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Summer Cosmos – A Host of *Cucumber mosaic virus*

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ABSTRACT

In order to identify the cause of virus disease-like symptoms developed naturally in Summer cosmos (Cosmos sulphureus) plants at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur campus, a study was conducted during March 2004 to August 2005. The natural symptoms in Summer cosmos were consisted of mosaic, yellowing, shoe-string and leaf curling along with severe stunting of the infected plants. The ailments were found to be sap transmissible. Gomphrena globosa and Chenopodium amaranticolor were found to be good local lesion hosts producing chlorotic local lesion in the inoculated plants. The virus isolates obtained from the infected G. globosa plant had wide host range including Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Ligominosae and Solanaceae. The dilution end point, thermal inactivation point and longevity in vitro were determined as 10^{-6}, 65^{0}C and 10 days, respectively. The host range test, dilution end point, thermal inactivation point and longevity in vitro suggested that the virus was identical to Cucumber mosaic virus (CMV). Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA) detected the virus as CMV. The results of the study revealed that the virus disease-like symptoms naturally manifested in summer cosmos plants was identified as CMV.

Key words: Summer cosmos, CMV, virus identification.

INTRODUCTION

Cosmos (*Cosmos sulphureus* Cav) is an ornamental flowering plant which belongs to the family Compositae. It is a native of Mexico from which spreads to the different parts of the world. Cosmos flowers contain triterpene alcohol which has got high medicinal value i.e. it is an anti-inflammatory substance (Akihisa *et al.* 1996). In Bangladesh summer cosmos is popularly grown in home garden, in front of different social and educational institution in summer season.

Typical virus disease- like symptoms as described by Brunt *et al.* 1997 were observed in summer cosmos plants growing at BSMRAU campus, Gazipur, Bangladesh. The symptoms of the infected plants consisted of mosaic, vein chlorosis, vein banding, curling and deformation of leaves as compared to apparently healthy neighbouring cosmos plants. The infected plants were severely stunted which yielded poor number of twisted and deform flowers. The growth reduction appeared as the production of small leaves clustering at the top of the plant. The severely infected plant tended to suffer from growth cessation and necrosis at the top. So far there is no report on summer cosmos virus disease in Bangladesh.

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Almost all plant viruses have ornamentals, weeds or other alternative natural hosts that provide a reservoir of viruses from which the economically important food and horticultural crop plants may become infected. The ecological and epidemiological studies of plant viruses have been emphasized the presence of such ornamentals, weeds or other alternate hosts grow around the crop field including the vector (Thresh 1981). Since the ornamentals, weeds or other alternate hosts contribute to the infection of crop plants in the field supply primary inoculum i.e. these act as initial sources of infection from which the viruses spread into or within a crop. It has been emphasized that the identification of initial sources of infection is of immensely important not only studying the epidemiological aspects of plant viruses, but also to formulate control measures or management practices against the virus diseases (Maramorosch and Harris 1981, Thresh 1980, Thresh 1982, Zitter and Simons 1980). In fact, plant virus research has been started very recently in Bangladesh; due to lack of trained manpower and proper facilities. Most of the crops even remain untouched in respect of plant virus disease diagnosis in the country. So the investigation of the virus diseases of ornamentals, weeds and other wild plants has not yet been started in Bangladesh. But the investigations of such plants are of immensely important to unfold the virus disease problems of crops.

Considering the facts this study was undertaken to identify the causal agent of virus disease-like symptoms in summer cosmos and to find out the host range of the causal agent.

MATERIALS AND METHODS

The experiment was conducted in the Plant Pathology Laboratory and insect proof net house of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, during March 2004 to August 2005. The symptomatological study was done on the naturally infected Summer cosmos (*Cosmos sulphuneus*) plants growing in BSMRAU campus by close and careful investigation. The photographs of the infected plants were also taken for further illustration. Several infected plants having identical virus disease-like symptoms were selected for the collection of samples. The collected samples were cut into small pieces using a razor blade and preserved at 4^0C in the plastic petridishes containing silica gel. A piece of blotter paper was put in each plastic petridishes to spread the cut sample on to it. The plastic petridishes were then wrapped with scotch tape to make it air tight (Bos 1969, Noordam 1973, Gibbs and Harrison 1979). The preserved samples were used when it was necessary.

