IDENTIFICATION AND MANAGEMENT OF VIRUSES INFECTING TOMATO IN HIMACHAL PRADESH

THESIS

BY

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IN

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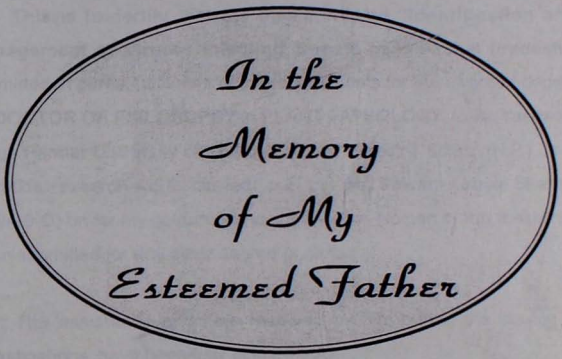
In the
Memory
of My
Esteemed Father

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CERTIFICATE-I

This is to certify that the thesis entitled **"Identification and Management of Viruses Infecting Tomato in Himachal Pradesh"**, submitted in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **PLANT PATHOLOGY** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan (H.P.) is a bonafide research work carried out by **Mr. Pawan Kumar Sharma (H-94-9-D)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received by him during the course of investigations have been fully acknowledged.

Place : Nauni, Solan
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CERTIFICATE-II

This is to certify that the thesis entitled **"Identification and Management of Viruses Infecting Tomato in Himachal Pradesh"** submitted by **Mr. Pawan Kumar Sharma (H-94-9-D)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan (HP), in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **PLANT PATHOLOGY** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the external examiner.

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(Pawan Kumar Sharma)

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INTRODUCTION

Tomato (*Lycopersicon esculetum* Mill.) is one of the most important vegetables grown throughout the world. In terms of value it comes next to potato and sweetpotato, but ranks first as processing crop in vegetables (Sandhu *et al.*, 1990). Tomato is known for its outstanding nutritional value. It is a rich source of vitamins A and C and has a pleasing taste.

In India, tomato is cultivated over an area of 50000 hectares with an annual production of 5000000 metric tons (Anonymous, 1997).

In Himachal Pradesh, the area under tomato cultivation is about 2570 hectares with an annual production of about 79057 metric tons (Anonymous, 1997), the midhills constituting the majority of the area, where it is grown as an off-season crop. The fruit is available from the months of June to October and finds ready market in Chandigarh, Punjab, Haryana, and even at Mumbai and Calcutta, where it can not be grown due to its specific agroclimatic conditons especialy high temperature during this period.

Tomato is prone to attack by a number of fungal, bacterial and viral diseases. Plant viruses are well known for causing tremendous economic losses to this crop. Generally, viruses inducing mosaic, curling and distortion type of symptoms on the leaves are encountered infecting tomato.

Although symptomatology is important in the study of plant virus diseases, yet symptomatology alone in itself is an inadequate criterion for exact identification of viruses especially in delineating the different strains of the same virus; as these may induce widely different symptoms in different cultivars and also unrelated viruses may cause similar symptoms in a common host. Identification of viruses, therefore, requires a great deal of caution and precise information on different biological and physico-chemical properties of the virus/virus strains under investigation. The various parameters employed in identification of virus/virus strains include information on such biological properties as symptomatology, host range, modes of transmission and biophysical properties. Besides, the knowledge of intrinsic properties such as particle morphology, serological properties especially the serological relationships etc. are of paramount importance in the proper identification and characterization of viruses/virus strains.

Thus an attempt has been made under the present investigations to study the different viruses and viral diseases infecting tomato in detail to save this economically remunerative vegetable crop from the ravages of viruses with the following objectives :

i) To record the prevalence, distribution of viruses and collection of virus isolates infecting tomato in major growing areas of Himachal Pradesh.

ii) Identification and characterization of virus isolates representing various localities in the state

iii) Management of viral diseases by using cultural methods and insecticidal sprays.

REVIEW OF LITERATURE

Tomato (*Lycopersicon esculentum* Mill.) has been reported to be infected by a large number of viruses both under natural and experimental conditions. There are about 36 viruses and their strains which are known to infect tomato as reported by Chenulu and Giri (1985). Majority of these viruses belong to 17 genera according to the latest classification proposed by Brunt *et al.* (1996).

FILAMENTOUS VIRUSES

i) Genus *Carlavirus*

Potato virus S (PVS) (Kuznetsova and Tursumetova, 1969)

Potato virus M (PVM) (Misra *et al.*, 1979)

Cowpea mild mottle virus (CPMV) (Brunt and Phillip, 1981)

ii) Genus *Potexvirus*

Potato virus X (PVX) (Gardner, 1927)

Family Potyviridae

iii) Genus *Potyvirus*

Potato virus Y (PVY) (Smith, 1949)

Tobacco etch virus (TEV) (Doolittle and Alexander, 1951)

Pepper veinal Mottle virus (PVMB) (Brunt and Kenten, 1971)

Peru tomato virus (PTV) (Raymer *et al.*, 1972)

Rod shaped viruses

i) Genus Tobamovirus

Tobacco mosaic virus (TMV) (Doolittle, 1928)

Tomato mosaic virus (ToMV) (Brunt, 1986)

Tomato double streak virus (TDSV) (MacNeil and Ismen, 1960)

ii) Genus Tobravirus

Tobacco rattle virus (TRV) (Paulus and Bald, 1963)

ISOMETRIC VIRUSES

i) Genus Caulimovirus

Cauliflower mosaic virus (CaMV) (Martelli and Quacquerelli, 1982)

ii) Genus Luteovirus

Tomato yellow top virus (Costa, 1949)

Tomato yellow net virus (Sylvester, 1954)

Beet western yellows virus (BWYV) (Duffus, 1973)

Potato leaf roll virus (PLRV) (Zitter and Tsai, 1981)

iii) Genus Necrovirus

Tobacco necrosis virus (TNV) (Thomas, 1972)

iv) Genus Tymovirus

Eggplant mosaic virus (EMV) (Barradas, 1983).

Family : Bromoviridae

v) Genus Alfamovirus

Alfalfa mosaic virus (AMV) (Zimmerman *et al.*, 1976)

vi) Genus Cucumovirus

Cucumber mosaic virus (CMV) (Chamberlain, 1934)
Tomato aspermy virus (TAV) (Blencowe and Caldwell, 1949)

vii) Genus Ilarvirus

Tobacco streak virus (TSV) (Martelli and Circulli, 1969)

Family : Bunyaviridae

viii) Genus Tospovirus

Tomato spotted wilt virus (TSWV) (Pittman, 1927).

Family : Comoviridae

ix) Genus Nepovirus

Tomato ring spot virus (ToRSV) (Samson and Imle, 1942)

Tomato black ring virus (TBRV) (Smith, 1945)

Tomato top necrosis virus (ToTNV) (Bancroft, 1968)

Family Rhabdoviridae

x) Genus Nucleorhabdovirus

Potato yellow dwarf virus (PYDV) (Black, 1937)

Tomato vein yellow virus (Maataoui *et al.*, 1985)

Family : Tombusviridae

xi) Genus Tombusvirus

Tomato bushy stunt virus (TBSV) (Smith, 1935)

GEMINATE PARTICLES

Family : Geminiviridae

i) Genus Geminivirus

Tomato yellow mosaic virus (ToYMV) (Debrot *et al.*, 1963)

Tomato leaf curl virus (ToLCV) (Yassin and Nour, 1965)

Tomato yellow leaf curl virus (TYLCV) (Cohen and Nitzany, 1966)

Curly top virus (CTV) (Bennett, 1971)

Tomato golden mosaic virus (TGMV) (Maytis *et al.*, 1975)

Tobacco leaf curl virus (TLCV) (Osaki and Inouye, 1981)

UNGROUPED VIRUSES

Pelargonium zonate spot virus (PZSV) (Gallitelli, 1982)

Tomato necrotic dwarf virus (Larsen *et al.*, 1984).

Since only three genera of viruses viz., *Tobamovirus*, *Tospovirus* and *Potyvirus* were encountered to infect tomato during the present investigations, therefore, for convenience sake the relevant information on these three particular genera has mainly been reviewed. Besides, the relevant literature dealing with other aspects of virus-host relationship and management studies undertaken during the present investigations has also been reviewed subsequently.

Genus *Tobamovirus*

Tomato mosaic virus and its strains

Tomato mosaic virus (ToMV)

Tomato mosaic virus (ToMV) has been reported to occur under natural conditions on tomato in different parts of the world such as United States (Clinton, 1909; Allard, 1916) and the Netherlands (Westerdijk, 1910). It is now believed to occur widely i.e. wherever tomato is grown (Broadbent, 1976; Hollings and Huttinga, 1976).

ToMV was earlier considered to be a strain of TMV because of its serological relationship with TMV (Van Regenmortel, 1975, 1981; Van De Walle and Siegel, 1976; Dawson *et al.*, 1979; Fraser and Loughlin, 1980; Fraser *et al.*, 1980; Burgyan and Gaborjanyi, 1984). However, since the two were readily distinguished by differences in host range, serological affinities and protein composition, ToMV has now been identified as a distinct virus (Harrison *et al.*, 1971; Fenner, 1976; Hollings and Huttinga, 1976; Gibbs, 1977). The major properties of ToMV and TMV were so similar, and their serological relationship so close, that ToMV hardly justified recognition as a distinct virus (Hamilton *et al.*, 1981). Nevertheless, it had since 1971 been increasingly accepted as a distinct virus because it failed to protect tomato plants from subsequent infection by TMV (MacNeill and Ismen, 1960; Rast, 1975) and is biologically distinct; moreover, it differed from TMV in its serological affinities and the composition and sequence of amino acids in its coat protein subunits.

Extensive surveys in several countries have established that TMV occurred comparatively rarely in tomato crops (MacNeill, 1962; Broadbent, 1962; Komuro *et al.*, 1966; Van Winckel, 1967a,b; Rast, 1975), presumably because it might be replicating very slowly and spread within plants much slower than ToMV (Komuro *et al.*, 1966; Jensen, 1969; Tomaru *et al.*, 1970).

Although tomato was by far the most important host of ToMV, it also occurred naturally in other plant species. It is one of atleast five *Tobamoviruses* infecting pepper (*Capsicum annuum* and *C. frutescens*) in widely scattered locations. It had been known to occur in crops in the United States (Miller and Thornberry, 1958; Knight *et al.*, 1962; Wetter, 1984), the United Kingdom (Fletcher, 1963), Italy (Conti and Masenga, 1977), France (Van Regenmortel, in Wetter, 1984), and Hungary (Csillery *et al.*, 1983). Besides tomato, ToMV had also been reported to infect other

crop plants such as potato (*Solanum tuberosum*) in Hungary (Juretic *et al.*, 1977; Horvath *et al.*, 1978), cherry (*Prunus avium and Prunus cerasus*), pear (*Pyrus communis*), apple (*Malus sylvestris*) and grape (*Vitis vinifera*) in the United States (Gilmer and Wilks, 1967a, b; Kirkpatrick and Lindner, 1964), and pear (*Pyrus calleryana*) in the Democratic Republic of Germany (Opel *et al.*, 1969). It induced severe stunting, distortion and necrosis of *Chenopodium murale* in the United States (Bald and Paulus, 1963).

Several isolates of ToMV had been reported to be serologically indistinguishable and had similar physico-chemical properties, and differed only slightly in the composition and sequences of their RNAs and proteins (Wang and Knight, 1967; Mosch *et al.*, 1973; Dawson *et al.*, 1975; Hollings and Huttinga, 1976). Nevertheless, many strains had been differentiated on the basis of symptoms which these strains induced in tomato and/or other species, and by their abilities to infect tomato genotypes containing genes for resistance (McRitchie and Alexander, 1963; Cirulli and Alexander, 1969; Pelham, 1972). Hence based on symptomatology and genotypic reactions the different strains have been placed in two groups as follows.

A. Symptomatologically distinct strains

1. Aucuba or yellow mosaic

This strain induced discoloration of fruits and development of bright yellow mottling, distortion and necrosis of leaves which, at maturity, may be almost white. This long recognized strain occurred sporadically, it infected only a few plants within a crop (Bewley, 1923; Henderson Smith, 1928; Kunkel, 1934).

