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Occurrence of *Cucumber mosaic virus* (CMV) in cucumber (*Cucumis sativus* L.) and sap transmission studies of the virus in southern districts of Tamil Nadu

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Abstract
Survey was conducted to record the incidence and severity of *Cucumber mosaic virus* disease *in* Cucumber in southern parts of Tamil Nadu. During Survey, the infected cucumber plants showed different symptoms *viz.,* green and yellow mosaic pattern, dark green mottling, leaf distortion, leaf crinkling, stunting of plants, pale green with dark green warts on fruit and malformed, misshaped fruits. Cucumber plants showing the above symptoms were collected from Srivenkateshwarapuram, Chennalpatti, Vadavallanadu and Anantha Nambi kuruchchi villages of Thoothukudi district, Pallayamkottai and Thalaikulam villages of Tirunelveli district, Pavoorchatram and Vaithiyalingapuram villages of Tenkasi district and Anjugramma village of Kanyakumari district. Among the surveyed area maximum disease incidence (67.5%) and severity (3.80) were recorded in Anantha Nambi Kuruchchi, while lowest disease incidence (14.5%) and severity (0.96) were recorded in Anjugrammam. The virus inoculum was extracted from CMV infected plants by using 0.1% phosphate buffer and inoculated in cowpea and cucumber plants. Systemic symptoms *viz.,* downward cupping, mosaic in cucumber and mosaic in cowpea were observed at 12, 21 and 23 days after inoculation respectively. Electron Microscopic observation of sap from CMV infected cowpea and cucumber plants showed 28 – 30 nm diameter size of spherical particle.

Keywords: *Cucumber mosaic virus*, sap transmission, electron microscopy, disease incidence

1. Introduction
Cucumber (*Cucumis sativus*) is the widely cultivated commercial vegetable crop grown in large greenhouses as well as small farms. Overall Cucurbitaceae family has 118 genera and 825 species among which 36 genera and 100 species are found in India (Chakravarthy 1982) [1]. This is the summer vegetable crop belongs to Cucurbitaceae family grown in tropical and subtropical areas of the country. It contains high water and consumed in immature stage which is suitable for vegetable salad preparation. Cucumber was originated in India and grows at a temperature of 20 ℃ to 24 ℃. Cucumber is rich in calcium, carbohydrates, phosphorous, vitamin B and C (Yawalkar 1985) [2].

China is the largest cucumber producer in the world with 58, 802, 433 MT. India ranks 27 by producing 146, 554 MT of cucumber in the world (FAOSTAT). In India cucumber is cultivated in an area of 111 ha with the production of 1638 MT. In India, cucurbitaceous crops contribute about 5.6% of the total vegetable production (Nagendran *et al.*, 2018) [3]. In Tamil Nadu cucumber is cultivated in an area of 57 ha with the production of 171 MT (NHB, 2020).

CMV is the most destructive and economically important plant virus which drastically affects the crop quality and yield of the cucumber plants (Scholthof *et al.*, 2011) [4]. The first mosaic disease in cucurbits was caused by Cucumber mosaic virus (Doolittle, 1916). *Cucumber mosaic virus* (CMV) has wider host range of 1200 different plant species and transmitted by 60 different species of aphids (Lecoq and Desbiez, 2012) [5]. Palukaitis and Garcia – Arenal (2003) [6] reported the sap and seed transmission of CMV. CMV belongs to the genus; *Cucumovirus.* family; *Bromoviridae* and it has an icosahedral, 29 nm diameter, tripartite genome of three single stranded positive sense RNA molecules comprising RNA -1, RNA-2 and RNA-3 that encodes four viral proteins (Gallitelli 2000) [7]. The infected cucumber plants recorded an average yield loss of 10-20% and maximum yield loss up to 100% (Zitter and Murphy, 2009) [8].

