The Bioscan 8(2): 437-441, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

BIOPHYSICAL CHARACTERIZATION, HOST RANGE AND TRANSMISSION STUDIES OF CUCUMBER MOSAIC VIRUS

V. D. CHANDANKAR*, M. K. MONDHE, P. R. BHOYAR, B. N. NINAWE[1] AND G. JADESHA[2]
[1]Department of Plant Pathology, PGI, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola - 444 104, M.S
[2]Department of Plant Pathology, Achyra N.G. Ranga Agriculture University, Rajendra Nagar, Hyderabad - 500 030, A. P.
e-mail: mondhem2@gmail.com

KEYWORDS
CMV
Mechanical inoculation
TIP
DEP
Longevity *in vitro*
Aphid transmission

Received on :
26.11.2012

Accepted on :
27.02.2013

***Corresponding author**

ABSTRACT

Cucumber Mosaic Virus a ubiquitous pathogen was isolated from infected cucumber infected plants for their identification and confirmation by procedure such as biophysical properties, host range and insect transmission studies. Virus strain isolated from cucumber infected plants showed Thermal Inactivation Point (TIP) at 60ºC, Dilution End Point (DEP) in between 10^{-4} to 10^{-5} and Longevity *in vitro* for two days at 28ºC-30ºC and 7 days at 6ºC-8ºC temperature. During host range studies on thirty one crops of different families; eleven crops belonging to Cucurbitaceae, Compositae, Chenopodiaceae, Amaranthaceae, Leguminosae and Solanaceae family showed viral disease expression. However, inoculum failed to induce symptoms on crops belonging to Crucifereae, Caricaceae and Malvaceae. CMV under this investigation was found to be transmissible by *Aphis gossypii* with 100 % efficiency, followed by *Aphis craccivora* , *Acyrthosiphum pisum* , *Dactynotus carthami* and *Aphis nerri* in non - persistent manner.

INTRODUCTION

Cucumber (*Cucumis sativus* L.), is a second most widely cultivated cucurbit after watermelon, is susceptible to various diseases such as mosaic, wilt, anthracnose, seedling blight, leaf spot, root rot, downy and powdery mildews. Among these, mosaic disease caused by the cucumber mosaic virus is an economically important and causes upto 40-60 % yield losses. CMV was first reported in 1916 as causal agent infecting cucumber plants (Doolittle, 1916). Cucumber plants may become infected at any stage of growth, from emergence of the seedling to crop maturity (Takanami, 1981). Incubation period for disease expression may range from 4 to 14 days depending upon inoculums load and host plant resistance. CMV infected cucumber plant displays a mottled leaf-pattern, with yellow and green areas, most conspicuous in young terminal leaves. The fruits on disease plants becomes mottled, distorted and malformed (Davis and Whitaker, 1962).CMV is widely prevalent plant virus because of its extensive host range which span over 800 plant species (Palukaitis *et al.,*1992) and is regarded as one of the most important viruses worldwide in field grown vegetables.(Tomlinson,1987). CMV is easily transmitted by mechanical inoculation as well as by more than 80 species of aphids in non-persistent manner (Palukaitis and Garcia-Arenal, 2003). It was reported that *Myzus persicae* and *Aphis gossypii* are among the more efficient vectors for this virus (Edwardson and Christie 1991, Palukaitis and Garcia-Arenal, 2003). It has been observed that several host of CMV play an essential role in virus epidemiology. Host range of the virus consists of more than 750 plant species, including vegetables, weeds and ornamental plants (Sikora, 2004) which serve as inoculum reservoirs from one growing season to the next. Therefore, the present investigations were undertaken with the objective of isolating CMV from Cucumber infected plants for their identity and confirmed by procedure such as host range, biophysical properties and insect transmission study.

MATERIALS AND METHODS

The present research work was carried out at Post Graduate Institute, Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M. S) during 2009-2011.

Pathogenicity assay

Leaf samples from naturally infected cucumber plants showing mosaic, leaf distortion, leaf puckering, vein clearing symptoms were collected from Horticulture field, Dr. PDKV, Akola, (M.S.) These samples were macerated in 0.1M potassium phosphate buffer (pH 7.5) supplemented with 0.1% sodium sulphite in a ratio of 1:10 (w/v) using a sterile pestle and mortar (Afreen *et al.*, 2009). The filtrate was inoculated on healthy young cucumber var. Pune Khira at cotyledon stage by leaf rub method using carborundum. The rub leaves were washed with a stream of tap water. Plants were kept at 28ºC in insect proof green house and disease expression was recorded. For control treatment carborundum dusted leaves were inoculated with phosphate buffer alone.

