

Natural Occurrence and Host Range Studies of *Cucumber Mosaic Virus* (CMV) Infecting Ornamental Species in the Rawalpindi-Islamabad Area of Pakistan

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Surveys were conducted during the spring and autumn of 2012 and 2013 to determine the presence, prevalence and distribution of *Cucumber mosaic virus* (CMV) on ornamental plants in the Rawalpindi-Islamabad area of Pakistan. A total of 1,783 symptomatic leaf samples (1,733 from ornamentals and 50 from weeds) were collected from 79 ornamental plant species in propagation sites such as nurseries, private gardens and public areas including parks and street-grown ornamentals. All the samples were subjected to double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using monoclonal antibodies for the detection of CMV. The disease incidence varied from season to season and nursery to nursery. The highest incidence of 46.47% and 35.01% of CMV was observed in public areas and propagation sites, respectively, during autumn 2012. On the other hand, the incidence was found to be 62.24% and 42.66%, respectively, in these sites during spring 2012. Similarly, the incidence was 59.28% and 47.36% during autumn 2013 and 66.5% and 51.69% during spring 2013 in public areas and propagation sites, respectively. Of the 79 ornamental plant species inoculated with gerbera isolate of CMV, 36 species were ELISA positive while the rest showed negative response. Among the tested weed flora of ornamentals, *Oxalis* spp., *Ixora* spp., *Cyprus rotundus* and *Portulaca somnifera* were also found to be infected with CMV.

Key Words: *Cucumovirus*, DAS-ELISA, monoclonal antibodies, ornamentals

Abbreviations: CMV – *Cucumber mosaic virus*, DAS-ELISA – double antibody sandwich enzyme-linked immunosorbent assay

INTRODUCTION

Floriculture is known to be a prospective business in Pakistan. The international demand for ornamentals has expanded rapidly in most countries of the world. Pakistan produces a large number of ornamental plants, and cut flowers make up the largest portion of this industry. The sale of ornamental cut flowers in Western Europe has increased up to \$12 billion, followed by the USA with \$6.9 billion and Japan with \$7.8 billion. The Netherlands contributes about 60% of the world's flower export. The other main flower-exporting countries are Colombia, Israel and Italy. In 1990, about 50% of the world's ornamental plants were exported by the Netherlands, followed by Denmark, Belgium, and Germany (Lawson 1996; Pautasso et al. 2010).

Pakistan's export of floriculture products is not encouraging due to many constraints. Among the

biotic stresses, viral infections cause serious economic losses to many ornamental plants worldwide. Ornamental plants, particularly those which are vegetatively propagated by rhizomes and bulbs, may also act as virus carriers (Flasinski et al. 1995). At least 125 different plant viruses have been reported to cause diseases in ornamental plants (Cohen 1995; Duarte et al. 2013; Ashfaq et al. 2014a). Viral infections not only directly and indirectly result in reduced growth and vigor (Ashfaq et al. 2015), but also in increased costs to maintain crop health, market value and quantity. In ornamental plants, losses due to viral infections can range from 10% to 100% (Brunt et al. 1996).

Cucumber mosaic virus (CMV) has a very broad host range of wild and cultivated plants with more than 1,200 known hosts, including some monocotyledons and a great number of dicotyledons (Chen 2003). CMV has also been reported to cause infection in cowpea, celery,

cucurbits, pepper, lettuce, tomato, chilies, banana, pasture legumes, kava and ornamental plants (Palukaitis et al. 1992; Flasiniski et al. 1995; Davis et al. 1996; Gafny et al. 1996; Latham et al. 1999; Iqbal et al. 2011; Ashfaq et al. 2014b). Several ornamental hosts of CMV play an essential role in virus spread and transmission.

Many developments have taken place in horticultural production in many countries of the world, but in Pakistan no attention has been paid on the various problems of the floriculture industry. The industry has been adversely affected by infection of various diseases, with CMV as the most important, among those that cause a drastic reduction in quantity and quality of flowers. The infected ornamental plants serve as sources of introducing viruses into other crops and countries.

In Pakistan, very little is known about the infection of ornamentals caused by viruses, therefore, the aim of the present work is to determine the incidence and host range of CMV on ornamental plants.