Mechanical inoculation test

The mechanical inoculation test was carried out following the method as described by Hill (1984). Three different plant species namely *Cosmos sulphureus, Chenopodium amaranticolor* and *Gomphrena globosa* were used in the test. The plants were grown in an insect proof net house. Sodium phosphate buffer p^H 7.1 was used to extract sap of infected summer cosmos leaves in the inoculation test. In each case 5g fresh leaves collected from the infected summer cosmos plants were macerated using 50 ml aforeside buffer for sap extraction. The leaves of the test plants were dusted with sterilized 600 mesh carborundum powder. The sap was then rubbed with finger on to the carborundum powder dusted leaves of the test plants. After rubbing with sap the inoculated leaves were carefully washed with sterilized distilled water using wash bottle. The inoculated plants were placed in the nethouse and cheeked everyday to detect the appearance of symptoms. The symptoms appeared in the inoculated plants were recorded and photographed for further illustration.

Local lesion isolation and propagation of virus

Single local lesion isolation was done by mechanical inoculating *G. globosa* to get pure isolate of the virus. Sap was extracted from the single local lesion of *G. globosa* with 0.02M sodium phosphate buffer (p^H 7.1) and inoculated to healthy *G. globosa* plants. The pure isolate of the virus was obtained by three successive inoculations in the local lesion hosts. Finally the sap extracted from local lesion was inoculated to the Summer cosmos plant for propagation of the virus. During virus isolation 600 mesh carborundum powder and 0.02M sodium phosphate buffer p^H 7.1 was used.

Host range test

Host range test was performed by inoculating 24 plant species belonging to six dicotyledonous plant families (Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitacceae, Leguminosae, Solanaceae). The test plant species were: *Gomphrena globosa, Chenopodium amaranticolor, Tagetes erecta, Cosmos sulphureus, Cosmos bipinnatus, Zinnia elegans, Cucumis sativus, Citrullus lanatus, Lagenaria siceraria, Trichosanthes anguina*, *Cucurbita moschata, Luffa acutangula, Luffa cylindrica, Benincasa hispida, Phaseolus vulgaris, Pisum sativum, Vigna unguiculata, Nicotiana glutinosa, Nicotiana tabacum, Nicotiana rustica, Lycopersicon esculentum*, *Physalis floridana, Petunia hybrida, Datura metel* and *Datura stramonium*.

Dilution end point test

The selected pure isolates of summer cosmos virus obtained from local lesion isolation on *G. globosa* were used for the determination of dilution end point of the virus following standard procedures by Noordam (1973). An amount of 1 gm of infected leaf sample was macerated in 10 ml of 0.02 M Sodium phosphate buffer (p^H 7.1) which was treated as original dilution. Using the original dilution, a series of eleven serial dilutions viz. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} and 10^{-11} was prepared using sap extracted from inoculation on test plants in 0.02M Sodium phosphate buffer p^H 7.1. These were inoculated to the *G. globosa, C, amaranticolor* and *C. sulpureus.* The inoculated plants were incubated in the net house and observed for symptoms development.

Thermal inactivation point test

The sap used in dilution end point test was also used for determining the thermal inactivation point test. The test was conducted following the method of Noordam (1973). The sap diluted series was 10^{-1} to 10^{-11} with 0.02M Sodium phosphate buffer, p^H 7.1. The diluted sap was heated in test tubes in a temperature control water bath for 10 min. The temperature range was 25^0C up to 90^0C with 5^0C interval. Untreated and treated sap was inoculated to *G. globosa, C. amaranticolor* and *C. sulpureus* for symptoms development.

Longevity *in vitro* (aging) test

Longevity *in vitro* (aging) was determined following the methods described by Noordam (1973). The sap of the infected leaves was diluted up to 10^{-8} and filtered through cheesecloth. The sap was then poured into test tubes at the rate of 2ml/tube and sealed with aluminum foil. The test tubes were kept at room temperature (30 ± 2^0C) in the laboratory rack. *G. globosa, C. amaranticolor* and *C. sulphureus* were inoculated with the sap sample everyday and continued up to 18 days. The inoculated plants were observed for symptom development.

