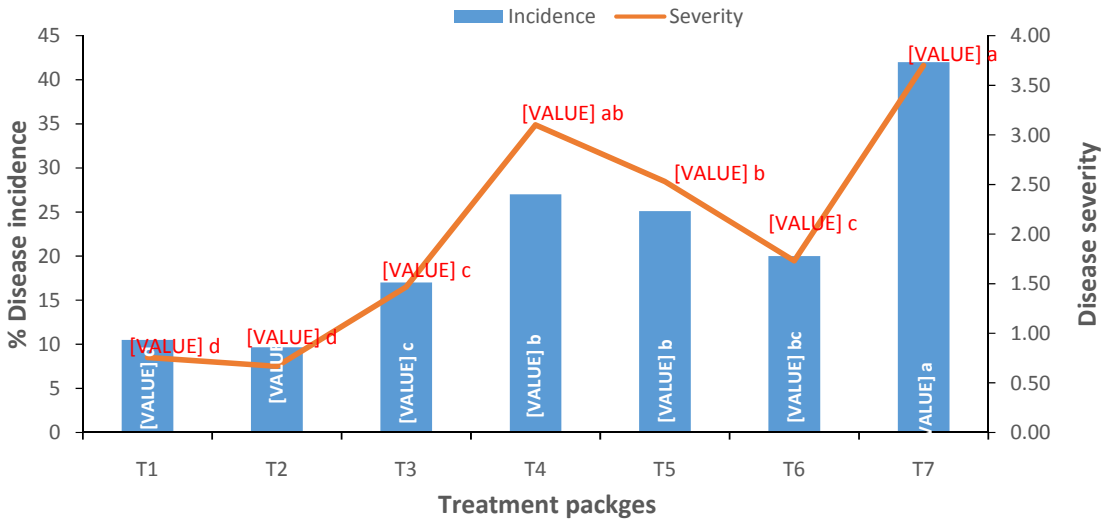

Fig. 7. Effect of different management packages on the incidence and severity of CMV in Cucumber. (T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays ofImidacloprid 0.1% at 15 days' interval Imidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

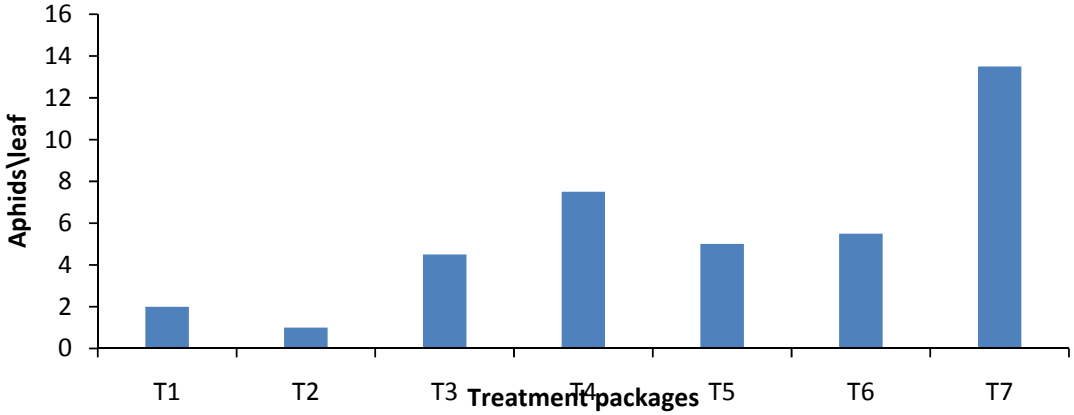

Fig. 8. Effect of different management packages on number of aphids/leaf. (T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays ofImidacloprid 0.1% at 15 days' intervalImidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

Relationship between aphid population and incidence of CMV

In the field trial it was found that the number of CMV infected plants were higher with the increase of aphid number per plant. The relationship was linear, positive and significant (R^2 = 0.7553) and could be expressed

by the regression equation Y= 3.6484x + 4.8546, where Y= incidence of CMV (%) and x =number of aphids per plant (Fig. 9). The R^2 value indicates that the spread of CMV in the field might be attributed by aphid population by 75.53 %.

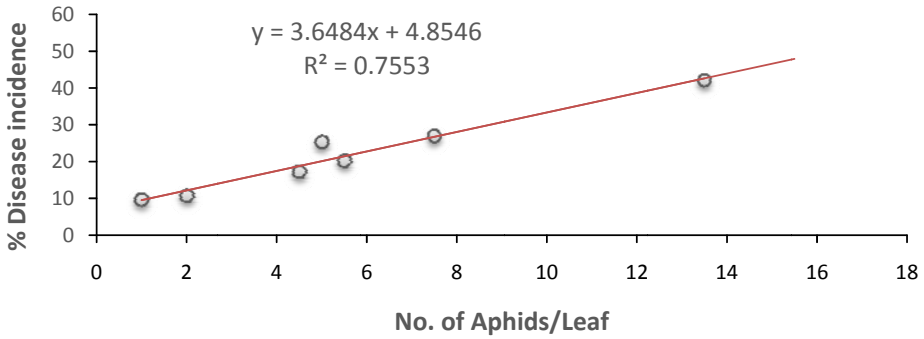

Fig. 9. Relationship between aphid population and percent disease incidence in different management options.