Biophysical properties of CMV isolate under investigations for Dilution End Point (DEP), Thermal Inactivation Point (TIP)

and Longevity *in vitro* (LIV) were determined as per standard methods described by Noordam (1973).

Dilution End Point (DEP)

The experiment was conducted to determine the infectivity of CMV sap after serial dilution. The standard virus leaf extract was diluted to get dilution varying from 10^{-1} to 10^{-8}. Standard leaf extract without dilution served as control (10^{1}). Periodical observations were recorded for the expression of disease symptoms for each dilution.

Thermal Inactivation Point (TIP)

Aliquot of 5mL standard CMV leaf extract in test tubes were subjected separately for 10 minutes to heat treatments starting from 40ºC to 95ºC with an interval of 5ºC in hot water bath. Immediately after heat treatment, the sap was cooled by passing tap water over the outer surface of the tube. A set of ten plants were inoculated for each temperature treatment. The control was maintained by inoculating the leaves of test plant with unheated standard CMV extract kept at room temperature (28ºC) and periodically observed for the expression of virus symptoms.

Longevity *in vitro*

CMV extracts were kept in two different condition viz., room temperature condition at 28ºC to 30ºC and refrigerated condition at 6ºC to 8ºC which were used to inoculate at a fixed period intervals *i.e.* 1, 4, 8, 12 and 24h and 2,3,4,5,6,7,8 and 9 days. Inoculated plants were maintained in insect proof cage house and observed periodically for expression of symptoms.

Host Range Study

To determine the host range and induced symptoms development, about 38 plants species to belonging to the nine families viz., Cucurbitaceae, Amaranthaceae, Solanaceae, Chenopodiaceae, Leguminaceae, Compositae, Malvaceae, Caricaeae and Crucifereae were selected (Table 3). Ten plants of each selected crops were mechanical inoculated and kept under control condition and induction of viral disease symptoms was recorded

Aphid transmission

Five aphid species viz., *Aphis gossypii*, *Aphis cracivora*, *Acyrthisyphon pisum*, *Dactynotus carthami* and *Aphis nerri* were collected from field grown Cotton, Cowpea, Dolichus bean, Safflower and Calotropis respectively. Individuals of aphids were reared onto healthy seedling of respective plant species in insect proof cages and left for reproduction. Several virus free adults of each aphid species were starved for two hrs (Dheepa *et al.*, 2010). Later on, they were allowed to feed for 20 min (acquisition period) on young cucumber mosaic virus infected leaves of the variety Pune Khira. Ten virulent aphids were released on each test plant and allowed to feed for overnight and thereafter killed by spraying Dimethoate. These plants were maintained in insect proof cage at 25-30ºC for 30 days and inspected daily for symptoms development.

RESULTS AND DISCUSSION

Pathogenicity assay

The CMV in the present investigation was found to be mechanically transmissible by sap inoculation on the cucumber cultivar, Pune Khira with 10 to 14 days of incubation time. However, considerable variation in incubation time of this virus was earlier reported by Bolton *et al.*, (1971). CMV inoculated cucumber plants manifest yellowing, mosaic, leaf distortion, leaf puckering, vein banding and stunted growth. Also, it was observed that inoculation at cotyledon stage gave undoubtedly disease symptoms than inoculation on to the older leaves. All the inoculated plants showed symptoms identical (Fig.1) to those observed under field condition. Similar results were recorded by Davis *et al.* (1996).

Biophysical characterization of CMV

Thermal Inactivation Point (TIP)

Table 1: Studies on Thermal Inactivation Point (TIP) of CMV

Sr. No.	Exposure temp.ºC	No. of plants inoculated	% Transmission
1	28ºC	10	100
2	40ºC	10	100
3	45ºC	10	90
4	50ºC	10	90
5	55ºC	10	70
6	60ºC	10	60
7	65ºC	10	00
8	70ºC	10	00
9	75ºC	10	00
10	80ºC	10	00
11	85ºC	10	00
12	90ºC	10	00
13	95ºC	10	00

Table 2: Studies on Dilution End Point (DIP) of Cucumber Mosaic Virus (CMV)