DAS-ELISA test

The virus-infected samples were tested by Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA) against the antisera of *Cucumber mosaic virus* (CMV), *Papaya ring spot virus (*PRSV)*, watermelon mosaic virus-2* (WMV-2), *Zucchini yellow mosaic virus* (ZYMV) and *Potato virus Y* (PVY) following the method as outlined by Clark and Adams (1977) with some modifications. In the test Patho Screen Kit presented by Agdia Incorporated, 30380 County Road 6, Elkhart, Indiana 46514 USA was used. The instructions written in the manual of Agdia Incorporated to perform the DAS-ELISA was followed. The sap was extracted from the leaf samples in extraction buffer at a 1:10 ratio (tissue weight: extraction buffer volume). The extracted sap was poured @ 100 μ 1 per well of the ELISA plate which was precoated with virus specific IgG. The plate was then incubated in a humid box at room temperature for 2 hr. After incubation the plate was washed with washing buffer and then enzyme conjugate was dispensed @ 100 μl per well. The plate was incubated and washed following the same procedure as mentioned before. The substrate solution was prepared following the instructions in the manual and used @ 100 μ1 per well. The plate was incubated in a humid box at room temperature for 60 min. Just after 60 min of incubation 50 μ1 of 3 M sodium hydroxide was added to each well to stop the reaction. The plate was then observed visually to detect the development of yellow color incase of positive reaction. The optical density (OD) values were measured with ELISA Reader EAR 400 FW (SIT-LABINSTRUMENTS) at 405 nm wavelength. The positive and buffer control were maintained.

RESULTS AND DISCUSSION

Symptomatological Study

The symptoms appeared in the naturally infected summer cosmos (*Cosmos sulphureus*) plants are noted in Table 1. The leaves of the infected plants developed mosaic accompanied with yellowing which was eventually visible as yellow mosaic symptom. The symptomatic leaves produced vein chlorosis along with mild curling. In many infected plants leaf distortion accompanied with shoestring were found as prominent symptoms. Necrosis of the infected leaves was also observed in severely infected plants. Leaf size of the infected plants was drastically reduced as compared to the healthy plants. The symptomatic plants produced small, twisted and deformed flowers as noted in Table 1. However it was exactly similar as shown in the inoculated plants (Figure 1A-D). Many flowers were found to be dried up before blooming. The petals of the infected flowers seemed to be irregular in size and shape. The color of the flowers of the infected plants was observed to be deep as compared to the flowers of the healthy plants. The infected plants were found to suffer from growth reduction which resulted severe stunting. The leaves of the infected plants were reduced in size and clustered at the top of the plants. The growth cessation of the infected plants was evident in all cases and the early infected plants were found to be half of the healthy counter parts. The symptom severity of the infected plants was evident as the development of necrosis at the top. All the symptoms what observed in the naturally infected summer cosmos plants were typical to the symptoms caused by plant viruses as described by Bos (1978) and Brunt *et al.* (1990). On the basis of the symptomatological study it might be concluded that the summer cosmos plants naturally developed symptoms might be due to infection of plant virus.

Figure 1. Inoculated Summer cosmos plant showing vein chlorosis, mosaic, yellowing, leaf reduction, leaf distortion, shoe-string and leaf necrosis (A – D).

Table 1. Virus disease-like symptoms in naturally infected Summer cosmos (*Cosmos sulphureus*) plants at BSMRAU campus

Plant parts	Symptoms
Leaves	The leaves of the infected plants developed mosaic accompanied with yellowing which was eventually visible as yellow mosaic symptom. The symptomatic leaves produced vein chlorosis along with mild curling. In many infected plants leaf distortion accompanied with shoe-string were found as prominent symptoms. Necrosis of the infected leaves was also observed in severely infected plants. Leaf size of the infected plants was drastically reduced as compared to the healthy plants. At the later stage the leaves of the infected plants seemed to be deformed.
Flowers	The symptomatic plants yielded small size, twisted and deform flowers. Many flowers were found to be dried up before blooming. The petals of the infected flowers seemed to be irregular in size and shape. The color of the flower of the infected plants was observed to be deep as compared to the flowers of the healthy plants.
Plants	The infected plants were found to suffer from growth reduction which resulted severe stunting. The leaves of the infected plants were reduced in size and clustered at the top of the plants. The growth cessation of the infected plants was evident in all cases and the early infected plants were found to be half of the healthy counter parts. The symptom severity of the infected plants was evident as the development of necrosis at the top.