Yield

All the management options reduced disease incidence and gave higher yield as compared to control (Table 5). The highest yield was found 13.07 ton/ha in treatment packages T2 (Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of imidacloprid 0.1% at 15 days' interval) which was statistically similar to T1 (Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem at 15 days' interval) but significantly higher from other management options (Fig. 10). The lowest yield (6.67 t/ha) was found in T7 (untreated control). The yield of other treatments ranged from 7.04 to 10.15 t/ha. The highest reduction of disease incidence was found 76.97% in treatment T2 which was statistically similar by T1 (75 %). Other treatment packages also reduce disease incidence at a considerable level (35.71-59.52 %). However, among the treatment packages, performance of packages T2 and T1 was the best.

Table 6. Effect of management packages on disease reduction and yield of cucumber

Treatments	Incidence	Reduction in disease incidence (%)	Yield T/ha	Yield increase T/ha
T1	10.50 d (18.88)	75.00	12.96 a	6.29
T2	9.67 d (18.05)	76.97	13.07 a	6.40
T3	17.00 c (24.31)	59.52	10.15 b	3.48
T4	27.00 b (31.29)	35.71	7.04 d	0.37
T5	25.10 b (30.06)	40.23	9.30 b	2.63
T6	20.00 bc (26.51)	52.38	8.52 c	1.85
T7	42.00 a (40.36)	-	6.67 d	-
LSD	4.76		0.87	
CV %	9.91		14.50	

* Means followed by same letter are not significantly different at 5% level by DMRT. Value within parenthesis are arcsine transformed value. (T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of

Imidacloprid 0.1% at 15 days' interval Imidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

Fig. Experiment plot

T_1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bioneem at 15 days interval

T2: Same as T_1, except spray with Imidacloprid 0.1%

T_3: Same as T_1, except spray with Imidacloprid 0.1%

T_4:Netting seedlings + 4 sprays of Imidacloprid 0.1% at 15 days' interval

T_5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval

T_6: Netting Seedling + Maize as barrier crop + straw mulch + 2 spray of Imidaclopri

Insect vector trapping by Sticky yellow trap

Fig. 10. Different management packages under field trial

Economic analysis

Results obtained from economic analysis of various treatments are presented in Table 6 and 7. All treatments more or less increase the gross return over control. However, grows return was highest in T2 followed by T1, T3, T5, T6 and T4. The lowest was obtained from Control. Marginal analysis has pointed out that all the management packages increase marginal benefit as well as marginal benefit cost ratio (MBCR) over control (Table 7). The highest MBCR was obtained from T2 and the lowest from T4. The results showed that additional investment of Taka 1 in T2 over control had additional income of Taka 3.17 and similarly Tk. 2.93 in T1, Tk. 1.61 in T3, Tk. 1.47 in T5, Tk. 1.31 in T6 respectively. Considering cost and return and MBCR from the economic analysis indicating that all the management packages except T4 (MBCR 1:0.85) were economically viable and maximum gain could be obtained from T2 (integration with netting seedlings, sticky yellow trap, polythene mulch and 4 spray with imidacloprid 0.1%).

Table 7. Cost and return in different management packages

Packages	*Var. Cost (Tk ha^{-1})	Yield (t ha^{-1})	**Gross return (Tk ha^{-1})
T_1	24000.00	12.96	194400.00
T_2	23000.00	13.07	196050.00
T_3	22000.00	10.15	152250.00
T_4	5000.00	7.04	105600.00
T_5	19000.00	9.30	139500.00
T_6	12000.00	8.52	127800.00
T_7 (Control)	-	6.67	100050.00

* Var. Cost: Cost that vary in different packages

** Whole Sell rate of cucumber @ TK 15.00/Kg

(T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays ofImidacloprid 0.1% at 15 days' interval Imidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

Table 8. Marginal analysis of different treatment packages

Packages	Gross return (Tk ha^{-1})	Var.Cost (Tk ha^{-1})	Gross margin (Tk ha^{-1})	Marginal benefit (Tk ha^{-1})	MBCR
T_1	194400.00	24000.00	170400.00	70350.00	1: 2.93
T_2	196050.00	23000.00	173050.00	73000.00	1: 3.17
T_3	152250.00	20000.00	132250.00	32200.00	1: 1.61
T_4	105600.00	3000.00	100600.00	550.00	1: 0.85
T_5	139500.00	16000.00	123500.00	23450.00	1: 1.47
T_6	127800.00	12000.00	115800.00	15750.00	1: 1.31
T_7 (Control)	100050.00	-	100050.00	-	-

