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Relationship of sunflower necrosis virus and its vector *Thrips palmi* (Karny)

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Abstract
An investigation was made to establish the virus-vector relationship for which transmission of sunflower necrosis virus through *Thrips palmi* (Karny) was carried out. The transmission of sunflower necrosis virus by thrips ranged from 13.33 to 23.33 per cent in the genotype tested and all genotype showed positive for DAC ELISA test. Further, results also revealed that vector *Thrips palmi* (Karny) could acquire the virus with an Acquisition Access Period (AAP) of 3 days from infected sunflower plants with 13.33 % transmission. Similarly, Inoculation Access Period (IAP) of 6 days was required for successful transmission of virus with 16.67 % transmission of virus. The percent transmission increased with increase in both acquisition and inoculation feeding period. Further, two thrips enough to acquire and transmit the virus from infected to healthy sunflower plant.

Keywords: sunflower necrosis virus, *Thrips palmi* (karny), acquisition access period (AAP)

Introduction
In India, oilseeds contribute significantly to national economy. Sunflower is an important oilseed crop and is one of the worlds leading edible oilseed crop and ranks third position. Sunflower is susceptible to several diseases caused by various agents including virus (Nagaraju, 1995) [1]. One such virus causing severe reduction in growth leading to yield loss is the *tobacco streak virus* (TSV). *Tobacco streak virus* belong to the serogroup *Illarvirus* sub group 1: genus *Illarvirus* and the family Bromoviridae, was first reported on *Nicotiana tabacum* (Van volten-Doting 1981) [2]. The virus is seed, pollen and thrips transmitted (Johnson *et al*. 1984 and Cupertno *et al*., 1984) [3, 4]. The disease was characterized by necrosis of leaves followed by necrosis on petioles, stem and floral calyx (Jain *et al*., 2000) [5].
The virus is transmitted through mechanical sap inoculation from infected plant to healthy one (Linga Reddy, 2003 and Ajith Prasad, 2004) [6, 7]. Where as, *Thrips palmi* (Karny) successfully transmitted the virus to sunflower plants (Aravind, 2002 and Lokesh, 2006) [8, 9]. Further, Among the different insect species, aphids (*Myzus persicae*), jassids (*Empoasca kerri)* and whiteflies *(Bemisia tabaci*) failed to transmit the virus (Halakeri, 1999) [10]. In order to investigate the virus-vector relationship of sunflower necrosis virus and *Thrips palmi*, the present experiments were conducted to find the same.

Material and Methods
Transmission of SNV through *Thrips palmi*
Different sunflower genotypes *viz*., KBSH-1, KBSH-44 and Morden were raised and maintained under glasshouse conditions in insect proof nylon mesh cages were used in transmission experiments. The healthy colonies of *T. palmi* maintained on sunflower, green gram, watermelon and peanut plants by weekly transfer of active nymphs were used for transmission studies.
Young leaves showing typical symptoms were kept in the Petriplate. Along the rim of Pertriplate, a thin layer of water was poured and about 20-30 nymphs were released onto such leaves using fine hairbrush. Nymphs fed on healthy leaves served as check. After three days 20-25 nymphs were transferred using fine hairbrush on to test plants at two leaves stage raised in insect proof wooden cages. The normal movement of thrips was observed to ensure that injury do not occur during the transfer. The plants were kept undisturbed allowing the nymphs to feed. The test plants were kept under observation upto 50 days for symptom expression.

Serological detection of sunflower necrosis virus by using DAC-ELISA

The plant samples from glasshouse were collected and tested by direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA) protocol with Tobacco streak virus antisera and alkaline phosphatase (ALP) enzyme and p-nitrophenylphosphate (pNPP) system was employed by following the standardized protocol in our laboratory.

Determination of acquisition access period (AAP)

Thrips were maintained by weekly transfer of active nymphs onto healthy sunflower plants raised inside wooden cages kept in the glasshouse. About 25-30 active nymphs were allowed to feed on young sunflower leaves showing typical disease symptoms of SNV, kept in a plastic container. The lid of the container were removed and replaced with 60-mesh size white nylon mesh. Wet cotton was placed at the edge of the petiole to prevent the leaf from drying. The nymphs were allowed to feed on infected leaf at different acquisition periods v*iz*., 1, 2, 3 and 4 days. Later the nymphs were transferred on to the test plants using fine hairbrush. The normal movement of thrips was observed to ensure that injury to thrips has not occurred during the transfer. The plants were observed up to 50 days for expression of symptoms.

Determination of inoculation access period (IAP)

About 25-30 nymphs were collected using fine hairbrush, which were kept for AAP of three days in a plastic container at room temperature. The viruliferous nymphs were then transferred onto sunflower test plants kept in wooden cages in glasshouse. Systemic insecticide (Imidachloprid 200 SL 0.05%) was sprayed onto the test plants at different inoculation periods of 1, 2, 4, 5, 6, 7, 8 and 9 days. The mortality of the vectors was ascertained and observations were recorded.

Determination of minimum number of thrips required to transmit the disease

Healthy nymphs were allowed to feed on to SNV infected sunflower leaves at room temperature. Different batches comprising 1, 2, 5, 20, 25 and 30 nymphs were collected and placed on each of the test plants (sunflower) raised in insect proof wooden cages. The normal movement of thrips was observed to assure that injury had not occurred during the transfer. The vector was allowed to feed on the test plants and observations were recorded.

