# Survey and molecular characterization of begomovirus, and assessment of yield losses caused by leaf curl disease of sunflower (*Helianthus annuus*)

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Received: 17 October 2023; Accepted: 06 September 2024

#### ABSTRACT

A comprehensive survey was conducted during winter (*rabi*) and rainy (*kharif*) seasons of 2019–20 to 2021–22 in the Kurnool, Nandyal, and Prakasam districts of Andhra Pradesh, focusing on the incidence of leaf curl disease in hybrid sunflower (*Helianthus annuus* L.) crop. The results revealed a high incidence of the disease, ranging from 40–94.5% across most of the surveyed hybrid varieties. Leaf curl-infected samples collected from the surveyed fields were analyzed by PCR using specific primers for the DNA-A component of the virus to confirm the presence of the pathogen. The PCR-amplified fragments were cloned and sequenced. Sequence analysis revealed that the sunflower isolate (Snf-AP) shared 99.2% nucleotide identity with the tomato leaf curl Karnataka virus (ToLCKV), which infects sunflower crops in Karnataka. This indicates a strong geographical and genetic connection between the viral strains affecting sunflowers in Andhra Pradesh and Karnataka. This genetic similarity is significant, as it suggests that the same or closely related viral strains are responsible for sunflower leaf curl disease across broader regions. The disease was found to affect sunflowers at all growth stages, with the highest incidence (42.3%) observed at the star bud stage. Infection at the star bud stage leads to substantial seed yield losses, with reductions of up to 82.8%. This level of damage underscores the economic impact of early infections, as yield losses of this magnitude can severely affect the profitability of sunflower farming. Additionally, a positive correlation was found between the whitefly population, weather parameters, and disease development.

Keywords: Leaf curl, PCR, Phylogenetic analysis, Sunflower, Survey, Yield losses

Sunflower (*Helianthus annuus* L.) is a major edible oilseed crop in India, valued for its high oil content and as a source of vegetable oil and protein. Due to its wide adaptability, sunflower has become an essential crop in various agro-climatic regions. However, its production has been hampered by both biotic and abiotic stresses, leading to yield stagnation. In the 2022–23 growing season, India produced 2.79 lakh tonnes of sunflower seeds from an area of 2.69 lakh hectares, with an average productivity of 1037 kg/ha (Directorate of Economics and Statistics, 2023). Despite its importance, sunflower cultivation faces growing challenges from plant diseases caused by different

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pathogens (Poornima et al. 2023). Of these one of the most significant being the sunflower leaf curl virus.

The leaf curl virus was classified under the begomovirus genus, is an emerging threat to sunflower production in India. It is transmitted by the whitefly, Bemisia tabaci, which has become a key vector in the spread of this pathogen (Govindappa et al. 2011). The disease impacts critical agronomic traits such as plant height, head diameter, seed weight, and oil content, particularly when the infection occurs early, around 30 days after sowing (DAS). Early infection has been shown to cause seed yield losses of up to 79.25% (Deepa et al. 2015), making it a serious concern for farmers. The virus primarily affects sunflower plants by stunting their growth, reducing seed set, and diminishing overall productivity. This decline in plant vigour and yield, especially when coupled with abiotic stresses like drought, further exacerbates the challenges faced by sunflower growers. In an earlier study conducted at the Regional Agricultural Research Station (RARS) in Nandyal district of Andhra Pradesh, disease incidence across 23 coordinated sunflower entries ranged from 0–18.8%, suggesting variability in resistance among different genotypes (Annual Report on Sunflower 2017–18). This variability points to the potential for breeding more resistant hybrids, which could help mitigate the effects of the virus. Given the growing prevalence of the sunflower leaf curl virus in Andhra Pradesh, the present investigation was initiated to survey the disease incidence, carry out molecular characterization of the virus, and assess yield losses caused by the infection at different stages of the crop's development.

### MATERIALS AND METHODS

Survey and collection of leaf curl samples of sunflower: A roving survey was conducted during winter (rabi) and rainy (kharif) seasons of 2019–20 to 2021–22 in the Kurnool, Nandyal, and Prakasam districts of Andhra Pradesh, focusing on the incidence of leaf curl disease in sunflower crop. Three mandals were selected from each district for the study. Within each mandal, five villages were surveyed. These villages are having distance of 5–10 km apart to ensure a representative sampling across the region. The surveys were conducted during the vegetative and flowering stages of the crop, which are critical periods for identifying infections based on visible symptoms. The selection of these stages is crucial as plants tend to show more pronounced phenotypic symptoms of infection, such as stunting, leaf curl, discolouration, or other deformities, during these growth phases. Leaf curl incidence was calculated as:

Disease incidence (%) = 
$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Leaf curl virus infected sunflower leaf samples were collected in polythene bags and labelled with required data from the farmers.