Sr. No.	Dilution	Percent Transmission
1	Crude Sap	100
2	1:10 (10^{-1})	100
3	1:100(10^{-2})	90
4	1:1000(10^{-3})	90
5	1:10,000(10^{-4})	70
6	1:100,000(10^{-5})	00
7	1:1000,000(10^{-6})	00
8	1:10,000,000(10^{-7})	00
9	1:100000000 (10^{-8})	00

Table 3: Longevity *in vitro* (LIV) of CMV

Sr. no.	Interval after extraction	No. of plants inoculated	Percent Transmission	
			Room temp.	6-8ºC
1	Control	10	100	100
2	4h	10	80	90
3	8h	10	70	80
4	12h	10	70	80
5	24h	10	60	70
6	2 days	10	50	70
7	3days	10	0	60
8	4days	10	0	50
9	5days	10	0	40
10	6days	10	0	30
11	7days	10	0	20
12	8days	10	0	0
13	9days	10	0	0

Table 4: Disease expression of CMV on different host plants

Sr. No.	Host plant andFamily	No. of plantsInoculated	No. of plants showing symptoms	Types of symptoms
I	Cucurbitaceae			
1	*Citrullus lunatus* (Watermelon)	10	-	No symptom
2	*Citrulus fistolusum* (Tinda)	10	-	No symptom
3	*Cucumis melo* (Muskmelon)	10	08	Mosaic
4	*Cucurbita moschata* (Pumpkin)	10	10	Mosaic
5	*Luffa acutagula* (Ridge gourd)	10	-	No symptoms
6	*Luffa cylindrical* (Smooth gourd)	10	-	No symptom
7	*Lagenaria ciceraria* (Bottlegourd)	10	-	No symptom
8	*Momordica charantia* (Bittergourd)	10	-	No symptom
II	Amaranthaceae			
1	*Gomphrona globosa*	10	08	Solid grey local lesion
III	Solanaceae			
1	*Capsicum annum* (Chilli)	10	09	Mosaic, fern leaf, stunting.
2	*Solanum melongona* (Brinjal)	10	-	No symptom
3	*Lycopersicon esculentum* (Tomato)	10	-	No symptom
4	*Datura metal*	10	-	No symptom
5	*Nicotiana glutinosa* L	10	09	Necrotic local lesion
6	*Nicotiana rustica* L	10	08	Necrotic local lesion
7	*Nicotiana tabaccum* L	10	09	No symptom
8	*Nicotiana tabaccum* var. *xanthi*	10	-	No symptom
9	*N. tabaccum* var. *sylvensis*	10	-	No symptom
10	*N. tabaccum* var. VT-1158	10	-	No symptom
11	*N. tabaccum* var. Samsun	10	-	No symptom
12	*N. tabaccum* var. White burley	10	-	No symptom
13	*N. tabaccum* var. Harrison special	10	-	No symptom
14	*N. tabaccum* var. Debeneyii	10	-	No symptom
IV	Chenopodiaceae			
1	*Chenopodium murale* L	10	09	Necrotic local lesion
2	*Chenopodium amaranticolor* L	10	08	Necrotic local lesion
V	Leguminaceae			
1	*Dolichus lablab*(Dolichus bean)	10	-	No symptom
2	*Vigna sinensis*(Cowpea)	10	-	No symptom
3	*Vigna radiata*(Green gram)	10	-	No symptom
4	*Glycine max* (Soybean)	10	-	No symptom
5	*Vigna mungo* (Black gram)	10	-	no symptom
6	*Phaseolus vulgaris* (French bean)	10	09	Brown local lesion
VI	Compositae			
1	*Helianthus annus* (Sunflower)	10	-	No symptom
2	*Carthamus tinctori*(Safflower)	10	08	Distortions of leaves
VII	Crucifereae			
1	*Brassica oleraceae* var. *capitata* (Cabbage)	10	-	No symptom
2	*Brassica oleraceae* var *botrytis* (Cauliflower)	10	-	No symptom
VIII	Cariacaeae			
1	*Carica papaya*(Papaya)	10	-	No symptom
IX	Malvaceae			
1	*Abelomoschus esculentus* (Okra)	10	-	No symptom

In the present study, it was observed that all the plants inoculated with untreated sap (Sap without heat treatments) as well as exposed to 40ºC for 10 minutes showed 100% virus transmission. The sap exposed to 45ºC and 50ºC temperature showed 90% CMV incidence. However, reduction in infectivity was observed when the sap was exposed to 55ºC and 60ºC temperature and showed 70 % and 60% virus incidence respectively. Virus could not withstand and failed to produce any symptoms at the temperature 65ºC and above. Therefore, the thermal inactivation point (TIP) of present CMV isolate lied between 60 – 65ºC as evident from complete inactivation of the virus in the sap treated at 65ºC for 10 minutes. Similar results for CMV was reported by Gahukar and Nariani (1982) in chilli with TIP at 60-62ºC.

