

# Serological diagnosis of crop and weed plants for the presence of Sunflower Necrosis Virus (SNV) through Direct Antigen Coated- Enzyme Linked Immuno Sorbent Assay (DAC-ELISA)

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## SUMMARY

Thirty nine crop plants and thirty seven weed plants belonging to different families were artificially inoculated with Sunflower Necrosis Virus (SNV) through sap and *Thrips palmi* (Karny), vector of SNV and were kept for symptom expression. These plants were then subjected to Direct Antigen Coated-Enzyme Linked Immuno Sorbent Assay (DAC-ELISA) analysis to detect the presence of the virus. Out of thirty nine crop plants, nine of them showed positive reaction, where as out of thirty seven weed plants, twenty four of them gave positive reaction indicating the presence of SNV in them. The above results thus proved that these positively reacted crop and weed plants act as collateral hosts to SNV and further help in the spread and survival of this virus during off season.

## Key words :

Sunflower  
necrosis virus,  
*Thrips palmi*,  
Crop plants,  
Weeds, DAC-  
ELISA

Sunflower (*Helianthus annuus* L.), a member of Asteraceae, is one of the important oilseed crops of the world and ranks third, after soybean and groundnut in area and production. Sunflower is reported to be susceptible to several diseases caused by various agents. However, off late a new virus disease on sunflower showing necrotic symptoms has been reported to occur around Bangalore (Anonymous, 1997 and Singh *et al.*, 1997). Surveys conducted in some parts have indicated that the disease incidence ranged from 5-80% and thus is of great importance (Anonymous, 2006). In general, the viral diseases are known to be associated with several crop plants and weeds causing symptoms specific on the main crop infected. SNV was reported to infect several crops and weed species present in and around sunflower fields (Ajith Prasad and Nagaraju, 2005 a and b and Lokesh, 2006). Thus, the objective of this study was to understand the sudden occurrence and spread of the disease and to determine the host range of the virus and thrips prevailed on them.

## MATERIALS AND METHODS

The experiment was carried out to detect the presence of SNV in different crops and

weed species. Ajith Prasad and Nagaraju (2005a) investigated and proved the transmission of SNV through mechanical inoculation and *T. palmi*. Accordingly, thirty nine healthy crops and thirty seven healthy weed plants were first inoculated with SNV through mechanical sap and its vector, *Thrips palmi* under lab conditions at two leaves stage. The inoculated seedlings were left undisturbed for symptom expression.

Prasada Rao *et al.* (2000) concluded that the causal virus of Sunflower Necrosis Disease (SND) was a strain of Tobacco Streak Virus (TSV) belonging to *Ilarvirus* group. This conclusion was based on the serological reaction with tobacco streak virus antiserum, molecular weight of virus coat protein and nucleic acid species analysis. Thus, for antigen coated enzyme linked immunosorbent assay (DAC-ELISA) Tobacco streak virus antiserum and alkaline phosphatase (ALP) enzyme and p-nitrophenylphosphate (pNPP) system were employed.

Crude plant extract was prepared in coating buffer using dilution of 1:100 (100 mg of leaf sample /1 ml buffer). The filtered extract was dispensed into each well of ELISA plate at the rate of 100 µl using a micropipette and the plate was incubated at 37° C for 1 hour.

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The contents of the plate were rinsed with PBS-Tween. The unoccupied spaces in the wells were blocked with milk protein or 2 per cent BSA and incubated for 1hr at 37°C. Antibody and alkaline phosphatase labeled anti goat IgG were used at 1:1000 and 1:10,000 dilutions and incubated at 37° C for 1 hour. Substrate p-nitrophenyl phosphate (PNP) was prepared in 10% diethanolamine (pH 9.8). 0.05mM of magnesium chloride was added to this solution. 100  $\mu$ l of the substrate was added to each

well and incubated for 30 min-1hr at 37°C. Change in the colour of the wells was observed using ELISA reader and OD value was recorded at 405nm.