Molecular characterization: Total DNA isolation from collected plant samples was done by Cetyltrimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle 1990). The pathogen infection was confirmed by PCR using specific primers to DNA-A component of begomovirus genome (Venkataravanappa et al. 2012). Further PCR amplified products were purified from agarose gel by using standard protocols and ligated into pTZ57R/T vector using Ins T/A clone PCR product cloning kit (Fermentas, city Germany) according to the manufactures instructions. The ligated product was transformed into Escherichia coli DH5a competent cells (Invitrogen Bioservices India Pvt. Ltd., Bengaluru, India). Three positive clones for each amplified fragment were selected, plasmids were isolated using Qiagen plasmid miniprep kit (Qiagen, Hilder, Germany) and sequencing was done using M13F/R primers at Medauxin Pvt. Ltd. Bengaluru, India.

Further, for detection of DNA satellites in infected sunflower plant samples were subjected to PCR amplification using universal primers specific to betasatellite and alphasatellite (Briddon *et al.* 2004).

Viral genome sequence analysis: The complete genome fragments of begomovirus infecting sunflower was assembled and subjected to ORF finder at NCBI to predicted coding region in the genome. Nucleotide

sequence similarity for complete genome was checked against GenBank sequences using BLASTn. The GenBank sequences showing maximum blast scores to begomovirus infecting different crops were retrieved and used for further analysis. The SEAVIEW and Bioedit (version 5.0.9) were used for sequence alignment (Hall 1999). Sequence identity matrices were generated by using Sequence Demarcation tool (SDT) (Muhire *et al.* 2014). Neighbour-joining method was applied to deduce phylogenetic tree using MEGA X software (Tamura *et al.* 2021).

Assessment of yield losses due to leaf curl virus: The field experiment was conducted during the winter (rabi) and rainy (kharif) seasons of 2019-2020 to 2021-2022 at the Regional Agricultural Research Station (Acharya N G Ranga Agricultural University, Guntur, Andhra Pradesh), Nandyal (15°27'N, 78°28'E), Andhra Pradesh. Hybrid KBSH-44 was used as a susceptible check in two plots (protected and unprotected), each with an area of 500 m<sup>2</sup>. The protected plot was treated with imidacloprid 600 FS @1 ml/kg, followed by two applications of diafenthiuron at 1.25 g/litre, sprayed at 30 and 45 days after sowing (DAS) to manage the whitefly population and sunflower leaf curl disease (Venkataramanamma et al. 2022). Leaf curl incidence and whitefly population (correlated with weather factors) were recorded from 30 DAS until seed setting at 10-days intervals, along with plant growth parameters and yield loss (Sastry and Singh 1973) in 10 randomly selected plants.

$$\text{Per cent yield loss } = \frac{ \begin{array}{c} \text{Yield of healthy plants} - \text{Yield} \\ \text{of diseased plants} \\ \hline \text{Yield of healthy plants} \\ \end{array} \times 100$$

The data pertaining to above parameters at different intervals collected from disease affected plants and healthy plants were subjected to t-test analysis to know the significance of disease.

### RESULTS AND DISCUSSION

Survey and collection of disease samples: During kharif 2021–22, the disease incidence was ranged from 1.0–40% on different sunflower hybrids (Table 1). Gowri hybrid in Prakasam district had the highest incidence of 40%, while Teja hybrid had the lowest of 1%. Whereas in case of rabi 2021-22, hybrids showed disease incidence ranging from 1.0–94.5%. Arun and Swathi hybrid in Kurnool and Nandyal district had the highest incidence more than 80%, while Kaveri, NDSH-1012, Super Raja hybrid had the less than 5% incidence. This may be due to the hybrids have different tolerance levels for sunflower leaf curl disease. The survey also showed maximum incidence was found on star bud and flowering stages in different hybrids and more incidences on some hybrids during *rabi*, than *kharif* season. Similarly, the literature survey showed that leaf curl disease incidence ranged from 2.65-30.6% on 12 different coordinated entries at Regional Agricultural Research Station (Acharya N G Ranga Agricultural University, Guntur, Andhra Pradesh), Nandyal, Andhra Pradesh (Venkataramanamma and Prabhakar 2020).