MATERIALS AND METHODS

Survey and Sample Collection

Different propagation sites (horticultural nurseries) and public and private areas (gardens and parks) of ornamental plants in the Rawalpindi and Islamabad areas were surveyed during the autumn and spring seasons of 2012 and 2013. Random sampling was done to collect ornamental plants showing characteristic symptoms of *Cucumber mosaic virus* (CMV), such as mosaic, mottling, necrosis, leaf distortion and stunting yellows, streak and color breaking, or other samples suspected to be infected by CMV. The collected samples were placed in plastic bags and brought to the Plant Virology Laboratory. All the samples were subjected to DAS-ELISA for confirmation of CMV infection.

Serological Test

DAS-ELISA (double antibody sandwich ELISA) was performed according to the method described by Clark and Adams (1977). CMV antibodies (Bioreba AG, Switzerland) were diluted in coating buffer at 1:200 and wells of the ELISA plate were coated (200 μ L per well). The plate was then incubated overnight at 4 °C and washed with PBS-Tween (washing buffer) three times at 5-min intervals.

CMV-infected samples were homogenized in extraction buffer and 200 μ L of the sap was applied to each well and the plate was incubated overnight at 4 °C. After incubation, the plate was washed with PBS-Tween for three to four times at 5-min intervals and blot-dried on paper towel. To each well, 200 μ L of diluted conjugated antibodies (1:200) was added before incubating the plate overnight at 4 °C followed by washing as mentioned above. Similarly, 200 μ L of freshly prepared substrate buffer containing *o*-nitrophenyl phosphate (75 μ g mL⁻¹) was added to each well before the plate was incubated at room temperature for 30 min.

The absorbance values (405 nm) were measured with an Automatic ELISA Reader (HER-480 HT Company (Illford) Ltd, UK). Samples were considered positive for CMV infection when the ELISA absorbance value was equal to two times or higher than the average of the absorbance value of the healthy tissue as well as the negative control. Commercial positive and negative controls (Bioreba) were included in the CMV ELISA kit. The reaction was stopped by adding 50 μ L of 3M NaOH to each well.

Disease Incidence

ELISA-positive samples were used to determine disease incidence, which was calculated based on the formula:

$$\text{Incidence of CMV (\%)} = \frac{\text{Number of ELISA positive samples of CMV}}{\text{Total number of samples tested}} \times 100$$

ELISA-positive samples were used as inoculum in subsequent studies.

Maintenance of CMV Source and Host Range Studies

A gerbera isolate of CMV was maintained and propagated in gerbera plants, and assayed by DAS-ELISA using CMV monoclonal antibodies. The leaves of these mechanically inoculated plants at 14 d post inoculation (dpi) were used for host range studies. The infected leaves were ground in 0.02 M phosphate buffer, pH 7.0, and strained through two layers of muslin cloth. At least three plants of each species were dusted with 600 mesh carborundum powder and inoculated with the aid of a forefinger. After inoculation, the plants were rinsed off with tap water to remove superfluous inoculum and kept in a protected place with sufficient illumination for

symptoms development. Symptom observations were recorded after 2 wk or longer and assayed by DAS-ELISA.

RESULTS

Symptomatology

The popularity of ornamentals among growers varies from season to season. The most popular ornamentals found in the nurseries, gardens, public and private parks of Rawalpindi and Islamabad include Chrysanthemum, Salvia, Geranium, Gilia, Gladiolus, Lily, Nasturtium, Phlox, Tulip, Zinnia, Rose, Gerbera, Jasmine, Dog flower and others. Among indicator plants inoculated with the infectious sap of gerbera isolate of CMV, only *Chenopodium amaranticolor* showed local lesion. *Nicotiana tabacum*, *Cucumis sativus* and *Lycopersicon esculentum* revealed systemic infection except *Datura* spp. which did not show any reaction to this isolate.

