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Occurrence of new streak virus in Okra and sap transmission studies of the virus in southern districts of Tamil Nadu

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Abstract
A new Streak Virus is prevalent in almost all Okra growing regions of southern districts in Tamil Nadu, but at a low to moderate severity. Symptoms of the virus in Okra include chlorotic spots with mild mosaic, veinal browning, chlorotic leaf blotches, distortion of leaves and fruits with malformation. Okra plants showing these symptoms were collected from different locations of Thiruvaikundam, Karungulam, Agaram, Killikulam, Palayamkottai, Surandai, Alangulam, Thovalai and Aralvaimozhi. The highest disease incidence was observed in Thiruvaikundam (48%), while the lowest in Vellamadam (16%). The virus inoculum was prepared in 0.1M Potassium phosphate buffer (pH 7.0) and inoculated into test plants (i.e., Okra and cowpea). Inoculated plants were observed 2-3 days after inoculation (DAI) for the development of symptoms. Symptoms like chlorotic spots, lesions, light and dark green patches were observed on Cowpea test plants. The infected Okra seedlings developed mosaic, distorted leaf and chlorotic symptoms after 15-20 days of inoculation.

Keywords: New streak virus, okra, disease incidence, sap inoculation

1. Introduction
"Okra" (*Abelmoschus esculentus* L.) is a valuable vegetable crop grown in tropical and subtropical regions around the world. Because of the multiple uses of the fresh leaves, buds, blossoms, pods, stems, and seeds, "okra" is known as a versatile crop. Carbohydrates are mainly present in the form of mucilage. The mucilage is highly soluble in water. Okra seeds contain about 20% protein and 40% oil. (Gemede *et al*., 2015) [3]. It is grown in northern India during the summer and as a winter crop in Maharashtra, Gujarat, Andhra Pradesh, Karnataka, and Tamil Nadu. It is unable to grow in steep hills or areas with extremely low temperatures. In 2019-20, India produced 6.46 million tonnes of okra, with an average yield of 12.28 tonnes per hectare (Panwar *et al*., 2019). There have been numerous reports of various pests and diseases affecting okra. Yellow vein mosaic virus (YVMV), Okra mosaic virus (OkMV), and Okra leaf curl virus (OkLCV) are the most common viruses that infect Okra. Yellow vein mosaic virus (YVMV) is the most serious viral disease of okra. Moreover, Okra has been severely impacted by a new disease in the states of Karnataka and Tamil Nadu during 2000 and 2001. The disease has also expanded to other states, notably Andhra Pradesh, Madhya Pradesh, Haryana, and Maharashtra. The disease is characterised by chlorotic spots, chlorotic leaf blotches, leaf distortion, chlorotic streaks, fruit distortion, and substantial yield losses of up to 63 percent. The causative virus causes chlorotic and necrotic lesions on *Vigna unguiculata* (C-152) and *Chenopodium amaranticolor*, as well as chlorotic local lesions and mosaic on *Cucumis sativus* and necrotic local lesions on *Gossypium hirsutum* and *Vigna mungo*, and also chlorotic local lesions and systemic necrosis on *Helianthus annuus.* (Reddy *et al*., 2007). In this study, the natural occurrence of the new virus on okra was observed and its sap transmission properties were documented and the confirmation of virus was done through Electron Microscopy.

2. Materials and Methods
2.1. Survey
To examine the prevalence of this viral disease in southern parts of Tamil Nadu, the Okra cultivating districts of Thoothukudi, Tirunelveli, and Kanyakumari were surveyed. In Thoothukudi district okra growing fields in Thiruvaikundam, Karungulam, Agaram and

Killikulam were surveyed. In Tirunelveli district, farmer's field in Palayamkottai, Surandai, Alangulam were observed for symptoms. In Kanyakumari district, farmer's fields in Thovalai and Aralvaimozhi were observed.

2.2. Sampling
Leaf samples from Okra plants showing symptoms such as Chlorotic spots with mild mosaic, chlorotic leaf blotches, distortion of leaves and fruits showing distortion and malformation were collected and brought to the laboratory to confirm virus infection using sap inoculation method and EM.

