Research Paper *Published Online: 18.10.2014, Vol. 02(01): 23-35, 2014*

An Investigation to Virus Like Diseases of Marigold

R. Sultana[1], A. M. Akanda[2], M. A. S. Haque[3], A. Majumdar[4] and M. A. Z. Al Munsur[4*]

Dept. of Agriculture Extension, Ministry of Agriculture (MOA), Khamarbari, Dhaka[1]
Dept. of Plant Pathology, BSMRAU, Gazipur[2]
International Development Enterprises, Bangladesh[3]
Dept. of Agriculture Extension, MOA, Khamarbari, Dhaka, Bangladesh[4]

Corresponding autor*: *zafurbau@gmail.com*

Abstract

A study was conducted to identify the cause of virus disease-like symptoms developed naturally in marigold (Tagetes erecta L.) plants in Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur.The natural symptoms in marigold consisted of yellowing, mosaic, shoestring, mottling and curling of leaves resulting sever stunting of the infected plants. The ailments were found to be sap transmissible to marigold inducing identical symptoms as observed in naturally infected plants. Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA) and Indirect Enzyme-Linked Immuno-Sorbent Assay (I-ELISA) detected the mixed infections of Cucumber mosaic virus (CMV) and Papaya ringspot virus (PRSV) from the naturally infected marigold plants. Chenopodium amaranticolor and Gomphrena globosa were found to be good local lesion host producing chlorotic and necrotic local lesions in the inoculated leaves respectively. The pure isolates obtained from the local lesion by using Chenopodium amaranticolor when used for host range test. It *was observed that the virus had wide host range which included the plants of Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Leguminosae, Malvaceae and Solanaceae. The dilution end point, thermal inactivation point and longevity in vitro were determined as 10^{-6}, 65C and 9 days, respectively. Moreover, the pure isolate obtained from the local lesion did not produce some symptoms like shoestring in marigold which observed in naturally infected marigold plants. The host range test, dilution end point, thermal inactivation point and longevity in vitro revealed that the purified isolate was the CMV. The results suggested that the virus was CMV, but not PRSV from the naturally infected marigold samples. The results of the study revealed that the virus disease-like symptoms manifested in marigold plants had mixed infection of CMV and PRSV.*

Key Words: *Investigation, Virus, Disease, Marigold, Tagetes erecta* L.

I. Introduction

Marigold (*Tagetes erecta* L.) belongs to Compositae family is a common, popular and important ornamental flowering plant. The marigold was first regarded with distrust due to its strong free flowering, short duration to produce marketable flowers, wide spectrum of attractive colour, shape, size and good keeping quality attracted the attention of flower growers. It is one of the most commonly grown flowers and extensively used on religious and social functions, in one forms or other (Bos and

Yadav 1998). The plant is grown in homes, gardens, fields, in front of community and social institution for aesthetic purpose. It is economically important for its showy flowers, tagetes oil and anti-nematicidal properties (Usman*et al*. 1972). Flowers are sold in the market as loose or after making into garlands. Other than cut-flower, marigold especially is used for beautification and also used in landscape plants due to its variable height and colour of flowers. It is highly suitable as a bedding plant. Both leaves and flowers are equally important from medicinal point of view. Leaf paste is used externally against boils and carbuncles. Leaf extract is good remedy for ear ache. Flower extract is considered as blood purifier, a cure for bleeding piles and is also a good remedy for eye diseases and ulcers (Bos and Yadav 1998).

Some of the marigold plants developed virus disease like symptoms consisting of yellowing, mosaic, vein chlorosis and mild curling. The infected plants were stunted yielding poor number of twisted and deformed flowers. The growth reduction appeared as the production of small leaves clustering around the main stem. The severely infected plants tended to cease growth showing necrosis at the top. So far there was no report available on marigold virus disease in Bangladesh. It appeared that marigold plant is naturally attacked by three different viruses' viz. *Cucumber mosaic virus* (CMV) as reported by Hanson *et al*. (1951), *Marigold mosaic virus* (MMV) and *Marigold mottle virus* as reported by Naqvi*et al.* (1981). CMV is identified as a member of cucumovirus which is characteristically transmitted by *Aphis gossypii*, *A. craccivora* and *Myzus persicae*. CMV was found to be seed transmitted in case of marigold plants. Naqvi *et al.* (1981) reported a potyvirus causing mosaic disease in marigold and they named it as *Marigold mosaic virus.* In fact marigold becomes an economically important year round ornamental plant in recent days in Bangladesh. It has even been cultivating commercially in some places of the country and its plays a vital role in flower business in Bangladesh. So the virus like disease what observed in marigold causing severe flower yield and quality deterioration need to be identified with systemic approach. It is immensely important to design management practices of the disease. As because vegetative propagation of the plant facilitates the early cultivation and produces better size flower. So, if the marigold is affected with virus (es) having natural crop hosts that must be threat of crop cultivation as well. The present research program was undertaken to identify the causal agent of virus disease-like symptoms of marigold and the crop hosts of the causal agent infecting marigold.

