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# Transmission and Host Range of Papaya Ring Spot Virus

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***Abstract*:** Papaya belongs to the family Caricaceae. The papaya ring spot virus disease (PRSV) is a well-known aphid and sap transmissible plant pathogenic virus in the genus *Potyvirus* and family *Potyviridae*. Among viral diseases, papaya ring spot virus is a wide spread pathogen that can cause up to 90% yield losses in papaya .The symptoms on mechanical transmission were characterized by vein clearing followed by chlorosis, yellow mosaic, blistering and leaf distortion. Later on necrotic spots developed leading to complete necrosis of leaves, petioles and stem. The result on aphid transmission revealed that, three aphid spp. *viz. Aphis gossypii, Aphis craccivora* and *Myzus persicae* transmit the virus in non - persistent manner from papaya (*Carica papaya*) to papaya. *Myzus persicae* was more efficient (90 %) than *Aphis gossypii* (80%). The, papaya ring spot virus was easily mechanically transmitted in papaya, cucurbits and some other plants. Experimental findings showed that the virus was successfully transmitted by the sap inoculation method in plants belonging to families *viz.* Caricaceae (*Carica papaya*), cucurbitaceae (*C. sativus, Cucurbita moschata*, *C. pepo, Luffa acutangula, L. cylindrica, Lagenaria siceraria, Memordica charantia)* with systemic mosaic mottling symptoms. However, plants of families Chenopodiaceae (*Chenopodium amaranticolor, Chenopodium quinoa*) produced local lesions.

***Keywords*:** Papaya ring spot virus, Transmission, Host range, *Myzus persicae* and *Aphis gossypii*

The importance of papaya in agriculture and in the World's economy is demonstrated by its wide distribution and substantial production in the tropical countries (Anonymous 2015). Many pathogens like viruses, fungi, bacteria and nematodes infect papaya, causing considerable losses in yield and deteriorate the quality of fruits. Besides these, a number of viruses belonging to Cucumo, Gemini, Ilar, Poty, Rhabdo, Tobra, and Tospo virus groups have been recorded on papaya. Important amongst them are papaya leaf curl virus, papaya ring spot virus and papaya mosaic virus. In India, papaya ring spot virus is the major viral disease causing considerable losses in yield and quality of fruits (Jensen 1949). At present papaya ring spot disease has assumed serious proportion and became a major constraint in papaya cultivation, thereby threatening the cultivation of papaya in India, including Maharashtra (Khurana and Bhargava 1970, Rao 1988 and Kale 1999). Symptoms consist of intense yellow mosaic on leaves, small shoestring-like new leaves, dark green and slightly sunken rings on the fruit, numerous oily- looking streaks on the stem and stunting of the plant. Trees infected at a very young age remain stunted and never produce any fruit (Kunkalikar et al 2006 and Reddy et al 2007). The virus was named as PRSV by DeBokx (1965). The virions are filamentous, non-enveloped and flexuous measuring 760-800 x 12 nm. Virus particles contain 94.5% protein and 5.5% nucleic acid. The protein component consists of the virus coat protein (CP), with molecular weight of about 36 kDa. The virus is naturally transmitted by the insect-vector aphids in a non- persistent manner, from papaya to papaya plants infecting all trees in an orchard within a few months. At present, sole cultivation of papaya has become more common after advent of improved varieties and hybrids. The area under this crop is continuously increasing because farmers prefer its cultivation due to its high yield potential, less water requirement and attractive prices in the market. The crop is emerging as an alternative cash crop to banana in Maharashtra. Considering the economic importance of crop and disease present investigation is carried out.

## MATERIAL AND METHODS

The present investigations were carried out in the glass house during 2016 at Department of Plant Pathology, College of Agriculture, Latur.

**Diseased samples:** The papaya ring spot disease samples were collected from the farmers' fields of various villages in Latur district, where papaya fields were found infected with PRSV.

**Transmission**

**Mechanical transmission:** For mechanical transmission, sap was extracted by crushing symptomatic leaves of diseased papaya plants with a mortar and pestle in a chilled 0.05M potassium phosphate buffer ($P^H$ 7.4) containing 0.02M 2-mercapto ethanol. Test plants were inoculated by conventional leaf rub method with a cotton swab. Carborandum powder (800 mesh) was used as an abrasive. Immediately after virus inoculation, the leaves of test plants were rinsed with tap water. Test plants used for mechanical inoculation were raised from virus free seeds in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture. Test plants were maintained in an insect-free glass house for 4- 6 weeks and observations

were recorded with respect of symptom development and incubation period.