Mechanical inoculation test

The mechanical inoculation test proved that the virus inciting symptoms naturally in the summer cosmos plants was readily sap transmitted to *Cosmos sulphureus, Chenopodium amaranticolor* and *Gomphrena globosa*. As shown in Figure 1 (A & B) the summer cosmos plant inoculated with sap extracted from naturally infected summer cosmos plants developed vein chlorosis, mosaic and yellowing. The inoculated plants showed leaf reduction, leaf distortion, shoe-string and leaf necrosis symptoms as shown in Figure 1 (C & D). *Chenopodium amaranticolor* inoculated plants developed necrotic local lesions as shown in Figure 2-A. Chlorotic local lesions were observed in the inoculated leaves of *Gomphrena globosa* when inoculated with the naturally infected samples of Summer cosmos (Figure 2-B). In all the cases the inoculated plants developed symptoms within 7-10 days of inoculation. The results of the mechanical inoculation test suggested that the virus causing symptoms in naturally infected Summer cosmos plants seemed to be mechanically inoculated.

Figure 2. Inoculated leaf of *Chenopodium amaranticolor* (A) and Gomphrena *globosa* (B) showing chlorotic local lesions and necrotic local lesions, respectively.

Isolation and propagation of virus

The inoculated summer cosmos plants produced symptoms identical to the naturally infected summer cosmos plants (Figure 3). The results suggested that the pure isolate of the virus obtained from local lesion isolation was the same, which naturally infected the Summer cosmos plant.

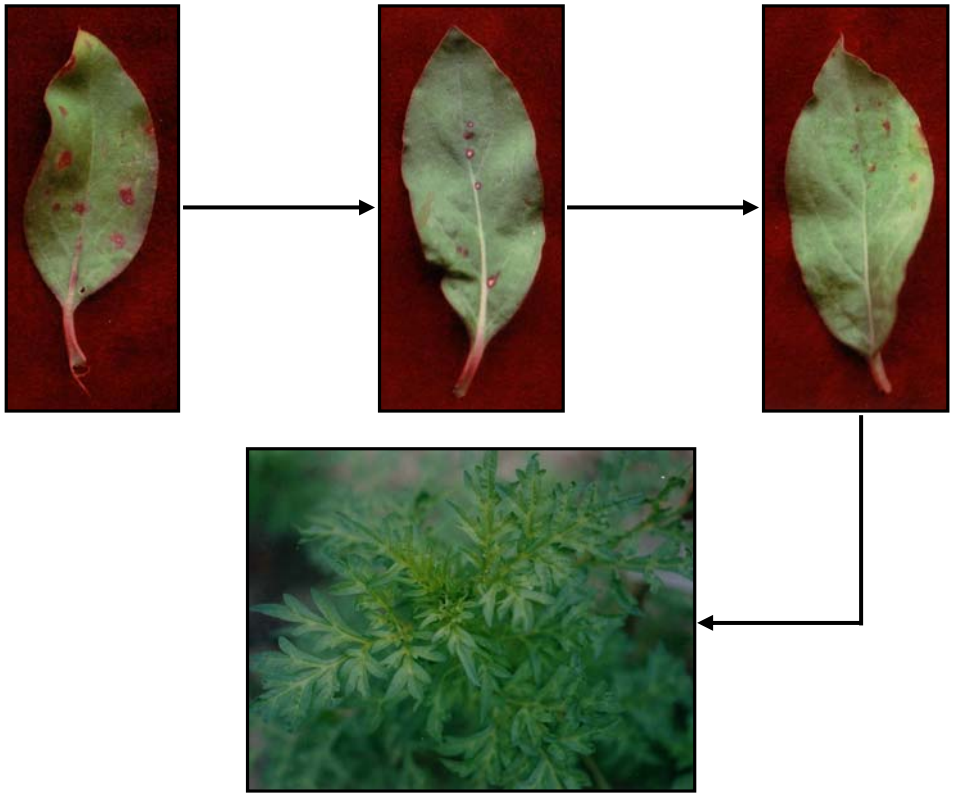

Figure 3. Pure isolate of the virus obtained from three successive inoculations on *G. globosa* and then Summer cosmos plant.