2. Common Mosaic

A common mosaic strain of ToMV induced a light and dark green mosaic and malformed leaves (Rast, 1975).

3. Corky Ringspot

Corky ringspot strain was first detected in 1982 in tomato cultivars, Jackpot blazer and Valerie in California. It induced mild to moderately severe leaf chlorosis and corky rings and lines on small and unevenly ripened fruits (Mayhew *et al.*, 1984).

4. Crusty fruit

Some isolates inducing yellow ringspot leaf symptoms in the Netherlands also induced on fruits formation of complete or incomplete rings of superficial corky tissue which, due to different growth rates of normal and affected areas, caused fruits to burst (Rast, 1975).

5. Dahlemense strain

Another strain of ToMV designated as Dahlemense strain extensively induced yellow leaf mottling in tomato (Melchers *et al.*, 1940).

6. Enation strain

Ainsworth (1937) reported enation strain of ToMV which induced enations on abaxial leaf surfaces, especially those with fern leaf symptoms. It also caused abnormal development and setting of flowers and thus caused heavy yield losses (Rast, 1967).

7. Black Fleck

This strain was reported by Hollings (1957). In addition to chlorosis, it induced black necrotic flecks on leaves.

8: MII-6

This was the best known strain for its widespread use in the mild strain protection of non-resistant tomato cultivars. It was obtained in 1968 as a nitrous acid mutant of a virulent Dutch strain, it induced virtually symptomless infection in inoculated tomato plants which were then often efficiently protected from the effects of more severe isolates (Rast, 1972).

9. Rosette

Rosette strain was reported by Price and Fenne (1951). It was an unusually virulent strain which caused severe distortion and stunting of infected plants.

10. Summer Necrosis

Summer necrosis strain induced leaf chlorosis and necrosis of petioles and stems when temperatures exceeded 30^{o}C. This necrosis was sometimes lethal, but surviving plants remained stunted and infected leaves exhibited chlorotic rings and malformation (Rast, 1975).

11. Yellow Atypical Mosaic

This strain had been studied extensively in the United States by Knight *et al.* (1962). It was separated from a mixture of two tobamoviruses isolated from naturally infected tomato (Miller and Thornberry, 1958).

12. Yellow Ringspot

Yellow ringspot strain induced yellow ringspots in the leaves of tomato and some related species (Rast, 1965, 1975). Leaf symptoms, however, were usually mild and fruits were rarely affected. Affected plants were often restricted to small areas of glasshouses in the Netherlands where temperatures were abnormally high.

13. Winter Necrosis

This strain initially induced leaf symptoms similar to those of the common mosaic strain, from late autumn to early spring. However, it caused necrosis of leaf bases, resulting in subsequent wilting and death of leaves. Immature fruits developed irregular necrotic blotches (Rast, 1975).

TOBACCO MOSAIC VIRUS AND ITS STRAINS

Tobacco mosaic virus (TMV)

The properties of a virus as well as of all living organisms are subject to variations giving rise to altered forms, even though the change may not be very conspicuous. A number of strains of TMV have been isolated from tobacco from time to time.

The wild strain vulgare (=**U**1) was the one that was most commonly found prevalent in field grown tobacco. It was presumably the same strain that was reported by Ivanowski in 1899. Mckinney (1929) succeeded in obtaining more virulent variants by choosing different parts of the infected plant as a source of inocula. The masked strain M, isolated by Holmes (1934) under high temperature conditions, was symptomless on Turkish (=samsun) tobacco, and its coat protein had the same overall amino acid composition as that of **U**1 strain.

The strain T14D1 was a variant of J14 isolated by Jenson (1937). The strain produced local lesions on Turkish tobacco and did not spread systemically. Subsequently, another strain designated as J14D1 was reported which was most probably a mutant of J14, was quite distinct since it not only produced local lesions on the initially inoculated leaves of Turkish tobacco, but also spread systemically, giving rise to yellow symptoms on the upper young leaves. The TMV mutant produced local necrotic lesions on java tobacco and it caused a severe systemic necrosis on young Turkish tobacco plants.

The yellow aucuba strain (YA) also caused necrotic symptoms on Java tobacco but spread systemically in Turkish tobacco, inducing a strong chlorosis (Bewley, 1924; Kunkel, 1934).

The green aucuba mosaic strain (GA) was isolated by Kunkel (1934) from YA-infected N. sylvestris plants that had been kept for 3 days at 35°C. On Turkish tobacco, it produced practically the same type of symptoms as the wild strain U1.

The spontaneous variant designated as 'flavum' isolated by Melchers (1940) and studied extensively by Aach (1958a, b) induced a strong chlorotic reaction on both the primary inoculated and systemically invaded leaves of Samsun tobacco. It did not invoke necrotic reaction on Java tobacco and severely retarded the growth of infected samsun plants. It also caused necrosis of the primary inoculated leaves.

Besides, the strains described above a number of strains of TMV have been isolated by different workers mostly on the basis of their reaction on different hosts other than tobacco. These strains were isolated from different hosts :

i) Cucumber (cucumber mosaic CV3) (Bewley, 1924)

ii) Cucumber (Cucumber mosaic 4, CV4) (Ainsworth, 1935).

iii) Plantago (Holmes's ribgrass, HR) (Holmes, 1941)

iv) Sunhemp (Southern sunhemp mosaic, SSM) (Capoor, 1950)

v) Orchid (Odontoglossum ringspot, ORSV) (Jensen and Gold, 1951)

vi) Cowpea (Cowpea mosaic, CPV) (Lister and Thresh, 1955).

vii) Cactus (Sammon's Opuntia, SOV) (Sammons and Chessin, 1961)

viii) Cucumber (Cucumber green mottle mosaic, CGMMV) (Inouye *et al.*, 1967)

Holmes's ribgrass strain, southern sunhemp mosaic, odontoglossum ringspot, are now considered as distinct viruses and are referred to as ribgrass mosaic virus, sunhemp mosaic virus, odontoglossum ringspot virus, respectively (Wetter, 1986; Varma, 1986; Edwardson and Zettler, 1986).

Rao and Reddy (1972) reported a new strain of TMV causing mosaic mottling on tomato. It caused local necrotic lesions on *Chenopodium amaranticolor, Capsicum frutescens, Datura stramonium, Nicotiana glutinosa, N. rustica* and *N. sylvestris*. The dilution end point of the virus was 10^{-3} to 10^{-4} and a thermal inactivation point between 50-55^{o}C.

Symptom variation had also been used as a criteria for delineating the different strains of TMV. Four such strains were identified on the basis of host range including *Solanum pennelli, Lycopersicon esculentum* (SMV-18) and *Lycopersicon peruvianum* (Rast, 1975).

Fernandez *et al.* (1976) differentiated the aucuba, yellow, common and distorting strains of TMV on the basis of differences in biophysical properties particularly longevity under *in vitro* conditions. *In vitro* these strains remained infective for 1.4, 3.2, 0.6 and 3.2 years, respectively.

Hollings (1978) reported three strains of TMV namely TMV-II-16, TMV-W and crusty fruit strain on the basis of reduction in tomato fruit yield. These strains reduced the yield by 8, 38 and 75 per cent, respectively.

Verma and Kumar (1980) reported a new strain of TMV from India on the basis of its sap transmissibility to the members of families Amaranthaceae, Chenopodiaceae and Solanaceae.

Dubey *et al.* (1981) described an unusual and novel strain of TMV infecting tomato in Solan area of Himachal Pradesh. It was aphid borne and was serologically related to 8 serotypes of TMV and 6 of ToMV. The virus isolated was transmitted by the aphid vectors *Myzus persicae, Brevicoryne brassicae* and *Aphis fabae*. It was seed borne in tomato. The thermal inactivation point was 94-95^{o}C, dilution end point 1:75000 to 1:80000 and longevity *in vitro* 40 days at room temperature and 14 weeks at 7^{o}C. In dried leaves infectivity was retained for 18 weeks.

Erkan and Yonganci (1983) isolated a strain of TMV from pepper. This strain was found to have distant serological relationship between tobacco and tomato strains of TMV. The virus particles were rod shaped (300 x 15 nm). The virus induced wilting, stunting, stem necrosis, defoliation, leaf and fruit deformation in pepper and latent infection in tomato.

Lanxiang *et al.* (1996) carried out a study on TMV strains of tomato in 30 provinces in China (1990-95). The authors identified TMV strains 0, 1, 2 and 1.2. Strain 0 was distributed most widely and predominantly in 19 provinces with an incidence of more than 50 per cent. Strain 1 accounted for more than 40 per cent of isolates in 11 provinces. The frequency of strain 1.2 was between 4 and 11.1 per cent in 6 provinces.

Genus: *Potyvirus*

Peru tomato virus

Raymer *et al.* (1972) reported Peru tomato virus from Peru. It caused distortion of leaves, stunting of plants and as a consequence of infection with the virus, there was tremendous reduction in yield. Accodring to Frioburg (1979) symptoms evoked by the virus on plants were mottle, epinasty, leaf crinkling and necrotic spotting. The host range was restricted to plant species belonging to the families *Solanaceae* and *Chenopodiaceae*. The virus was transmitted by *Myzus persicae* but not by seed. Fernandez and Fulton (1980) reported that the virus had thermal inactivation point (TIP) between 55-60^{o}C and dilution end point (DEP) betwen 10^{-5}-10^{-6}. The longevity *in vitro* was 10-21 days. Frioburg (1979) also reported that Peru tomato virus was serologically distantly related to henbane mosaic, pepper mottle, pepper veinal mottle, potato A, potato Y, tobacco etch and tobacco vein mottling viruses. The virus particles were flexuous rods with a modal length of 750 nm and diameter of 12 nm. Fernandez and Fulton (1980) found normal length of virus particles between 741 and 746 nm.

Potato virus Y

Smith (1949) described the symptoms, methods of spread and hosts of potato virus Y associated with tomato plants in England. Conover and Fulton (1953) also reported the occurrence of PVY alone or in combination with TMV in field tomatoes in Florida (USA). Potato virus Y in tomato caused leaf necrosis, downward rolling of leaflets and petioles, and stunting of plants. Limasset (1955) found PVY to be the most predominant virus infecting tomatoes.

Sakimura (1953) isolated PVY from tomato in Hawaii and found it to be mechanically transmissible to egg plant, *Solanum nodiflorum, Nicotiana glutinosa, N. rustica, Physalis peruviana, Chilli (Capsicum frutescens)*, petunia, *Nicandra physaloides* and tomato. The virus had a thermal inactivation point (TIP) between 53^{o}C and 56^{o}C after 10 min. exposure, dilution end point (DEP) between 1:300 and 1:1000 and longevity *in vitro* (LIV) between 48-72 hours at 25-29^{o}C and 66-82 days at 0-3^{o}C.

Simmonds (1956) presented evidence that tomato leaf shrivel disease was caused by a strain of PVY. The same virus in association with aucuba strain of TMV caused 'yellow shrivel' and with CMV, it produced 'fern leaf' symptoms (Francki *et al.*, 1979). In mixed infection with tomato aspermy virus, it retarded the growth of tomato plants. The leaflets in such cases were either reduced to tiny protuberances or completely absent (Schmelzer and Wolf, 1975).

The presence of PVY on tomato was also reported from Israel (Nitzany and Wilkinson, 1960), Turkey (Ozalp, 1961), Mediterranean region (Zimmerman and Pilowsky, 1975), Hungry (Tobias and Andrasfaly, 1984), Cyprus (Ioannou, 1985), Crete (Avgelis, 1986), China (Feng and Cai, 1987), Italy (Grassi and Gallitelli,

1990), Greece (Kyriakopoulou *et al.*, 1991) and Japan (Ushiyama *et al.*, 1991). However, Tobacco veinal necrosis disease caused by PVY^N strain was observed to be very destructive and could cause a complete loss of crop. In pepper and tomato heavy losses were estimated especially when potato virus Y was present together with other viruses (Edwardson, 1974).

Gracia and Feldman (1977) reported 16 per cent incidence of PVY in tomato from Mendoza province. Hassan *et al.* 1993) from Pakistan also reported 20 per cent seedlings infection in tomato due to potato virus Y.