Table 1 Survey for the leaf curl diseases of sunflower

District name	Hybrid name	Stage of the crop	Leaf curl in	cidence (%)	Preceding crops
		-	Average	Range	followed by farmers
		Kharif 20	021–22		
Nandyal	Ganga kaveri	Star bud	3.50	3.0-4.0	Black gram
	(GK-2002)	Flowering	10.0	4.1-12.0	Groundnut
		Seed setting	10.0	4.2-12.0	Maize
	Sunbred-275\	Star bud	2.00	1.0-4.0	Black gram
		flowering	5.00	3.0-7.0	Groundnut
		Seed setting	5.50	3.1-8.0	Maize
Prakasam	Gowri	Star bud	30.5	28-31	Redgram
		Flowering	40.0	35-42	Black gram
		Seed setting	40.0	35–43	Sunflower
	Teja	Star bud	1.00	1.0-1.1	Redgram
		Flowering	1.20	1.0-1.6	Black gram
		Seed setting	1.20	1.0-1.6	Sunflower
Kurnool	Ganga kaveri	Star bud	1.50	1.0-2.0	Maize
	(GK-2002)	Flowering	2.50	2.0-4.0	Black gram
		Seed setting	2.70	2.0-5.0	
	GHS 4455	Star bud	1.20	1.0-1.5	Cotton
		flowering	2.00	1.0-4.0	Maize
		Seed setting	2.10	1.0-4.0	Bengalgram
		Rabi 20	19–20		
Kurnool	Arun	Star bud	42.5	35-60	Maize
		flowering	73.4	60-80	Redgram
		Seed setting	87.5	80-100	Onion
	GHS 4455	vegetative	1.20	1.0-2.0	Maize
		Flowering	2.20	2.0-3.0	Redgram
		Seed setting	2.30	2.0-3.1	
	Kaveri	Star bud	3.20	3.0-4.0	Maize
		Flowering	5.50	3.0-6.0	Redgram
		Seed setting	5.50	3.0-6.0	
	NDSH-1012	Star bud	2.50	2.0-2.8	Maize
		Flowering	3.50	3.0-5.0	Redgram
		Seed setting	3.50	3.0-5.0	
Nandyal	Swathi	Star bud	45.6	40-60	Maize
		Flowering	80.0	65.5-82	Chillies
		Seed setting	94.3	90-95	
	Arun	Star bud	70.0	40-80	Chillies
		flowering	88.0	70–90	Sunflower
		Seed setting	94.5	90–98	Groundnut
	Super Raja	Star bud	0.50	0.4-1.0	Maize
	- <del>-</del>	Flowering	0.80	0.6-1.2	
		Seed setting	0.80	0.6-1.2	

During survey the predominant symptoms observed was small size, malformed leaves, thickening and brittleness of leaves, irregular and thickened veins, enations and upward leaf curling, the infected plants were identified (Supplementary Fig. 1). Yellow discolouration of emerging leaves, severe reduction in leaf size and stunting were also observed. No ear head was erected in case of early infection and small heads were observed in later infected plants.

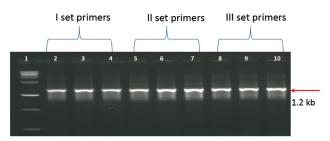


Fig. 1 Gel picture showing PCR amplification of coat protein, rep and intergenic region of DNA-A components by using three sets of specific primers.

Lane 1: Lambda DNA/Eco R1 + Hind 3 marker; Lane 2–10 virus infected sunflower samples amplified product of 1.2 kb using three sets of primers.

Detection of begomovirus in sunflower samples: The complete genome sequence of begomovirus was amplified using three sets of overlapping primers which cover the entire genome of DNA-A component of virus. The primer pairs (OY 2395F/OY 680R, GEMA 1232F/BEG2350R, MKBEGF4/MKBEGR5) yielded an amplicon size of 1.2 kb in size for the DNA-A component (Venkataravanappa et al. 2012). There was no amplification in the samples collected from non-symptomatic plants. The PCR amplificons were cloned and sequenced. The raw sequence data (DNA-A component) obtained from the begomovirus infecting sunflower were assembled by using different programs (Bioedit, Clustal X2 and Sea View) and the consensus sequenced was deposited in NCBI database (Accession number OR541130) (Fig. 1). The attempts to amplify the sub genomic components betasatellite (DNAB) and alphasatellite resulted no amplification indicating that virus is not associated with the satellites.

