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Host range and status of Tospovirus on chilly / Capsicum in Karnataka

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Abstract

Survey conducted during 2014-15 and 2015-16 on the incidence of Tospovirus and its vector in eight districts of Karnataka on chilly / Capsicum revealed the occurrence of disease in the range of 0 to 16.5 per cent. Highest disease incidence was recorded in Raichur and least in Chamarajanagar district. RT-PCR analysis of the collected infected samples and thrips (vector) showed positive to Tospovirus. The virus was found transmitted to different hosts *viz.,* tomato, cowpea, groundnut, brinjal, beans, potato, Ipomea, petunia and to different weed species.

Chilly (*Capsicum annuum* L.) an important spice crop in India and is grown for its pungent fruits which are used both green and ripe to impart pungency to the food. Chilly suffers from a large number of viral, bacterial, nematode and phytoplasma diseases. Among them it is highly susceptible to a large number of viruses. In the tospoviruses genus *Groundnut bud necrosis virus* (GBNV) (Satyanarayana *et al*., 1996a, b; Hemalatha *et al*., 2008, Anjaneya Reddy *et al.,* 2008) and *Capsicum chlorosis virus* (CaCV) (Krishnareddy *et al*., 2008) are emerging as a significant limiting factor in the sustainable production of chilly in India.

Roving survey was conducted during summer 2014 -16 in different districts of Karnataka to assess the status of Tospovirus incidence on chilly\Capsicum. A minimum of 5 fields were selected randomly at each location for assessing the disease incidence. The percent disease incidence was calculated. The infected leaves and thrips samples collected during the survey were tested for the virus through RT-PCR.

Total RNA was extracted from collected leaf samples and thrips by TRI reagent and chelex 100 biorad methods respectively (Boonhan *et al.,* 2002). Total RNA isolate was reverse transcribed for cDNA synthesis and this cDNA was used as template for PCR by using primer specific for tospovirus. The 25µl of PCR reaction mixture consisted of dNTPs, $Mgcl_2$, 10X Taq buffers, primers, Taq polymerase enzyme, cDNA template and water to make up the volume. The amplified PCR product was separated in one per cent agarose gel containing ethidium bromide in 1X TBE buffer. The amplified virus specific N gene product visualized in gel documentation unit.

For determination of host range, virus was inoculated to different hosts *viz., Vigna mungo*, *Vigna radiata*, *Dolichos lablab, Arachis hyopogaea*, *Cajanus cajan*, *Cicer arietinum*, *Vigna unguiculata, Phaseolus vulgaris, Glycine max, Pisum sativam*, *Cucumis sativus, Citrullus lanatus, Nicotiana tabacum, Nicotiana glutinosa, Lycopersicon esculantum*, *Solanum tuberosum* and *Solanum nigrum*.

Plants of each species were raised in polyethylene bags. Plants were inoculated at primary leaf stage with standard extract of virus by mechanical

sap inoculation. In each plant species, 10 plants were inoculated and one set of un-inoculated plants were maintained as control. The inoculated plants were kept in the insect proof glass house and examined periodically for symptom expression. The plant species inoculated were tested by ELISA to confirm the presence of virus. The symptoms expressed by the different plant species were recorded.

The results of roving survey on the incidence of Tospovirus disease of chilly / Capsicum crop in the districts of Karnataka *viz.,* Bengaluru rural, Mandya, Chamarajanagar, Kolar, Davangere, Haveri, Raichur and Tumkur are presented in Table I. The vector observed was *Thrips palmi* and *Scirtothrips dorsalis*

Among the different districts surveyed, the maximum disease incidence was recorded in Raichur (16.5%) followed by Haveri (15.5%),Tumkur (14%), Mandya (12%), Davangere (10%), Bengaluru rural (9%), Kolar (8%) and Chamarajanagar (8%) districts. The infected plants exhibited symptoms like cholotic and necrotic ring spot followed by mosaic type in older leaves. In addition, die back, reduction in leaf size and upward curling of apical tip of leaves was observed. Necrotic streak was seen on stem and chlorotic ring spot on fruits. Thrips (*Thrips palmi* and *Scirtothrips dorsalis*) were invariably found in every infected field surveyed.

The PCR amplification using N gene specific primer for tospovirus resulted in the amplification of 800bp DNA fragment from virus infected samples only. Where as in case of thrips, successful detection of virus specific product observed only when a group of 5 thrips were used for detection and a clear DNA fragment of 800bp was amplified.

Among the test plant species inoculated with the crude sap, *Arachis hyopogaea, Phaseolus vulgaris, Dolichos lablab*, *Glycine max, Pisum sativa, Solanum tuberosum* and *Citrullus lanatus* showed systemic symptoms like mosaic with chlorotic ring spot

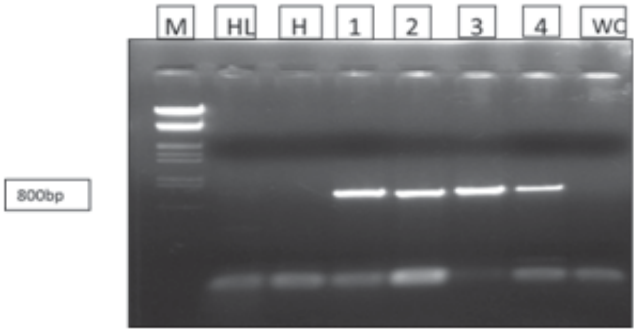

Plate1: Amplification of N gene in tospovirus infected leaf and thrips samples
Lanes M: DNA marker
Lane 1 and 2: Tospovirus leaf samples
Lane 4and 5: Tospovirus thrips samples
HL: healthy leaf sample
HT: healthy thrips sample
WC: water control

after ten days of inoculation. Localized chlorotic followed necrotic lesions were observed on leaves of *Vigna unguiculata*, different species of Tobacco, *Datura stramonium*, *Ipomoea marginata*, *Petunia hybrida* and *Chenopodium amaranticolor* four to five days after inoculation. The results are presented in Table II.