II. Materials and Method

The experiment was carried out in the Plant Pathology laboratory and insect proof net house of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur from Sept. 2003 to April 2004.

Symptomatological study on the naturally infected marigold plants: The marigold plants grown at a house of Joydebpur town, Gazipur developed virus disease like symptoms were examined. The symptoms expressed in naturally infected marigold plants were observed carefully and the ailments developed in the leaves and flowers of the plants were noted. The photographs of the symptoms were taken for further illustration.

Sample collection and preservation: Some of the naturally infected marigold plants produced distinct and identical symptoms were uprooted with proper care and transplanted in earthen pots and these were used to collect sample for further study. Other than these plants, the leaf samples were also collected from the other symptomatic plants showing similar symptoms. The collected leaves were cut into small pieces and preserved at 4°C in the plastic petridishes containing silica gel. A piece of blotter paper was put on the silica gel in each plastic petridish to spread the cut sample on to it. The plastic petridishes were then wrapped with scotch tape to make it air tight before being placed for preservation (Noordam 1973, Gibbs and Harrison 1979). The preserved samples were also 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 such as *Tagetes erecta*, *Chenopodium amaranticolor* and *Gomphrena globosa* were inoculated for the test. The plants were grown in the five inches earthen pots. In each pot manure and fertilizers were used as per recommendation (Akanda 1991). The irrigation and other necessary care were done to ensure the normal growth of the plants. Three different buffers namely 0.01M Potassium phosphate buffer pH 7.0, Sodium phosphate buffer pH 7.0 and Sodium potassium phosphate buffer pH 7.0 were used for extracting the sap from the symptomatic leaves of infected marigold plants. A total of 5g fresh leaves was collected from the infected marigold plants in each case and macerated using 50ml aforeside buffers for sap extraction. The leaves of the test plants were dusted with sterilized 600 mesh carborundum powder before inoculation. The dusted leaves were then rubbed with finger soaked in the sap. After rubbing with sap the inoculated leaves were carefully washed with sterilized water using wash bottle. Then the inoculated plants were placed in an insect proof net house and checked everyday to detect the appearance of symptoms. The symptoms appeared in the inoculated plants were noted and photographs of the symptoms were taken for interpretation of the ailments.

Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA): DAS-ELISA as outlined by Clark and Adams (1977) was employed to test the samples against the antibodies of *Cucumber mosaic virus* (CMV), *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* 2 (WMV 2) and *Zucchini yellow mosaic virus* (ZYMV) with some modifications. In the test PathoScreen Kit prepared by Agdia Incorporated, 30380 County Road 6, Elkhart, Indiana 46514 USA was used. The methods and application procedures written in the manual of Agdia Incorporated were followed in performing DAS-ELISA. The leaves of the naturally infected marigold plants and inoculated marigold plants were collected and the sap was extracted at the ratio of 1:10 (tissue weight: extraction buffer volume). The extracted sap was poured @ 100 □ l per well ofthe ELISA plate which was precoated with virus specific IgG. The plate having sap was then incubated for 2 hrs.in a humid box at room temperature. The plate was washed for 5-6 times with washing buffer after incubation and then enzyme conjugate was dispensed @ 100 □ l per well. The procedures as mentioned before were applied for incubation and washing of the plates. The substrate solution was prepared following the instructions of the manual then used @ 100 □ l per well. The plate was incubated in a humid box at room temperature for 60 min. The reaction was stopped by pouring 50 □ l of 3M sodium hydroxide per well of microtitre plate just after 60 min of incubation. In case of positive reaction yellow color was developed in the plate which was observed visually. The ELISA Reader EAR 400 FW was used to measure the optical density (OD) values of the well at 405 nm wavelength. The positive and buffer control were maintained.

Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA): I-ELISA was performed following the procedures written in PathoScreen Kit. I-ELISA was carried out for the detection of PVY. However, procedures indicated in Agdia Incorporated manual was followed, although the principles were the same as developed by Clark and Adams (1977). In I-ELISA the sample extract was dispensed @ 100 □ l per well of the ELISA plate which was not precoated with IgG as in DAS- ELISA. The virus specific IgG was poured @ 100 □ l per well after washing the samples. Incubation, washing and subsequent other steps were followed as same as DAS- ELISA.

Local lesion isolation: The local lesion isolation was done using *C. amaranticolor* plants. The leaves of *C.amaranticolor* were mechanically inoculated following the methods as described before. For the purpose, the sap was extracted taking the leaves of naturally infected marigold plants. After 5-8 days of inoculation single local lesions were developed in the leaves of *C. amaranticolor*plants. A sterilized razor blade was used for cutting the single local lesion and placed in a mortar containing a drop of 0.01M Potassium phosphate buffer pH 7.0 and macerated the lesion with a pastle. The sap was then mechanically inoculated to the healthy *C. amaranticolor* leaves. Four successive inoculations were done in the local lesion host for obtaining the pure isolate of the virus. Finally, the local lesion

extracted sap was also inoculated to the marigold plants to observe the symptoms development. The pure isolate of the virus was preserved at 4^0C on silica gel following the method described earlier and also maintained in marigold plant for further study. During virus isolation 600 mesh carborundum powder and 0.01M Potassium phosphate buffer pH 7.0 was used. If it was not otherwise stated the entire works were conducted in an insect proof net house.

Host range test: In all 34 plant species belonging to eight dicotyledonous plant families (*Amaranthaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Leguminosae, Malvaceae,* and *Solanaceae*) and one monocotyledonous family (Gramineae) were mechanically inoculated to perform the host range test. The test plant species were *G. globosa, C. amaranticolor, T. erecta, Brassica oleracea* var. *capitata, B. oleracea* var. *botrytis, Cucumis melo, C. sativus, Citrullus lanatus, Luffa cylindrica, L. acutangula, Cucurbita moschata, Lagenaria siceraria, Benincasa hispida, Momordica charantia, Oryza sativa, Triticum aestivum, Zea mays, Glycine max, Phaseolus vulgaris, Pisum sativum, Lablab purpureus, Vigna sinensis, V. unguiculata, Abelmoschus esculentus, Hibiscus cannabinus, H. rosa-sinensis, Capsicum annuum, Solanum melongena, Lycopersicon esculentum, Datura metel, D. stramonium, Physalis floridana, Nicotiana tabacum* and *N. glutinosa*. The virus inocula were prepared by macerating virus infected leaves of marigold prior inoculated with the pure isolate of virus obtained from local lesion isolation in 0.01M Potassium phosphate buffer pH 7.0. The carborundum powder (600 mesh) dusted leaves of the test plants were rubbed with finger dipped in extracted sap. After inoculation test, plants were placed in an insect proof net house and observation was made up to six weeks for symptom development. The leaves of the asymptomatic plants were used as samples for back inoculation to marigold / *C. amaranticolor*for further confirmation of the virus host.

Dilution end point test: The dilution end point of the virus was determined using the method as described by Noordam (1973). The leaves of the marigold plants inoculated with the pure isolated viruswere used for dilution end point determination test. 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 infected marigold leaves. The dilutions were prepared using 0.01M Potassium phosphate buffer pH 7.0. The sap was inoculated to the *G. globosa,C. amaranticolor* and *T. erecta* plants. The inoculated plants were maintained in an insect proof net house to observe the appearance of the symptoms.

Thermal inactivation point test: The sap used in dilution end point test was also used for thermal inactivation point test. The test was conducted following the method of Noordam (1973). A single dilution 10^{-3} was heated in test tubes in a temperature control water bath for 10 min in each case. The temperature range was 25^0C to 90^0C with 5^0C interval. For symptoms development the untreated and treated sap were inoculated to *G. globosa,C. amaranticolor* and *T.erecta*.

Longevity *in vitro* (aging) determination: Longevity *in vitro* (aging) was determined following the methods described by Noordam (1973). The sap of the infected leaves was diluted upto 10^{-3} and filtered through cheese cloth. After pouring the test tubes by extracted sap at the rate of 2ml/ test tube, the tubes were sealed with aluminium foil. Then the test tubes were kept at room temperature (30 ± 2^0C) in the laboratory rack. The sample was inoculated to *G. globosa,C. amaranticolor* and *T. erecta* every day and continued upto 18 days. The inoculated plants were observed for six weeks to notice the development of the symptoms.