2. Materials and Methods

2.1. Survey

To record the occurrence of *Cucumber mosaic virus* disease in southern parts of Tamil Nadu, the Cucumber growing districts of Thoothukudi, Tirunelveli, Tenkasi and Kanyakumari were surveyed. In Thoothukudi district, survey was conducted in Srivenkateshwarapuram from Sathankulam block and Chennalpatti, Vadavallanadu and Anantha Nambi kuruchchi from Karungulam block. In Tirunelveli district survey was conducted in Palayamkottai village of Palayamkottai block and Thalaikulam village of Nanguneri block. In Tenkasi district, Pavoorchatram village of Keelapavoor block and Vaithiyalingapuram village of Alangulam block were surveyed. In Kanyakumari district, Anjugrammam village of Agastheeswaram block was surveyed.

2.2. Disease evaluation

2.2.1. Assessment of disease incidence

Cucumber Mosaic Virus disease incidence was assessed by the number of visibly diseased plants, usually in relation to the total number of plants assessed and expressed as the proportion or percentage of plants infected with CMV. Each and every field was observed with 25 plants at random and scored as infected and healthy plants. Percent disease incidence was calculated by using the following formula

$$\text{Percent Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

2.2.2. Assessment of disease severity

It refers to the degree of symptom expression and was assessed based on 0 – 4 scale as follows (Monma and Sakata 1997) [9].

Symptom description	Scale
No symptoms	0
Mild mosaic, no leaf distortion	1
Strong mosaic, mild leaf distortion	2
Severe mosaic and distortion	3
Severe mosaic, distortion and stunting	4

Randomly 25 plants were assessed for the disease severity index by using the following formula,

$$\text{Severity index} = \frac{\sum (\text{symptom index X Number of plants with each symptom index})}{\text{Total number of plants}}$$

2.3. Sampling

Leaf samples from cucumber plants showing green and yellow mosaic pattern, dark green mottling, leaf distortion, leaf crinkling and fruits showing pale green with dark green warts and malformation, miss happening were collected and brought to the laboratory to confirm the virus infection by using sap inoculation and Electron Microscopy (EM).

2.4. Characterization of Cucumber Mosaic Virus by sap inoculation

Sap transmission is the most practical way of studying the plant viruses biologically, virus inoculum was prepared in such a way that the leaves showing respective symptoms were weighed approximately one gram and equal quantity of 0.1 M Phosphate buffer (pH 7.0) containing 0.1% mercaptoethanol was added and macerated well. Then the sap was inoculated in the test plants *viz.,* cowpea and cucumber. To facilitate the virus entry carborundum powder which act as an abrasive was rubbed on the leaves before inoculation, to make minute injuries on plant leaf surfaces. After, 2-3 minutes of inoculation, the leaves were rinsed with water to remove excess inoculum and abrasive. Uninoculated seedlings were also kept for each test plant as control to compare disease severity. All inoculated and uninoculated plants were kept in insect proof cage and maintained in glass house until the symptom expression (Kavyashri *et al.,* 2016) [10]

2.5. Electron Microscopy

The infected plant sample was macerated in sodium phosphate buffer pH 7.5 at 1:3 (w/v) proportion. It was centrifuged at 12, 000 rpm for 10 min. After that, supernatant was collected and examined under a Transmission Electron Microscope to assess the shape and size of the virus particles causing mosaic symptom. To assess viral particles in suspicious leaves, a drop of supernatant was spread over carbon-coated grids and let to settle for 2-3 minutes. To remove the excess sample, blotting paper was used. 3 -5 drops of 1% Uranyl acetate solution were applied on the grids for Negative staining. The additional stain was drained by touching the blotting paper strip to the grid edge. The grids were dried in desiccator for 15 - 30 min and examined under Electron microscope at various magnifications with JOEL 100 S Transmission Electron Microscope at TNAU, Coimbatore.

