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Biophysical Properties of CMV Transmission in *Cucurbita pepo* (Pumpkin) in Dehradun Uttarakhand

Shivani Jasrotia* and Nirmala Koranga

Department of Botany, D.B.S.(P.G) College, Dehradun, Uttarakhand, India

**Corresponding author*

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A B S T R A C T

Cucumber mosaic virus is omnipresent infectious pathogenic source to various vegetables including cucurbitaceous crops. In the present study the virus is isolate from infected from infected pumpkin plant for transmission. Survey was done from Distt. Dehradun during December 2018 to May 2019 to identify the occurrence of CMV on pumpkin. Leaf samples were collected from the field area and subjected to lab to investigate the presence of CMV by process of inoculating sap from infected plant of pumpkin to healthy vigorously raised seedlings of pumpkin planted in pots for experiment. The infected plant show symptoms like hypertrophy, stunting, mosaic pattern on leaves, small leaf size and chlorosis. Biophysical properties of CMV in *C. pepo* were determined by performing experiment. Thermal inactivation point (TIP)was 70º in mild isolate and 75º in severe isolate, Dilution end point (DEP) was 1:100,000 (10^{-5}) in mild isolate and 1:5,00,000 in severe isolate, Longevity 'in vitro' (LIV) of 5 days with 40% infection in mild isolate and 6 days with 20% infection in severe isolate was found. Longevity in vivo of9 days with 20% infection in mild isolate and 14 days with 20% infection in severe isolate was found.

Introduction

C. pepo (Pumpkin) belongs to cucurbitaceae family grown worldwide. Pumpkin plants are mostly infected by disease caused by viruses among these CMV cause majority of the infection in pumpkin causing severe economic loss that is ruinous to agronomist and local farmers. CMV infect more than 1200 plant species among 100 different plant species (Zitter and Murphy 2009). In India CMV incidence was earlier recorded in pumpkin. Infected pumpkin plant show symptoms including mild and severe. Mild symptoms include vein banding, chlorosis, Mosaic leaves, reduction in growth while severe symptom include stunted growth, leaf distortion, leaf margin curve down along with dark green patches on leaf surface (Sandu and Kamg, 2007). Cucumber mosaic virus CMV has broad host

range among many known viruses (Palukaitis and Garcia- Arenal 2003b; Garcia- Arenal and Palukaitis, 2008) and was firstly recorded as the source of infecting cucurbits (Doolittle, 1916). Cucumis virus-3 was reported in India by Goel *et al.*, (1973), Qureshi *et al.*, (1978), Park *et al.*, (1990). In India Rao *et al.*, (1984) reported Cucumber green mottle mosaic virus in few cucurbits. Koranga (2018) reported CMV in cucurbit *Lagenaria siceraria* in western Himalayas on basis of Physical properties. Fiedorow (1993) recorded CMV susceptibility to cultivars of *C. pepo* and other cucurbits. Chandankar *et al.*, (2013) reported CMV on the basis of Biophysical characterization, host range and transmission in India. The objective of present study were to identify the virus from infected plant of pumpkin and characterize on the basis of Biophysical properties in Dehradun.

Materials and Methods

Site selection

Present research was done in D.B.S P.G College Dehradun during 2018-2019. Survey was conducted in local sites of Dehradun. Leaves of pumpkin plant showing infection of CMV were collected from the selected sites and were subjected to laboratory for further experiment. Infected leaves show mild and severe symptoms like yellowing of leaves, leaf distortion, leaf upward rolling and leaf deformation.

Pathogenic isolate

Infected sample collected from the field were crushed in sterilized mortal and sap was collected by filter through muslin cloth. Sap was collected in watch glass and mixed with carborandum (silicon carbide) powder (600 mesh).