## RESULTS AND DISCUSSION

Among various crop plants tested, the crop plants viz., *Helianthus annuus* (cv. KBSH-44), *Citrullus lanatus* (cv. ARKA MANIK), *Cucurbita moschata* (cv. ARKA SURYAMUKHI), *Arachis hypogaea* (cv. JL-24), *Lablab*

**Table 1: Detection of SNV by DAC-ELISA in crop plants inoculated through sap**

Sr. No.	Crop species		Variety	OD value at 405 nm		Reaction
	Scientific name	Common name		Healthy	Infected	
1.	<i>Helianthus annuus</i>	Sunflower	KBSH-44	0.147	1.912	Positive
2.	<i>Carthamus tinctorius</i>	Safflower	C-18	0.134	0.126	Negative
3.	<i>Guizotia abyssinica</i>	Niger	S-26	0.312	0.394	Negative
4.	<i>Tagetes erecta</i>	Marigold	Chinthamani	0.014	0.012	Negative
5.	<i>Zinnia elegans</i>	Zinnia	ZE-231	0.023	0.013	Negative
6.	<i>Brassica nigra</i>	Mustard	Kanakpura local	0.142	0.134	Negative
7.	<i>Brassica oleracea</i> var. <i>gongylodes</i>	Knol khol	KO-04-890	0.241	0.124	Negative
8.	<i>Brassica oleracea</i> var. <i>Capitata</i>	Cabbage	B0-01-001	0.023	0.014	Negative
9.	<i>Raphanus sativus</i>	Radish	Mino long	0.023	0.035	Negative
10.	<i>Cucumis sativus</i>	Cucumber	PS -24	0.183	0.155	Negative
11.	<i>Citrullus lanatus</i>	Watermelon	Arka Manik	0.131	1.935	Positive
12.	<i>Cucumis melo</i>	Musk melon	Deepti	0.140	0.153	Negative
13.	<i>Cucurbita moschata</i>	Pumpkin	Arka Suryamukhi	0.129	1.124	Positive
14.	<i>Lagenaria siceraria</i>	Bottle gourd	PSPR	0.341	0.304	Negative
15.	<i>Luffa acutangula</i>	Ridge gourd	Arka Sujata	0.139	0.121	Negative
16.	<i>Momordica charantia</i>	Bitter gourd	Arka Harit	0.218	0.301	Negative
17.	<i>Trichosanthes cucumerina</i>	Snake gourd	C0-2	0.021	0.013	Negative
18.	<i>Ricinus communis</i>	Castor	DCS-9	0.124	0.183	Negative
19.	<i>Arachis hypogaea</i>	Groundnut	JL-24	0.138	1.119	Positive
20.	<i>Glycine max</i>	Soybean	KB-79	0.131	1.193	Positive
21.	<i>Cajanus cajan</i>	Pigeon pea	TTB-7	0.102	0.133	Negative
22.	<i>Pisum sativum</i>	Pea	KPMR-1	0.120	0.109	Negative
23.	<i>Vigna radiate</i>	Green gram	Pusa Baisakhi	0.295	0.312	Negative
24.	<i>Vigna mungo</i>	Black gram	T-9	0.032	0.036	Negative
25.	<i>Lablab purpureus</i>	Field bean	HA-3	0.091	1.021	Positive
26.	<i>Macrotyloma uniflorum</i>	Horse gram	PHG-9	0.292	1.128	Positive
27.	<i>Phaseolus vulgaris</i>	Common bean	Topcrop	0.230	0.235	Negative
28.	<i>Crotalaria juncea</i>	Sunhemp	Local	0.209	0.255	Negative
29.	<i>Cicer arietinum</i>	Chickpea	A-1	0.104	0.126	Negative
30.	<i>Vigna unguiculata</i>	Cowpea	C-152	0.134	1.309	Positive
31.	<i>Cyamopsis tetragonoloba</i>	Cluster bean	Pusa Navadhar	0.144	0.123	Negative
32.	<i>Abelmoschus esculentus</i>	Bhendi	Arke Komar	0.158	0.131	Negative
33.	<i>Sesamum indicum</i>	Sesamum	KL-31	0.281	0.242	Negative
34.	<i>Capsicum annum</i>	Chilli	Pusa Jwala	0.202	0.219	Negative
35.	<i>Praecitrullus fistulosus</i>	Round melon	Arka Tinda	0.101	0.121	Negative
36.	<i>Lycopersicon esculentum</i>	Tomato	Pusa Ruby	0.016	0.012	Negative
37.	<i>Nicotiana tabacum</i>	Tobacco	Xanthi	0.231	1.144	Positive
38.	<i>Gossypium hirsutum</i>	Cotton	Varalakshmi	0.025	0.012	Negative
39.	<i>Solanum melongena</i>	Brinjal	BB-12	0.145	0.134	Negative

