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Different modes of transmission of mosaic disease of pumpkin (*Cucurbita moschata*)

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Abstract
Among the all cucurbitaceous family pumpkin is one of most important crop which having highly nutritious value. About the nutritional status of pumpkin contains, protein 1.2 g, dietary fibre 0.6 g, carbohydrates 7.5 g as well as pumpkin is a good source of Vitamin-A which is letter generated in body. Among the various problems involved in the cultivation of pumpkin crop insect, pest and diseases are of prime importance. In which diseases are the major problem in pumpkin cultivation. Among the all diseases the virus diseases are most significant pathogens of cucurbits (cucumber, watermelon, melon and pumpkins). Present investigation was undertaken in Kharif------ for the study the various modes of transmission of mosaic disease of pumpkin. There are various modes of transmission was studied such as mechanical or sap, seed and insect (aphid and whitefly). In present investigation mechanical transmission study indicated that only the leaf extract from isolate I recovered the virus whereas the extract from isolate II does not. Seed transmission study indicated that, all the virus isolates were not transmissible through seeds. In insect transmission study it was found that, out of Isolate I and Isolate II only virus isolate I was transmitted by aphids. The aphid species *M. persicae* used for virus transmission proved to be most efficient vector which transmitted virus isolate I, transmission percentage was 40-100 per cent. Study also indicated that, even a single viruliferous adult of *M. persicae* was able to acquire and transmit the virus up to 40-50 per cent in Isolate I. On the other hand, Isolate II was transmitted through whitefly only. No transmission was shown by virus Isolate I. The whitefly, *Besimia tabaci* was found to be efficient vector for transmission of virus isolate II. It is also stated that, single whiteflies were able to transmit the virus with 30 per cent transmission efficiency.

Keywords: pumpkin, mosaic, transmission, mechanical or sap, seed, insect, aphid, whitefly

1. Introduction
Pumpkin (*Cucurbita moschata* Duch) is an important crop species for Indian agriculture of *Cucurbitaceae* family and is grown widely throughout the year. Pumpkins are thought to have originated in North America. Pumpkins are grown all round the world for variety of regions ranging from agricultural purposes to marketable and ornamental sales. It is most common rather cheaper and popular vegetable in all states in India as well as in world. The dietary status of pumpkin contains about 1.2 g of protein, 0.6 g dietary fibres and 7.5 g carbohydrates. It also contains 24.4 mg calcium, 13.9 mg magnesium, 51 mg phosphorous and 394 mg potassium. Pumpkin is an important article of diet, the main nutrients are lutein and both alpha and beta carotene, the latter which generates vitamin A in body. Demand for year-round production of pumpkin in many parts of the world has led to in a serious prevalence of the pest and diseases of pumpkin crop. Among the various problems involved in the cultivation of pumpkin crop insects, pests and diseases are of prime importance. Among the major diseases, diseases triggered by viral pathogen are one of major risk in pumpkin cultivation which can cause mare economic losses of crop plant. Viruses are the most important pathogens of cucurbits (cucumber, watermelon, melon and pumpkins) belonging to the family *Cucurbitaceae*. More than 30 infectious viruses causing destructive symptoms and considerable economic losses were reported on these plants (Zitter *et al.* 1996). It is usually not easy to find out appropriate control measures to lessen the amount of destruction. Viruses cause extensive losses in cucurbit crops. Zucchini yellow mosaic virus known to be one of the most destructive viruses of pumpkins (Dukia, 2001). Mosaic disease of pumpkin not easy to control completely, due to its different modes of transmission. It is useful to understand the various modes of disease transmission, it's better for the disease management also.

Considering all above fact present study on different modes of transmission of mosaic disease of pumpkin was undertaken to find out mosaic disease transmission vector in Kharif-…….. at glass house and field condition of department of Plant pathology and Agril. Microbiology at MPKV. Rahuri.

Material and Methodology
1) Mechanical transmission
a. Preparation of inoculum
The inoculum was prepared from young infected leaves showing prominent virus symptoms of individual isolates of pumpkin. The leaves from each is olates were collected, washed thoroughly in running tap water to remove dirt, immediately wiped off excess water with the help of blotting paper and weighed on the chemical weighing balance. The inoculum was prepared by grinding young infected leaves in sterilised mortar and pestle in chilled 0.1 M phosphate buffer, pH 7.0.The buffer was added at the rate of 1ml per gm. of infected tissue 1:1(w/v) basis while

macerating. The resulting pulp crude sap after maceration was obtained by squeezing through a double layered muslin cloth. This extract was used as "standard inoculum" for further studies.