Mechanical inoculation of sap

Sap was inoculated on young leaves of seedlings of pumpkin growing in experiment field area by gently rubbing with fingertip dipped in sap. Two leaf stage were selected for inoculation. Immediately after rubbing, leaves were washed with water to remove excess of sap. 20 seedlings of pumpkin were grown in experimental field for inoculation out of which 10 were inoculated with mild and 10 were inoculated with severe infectious sap.

Biophysical Properties

Biophysical characterization of CMV isolate in pumpkin under examination for Dilution end point (DEP), Thermal inactivation point (TIP), Longivity 'in vitro' (LIV) and Longivity 'in vivo' (LIVo) were defined according to standard procedure reported by Noordam (1973).

Dilution end point (DEP)

Dilution end point (DEP) is the dilution of sap from a virus infected plant which is still infectious, but is usually given as the range between this dilution and the next one at which the infectivity is lost. Diseased developing leaves of systemically infected pumpkin plant were collected and subjected to laboratory. Saps from diseased leaves were taken out by grinding them in mortar and pestle. Strain the sap through muslin cloth.

Dilute the sap with distilled water to prepare dilutions of different concentration in series viz. control (normal sap), 1:10,1:100, 1:1000, 1:50,000, 1:1,00,000, 1:5,000,000, for each virus isolate (mild and severe) to check the dilution end point. Eight test tubes were positioned in series in test tube rack number them as 1-8. Except one others are used for making different dilutions.

Eight lots were taken for 10 pumpkin seedlings for each isolates. Lot 1 was inoculated with normal sap (Undiluted) and other lots from 2-7 were inoculated with dilution of different concentration respectively. Excess of inoculation of different concentration in plant is washed and were placed separately in insect proof conditions and kept for observation up to 10-20 days after inoculation. Observe the symptoms periodically and note down the number of infected seedling in different lot.

Thermal inactivation point (TIP)

Thermal inactivation point is temperature at which infectivity of virus is lost. To ascertain the thermal inactivation point young infected leaves of pumpkin showing symptoms were gathered and mashed in sterilized mortar and pestle. Filter the sap through muslin cloth. Pour 2ml of the crude sap in different test tubes with help of sterilized pipette and number them as 1-8. First test tube is taken at normal room temperature as control while test tube from 2-8 were heated for 10 minutes at different temperature respectively in water bath. After subjection to different temperature for 10 minutes take out test tubes from water bath and immediately cooled in running normal tap water. Ten pumpkin seedlings were taken in eight lots. Seedlings of lot 1 is inoculated with normal sap (untreated) while lot 2- 8 were inoculated with treated sap of test tubes respectively. The inoculated plants were showered with water spray and placed inside insect proof condition. Observation is done up to 10-20 days after inoculation. Observe the expressed symptoms periodically and note down the number of infected seedling in different lot.

Longevity 'in vitro' (LIV)

Experiments on seedlings of test plant were organized to check the infectivity of crude sap. Longevity in vitro (LIV) is the time in durations of hours, days, and weeks to determine the inactivation period of virus at which infectivity rate decline.

Diseased leaves of infected plant showing mosaic symptoms were taken and crumble them to get extract. About 20ml of sap were taken in each case (mild and severe) and pour them into separate conical flask. Kept this conical flask in dark. Experiment is conducted at room temperature (30ºC-32ºC). First experiment is done in controlled condition while other experiments were done after fixed time interval of 24 hours for 9 days Inoculate the test plant with extracted sap and then kept them in glass house under observation. Observe the symptoms periodically and note them.

Longevity 'in vivo'

To determine the length of time to which two virus isolates might survive in plant tissue under 'in vivo' conditions young leaves infected with each isolate showing prominent symptoms were plucked and stored separately in dark place at room temperature (30ºC-35ºC). Everyday 10 pumpkin seedlings were inoculated with each isolate taking the inoculum from stored leaves. Inoculations were made up to 15 days. Every lot of seedlings was observed up to 15-20 days after inoculation.