Host range test

The summarized results of the host range test are noted in Table 2. It was observed that the plant species belonging to six different families namely Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Leguminosae and Solanaceae were found to produce various types of symptoms upon mechanical inoculation of purified isolate of the virus. Among the plant species of Compositae and Cucurbitaceae, *Zinia elegans* and *Cytrullus lanatus,* respectively did not develop any symptom when mechanically inoculated with the virus infected summer cosmos. The symptoms developed in some of the inoculated plants are listed in Table 2. Necrotic local lesions developed on the inoculated leaves of *Gomphrena globosa* and *Pisum sativum*, while chlorotic local lesions developed in *Chenopodium amaranticolor, Datura stramonium, Datura metel* and *Physalis floridana*. Systemic yellow mosaic was observed in *Tagetes erecta* and *Cosmos sulphureus* while mosaic was recorded in *Cosmos bipinnatus*. Systemic mosaic was recorded as symptom on the inoculated leaves of *Cucumis sativus, Luffa cylindrica, Cucurbita moschata, Lagenaria siceraria, Luffa acutangula, Benincasa hispida, Trichosanthes anguina, Phaseollus vulgaris, Vigna unguiculata, Lycopercicon esculentum, Nicotiana glutinosa, Nicotiana tabacum* and *Nicotiana rustica.* Sultana (2004) got the similar result in host range test when she worked with the yellow mosaic symptom of marigold and finally identified the virus as *Cucumber mosaic virus*. The results of the present study demonstrated that the virus under present investigation have wide host range and the virus might be *Cucumber mosaic virus* as reported by Purcifull *et al.* (1984), Brunt *et al.* (1990) and Francki *et al.* (1979).

Table 2. Host range test

Family	English name	Botanical name	Symptom*
Amaranthaceae	Button flower	*Gomphrena globosa*	nll
Chenopodiaceae	Chenopodium	*Chenopodium amaranticolor*	cll
Compositae	Marigold	*Tagetes erecta*	ym
	Summer cosmos	*Cosmos sulphureus*	ym
	Winter cosmos	*Cosmos bipinnatus*	m
	Zinnia	*Zinnia elegans*	-
Cucurbitaceae	Cucumber	*Cucumis sativus*	sm
	Water melon	*Citrullus lanatus*	-
	Sponge gourd	*Luffa cylindrica*	sm
	Pumpkin	*Cucurbita moschata*	sm
	Bottle gourd	*Lagenaria siceraria*	sm
	Ridge gourd	*Luffa acutangula*	sm
	Wax gourd	*Benincasa hispida*	sm
	Snake gourd	*Trichosanthes anguina*	sm
Leguminosae	Bush bean	*Phaseollus vulgaris*	sm
	Pea	*Pisum sativum*	nll
	Cowpea	*Vigna unguiculata*	sm
Solanaceae	Tomato	*Lycopersicon esculentum*	sm
	Datura	*Datura stramonium*	cll
	Datura	*Datura metel*	cll
	Physalis	*Physalis floridana*	cll
	Tobacco	*Nicotiana glutinosa*	sm
	Tobacco	*Nicotiana tabacum*	sm
	Tobacco	*Nicotiana rustica*	sm

*nll= necrotic local lesion, cll= chlorotic local lesion, ym= yellow mosaic, sm= systemic mosaic, - = negative response.

Dilution end point test

The crude sap was extracted from the symptomatic Summer cosmos plant inoculated with the pure isolate and then inoculated on *Cosmos sulphureus, Gomphrena globosa* and *Chenopodium amaranticolor*. The results of the dilution end point test are summarized in Table 3. It was observed that the virus in crude extracted sap remained infective up to 10^{-6} in successive mechanical inoculation. It has been reported that the dilution end point of *Cucumber mosaic virus* (CMV) was 10^{-6} when inoculated on *Tagetes erecta* as reported by Brunt *et al.* (1990), Francki *et al.* (1979) and Purcifull *et al.* (1984). The results of the present study in respect of dilution end point determination suggested that the virus might be CMV.

Table 3. Dilution end point test

Dilution range	Inoculation response on *		
	C. sulphureus	*G. globosa*	*C. amaranticolor*
Undiluted	+	+	+
10^{-1}	+	+	+
10^{-2}	+	+	+
10^{-3}	+	+	+
10^{-4}	+	+	+
10^{-5}	+	+	+
10^{-6}	+	+	+
10^{-7}	-	-	-
10^{-8}	-	-	-
10^{-9}	-	-	-
10^{-10}	-	-	-
10^{-11}	-	-	-

* + = Symptom appeared, - = No symptom

Thermal inactivation point test

The results of the thermal inactivation point test are presented in Table 4. Three different plants namely *Cosmos sulphureus*, *Gomphrena globosa* and *Chenopodium amaranticolor* were used in

the test. The extracted crude sap of infected Summer cosmos leaves retained infectivity when boiled up to 65^0C for 10 minutes. Singh *et al.* (1999) reported that the thermal inactivation point of a strain of *Cucumber mosaic virus* (CMV) was detected as 60^0C while they were working with marigold plant. They used the crude sap from *Nicotiana glutinosa* plant instead of marigold. Brunt *et al.* (1990) noted the thermal inactivation point of CMV as 55 to 70^0C. The slight variation of thermal inactivation point occur might be due to variation of isolates, assay host and the plant sample used for inoculation and sap extraction. However the results of our study suggested that the virus under the present investigation might be a strain of CMV.