III. Results and Discussion

Symptomatological study on the naturally infected marigold plants

The leaves of the infected plants developed yellow mosaic symptoms. The symptomatic leaves showed vein chlorosis along with mild curling. The shoestring and leaf distortion types of symptoms were also

present with deformation. Downward curling at the edge of the leaves was observed. Leaf size was reduced to a great extent as compared to apparently healthy plants. The size and shape of the flowers were small and irregular as compared to healthy ones. The color of the flowers seemed to be dull. The flowers were not blossomed fully. The growth reduction resulted severe stunting of the infected plants was commonly observed. The severity of this disease was evident with the development of necrosis at the top.

The symptoms observed in the naturally infected marigold plants seemed to be typical virus disease like symptoms as described by Bos (1969). These symptoms developed in naturally infected marigold plants were found to be identical to either *Cucumber mosaic virus* (CMV) or potyvirus as described by Hanson *et al.* (1951), Sang and Varma (1975), Joshi and Dubey (1975), Naqvi*et al.* (1981), Usman*et al.* (1981) and Rahman and Rao (1992). On the basis of this symptomatological study it was concluded that the marigold plants naturally developed symptoms might be due to infection of any virus(es).

Plate 1. Naturallyinfected marigold plant showing vein chlorosis with mild curling (A-C)

Plate 2. Naturallyinfected marigold plant showing shoestring,leaf distortion, deformation and downward curling (A-C)

Mechanical inoculation test

The virus causing symptoms naturally in the marigold plant was proved to be sap transmitted to *T. erecta*, *G.globosa*, *C.amaranticolor*, but did not show any symptom when inoculated on *H.rosa-sinensis*. The sap inoculated marigold plant produced yellow mosaic, vein-chlorosis, leaf curling and shoestring symptoms along with clustering of leaves and growth cessation symptoms as shown in Plate 3. The inoculated *C.amaranticolor* plant developed chlorotic local lesions which turned into dark brown color at the later stage which was surrounded by yellow color (Plate 4). *G.globosa* inoculated plants were found to develop necrotic local lesion on leaves (Plate 5). *H.rosa-sinersis* failed to develop symptoms after inoculation and the virus was not recovered on back inoculation.

It was observed from the mechanical inoculation test that among the three buffers potassium phosphate buffer pH 7 was most effective buffer used for mechanical inoculation (Table 1). The rest of the two buffers were showed similar performance in case of *T.erecrta* only. So the Potassium phosphate buffer was used in all the subsequent inoculation tests. Similar results were reported by Singh *et al.* (1999) while working with a strain of CMV he used 0.05M Phosphate buffer pH 7.0. It was not clear that he used Potassium phosphate buffer. Lot *et al.* (1972) and Clark *et al.* (1974) noted also similar results

while they worked with CMV. The results of the mechanical inoculation test suggested that the virus causing symptoms in naturally infected marigold plant might be a virus identical to CMV.

Plate 3. Inoculated marigold plant showing yellow mosaic, vein-chlorosis (A), leaf curling (B), shoestring and clustering of leaves (C)

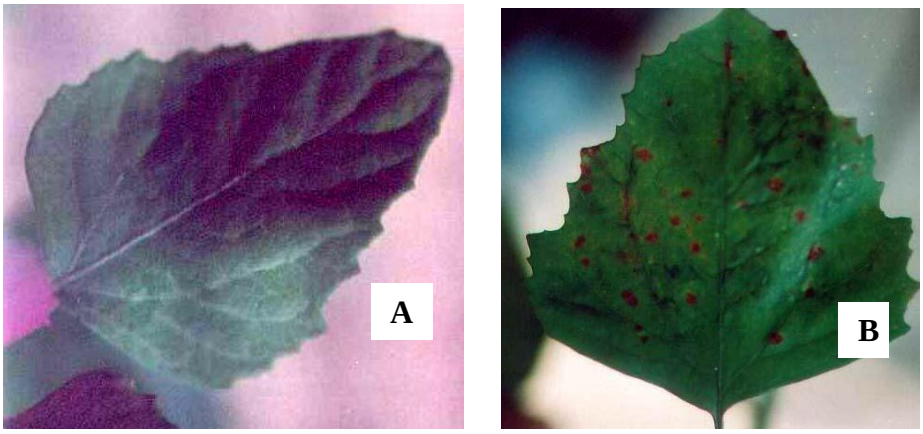

Plate 4. Healthy leaf of *Chenopodium amaranticolor* (A) and inoculated leaf showing chlorotic dark brown local lesions surrounded by yellow color (B)

