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BIO-PHYSICAL PROPERTIES OF THE PAPAYA RINGSPOT VIRUS CAUSING RINGSPOT DISEASE IN PAPAYA (*Carica papaya* L.)

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ABSTRACT: Papaya ring spot virus (PRSV), a member of *Potyviridae*, is one of the devastating virus of the papaya and causes yield loss more than 90 per cent. It has proved as major constraint for successful cultivation of this crop in the tropical and sub tropical countries. The virus contains ribonucleic acid (RNA) with filamentous particle. The dilution end point of papaya ringspot virus was recorded between 1 x 10^{-3} to 1 x 10^{-4} thermal inactivation point between 50–55°C and longevity *in vitro* between 8 to 10 hrs.

Keywords: *Papaya ringspot virus (PRSV), dilution end point (DIP); thermal inactivation point (TIP); longevity in vitro (LIV)*

Papaya (*Carica papaya* L.) is one of the most important fruit crops grown, in India. Papaya ring spot virus (PRSV) is one of the devastating virus and major constraint in the successful cultivation of this crop in the tropical and sub tropical countries. The virus was reported to cause about 70% yield loss in tropical and subtropical regions with over 90% disease incidence (Singh *et al.,* 8; Singh, 6. This disease was first described by Lindner *et al*. (3) and viral nature was described and named the papaya ringspot virus by Jensen (1). Papaya ring spot virus disease has been reported by various workers in different names viz., papaya distortion ringspot virus, papaya mosaic virus, papaya leaf reduction virus and watermelon mosaic virus-1 (WMV-1). PRSV has two major types (Type-P and Type-W) which are serologically indistinguishable. Type P isolate infects papaya and several members of melon family and occurs in tropical and sub tropical areas of the world, including India (Purcifull *et al.,* 4; Singh, 7). Whereas, type W isolates have been reported in cucurbits in many areas of the world. Incidence of PRSV in India is as high as 99 per cent (Verma, 9). In Uttar Pradesh, PRSV is one of the devastating virus of the papaya and causes significant damage. Ninety per cent PRSV disease was recorded in Eastern Uttar Pradesh (Khurana, 2; Singh *et al.,* 8). PRSV is transmitted in a non persistent manner by several species of aphids. *Myzus persicae* Sulzer and *Aphis gossipii* are the most efficient vector of the virus and is responsible for the spread of the disease in nature. The virus is also transmitted by *Cuscuta reflexa* Roxb. and mechanically. Therefore, study was undertaken to find out the biophysical properties of the virus.

MATERIALS AND METHODS

Bio-Physical properties *i.e.,* thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) of papaya ringspot virus of papaya were studied

Thermal inactivation point (TIP)

Young infected leaves of papaya with typical symptoms were collected and ground in a mortar in 0.1M phosphate buffer (pH, 7.0) of 1:1 ratio (w/v). The slurry was squeezed through muslin cloth. Sap was centrifuged at 3000 rpm for five minutes and supernatant was collected. The supernatant was distributed in thin walled test tubes by pouring 2 ml of sap in each tube with the help of a pipette, without touching the sides of the tubes. The samples were heated at 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 and 80°C temperatures in water bath. The water bath was filled with water until the level was at least 3 cm above the level of the sap in the test tube. One test tube was placed in the rack of water bath when water temperature was reaches at

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30°C (lowest). A thermometer was placed in water bath close to test tube at same level. The temperature in each case was maintained for 10 minutes. Test tube was removed from water bath after 10 minutes and cooled in running water. After heating the water bath to the next temperature treated a second tube in the same manner. When all test tubes were treated at specified temperatures, the leaves of *Chenopodium amaranticolor* were inoculated with each sample separately, including one untreated control, kept at ambient temperature (20±°C). Regular observations were recorded for the appearance of symptoms in different treatments.

Dilution End Point (DEP)

The inoculum (sap) was prepared as earlier and two ml sap was pipetted to each test tube and the tubes were closed with aluminium foil. Dilutions were made in a series like undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. Eight test tubes were placed in a row in a test tube stand. Second of these test tubes were filled with 9 ml water with help of a pipette. One ml sap was transferred in the second test tube to make dilution 10^{-1}. Sap was mixed thoroughly with water in test tube and 1 ml of this dilution (10^{-1}) was transferred to the third test tube to be make the dilution (10^{-2}). This procedure was repeated till 10^{-7}. The leaves of *Chenopodium amaranticolor* were inoculated with sap at different dilutions to test infectivity. There were five replicates for each dilution level. Symptoms were observed after 10-15 days and data were recorded for each treatment separately.

Longevity *in vitro* (LIV)

Longevity *in vitro* is a time expressed in days, weeks, hours for which the virus in crude juice kept at room temperature remains infective. It is usual to store the crude juice in closed tubes and to lost a sample on test plants at a series of intervals. The inoculum was prepared as earlier and two ml sap was pipetted to each test tube and the tubes were closed with a stopper or aluminium foil. Tubes were stored at room temperature for 2, 4, 6, 8, 10, 12, 14, 16 and 18 hrs. After the specified duration of storage the samples were inoculated on the leaves of *Chenopodium amaranticolor*. Regular observations were made for the appearance of symptoms and data were recorded from each plant separately.

RESULTS AND DISCUSSION

Thermal inactivation point

It is clear from the observations and data presented in Table 1 that the virus was found active at a temperature up to 50°C but it was inactivated at 55°C which indicated that the virus was inactivated between 50 and 55°C as the sap treated at 55°C for ten minutes could not produce any lesion on *Chenopodium amaranticolor* plants. The loss of infectivity of virus is increased at above 40°C.

Table 1: Thermal inactivation point of papaya ringspot virus.

Temperature (°C)	Average no. of local lesion on *Chenopodium amaranticolor* leaves
30	30.65
35	26.50
40	23.45
45	15.45
50	6.40
55	No lesions
60	No lesions
65	No lesions
70	No lesions
75	No lesions
80	No lesions

Dilution end point

Data presented in Table 2 indicated that the virus remained infective in sap extracted from diseased leaves of papaya at 1: 1000 dilution but not at 1: 10000 dilution, which indicated the dilution end point between 1: 1000 and 1: 10000.

Table 2: Dilution end point of papaya ringspot virus.

Dilution (Concentration)	Average no. of local lesion on *Chenopodium amaranticolor* leaves
1:1	26.65
1:10	16.70
1:100	9.05
1:1000	3.10
1:10000	No lesions
1:100000	No lesions
1:1000000	No lesions
1:10000000	No lesions

Longevity in vitro

A perusal of the data presented in Table 3 reveals that virus was infectious up to 8 hrs of storage at room temperature and it was inactivated after 10 hrs of storage. The longevity of virus was recorded between 8 and 10 hrs at room temperature.

Table 3: Longevity *in vitro* of papaya ringspot virus.

Duration (hrs.)	Average no. of local lesion on *Chenopodium amaranticolor* leaves
0	32.25
2	25.50
4	20.25
6	11.65
8	6.70
10	No lesions
12	No lesions
14	No lesions
16	No lesions
18	No lesions

Dilution end point of papaya ringspot virus was recorded between 1 x 10^{-3} to 1 x 10^{-4}, thermal inactivation point between 50–55°C and longevity *in vitro* between 8 to 10 hrs. Similar results were reported by Singh (6); Sharma *et al*. (5) and Wu *et al.* (10).

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