Most of the strains of potato virus Y were reported to cause mild mottling in pepper, tobacco and tomato hosts. Early infection of tobacco with potato virus Y was observed to decrease its yield to an extent of 30 per cent (Sievert, 1978).

Potato virus Y reported by de Bokx and Huttinga in 1981 had a thermal inactivation point (TIP) between 50 and 62°C, the dilution end point (DEP) between 10^{-1} and 10^{-6}, and the longevity *in vitro* (LIV) at 18-22°C was 7-50 days. Most of the potyviruses were known to have a TIP between 50-75°C (usually 55-60°C), DEP between 10^{-1} and 10^{-4} and longevity in sap 1-50 days but usually 2-4 days (Hollings and Brunt, 1981).

The modal length of potato virus Y particles in purified preparations and under dip proparations was 684 nm and 730 nm respectively, as reported by Delgado-Sanchez and Grogan (1966). Potato virus Y particles were flexuous filaments and about 11 nm width and were helically constructed with a pitch of about 3.3 nm (Varma *et al.*, 1968). de Bokx *et al.* (1975) observed the modal length of virus particles to be 740 nm in clarified sap of tobacco cultivar white burley. Savino

and Franco (1980) also found filamentous particles of potyvirus group in tomato infected by a virus disease.

Behl *et al.* (1987) reported a severe outbreak of shoe string desease of tomato in Solan area of Himachal Pradesh caused by necrotic strain of PVY.

Potato virus Y strains

de Bokx (1961) and de Bokx and Piron (1978) grouped several strains of PVY according to the local and systemic symptom produced on *Nicotiana tabacum* (cvs. white burley and Samsun NN), *Physalis floridana* and *Solanum tuberosum* cv. Duke of York.

Potato virus Y^0 group (common strains)

These strains mainly induced severe systemic crinkle symptoms, rugosity or leaf drop streak in potato, systemic necrosis in *Physalis floridana* and systemic mottling in tobacco.

Potato Virus Y^CGroup (Stipple Streak Strains, Including Potato Virus C)

Potato virus C itself was not transmitted through the aphid vector *Myzus persicae* (Watson, 1956), but other strains of this group were easily transmitted by *Myzus persicae* (de Bokx and Piron, 1978). Many potato cultivars were hypersensitive to the strains in this group (Calvert *et al.*, 1980). Susceptible cultivars showed systemic mosaic or stipple streak. Symptoms in tobacco and *Physalis floridana* were similar to those induced by PVY^O strains (de Bokx and Huttinga, 1981).

Potato Virus Y^N (Tobacco Veinal Necrosis Strains)

These strains induced systemic veinal necrosis in tobacco, systemic mottling in *Physalis floridana* and very mild mottling in almost all potato cultivars.

TOMATO SPOTTED WILT VIRUS

The disease was first reported from Australia in 1927 by Pittman. It was transmitted by *Thrips tabaci.* Later, the disease was recorded from Wisconsin (USA) (Doolittle and Summer, 1931). The young leaves of tomato developed typical bronze markings. Sometimes the young plants died due to infection. As reported by Berkeley (1935), the disease was present in Ontario (Canada) since 1931 but was not studied till 1934. The virus was inactivated by heating at 45°C and could survive in sap from 1.5 to 2 hours (Norris, 1943).

Best and Gallus (1950, 1953) characterized six strains of TSWV on the basis of three indicator plants viz., *Lycopersicon esculentum* (Var. Dwarf Champion), *Nicotiana glutinosa and Nicotiana tabacum* (Var. Blue pryor). The diagnostic markers (symptoms) by which strains were distinguished from each other were based on the rules that each marker must be readily distinguishable from other markers used. These strains were designed by letters A, B, C1, C2, D and E, respectively. All these strains move systemically in *Lycopersicon esculentum* (var. Dwarf champion) and in this plant the strains may be classified broadly into three main groups according to the systemic symptoms developed. Those producing severe necrosis alongwith the formation of brown (or purple) pigment (strains A, B and D); those producing only very mild surface necrosis unaccompanied by pigmentation, often in the form of ringspot or parallel line patterns (strain C1, C2); and those in which there is neither visible necrosis nor pigmentation (strain E).

During January and February, 1980, tomatoes in Uruguay were very severely affected by a virus disease. Infected plants were stunted and the leaves turned yellow, numerous necrotic spots and ringspots were evident on the young leaves. The disease was found to be caused by TSWV (Tezuka and Lasa, 1982).

Tomato spotted wilt virus was also isolated from tomato in Andhra Pradesh. It was identified on the basis of symptoms, transmission by thrips (*Scirtothrips dorsalis*), in vitro properties, particle morphology and serology. It caused bronzing and necrosis on tomato (Rao *et al.*, 1981).

Allen and Broadbent (1986) reported the epidemic of tomato crop in Eastern Canada caused by TSWV. They found both the strains i.e. tip blight and mild strains responsible for the epidemic.

A type strain of TSWV was isolated from tomatoes in Himachal Pradesh. This strain caused bronzing of leaves and ring spotting of tomato fruit (Khurana *et al.*, 1990).

Tomato plants growing in a greenhouse in South Finland in 1989 were observed exhibiting symptoms of wilt, terminal dieback and leaf mottling. ELISA tests and electron microscopy revealed that the disease was caused by tomato spotted wilt virus (TSWV) (Lemmetty, 1991). Tomato plants on mechanical inoculation with TSWV showed that the serological activity of the virus by DAS ELISA was correlated with the host plant, the time after inoculation and part of the plant tested. In dwarf tomato cultivar the virus reached its highest A_{405} between 8 and 21 days and later on 49 days after inoculation. The virus was not detectable 35-42 days after inoculation (Kamiska, 1996).

CYTOPATHOLOGICAL STUDIES

Viruses induce changes in the cytoplasm of the infected cells. These changes manifest themselves as inclusion bodies with characteristic ultra structures which are quite specific to the virus or virus group. McWhorter (1965) commented that viruses sign the cells of the plants they inhabit and that learning how to read these signatures can reveal the nature of the virus. The subject of plant virus induced inclusions has been reviewed by various workers from time to time (Smith, 1958; Goldin, 1963; Mcwhorter, 1965; Thaler, 1966; Rubio-Huertos, 1972; Martelli and Russo, 1977; Christie and Edwardson, 1977; and Martelli and Russo, 1984).

Inclusion bodies have been classified according to diverse criteria. The more recent trend is to classify the inclusions on the basis of morphology, ultrastructure and distribution within the cell. Based on these criteria, it has been found that inclusions induced by the viruses belonging to different groups viz. potex, poty, tobamo, como, nepo, tymo, cucumo, caulimo, plant rhabdo, plant reo and gemini viruses are quite characteristic to the virus group (Martelli and Russo, 1984), besides, inclusions induced by some viruses are quite characteristic to the virus itself.

1. Genus *Potyvirus*

Viruses belonging to *Potyvirus* induced inclusion bodies called cylindrical inclusions in the cytoplasm of the infected cells which are characteristic of this genus. These cylindrical inclusions are made up of proteinaceous sheets radiating from central axis and curve around it. The shape of the inclusion body observed in ultrathin sections depends upon the plane of section cutting. In cross section they appear as pinwheel (Fenner, 1966), scrolls or laminated aggregates. In longitudinal section they appear as bundle of lamillae, tubes and laminated aggregates corresponding to pinwheel and scrolls. David-Ferreira and Borges (1958) appear to

be the first to observe cylindrical inclusions with electron microscope in PVY infected cells. A variety of intracellular structures were subsequently reported in the cells infected by different potyviruses by Cremer *et al.* (1960). Edwardson (1966a,b) showed that various structures represent different aspects of the same type of inclusions. Potyvirus inclusions have been dealt in detail by Edwardson (1974). Members of potyviruses can be grouped into sub-divisions on the basis of morphology of the cylindrical inclusions induced by them. Thus, Edwardson (1974) proposed a classification of potyviruses into three groups, based on the type of structures attached to the central portion of pinwheal inclusions :

a) Scrolls or tubes (in longitudinal sections) :

Sub-division I (12 viruses).

b) Laminated aggregates :

Sub-division II (11 viruses) and

c) Scrolls, tubes and laminated aggregates :

Sub-division III (17 viruses).

2. Tomato spotted wilt *Tospovirus*

In thin section studies the characteristics TSWV particles were reported to aggregate in the cisternae of endoplasmic reticulum (ER). This type of inclusion had been reported associated with TSWV in a variety of infected tissues beginning with Ie's (1964) studies and continuing with those of Bertaccini and Bellardi (1990) and Khurana and Co-workers (1990), and was considered unique for TSWV infections. Aggregation of virus particles between ER membranes is not a property that is unique to TSWV. Particle aggregates have been described as occurring only

in the cytoplasm of TSWV infected cells by all investigators except Kitajima (1965), who reported individual as well as aggregated particles enclosed in ER and also aggregates of particles between membranes of the nuclear envelope in tobacco root cells infected with the Vira-Cabeca strain.

Milne (1970), Ie (1971), and Francki and Hatta (1981) described electron-opaque proteinaceous material in the cytoplasm of TSWV-infected cells. This material was interpreted to be viroplasm. TSWV-induced inclusions had also been studied in the light microscope. Living cells of infected tobacco were observed by Bald (1962) to contain loose aggregates which increased in size and fused into large inclusions. Kobatake and co-workers (1976) reported induction of a cytoplasmic inclusion larger than nuclei in infected *Datura stramonium* epidermis stained with Phloxine-Methylene Blue.

EPIDEMIOLOGY

Epidemiological information is essential to develop truly effective management strategies. However, the epidemiology is exceptionally difficult to elucidate and predict because it is influenced by so many interlocking activities, only some of which are known and measurable. In case of plant virus diseases, it depends on the survival systems of viruses, their vectors, spreading kinetics and interaction between host, vector, viruses and the prevailing meteorological conditions. The relevant literature dealing with virus vector and environmental factors studied under present investigations has been reviewed under the following paragraphs.

Virus vectors and environmental factors

The virus-vector relationship relating to aphid vectors has been extensively studied by various workers (Watson, 1936, 1938, 1940; Watson and Roberts,

1939). Potato virus Y has been reported to be transmitted by atleast 25 species of aphids in a non-persistent manner and *Myzus persicae* was observed to be the most efficient vector. A few other aphids which transmit the virus are *Aphis fabae, Macrosiphum euphorbiae, Myzus certus, Phorodon humuli, Rhopalosiphum insertum,* and *R. padi* (Kennedy *et al.*, 1962; Kostiw, 1978; Van Hoof, 1980 and Sigvald, 1983).

In studies using yellow pan traps to estimate the number of aphids in late May-early August in plots of the tomato variety Mokka grown for seed, the progress of infection was followed by sap inoculation of herbaceous indicators. Aphid activity was greater in June than in July, but aphid transmitted infection was first noted in July. In all cases the pathogen was CMV. Tobacco mosaic virus infection was noted consistently from early June to early August, incidence being 10-20 per cent (Basky, 1983).

Studies carried out on the epidemiology of CMV in the Philippines revealed that the highest population of trapped *Aphis gossypii* a vector of CMV-Y occurred during the rainy season at an optimum wind speed of 50 km/d, solar radiation of 400 lx/d, rainfall of 4-6 mm/d, average temperature of 27.8^{o}C and in 65 days old tomato plants. The highest incidence of CMV-Y occurred in 75 days old tomato plants. Optimum conditions for infection of CMV-Y were temperature of 29^{o}C, solar radiation of 460 lx/d and sunshine duration of 85 min/d. These prevailing weather conditions were more favourable to the activity of the aphid vector during the wet season than during the dry season (Xuan *et al.*, 1988).

Cucumber mosaic virus has been recognized as a major factor responsible for tomato epidemics in Southern Italy. The environmental factors played a major role in the establishment of epidemics. A mild winter allowed survival of spontaneous hosts of CMV and increased crowding of aphid colonies on some

plants. This forced alate aphids to move to tomato seedlings which, in turn, were transferred earlier than usual. High temperatures, since the beginning of March forced growers to open plastic tunnels to tomato beds. Through these openings viruliferous aphids could infect young tomato plants earlier. An increasing number of tomato cropping areas, influenced by hybrids which substituted old local varieties, produced a continuum of new genotype (Gallitelli *et al.*, 1990).