**Aphid transmission:** For aphid transmission, *Aphis craccivora* Koch., *Aphis gossypii* Glov, and *Myzus persicae* Sluz, raised from single aphid colony were used. For raising aphid colony, the healthy leaves of cotton (*Gossypium hirsutum* L.) and groundnut (*Arachis hypogea* L.) were placed in a Petri dishes on slightly wet filter paper and an apterous form of aphids were transferred separately with small camel hair brush to the leaves. Petri dishes were closed for 8 hours and the newly born aphids were used for transmission studies. The apterous forms of aphids were transferred to clean Petri dishes for 2 hours for fasting. This was followed by an acquisition feeding of 40 to 60 seconds on virus infected detached leaves of source plant. Aphids were allowed to make only brief probes of 40 to 60 seconds duration. Aphids still in probing position at 40 seconds were picked up with camel hair brush and transferred in batches of 25 to healthy test plants for inoculation feeding of four hours. The test plants were kept in muslin cages. Later, aphids were killed by spraying with 0.02 per cent imidacloprid (17.8 EC) insecticide and plants were maintained in an insect free glasshouse for three to four weeks. Observations were recorded for the symptoms on test plants.

**Host range:** For host range studies, plant species belonging to the different families *viz.* Cucurbitaceae, Chenopodiaceae and Solanaceae were raised from healthy seeds in earthen pots containing steam sterilized soil, sand and compost mixture (2:1:1) and maintained in an insect free glass house. Ten plants of each host species were inoculated with the sap extracted from virus infected papaya (Cv. Red lady) plants by conventional leaf rub method and aphid transmission also done simultaneously. All plants were inoculated on the first leaf or fully expanded leaves. The inoculated plants were kept for observation for 4-6 weeks along with the control plants

The following species were used as test plants in host range studies. Family / Host species Amaranthaceae (*Amaranthus caudatus* L), Chenopodiaceae (*Chenopodium album* L*., Chenopodium amaranticolor*), Compositae (*Helianthus annuus* L.), Cruciferae (*Raphanuns sativus* L.), Cucurbitaceae (*Luffa actungula* L*., Momordica charantia* L*., Cucumis sativus* L,*. Lagenaria siceraria, Cucurbita pepo* L*., Cucurbita moschata*), Leguminoceae (*Phaseolus vulgaris* L*, Pisum sativum* L*., Vigna mungo, Vigna radiate, Cajanus cajan*), Malvaceae (*Abelmoschus esculenttus,*) and Solanaceae (*Capsicum annum* L, *Nicotina tabacum* L*., Nicotina glutinosa* L*., Nicotiana xanthi*)

## RESULTS AND DISCUSSION

**Collection of PRSV samples:** The PRSV infected papaya samples collected exhibited the symptoms *viz.,* severe mosaic, leaf distortion, shoe stringing and fruits with ring spot.

**Isolation and maintenance:** All the inoculated papaya Cv. Red Lady seedlings showed the PRSV symptoms, which were used as a source of virus inoculum for further studies.

**Symptomatology:** All the ten inoculated plants showed symptoms within 2 to 3 weeks after inoculation. The initial symptoms observed varied from chlorotic mottling of the leaves to severe rugosity. Infected plant showed chlorosis on the youngest leaves, vein clearing rugosity and mottling of leaf lamina interveinal puckering or bulging of the leaf tissues on the upper surface of young leaves (Plate I) . In the severe cases filiform shoe string and distinct chlorotic streak were found on the leaf tendrils. Most of the field surveyed revealed characteristic symptoms of papaya ring spot virus. Various types of symptoms like mild to severe mosaic, mottling, ring spot on fruits, leaves and stems, distortion of fruits, leaves and stems, filiform leaf, shoestring leaf, vein curling, vein distortion, puckering, leaf curling, leaf rolling, fruit yellowing, vein zigzag and stunting growth of plants were observed during collection of PRSV samples. Several workers have described same type of symptoms for PRSV in mechanical transmission and in field. (Kshirsagar 2014, Surwade 2014, Singh et al 2017).