Genome structure of begomovirus infecting sunflower: The complete genome (DNA-A) of sunflower isolate (Snf-AP) was 2793 nt in length and showed typical genome organization like other monopartite begomoviral genomes by encodes six ORFs (V1, V2, C1, C2, C3 and C4) both sense and antisense strand reported so far. The Intergenic region (IR) contained one directly repeated sequence or iteron, the TATAbox, stem-loop and nanonucleotide sequence, TAATATTAC, which is required for transcription and viral genome replication (Supplementary Fig. 2).

Complete genome sequence of the begomovirus infecting sunflower (Snf-AP) from the present study was compared with corresponding region of 48 begomoviruses retrieved from the NCBI database by using SDT. The analysis showed that Snf-AP isolate shared maximum nucleotide identity of 99.2% with the DNA-A component of tomato leaf curl Karnataka virus (ToLCKV) in India infecting sunflower (JX678965). Further sequence of Snf-AP isolate also shared less than 88% nucleotide identity with several other begomoviruses associated with the diverse crops in India. This result was supported by two-dimensional colour coded matrix generated by using SDT (Fig. 2).

The phylogenetic tree was built using MEGA 11 using neighbour joining tree method by utilizing nucleotide sequence of Snf-AP clone and other selected begomoviruses retrieved from NCBI database. The analysis revealed that Snf-AP clone is closely cluster with ToLCKV, previously reported in various crops such as *Zinnia* spp., Cockscomb, and sunflower (MK965196, OP905627, KX219744, JX678965, and JX678965) (Fig. 3). Further comparison of coding regions of Snf-1 clone showed the highest nucleotide identity of coat protein (CP), Pre-coat (AV2), Rep (C1), TrAP (C2), REn (C3), and C4 at the protein level are aligned with an isolate of ToLCV that infects sunflowers.

The begomovirus associated with sunflower leaf curl disease was identified through complete genome sequencing and phylogenetic analysis revealed that the virus was closely related to tomato leaf curl virus (ToLCV) infecting sunflower (JX678965) in India. As per the begomoviruses spp. demarcation criteria (91% nucleotide identity; Walker *et al.* 2020), the begomovirus infecting sunflower is a variant of ToLCKV infecting sunflower in India (Govindappa *et al.* 2011, Vanitha 2013, Latha and Kumar 2018).

Assessment of yield losses: During the experimental period, the data was collected from 30 DAS at 10-days interval from 30, 40, 50, 60 and 70 DAS. Usually, symptoms

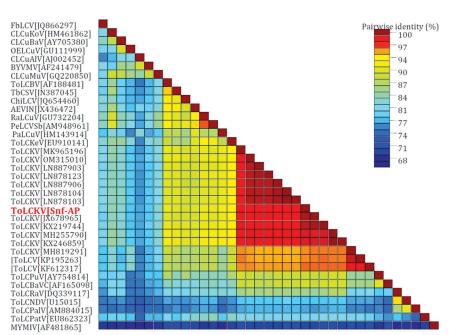


Fig. 2 Two-dimensional colour-coded matrix of pairwise identity scores of the ToLCKV with other selected begomoviruses from GenBank were generated by using the Sequence demarcation tool.

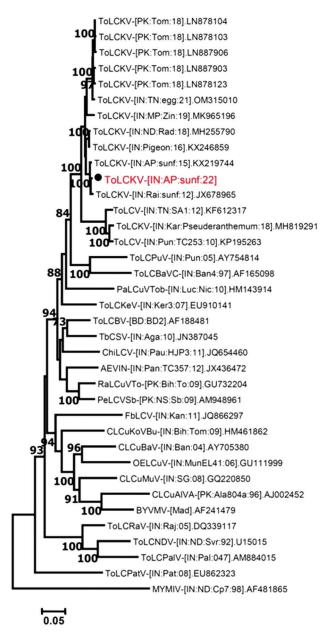


Fig. 3 Dendrogram showing the relationship of the ToLCKV (Snf-AP) infecting sunflower with selected begomoviruses.

started to appear from 22–30 DAS and these plants died prematurely.