Results and Discussion

In the present study on transmission of sunflower necrosis virus (SNV) through *Thrips palmi.* The vector of SNV successfully transmitted SNV to test plants. The results revealed that the transmission of SNV by thrips ranged from 13.33-23.33 per cent in all the genotypes tested, Highest mean per cent transmission was observed in KBSH-44 (23.33), followed by Morden (20.00) and KBSH-1(13.33) with an average transmission range of 18.88 per cent (Table 1) and the symptoms of curling and necrosis were observed. Successful transmission of SNV through thrips was also reported by Shivasharanayya (2000), Ajith Prasad (2004) and Aravind (2002) [11, 7, 8]. The symptoms produced upon inoculation through thrips were similar to the symptoms reported in sunflower under field conditions (Nagaraju *et al.*, 1998; Halakeri, 1999; Shivasharanayya, 2000, and Aravind, 2002) [12, 10, 11, 8] and also through thrips under laboratory conditions (Linga Reddy, 2003 and Ajith Prasad, 2004) [6, 7].

Serological detection of sunflower necrosis virus (SNV) by using DAC-ELISA, the results obtained in all the genotypes showed a positive reaction with TSV antisera indicating the presence of SNV in the leaf samples. These results thus prove the transmissible nature of SNV through *Thrips palmi*. Confirmation of successful transmission of sunflower necrosis virus through thrips by using DAC-ELISA was also reported by Linga Reddy (2003) [6] and Lokesh (2006) [9].

In the present study, investigation was made to establish the virus -vector relationship. Whereas, acquisition access period of 3 and 4 day resulted in successful transmission (13.33 % and 23.33%) of the virus (Table 2). Similar results have been reported by Shivasharanayya (2000) [11], who found that thrips could acquire the virus and become viruliferous only when they feed for three days on the source plant.

The experiment carried out to determine the Inoculation Access Period (IAP) revealed that IAP of 6 days was necessary for successful transmission of the virus with the mean percent transmission of 16.67. IAP of less than six days was found insufficient for transmission of the virus (Table 3). Similar results were reported by Shivasharanayya (2000) [11] and Aravind, (2002) [8].

Further, to Determination of minimum number of thrips required to transmit SNV. The results revealed that two thrips was able to acquire and transmit the virus with mean per cent transmission of SNV 6.67 (Table 4). Shivasharanayya (2000) [11] reported that the vector, *Thrips palmi* was successful in transmitting the disease.

Table 1: Transmission of SNV through *Thrips palmi*

Sl. No.	Cultivar	Replication	No. of plants		Transmission (Per cent)	Mean transmission (Per cent)	DAC ELISA reaction
			Inoculated	Infected			
1.	KBSH-1	I	10	1	10	13.33	Positive
		II	10	1	10		
		III	10	2	20		
2.	KBSH-44	I	10	3	30	23.33	Positive
		II	10	2	20		
		III	10	2	20		
3.	Morden	I	10	1	10	20.00	Positive
		II	10	2	20		
		III	10	3	30		
	Mean transmission (%)					18.88	

Table 2: Determination of Acquisition Access Period (AAP) of sunflower necrosis virus by *Thrips palmi*

S. No.	AAP	Number of plants		Transmission (Per cent)	Mean transmission (Per cent)
		Inoculated	Infected		
1.	10 hours	10 10 10	-	-	-
2.	20 hours	10 10 10	-	-	-
3.	24 hours	10 10 10	-	-	-
4.	48 hours	10 10 10	-	-	-
5.	72 hours	10 10 10	1 1 2	10 10 20	13.33
6.	84 hours	10 10 10	1 2 2	10 20 20	16.66
7.	96 hours	10 10 10	2 3 2	20 30 20	23.33

Table 3: Determination of Inoculation Access Period (IAP) of sunflower necrosis virus by *Thrips palmi*

S. No.	IAP (days)	Number of plants		Transmission (Per cent)	Mean transmission (Per cent)
		Inoculated	Infected		
1.	1	10 10 10	- - -	- - -	- - -
2.	2	10 10 10	- - -	- - -	- - -
3.	4	10 10 10	- - -	- - -	- - -
4.	5	10 10 10	- - -	- - -	- - -
5.	6	10 10 10	2 1 1	20 10 10	16.67
6.	7	10 10 10	2 1 1	20 10 10	16.67
7.	8	10 10 10	2 2 2	20 20 20	20.00
8.	9	10 10 10	2 2 2	20 20 20	20.00

Note: AAP - 3 days

Table 4: Determination of minimum number of thrips required to transmit SNV

S. No.	Number of thrips per plant	Number of plants		Transmission (Per cent)	Mean transmission (Per cent)
		Inoculated	Infected		
1.	1	10 10 10	0 0 0	-	-
2.	2	10 10 10	1 0 1	10 00 10	06.67
3.	5	10 10 10	1 1 1	10 10 10	10.00
4.	10	10 10 10	2 1 1	20 10 10	13.33

5.	20	10 10 10	2 2 2	20 20 20	20.00
6.	25	10 10 10	3 2 2	30 20 20	23.33
7.	30	10 10 10	3 3 2	30 30 20	26.67

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