3. Results and Discussion

3.1 Survey

Roving survey was conducted in vegetable growing areas of Thoothukudi, Tirunelveli, Tenkasi and Kanyakumari districts of Tamil Nadu to access the incidence and severity of *Cucumber mosaic virus* disease. In each field, 25 plants were observed for the virus infection and recorded the incidence & severity. Survey revealed the presence of CMV in southern districts of Tamil Nadu *viz.,* Thoothukudi, Tirunelveli, Tenkasi and Kanyakumari. In the surveyed area, percent disease incidence and disease severity ranged from 14.5% to 67.5% and 0.96 to 3.80 respectively. During survey, maximum disease incidence of 67.5% and disease severity of 3.80 was recorded in Anantha Nambi Kuruchi followed by Srivenkateshwarapuram (62.5%; 3.28), Vadavallanadu (60%; 3.08), Pavoorchatram (57.5%; 2.96), Thalaikulam (55%; 2.65), Vaithiyalingapuram (47.5%; 2.40), Pallayamkottai (25%; 1.80), Chennalpatti (22.5%; 1.56), Anjugrammam (14.5%; 0.96) [Table1; Plate1; Fig1; Fig2].

3.2. Symptomology

During survey *Cucumber mosaic virus* infected plants were showed different symptoms *viz.,* green and yellow mosaic pattern, dark green mottling, leaf distortion, leaf crinkling, stunting of plants, pale green with dark green warts on fruit and malformed, misshaped fruits (Plate2).

3.3. Sap transmission

The test plants *viz.,* cowpea and cucumber were raised in small pots containing sterilized mixture of soil + sand + FYM (2:1:1) and maintained in glass house of Department of Plant Pathology, AC&RI, Killikulam. Ten plants were raised for

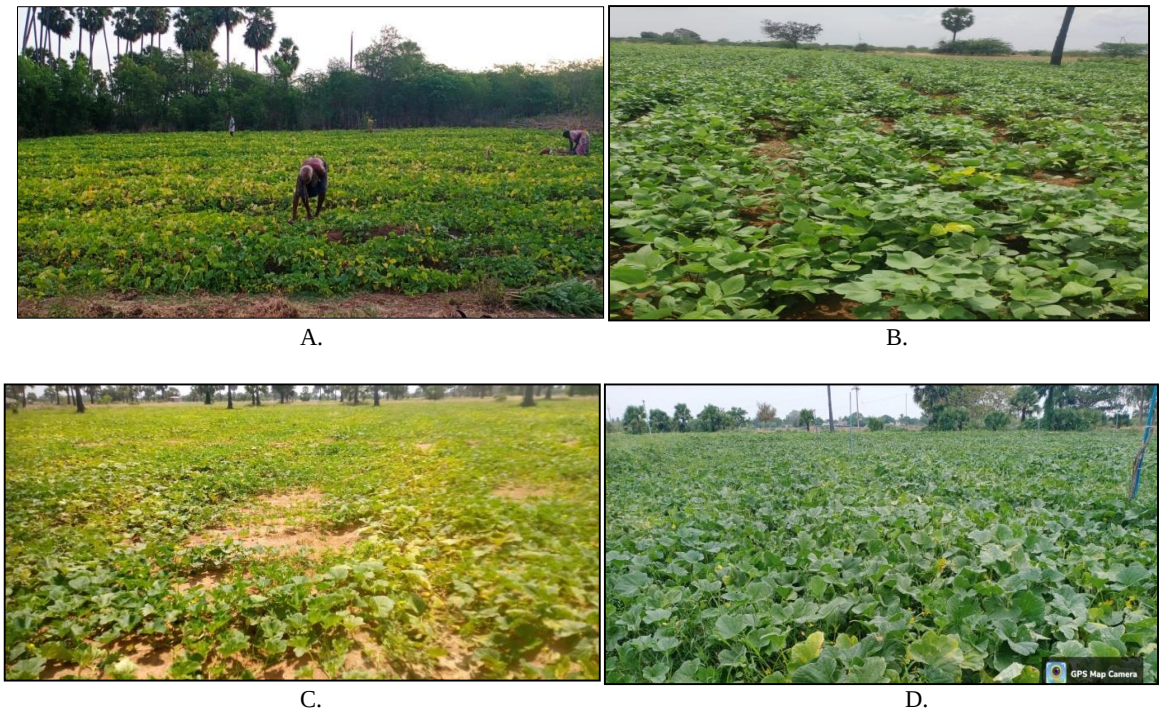
each test plant and single seedling was maintained in each pot. The test plants were mechanically inoculated with the sap extracted from cucumber mosaic virus infected plants. Systemic infection *viz*., downward cupping, mosaic on cucumber and mosaic on cowpea were seen on 12, 21 and 23 days after inoculation respectively. The CMV inoculum was maintained in cucumber plants by sap inoculation under glass house condition (Plate 3).