b. Inoculation procedure

Well grown and healthy pumpkin seedlings were selected for inoculation. Selected plants were watered at least half an hour earlier before they were inoculated, so that the leaves may remain turgid at the time of inoculation. Before inoculation, 600 mesh carborundum powder or celite an abrasive was dusted uniformly on the upper surface of the leaves of the test plants. The cotyledons leaves of test plants were inoculated gently by rubbing the upper surface of the cotyledons leaves with the forefinger dipped in the inoculum. The plants species from *Chenopodiaceae* and other families were inoculated at 5-6 leaf stage. However species of *Cucurbitaceae* families were inoculated on cotyledons leaves. After inoculation, the inoculated leaves were washed immediately in the stream of tap water to remove excess inoculum. The inoculated seedlings were labelled properly and kept for observation in the glasshouse. The control plants were treated similarly using neutral phosphate buffer solution only. The inoculated plants were observed periodically and observations were recorded as and when the symptoms appeared.

2) Seed transmission

For seed transmission studies, the seedlings of pumpkin were inoculated with all the two isolates and kept under glasshouse for symptom expression and the collection of infected fruits on maturity was done. The fruits were harvested; seeds were extracted and dried under shade. The seeds collected from infected plants of each seedling and of each virus isolate were tested separately under glasshouse for seed transmission study. Twenty seeds of respective isolates of pumpkin were sown separately in earthen pots in an insect proof glasshouse and the seedlings were kept under observation for about three months. Observations on germination per cent and development of virus symptoms were recorded. For control, seeds collected from the healthy fruits were also sown simultaneously. The number of seeds germinated and the number of plants exhibiting virus symptoms were recorded.

3) Insect Transmission

i. Maintenance of whitefly culture

The type culture of whitefly (*Bemisia tabaci,* Genn.) was collected from the Vegetable Improvement Scheme, Department of Horticulture, MPKV., Rahuri and was maintained on cucumber, tabacco (*N. tabaccum*) and also on brinjal plants grown in insect proof glasshouse.

ii. Collection of whiteflies

An aspirator made with a test tube (big size) was used for the collection of whiteflies. By slowly turning the leaves slightly upwards the whiteflies were sucked into the aspirator. The whiteflies were later transferred into the cages.

iii. Rearing cage for whitefly

A wooden cage (60 x 60 x 60 cm) was constructed and muslin cloth was fixed on three sides with adhesive, fevicol and the top and the front was covered with glass. The front glass can be easily moved on the grooves made in the wooden framework. This frame was kept on the wooden rectangular base. In each cage healthy brinjal, tobacco plants were grown in earthen pots and used for maintenance of pure culture of Whitefly (*Bemisia tabaci*, Genn.)

iv. Cages used for acquisition of virus

The 20 cm long plastic bottle with 7.5 cm diameter at one end and tapering towards the narrow mouth was used to prepare cage for acquisitions. The bottom portion of such bottles was removed with the help a sharp knife and was covered with muslin cloth. The whiteflies were collected into the bottle and the infected pumpkin branch was inserted into the bottle through the narrow mouth and then closed with cotton plug. In all the experiments 24 hours acquisition access feeding period was given. After the acquisition access period, the viruliferous whiteflies were taken and were used for inoculation.

v. Cages used for inoculation of seedlings

A cylindrical shaped iron cage about 80cm long and 20cm in diameter was taken. A white coloured clean muslin cloth is then wrapped around the iron cage and stitches using thread. A small grove of about 1cm was made on the cages, which helped to release the viruliferous whiteflies into the cages placed over the seedlings, bigger sized cages were used or cages were plugged with cotton after inserting the young leaflets into the tube and the cages were tied to bamboo with a rubber band.

vi. Transmission (Inoculation Method): Whitefly transmission

Insect transmission studies were made by whitefly (*Bemisia tabaci* Genn.). About 200-500 adults of *Bemisia tabaci*, Genn. were collected from rearing cages and starved for 1 hr. and then released into acquisition cages and later pumpkin infected branch was inserted and allowed to feed for 24 hr. as an acquisition access period. The viruliferous whiteflies were onto healthy pumpkin seedlings access small plastic tubes (8 cm height) and allowed to feed for 24 hr. as an inoculation access period. After inoculation the whiteflies were sprayed with 0.15 percent dimethoate to kill the whiteflies.

vii. Rate of transmission

Healthy *B. tabaci* Genn. were collected in an acquisition cages with the help of an aspirator and pumpkin infected twig was inserted inside the bottle and whiteflies were allowed to feed for 24 hr. After acquisition the whiteflies were released on to healthy seedlings at the rate of ten insect per seedling, with the help of an aspirator. Plastic cages (8 cm x 3 cm) were used for confining whiteflies. After 24 hr. of inoculation access period whiteflies were killed by spraying with 0.15 per cent dimethoate and kept in insect proof glasshouse for symptoms development.

viii. Minimum number of whiteflies required for transmission

Healthy *B. tabaci*, Genn. were starved for 1 hr. and allowed for 24 hr. of acquisition access on infected pumpkin plants. The whiteflies were then transferred to young healthy pumpkin seedlings in batches of 1, 2, 3, 4, 5 and 15 separately. Ten plants were inoculated in each treatment. Whiteflies were given inoculation access period of 24 hr.