Butter and Rataul (1977) reported 8 days as incubation period of TLCV in August and 90 days in winter. The acquisition threshold for the vector was 31 minutes; it resulted in 3 per cent transmission. An acquisition access of 24 hours for a female *Bemisia tabaci* on a virus source resulted in 30 per cent transmission. A minimum feeding of 32 minutes was required by a viruliferous white fly to cause infection on tomato test plants, this gave 4 per cent transmission.

In a study the minimum acquisition and inoculation feeding periods required by *Bemisia tabaci* to transmit TLCV were found to be 30 minutes each. After acquisition the vector required another 6 hours to become viruliferous. A single whitefly transmitted the virus and remained infective throughout its life (Reddy and Yaraguntaiah, 1981).

Management of virus diseases of tomato

The various approaches employed by different workers for the management of tomato viruses in different parts of the world includes modifications of the various cultural practices, control of insect vectors and planting resistant cultivars.

A. MODIFICATION OF CULTURAL PRACTICES

i) Effect of dates of sowing

Saklani and Mathai (1977) recorded observations on the occurrence of tomato leaf curl virus symptoms on tomato cv. Pusa Ruby planted at an interval of 15 days. The optimum planting period was Ist October to 16th December, followed by Ist January to Ist March. Planting in these periods developed symptoms after 132-162 days and 66 to 102 days, respectively, compared with 25-45 days when planting was done during the rest of the year.

Investigations carried out on yellow dwarf disease of tomato caused by tobacco leaf curl virus revealed that there were 3 annual peaks of the vector whitefly, in late May to early June, early June to mid July, and mid August to late August. The disease normally appeared first in mid July, the numbers of affected plants increasing rapidly during August (Kobatake *et al.*, 1981).

Basky (1983) while investigating the progress of infection of CMV using yellow pan traps to estimate the number of aphids in late May-early August in plots of the tomato variety Mokka grown for seed found that the aphid activity was greater in the month of June than in July.

An epidemiological study of TYLCV in a coastal region of South Eastern Cyprus revealed that the incidence, severity and rate of spread of the disease were maximum in crops transplanted during summer and early autumn, when vector populations were also highest, while crops transplanted in winter and early spring (December-March) escaped infection completely. Consequently, timing of transplanting could be an effective means for cultural disease management (Ioannou and Iordanou, 1985).

Investigations carried out on epidemiology of TLCV in South India revealed that from July to November the incidence ranged from 17 to 53 per cent and from February to May upto 100 per cent of the crops were infected. In sequential sowings 90 to 100 per cent of plants were infected in plots sown between the end of January and the end of May. Infection in plots sown later was progressively less (Saikia and Muniyappa, 1989).

It was observed in Southern Turkey that TYLCV incidence could be considerably reduced by planting the fall crop a few weeks later i.e. at the beginning of October, instead of the usual planting in August/September when whitefly populations are highest (Abak *et al.*, 1991). Similarly in the Jordan valley, disease incidence is lower when the fall crop is planted later (Kasrawi, 1991).

Navas *et al.*, 1991 recorded that *Frankliniella occidentalis* also transmitted tomato spotted wilt virus besides *Thrips tabaci* and *F. fusca* whith occurred in higher densities in South Eastern USA during the month of May, whereas their densities were quite low at other times especially during autumn cropping season.

The rate of infection by tomato yellow leaf curl virus was recorded in 4 monthly plantings, September 1991-January, 1993, and populations of the vector, *Bemisia tabaci*, were monitored in the Sultanate of Oman. The results indicated that infection can be reduced by sowing in August and planting in September, or by sowing in December and planting in January, with nursery protection under insect proof conditions (Zouba *et al.*, 1993).

ii) Use of reflective/plastic mulches

Repelling insect vectors, primarily aphids but also whiteflies, from fields by covering the soil with aluminium sheets or black or transparent polythene sheets or

by mulching the soil with some reflective material leads to delayed virus infection, decreased incidence, and increased yield. Various types of mulches employed for this purpose are aluminium foil, clear polythene, grey plastic, yellow polythene and organic mulches.

Covering the soil with fresh wheat straw had been observed to decrease TYLCV incidence (Cohen and Melamed-Madjar, 1974). In the Negev area, overwintering crops of greenhouse tomatoes are sown in September. At the beginning of growing season, crops often become infected by tomato yellow leaf curl virus, transmitted by *Bemisia tabaci.* Tomato plants covered for the first month with perforated polyethylene or polypropylene to protect them from *B. tabaci* whiteflies are less infected with virus than uncovered plants sprayed with insecticide (Berlinger *et al.*, 1983).

Suwwan *et al.* (1988) studied the effect of 5 mulch types, i.e. silver (aluminium treated, reflective, plastic, black plastic, paper, white/black plastic and black/white plastic were evaluated in terms of their effect on growth, yield, fruit quality and incidence of TYLCV. It was recorded that total marketable yields were significantly increased by all mulch treatments. Incidence of TYLCV was reduced by silver plastic mulch.

Greenough *et al.* (1990) reported that the plastic film mulches affect thrips immigration and TSWV incidence in tomato. In comparison with non-mulched treatment, aluminium surfaced mulch reduced the numbers of trapped thrips by 68 per cent and the incidence of TSWV by 64 per cent in tomato.

In field trials conducted in Florida (USA) in tomatoes, aluminium painted plastic mulch, whether with the entire exposed mulch surface painted or with lateral strips painted, was generally equivalent to aluminium plastic film and superior to

non-painted plastic with respect to the numbers of aphids and thrips collected from water dish traps or to the number of aphids on foliage. Aluminium painted mulch resulted in fewer tomato plants infected with aphid transmitted viruses compared with non-painted mulch (Kring and Schuster (1992).

Cohen and Mela-med Madjar (1978) reported that soil mulching with yellow polythene sheets delayed spread of tomato yellow leaf curl virus on tomato for atleast 20 days. A combination treatment of azinphos methyl starting 20 days after germination was found to be the most effective in preventing virus spread.

Mauromicale *et al.* (1996) carried out investigations to know the effect of mulching with polyethylene sheets of different colours (black, transparent white and reflective aluminium colour) on the spread of *Bemisia tabaci*, the vector of TYLCV and the production behaviour of tomatoes grown in greenhouses in 2 different cycles (Transplant dates, 25 March and 31 August). Mulching with the aluminilum coloured polyethylene caused a delay in the infestation rate of *Bemisia tobaci* and, as a consequence delay of infection by TYLCV. Mulching with black or transparent polyethylene decreased levels of *B. tabaci* infestation and TYLCV infection below those using aluminium coloured polyethylene.

B. INSECTICIDAL SPRAYS

Prevention or reduction of virus infection, particularly for the viruses transmitted by aphids and whiteflies, various chemicals have been tried with varying degrees of success in managing the virus diseases in plants.

Seed bed applications of the systemic insecticides aldicarb, carbofuran, disulfoton, fenamiphos and terbufos control tomato spotted wilt virus disease of

tomatoes as effectively as repeated foliar applications of endosulfan for upto 12 weeks (Treverrow and Mutton, 1990).

Field tests have shown that the non-residual Krishi oil is effective against the TLCV vector, *Bemisia tabaci* and control the spread of the disease (Sastry *et al.*, 1978).

Reddy and Yaraguntaiah (1981) found Carbofuran to be very effective in reducing the incidence of tomato leaf curl disease and increasing yields. Nursery treatment + main plot treatment, and nursery treatment + seedling soaking reduced the whitefly (*Bemisia tabaci*) vector populations and disease incidence, and increased yield.

Fortnightly applications of Ripcord (Cypermethrin) 40 per cent e.c. at 5 ml/100 l controlled thrips (vectors of TSWV) and reduced the incidence of TSWV in tomatoes (cv. Ronita) (Nunez, 1981).

Yassin (1975) observed significant decrease in TYLCV incidence and significant increase in yield after weekly sprays of omethoate, malathion, or mavinphos when used either in the seedbed or after transplanting.

Use of insecticides permethrin, methidathion, and pyrimiphos methyl when applied in combination with mineral oils resulted in 2-3 fold increases in tomato yield and a reduction in virus incidence (Hipar or Sunoco) (Sharaf and Allawy, 1981).

Incidence of TLCV transmitted by *Bemisia tabaci* is minimized by initial root dip of tomato seedlings (cv. Pusa ruby) in a 0.1 per cent carbofuran solution for 1 h

followed by 2 foliar sprays of Agricultural spray oil first at 20 days after transplanting and 2nd, 10 days after that (Sastry, 1989).

The incidence of cucumber mosaic virus (CMV) which is transmitted by aphid vectors had been reported to be reduced by a number of insecticides.Endosulfan 35 EC was found to be the most effective treatment followed by methomyl 18 L, aldicarb 15G and carbofuran 34 (Xuan *et al.*, 1988). Similarly, incidence of CMV has also been reported to be reduced by application of mineral oils (Gallitelli *et al.*, 1990).

C. SOURCES OF RESISTANCE

Sources of resistance to PVY are available in *Lycopersicon* species. Among tomato breeding lines and lines of wild *Lycopersicon species*, sources of resistance have been found in the lines PI 128660 which usually reacts as symptomless and with low virus concentrations and PI 247087 was fond to be immune (Pandey and Summanwar, 1982).

In a study conducted at Taiwan none of 19 wild tomato lines (*Lycopersicon pimpinellifolium, L. peruvianum and L. hirsutum*) were found resistant following two consecutive inoculations with the PVY. However, the *L. hirsutum* lines VLIII and 3683, previously found to be resistant, were again confirmed to be resistant (Anonymous, 1987).

Silva *et al.* (1986) screened 31 lines or cultivars of *Lycopersicon* species for resistance against TMV under glasshouse conditions. The tomato lines LA 1221 and LA2088 were resistant to TMV. Several *L. pimpinellifolium* lines were moderately resistant to 1 or more isolates of TMV.

At AVRDC, Taiwan eight cultivated and wild tomato lines with resistance to TYLCV were screened against a Taiwan isolate of TYLCV. *Lycopersicon peruvianum* INRA Sal (VL15) and *L. esculentum* VL81 and VL82 were found to be the most resistant (Anonymous, 1987).

Erkan (1987) screened 21 cultivars of tomato against ToMV. None of the cultivars tested was completely resistant. Cardinal, Dineb, E.U.Z.F. 2271, HI706, Rigel, Royal Chico, T_2 Imp, Titanom and ventura were less susceptible than others.

Resistance to CMV is available in *Lycopersicon spp.* CMV was inoculated in *Lycopersicon hirsutum*, 5 accessions of *L. peruvianum* and a number of tomato lines. A high degree of resistance was seen in *L. peruvianum* line LA2388G1 567 and in a tomato line derived from a cross with *L. chilense* (Stamova *et al.*, 1990).

Behl and Chowfla (1989) screened one hundred fifteen cultivars/lines of tomato against PVY in Himachal Pradesh. None of the lines was immune to the disease. HC238, and S-12 were highly resistant, Sabour prabha, EC42662-1, EC-16340, EC-16022 and EC14166 were moderately resistant and the remaining lines were highly susceptible or moderately susceptible. It was concluded that HC238 and S-12 can be used as donors in breeding for disease resistance

Sources of resistance against leaf curl virus are available in tomato. The accessions LE812 from AVRDC and LE376 from Ludhiana are virtually free of the disease when grown under field conditions (Shoba and Arumugam, 1990).

In a study, 1201 tomato cultivars, breeding lines and accessions from 9 *Lycopersicon* species were screened for TYLCV under field and laboratory conditions. Two lines of *L. hirsutum* (PI390658 and PI390659) and 2 lines of *L.*

peruvianum (PI127830 and PI127831) were resistant to TYLCV infection (Muniyappa *et al.*, 1991).

Moustafa and Nakhla (1992) evaluated six cultivars of tomato against a local strain of TMV under greenhouse and field conditions. Mormor and Mocimor were classified as resistant and mobaci, moperou and Piermor as tolerant.

Abou-Jawdah *et al.* (1996) tested 42 tomato genotypes for resistance to TYLCV. TY-King, W3093, W3098 and Fima showed milder symptoms and produced significantly higher yields than susceptible controls.