3.4. Electron Microscopy

Leaf dip preparation of sap of CMV inoculated cowpea and cucumber plant samples from southern districts of Tamil Nadu revealed the presence of spherical viral particle measuring 28-30 nm under EM (Plate 4). Similarly, Jalender *et al*., (2017) [11] were also observed the spherical particle of CMV with the diameter of 28.5 nm from tomato under Transmission Electron Microscope.

Table 1: Survey for the occurrence of *Cucumber mosaic virus* disease in cucumber in southern districts of Tamil Nadu

S. No.	District	Place		Disease Incidence (%)	Disease Severity
		Block	Village		
1.	Thoothukudi	Sathankulam	Srivenkateshwarapuram	62.50	3.28
		Karungulam	Chennalpatti	22.50	1.56
			Vadavallanadu	60.00	3.08
			Anantha Nambi kuruchchi	67.50	3.80
2.	Tirunelveli	Palayamkottai	Pallayamkottai	25.00	1.80
		Nanguneri	Thalaikulam	55.00	2.65
3.	Tenkasi	Keelapavoor	Pavoorchatram	57.50	2.96
		Alangulam	Vaithiyalingapuram	47.50	2.40
4.	Kanyakumari	Agastheeswaram	Anjugrammam	14.50	0.96



A. B. C. D.

Plate 1: Survey for the incidence of CMV disease in southern parts of Tamil Nadu a) Thoothukudi b) Tirunelveli c) Tenkasi d) Kanyakumari