*purpureus* (cv. HA-3), *Macrotyloma uniflorum* (cv. PHG-9), *Vigna unguiculata* (cv. C-152), *Nicotiana tabacum* (cv. XANTHI) and *Glycine max* (cv. KB-79) showed positive reaction indicating the presence of virus both under artificial sap and thrips inoculation (Table 1 and 2). The results were in accordance with Linga Ready and Nagaraju (2006) who reported that groundnut, cowpea, cucumber and horse gram could also serve as source

plant for the sunflower necrosis virus.

Among various weeds tested, twenty three weeds viz., *Lagascea mollis*, *Alternanthera sessilis*, *Commelina benghalensis*, *Crotalaria spectabilis*, *Euphorbia hirta*, *Cassia obtusifolius*, *Ocimum sanctum*, *Sida rhombifolia*, *Oxalis corniculata*, *Physalis minima*, *Galinsoga parviflora*, *Euphorbia geniculata*, *Solanum nigrum*, *Phyllanthus niruri*,

**Table 2 : Detection of SNV by DAC-ELISA in crop plants inoculated through thirps**

Sr. No.	Crop species			OD value at 405 nm		Reaction
	Scientific name	Common name	Variety	Healthy	Infected	
1.	<i>Helianthus annuus</i>	Sunflower	KBSH-44	0.156	1.235	Positive
2.	<i>Carthamus tinctorius</i>	Safflower	C-18	0.111	0.121	Negative
3.	<i>Guizotia abyssinica</i>	Niger	S-26	0.311	0.320	Negative
4.	<i>Tagetes erecta</i>	Marigold	Chinthamani	0.245	0.125	Negative
5.	<i>Zinnia elegans</i>	Zinnia	ZE-231	0.221	0.231	Negative
6.	<i>Brassica nigra</i>	Mustard	Kanakpura local	0.214	0.363	Negative
7.	<i>Brassica oleracea</i> var. <i>gongylodes</i>	Knol khol	KO-O4-890	0.351	0.213	Negative
8.	<i>Brassica oleracea</i> var. <i>capitata</i>	Cabbage	B0-01-001	0.215	0.321	Negative
9.	<i>Raphanus sativus</i>	Radish	Mino Long	0.265	0.151	Negative
10.	<i>Cucumis sativus</i>	Cucumber	PS -24	0.121	0.122	Negative
11.	<i>Citrullus lanatus</i>	Watermelon	Arka Manik	0.234	1.154	Positive
12.	<i>Cucumis melo</i>	Musk melon	Deepti	0.261	0.213	Negative
13.	<i>Cucurbita moschata</i>	Pumpkin	Arka Suryamukhi	0.327	1.541	Positive
14.	<i>Lagenaria siceraria</i>	Bottle gourd	PSPR	0.215	0.251	Negative
15.	<i>Luffa acutangula</i>	Ridge gourd	Arka Sujata	0.222	0.223	Negative
16.	<i>Momordica charantia</i>	Bitter gourd	Arka Harit	0.156	0.166	Negative
17.	<i>Trichosanthes cucumerina</i>	Snake gourd	C0-2	0.321	0.313	Negative
18.	<i>Ricinus communis</i>	Castor	DCS-9	0.201	0.213	Negative
19.	<i>Arachis hypogaea</i>	Ground nut	JL-24	0.342	1.345	Positive