Plate 5. Healthy leaf of *Gomphrena globosa* (A) and inoculated leaf showing necrotic local lesion on leaves (B)

Table 1. Performance of three buffers used in mechanical inoculation test

Plants	Buffer used *		
	K-K	Na-Na	Na-K
Tagetes erecta	+	+	+
Gomphrena globosa	+	-	-
Chenopodiumamaranticolor	+	-	-
Hibiscus rosa-sinensis	-	-	-

* K-K - 0.01M Potassium phosphate buffer pH 7.0, Na-Na - 0.01M Sodium phosphate buffer pH 7.0 and Na-K -0.01M Sodium potassium phosphate buffer pH 7.0

Serological test

Both the samples were found to be reacted positively against the antibodies of two different viruses namely *Cucumber mosaic virus* (CMV) and *Papaya ring spot virus* (PRSV) (Table 2). The negative reactions were observed against *Watermelon mosaic virus* 2 (WMV 2), *Zucchini yellow mosaic virus* (ZYMV) and *Potato virusY* (PVY). The results suggested that the naturally infected marigold plants produced virus disease-like symptoms due to double infection of CMV and PRSV. The natural occurrence of CMV in marigold was reported by Hanson *et al*. (1951), Joshi and Dubey (1972) and Sang and Varma (1975). But there was no report on the natural infection of PRSV in marigold, although Naqvi*et al*. (1981), Lockhart (1989) and Rahman and Rao (1992) reported the occurrence of potyvirus in marigold. However, they did not specify the name of the potyvirus infecting marigold in their report.

Table 2. Results of DAS-ELISA and I-ELISA

Sample	Reaction against the antibodies of *									
	CMV		PRSV		WMV 2		ZYMV		PV Y	
	VO	OD	VO	OD	VO	OD	VO	OD	VO	OD
Naturally infected marigold leaves	+	1.563	+	0.229	-	0.086	-	0.138	-	0.146
Inoculated marigold leaves	+	1.405	+	0.416	-	0.085	-	0.146	-	0.138
Control	+	1.849	+	0.538	+	2.472	+	3.00	+	0.971

*VO –visual observation, OD – optical density, + Positive response, -Negative response, CMV- *Cucumber mosaic virus*, PV Y – *Potato virus* Y, PRSV- *Papaya ring spot virus*, ZYMV- *Zucchini yellow mosaic virus*, WMV 2- *Watermelon mosaic virus* 2.

Local lesion isolation

The local lesion isolation was accomplished by using *C.amaranticolor* followed inoculating the pure isolate in marigold plant (Plate 6). The pure isolate of the virus obtained from successive four inoculated *C.amaranticolor* plants taking single lesion in each step when inoculated to the marigold plant, it was observed that yellow mosaic and curling of leaves developed (Plate 6). The marigold plants did not produce shoestring symptoms and severity of the symptom was not similar to the either original ones or the inoculated marigold plants. Moreover, the chlorotic local lesions what observed in *C.amaranticolor* were similar to CMV as reported by Brunt *et al*. (1997) and Franck*et al*. (1979). The results demonstrated that the another virus i.e., PRSV detected by DAS-ELISA from the original sample as well as from the marigold symptomatic plants inoculated with original sample did nit produce local lesion in *C. amaranticolor*. So the pure isolate seemed to be the CMV only i.e., CMV

was recovered through local lesion isolation excluding PRSV. Moreover, it could be referred here that *C. amaranticolor* produced local lesion when inoculated with PRSV, but not for all isolates of the virus as reported by Purcifull*et al.* (1984) and Brunt *et al*. (1990). The purified isolate did not produce shoestring and leaf distortion type of symptoms those were regarded as characteristics symptoms of PRSV usually developed in most of the hosts infected by the virus indeed Purcifull*et al.* (1984).