**Transmission studies**

**Mechanical transmission:** The results on sap inoculation indicated that, the virus was readily transmitted by mechanical means under artificial conditions. Cultivar Red lady was mechanically inoculated using 0.1 M potassium phosphate buffer and started developing symptoms 15 days after inoculation. The symptoms always started on newly emerged leaves of papaya seedlings, showing vein clearing, chlorotic spots and chlorotic rings. Later these plants produced varied types of symptoms including leaf reduction to shoestring, leaf distortion, puckering, mosaic pattern and stunted growth. Similar type of results of mechanical transmission i.e. symptoms on leaves, stem and fruits of infected papaya plants WERE reported by several workers. (Roy et al 1999, Reddy et al 2007 and Singh et al 2017)

**Aphid transmission:**. The three aphid spp. *Aphis gossypii, Aphis craccivora* and *Myzus persicae* transmit the virus in non-persistent manner from papaya (*Carica papaya*) to papaya. (Table 1, Fig. 1) *Myzus persicae* was more efficient (90 percent) than *Aphis gossypii* (80%) and *Aphis craccivora* in transmitting the virus. The appearance of symptoms was fast in case of plants inoculated with *Myzus persicae* as compared with plants inoculated with *A.gossypii* and *A.craccivora.* Similar results regarding *Myzus persicae* as efficient vector were observed by Reddy et al (2007) and Gude et al (2008). The transmission efficiency of aphids i.e. *Myzus persicae* and *Aphis gossypii* was dependent on the number of aphids used / test plant (Table 2, Fig 2 & 3). The percent transmission varied from obtained was 10 to 90%, when inoculated with 1-18 aphids/test plants (*Myzus persicae)* were used. Similarly, when we used same no. of aphids / plant the per cent transmission obtained was 10-

80% by *Aphis gossypii*. Similar results regarding transmission efficiency of *Aphis craccivora* and *Myzus persicae* was earlier reported by Reddy, (2007). Kalleshawaraswamy) 2008) reported that *Myzus persicae* (56%) and *Aphis gossypii* (53%) were significantly more efficient in transmitting PRSV than *A. craccivora* (38%). The systemic symptoms produced on cultivar Red lady of papaya by aphid inoculation were similar to those produced on same cultivar by sap- inoculation.

Fig. a. Leaf distortion

Fig. b. Blistering of leaves

Fig. c. Local lesion on leaves

Fig. d. Mosaic motteling

**Plate I.** Symptoms of papaya ring spot virus disease produced by mechanical transmission on papaya seedlings

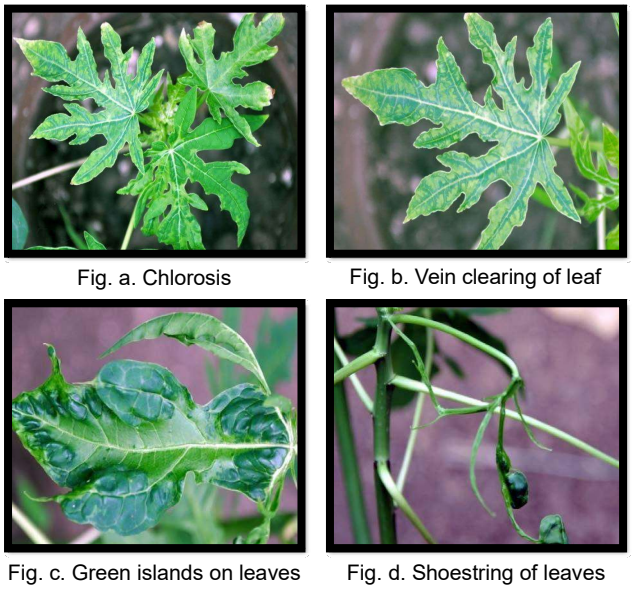

Fig. a. Chlorosis

Fig. b. Vein clearing of leaf

Fig. c. Green islands on leaves

Fig. d. Shoestring of leaves

**Plate II.** Symptoms of papaya ring spot virus disease produced by mechanical transmission on papaya seedlings

**Host range of papaya ring spot virus:** The papaya ring spot virus was easily mechanically transmitted in papaya, cucurbits and some other plants. Papaya ring spot virus infected only 9 plant species and failed to infect 12 plant species. Out of 9 plant species infected 2 from Chenopodiaceae, 6 from Cucurbitaceae and one from

**Plate III.** Symptoms of papaya ring spot virus disease produced by aphid transmission

**Table 1.** Aphid transmission of the virus causing papaya ring spot virus in papaya Cv. Redlady

| Aphid species | Transmission (%) | Reaction on PRSV | |
|---|---|---|---|
| | | Local | Systemic |
| *Aphis gossypii* | 70 | ------ | Vc, MMo, Ld, Ss |
| *Myzus persicae* | 90 | ------ | Vc, MMo ,Ld, Ss |
| *Aphis craccivora* | 50 | ------ | Vc, MMo, Ld, Ss |