20.	<i>Glycine max</i>	Soybean	KB-79	0.196	0.164	Negative
21.	<i>Cajanus cajan</i>	Pigeon pea	TTB-7	0.315	0.321	Negative
22.	<i>Pisum sativum</i>	Pea	KPMR-1	0.211	0.213	Negative
23.	<i>Vigna radiate</i>	Green gram	Pusa Baisakhi	0.132	0.133	Negative
24.	<i>Vigna mungo</i>	Black gram	T-9	0.338	0.322	Negative
25.	<i>Lablab purpureus</i>	Field bean	HA-3	0.219	1.002	Positive
26.	<i>Macrotyloma uniflorum</i>	Horse gram	PHG-9	0.227	1.512	Positive
27.	<i>Phaseolus vulgaris</i>	Common bean	Topcrop	0.231	0.211	Negative
28.	<i>Crotalaria juncea</i>	Sunhemp	Local	0.252	0.211	Negative
29.	<i>Cicer arietinum</i>	Chickpea	A-1	0.214	0.217	Negative
30.	<i>Vigna unguiculata</i>	Cowpea	C-152	0.112	1.241	Positive
31.	<i>Cyamopsis tetragonoloba</i>	Cluster bean	Pusa Navadhar	0.322	0.326	Negative
32.	<i>Abelmoschus esculentus</i>	Bhendi	Arke Komar	0.231	0.213	Negative
33.	<i>Sesamum indicum</i>	Sesamum	KL-31	0.156	0.126	Negative
34.	<i>Capsicum annum</i>	Capsicum	Pusa Jwala	0.216	0.215	Negative
35.	<i>Praecitrullus fistulosus</i>	Round melon	Arka Tinda	0.366	0.326	Negative
36.	<i>Lycopersicon esculentum</i>	Tomato	Pusa Ruby	0.156	0.145	Negative
37.	<i>Nicotiana tabacum</i>	Tobacco	Xanthi	0.245	1.442	Positive
38.	<i>Gossypium hirsutum</i>	Cotton	Varalakshmi	0.321	0.321	Negative
39.	<i>Solanum melongena</i>	Brinjal	BB-12	0.216	0.265	Negative

*Malvestrum coromandelianum*, *Ageratum conyzoides*, *Achyranthus aspera*, *Abutilon indicum*, *Ocimum canum*, *Crotalaria striata*, *Bidens pilosa*, *Stachytarpetta indica*, *Acanthospermum hispidum* and *Xanthium strumarium* showed positive reaction. Thus, from the above experiment, presence of SNV is confirmed in the above mentioned weeds when the virus is inoculated through sap and thrips (Table 3 and 4). Similar results were observed by Lokesh (2006) who reported positive reaction in *Euphorbia geniculata*, *Galinsoga*

*parviflora*, *Phyllanthus niruri*, *Malvestrum coromandelianum*, *Achyranthus aspera*, *Abutilon indicum*, *Ocimum canum*, *Crotalaria striata*, *Bidens pilosa*, *Stachytarpetta indica*, *Acanthospermum hispidum*, *Ageratum conyzoides* through DAC-ELISA. Similar confirmations through DAC-ELISA to the presence of SNV in weed species like *Ocimum sanctum*, *Gomphrena decumbense*, *Acanthospermum hispidum* and *Alternanthera sessilis* were made by Linga Reddy (2003) which was naturally infected by SNV under field