Incidence of CMV in Autumn

The highest incidence of CMV in the autumn of 2012 and 2013 was 46.5% and 59.3% respectively, in public and private gardens and parks followed by horticultural nurseries (35.01% and 47.4%). Of the 19, only 7 ornamental species comprising 104 and 108 leaf samples collected from propagation sites in the autumn of 2012 and 2013, respectively, showed positive reaction against CMV with the highest incidence in lily plants (100%) and the lowest incidence in Arabian jasmine (20–53.3%) (Table 1). On the other hand, in the autumn of both 2012 and 2013, only 11 ornamental plant species, consisting of 198 leaf samples collected from public and private gardens and parks, showed 100% incidence of CMV in lily, chrysanthemum, euphorbia and petunia plants, while the lowest incidence was recorded in geranium (33.3%) and Arabian jasmine (22.2%) plants (Table 2).

Ornamental plants grown in public areas (public and private gardens and parks) were heavily infected with CMV compared with those ornamentals grown in propagation sites (Tables 1 and 2). In the autumn of both 2012 and 2013, gerbera plants showed maximum incidence ranging from 88% to 94% in propagation sites and public areas, followed by dog flower and geranium plants with >86% incidence. It was also observed that CMV caused infection only in bougainvillea (90–

Table 1. Incidence (%) of *Cucumber mosaic virus* (CMV) in ornamental plant species grown in the different propagation sites.

Host Plant	% Incidence of CMV			
	Spring 2012	Autumn 2012	Spring 2013	Autumn 2013
Rose	0.0	0.0	0.0	0.0
Dog Flower	86.6	85.1	86.2	100.0
Ficus	0.0	0.0	0.0	0.0
Bougainvillea	0.0	20.0	0.0	100.0
Chrysanthemum	62.5	100.0	63.1	55.5
Delphinium	0.0	0.0	0.0	0.0
Euphorbia	0.0	0.0	0.0	100.0
Gerbera	88.0	100.0	88.4	100.0
Gomphrina	0.0	0.0	0.0	71.4
Chinese rose	0.0	0.0	0.0	0.0
Arabian Jasmine	20.0	0.0	53.3	88.2
Lily	100.0	80.0	100.0	55.5
Marigold	0.0	12.5	0.0	0.0
Geranium	64.8	0.0	65.7	94.2
Petunia	75.0	89.2	76.9	100.0
Salvia	0.0	0.0	0.0	60.0
Money Plant	0.0	0.0	0.0	0.0
Begonia	0.0	0.0	0.0	0.0
Dahlia	0.0	0.0	0.0	0.0

Table 2. Incidence (%) of *Cucumber mosaic virus* (CMV) in ornamental plant species grown in public areas.

Host Plant	% Incidence of CMV			
	Spring 2012	Autumn 2012	Spring 2013	Autumn 2013
Dog Flower	42.1	91.7	46.6	76.9
Bougainvillea	100.0	66.6	90.0	100.0
Marigold	0.0	0.0	0.0	0.0
Chrysanthemum	100.0	65.0	100.0	81.2
Cineraria	0.0	75.0	0.0	85.7
Dahlia	0.0	0.0	0.0	40.0
Euphorbia	100.0	26.7	100.0	25.0
Gerbera	94.1	100.0	100.0	100.0
Arabian Jasmine	50.0	61.1	22.2	92.9
Lily	100.0	66.6	100.0	90.0
Geranium	33.3	100.0	33.3	100.0
Petunia	100.0	70.0	100.0	50.0
Ficus	0.0	0.0	0.0	0.0
Ulta Ashok	0.0	0.0	0.0	0.0
Chinese Rose	71.4	100.0	70.5	100.0
Money Plant	0.0	0.0	0.0	0.0
Duranta	77.7	100.0	72.7	83.3
Statice	0.0	0.0	0.0	0.0

100%) and euphorbia (100%) plants in public areas, and interestingly these plants were found to be free of CMV infection in the propagation sites. The rest of the ornamental plant species, viz. rose, ficus, delphinium, gomphrena, ulta ashok, marigold, salvia, statice, money plant, begonia and dahlia, were found to be free of CMV infection.