MATERIAL AND METHODS

INCIDENCE AND DISTRIBUTION OF VIRUS DISEASES

An extensive survey of different tomato (*Lycopersicon esculentum* Mill) growing districts : Solan, Sirmour, Shimla, Kullu and Mandi of Himachal Pradesh was conducted during the year 1996 and 1997 to determine the distribution and incidence of virus diseases. Incidence counts were made by choosing 4-5 different locations in the field at random and observations recorded by counting the number of healthy and diseased plants.

The per cent disease incidence was calculated by using the following formula:

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

COLLECTION AND MAINTENANCE OF VIRUS ISOLATES

The cultures of various isolates were collected from different tomato growing localities of the above mentioned districts. The isolates collected on the basis of symptoms were maintained on healthy seedlings of tomato variety 'Naveen' by mechanical inoculation under insect proof glasshouse conditions.

RAISING AND MAINTENANCE OF TEST PLANTS

Healthy seedlings of tomato and other test plants species were raised in earthen pots filled with sterilized soil and FYM mixture (3:1 ratio V/V). Fifteen to

thirty days old seedlings of test plants at 2-3 leaf stage were used for inoculation whereas, plant species belonging to families cucurbitaceae and leguminosae were inoculated at cotyledonary and primary leaf stages, respectively, whereas the plants of other families were inoculated at 4 to 5 leaf stages. The pots containing test plants were maintained under insect proof screen/glasshouse where the temperature varied between 25-30^{o}C during the period of experimentation.

PREPARATION OF INOCULUM

The inoculum of each isolate was prepared from the young leaves of infected plants showing pronounced symptoms. Leaves were harvested and washed thoroughly first with tap water and then with distilled water to remove the extraneous material. These were then dried by keeping them between the folds of blotting papers. The dried leaves were chopped into small bits with sterilized scissors and were triturated in phosphate buffer (0.1 M pH 7.0) in a sterilized pestle and mortar. The slurry thus obtained was strained through two layers of muslin cloth in sterilized Petri-dish. The standard extract thus prepared was used for mechanical inoculations. The inoculum extraction of isolate Tom-4 and its inoculation on tomato was carried out at low temperature. The inoculum was prepared in phosphate buffer (pH 7.0, 0.01 M) containing the reducing agent sodium sulphite. The tomato plants to be inoculated were predisposed to infection by keeping them in a dark room for 24 hours before inoculation.

TRANSMISSION

Mechanical transmission

Healthy test plants of the same age and uniform size were selected for mechanical transmission studies. Test plants of tomato were inoculated at 2-3 leaf stage by the leaf rub method. The standard extract was applied by rubbing the sap

with fore finger or by cotton swab method. Inoculated leaves were washed thoroughly with distilled water immediately after inoculation to eliminate the excess of inoculum and abrasive from the leaf surface. During mechanical transmission test every possible care was taken to avoid lethal injury to the leaves by abrasive or through hand pressure.

Seed Transmission

To check the possibility of seed-borne nature of different representative isolates, 1000 seeds from the plants inoculated artificially with each isolate under glasshouse conditions were collected during 1996. These seeds were sown during the growing season of 1997 in pots having sterilized potting mixture. The plants thus raised were allowed to grow under insect proof conditions and were observed for symptom expression upto 3 to 5 weeks. Seedlings raised from seeds collected from healthy plants were kept as control.

Transmission by aphids

For aphid transmission tests, virus free colonies of most commonly encountered aphid species viz., *Myzus persicae Sulz., Aphis craccivora* Koch and *Brevicoryne brassicae* Linn., were examined for their possibility to act as vectors. Non-viruliferous apterous form of each aphid species was removed from its colony with gentle tapping and by moist camel hair brush in separate Petri-dishes. These were then given one hour preacquisition fasting, and acquisition access for 30 minutes on young systemically infected leaves cut into small square pieces of 2 cm kept on moist blotting paper in separate Petri-dishes. After the acquisition access, sections of leaf tissue infested with 5 aphids were placed on leaf of the healthy tomato plants. For each isolate, ten plants were inoculated and kept in separate insect proof cages. After 24 hours of inoculation access, the plants were sprayed

with 0.1 per cent malathion to kill the aphids. These plants were observed for 3 to 5 weeks for symptom development under glasshouse conditions.

HOST RANGE

For host range studies plant species belonging to different families were grown in pots under insect proof glasshouse as stated earlier. Ten plants of each species were inoculated with standard extract of the virus and were kept under observation for 3-5 weeks for development of symptoms. The plants which did not show any apparent symptoms were back inoculated on the original host.

BIOPHYSICAL PROPERTIES

Biophysical properties of the isolates under investigation such as thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) were determined as per standard methods described by Noordam (1973).

Thermal inactivation point (TIP)

For determining the thermal inactivation point, standard extracts were prepared in 0.1 M phosphate buffer (pH 7.0). Two ml of the standard extract was pipetted in each thin walled test tube, which were then subjected to different temperatures ranging from 30 to 90^{o}C in thermostatically controlled waterbath. The test tubes were kept immersed in hot waterbath at particular temperature for 10 minutes for each treatment and were continuously shaken during the heating treatment. Heated tubes were placed in ice cold water. The inoculum thus obtained was inoculated on test plants starting from the highest temperature, coming down to the lowest ones. Non-treated extract served as control.

Dilution end point (DEP)

Different dilutions of inoculum were prepared in test tubes by adding calculated amount of 0.1 M phosphate buffer (pH 7) to the crude extract. The dilutions tested were 1:1, 1:10, 1:100, 1:1000, 1:10000 and 1: 100000. The diluted sap was inoculated on the test plants starting with the highest dilution to the lowest ones. Undiluted crude sap served as control.

Longevity *in vitro* (LIV)

The retention of the virus infectivity *in vitro* was measured by storing the standard extract of the virus isolates at room temperature (22-26^{o}C). The first inoculation was done immediately after preparation of the extract followed by inoculations at different intervals of storage. Sap extracted from healthy leaves and similarly kept at room temperature served as control.

SEROLOGY

Enzyme linked immunosorbent assay (ELISA) tests were conducted for the identification of different tomato virus isolates. Direct antigen coating (DAC) ELISA method given by Hobbs *et al.* (1987) was followed. The general procedure followed for carrying out the DAC ELISA test is as under:

1. The plant extracts were prepared in coating buffer and 200 µl of plant extract was added to each well of the microtitre plate. The plate was incubated at 37^{o}C for 1 hour.

2. After emptying the plate, it was washed by flooding the wells with PBS-T thrice for 3 minutes each. The residual liquid was drained by tapping the plate on a paper towel.

3. 200 µl of antiserum diluted in antibody buffer was added to each well and incubated at 37°C for 2 hours.

4. The plate was again washed thrice with washing buffer.

5. 200 µl of alkaline phosphatase labelled antirabbit IgG diluted in substrate buffer was added to each well. The plate was incubated overnight at 4°C.

6. The plate was washed thrice with washing buffer.

7. 200 µl of freshly prepared para-nitrophenyl phosphate (pNPP) substrate was then added to each well.

8. The plate was kept at room temperature for 2 hours.

9. The substrate turned yellow in case of positive reaction.

10. Reaction was stopped using 50 µl of 3M NaOH per well.

ELECTRON MICROSCOPY

For determining the size and shape of the virus particles of different isolates, dip method was used.

Dip preparations

Negatively stained dip preparations were prepared as follows : a) From epidermal strips according to the method of Hitchborn and Hills (1965) and b) From clarified virus concentrates. The isolate suspected to be tomato spotted wilt virus was examined after fixation with glutaraldehyde.

a) Epidermal strips (Hitchborn and Hills, 1965)

A micro drop of negative stain (1% phosphotungstic acid, pH 7.0) was placed on a collodion coated and carbon backed 300-400 mesh copper grid. Lower epidermal peelings of the infected leaf, with the ruptured cells facing the grid were

gently slided over the stain drop several times. The excess stain was then sucked up with a piece of Whatman No.1 filter paper. The grid thus prepared was screened under the transmission electron microscope at 80 kV.

b) Clarified virus concentrate (CVC)

Virus isolate(s) were partially purified according to the procedure described by Christie *et al.* (1987). The procedure was slightly modified. Some isolates showed a relatively very poor concentration of flexuous particles in dip preparation, so they were processed as follows for obtaining CVC.

One gram of young leaf tissue from systemically infected plant (3-4 weeks after inoculation) was grounded in 2 ml of 0.1 M potassium phosphate buffer pH 7.2 containing 0.1 M EDTA. Slurry so obtained was filtered through cheese cloth. The filterate was stirred with equal volume of the mixture of n-butanol and chloroform (1:1 v/v) for 10 minutes and was centrifuged at 10,000 g for 5 minutes. The resultant pellet was discarded and to the supernatant (aqueous phase), PEG-6000 and NaCl were added to make the final concentration 6 per cent and 0.125 M, respectively. The contents were shaken for 30 minutes and then centrifuged at 10,000 g for 10 minutes to pellet the virus precipitate. Supernatant was discarded and the liquid sticking to the inner walls of the centrifuge tube was wiped with filter paper to remove as much of the liquid as possible. The pellet was resuspended in 0.1 ml of the extraction buffer by thoroughly disintegrating the precipitate in the buffer and then allowing proper resuspension by leaving at $4^{o}C$ overnight. The suspension so obtained was called CVC (clarified virus concentrate). For the preparation of negatively stained grids from CVC, the following procedure was adopted.

Small drops (50 µl) of the CVC of different isolates were placed on parafilm in a humid Petridish. Copper grids (300 mesh) coated with collodion film were floated

on the drop (film side down) for 5 minutes at room temperature. They were then washed with 30 drops of distilled water, drained by touching edge (vertically) with filter paper and negatively stained with 4-5 drops of 2 per cent aqueous uranyl acetate. They were then drained and air dried and screened as above.

Processing of isolate Tom-4

Samples were processed according to the method of Iwaki *et al.* (1984) with modifications (Khurana *et al.*, 1990). Symptomatic area of the leaf was excised and cut into fine bits (1 mm wide) and were fixed with 3 per cent phosphate buffered glutaraldehyde for 2 h at room temperature. Then fixative was filtered out and bits were given two washes with the phosphate buffer (0.1 M Sorenson's phosphate buffer, pH 7.2) to thoroughly remove the fixative. The bits were wiped with filter paper to remove excess fluid and then 0.1 g of these bits were triturated with 1 per cent phosphotungstic acid, pH 7.0. The slurry was transferred to an eppendorf tube. The tube was left undisturbed overnight at 4^{o}C for sedimentation of particulate materials. A drop of the clear fluid was taken and a grid floated over it for 5 minutes at room temperature. The grid was drained by touching its edge with filter paper and allowed to air dry. Then it was screened with electron microscope.

ISEM

Immunosorbent electron microscopic studies were conducted by using antisera to the various tobamo viruses namely TMV strains U1, D, Tom, Bringal, P11, P14, Cucumber green mottle mosaic virus and potyviruses namely leaf distortion virus of datura, Peru tomato, papaya ring spot, tobacco etch, turnip mosaic, zucchini yellow mosaic, BCMV, PVA, PVX, PVY, PVS, PVM and tomato spotted wilt virus. Both trapping and decoration were studied employing the procedure described by Garg and Khurana (1988) and Khurana and Garg (1989). Titres of differnt antisera were brought down to 1:25 by dilution with 0.86 per cent

NaCl. These diluted antisera were used both for trapping and decoration. The CVC obtained in the preceding section were diluted 20 times with the extraction buffer before use. First virions were trapped (trapping) on antiserum coated grids and then they were made to bind antibodies (decoration).

Particle morphology of isolates Tom-1, Tom-2, Tom-3, Tom-4, Tom-5, Tom-6 and Tom-7

Young infected leaf was cut with a sharp razor blade and the cut end was repeatedly dipped in a drop of 2 per cent aqueous uranyl acetate placed on to an electron microscope grid coated with collodion and fixed to a sticky surface. The drop was allowed to stay on the grid for 5 minutes and then drained with a piece of filter paper. The grid was then allowed to air dry and then screened with JEOL 100S Transmission Electron Microscope at 80 kV.