**Table 3: Detection of SNV by DAC-ELISA in weeds inoculated through sap**

Sr. No.	Weeds	Family	OD value at 405 nm		Reaction
			Healthy	Infected	
1.	<i>Lagascea mollis</i>	Asteraceae	0.131	1.122	Positive
2.	<i>Parthenium hysterophorus</i>	Asteraceae	0.165	0.131	Negative
3.	<i>Sonchus oleraceus</i>	Asteraceae	0.321	0.322	Negative
4.	<i>Alternanthera sessilis</i>	Amaranthaceae	0.032	0.954	Positive
5.	<i>Amaranthus spinosus</i>	Amaranthaceae	0.265	0.121	Negative
6.	<i>Commelina benghalensis</i>	Commelinaceae	0.214	1.124	Positive
7.	<i>Ipomoea pandurata</i>	Convolvulaceae	0.132	0.132	Negative
8.	<i>Crotalaria spectabilis</i>	Euphorbiaceae	0.311	1.531	Positive
9.	<i>Euphorbia hirta</i>	Euphorbiaceae	0.125	0.988	Positive
10.	<i>Cassia obtusifolius</i>	Fabaceae	0.111	0.985	Positive
11.	<i>Ocimum sanctum</i>	Labiatae	0.121	1.321	Positive
12.	<i>Sida rhombifolia</i>	Malvaceae	0.311	1.121	Positive
13.	<i>Oxalis corniculata</i>	Oxalidaceae	0.121	0.955	Positive
14.	<i>Portulaca oleraceae</i>	Portulacaceae	0.124	0.231	Negative
15.	<i>Physalis minima</i>	Solanaceae	0.131	0.987	Positive
16.	<i>Galinsoga parviflora</i>	Asteraceae	0.321	1.101	Positive
17.	<i>Eupatorium odoratum</i>	Asteraceae	0.213	0.121	Negative
18.	<i>Euphorbia geniculata</i>	Euphorbiaceae	0.183	1.941	Positive
19.	<i>Solanum nigrum</i>	Solanaceae	0.243	1.123	Positive
20.	<i>Phyllanthus niruri</i>	Euphorbiaceae	0.153	1.942	Positive
21.	<i>Malvestrum coromandelianum</i>	Malvaceae	0.147	1.231	Positive
22.	<i>Ageratum conyzoides</i>	Asteraceae	0.164	1.211	Positive
23.	<i>Polygonium plebium</i>	Polygonaceae	0.311	0.321	Negative
24.	<i>Datura stramonium</i>	Solanaceae	0.411	0.321	Negative
25.	<i>Tridax procumbense</i>	Asteraceae	0.121	0.223	Negative
26.	<i>Achyranthes aspera</i>	Amaranthaceae	0.139	1.235	Positive
27.	<i>Abutilon indicum</i>	Malvaceae	0.121	1.651	Positive
28.	<i>Ocimum canum</i>	Labiatae	0.151	1.673	Positive
29.	<i>Argemone mexicana</i>	Papaveraceae	0.341	0.231	Negative
30.	<i>Crotalaria striata</i>	Fabaceae	0.124	0.989	Positive
31.	<i>Bidens pilosa</i>	Asteraceae	0.111	0.974	Positive
32.	<i>Borreria stricta</i>	Rubiaceae	0.101	0.132	Negative
33.	<i>Stachytarpetta indica</i>	Verbenaceae	0.110	1.654	Positive
34.	<i>Leucas aspera</i>	Labiatae	0.254	0.213	Negative
35.	<i>Acanthospermum hispidum</i>	Asteraceae	0.134	0.986	Positive
36.	<i>Synedrella nodiflora</i>	Asteraceae	0.111	0.132	Negative
37.	<i>Xanthium strumarium</i>	Asteraceae	0.152	1.121	Positive