Results and Discussion

Dilution end point (DEP)

It has been noticed that expression of symptom delayed gradually after a certain level of increasing dilutions (each).From the observations collected from two trials after two weeks (20 days) of inoculation showed that loss its infectivity in mild isolate is at a dilution of 1:100,000 (10^{-5}). While loss in infectivity in severe isolate is at higher dilution i.e 1:5,00,000. The results are given in table 1.

Thermal inactivation point(TIP)

During experiment it was found that the lot with untreated sap show 100% of transmission of virus infection. The mild isolate lost is infectivity at 70 ºC while severe isolate lost its infectivity at 75 ºC. It is evident from data that virus is able to produce symptoms up to thermal treatment of 70 ºC and above that virus could not withstand (Table2)

Longevity 'in vitro'

In present study the data obtained from two trials indicates that with increasing storage period (days) in two isolate the infectivity decrease. Infection retained by virus in mild isolates was up to 5^{th} days with 40% of transmission i.e the virus was activated/ infectious up to 5^{th} days at room temperature after that i.e on 6^{th}days no infection was noticed.

Table.1 Dilution end point of mild and severe isolates of pumpkin mosaic virus.

Dilutions	No. of plant infected out of 10 inoculated				Total no. of plant infected out of 20 inoculated	
	Trial I		Trial II			
	Mild	Severe	Mild	Severe	Mild	Severe
Control	10	10	10	10	20	20
1:10(10^{-1})	10	8	9	10	19	18
1:100(10^{-2})	8	7	9	9	17	16
1:1000(10^{-3})	6	4	4	6	10	10
1:10,000 (10^{-4})	3	3	2	5	5	8
1:50,000	1	2	1	2	2	4
1:1,00,000(10^{-5})	-	1	-	1	0	2
1:5,00,000	-	-	-	-	-	-

Table.2 Thermal inactivation point of pumpkin mosaic virus (mild) and severe isolates.

Temperature in ºC	No. of plant infected out of 10 inoculated				Total no. of plant infected out of 20 inoculated	
	Trial I		Trial II			
	Mild	Severe	Mild	Severe	Mild	Severe
Control	10	10	10	10	20	20
45 ºC	9	9	8	10	17	18
50 ºC	7	9	7	7	14	15
55 ºC	5	6	5	6	10	12
60 ºC	3	5	2	4	5	10
65 ºC	1	2	1	2	2	7
70 ºC	0	1	0	1	0	3
75 ºC	0	0	0	0	0	0

Table.3 Longevity ‘in vitro’ of pumpkin mosaic virus (mild) and severe isolates.

Storage (days)	No. of plant infected out of 10 inoculated				Total no. of plant infected out of 20 inoculated	
	Trial I		Trial II			
	Mild	Severe	Mild	Severe	Mild	Severe
Control	10	10	10	10	20	20
8hr	9	10	7	9	16	19
16hr	7	9	7	8	14	17
24hr	7	8	7	7	14	15
2 days	7	7	5	7	12	14
3 days	7	6	5	7	12	13
4 days	6	6	4	6	10	12
5 days	5	4	3	6	8	10
6 days	0	1	0	1	0	2
7 days	0	0	0	0	0	0
8 days	0	0	0	0	0	0

Table.4 Longevity 'in vivo' of pumpkin mosaic virus (mild) and severe isolates.

Storage (days)	No. of plant infected out of 10 inoculated				Total no. of plant infected out of 20 inoculated	
	Trial I		Trial II			
	Mild	Severe	Mild	Severe	Mild	Severe
Control	10	10	10	10	20	20
1	9	10	10	9	19	19
2	9	9	9	8	18	17
3	8	8	9	8	17	16
4	7	8	7	5	14	13
5	6	6	5	6	11	12
6	5	6	4	5	9	11
7	4	5	4	5	8	10
8	3	5	2	3	5	8
9	1	5	1	3	2	8
10	0	3	1	3	0	6
11	0	3	0	2	0	5
12	0	2	0	2	0	4
13	0	2	0	1	0	3
14	0	1	0	1	0	2
15	0	0	0	0	0	0