IEM studies of isolates Tom-1, Tom-2, Tom-3, Tom-4, Tom-5, Tom-6 and Tom-7

Trapping

Antisera to the earlier mentioned tobamoviruses and potyviruses were diluted with normal saline (0.86 % NaCl solution) to a microprecipitin titre of 1:25. Microdrops of the diluted antiserum were placed on parafilm fixed to a glass slide which in turn was placed in a humid Petridish. Collodion film coated copper grids (300 mesh) were floated on these drops for 5 minutes at room temperature. In the mean time microdrops of the clarified virus extract were placed on parafilm fixed on another glass slide which in turn was placed in another humid Petridish. Grids after 5 minutes were washed with distilled water drops, then drained by touching their edges with filter paper. They were then immediately floated on drops of clarified virus extract and incubated for 1 hour at 37^{o}C. The grids were then washed with distilled water, drained by touching with filter paper and negatively stained with

2 per cent aqueous uranyl acetate solution. They were finally drained by touching edge with filter paper, allowed to air dry and then screened with JEOL 100S Transmission Electron microscope. This procedure completed what is called as trapping.

Decoration

To study the seroaffinity of the virus isolates with tobamo and potyviruses, the grids with trapped virions but without negative staining were floated on another set of microdrops of the antisera and incubated for another 1 hour at 37°C. They were then washed with distilled water, drained and finally stained with 2 per cent aqueous uranyl acetate. The grids were allowed to air dry after draining by touching with filter paper and were then ready for screening with electron microscope.

Source of antisera

Antisera of tobamo viruses and some of those of potyviruses were obtained from D.Z. Maat, the Netherlands through Prof. Khalid Mahmood, Aligarh Muslim University, Aligarh. Some of the antisera were those available in the EM laboratory of CPRI, Shimla and rest were purchased from Agdia Inc., Elkhart, U.S.A.

Cytopathological studies

These were carried out as per the method described by Ronald (1978) and Khurana and Singh (1986). Five small bits (1 x 4 mm) of infected leaf from each isolate were taken and immediately transferred to 3 per cent glutaraldehyde solution prepared in 0.1 M phosphate buffer pH 7.2. The samples were kept in this fixative for 2 hours at room temperature. The fixed samples were washed with distilled water, giving two changes at 1/2 hour interval. These were then transferred to one

per cent osmium tetraoxide (OsO4) solution prepared in 0.1 M phosphate buffer, pH 7.2 and were kept for two hours at 4°C.

Enblock staining

After OsO4 fixation, sample bits were washed with distilled water and 2 per cent aqueous uranyl acetate solution was poured into the vials to cover the bits. The stoppered vials were then transferred to refrigeration for overnight. The uranyl acetate solution was then drained out and sample bits were then dehydrated in the graded acetone series as follows:

Acetone (%)	Period of treatment (minutes)
25	10
50	10
70	10
90	10
100	20
	(2 changes)

After this, the samples were transferred to propylene oxide. Two changes each of 30 minutes of propylene oxide were given. Samples were then shifted to the following SPURR's low viscosity resin mixture and propylene oxide series.

Preparation of resin mixture

	Resin/chemical	Quantity
1.	VCD (venylcyclohexine dioxide) (resin)	10 ml
2.	NSA (Nonenyl succinic anhydride)(hardner)	26 ml
3.	DER (Flexibilizer)	6 ml

The above three constituents were thoroughly mixed and to this 0.4 ml of DMAE (dimethyl amino ethanol in an acceletor) was added and mixed properly. One portion of above mixture was diluted with 3 parts of propylene oxide and tissues from propylene oxide were transferred to 1:3 resin and propylene oxide mixture prepared above for 2 hours. After this, caps of the vials were taken off and propylene oxide was allowed to evaporate in a framehood till almost only resin mixture was left. Then tissues were shifted to fresh pure resin mixture for another 2 hours. The tissues were finally embedded in fresh resin mixture in plastic moulds, after giving proper orientation to the tissues. Resin mixture was then cured at 70°C for 16 hrs. The cured blocks were removed from the moulds and trimmed with the help of stereobinocular.

Ultrathin sectioning

Ultra-thin sections of the tissues were obtained with glass knives using LKB ultraton NOVA ultramicrotome. The silver golden interference colour ribbons of ultrathin section (approxmiately 80 nm) were lifted on copper grids. These sections were then stained first with saturated alcoholic solution of uranyl acetate (ethanolic) and then with lead citrate as follows:

i) Preparation of stains

Saturated ethanolic uranyl acetate staining solution was prepared by shaking excess amount of uranyl acetate in 50 per cent ethanol solution in water. After 10 minutes of vigorous shaking, solution was centrifuged at 5000 g for 10 minutes, supernatant thus obtained was decanted off and used as the stain.

ii) **Lead citrate solution**

Lead citrate solution was prepared from the following solutions:

Solution A:	Trisodium citrate (37.7% solution in distilled water)
Solution B:	Lead nitrate (33.1% solution in distilled water)
Solution C:	1 N NaOH (sodium hydroxide solution)

In a clean beaker, above solutions were added in the following sequence and amounts : 16 ml of distilled water + 3 ml of solution A, stirred the solution and added 2 ml of solution B, again mixed the contents, added 4 ml of solution C. Dissolved the precipitates by shaking. The solution was then filtered through Whatman filter paper No.1. The solution was then diluted in 1:4 ratio with 1N NaOH and diluted solution was used as stain.

Staining of ultrathin sections

Drops of ethanolic uranyl acetate stain solution were placed on parafilm. Grids carrying ultrathin sections were immersed in the stain drops and covered with black cover. Staining was continued upto 20 minutes at 22-25^{o}C. The grids were then washed in excess of 50 per cent ethanol, followed by excess of distilled water. The grids were drained by touching their edge with filter paper and were again floated on lead citrate solution drops placed on parafilm on a glass slide placed in a Petridish containing pellets of sodium hydroxide. Staining in this solution was allowed for 10 minutes at 25^{o}C. The grid were lifted and washed with excess0.1 N NaOH solution followed by distilled water. The grids were then drained, air dried and examined under electron microscope for the presence of virus induced sub cellular alterations.

EPIDEMIOLOGY

A field trial to find out the correlation between the disease incidence and the prevailing meteorological conditions and insect vector population was laid out at the experimental field of the department of Vegetable Crops at Nauni, Solan during the years 1996 and 1997 cropping seasons.

The observations for disease incidence were made on 1000 randomly selected plants each year for the two cropping seasons of 1996 and 1997. The independent variables chosen for correlation studies were temperature, relative humidity, cumulative rainfall, wind velocity, sunshine hours and vector population. Data on meteorological variables (maximum/minimum temperature, relative humidity, cumulative rainfall, wind velocity, sunshine hours) were recorded daily but an average of seven days was taken for analysis of data.

For taking vector counts, galvanized metallic pan traps (30 x 30 x 10.6 cm) painted with bright yellow paint were kept within the crop. The sticky traps were positioned approximately one metre above the crop in an inclined position (about 80^{o}). The aphid count and disease incidence were recorded at weekly interval and expressed as percentage increase in every seven days.

Simple regression of dependent variable (disease incidence) with each of the independent variable (temperature, RH, rainfall, wind velocity, sunshine hours and vector population) were examined first and then multiple regression were built up with the factor which primarily influenced the disease development (Gomez and Gomez, 1983).

MANAGEMENT

Effect of different dates of planting on disease incidence and yield

To study the effect of different dates of planting on virus disease incidence in tomato, a field trial with five different planting dates was carried out at the experimental field of the Department of Mycology and Plant Pathology at Nauni, Solan during the years 1996 and 1997 cropping seasons in a randomized block design with four replications. The plot size was kept 2.9 m x 1.1 m each plot accommodated 4 rows of tomato with row to row and plant to plant distance of 90 cm and 30 cm, respectively.

Observations on disease incidence as well as yield for each planting date were recorded and analysed statistically.

Effect of insecticidal sprays on the disease incidence and crop yield

To study the effect of different insecticides on virus disease(s) incidence and yield of tomato, field trials with four different insecticides (Malathion 50 EC) (0.05%), (Monocrotophos 36 SL) (0.02%), (Metasystox 25 EC) (0.025%) and (Rogor 30 EC) (0.03%) were carried out at the experimental field of the Department of Mycology and Plant Pathology at Nauni, Solan during 1996 and 1997 cropping seasons in a randomized block design with four replications. The plot size was ket 2.9 m x 1.1 m. The row to row and plant to plant distance was 90 cm x 30 cm. The insecticidal sprays were done at an interval of ten days each.

Observations on disease incidence as well as yield for each insecticide were recorded and analyzed statistically.

Effect of polythene mulches on disease incidence and crop yield

Experiments to examine the effect of polythene mulches of different colours (yellow, white, black and green) were conducted during the years 1996 and 1997 in the experimental farm of the department of Mycology and Plant Pathology at Nauni, Solan. The trials were laid out in a randomized block design with four replications with plot size of 2.9 m x 1.1 m. The planting was done at a spacing of 90 cm x 30 cm. After a week of transplanting, polythene mulches were spread by making holes in it for the plants. All other agronomical operations needed for the crop were kept same for all the treatments. Data on yield and disease incidence in different treatments were recorded and analyzed statistically.

SCREENING OF TOMATO GERMPLASM

The germplasm of tomato (*Lycopersicon esculentum*) was screened to evaluate their comparative susceptibility or resistance against the most prevalent virus. The seeds of each collection were sown in earthen pots having sterilized potting mixture under insect proof glasshouse conditions. Ten plants of each variety/collection/line were inoculated mechanically with the standard extract of the virus at 2-3 leaf stage. The infected and healthy plants were counted and percentage of infection was calculated. Reaction of different varieties/collections/ lines were recorded by using the following rating:

Per cent infection	Rating
0-5	Highly resistant (HR)
5-10	Resistant (R)
10-20	Moderately susceptible (MS)
20-30	Susceptible (S)
Above 30	Highly susceptible (HS)

EXPERIMENTAL RESULTS

During the present investigations, studies on the occurrence, distribution and identification of different virus isolates associated with the viral diseases of tomato in different districts of Himachal Pradesh were undertaken. Besides, attempts were also made for the management of virus disease (s) through modification of cultural practices such as different dates of sowing, use of polythene mulches and by insecticidal sprays. The available tomato germplasm was also screened to find the sources of resistance, if any. Field experiments on the management aspects were conducted at the experimental farms of the Departments of Mycology and Plant Pathology and Vegetable Crops at the main campus of the University. In addition, epidemiological studies to correlate the disease incidence with different weather parameters and vector populations were also carried out. Results of these investigations are presented in the ensuing paragraphs

SURVEYS FOR THE OCCURRENCE AND DISTRIBUTION OF VIRAL DISEASE(S)

Surveys of different tomato growing localities of Solan, Sirmour, Shimla, Mandi and Kullu districts of Himachal Pradesh were undertaken during the cropping seasons of 1996 and 1997 to record the prevalence and incidence of virus disease(s) in tomato. The incidence of the disease was calculated by counting the number of diseased and healthy plants and per cent disease incidence was worked out as per the equation given in Materials and Methods. The data on disease incidence are presented in Table 1.

Table 1. **Incidence of virus disease(s) in different tomato growing areas of Himachal Pradesh**

District	Locality surveyed	Per cent disease incidence	
		1996	1997
Solan	Baran	15	23
	Basal	60	52
	Bairity	50	45
	Baknaghat	40	50
	Chhamrog	50	47
	Chambaghat	62	70
	Dharja	50	57
	Gaura	10	18
	Ghatti	69	63
	Gamberpul	71	65
	Kotli	10	14
	Kandaghat	62	51
	Kothi	80	72
	Kyar	25	34
	Kalaghat	47	43
	Kiarighat	35	39
	Lavighat	62	55
	Giripul	20	15
	Majholi	30	26
	Nauni	75	68
	Pandah	40	33
	Shalna	50	53
	Shulman	43	45
Sirmour	Bowli	15	21
	Dhanech	60	64
	Gadshaya	30	20
	Joharji	10	18
	Lakhot	36	31
	Maloti	37	32
	Maryog	45	52
	Narag	23	28
	Naina Tikkar	25	30
	Pabiana	58	55
	Ratoli	50	54
	Sattiwala	33	39
	Shinaghat	44	37
	Dilman	30	26

Contd.....