Incidence of CMV in Spring

During spring 2012 and 2013, only 203 samples out of 432 with an average incidence of 46.9% showed the presence of CMV as confirmed by ELISA, while the rest of the samples did not reveal any infection of CMV in propagation sites. Survey results

indicated that the highest CMV infection in ornamental plants in public areas occurred in both spring and autumn seasons (Table 3). Moreover, the frequency and incidence of CMV, as shown in spring surveys (Tables 1 and 2) revealed the highest incidence in gerbera (100%) and the lowest in bougainvillea (20–50%) plants in propagation sites. Bougainvillea plants showed dramatic changes in virus incidence which varied from 20% to 100% from season to season.

Dog flower (76.9–91.7%) and Arabian jasmine (61.1–92.9%) grown in public and private areas (gardens and parks) were heavily infected with CMV when compared with ornamentals grown in propagation sites. The rest of the ornamental plant species, viz. rose, ficus, ulta ashok, delphinium, marigold, salvia, statice, money plant, begonia and dahlia were found to be free from CMV infection.

Host Range Studies

Out of the 79 ornamental species subjected to inoculation of gerbera isolate of CMV, only 36 species showed positive response to CMV, while the rest (43 species) were negative to CMV (Table 4).

DISCUSSION

Viruses have always been a tremendous threat to ornamental plants worldwide (Daughtrey et al. 1997; EPPO 1999; Parrella et al. 2003; Mandal et al. 2012), and this threat has had a great impact on the floriculture industry in terms of economic losses due to drastic reduction of quality and quantity of flowers. In Pakistan, a high incidence of viruses has been observed in ornamental plants. The present study reported high incidences of CMV in different ornamental plants in Rawalpindi and Islamabad during autumn and spring of 2012 and 2013. These

results are in agreement with those of other workers who had also reported CMV in most of the ornamental plant species (Horvath et al. 2006; Ghotbi and Shahraeen 2012). The increased incidence in the spring season is attributed to increased aphid population during spring compared with the much lower population in autumn.

It was also commonly observed that the aphid population increased rapidly in perennial hosts that harbor CMV in the warmer spring season as the virus spread rapidly to crops that are tender, young and attractive to migrating aphids. However, the increased disease incidence in ornamental plants under non-nursery conditions might be due to lack of monitoring under such conditions. Unfortunately, horticultural nurseries are also not free from infection possibly due to lack of proper supervision of nursery workers. Another reason for the persistence of infection is that most of the vegetatively propagated ornamentals, such as bulb and rhizomes, acted as virus reservoirs and transport conveyance of infested materials by human activities. A similar observation has been made by Flasiniski et al. (1995).

With reference to ornamental plant species, White (1946) first reported CMV to cause a disease of gladiolus in Tasmania. Since then, its occurrence has been noted in Wisconsin (Bridgmon 1951), Canada (Berkeley 1951), Oregon (McWhorter 1957), New Zealand and England (Doddall 1928; Chamberlain 1954). Recently this virus has emerged in Pakistan to become a threatening disease of ornamental plant species in nurseries in Rawalpindi and Islamabad as well as on ornamental plant species grown under non-nursery conditions. Surveys revealed that symptoms in non-nursery plants were much more severe than those observed under nursery conditions. Increase in disease

Table 3. Ornamental plants infected with *Cucumber mosaic virus* (CMV) in different seasons in Pakistan.

Season	Propagation Sites	Public Areas	Total	CMV Infected Ornamentals
Spring 2012	96/225	122/196	218/421	Lily, Gerbera, Dog flower, Petunia, Chrysanthemum, Arabian jasmine, Bougainvillea, Euphorbia, Geranium, Chinese Rose, Duranta, Cineraria
Spring 2013	107/207	133/200	240/407	Lily, Gerbera, Dog flower, Petunia, Chrysanthemum, Dahlia, Motiya, Bougainvillea, Euphorbia Geranium, Chinese Rose, Duranta
Autumn 2012	104/297	99/213	203/510	Lily, Gerbera, Dog flower, Cineraria, Marigold, Petunia, Chrysanthemum, Motiya, Bougainvillea, Euphorbia Geranium, Chinese Rose, Duranta
Autumn 2013	108/228	99/167	207/395	Lily, Rose, Gerbera, Dog flower, Cineraria, Gomphrina, Petunia, Chrysanthemum, Motiya, Salvia, Bougainvillea, Duranta, Euphorbia Geranium, Chinese Rose
Total	415/957	453/776	868/1733	

Table 4. Reaction of ornamental test plants to mechanical inoculation with *Cucumber mosaic virus* (CMV).