(MBCR: Marginal benefit cost ratio)

(T1: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays of Bio-neem 0.2% at 15 days' interval; T2: Netting Seedling + sticky yellow trap +Polythene mulch + 4 sprays ofImidacloprid 0.1% at 15 days' interval Imidacloprid 0.1%; T3: Netting Seedling + sticky yellow trap + Polythene mulch + 2 sprays of Imidacloprid 0.1% at 20 days' interval; T4: Netting Seedling + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T5: No netting +Polythene mulch + 4 sprays of Imidacloprid 0.1% at 15 days' interval; T6: Netting Seedling + Maize as barrier crop + sticky yellow trap + straw mulch + 2 spray of Imidacloprid; T7: Control)

Different management packages caused 35.71-76.97 % reduction in disease incidence and increase yield 0.37-6.40 ton/ha (Table 5). In the present investigation, treatment packages comprising with Netting Seedling, sticky yellow trap, Polythene mulch and 4 sprays of Imidacloprid 0.1% /Bio-neem at 15 days interval (T2 & T1) were found better than any other packages in terms of disease suppression and yield improvement (Table 5& Fig. 10). Successful application of integrated management for CMV has also been postulated in the review by Hooks and Fereres (2006). Among the treatment packages, T4 was found less effective. This is obvious, because the non-persistent manner of virus transmission like CMV. Only use of insecticides is not always effective as the aphids become irritated and therefore jump from leaf to leaf or plant to plant in an attempt to avoid the insecticides, subsequently infecting healthy plants because the acquisition and inoculation time is very short. For this, aphids are capable to inoculate healthy plants within few seconds. That is why disease incidence and severity was high as compared to other packages and ultimately reduce the yield (Fig. 10 and Table 5). Because of the very short time needed to transmit a virus, aphids are capable of transmitting NPVs (Non-persistent viruses) prior to being killed by an insecticide. This observation is an agreement with the findings of Hooks *et al*. (2007).

Again treatment T2 gave higher yield than T1 although statistically similar. It might be due to less suppression of aphids by Bio-neem as compared to Imidacloprid 0.1 %. However, in case of diseases incidence and yield both the packages more or less similar. However, the better result was achieved from the treatment packages T2 and

T1. It might be due to sticky yellow trap acted as continuous "spread breakers" by attracting aphids and preventing the colonization on the cucumber leaves and insecticidal sprays further suppressed disease spread. The finding is also the conformity of the previous findings of Anandam and Doraiswamy 2002 in case of non-persistent virus like CMV. The environmental point of view spray with Bio-neem, integration with sticky yellow trap, netting seedling and use polythene mulch during winter season may be a good option to reduce CMV incidence and increase yield of cucumber.

Economic analysis revealed that profit varies depending on the management packages. Results of the present investigation indicate that T2 is the best treatment in terms of economic gain. It has got chemical back up in addition to sticky yellow trap. So that successful control was achieved against aphid vector which reduced incidence and severity of CMV. Furthermore, polythene mulch increases the soil temperature that enhance the growth and development of cucumber as well as suppress weeds in the field. Therefore, higher yield was achieved from that treatment. From environmental point of view T1 may be used. Because it has got botanical insecticide (Bio-neem) instead of chemical which is environmentally safe although marginal benefit cost ratio (MBCR) was little lower than T2. Although the variable cost of T2 and T1 (Tk 24000 and 23000) is higher but the treatments are cost effective considering return for additional cost.

Effect of CMV on yield depends on a number of factors, including plant age and growth stage when infected, viruliferous vector population, environmental conditions etc. (Agrios*et al*. 1988; and Rahman *et al.* 2008). Results of the present investigation demonstrate that CMV of cucumber may be effectively managed through integration of netting seedlings, use sticky yellow trap, polythene mulch (winter season) and spray of imidacloprid 0.1% or Bio-neem. This is the first report of an integrated management of Cucumber mosaic virus (CMV) of cucumber in Bangladesh. Further trial is needed for more confirmation.