During *rabi* 2019–20 (Table 2), leaf curl disease incidence was recorded as 4.2%, 9.93%, 14.8%, 15.5% and 10.5% at 30, 40, 50, 60 and 70 DAS respectively and the cumulative incidence was 54.8%. Mean whitefly population was 7.37 at 30 DAS, 6.5 at 40 DAS, 5.37 at 50 DAS, 7.25 at 60 DAS and 5.75 at 70 DAS in an untreated plot. Plant height was recorded as 67 cm (at 30 DAS), 144 cm (at 70 DAS) and 153.5 cm (healthy plants). Inter nodal length varied from 2.3 cm (infected at 30 DAS) to 4.5 cm (infected at 70 DAS) and 5.1 cm was observed in healthy plants. Head diameter recorded as 4.6 cm, 6.6 cm, 8.8 cm, 11.0 cm and 13.6 cm at 30, 40, 50, 60 and 70 DAS,

respectively and 15.3 cm in healthy plants. For plant height, inter nodal length and head diameter t-test analysis showed significant differences in infected plants with healthy plants at different intervals except at 70 DAS, where non-significant differences were observed.

Leaf curl infected plants produced 8 filled seeds (at 30 DAS), 735 filled seeds (at 70 DAS) and 876 healthy plants. Percentage of seed yield losses 88.6% at early stages of virus infection (30 DAS), 67% at 40 DAS, 47.9% at 50 DAS, 32% at 60 DAS and lower incidence of 8.69% at 70 DAS was recorded. This was confirmed by t-test analysis for % of seed yield losses at 30, 40, 50 and 60 DAS and 70 DAS.

During *rabi*, 2020–21, leaf curl incidence was recorded as maximum (8.33%) at 50 DAS and minimum (0.8%) at 70 DAS (Table 2). Mean whitefly population is ranged from 1–1.69/plant was recorded. Percentage of seed yield losses observed was maximum (81.5%) at 30 DAS.

During the year 2021–22, disease incidence was recorded as 54.37% at 70 DAS (Table 2) and whitefly population ranged from 1.7–2.8/plant was recorded. Seed yield losses were 78.3% at 30 DAS and minimum of 11.6% was observed at seed setting stage.

Pooled data analysis revealed that highest incidence (14.5%) was observed between 40–50 DAS (star bud stage), followed by 11.8% at 60 DAS (flowering), and lowest (4.1%) at 30 DAS (vegetative stage) (Table 3). At 30 DAS infected plants had a minimum height of 67.8 cm and a maximum of 144.7 cm at 70 DAS. Inter-nodal length, head diameter and total seeds were significantly affected at 30 DAS. Seed yield losses were maximum (82.8%) at 30 DAS. The number of filled seeds and weight were higher in plants infected at 70 DAS compared to those at 30 DAS.

Deepa *et al.* (2015) and Ifthikar *et al.* (2021) also reported substantial yield losses at early stages, corroborating these findings. Vindyashree *et al.* (2015) reported that leaf curl virus requires a short incubation period of 2–3 weeks for symptom expression in crops like sunflower, tomato and tobacco. Adult plant resistance and reduced vector activity play a major role for less disease incidence at later stage of crop.

In 2020–21, leaf curl (17.6%) and whitefly population (1-1.69%) were notably lower compared to the other two years (2019-20 and 2021-22) which might be due to increased rainfall, affecting whitefly population negatively. Similarly, high temperatures, low humidity, and insufficient rainfall favoured more vector population in other two cropping periods. Similar studies on the effect of geographical and climatic variations, cropping seasons, and natural enemy presence were reported (Mubeen et al. 2017, Saeed et al. 2018, Lobin et al. 2022). During correlation and regression analysis, significant positive correlation was found between whiteflies and relative humidity-I (-0.62), Tmax (0.46) whereas non-significant, positive correlation with Tmin (0.37) and negative non-significant with RH-II (-0.24) and rainfall (-0.31). Similarly, significant positive correlation was observed between whitefly population and leaf curl incidence in this experiment.