Sr. No.	Host Name		Symptoms	ELISA positive
	Common Name	Scientific Name		
1	Begonia	<i>Begonia</i> spp.	Mo, Mot	—
2	Arabian Jasmine (Chambeli)	<i>Jasmine sambac</i>	Mo	+
3	Berry (Deadly night shade)	<i>Atropa balladona</i>	MM	—
4	Viscaria	<i>Viscaria</i> spp.	Mo	—
5	Rose	<i>Rosa sinensis</i>	Chl	—
6	Geranium	<i>Pelargonium hortorum</i>	Mo	+
7	Bougainvillea	<i>Bougainvillea spectabilis</i>	Mo, Mot	+
8	Dog Flower	<i>Antrrhinum majus</i>	Mo	+
9	Lily	<i>Lilium</i> spp.	Str, Y	+
10	Chrysanthemum	<i>Chrysanthemum</i> spp.	SM	+
11	Delphinium	<i>Delphinium</i> spp.	MM	—
12	Euphorbia	<i>Euphorbia pulcherrima</i>	SM	+
13	Gul-e-Asharfi	<i>Calendula officinalis</i> Linn.	Y	+
14	Marigold	<i>Calendula</i> sp.	Ld	—
15	Date Palm	<i>Phoenix dactylifera</i>	Mo	—
16	Long leaf ficus	<i>Ficus bennendijkii</i>	MM	—
17	Money plant	<i>Epipremnum aureum</i>	Mo	—
18	Elephanta	<i>Calocasia</i> spp.	Mo, Mot	—
19	Cactus	<i>Cactus capsula</i>	Mo	—
20	Petunia	<i>Petunia hybrid</i>	SM, Y	+
21	Dianthus	<i>Dianthus caryophyllus</i>	Y	—
22	Cineraria	<i>Cineraria</i> spp.	SM	—
23	Chinese rose	<i>Hibiscus rosa-sinensis</i>	Mo	+
24	Aster	<i>Aster</i> spp.	Mo	—
25	Primula	<i>Primula</i> spp.	Y	—
26	Dahlia	<i>Dahlia hybrid</i>	Mo	—
27	Salvia	<i>Salvia splendens</i>	SM	+
28	Phlox	<i>Phlox drummondii</i>	Mo	—
29	Golden flax	<i>Lilium flavum</i>	Mot	+
30	Draunta	<i>Draunta repens</i>	Y, Stu	+
31	Calendula	<i>Calendula</i> spp.	Y	+
32	Sweet peas	<i>Lathyrus odoratus</i>	SM	+
33	Ranunculus	<i>Ranunculus asiaticus</i>	Y	—
34	Aucuba	<i>Aucuba</i> spp.	Ld	—
35	Syngonium	<i>Syngonium</i> spp.	Mo	—
36	Sweet lime	<i>Citrus limettioides</i>	Y	—
37	Gerbera	<i>Gerbera jamesonii</i>	Mo, Mot	+
38	Helichrysum	<i>Helichrysum</i> spp.	Y	—
39	Calceolaria	<i>Calceolaria</i> spp.	Y, Mo	—
40	Gul-e-banafsha	<i>Viola odorata</i>	Mo, Mot	+
41	Raphia	<i>Raphia</i> spp.	SM	+
42	Chandana	<i>Tabernaemontana coronara</i> Wild	Mo	+
43	Zinnia	<i>Zinnia</i> spp.	Mot, Lc	+
44	Golden Cane palm	<i>Dypsis lutescens</i>	Str	+
45	Gladiolus	<i>Gladiolus hortulanus</i>	Str	+
46	Circus flower	<i>Circus</i> spp.	Y	+
47	Sadabahar	<i>Vinka rosia</i>	Mo	—
48	Avocado	<i>Persea americana</i>	Mo	—
49	Jangli Toot	<i>Broussonetia papyrifera</i>	Mo	—
50	Gomphrina	<i>Gomphrina globosa</i>	Mot	+
51	Coleus	<i>Coleus blumei</i>	Mo	+
52	Poinsettia	<i>Euphorbia pulcherrima</i>	Mot	+
53	Khatti	<i>Citrus jambhiri</i>	Mo	+
54	Night blooming jasmine	<i>Cestrum nocturnum</i>	Y	—
55	Jhumka lata flower	<i>Passiflora incarnate</i>	Mot	—
56	Gardenia	<i>Gardenia</i> spp.	SM	—
57	Hijal	<i>Barringtonia acutangula</i>	Mo	—
58	Gul-e-cheen (Frangi pani)	<i>Plumeria obtuse</i>	Mot	—
59	Chinese tallow	<i>Sapium sebiferum</i>	Mo	—
60	Jaman	<i>Eugenia jambolana</i>	Mot	—
61	Golden shower tree	<i>Cassia fistula</i>	Y	—
62	Chinese trumpet creeper	<i>Tecoma gandiflora</i>	SM	—
63	Balladona lily	<i>Amaryllis balladona</i>	Mot	+
64	Indian devil	<i>Alestonia scholaris</i>	Mo	—
65	Pumpkin (Kadu petha)	<i>Cucurbita maxima</i>	Mot	—
66	Magnolia	<i>Magnolia</i> spp.	Mo	—

