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Relationship of *cucumber mosaic virus* with *Aphis gossypii* Glover

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Abstract
Cucumber mosaic virus (CMV) was transmitted more efficiently by *Aphis gossypii*. Pre-acquisition starvation for four hours was beneficial. Around 20 and 30 minutes of acquisition access period with 20 min of inoculation feeding period was required for transmitting 100 per cent CMV virus, when 10 aphids per plant were released. Thirty min. acquisition feeding period and one hour of inoculation feeding period showed maximum per cent of CMV transmission. Minimum 10 aphids were required for the 100 per cent transmission of virus. Feeding aphids lost infectivity after one hour. The studies indicated non-persistent relationship of CMV with *A. gossypii*.

Keywords: *Aphis gossypii*, Pre-acquisition starvation, acquisition access period, non-persistent

1. Introduction
Cucumber (*Cucumis sativus* L.) is infected by several types of viruses. Out of these, cucumber mosaic virus (CMV) was first reported in the year 1934 by Price hence the name cucumber mosaic virus (Price, 1934) [7]. The CMV belongs to group IV of (+) ssRNA with family: *Bromoviridae* and genera: *Cucumovirus* (Fauquet *et al*., 2005) [2]. During present investigation, virus-vector relationship of CMV was studied.

2. Material and Methods
The CMV culture was maintained in an insect prof cage by mechanical sap inoculation on healthy cucumber plants. The aphids collected from field and later transferred to a healthy cotton plant were kept in insect proof cage for multiplication, after one hour starvation. The aphid was identified as *Aphis gossypii* (Glover) (Hemiptera: Aphididae) by ICAR-NBAIR, Bangalore. The new generation (aviruliferous) aphids were transferred to other healthy plants of cotton in insect proof cages for reproduction and used for further study.
The wingless female adult aphids were used for transmission studies. About 15 days old young seedings of cucumber were used as test plants. During pre-acquisition starvation period, aphids were placed in a petri plate under dark condition. For acquisition of virus, CMV infected leaves were kept in a petri plate contain aphids. Then those were transferred to healthy cucumber plants after acquisition. After feeding period the aphids were sprayed with acetamiprid 0.2 g/lit, to avoid their further multiplication. Later plants were kept in an insect proof net house for expression of symptoms (Plate 1).
The preliminary work was carried out with different acquisition access, acquisition feeding, inoculation feeding and inoculation threshold periods and number of aphids required for transmission of CMV to cucumber through *A. gossypii.* Initially four hours of pre-acquisition fasting period was given to aphids. The number of insects per plant transferred was ten in number (Plate 2).

2.1 Acquisition access period (AAP)
The pre-acquisition starved aphids were allowed to feed on infected cucumber leaves showing CMV symptoms. The aphids were exposed to different acquisition feeding time on infected plant leaves (10, 20, 30 min, 1, 2, 5 and 10 h.). After each acquisition feeding period, the aphids were transferred to 15 days old healthy cucumber seedlings, at the rate of 10 aphids per plant. Ten plants were inoculated. After 20 min. of feeding, aphids were killed by sprayed acetamiprid @ 0.2 g/lit, to avoid their multiplication. The inoculated plants were kept in an insect proof cages and diseases symptoms were observed daily.

2.2 Acquisition feeding period (AFP) and inoculation feeding period (IFP)

To calculate AFP, the aphids were fed on CMV infected leaves at different time intervals. The aphids were then transferred to cucumber seedlings, at the rate of 10 aphids per plant and were allowed for different IFP. After giving required IFP, aphids were killed by sprayed acetamiprid @ 0.2 g/lit. The inoculated plants were kept in an insect proof cages for symptom development.

2.3 Inoculation threshold period (ITP)

Minimum initial time period needed to acquire a virus and inoculate it to the virus free plant was recorded.

2.4 Number of aphids required for CMV transmission

After giving a minimum AAP of 20 min. healthy cucumber plants were inoculated with number of aphids *viz*., 1, 2, 3, 5, 10, 15 and 20 aphids per plant. These aphids were allowed to feed for 20 min and then killed by sprayed acetamiprid @ 0.2 g/lit and the plants were kept in insect proof cages for symptom development.

The per cent disease incidence of inoculated plants, number of aphids required for virus transmission, time received to transmit the virus were recorded. The aphids inoculated plants showing symptoms were tested for CMV by double antibody sandwich -enzyme linked immunosorbent assay (DAS-ELISA) as described by Kavyashri, 2014 [5].

3. Results and Discussion

3.1 Acquisition access period (AAP)

A. gossypii was able to transmit 100 per cent virus with minimum AAP of 20 and 30 min. During the period of 10 min. of AAP, the per cent transmission of virus was 80 per cent, while in case of 1, 2, 5 and 10 h of AAP the infection was 80, 60, 40 and 30 per cent respectively. The number of days required for expression of symptom, varied from 10 to 32 days (Table 1). However, the transmission efficiency decreased with the increase in AAP. Longer access period resulted in poor viral acquisition and inoculation efficiencies.