District	Locality surveyed	Per cent disease incidence	
		1996	1997
Shimla	Junga	19	22
	Kiar	10	15
	Kiari	20	17
	Panjoli	10	13
	Shathali	20	17
	Shoghi	42	47
Mandi	Nerchowk	49	35
	Nagwain	27	15
	Oat	36	40
	Panarsa	16	20
	Sundernagar	45	43
	Takoli	33	36
	Bhangrotu	35	29
Kullu	Badah	23	18
	Bajaura	25	19
	Kalali	27	30
	Jhiri	31	24
	Mohal	15	17
	Sharabai	15	23
	Shamshi	32	29

The data presented in Table 1 indicated a considerable variation in disease incidence in both the years of survey (1996-97) at different locations. The disease incidence ranged from 10 to 80 per cent during 1996 and 13 to 72 per cent during 1997. However, the incidence within the districts in both the years varied from 10 to 80 per cent and 14 to 72 per cent in Solan; 10 to 60 per cent and 18 to 64 per cent in Sirmour, 19 to 42 per cent and 17 to 47 per cent in Shimla, 16 to 49 per cent and 15 to 40 per cent in Mandi, and 15 to 32 per cent and 17 to 30 per cent in Kullu during 1996 and 1997, respectively. It was observed during surveys that Naveen, Nutan, Meenakshi-99 and other unidentified hybrids of tomato were grown by the growers. The most common symptoms encountered on infected plants were mosaics (green and yellow mosaic), shoestringing, blistering, crinkling, curling of leaves and stunting of plants.

COLLECTION, MAINTENANCE AND GROUPING OF TOMATO VIRUS ISOLATES

Twenty one isolates designated as T_1 to T_{21} (Table 2) were collected from the aforesaid tomato growing localities representing different districts of Himachal Pradesh. These isolates were maintained on healthy seedlings of tomato variety Naveen under insect proof glasshouse conditions. On the basis of their reaction on certain indicator plants (Table 3), these isolates were further categorized into 7 groups (Table 4). A representative isolate from each group was selected and redesignated as Tom 1 to Tom 7 for further detailed investigation on symptomatology, transmission, host range, electron microscopy, cytopathology and serology.

SYMPTOMATOLOGY

A detailed account of symptomatology evoked by representative isolates is presented in the following paragraph. However, for comparison sake the main differentiating symptoms of each isolate have also been tabulated in Table 5.

Table 2. Isolates and respective localities in different districts of Himachal Pradesh

District	Locality	Isolate	Prominent symptoms
Solan	Kandaghat	T_1	SS
	Chambaghat	T_2	LY, VC
	Dharja	T_3	LB
	Nauni	T_4	MM, LD
	Bareti	T_5	RLL, MM
	Kothi	T_6	SM
	Ghatti	T_7	LP
	Lavighat	T_8	NGTP
Mandi	Nerchowk	T_9	LR
	Sundernagar	T_{10}	MoM, PS
	Bhangrotu	T_{11}	PGVB
	Takoli	T_{12}	LBr
Sirmour	Shinaghat	T_{13}	LMo
	Narag	T_{14}	MM, GVB, LD
	Sattiwala	T_{15}	SDLL
	Dilman	T_{16}	PS, LB
	Lakhot	T_{17}	PS, DCL

Contd....

District	Locality	Isolate	Prominent symptoms
Kullu	Mohal	T_{18}	LY
	Sarabai	T_{19}	LC
	Kalali	T_{20}	SM, RLL
	Jhiri	T_{21}	PMoM, FRL, LP

Acronyms used to describe the symptoms

DCL	=	Downward curling of leaves
FRL	=	Forward rolling of leaves
GVB	=	Green vein banding
LB	=	Leaf blistering
LBr	=	Leaf bronzing
LD	=	Leaf distortion
LP	=	Leaf puckering
LM	=	Leaf mottling
LR	=	Leaf rolling
LC	=	Leaf crinkling
LY	=	Leaf yellowing
MM	=	Mild mosaic
MoM	=	Mosaic mottling
NGTP	=	Necrosis of growing tips of plants
PGVB	=	Pronounced green vein banding
PMoM	=	Pronounced mosaic mottling
PS	=	Plants stunted
RLL	=	Reduction of leaf lamina
SM	=	Severe mosaic
SDLL	=	Severe distortion of leaf lamina
SS	=	Shoe-stringing
VC	=	Vein clearing

Table 3. Reaction of different tomato virus isolates on indicator plants

District	Locality	Isolate No.	Indicator/reaction								
			Nicotiana glutinosa	*Nicotiana tabacum white burley*	*Nicotiana debneyi*	*Nicotiana rustica*	*Datura stramonium*	*Datura metel*	*Chenopodium murale*	*Chinopodium quinoa*	*Chenopodium amaranticolor*
Solan	Kandaghat	T_1	+	-	+	-	-	+	+	-	+
	Chambaghat	T_2	+	+	-	-	-	+	+	-	+
	Dharja	T_3	+	+	+	+	-	-	-	-	+
	Nauni	T_4	+	+	-	+	+	-	-	-	-
	Bareti	T_5	+	-	+	-	-	+	+	-	+
	Kothi	T_6	+	+	-	+	+	-	-	-	-
	Ghatti	T_7	-	+	-	-	-	+	-	-	+
	Lavighat	T_8	+	+	-	+	+	-	-	+	+
Mandi	Ner Chowk	T_9	-	+	-	-	-	+	+	+	+
	Sundernagar	T_{10}	+	+	-	-	-	+	+	-	+
	Bhangrotu	T_{11}	-	+	-	-	-	+	+	+	+
	Takoli	T_{12}	+	+	-	+	+	-	-	+	+
Sirmour	Shinaghat	T_{13}	+	+	-	+	+	-	-	-	-
	Narag	T_{14}	-	+	-	-	-	+	+	+	+
	Sattiwala	T_{15}	+	+	+	+	-	-	-	-	+
	Dilman	T_{16}	+	-	+	-	-	+	+	-	+
	Lakhot	T_{17}	+	+	-	+	+	-	-	-	-
Kullu	Mohal	T_{18}	+	+	+	-	-	-	-	-	+
	Sarabai	T_{19}	+	+	-	+	+	-	-	-	+
	Kalali	T_{20}	+	+	+	+	-	-	-	-	-
	Jhiri	T_{21}	-	+	-	-	-	+	-	-	+

\- = *No reaction*

\+ = *Expression of symptoms*

Table 4. Grouping of tomato virus isolates on the basis of their reaction on indicator plants

Re-designation of the representative isolate	Isolate(s)	Representative isolate
Tom-1	T_1, T_5, T_{16}	T_1
Tom-2	T_2, T_{10}	T_2
Tom-3	T_4, T_6, T_{13}, T_{17}, T_{19}	T_4
Tom-4	T_8, T_{12}	T_8
Tom-5	T_9, T_{11}, T_{14}	T_{14}
Tom-6	T_3, T_{15}, T_{18}, T_{20}	T_{15}
Tom-7	T_7, T_{21}	T_{21}

Table 5. Comparative symptomatology of different tomato virus isolates

Isolate	Differentiating symptoms	Incubation period (days)
Tom-1	Shoe- stringing and inward rolling of leaves	8-10
Tom-2	Yellowing, vein clearing and blistering of leaves	9-10
Tom-3	Mosaic and distortion of leaves into tendril like structures	17-18
Tom-4	Drying and necrosis of leaves and death of growing tips of plant	23-25
Tom-5	Mild mosaic, with vein banding and slight distortion of leaf lamina	12-24
Tom-6	Green vein banding and blisters on leaves	13-16
Tom-7	Mosaic mottling with inward rolling and puckering of the leaves	10-14

ISOLATE Tom-1

The first noticeable symptom produced by this isolate was in the form of mild mosaic on young infected leaves of tomato plants after 8-10 days of inoculation. Some of the newly emerged leaves were in the form of threads. Rest of the leaves exhibited reduction of leaf lamina alongwith inward rolling of leaves. These leaves later turned into thread like structures. Development of blisters on the leaf lamina also took place. The most prominent and striking symptoms of this isolate were shoe stringing and inward rolling of leaves (Fig. 1A and B). Infected plants were stunted and had less number of flowers as compared to healthy ones and the fruits were small sized.

ISOLATE Tom-2

Initial symptom produced by this isolate was in the form of mild mosaic which appeared after 9-10 days of inoculation. Subsequently, there was clearing of the veins followed by general yellowing of the leaves alongwith distortion of leaf lamina and formation of blisters on certain infected leaves (Fig. 2).

ISOLATE Tom-3

The initial symptoms evoked by this isolate were in the form of mild mosaic after 17-18 days of inoculation. Gradually vein clearing and occasional downward curling of leaves took place. In advanced stages of infection leaves were distorted and reduced to tendril like structures. The leaves on lateral branches of such plants exhibited mosaic symptoms only. The infected plants were stunted in growth (Fig. 3).

1A

1B

2

ISOLATE Tom-4

This isolate was not found to be easily sap transmissible by the usual sap transmission procedure followed for other isolates. Its inoculum extraction and inoculation was carried out at a low temperature as given in Materials and Methods. The isolate produced initial symptoms of the disease as thickening of the veins of younger leaves after 23-25 days of incubation period. The inoculated leaves also exhibited leaf curl symptom. These leaves also produced bronzing effect followed by numerous small, dark spots. Young growing tips of the infected plants exhibited severe necrosis. The infected plants produced less fruits as compared to healthy ones. The mature fruits exhibited characteristic ring pattern on their surface. The differentiating symptoms of this isolate have been exhibited in Fig. 4-6.

iSOLATE Tom-5

Initial manifestation of the symptoms of this isolate was in the form of mild mosaic pattern on the leaves after 12-14 days of inoculation, followed by mild green vein banding and slight distortion of the leaves (Fig. 7). The leaves were curled downwards.

ISOLATE Tom-6

The symptoms induced by this isolate appeared 13-16 days after inoculation in the form of perceptible green vein banding on the leaves (Fig. 8), followed by the appearance of dark green blisters. Inoculated plants showed delayed flowering and fruiting.

PLATE-II

Fig.3 Infected tomato plants exhibiting acute distortion of leaves including shoe-stringing (Isolate Tom-3)

Fig.4 Bud necrosis symptoms of naturally infected tomato plants (Isolate Tom-4)

3

ISOLATE Tom-7

This isolate produced mosaic mottling pattern on the leaves after 10-14 days of inoculation. However, the most striking symptom of this isolate was puckering and slight rolling of leaves (Fig. 9). The infected plants were stunted and produced small sized fruits.

The symptoms observed under the glass house conditions were also noticed to be prevalent under natural conditions during surveys of different tomato growing localities.

HOST RANGE

Thirty one plant species belonging to eight different families, i.e. Amaranthaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Leguminosae, Malvaceae and Solanaceae were raised under insect proof glasshouse conditions to determine the host range of the virus. Ten plants of each species were inoculated mechanically with the standard inocula of each isolate. Inoculated plants were observed for 3 to 5 weeks for the appearance of symptoms. The plants exhibiting no symptoms were back inoculated to tomato cultivar Naveen to detect latent infection if any.