Table 4. Continued...

Sr. No.	Host Name		Symptoms	ELISA positive
	Common Name	Scientific Name		
67	Jetropha	<i>Jetropha parduraefolia</i>	Mot	+
68	Ulta Ashok	<i>Polyalthia longifolia</i>	Mo	—
69	Aatrobatro	<i>Artabotrys odoratissimus</i>	Mot	—
70	Wool flowers	<i>Celosia</i> spp.	Y	+
71	Freesia	<i>Freesia</i> spp.	Y, Mo	—
72	Sunflower	<i>Helianthus annuus</i>	Mo	+
73	Hoya	<i>Hoya carnosia</i>	Mo	+
74	Cosmos	<i>Cosmos</i> spp.	Mot	+
75	Anthurium	<i>Anthurium</i> spp.	Mo	—
76	Rud bekia	<i>Rud bekia</i> spp.	Y, Mot	—
77	Ixora	<i>Ixora</i> spp.	Mo	+
78	Oxalis	<i>Oxalis</i> spp.	Mo	+
79	Portulaca	<i>Portulaca</i> spp.	Y, Mot	—

Mo – Mosaic, Y – Yellowing, Mot – Mottling, SM – Systemic Mosaic, Stu – Stunting, Lc – Localized chlorosis, Ld – Leaf distortion, Str – Streaking, MM – Mild Mosaic

severity under non-nursery conditions is probably due to improper care. With proper supervision of nursery workers, plants under nursery conditions may also show disease severity but to a much lesser extent than that under uncontrolled conditions.

Many important ornamentals fall under the broad host range of CMV which is known to infect more than 1000 species in over 100 plant families and can cause significant economic losses in many ornamental, horticultural and vegetable crops. In the present study, out of 79 ornamental plant species inoculated with gerbera isolate of CMV, only 36 species showed positive reaction to ELISA test. Among the tested weed flora of ornamentals, *Oxalis* spp., *Ixora* spp., *Cyprus rotundus* and *Portulaca somnifera* were found to be infected with CMV. The results are in agreement with those of other workers who also reported various ornamentals and weeds as hosts of CMV (Chupp and Sherf 1960; Anderson et al. 1992; Jung et al. 2000; Verma et al. 2004a & b; Agrios 2005; Iqbal et al. 2011).

CONCLUSION

These observations suggest that CMV is present in almost all nurseries of the Rawalpindi-Islamabad area. It was also observed that the incidence of disease was higher in public areas in spring than in autumn. The presence of this potentially destructive virus on ornamentals could pose a serious threat to the floriculture industry. Therefore, necessary strategies should be adopted to prevent the widespread occurrence of this virus. Further surveys are also needed to determine its incidence, prevalence and the resulting economic losses both in propagation and public sites in other areas of Pakistan.

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