Table 2 Leaf curl incidence and its effects on plant growth and yield contributing parameters in sunflower

Age of	Lea	Leaf curl	Whitefly			After har	er harvesting			Total seeds	spea	Filled seeds	eeds	III filled		Filled seeds	seds	Per cent of	t of	Oil (%)	(0)
the crop at infection	Incidence	Incidence Cumulative	popula- tion	Plant height (cm)	height n)	Internode length (cm)	ode (cm)	Head diameter (cm)	umeter )					seeds	80	weight	<del>+</del>	seed yield losses	eld		
									2019–20	0											
30 DAS	4.20	4.20	7.37	0.79	$\infty$	2.30	S	4.60	S	70.0	$\infty$	8	S	62	NS	0.38	S	9.88	$\infty$	30.2	S
40 DAS	9.93	14.1	6.50	84.0	$\infty$	3.60	S	09.9	S	221	$\infty$	73	$\infty$	148	S	3.50	S	0.79	S	33.0	S
50 DAS	14.8	28.9	5.37	103.0	$\infty$	3.70	S	8.80	S	413	$\infty$	215	S	198	S	8.80	S	47.9	S	35.5	S
60 DAS	15.5	44.3	7.25	127.8	NS	4.20	S	11.0	S	640	$\infty$	435	S	205	S	17.9	S	32.0	S	36.8	SN
70 DAS	10.5	54.8	5.75	144.0	NS	4.50	NS	13.6	NS	805	NS	735	NS	20	SN	29.6	NS	8.69	1	37.4	SN
Healthy	ŀ		3.50	153.5	1	5.10	1	15.3	ŀ	928	1	824	1	52	1	33.7	1	;	1	37.8	!
									2020-21	_											
30 DAS	2.87	2.87	1.00	69	$\infty$	2.50	S	4.3	S	92	$\infty$	17	S	75	NS	9.0	S	81.5	S	32.0	S
40 DAS	5.33	8.20	1.38	83	$\infty$	3.50	S	0.9	S	180	$\infty$	78	S	102	S	3.73	S	9.99	S	34.5	S
50 DAS	8.33	13.7	1.69	108	$\infty$	3.87	S	8.9	S	422	S	218	S	204	S	8.92	S	48.3	S	36.0	SN
60 DAS	3.14	16.8	1.60	123	S	4.55	NS	11.6	S	675	S	485	$\infty$	190	$\infty$	19.5	S	28.1	S	37.2	SN
70 DAS	08.0	17.6	1.00	146.4	SZ	5.08	NS	12.8	NS	800	SZ	720	SZ	08	SZ	8.62	NS	10.0	SZ	37.6	SN
Healthy	ŀ	ł	0.5	154	1	5.10	ŀ	13.6	1	925	1	850	1	75	1	34.0	1	8.8	1	37.6	1
									2021–22	6											
30 DAS	5.13	5.13	1.7	67.5	S	2.60	S	4.50	S	92	S	16.5	S	59.5	S	78.3	S	0.62	S	30.5	S
40 DAS	6.97	15.1	1.9	85.0	S	3.45	S	6.72	S	190	S	08	S	110	SN	57.9	S	3.67	S	32.2	S
50 DAS	20.3	35.4	2.5	108	$\infty$	3.90	S	8.80	S	400	$\infty$	210	$\infty$	190	S	47.5	S	8.59	S	33.6	S
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60 DAS	16.9	52.3	2.8	130	S	4.20	S	11.9	S	620	S	440	S	180	S	29.0	S	18.0	S	34.8	S
70 DAS	2.1	54.4	2.4	143.7	SZ	4.64	NS	12.4	SN	098	SZ	092	SZ	100	SN	11.6	SN	31.1	SZ	35.5	SZ
Healthy	1	1	1.13	152	1	5.20	:	14.0		950	S	864		98	1	9.05	:	35.3	1	35.9	1
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DAS, Days after sowing; S, Significant; NS, Non-significant (t-test).