After inoculation access period of 24 hr. the whiteflies were killed by spraying 0.15 per cent dimethoate and plants were kept in insect proof glasshouse for symptoms production.

ix. Aphid transmission

Aphid transmission studies were conducted to find out the aphid vectors for the transmission of pumpkin viruses. The colonies of non- viruliferous aphids were maintained on host plant viz., *Myzus persicae* Sulz. on brinjal, whitefly (*Bemisia tabaci* Gen.) on tobacco (*Nicotina tabacum* L.) cv. White Burley, in an insectary in small muslin covered cages. These insects were identified from Department of Agricultural Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri. Young 12-15 days old plants of pumpkin were used as a test plant. Wingless adult females were employed in all insect transmission experiments. Aphids were gently disturbed on their feeding leaf by slightly touching their antennae which made them to withdraw their stylets. They were carefully picked up and transferred with the moistened tip of a camel hair brush. During starvation periods, insects were placed in the test tubes after collecting from their colonies by means of a camel hair brush prior to each test, the aphids were starved for 120 min. and by keeping them in a big glass tube covered with muslin cloth. For 15 min. acquisition feeding, aphids were placed on virus infected detached young leaves, separately in a small, clean Petri plate. After acquisition feeding on virus source plant, the insects were liberated 30 min. for inoculation feeding on healthy young pumpkin plants, thus the test plants were completely covered with celluloid cages and protected from other sources of infection. After inoculation feeding, plants were sprayed with 0.025% methyl demeton to kill the insects. The plants were observed for 50 days for symptoms development.

x. Number of aphids required for virus transmission

Per cent virus transmission of various pumpkin virus isolates by varying number viz., 1, 5, 10, 15, 20, 25, 30 of viruliferous aphids of *M. persicae* were transferred to individual test seedling of pumpkin and aphids were allowed for pre-acquisition fasting for 2hr. and acquisition feeding for 15 min. on various isolates of virus source separately and then transferred to healthy young pumpkin plants for transmission feeding for 30 min. after which they were killed with 0.025 % methyl demeton solution. Twenty seedlings were exposed to each group.

Results

1. Transmission by mechanical sap inoculation

Virus inoculation was prepared from the leaves extracts of isolate I and isolate II and used to recover the virus. Inoculation was done with these-extract after a specific incubation period symptoms were only shown by virus isolate I. No symptoms were shown by virus isolate II (Table 1). Hence the results on mechanical transmission indicated that only the leaf extract from isolate I recovered the virus whereas the extract from isolate II does not.

Table 1: Mechanical transmission studies of Isolate I and II.

Sr. No	No. of Sap inoculated seedling	Isolate I		Isolate II	
		Infected/ Inoculated	Per cent transmission	Infected/ Inoculated	Per cent transmission
1	10	5/10	50	0/10	00
2	10	7/10	70	0/10	00
3	10	4/10	40	0/10	00
4	10	6/10	60	0/10	00
5	10	10/10	100	0/10	00
6	10	10/10	100	0/10	00
7	10	9/10	90	0/10	00

2. Transmission through seeds

To determine the possibility of seed transmission of virus isolates I and II were tested. Twenty seeds from infected fruit of each isolate were tested. The seedlings were grown in control glass house conditions and were under observation for two month (Table 2). Since no symptoms were shown by the seedlings with concern to mosaic disease, the study indicated that, all the virus isolates were not transmissible through seeds.

3. Insect transmission

3.1. Transmission through aphids: It was found that out of Isolate I and Isolate II only virus isolate I was transmitted by aphids. The aphid species *M. persicae* used for virus transmission proved to be most efficient vector which transmitted virus isolate I. The transmission percentage was 40-100 % (Table 3).

3.2. Number of aphids required to transmit the virus from Isolate I by *M. persicae*: The results presented in Table 3 indicated that, even a single viruliferous adult of *M. persicae* was able to acquire and transmit the virus up to 40-50 per cent in Isolate I. *M. persicae* could not acquire and transmit the isolate II.