Fig.1 Percentage transmission of dilution end point (DEP) of mild and severe isolates

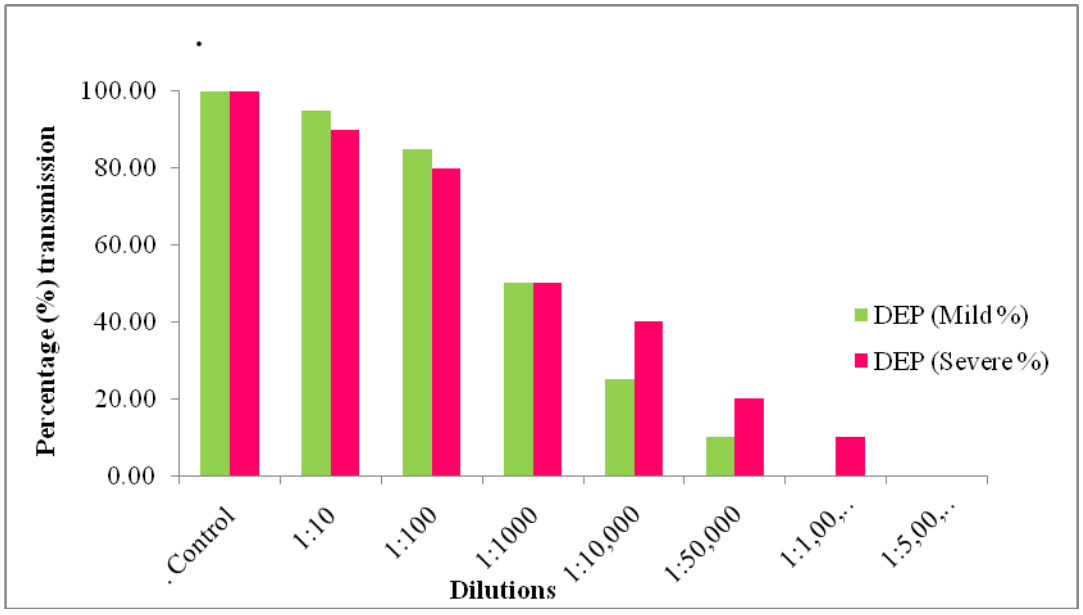


Fig.2 Percentage transmission of thermal inactivation point (TIP) of mild and severe isolates.