These results were supported by those reported by Manjunatha and Byadgi (2018) [6] while studying transmission efficiency of *A. craccivora* and *A. gossypii*. The virus transmitted within 20 min of acquisition and 10 to 15 min inoculation feeding period, showed 100 per cent infection, and the symptoms of CMV expressed within 30 days. Sylvester and Osler (1977) [11] observed more transmission with increase in length of inoculation access period, but the rate of transmission, decreased exponentially with time.

3.2 Acquisition feeding period (AFP) and inoculation feeding period (IFP)

The *A. gossypii* were allowed to acquire CMV virus from cucumber leaf then transferred to healthy cucumber plants (10 aphids/plants) for IFP at different time intervals. It showed minimum acquisition and inoculation feeding periods required for the successful transmission of virus. At 10 min AFP and 30 min IFP 70 per cent infection was observed. With 20 min AFP, 90 per cent infection was observed with 30 min of IFP. Whereas 30 min and one hour of AFP showed 100 per cent infection was seen. At AFP of 1, 2, 5 and 10 h highest infection was observed 80, 30, 20 and 10 per cent with 30 min of IFP as shown in Table 2.

With the increase in the AFP the per cent infectivity decreased as aphids lost infectivity after feeding for an hour. Sravika *et al.* (2018) [9] observed decrease in per cent transmission of the virus due to formation of salivary sheath.

Similar type of work was undertaken by Kavyashri and Nagaraju (2014) [5], with the AFP of 20 min, when 10 viruliferous aphids were transferred to each of gherkin plants. At 20 min IFP they observed 100 per cent infection. Swenson and Marsh (1967) [10] also reported that aphid (*A. gossypii*) will retain virus for less than one hour upon feeding the source.

Aphid (*A. gossypii*) transmission was achieved in bitter gourd after 24 h of acquisition feeding period and 24 h of inoculation feeding period. Seven days after inoculation, the 28 plants out of 30 inoculated plants were checked for cucurbit aphid borne yellows virus (CABYV) by PCR for confirmation (Sangeetha *et al*., 2019) [8].

3.3 Inoculation threshold period (ITP)

Minimum AFP of 30 min with one hour of IFP is required by an aphid to transmit maximum (100%) infection. successfully with a minimum inoculation feeding period of one hour is called as inoculation threshold period of the aphid (*A. gossypii*).

Bhargava and Khurana (1969) [1] also reported minimum inoculation threshold period of *A. gosspyii* (5 min) of AFP. When coupled with one hour inoculation feeding period it gave 100 per cent infection for papaya mild mosaic virus.

3.4 Determination of number of aphids required for virus transmission

Fifteen days old cucumber seedlings were inoculated with different number of aphids *viz*., 1, 2, 3, 5, 10, 15 and 20 to determine optimum number of aphids required for 100 per cent transmission of CMV. The efficiency of transmission varied with respect to number of aphids. Hundred per cent transmission was obtained when 10, 15 and 20 aphids per plant were used. When 1, 2, 3 and 5 aphids were used for inoculation, the per cent transmission was 20, 40, 60 and 90 per cent. The number of days taken for symptom expression varied from 10 to 25 days (Table 3). Increase in number of viruliferous vector per plant increased efficiency of transmission.

The results obtained by Kalleshwaraswamy and Krishna Kumar (2008) [3] showed single aphid inoculation which indicated that *A. gossypii* (53%) was more efficient in transmitting papaya ring spot virus (PRSV) to papaya plants. PRSV transmission efficiency was 100 per cent when a group of five aphids per plant were used. Kavyashri (2014) [4] also reported that hundred per cent transmission of CMV on gherkins when 10 aphids per plant were released and when single aphid was used for inoculation the per cent transmission was 20 per cent.

Table 1: Standardization of different acquisition access period (AAP) on transmission of cucumber mosaic virus in cucumber by *A. gossypii*

Time for acquisition	Transmission (%)	No. of days for symptom expression
10 Min	80	10
20 Min	100	10
30 Min	100	10
1 h	100	20
2 h	80	24
5 h	60	30
10 h	40	32

Table 2: Standardization of virus vector relationship on transmission of cucumber mosaic virus in cucumber by *A. gossypii*

Inoculation feeding period (IFP)	Disease per cent infection at different acquisition feeding period (AFP)						
	10 min	20 min	30 min	1 h	2 h	5 h	10 h
10 min	40	50	60	30	10	10	00
20 min	50	80	80	50	20	10	10
30 min	70	90	90	80	30	20	10
1 h	30	90	100	70	20	10	00
2 h	30	50	70	30	20	10	00
5 h	20	30	50	20	10	10	00
24 h	00	20	40	10	10	00	00
48 h	00	10	20	10	00	00	00