Table 3 Pooled analysis of leaf curl incidence and assessment of yield losses by sunflower leaf curl virus in sunflower crop for experimental period

Age of	Inci-		White			After harvesting	vesting			Total	 	Filled	   p	III filled	eq	Per cent of	it of	Filled seeds	seeds	Oil	
the crop at infection	dence	the crop at dence incidence infection (%) (cumulative)	Hies	Plant height (cm)	neight 1)	Internode length (cm)	node (cm)	Head diameter (cm)	umeter .)	seec	S	seed	S	seeds	Si	seed yield losses	ss	weight	;ht	(%)	
30 days	4.10	4.10	3.36 67.8	67.8	S	2.46	S	4.47	S	0.67	S	13.8	S	65.5	NS	82.8	S	0.53	S	30.9	S
40 days	8.40	12.5	3.26	84.0	S	3.51	S	6.44	S	197	$\infty$	77.0	$\infty$	120	S	60.5	S	3.63	S	33.2	S
50 days	14.5	26.0	3.18	106.3	S	3.82	S	8.83	S	412	$\infty$	214.3	$\infty$	197.3	$\infty$	47.9	S	8.77	$\infty$	35.0	NS
60 days	11.8	37.8	3.90	126.9	SS	4.31	S	11.5	S	645	$\infty$	453.0	$\infty$	224	$\infty$	34.7	S	15.4	$\infty$	36.3	NS
70 days	4.50	42.3	3.00	144.7	SS	4.74	SN	12.4	SN	808	NS	738.3	NS	83.3	NS	14.3	:	27.7	NS	36.8	NS
Healthy	ł	ŀ	1.78	1.78 153.1	1	5.13	1	14.3	1	917	1	846.0	1	71.0	1	69.7	:	32.1		37.1	1
פר מארו	90	DAC Der after action of Circuit and Man Man Simifornia (+ 1004)	F. Cont.	NIC NIC	1:00:0	1 1) 10005	(+00)														

DAS, Days after sowing; S, Significant; NS, Non-significant (t-test).

With above weather parameters linear regression equation was developed, as follows:

y = 0.48-0.48 Tmax + 0.33 Tmin + 0.3 RH-I-0.22 RH-II-0.06rainfall (R<sup>2</sup>=0.75)

From the above equation, significant factor RH-I was taken into consideration for step wise regression and the equation is as follows:

$$y = 20.05 + 0.27RH-I (R^2=0.39)$$

This is in accordance with Sharma *et al.* (2017) findings of a positive correlation between adult whitefly population and temperature (max, min), sunshine hours, and a negative correlation with humidity (max, min) and rainfall in tomato crop, they established a regression equation with key weather parameters, yielding a R<sup>2</sup> value of 0.89. In contrast, the current study revealed a significant positive correlation between whiteflies and RH-I. On the other hand, Ghante *et al.* (2020) reported a non-significant negative relationship between whiteflies and maximum temperature, as well as bright sunshine hours. Additionally, they observed a non-significant yet positive correlation between whitefly population and rainfall, morning relative humidity, and evening relative humidity.

The analysis showed that sunflower leaf curl disease in India is caused by a whitefly-transmitted begomovirus (ToLCKV), resulting in a maximum leaf curl incidence of 42.3% at the star bud stage, followed by flowering. Early-stage infection (30 DAS) leads to a significant seed yield loss of 82.8%, while later-stage infection (70 DAS) results in a 10.3% loss. There's a notable positive correlation between whitefly population and RH-I. This study will aid in designing disease management strategies.

## REFERENCES

Annual Report of Sunflower. 2017–18. ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad, pp. 161–62.

Briddon R W, Bull S E, Amin I, Mansoor S, Bedford I D, Rishi N, Siwatch S S, Zafar Y, Salam A M and Markham P G. 2004. Diveristy of DNA 1: A satellite-like molecular associated with monopartite begomovirus-DNA β complexes. *Virology* **324**(1): 462–74.

Deepa, Gururaj S, Govindappa M R, Naik M K and Suresh S R. 2015. Estimation of yield loss in sunflower due to sunflower leaf curl disease at different stages of crop growth. *International Journal of Plant Protection* **8**(1): 138–41.

Directorate of Economics & amp; Statistics. 2023. https://desagri.gov.in

Doyle J J and Doyle J L. 1990. Isolation of plant DNA from fresh tissue. *Focus* **12**: 13–15.

Ghante V N, Duttarganvi S, Umesh M R and Kulkarni V. 2020. Influence of weather parameters on population of whitefly, *Bemisia tabaci* in sunflower. *Journal of Entomology and Zoology Studies* 8(6): 1729–34.

Govindappa M R, Shankar goud I, Shankarappa K S, Wickramaarachchi W A R T and Anjaneya Reddy B. 2011. Molecular detection and partial characterization Begomovirus associated with leaf curl disease of sunflower (*Helianthus annus*) in southern India. *Plant Pathology Journal* 10: 29–35.