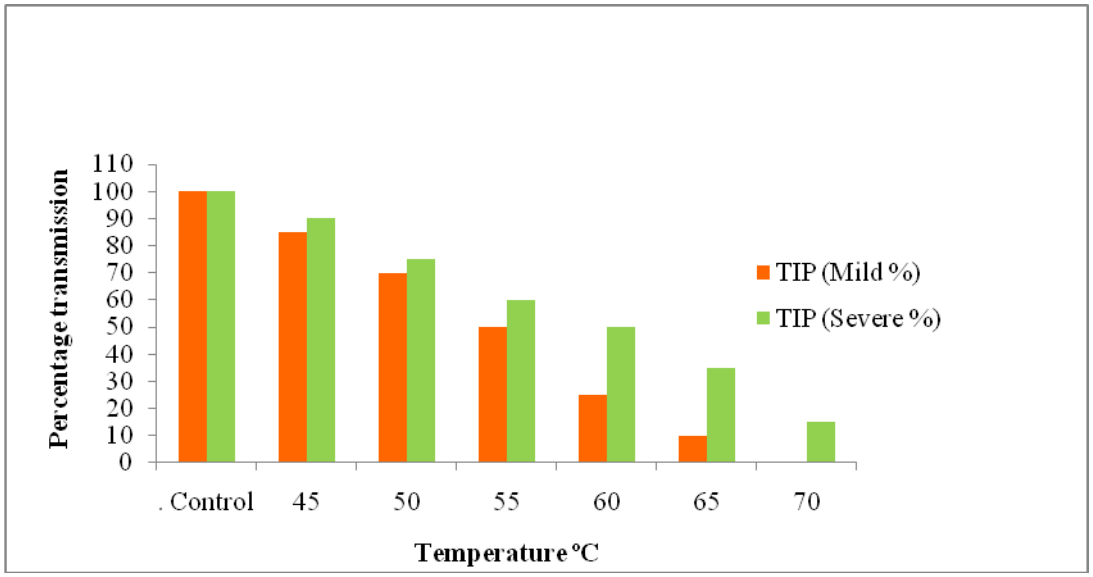


Fig.3 Percentage transmission of LIV mild and severe isolates.

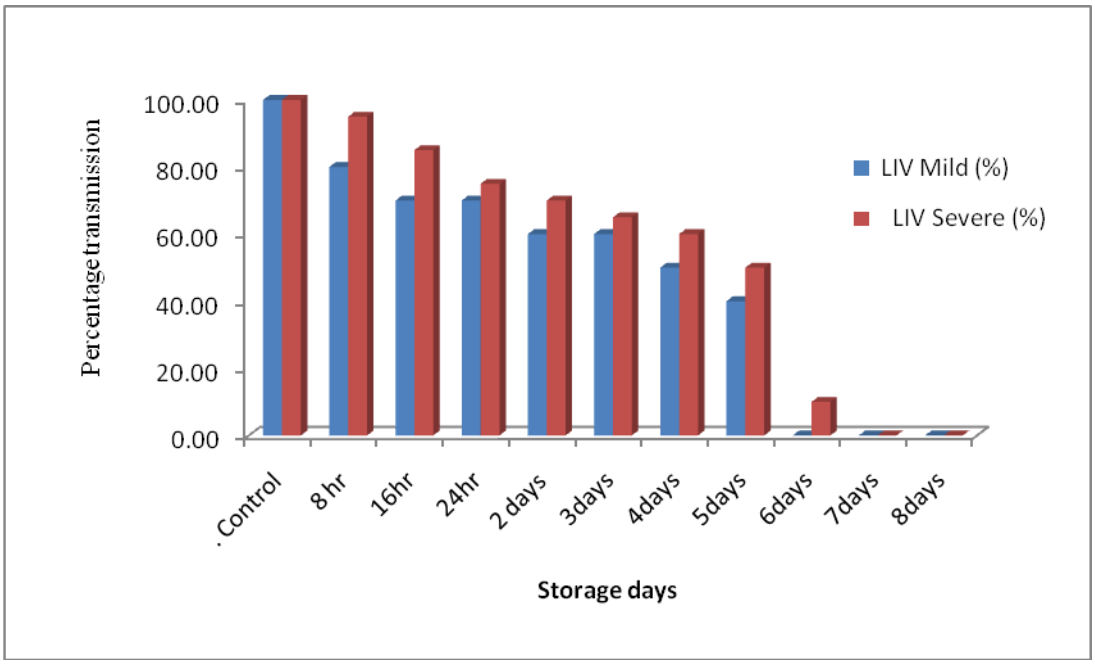
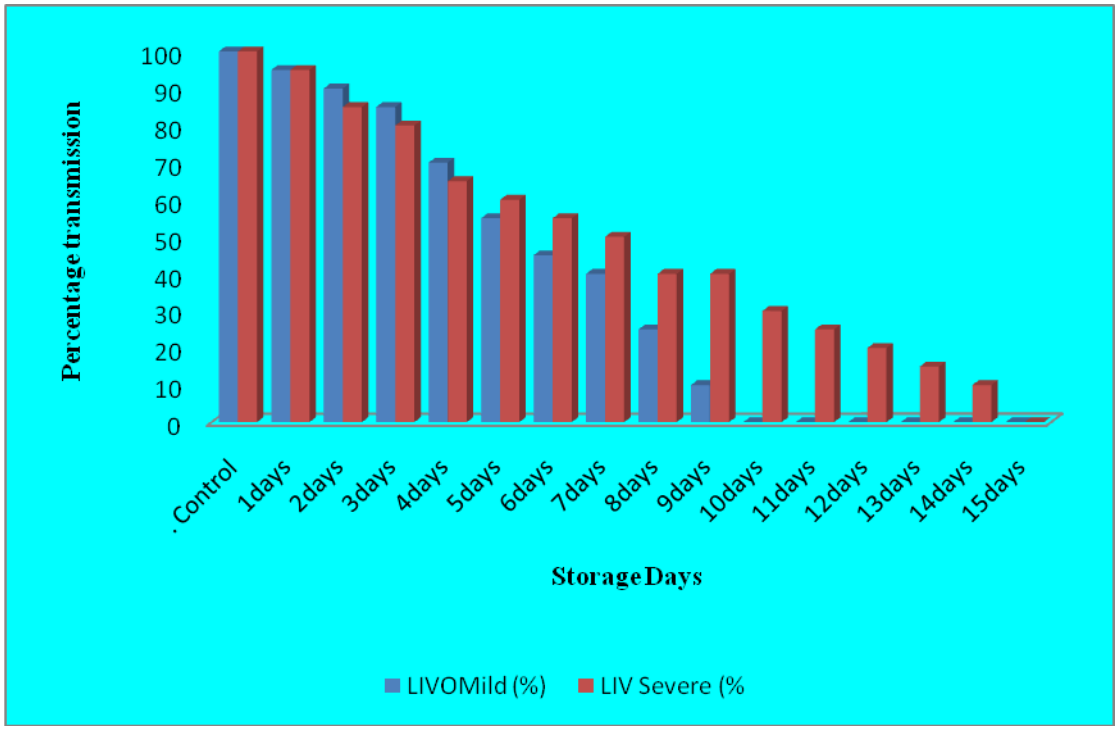