Dilution End Point (DEP)

Assay plants inoculated with the crude sap diluted to $10^{-1}$to 10^{-4} produced mosaic, leaf distortion and leaf puckering symptoms. However, sap failed to produce symptoms with extract diluted to 10^{-5} and above. Therefore, it is evident from the data in Table 2 that dilution end point of the test virus ranged between 1:10000 and 1:10,0000 (10^{-4} and 10^{-5}). A number of workers have also reported the dilution end point of CMV to be ranging between 10^{-4} and 10^{-5}. (Vasudeva *et al.,* 1949; Mueller, 1966 and Gahukar and Nariani, 1982)

Longevity *in vitro* (LIV)

It is clear from the data presented in Table 3 that the CMV under investigation retained infectivity for a period of 2 days with a transmission 50 % virus transmission under room temperature (28ºC to 30ºC). The data presented in Table 3 revealed that the ageing of isolate was delayed when infected

Table 5: Aphid transmission study

Sr. No.	Aphids spp	No. of aphids/ plants	Percent Transmission	Incubation period	Types of symptoms
1	*Aphis gossypii*	10	100	13	Mosaic and leaf puckering
2	*Aphis craccivora*	10	80	14	Mosaic and yellowing
3	*Acyrthisiphum pisum*	10	90	13	Mosaic and vein banding
4	*Dactynotus carthami*	10	80	14	Mosaic
5	*Aphis nerri*	10	70	13	Mosaic and vein banding

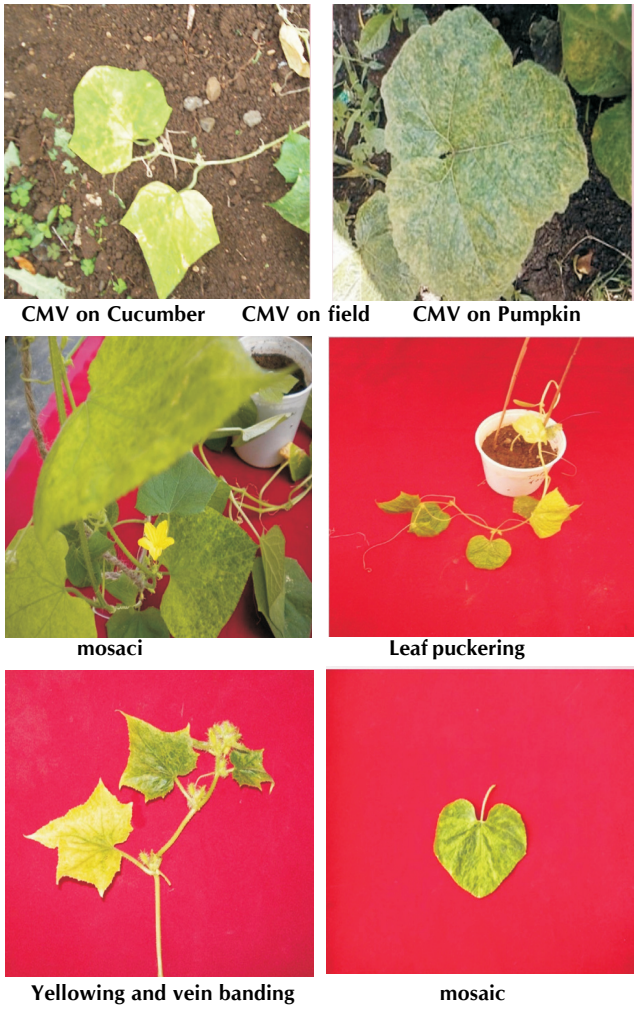

Figure 1: CMV infection under *in vivo* and *in vitro* condition.

sap was stored at freezing temperature (6ºC to 8ºC) and retained its infectivity for 7 days resulting in transmission of about 20%.