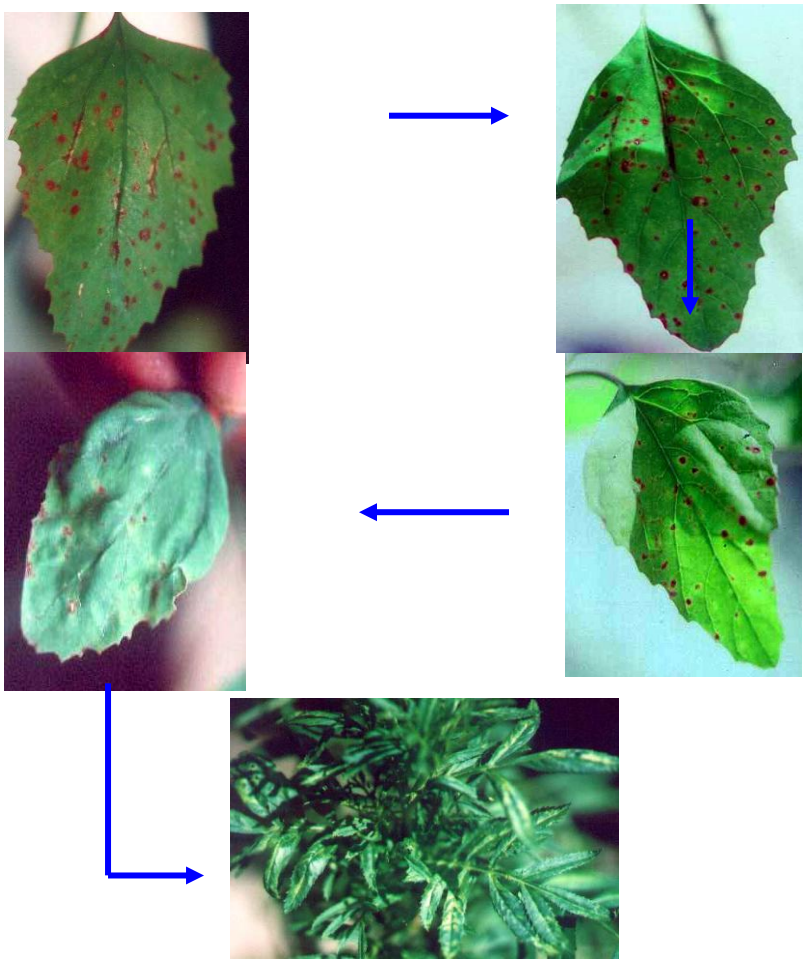

Plate 6. Pure isolate of the virus obtained from four successive inoculation on *C.amaranticolor* and then marigold plants

Host range test

It was observed that the plant species belonging to seven different families namely Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Leguminosae, Malvaceae and Solanaceae were found to response positively upon mechanical inoculation and produced various types of symptoms. The plant species of Cruciferae and Gramineae did not develop any symptom when mechanical inoculation was done using virus infected marigold sample. The inoculated *C. amaranticolor*, *D. metel* and *D. stramonium* developed chlorotic local lesion, while necrotic local lesion was observed in *G. globosa* and *P. vulgaris*. The yellow mosaic was observed in marigold plants. Systemic mosaic was induced in musk melon, cucumber (Plate 7 A), watermelon, snake gourd (Plate 7 B), pumpkin (Plate 7 C), bottle gourd, ridge gourd (Plate 8 A), wax gourd (Plate 8 B), okra (Plate 8 C), *N. tabacum*, *N. glutinosa*, *Glycine max*, *Pisum sativum*, *Vigna sinensis*, *V. unguiculata*, *Capsicum annuum*, *Solanum melongena*, *Lycopersicon esculentum* and *Physalis floridana* upon inoculated with the sample of infected marigold. The results suggested that the virus under investigation had wide host range and identical to CMV as reported by Purcifull*et al*. (1984), Brunt *et al.* (1990) and Franckiet *al.* (1979).

Plate 7. Inoculated leaves showing systemic mosaic on *Cucumis sativus* (A),*Luffa cylindrica* (B) and *Cucurbita moschata* (C)

Plate8. Inoculated leaves showing systemic mosaic on *Luffa acutangula* (A), *Benincasa hispida* (B) and *Abelmoschus esculentus* (C)

Dilution end point determination

The extracted crude sap infected marigold leaves was inoculated on *G. Globosa, C. amaranticolor*and *T. erecta.* It was observed that the virus remained infective up to 10^{-5} in successive mechanical inoculation test (Table 4). It was reported that the dilution end point of CMV was 10^{-6} when inoculated on *T. erecta,* but the dilution end point of PRSV was 10^{-3} as reported by Brunt *et al.* (1997). In respect to dilution end point, the virus under the present study was almost identical to CMV, but different than that of PRSV. So, the results suggested that the virus might be CMV.