- Hall T A. 1999. Bio Edit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids Symposium Series Abbreviation 41: 95–98.
- Iftikhar Y, Mubeen M, Sajid A, Zeshan M A, Shakeel Q, Abbas A, Bashir S, Kamran M and Anwaar H. 2021. Effects of tomato leaf curl virus on growth and yield parameters of tomato crop. *Arab Journal of Plant Protection* **39**(1): 79–83.
- Latha P and Kumar M S. 2018. Molecular detection and characterization of leaf curl and phyllody associated with sunflower in Andhra Pradesh. *Journal of Emerging Technologies and Innovative Research* **5**(1): 526–36.
- Lobin K K, Jaunky V C and Taleb-Hossenkhan N. 2022. A metaanalysis of climatic conditions and whitefly *Bemisia tabaci* population: Implications for tomato yellow leaf curl disease. *Journal of Basic and Applied Zoology* **83**: 57. https://doi. org/10.1186/s41936-022-00320-8
- Mubeen M Y, Iftikhar M I, Ullah Q, Shakeel M, Aatif I and Bilqees. 2017. Incidence of okra yellow vein mosaic disease in relation to insect vector and environmental factors. *Environment and Ecology* **35**: 2215–220.
- Muhire B M, Varsani A and Martin D P. 2014. SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. *Plos One*. https://doi.org/10.1371/journal.pone.0108277
- Poornima, Kulkarni V V, Ghante V K and Umesh M R. 2023. Present status of major diseases of sunflower in northern-east dry zone of Karnataka. *Journal of Oilseeds Research* **40**(Special Issue): 47–48.
- Saeed F, Afzaal M, Niaz B, Arshad M U, Tufail T, Hussain M B and Javed A. 2018. Bitter melon (Momordica charantia): A natural healthy vegetable. International Journal of Food Properties 21(1): 1270–90. https://doi.org/10.1080/1094291 2.2018.1446023
- Sastry K S and Singh S J. 1973. Assessment of losses in tomato by tomato leaf curl virus. *Indian Journal of Mycology and*

- Plant Pathology 3: 50-54.
- Sharma D, Maqbool A, Jamwal V V S, Srivastava K and Sharma A. 2017. Seasonal dynamics and management of whitefly (*Bemesia tabaci* Genn.) in tomato (*Solanum esculentum* Mill.). *Brazilian Archives of Biology and Technology*. https://doi.org/10.1590/1678-4324-2017160456
- Tamura K, Stecher G, Peterson D, Filipski A and Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis. *Molecular Biology and Evolution* 38(7): 3022–27. doi:10.1093/molbev/msab120
- Vanitha L S, Shankarappa K S, Rangaswamy K T, Wickramaarachchi and Govindappa M R. 2013. Complete nucleotide sequence of tomato leaf curl Karnataka virus and β satellite molecule associated with leaf curl disease on sunflower in India. *Plant Pathology Journal* 12: 19–25.
- Venkataramanamm K and Prabhakar K. 2020. Field evaluation of coordinated entries of sunflower for important diseases in Andhra Pradesh. *Journal of Agricultural Sciences* 6(2): 106–10.
- Venkataramanamma K, Neelima S, Prabhakar K and Lakshmi Kalyani D. 2022. Management of leaf curl disease of sunflower under field conditions. *Agricultural Research Journal* **59**(3): 447–52. DOI No. 10.5958/2395-146X.2022.00067.9
- Venkataravanappa V, Reddy C N L, Jalali S and Krishna Reddy M. 2012. Molecular characterization of distinct bipartite begomovirus infecting bhendi (*Abelmoschus esculentus* L.) in India. *Virus Genes* 44: 522–53. 5 DOI 10.1007/s11262-012-0732-y
- Vindyashree M, Govindappa M R, Ghante V N, Aswathanarayana D N and Shankergoud I. 2015. Biological and molecular evidences on host range of leaf curl begomovirus disease of sunflower (*Helianthus annuus* L.). *Journal of Applied and* Natural Science 7(1): 381–87.
- Walker P J, Siddell S G and Lefkowitz E J. 2020. Changes to virus taxonomy and the statutes ratified by the international committee on taxonomy of viruses. Archieves of Virology 165: 2737–48.