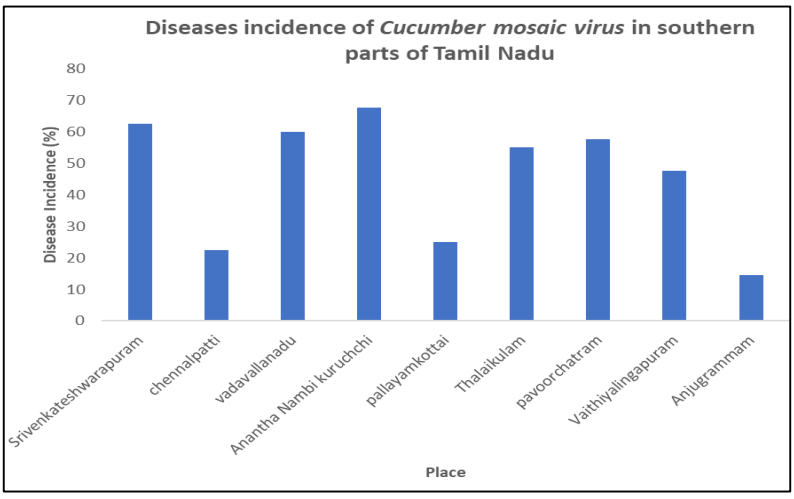


Fig 1: Disease incidence of CMV in southern parts of Tamil Nadu

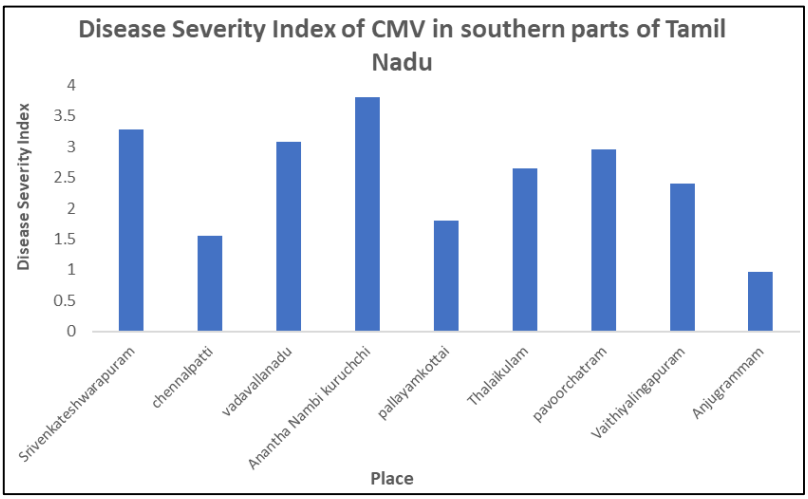


Fig 2: Disease Severity index of CMV in southern parts of Tamil Nadu

A. B. C.

Plate 2: Symptoms of *Cucumber mosaic virus* disease in Cucumber. a) Mosaic pattern b) Leaf crinkling c) Malformed and misshapen fruits

A. B. C.

Plate 3: Sap transmission of *Cucumber mosaic virus* a) downward cupping on cucumber b) mosaic on cucumber c) mosaic on cowpea

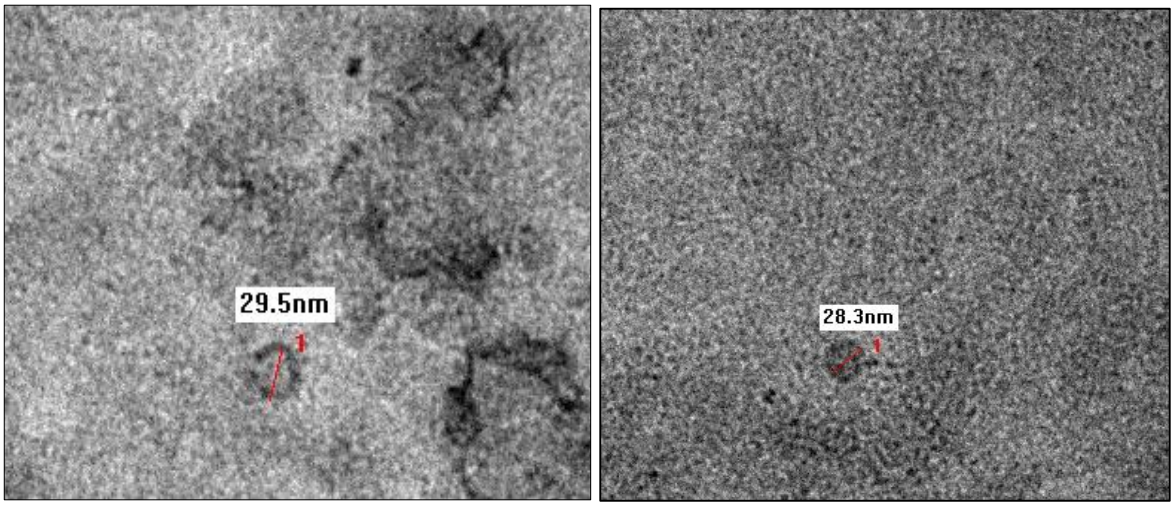


Plate 4: Electron microscopy of CMV infection a) Cucumber b) Cowpea

4. Conclusion

The disease incidence was observed in vegetable growing areas of southern parts of Tamil Nadu. Among the different districts surveyed, maximum disease incidence and disease severity of *Cucumber mosaic virus* was observed in Thoothukudi district. In the present study, CMV produced systemic symptoms of downward cupping, mosaic on cucumber and mosaic on cowpea. In severe cases, infected plants become stunted and dried. Electron microscopic observation of spherical particle with the size of 28 to 30 nm diameter from the sap of infected cowpea and cucumber plants revealed that the virus was belonging to the *Cucumovirus* group. Similar finding was reported by Jalender *et al.*, (2017) [11]. They observed 28.5 nm diameter spherical particle from the CMV infected tomato plants under EM.

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