Vc = Vein clearing MMo= Mild mosaic
Ld = Leaf distortion Ss = Shoe string

**Table 2.** Efficiency of *Myzus persicae* and *Aphis gossypii* vectors in transmitting the papaya ring spot virus in Cv. Redlady

| No. of aphids /plant | *Aphis gossypii* | *Myzus persicae* |
|---|---|---|
| | Transmission (%) | Transmission (%) |
| 1 | 10 | 10 |
| 2 | 10 | 20 |
| 4 | 20 | 30 |
| 6 | 40 | 50 |
| 8 | 50 | 60 |
| 10 | 60 | 70 |
| 12 | 70 | 90 |
| 14 | 70 | 80 |
| 16 | 70 | 90 |
| 18 | 80 | 90 |

Caricaceae. The virus was successfully transmitted by sap inoculation method in plants belonging to families Caricaceae *viz.* (*Carica papaya*) and Cucurbitaceae (*C. sativus, Cucurbita moschata*, *C. pepo, Luffa acutangula, L. cylindrica, Lagenaria siceraria, Memordica charantia)* with systemic mosaic mottling and leaf distortion symptoms. However, plants of families Chenopodiaceae (*Cheno podium amaranticolor, Chenopodium quinoa*) produced local lesions. Similar results were reported by Kumar et al (2014). The virus under study did not produce any symptoms on *Nicotiana xanthi, Nicotiana glutinosa, Nicotiana tabaccum*, *Capsicum annum* L., *Abelmoschus esculentus*, *Vigna mungo*, *Vigna sinensis* and *Pisum sativum* , which indicated their non-host status. Similar results on host range was reported by Tripathi et al (2008), Kumar et al (2014), Muske et al (2014) and Singh et al (2017). Muske et al (2017) also reported that Zucchini is an indicator plant where as it helps in the early and accurate virus indicator prominently than other host plant. Many cucurbitaceous plants were reported as natural hosts of papaya ring spot virus. (Singh et al 2017).

**Table 3.** Host range of papaya ring spot virus isolate

| Family / Host species | Main symptoms |
|---|---|
| Amaranthaceae | |
| *Amaranthus caudatus* L. | -- |
| Chenopodiaceae | |
| *Chenopodium album* L. | LL |
| *Chenopodium amaranticolor* | LL |
| Compositae | |
| *Helianthus annus* | -- |
| Cruciferae | |
| *Raphanuns sativus* L. | -- |
| Cucurbitaceae | |
| *Luffa actungula* L. | VC,M,MO |
| *Memordica charantia* L. | MO, M |
| *Cucumis sativus* L. | VC,M, MO,LD |
| *Lagenaria siceraria* | VC,SM,BL,GVB,C,LD |
| *Cucurbita pepo* L. | VC,BL,SM,LD |
| *Cucurbita moschata* | MO, M |
| Leguminoceae | |
| *Phaseolus vulgaris* L. | -- |
| *Pisum sativum* L. | -- |
| *Vigna mungo* | -- |
| *Vigna radiate* | -- |
| *Cajanus cajan* | -- |
| Malvaceae | |
| *Abelmoschus esculenttus* | -- |
| Solanaceae | |
| *Capsicum annum* L. | -- |
| *Nicotina tabacum* L. | -- |
| *Nicotina glutinosa* L. | -- |
| *Nicotiana xanthi* | -- |
| Caricaceae | |
| *Carica papaya* | VC,SM,BL,GVB,C,LD,SS |

VC- Vein clearing
M- Mosaic
LL- Local lesions
SM- Severe mosaic
BL- blistering
LD-Leaf distortion
GVB- Green vein banding
-- Non host
C - chlorosis
Mo- Mottling
SS- Shoe string

Fig . a. Bitter gourd leaf showing mottling

Fig. b. Mosaic and blisters on Cucurbita pepo

Fig.c. *Chenopodium amaranticolor* showing local lesion

Fig. d. Papaya showing local lesion

**Plate IV.** Host range of papaya ring spot virus

Fig a. Mild mosaic and blister

Fig b. Leaf distortion

Fig c. Leaf exhibiting vein clearing and green vein banding

Fig d. Mosaic symptom

**Plate V.** Host range of papaya ring spot virus

## CONCLUSIONS

Thus, from the results obtained on various aspects during present investigation on papaya ring spot virus disease of papaya, it is concluded that, Based on transmission (insect and mechanical), host range and symptomatology, the virus under present study transmitted by aphids (*M. persicae, A. gossypii, A. craccivora*) and mechanical means. Papaya ring spot virus is restricted to the families such as Caricaceae, Cucurbitaceae and Chenopodiaceae.

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