Thermal inactivation point determination

The extracted crude sap of infected marigold leaves remained infective when boiled up to 65°C for 10 minutes and the test was carried out by inoculating *T. erecta*, *G. globosa*, *C. amaranticolor* (Table 5). Singh *et al.* (1999) reported that thermal inactivation point of the strain of cucumber mosaic cucumovirus was 60°C while they were working with marigold plant. They used the sap from *N. glutinosa* instead of marigold. Moreover, the thermal inactivation of PRSV was reported to be 54-60°C (Purcifull*et al.* 1984, Brunt *et al.* 1997). Brunt *et al.* (1997) noted the thermal inactivation point of CMV as 55-70°C. The slight variation might be due to variation of isolates, assay host and the plant sample used for inoculation. However, the results of the present study suggested that the virus might be a strain of CMV.

Table 3. 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
Cruciferae	Cabbage	*Brassica oleracea var. capitata*	-
	Cauliflower	*Brassica oleracea var. botrytis*	-
Cucurbitaceae	Muskmelon	*Cucumis melo*	CS, SM
	Cucumber	*Cucumis sativus*	SM
	Water melon	*Citrullus lanatus*	-
	Snake gourd	*Luffa cylindrical*	SM
	Pumpkin	*Cucurbita moschata*	SM
	Bottle gourd	*Lagenaria siceraria*	SM
	Ridged gourd	*Luffa acutangula*	SM
	Wax gourd	*Benincasa hispida*	SM
	Bitter gourd	*Momordica charantia*	-
Gramineae	Rice	*Oryza sativa*	-
	Wheat	*Triticum aestivum*	-
	Corn	*Zea mays*	-
Leguminosae	Soybean	*Glycine max*	CS, SM
	Bush bean	*Phaseolus vulgaris*	NLL
	Pea	*Pisum sativum*	NLL
	Country bean	*Lablab purpureus*	-
	Blackgram	*Vigna sinensis*	NLL
	Cowpea	*Vigna unguiculata*	SM
Malvaceae	Okra	*Abelmoschus esculentus*	CS, SM
	China rose	*Hibiscus rosa-sinensis*	-
	Kenaf	*Hibiscus cannabinus*	-
Solanaceae	Chilli	*Capsicum annuum*	SM
	Brinjal	*Solanum melongena*	SM
	Tomato	*Lycopersicon esculentum*	SM
	Datura	*Datura metel*	CLL
	Datura	*Datura stramonium*	CLL
	Physalis	*Physalis floridana*	CLL
	Tobacco	*Nicotiana tabacum*	SM
	Tobacco	*Nicotiana glutinosa*	SM

*NLL -Necrotic local lesion, CLL -Chlorotic local lesion, YM- Yellow mosaic, SM -Systemic mosaic, CS -Chlorotic spots, and - Did not react

Table 4. Dilution end point test

Dilution range	Inoculation response on*		
	T. erecta	*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 and - = No symptom

Table 5. Thermal inactivation point test

Temperature range (°C)	Inoculation response on*		
	T. erecta	*G.globosa*	*C.amaranticolor*
Untreated	+	+	+
25	+	+	+
30	+	+	+
35	+	+	+
40	+	+	+
45	+	+	+
50	+	+	+
55	+	+	+
60	+	+	+
65	+	+	+
70	-	-	-
75	-	-	-
80	-	-	-
85	-	-	-
90	-	-	-

***** + = Symptom appeared and - = No symptom

Longevity *in vitro*

The sap of the symptomatic leaves of marigold plants were found to retain infectivity upto 9 days when stored at room temperature (30 ± 2°C) when tested by inoculating *T. erecta*. But when tested by inoculating *C. amaranticolor* and *G. globosa* the infectivity was found to persist upto 8 days (Table 6). As reported by Brunt *et al*. (1997) the CMV remained infective in crude sap from 1 to 10 days. However, they did not mention the name of the plant used for sap extraction and inoculation. Purcifull*et al*. (1984) determined the longevity *in vitro* of PRSV as 8 hrs. The results of the present study suggested that the virus under investigation was close to CMV in respect to its longevity *in vitro*.

Table 6. Longevity in vitro (aging) test

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

***** **+** = Symptom appeared and **-** = No symptom

IV. Conclusion

It can be concluded from the experiment that the virus disease-like symptoms naturally developed in marigold plants were mechanically sap transmissible. DAS-ELISA and I-ELISA detected the mixed infection of CMV and PRSV from the naturally infected marigold plants. The bioassay revealed that the purified isolate was the CMV. The results suggested that the virus was CMV, but not PRSV from the naturally infected marigold samples. The results of the study revealed that the virus disease-like symptoms manifested in marigold plants had mixed infection of CMV and PRSV.