12. Research highlight/findings:

- Determined incidence of *Cucumber mosaic virus* (CMV) (10.75-28.5%) in major cucumber growing areas of Bangladesh through survey.
- Identified CMV from like mild mosaic, mosaic, mosaic & stunting, mosaic & curling and leaf narrowing symptoms by DAS-ELISA and molecular tools (RT-PCR).
- Determined *Cucumber mosaic virus* subgroup IB infecting Cucumber in Bangladesh which are similar to accession JF270606 & EF153734 based on phylo genetic grouping.
- Strong positive correlation between vector (aphids) and CMV infection was observed in developing CMV disease in the cucumber field.
- Developed effective management option for CMV of Cucumber-

Integration of netting seedling, sticky yellow trap, polythene mulch and 4 sprays of Imidacloprid / Bioneem at 15 days interval effectively reduced CMV incidence and increase yield of Cucumber.

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B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	8	265000.00	8	265000.00	Achieved
(b) Lab &field equipment	2	17000.00	2	17000.00	Achieved
(c) Other capital items	N/A	N/A	N/A	N/A	

2. Establishment/renovation facilities:

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
N/A	N/A	N/A	N/A	N/A	

3. Training/study tour/ seminar/workshop/conference organized:

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	N/A	N/A	N/A	N/A	
(b) Workshop	N/A	N/A	N/A	N/A	

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)
A. Contractual staff salary	247549.00	247549/-	247549/-	0	100
B. Field research/lab expenses and supplies	1166556.00	1166556/-	1166556/-	0	100
C. Operating expenses	27620.00	276280/-	275700/-	0	100
D. Vehicle hire and fuel, oil & maintenance	200000.00	200000/-	200000/-	0	100
E. Training/workshop/seminar etc.	-	-	-	0	-
F. Publications and printing	49300.00	24300/-	24300/-	0	47.56
G. Miscellaneous	110000.00	110000/-	110000/-	0	100
H. Capital expenses	435000.00	435000/-	435000/-	0	100

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	**Major technical activities performed in respect of the set objectives**	**Output(i.e. product obtained, visible, measurable)**	**Outcome(short term effect of the research)**
Detection of Cucumber mosaic virus strain infecting cucumber in Bangladesh using molecular tools.	i) Survey & virus sample collection ii) Virus detection by TEM, DAS-ELISA, iii) RNA extraction and RT-PCR, amplification of DNA fragments & sequencing.	-Identified five CMV biotypes like mild mosaic, mosaic, mosaic & stunting, mosaic & curling and leaf narrowing.- Find out Cucumber mosaic virus subgroup IB infecting Cucumber in Bangladesh based on phylogenetic grouping	Accurate detection of CMV using moleculartools inBangladesh thatfacilitate furthermolecular study of different plant viruses.
Study the virus-vector relationship in development of	i) Data record on CMV infected plants ii) Observe vectorpopulation on	Strong positive correlation between vector (aphids) and CMV infection was observed in	Help in adopting virus management strategies through vector control

viral disease in the cucumber field.	leaf	developing CMV disease in the cucumber field.	
To develop potential management options for minimizing CMV infection through integrated approach.	i) Six treatment packages with a control were evaluated through field trial. ii) CMV incidence, disease severity and yield were recorded under different treatment packages.	Management option developedfor CMV of Cucumber-"Integration of netting seedling, sticky yellow trap, polythene mulch and 4 sprays of Imidacloprid /Bio-neem at 15 days interval effectively reduced CMV incidence and increase yield of Cucumber"	

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication	1	-	
Information development			
Other publications, if any			

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

"Integration of netting seedling, sticky yellow trap, polythene mulch and 4 sprays of Imidacloprid (0.1%) or Bioneem (0.2%) at 15 days interval effectively reduced CMV incidence and increase yield of Cucumber"

ii. **Generation of new knowledge that help in developing more technology in future**

The knowledge of the present investigation helps in developing more effective management technology regarding the non-persistent viruses in Bangladesh.

iii. Technology transferred that help increased agricultural productivity and farmers' income

The developed technology may help increased cucumber production that enhance farmer's income.

iv. Policy Support

The findings of the present project may assist the policy makers of the agricultural sectors for planning their future research directions regarding plant viruses for sustainable food and nutrition security in Bangladesh.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):N/A

ii) Field Monitoring (time& No. of visit, Team visit and output):

Monitoring team	Date(s) of visit	Total visit till date (No.)	Remarks
Technical Division/ Unit, BARC	14/03/2018	1	
PIU-BARC, NATP-2	14/03/2018	1	

Internal Monitoring (BARI)	06/02/2018, 19/02/2018	2	Satisfactory

H. Lesson Learned/Challenges (if any)

i) Delayed fund release hindered smoothly run the project activities.
ii) Unstable electricity supply hampered the lab work especially molecular work and also storage of molecular chemicals
iii) Timely release of fund is essential for better achievement of the project.

I. Challenges (if any)

The supply of molecular chemicals and backup supports for molecular work still time consuming.

Signature of the Principal Investigator Date ……. Seal	Counter signature of the Head of the organization/authorized representative Date ……. Seal