Table 4. Thermal inactivation point test

Temperature range (^{0}C)	Inoculation response on *		
	C. sulphureus	*G. globosa*	*C. amaranticolor*
Untreated	+	+	+
25	+	+	+
30	+	+	+
35	+	+	+
40	+	+	+
45	+	+	+
50	+	+	+
55	+	+	+
60	+	+	+
65	+	+	+
70	-	-	-
75	-	-	-
80	-	-	-
85	-	-	-
90	-	-	-

* + = Symptom appeared, - = No symptom

Longevity *in vitro* test

The crude sap of the symptomatic leaves of Summer cosmos plants retained infectivity up to 10 days at room temperature (30 ± 2^0C). The results of the longevity *in vitro* test are summarized in Table 5. As reported by Brunt *et al.* (1990) the *Cucumber mosaic virus* (CMV) remained infective in crude sap from 1 to 10 days. However they did not mention the name of the plant species used for sap extraction and inoculation. The results of the present study suggested that the virus under investigation might be the CMV in respect to its longevity *in vitro* test.

Table 5. Longevity *in vitro* test

Longevity period (day)	Inoculation response on *		
	C. sulphureus	*G. globosa*	*C. amaranticolor*
0	+	+	+
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	+
10	+	+	+
11	-	-	-
12	-	-	-
13	-	-	-
14	-	-	-
15	-	-	-
16	-	-	-
17	-	-	-
18	-	-	-

* + = Symptom appeared, - = No symptom

Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay

The results of Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA) are noted in Table 6. Both the samples i.e. naturally infected and mechanically infected Summer cosmos plants were found to be reacted positively against the antisera of *Cucumber mosaic virus* (CMV). The negative reaction was observed against the antisera of *Papaya ring spot virus* (PRSV), *Watermelon mosaic virus-2* (WMV-2), *Zucchini yellow mosaic virus* (ZYMV) and *Potato virus-Y* (PVY). The natural occurrence of CMV in marigold was reported by Hanson *et al.* (1951), Josshi and Dubey (1972) and Sang and Varma (1975). Sultana (2004) detected the natural infection of marigold plant collected from Joydebpur, Gazipur area. The results of the DAS-ELISA suggested that the Summer cosmos plant collected from Bangabandhu Sheikh Mujibur Rahman Agricultural University campus, Joydebpur, Gazipur was infected with CMV.

Table 6. Results of DAS-ELISA

Sample	Reaction against the antibody of *									
	CMV		PRSV		WMV-2		ZYMV		PVY	
	VO	OD	VO	OD	VO	OD	VO	OD	VO	OD
Naturally infected Summer cosmos leaves	+	1.65	-	0.05	-	0.17	-	0.13	-	0.16
Mechanically inoculated Summer cosmos leaves	+	1.45	-	0.11	-	0.14	-	0.11	-	0.13
Positive control	+	1.94	+	0.99	+	1.20	+	1.05	+	1.11
Negative control	-	0.08	-	0.16	-	0.19	-	0.15	-	0.14

* VO = Visual observation, OD = Optical density, + = Positive response, - = Negative response, CMV = *Cucumber mosaic virus*, PRSV = *Papaya ring spot virus*, WMV-2 = *Watermelon mosaic virus-2*, ZYMV = *Zucchini yellow mosaic virus,* PVY = *Potato virus-Y*

CONCLUSION

The results obtained in the study revealed that the virus disease-like symptoms naturally developed in Summer cosmos were sap transmissible and the bio-assay (host range test, dilution end point, thermal inactivation point, longevity *in vitro*) suggested that the virus infecting Summer cosmos was similar to CMV. Finally, Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA) detected the virus from Summer cosmos as CMV. It is the first report of CMV infecting Summer cosmos in Bangladesh. However, an in-depth study needs to be done with the naturally infected Summer cosmos since CMV causes major diseases of Cucurbits and many other crops.

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