V. Acknowledgements

The authors express their immense indebteness and profound gratude to Prof. Dr. Abdul Mannan Akanda, Dept. of Plant Pathology, Bangabandu Sheikh Mujibur Rahman Agricultural University, Gazipur. The autors extend their heartful thanks to Prof. Dr. Khurshid Alam Bhuiyan and Prof. Dr. Jalal Uddin Ahmed for cooperation and encouragement. The author also extends their coordial thanks to the laboratory staff of Plant Pathology, Dept. of BSMRAU, Gazipur.

VI. Reference

Akanda, M. A. M. (1991). Studies on the virus and Mycoplasma diseases of crops in Bangladesh. Ph. D. Thesis. Kyushu Univ., Japan. 180 pp.

Bos,T. K. and L. P. Yadav (1998). Commercial Flowers. Naya Prokash, 2069 Bidhan Sarani, Calcutta700 006, India.713-731.

Brunt, A., K. Crabtree and A. Gibbs.(1990). Viruses of Tropical Plants. Redwood Press Ltd., Melksham, Wilshire, UK. 293-297 pp.

Brunt,A., K. Crabtree, M. J. Dallwitz; A. J.Gibbs and L. Watson (1997). Viruses of Plants. University Press, Cambridge, UK.650-654 pp.

Clark, G. L., K. W. C. Peden and Symons, R. H. (1974). Virology 62:434.

Clark, M. F. and A. N. Adams (1977). Characteristics of the microplate method of ELISA for detection of plant viruses. J. Gen. Virol. 34: 475-483.

Francki, R. I. B., D. W. Mossop and T. Hatta. (1979). CMI / AAB. Description of Plant Viruses. No. 213: 6.

Gibbs, A. J. and B. D. Harrison (1979). Plant virology- the principles. Edward Arnild. London. 467 pp.

Hanson, H. R., H. R. Weber and Troelsen Johansan, G. (1951). Plant disease in Denmark in 1949. Annual survey of data collected by the state phytopathological service, Lyngby. T. Planteavl. 55:1-81.

Hill, S. A. (1984). Methods in Plant Virology.Vol. I. Backwell, London, 167 pp.

Joshi, R. D. and L. N. Dubey (1972). Studies on a mosaic disease of marigold (*Tagetes erecta* L.) in U. P. Science and Cult. 38:147-148.

Lockhart, B. E. L. (1989). Recurrence of natively occurring *Potato yellow dwarf virus* in Minnesota.Plant Disease 73: 321-323.

Lot, H., J. Marrou., J. B. Quiot and C. Esvan. (1972). Annls. Phytopath. 4:25 (Appeared in CAB Abstract).

Moini, A. A. and K. Izadpanah. (2000). New hosts for *Tomato spotted wilt virus* in Tehran. Indian J. Plant Pathol. 36:3-4.

Naqvi, Q.A. and K. Mahmood. (1978). Purification and properties of *Brinjal mild mosaic virus*. Phytopathol. Z. 93:86-89.

Naqvi, Q.A., S. Hadi and K. Mahmood. 1981. *Marigold mottle virus* in Aligarh, India. Plant Disease 65:271-275.

Noordam, D. (1973). Identification of plant viruses- methods and Experiments. Centre for Agricultural Publishing and Documentation, Wageningen, the Netherlands. 207 pp.

Purcifull, D. E., J. R. Edwardson, E. Hiebert and D. Gonsalves. 1984. CMI / AAB. Description of Plant Viruses. No. 292: 7.

Rahaman, M. A. and N. G. Rao. (1992). A mosaic disease of marigold caused by a potyvirus. Indian J. Plant Protection 20(2): 239-240.

Sang, A. and A. Varma (1975). *Marigold mosaic virus*. Phytopathol. Z. 84:10-17.

Singh Doomar, Q. A. Naqvi and I. D. Garg.(1999). A strain of cucumber mosaic cucumovirus causing mosaic in marigold in India. Indian Phytopathology 52: 2, 114-117.

Usman, K. M., G. Ramakrishnan and T. K. Kandaswamy (1972). A note on the occurrence of mosaic disease on marigold, *Tagetes erecta* Linn. Science and Culture 38: 489.

Citation for this article (APA Style):

Sultana, R., Akanda, A. M., Haque, M. A. S., Majumdar, A., Munsur, M. A. Z. Al., 2014. An Investigation to Virus Like Diseases of Marigold. *Journal of Bioscience and Agriculture Research, 02(01), 16-23*. Retrieved October 18, 2014, from http://www.journalbinet.com/current-issue-jbar-1.html.