This nature of CMV in extracted sap has been widely known and reported by a number of workers from different parts of the world (Gahukar and Nariani(1982), Xu and Barnett, (1984) Shrivastava *et al.*, (1992) and Singh *et al.* (1999).

Host range study

Among 38 plant species belonging to nine families tested for cucumber mosaic virus, 11 plant species expressed visible symptoms as described in Table 4. Both the species *Cucumis melo*, *Cucurbita moshcata* from Cucurbitaceae showed mosaic like symptoms. Whereas, *Capsicum annum* showed mild mosaic with fern leaf like symptoms. Agrios *et al*. (1985) reported that infection of CMV on *Capsicum annum* develop necrotic ring and oak leaf like pattern. Necrotic local lesions were observed on *Nicotiana glutinosa* and *N. rustica* of Solanaceae, *Chenopodium murale* and *C. amaranticolor* of Chenopodiaceae. However, *Gomphrona globosa*, *Beta vulgaris* from Chenopodiaceae and *Phaseolus vulgaris* from leguminosae showed chlorotic local lesion. *Carthamus tinctori* showed distortion of leaves. Similar symptoms were recorded by Mathur *et al*. (1966). Thus, Mathur *et al*. (1966) observed necrotic local lesion on *N. glutinosa, N. tabacum* and *C. amaranticolor*, where as chlorotic local lesion on *Beta vulgaris*.

The present investigation is in agreement with findings of several workers like, Anjanneyulu and Apparao (1967) who observed mosaic symptoms on *Cucumis melo*, Quiaoit and Fulton (1966) recorded local lesion on *C. amaranticolor, C. quinoa* and *C.murale* after CMV inoculation. Similarly, Teakle *et al*. (1963) reported small local lesion on *C. amaranticolor*.

Aphid transmission study

In the current study, CMV was transmitted by all aphids species tested (Table 5 Among them, *Aphis gossypii* was the most efficient and showed 100% transmission followed by *Acyrthisiphum pisum, Aphis craccivora, Dactynotus carthami* and *Aphis nerri* and showed 90, 80, 80 and 70 per cent CMV incidence on inoculated plants respectively. These results revealed that CMV is aphid transmissible and agreed with the data recorded by Rao (1980). Inoculation of virulent aphid on Cucumber cultivar, Pune Khira develops the visible symptoms within 13-14 days.

The results of aphids transmission studies was supported by the finding of Hobbe *et al*. (2000), Gildow *et al*. (2008), Badak *et al*. (2009) and Dheepa and Paranjothi, (2010).

Since there are no efficient chemicals treatments to protect plants from virus infection especially those transmitted by vectors like Aphid species in non-persistent manner and once the plant infected, one can do nothing except eradicating the infected plants. For these reasons it may be time to develop an efficient control strategy to avoid possible virus problems. One of the most important elements of this strategy is the determination of virus sources, mode of transmission, host range including weeds. Environmental conditions may play an important role in symptom expression. So, the elimination of CMV sources, secondary hosts, vectors and crop rotation by excluding virus host range will help in management of CMV at field level.

REFERENCES

Afreen, B., Khan, A. A., Naqvi, Q. A., Kumar, S., Pratap, D., Snehi, S. K. and Raj, S. K. 2009. Molecular identification of a *Cucumber mosaic virus* subgroup II isolate from carrot(*Daucus carota*) based on

RNA3 genome sequence analyses. *J. Plant Dis. Protect.* **116(5):** 193-199.

Agrios, G. N., Walker, M. E. and Ferro, D. N. 1985. Effect of CMV inoculation at successive weakly intervals on growth and yield of pepper plant. *Plant Dis.* **69:** 52-55.

Anjaneyulu, A. and Apparao, A. 1967. Natural occurrence of cucumber mosaic virus on chili in India. *Indian Phytopath.* **20:** 380-381.

Bolton, A. T. and Nuttall, V. W. 1971. A highly virulent strain of Cucumber mosaic virus occurring in cucumber in Eastern Ontario**.** *Can. Plant Dis. Surv.* **51(4):**138-141.

Badak, K. S., Sawant, D. M. and Deshmukh, G. D. 2009. Transmission of groundnut mosaic virus by aphids species. *J. Plant Dis. Sci.* **4(1):** 99-103.