2.3. Disease Evaluation
The intensity of Tobacco Streak virus of Okra in Southern districts was evaluated by disease incidence levels. Random samples were taken in each field, and the number of plants affected over the total number of plants was counted and reported as a percentage of disease incidence using the formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} x\ 100$$

2.4. Characterization of New Streak virus by sap inoculation:
Mechanical sap transfer is the most practical way for studying 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 0.1M Phosphate buffer (pH 7.0) was added and macerated well. Then, the sap was clarified and inoculated in the test plants (Cowpea and Okra) which were at two-leaf stage. In order to facilitate the virus entry in leaves, celite or carborundum powder which acts as an abrassive was rubbed on the leaves to make minute injuries on plant leaf surfaces. The leaves were properly rinsed with water immediately to eliminate excess inoculum and abrassive. Uninoculated seedlings were also kept for each test plant to compare symptom severity i.e.control. All inoculated plants were kept in the glass house with proper labelling until symptoms appeared (Hull *et al*., 2009) [4].

2.5. Electron Microscopy
The suspected plant sample was ground in Sodium phosphate buffer pH 7.5 at 1:3 (w/v) proportion. After centrifuging for 10 minutes at 12000 rpm, the supernatant was collected and examined under a Transmission Electron Microscope to determine the shape and size of the virus particles generating chlorotic symptoms in the diseased sample. A drop of supernatant was poured on the carbon-coated grids and left to settle for 2-3 minutes to evaluate viral particles in suspected leaves. Blotting paper was used to remove the excess sample. A little droplet of dye (1% uranyl acetate) was applied on top of it and left for 2-3 minutes. By touching the blotting paper strip to the grid's edge, the extra stain was drained. The grids were dried in a dessicator for 15-30 minutes before being viewed at various magnifications with a JOEL 100 S transmission electron microscope (TNAU, Coimbatore). The photographs of the virus particles magnified were taken.

3. Results and Discussion
3.1. Survey
Roving survey was carried out in vegetable growing tracts of Thoothukudi, Tirunelveli and Kanyakumari districts to assess the incidence and severity of the disease. In each field, twentyfive plants were taken into consideration and viral symptoms were accounted. Moreover, disease incidence was recorded and infected samples were collected for further investigation. Survey revealed the presence of this new viral disease in all Southern districts of Tamil Nadu *viz*., Thoothukudi, Tirunelveli and Kanyakumari. The per cent disease incidence ranged from 16-48%. Per cent disease incidence was high in Thiruvaikundam (48%) followed by Karungulam (44%) and Killilulam (40%).