Table 3: Determination of number of aphids required for virus transmission of cucumber mosaic virus in cucumber

No. of aphids used	Transmission (%)	No. of days for symptom expression
1	20	25
2	40	23
3	60	20
5	90	18
10	100	15
15	100	15
20	100	10

Aphids culture maintained in insect proof wooden cages on cotton (A) and cucumber plants (B)

Stereomicroscopic view of *Aphis gossypii*

Plate 1: Maintenance of CMV culture in cucumber plants through sap inoculation in net house

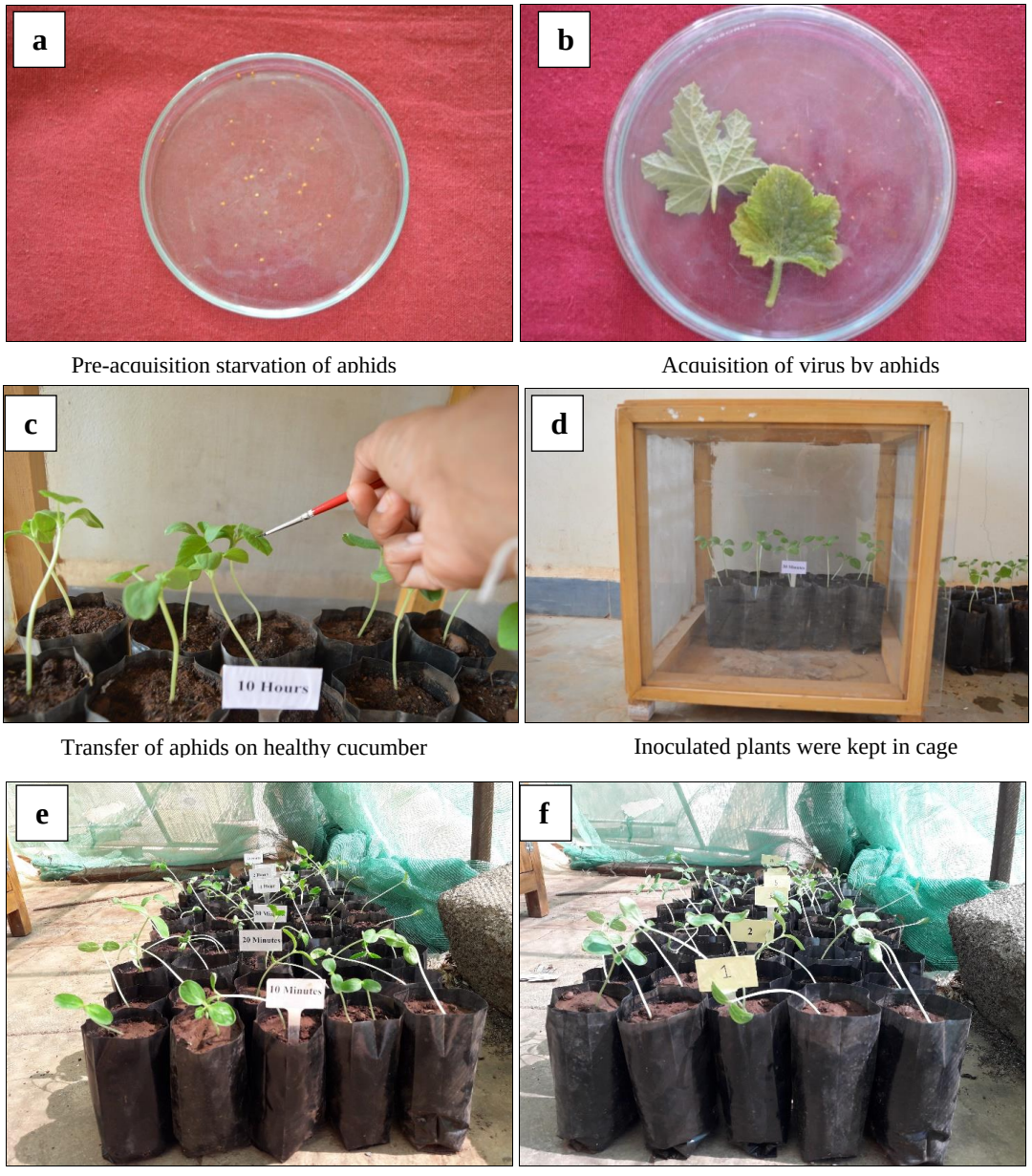

Plate 2: Virus vector relationship. a) four hours of pre-acquisition starvation was given to aphids; b) CMV symptomatic leaves kept for virus acquisition by aphids; c) transfer of aphids to healthy cucumber seedlings; d) aphids transferred plants were kept in cages still inoculation periods end; d) and e) inoculated plants were kept in net house for symptoms expression.

4. Acknowledgment
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