Fig.4 Percentage transmission of LIVo mild and severe isolate



Infection retained in case of severe isolate of virus was noticed up to 6th day with 20% infection at room temperature and on 7th day no infection was noticed at room temperature. During experiment 100% virus infection was observed in untreated lot by both isolates (mild and severe). Mild isolates gave only 40% infection on 5th day of storage period and after this period no infection was obtained that is the infection was ceased on 6th day. 20% infection was observed by severe isolate on 6th day which ceased thereafter (7th day) (Table 3)

Longevity 'in vivo'

In present study the data obtained from two trials indicates that with increasing in -vivo storage periods (days) in two isolate the infectivity of two isolate decreases. Infection retained by virus in mild isolates in -vivo storage was up to 9th days with 20% of transmission i.e the virus was activated/ infectious up to 9th days at room temperature after that i.e on 10th days no infection was noticed. Infection retained in case of severe isolate of virus was noticed up to 14th day with 20% infection at room temperature and on 15th day no infection was noticed at room temperature. During experiment 100% virus infection was observed in untreated lot by both isolates (mild and severe). Mild isolates gave only 20% infection on 9th day of storage period and after this period no infection was obtained that is the infection was ceased on 10th day. Severe isolate show 40% infection of seedlings inoculated with stored sap by 9th days. 20% infection was observed by severe isolate on 14th day which ceased thereafter (15th day). (Table 4). Results from biophysical properties studies viz. Dilution end point(DEP), Thermal inactivation point(TIP), Longivity in 'vitro'(LIV), Longivity in 'vivo' (LIVo) were similar to earlier findings elsewhere (Vasudeva *et al.*, 1949., Gahukar and Nariani 1982., Parvin *et al.*, 2007; Chandankar *et al.*, 2013; Koranga 2018; Ahamedemujtaba *et al.*, 2019).

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