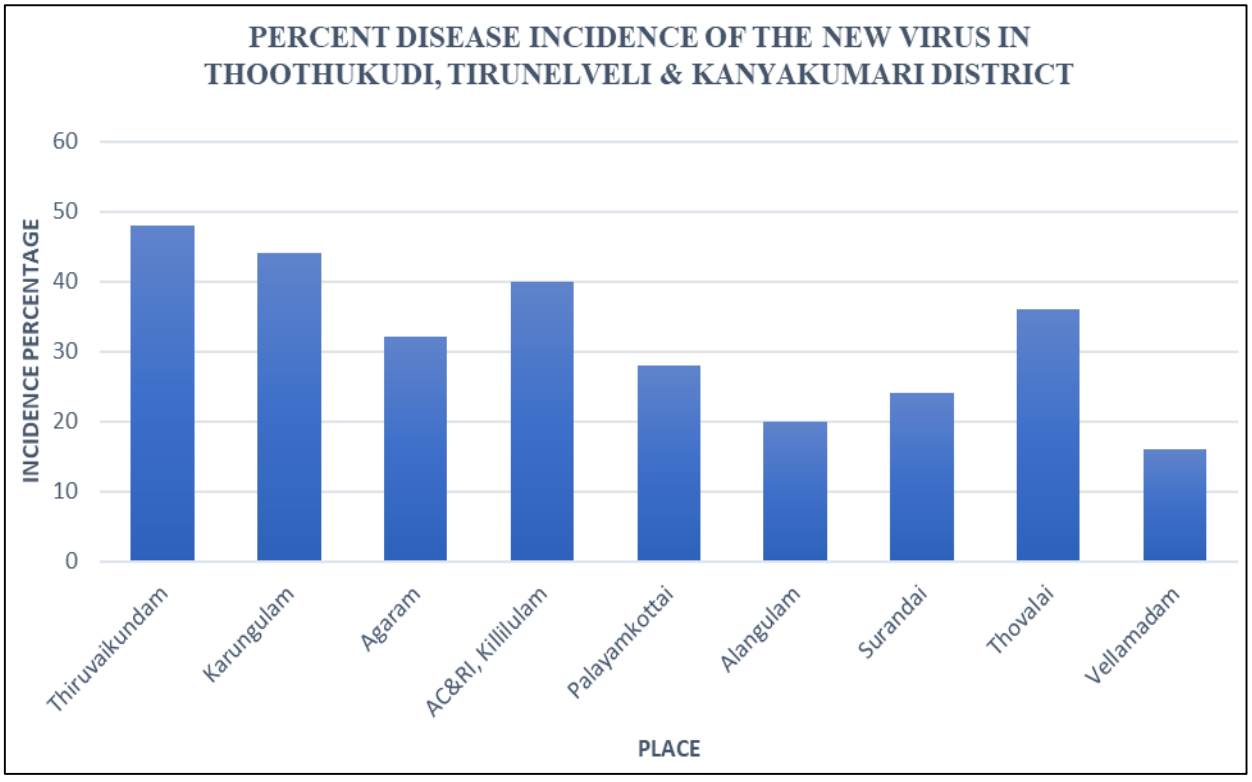


Fig 1: Bar chart indicating Percent Disease Incidence in different regions.

Table 1: Tobacco streak virus incidence in Southern districts of Tamil Nadu

S. NO	District	Locality	Variety/Hybrid	Disease Incidence
1.	Thoothukudi	Thiruvaikundam	Suraksha	48
		Karungulam	COBH 1-Hybrid	44
		Agaram	Suraksha	32
		AC&RI, KilliKulam	COBH 1-Hybrid	40
2.	Tirunelveli	Palayamkottai	Suraksha	28
		Alangulam	CO-2	20
		Surandai	CO-2	24
3.	Kanniyakumari	Thovalai	COBH 1-Hybrid	36
		Vellamadam	Sushithra	16

3.2. Symptomatology

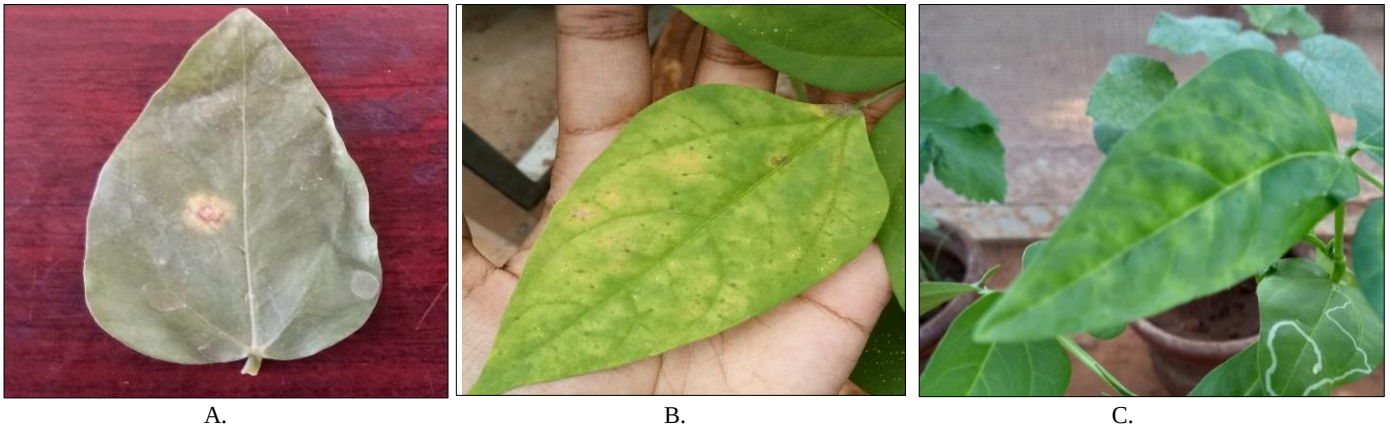
Observed symptoms in the Okra field revealed that the disease mostly affects the leaves and also results in mis-shapen, or malformed fruits. Chlorotic spots, distortion of leaves, chlorotic streaking, distortion of fruits were the symptoms observed in the field. Other symptoms include stunting, veinal necrosis, leaf mosaic (mottling), dwarfed plants and stem discoloration. The survey results are in agreement with the findings of Reddy *et al.*, (2007).

A. B. C. D.

Fig 2: Symptoms exhibited by *Tobacco streak virus* infected Okra plants. a) Infected Okra plants exhibiting chlorotic spots b) Okra plants showing chlorotic streaks and mottling c) Veinal necrosis in Okra leaves d) Deformed Okra fruits

3.3. Sap transmission

Seeds of test plants namely cowpea (CO7)) and Okra (COBH4) were used for sap transmission studies. Test plants were raised in glass house (Dept of Plant Pathology, AC&RI, Killikulam) in small pots containing sterilized mixture of soil+sand+FYM (2:1:1). One seedling was planted in each pot. The test plants were mechanically inoculated with the virus infected Okra plants by using 0.1M Phosphate buffer. In cowpea, local lesions were seen 5-6 days after inoculation as mild chlorotic spots and later it turned to necrotic lesions. Moreover, yellowing of leaves were observed systemically on cowpea leaves after 15-20 days of inoculation. The infected Okra seedlings developed mosaic, distorted leaf and chlorotic symptoms after 20 days of inoculation which were identical to the symptoms observed in the field. Then these single lesion and symptomatic parts of infected leaves were transferred to cowpea plants mechanically by sap inoculation and maintained under glass house condition.