**Table 4: Detection of SNV by DAC-ELISA in weeds inoculated through thrips**

Sr. No.	Weeds	Family	OD value at 405 nm		Reaction
			Healthy	Infected	
1.	<i>Lagascea mollis</i>	Asteraceae	0.212	1.214	Positive
2.	<i>Parthenium hysterophorus</i>	Asteraceae	0.264	0.321	Negative
3.	<i>Sonchus oleraceus</i>	Asteraceae	0.213	0.211	Negative
4.	<i>Alternanthera sessilis</i>	Amaranthaceae	0.151	1.166	Positive
5.	<i>Amaranthus spinosus</i>	Amaranthaceae	0.211	0.225	Negative
6.	<i>Commelina benghalensis</i>	Commelinaceae	0.151	1.332	Positive
7.	<i>Ipomoea pandurata</i>	Convolvulaceae	0.126	0.121	Negative
8.	<i>Crotalaria spectabilis</i>	Euphorbiaceae	0.166	1.316	Positive
9.	<i>Euphorbia hirta</i>	Euphorbiaceae	0.156	1.212	Positive
10.	<i>Cassia obtusifolia</i>	Fabaceae	0.116	1.345	Positive
11.	<i>Ocimum sanctum</i>	Labiatae	0.136	1.156	Positive
12.	<i>Sida rhombifolia</i>	Malvaceae	0.663	1.641	Positive
13.	<i>Oxalis corniculata</i>	Oxalidaceae	0.326	1.311	Positive
14.	<i>Portulaca oleraceae</i>	Portulacaceae	0.215	0.321	Negative
15.	<i>Physalis minima</i>	Solanaceae	0.124	1.245	Positive
16.	<i>Galinsoga parviflora</i>	Asteraceae	0.321	1.551	Positive
17.	<i>Eupatorium odoratum</i>	Asteraceae	0.134	0.161	Negative
18.	<i>Euphorbia geniculata</i>	Euphorbiaceae	0.115	0.999	Positive
19.	<i>Solanum nigrum</i>	Solanaceae	0.152	1.620	Positive
20.	<i>Phyllanthus niruri</i>	Euphorbiaceae	0.262	1.321	Positive
21.	<i>Malvestrum coromandelianum</i>	Malvaceae	0.134	1.111	Positive
22.	<i>Ageratum conyzoides</i>	Asteraceae	0.101	0.989	Positive
23.	<i>Polygonium plebium</i>	Polygonaceae	0.123	0.136	Negative
24.	<i>Datura stramonium</i>	Solanaceae	0.156	0.264	Negative
25.	<i>Tridax procumbense</i>	Asteraceae	0.223	0.254	Negative
26.	<i>Achyranthes aspera</i>	Amaranthaceae	0.123	1.211	Positive
27.	<i>Abutilon indicum</i>	Malvaceae	0.166	0.982	Positive
28.	<i>Ocimum canum</i>	Labiatae	0.316	1.636	Positive
29.	<i>Argemone mexicana</i>	Papaveraceae	0.164	0.166	Negative
30.	<i>Crotalaria striata</i>	Fabaceae	0.136	1.611	Positive
31.	<i>Bidens pilosa</i>	Asteraceae	0.361	1.235	Positive
32.	<i>Borreria stricta</i>	Rubiaceae	0.321	0.166	Negative
33.	<i>Stachytarpetta indica</i>	Verbenaceae	0.156	0.998	Positive
34.	<i>Leucas aspera</i>	Labiatae	0.216	0.123	Negative
35.	<i>Acanthospermum hispidum</i>	Asteraceae	0.311	1.262	Positive
36.	<i>Synedrella nodiflora</i>	Asteraceae	0.162	0.156	Negative
37.	<i>Xanthium strumarium</i>	Asteraceae	0.133	1.225	Positive

conditions.

The study thus reveals that crop plants and weed species act as reservoirs for both virus and thrips and thus help in the spread and survival of the virus especially during offseason.

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