Table 2: Seed transmission of different Pumpkin virus isolates through infected seed

Cultivars	No. of seeds sown	No. of seed germinated	Germination %	No. of plants showing symptoms	% transmission
Isolate I					
1	20	17	85	00	00
2	20	15	75	00	00
3	20	16	80	00	00
Isolate II					
1	20	12	60	00	00
2	20	14	70	00	00
3	20	13	65	00	00

Table 3: Effect of number of aphids *M. persicae* on transmission of various pumpkin virus isolates

Sr. No.	No. of aphids	Isolate I		Isolate II	
		Infected/ Inoculated	Per cent transmission	Infected/ Inoculated	Per cent transmission
1	1	4/10	40	0/10	00
2	4	5/10	50	0/10	00
3	7	8/10	80	0/10	00
4	10	10/10	100	0/10	00
5	13	10/10	100	0/10	00
6	15	10/10	100	0/10	00
7	20	9/10	90	0/10	00

Further it was noticed that virus transmission increased with increase in aphid number up to 10-15 aphids per test plant and maximum 100 per cent transmission was recorded in isolate I when the population of aphids per test plant was increased up to 10.

3.3. Transmission through whiteflies: Isolate II was transmitted through whiteflies. No transmission was shown by virus Isolate I. The whitefly *Besimia tabaci* was found to be efficient vector for transmission of virus isolate II (Table 4).

3.4. Number of whitefly required to transmit the virus from Isolate II: The result showed in Table 4 resulted that, the single whiteflies were able to transmit the virus with 30 per cent efficiency. A 100 per cent efficiency of transmission was achieved with five or more whiteflies per test plant. An acquisition access period of 24 hr. was required for 100 per cent transmission efficiency, whereas 24 hr. inoculation access period resulted in 100 per cent transmission. Effect of number of whiteflies was determined by giving 24 hrs. of each AAP and IAP 15-20 days old pumpkin seedlings were used.

Table 4: Effect of whitefly *Bemisia tabaci* on transmission of various pumpkin virus isolates

Sr. No.	No. of whiteflies	Isolate I		Isolate II	
		Infected/ Inoculated	Per cent transmission	Infected/ Inoculated	Per cent transmission
1	1	0/10	00	3/10	30
2	2	0/10	00	4/10	40
3	3	0/10	00	6/10	40
4	4	0/10	00	9/10	90
5	5	0/10	00	10/10	100
6	8	0/10	00	10/10	100
7	10	0/10	00	10/10	100

Discussion

1. Transmission by mechanical sap inoculation:- In present studies, the extract prepared from virus isolate I of pumpkin by using mechanical sap inoculation method indicated that pumpkin virus isolates were transmitted through sap from pumpkin to pumpkin and to other Cucurbitaceous hosts. These results are in agreement with those reported by several other workers, Honda *et al.,* 1989; Yeh *et al.,* 1984 and Chakraborty *et al.,* 1997. The pumpkin virus isolate II was not mechanically sap transmissible to pumpkin to pumpkin and to other host plant but was transmissible by whitefly, *Bemisia tabaci*. Similar results were given by Capoor and Ahmad, 1975; Jayashree *et al.,* 1999.

3. Transmission through seeds

In seed transmission studies, none of the tested seedlings showed the symptoms of pumpkin virus isolate I and II there by study indicated that, the pumpkin virus isolates I and II were not transmitted through seeds in pumpkin. Similar types of results have been reported by Honda *et al.,* 1989 and Stanley *et al.,* 2001.

3. Transmission through insect

i. Aphid transmission of virus isolate I

Lecoq *et al.,* (2001) [6] observed that, moroccan watermelon mosaic virus was efficiently transmitted by two aphids species, *M. persicae* and *Aphis gossypii* in a non-persistent manner. *M. persicae* was found most efficient vector for transmission of isolate I. Similar results have been reported by other workers Makkouk and Lesemann, (1980) [7], Ilhe *et al.,* (2014) [4] for transmission of Watermelon mosaic virus - 1.

In the studies of vector population required for virus transmission, it was observed that single viruliferous adult of *M. persicae* was able to acquire and transmit the virus isolate I. The per cent virus transmission by single aphids up to 40-50 per cent. The transmission of virus isolate I by *M. persicae* was increased with increase in number of aphids up to 10 aphids per test plants and maximum transmission percentage 100 per cent was observed when the population of aphids per test plant was increased up to 10. These results are in agreement with the findings of Makkouk and Lesemann (1980) [7] and Lecoq *et al.,* (2001) [6].

ii. Whitefly transmission of virus isolate II

The virus isolate was found to be transmitted by whitefly only and not by mechanical sap transmission or aphids. Similar result also stated by Jayashree *et al.,* 1999.

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