A. B. C.

D. E. F.

Fig 3: Manifestation of TSV symptoms on cowpea (CO7) and Bhendi (COBH4). a) Inoculated cowpea plants showing chlorotic local lesions b) Systemic infection (I.E) yellowing exhibited by inoculated cowpea cultivars c) Chlorotic mottling symptoms in cowpea formed 15-20 days after inoculation d) Inoculated Bhendi plants exhibiting chlorotic patches e) Inoculated Bhendi plants showing marginal yellowing f) Inoculated plants showing chlorotic spots

3.4. Electron Microscopic Observation

Leaf-dip preparations of sap inoculated cowpea and Okra plant samples from southern districts of Tamil Nadu revealed the presence of two types of particles namely isometric and quasi spherical particles measuring 25-35 nm. Similar to the above findings Cook *et al.*, (1999) [2] also observed the spherical particle of TSV having a diameter of about 28- 30 nm under EM.

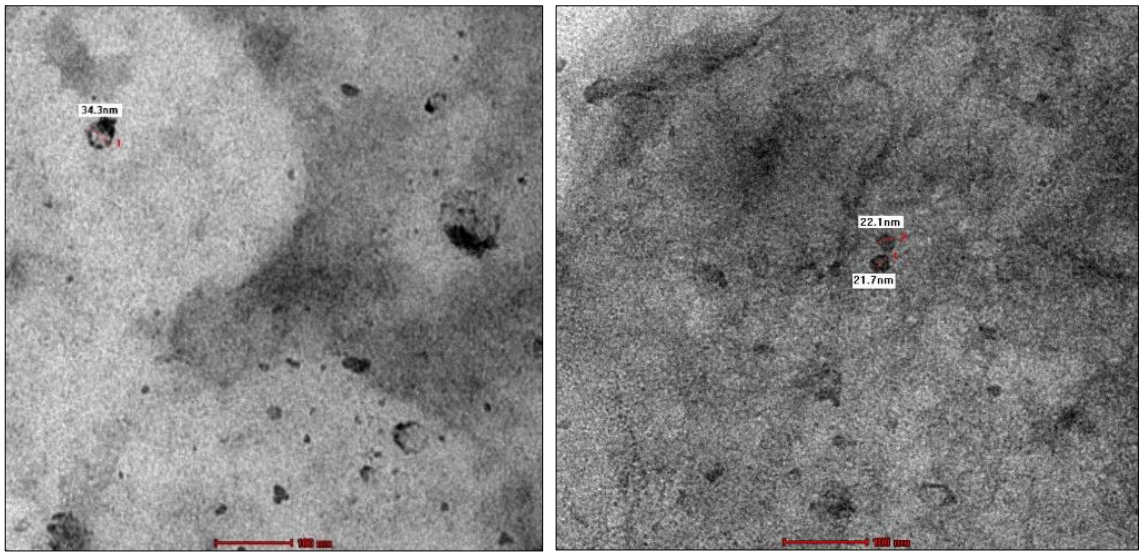


Fig 4: Leaf-dip preparation from sap inoculated cowpea and Okra samples showing spherical virus particles. Bar represents 100 nm

4. Conclusion

The disease incidence of this new viral disease was observed in southern districts of Tamil Nadu where Okra is grown. The disease incidence was high in Thiruvaikundam. The disease is newly emerging in some commercial fields where clear chlorosis type of symptoms on major leaves of plants appeared.

In the present study, the new virus produced various symptoms on cowpea seedlings which included necrotic lesions, systemic veinal necrosis, necrotic streaks on stem, finally leading to complete drying of leaves. The particle morphology and size suggest that the virus belongs to Ilarvirus group (Bol & John, 1999) [1]. An Ilarvirus that causes leaf necrosis and chlorosis that is antigenically related to tobacco streak virus (TSV) has been found infecting various other crops. (Jagtap *et al*., 2012; Jain *et al*, 2005; Kumar *et al*., 2007; Sivaprasad *et al*., 2010; Bhaskara Reddy *et al*., 2012) [5, 6, 11, 10]. Further research on serological and molecular aspects is being taken out. Such research could aid in the development of measures to combat the emerging viral infections that are affecting Okra.

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