Similar type of survey was conducted by Kunkalikar *et al.* (2010) between 2002 and 2009 in the major vegetable-growing areas of India. Groundnut bud necrosis virus (PBNV), Watermelon bud necrosis virus (WBNV), Capsicum chlorosis virus (CaCV), and Iris yellow spot virus (IYSV) were documented widely in tomato and chilly / Capsicum in 14 states representing southern, northwestern, north-eastern, and central regions.Symptoms induced by the virus in most cases were similar to those caused by GBNV (Umamaheswaran, *et al.,* 2003). The virus caused only chlorotic and necrotic lesions on plant species belonging to the family Amaranthaceae and Chenopodiaceae. Both localized as well as systemic infections were observed on plants belonging to Fabaceae and Solanaceae. The virus induced chlorotic and/or necrotic lesions followed by veinal necrosis,

TABLE I

Incidence of tospovirus on chilly/Capsicum in different districts of Karnataka during 2014-16

Districts	Name of the Location	Variety / Line	Per cent disease Incidence (%)
Bengaluru rural	Shivakote	Local variety	1.5 to 5
Chikkabelekere	Arka Meghana	0 to 1.5	
Guniagrahara	Local variety	1.5 to 3	
Hesaragatta	Local variety	03 to 6	
Tirumalapura	Local variety	5.5 to 9	
Doddabellapura	Hybrid	2.0 to 6	
Chamarajnagar	Bheemanabeedu	Namdhari	2.2 to 8
Bommalapura	Local variety	5.5 to 7	
Chennamallipura	Myhico	2.0 to 5	
Hasaguli	Hybrid	1 to 3	
Hosuru	Local variety	2 to 06	
Mandya	Jakkanahalli	Indra	5.7 to12
Nagamangala	Indra	3 to 8	
Pandavapura	Indra	1.5 to 4	
Kardkere	Local variety	4.5 to 13	
Shettahalli	Local variety	2.5 to 07	
Tumkur	Sira	Namdhari	6.5 to 11
Agrahara	Local variety	3.5 to 09	
Dasarahalli	Hybrid	2.5 to 07	
Golahalli	Hybrid	6.6 to 14	
kallahalli	Local variety	3.5 to 6	
Kolar	Kolar	Local variety	2.5 to 7
Chintamani	Local variety	4 to 6.5	
Mulabagilu	Local variety	3 to 5.4	
Devapalli	Local variety	1.5 to 6	
Sreenivaspura	Local variety	2.5 to 8	
Davangere	Anaji	Bejo hybrid	5.5 to 10
Devikere	Devenurdelux	1.5 to 7.5	
Hebbalu	Namdhari	2.5 to 6.7	
Kurki	Local variety	4.5 to11.5	
Haveri	Dandgthalli	Local variety	2.7 to 10.5
Halageri	Local variety	3.8 to 12.8	
Masang	Local variety	2.5to14.5	
Tuminkatti	Local variety	5.5 to 15.5	
Raichur	Aroli	Local variety	3.5 to 12.5
Daganur	Local variety	7.5 to 16.5	
Dinni	Local variety	5.5 to 14.5	
Udamagal	Local variety	7.5 to 13.5	

TABLE II

Host range of Groundnut bud necrosis virus infecting chilly / Capsicum

Name of the host	No. of plants inoculated	No. of plants infected	Transmission (%)	Symptoms observed	ELIS Adetection
Leguminosae					
Arachis hyopogaea	10	10	100	CRS	+
*Cajanus cajan (*L.)Millsp	10	0	0	-	-
Cicer arietinum L.	10	-	-	-	-
Vigna unguiculata	10	10	100	CLL and NLL	+
Phaseolus vulgaris	10	10	100	CRS	+
Dolichos lablab	10	10	100	CRS	+
Glycine max (L.) Merr.	10	10	100	NS	+
Vigna mungo (L.)Hopper	10	-	-	-	-
V. radiate (L)Wilzek	10	-	-	-	+
Pisum sativa L.	10	10	100	M	+
Cucurbitaceae					
Citrullus lanatus L.	10	10	100	M	+
Cucumis sativus L.	10	-	0	-	-
Convolvulaceae					
Ipomoea marginata L.	10	9	90	CS	+
Amaranthacea					
Amaranthus viridis L.	10	5	50	CS	+
Solanaceae					
Nicotiana tabacum L.	10	10	100	CRS	+
Nicotiana glutinosa L.	10	10	100	CRS	+
Nicotiana benthamiana L.	10	10	100	M	+
Lycopersicon					
esculantum Mill.	10	10	100	M and CRS	+
Solanum nigrum L.	10	10	10	M and CRS	+
Solanum tuberosam L.	10	10	100	NRS	+
Petunia hybrid L.	10	9	90	NRS	+
Datura stramonium L.10	10	100	CS	+	
Chenopodiaceae					
Chenopodium amaranticolor L.	10	10	100	CLL and NLL	+

CRS : Concentric Ring spot, CLL : Chlorotic local leasion, NLL : Nectrotic local leasion, NS:Necrotic spot, M: Mosaic NS : Necrotic spot, CS: chlorotic spot

stem necrosis, leaf deformation and bud necrosis in *Arachis hypogaea, Vigna mungo*, *V. radiata*, *V. unguiculata*, *Macrotyloma uniflorum* and *Physalis floridana*.

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