Davis, R. I., Brown, J. F. and Pone, S. P. 1996. Causal relationship between cucumber mosaic cucumovirus and kava dieback in south Pacific. *Plant Dis.* **80:**194-198.

Davis, G. N. and Whitaker, T. W.1962. Cucurbits Botany, cultivation and utilization. p.162-165.

Dheepa, R. and Paranjothi, S. 2010. Transmission of cucumber mosaic virus infecting banana by aphid and mechanical methods. *Emir.J.Food Agri.* **22(2):**117-129.

Doolittle, S. P. 1916. A new infectious mosaic disease of cucumber. *Phytopath.* **6:** 145-147.

Edwardson, J. R. and. Christie, R. G. 1991. CRC Handbook of viruses infecting legumes. *CRC Press, Boca Raton, University of Florida,* USA. pp. 293.

Sikora, E. J. 2004. Cucumber Mosaic Virus *Plant Disease Notes, July 2004,* ANR-868.

Gahukar, K. B. and Nariani, T. K. 1982. Studies of aphids borne mosaic disease of chili. *Ind. Phytopath.* **35(1):** 73-79.

Gildow, F. E., Shah, D. A., Sacket, W. M., Butzlernault, B. A. and Fleischer, S. J. 2008. Transmission efficiency of CMV by Aphids associated with virus epidemic in snap bean. *Phytopath.* **98:**1233-1241.

Hobbs, H. A., Eastburn, D. M., D'Arcy, C. J., Kindhart, J. D., Masiunas, J. B., Voegtlin, D. J., Weinzierl, R. A. and McCoppin, N. K. 2000. Solanaceous weeds as possible sources of Cucumber mosaic virus in Southern Illinois for aphid transmission to pepper. *Plant Dis.* **84:** 1221-1224.

Mathur, S. B., Mishra, M. D and Tiwari, V. P. 1966. A new strain of tobacco mosaic virus affecting chili pepper variety orange. *Plant Dis. Reporter* **50(8):** 619-622.

Mueller, W. C. 1966. Cucumber mosaic virus caused of a mosaic disease of canna. *Plant Dis. Reporter* **50(12):** 899-901.

Noordam, D. 1973. Identification of plant viruses: methods and experiments. *Centre Agricultural Publications, Documentation (Pudoc), Wageningen*. p.207.

Palukaitis, P. and García-Arenal, F. 2003. *Cucumoviruses. Adv. Virus Res.* **62:** 241–*323.*

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G. and Francki, R. I. B. 1992. Cucumber mosaic virus. *Adv. Virus. Res.* **41:** 281-284.

Rao, D. G. 1980. Studies on a new strain of banana mosaic virus in South India. In: Proc. National Seminar on Banana Production Technology. C. R. Muthukrishnan and J. B. M. Abdul Khader (Eds.). *Tamilnadu Agricultural University,* Coimbatore, India. pp.155-159.

Quiaoit, A. R. and Fulton, R. W. 1966. A disease of *Rhamnus cathactica* caused by cucumber mosaic virus. *Plant Dis. Reporter* **50(8):** 613.

Shrivastava, K. M. Raj, S. K and Singh, B. P. 1992. Properties of CMV strain naturally infecting chrysanthemum in India. *Plant Dis.* **76:** 474-477.

Singh, D., Naqvi. Q. A. and Garg, D. 1999. A strain of cucumber mosaic cucumovirus causing mosaic virus in marigold in India. *Ind. Phytopath.* **52(2):**114-117.

Takanami, Y. 1981. A striking change in symptoms on cucumber mosaic virus infected tobacco plants induced by a satellite RNA. *Virology.* **109:** 120-126.

Teakle, D. S., Gill, C. C., Tayler, R. H. and Raube, R. D.1963. Cucumber mosaic virus in passiflora in California. *Plant Dis. Reporter* **47(7):** 677-678.

Tomlison, T. A. 1987. Epidemiology and control of virus diseases of vegetables. *Annals of Applied Bio.* **110:** 661-681.

Vasudeva, R. S. Raychoudhary, S. P. and Singh, J. 1949. A new strain of cucumis virus. *Ind. Phytopath.* **2:** 180-185.

Xu, Zeyong and Barnett, O. W. 1984. Identification of cucumber mosaic virus strain from natural peanut in China. *Plant Dis*. **68:** 386-389.