Results of the host range experiment are tabulated in Table 6. A perusal of the data revealed that all the isolates except isolate Tom-3 infected one member or the other of families Chenopodiaceae and Solanaceae only. Isolate Tom-3 did not infect any member of family Chenopodiaceae. Isolate Tom-4 infected *Vigna sinensis* in Leguminosae and *Gomphrena globosa* Amaranthaceae families. Different type of symptoms expressed were in the form of local lesions, mosaic, mottling, blisters, necrosis, vein clearing, yellowing and distortion of leaf lamina. The local lesions produced initially, coalesced later on and ultimately the whole leaf

PLATE-III

Fig.5 TSWV infected tomato plant exhibiting symptom on fruits (Isolate Tom-4)

Fig.6 Tomato fruits showing typical ring-spotting and yellow hallow (Isolate Tom-4)

Fig.7 Mild mosaic, green vein banding and distortion of leaf lamina (Isolate Tom-5)

7

6

Table 6. Host range of different tomato virus isolates

Family/ Host species	Isolate						
	Tom-1	Tom-2	Tom-3	Tom-4	Tom-5	Tom-6	Tom-7
AMARANTHACEAE							
Amaranthus caudatus	-	-	-	-	-	-	-
Gomphrena globosa	-	-	-	M	-	-	-
CHENOPODIACEAE							
Chenopodium amaranticolor	ChLL	LL	-	MM	LL	ChLL	ChLL
C. murale	LL	NS	-	-	LL	-	-
C. quinoa Wild	-	-	-	MM	ChLL	-	-
COMPOSITAE							
Lactuca sativa	-	-	-	-	-	-	-
Zinnia elegans Jacq.	-	-	-	-	-	-	-
CRUCIFERAE							
Brassica oleracea var. *capitata*	-	-	-	-	-	-	-
Brassica oleracea var. *gongylodes*	-	-	-	-	-	-	-
Brassica rapa	-	-	-	-	-	-	-
Raphanus sativus	-	-	-	-	-	-	-
CUCURBITACEAE							
Cucumis sativus	-	-	-	-	-	-	-
LEGUMINOSAE							
Cicer arietinum	-	-	-	-	-	-	-
Vigna sinensis	-	-	-	MCNLL	-	-	-
Lens esculenta	-	-	-	-	-	-	-
Phaseolus vulgaris	-	-	-	-	-	-	-
Phaseolus mungo Var. *radiatus*	-	-	-	-	-	-	-
Pisum sativum	-	-	-	-	-	-	-
Trigonella foenum graecum	-	-	-	-	-	-	-
Vicia faba	-	-	-	-	-	-	-
MALVACEAE							
Abelmoschus esculentus	-	-	-	-	-	-	-
SOLANACEAE							
Capsicum annuum	-	-	MM	-	-	-	-
Datura metel	-	-	-	-	N	-	N
D. metel Var. *festuosa*	MM, VCL	N, MM	-	-	-	MM,Y, LD	-
D. stramonium	-	-	-	W	-	-	-
Nicandra physaloides	-	-	LD, M	-	NS, CR, Y, VN	NS	NS,CC,Y
Nicotiana debneyi	MM, Y	-	-	-	-	MM, Y	-
N. glutinosa	LL	NS	SM	LN	-	LL	-
N. tabacum var. white burley	-	N	M,VCL	MM	NS, CR	NS	NS
N. rustica	-	-	MM	LM	-	SM, LB	-
Solanum khasianum	-	-	-	-	-	-	-

Acronyms used to describe the reactions

ChLL	=	Chlorotic local lesions	LM	=	Leaf mottling
LL	=	Local lesions	M	=	Mosaic
MM	=	Mild mosaic	LN	=	Leaf necrosis
MCNLL	=	Magenta coloured necrotic local lesions	CR	=	Concentric rings
SM	=	Severe mosaic	Y	=	Yellowing
N	=	Necrosis	VN	=	Vein necrosis
NS	=	Nectotic spots	VCL	=	Vein clearing
LD	=	Leaf distortion	W	=	Wilting
LB	=	Leaf blistering	-	=	No reaction

PLATE-IV

Fig.8 Green vein banding and small blisters on leaves (Isolate Tom-6)

Fig.9 Mosaic mottling with inward rolling and puckering of leaves (Isolate Tom-7)

B

9

turned necrotic. It was interesting to note that none of the isolates could infect the test plants belonging to the families Cruciferae, Cucurbitaceae, and Malavaceae (Fig. 10-34).

TRANSMISSION

I) Sap

The standard extract of the virus prepared from infected leaves of tomato variety Naveen showing prominent symptoms of all the isolates were inoculated separately on young healthy seedlings of Naveen. The inoculated plants were kept under observation for 3 to 5 weeks for the development of symptoms.

The results of the mechanical sap inoculation experiments revealed that all the isolates were easily sap transmissible (Table 7). Isolates Tom-I, Tom-5 and Tom-7 exhibited the highest transmission of 100 per cent each whereas Isolate Tom-4 showed the lowest transmission of 60 per cent.

ii) Seed transmission

Since certain viruses have been reported to be seed-borne in nature, therefore, it became important to determine whether the present isolates could be transmitted through seeds or not. The seed collected from the infected plants were sown in pots in the glasshouse. Observations were made on the development of symptoms on these plants upto 3 to 5 weeks after germination. None of the isolates were found to be seed transmitted (Table 9).

iii) Transmission by aphids

Three aphid species viz., *Myzus persicae Sulz.; Aphis craccivora Koch and Brevicoryne brassicae Linn.*, were tested for the transmission of different isolates.

PLATE-V

Fig.10 *Chenopodium amaranticolor* leaf showing numerous chlorotic local lesions (Isolate Tom-1)

Fig.11 *Chenopodium murale* leaf showing few chlorotic local lesions (Isolate Tom-1)

Fig.12 Mild mosaic and vein clearing of leaves of *Datura metel var. festuosa* (Isolate Tom-1)

Fig.13 Leaf of *Nicotiana glutinosa* showing chlorotic lesions (Isolate Tom-1)

Fig.14 *Chenopodium amaranticolor* leaf exhibiting pinhead sized chlorotic local lesions (Isolate Tom-2)

Fig.15 Necrosis of *Chenopodium murale* leaf (Isolate Tom-2)

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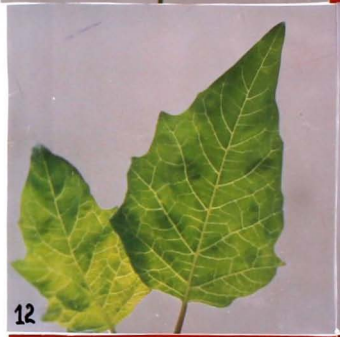
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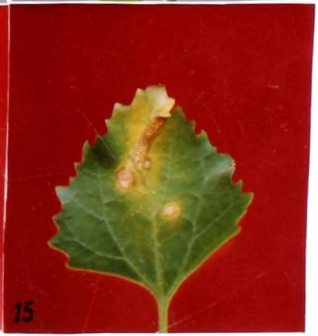
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A perusal of the data in Table 8 revealed that all the aphid species tested failed to transmit the isolates Tom-1, Tom-2, Tom-4, Tom-5, Tom-6 and Tom-7. Only the isolate Tom-3 was transmitted by aphids. *Myzus persicae* exhibited high degree of transmission (90%) followed by *Aphis craccivora* (30%). *Brevicoryne brassicae* failed to transmit any of the isolates.

BIOPHYSICAL PROPERTIES

Although the study of biophysical properties has outlived their usefulness due to their limited nature in diagnosis, yet they are quite useful in separating the mixture of viruses and for designing purification schemes. Thus, the biophysical properties such as thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) of each isolate were determined according to the standard procedures described in Materials and Methods. The results are outlined in the following paragraphs.

i) Thermal inactivation point (TIP)

Data set out in Table 10 revealed that the thermal inactivation point (TIP) of isolates Tom-1, Tom-2 and Tom-6 ranged between 80-85^{o}C and that of isolates Tom-5 and Tom-7 between 75-80^{o}C; of isolate Tom-3 between 55-60^{o}C. Isolate Tom-4 exhibited the lowest thermal inactivation point. It was between 40-45^{o}C.

ii) Dilution end point (DEP)

The dilution end points of different isolates were determined by serial dilutions of the infectious sap which was inoculated on the test plants. The results of the experiment are presented in Table 11. It is evident from the data that the dilution end point of all the isolates except for isolate Tom-1 ranged between 1 : 1000 and

Table 7. Transmission of tomato virus isolates through sap

Isolate	No. of plants inoculated	No. of plants infected	Per cent transmission
Tom-1	20	20	100
Tom-2	20	18	90
Tom-3	20	15	75
Tom-4	20	12	60
Tom-5	20	20	100
Tom-6	20	19	95
Tom-7	20	20	100

Table 8. Transmission of tomato virus isolates by aphids

Aphis species	Virus isolate/per cent transmission						
	Tom-1	Tom-2	Tom-3	Tom-4	Tom-5	Tom-6	Tom-7
Myzus persicae	0	0	90	0	0	0	0
Aphis craccivora	0	0	30	0	0	0	0
Brevicoryne brassicae	0	0	0	0	0	0	0

Table 9. Comparative mode of transmission of tomato virus isolates

Isolates	Mode of transmission				
	Sap	Seed	*Myzus persicae*	*Aphis craccivora*	*Brevicoryne brassicae*
Tom-1	+	-	-	-	-
Tom-2	+	-	-	-	-
Tom-3	+	-	+	+	-
Tom-4	+	-	-	-	-
Tom-5	+	-	-	-	-
Tom-6	+	-	-	-	-
Tom-7	+	-	-	-	-

Table 10. **Thermal inactivation point of tomato virus isolates**

Temperature (°C)	Isolate/per cent transmission						
	Tom-1	Tom-2	Tom-3	Tom-4	Tom-5	Tom-6	Tom-7
30	100	100	70	25	100	100	100
35	100	100	50	15	100	100	100
40	100	100	40	5	90	100	100
45	100	100	20	0	80	100	90
50	80	80	10	0	60	90	70
55	70	60	5	0	50	70	50
60	60	50	0	0	45	60	45
65	55	45	0	0	40	45	30
70	45	40	0	0	35	40	25
75	30	25	0	0	15	30	10
80	20	10	0	0	0	15	0
85	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0

Table 11. **Dilution end point of tomato virus isolates**

Treatment (Dilution)	Isolate/per cent transmission						
	Tom-1	Tom-2	Tom-3	Tom-4	Tom-5	Tom-6	Tom-7
Crude sap	100	90	80	50	100	90	100
1 :1	90	80	70	40	90	70	90
1 : 10	70	80	60	35	80	50	60
1 :100	40	50	40	20	70	30	40
1 :1000	25.	30	20	10	50	20	10
1: 10000	10	0	0	0	0	0	0
1 : 100000	0	0	0	0	0	0	0

PLATE VI

Fig.16 *Datura metel* plant showing leaf necrosis (Isolate Tom-2)

Fig.17 *Nicotiana glutinosa* plant exhibiting necrotic spots on inoculated leaves (Isolate Tom-2)

Fig.18 Acute stunting and necrosis of *Nicotiana tabacum* var. white burley leaves (Isolate Tom-2)

Fig.19 *Chenopodium amaranticolor* leaf showing numerous pinhead sized chlorotic lesions (Isolate Tom-5)

Fig.20 *Chenopodium murale* leaf showing chlorotic spots (Isolate Tom -5)

Fig.21 *Chenopodium quinoa* leaf showing chlorotic local lesions (Isolate Tom-5)

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1 : 10000, for which the DEP ranged between 1 : 10000 and 1 : 100000. All the isolates except for Tom-1 were rendered innocuous when diluted 10^{-4} times. Isolate Tom-1 became non-infections when diluted 10^{-5} times.

iii) **Longevity *in vitro* (LIV)**

The longevity *in vitro* of the isolates was determined by storing the infectious sap at room temperature (22-26^{o}C) as per the procedure given under Materials and Methods. The results of the experiment presented in Table 12 revealed that the longevity *in vitro* of isolates Tom-1, Tom-6 and Tom-7 ranged between 60 and 70 days. These isolates showed infectivity upto 60 days when stored at room temperature. Isolates Tom-2 and Tom-5 exhibited longevity *in vitro* between 70 and 80 days, the infectivity was retained upto 70 days at room temperature. Isolate Tom-3 had a short longevity *in vitro* ranging between 96 and 120 hours. This isolate was infectious when stored upto 96 hours at room temperature. Isolate Tom-4 recorded the lowest LIV. It retained its infectivity when stored upto 5 hours at room temperature. The infectivity was lost when it was stored for 7 hours at room temperature.

SEROLOGY

ELISA

Direct antigen coating (DAC) ELISA system was used for serological indexing against all the seven representative tomato virus isolates. The experiment was repeated thrice to examine the reproducibility of the results.

PLATE-VII

Fig.22 *Datura metel* plant showing necrosis of the lower leaves (Isolate Tom-5)

Fig.23 *Nicandra physaloides* leaf showing large necrotic spots alongwith necrosis of veins and veinlets (Isolate Tom-5)

Fig.24 *Nicotiana tabacum* var. white burley leaf exhibiting necrotic ring spotting (Isolate Tom-5)

Fig.25 *Chenopodium amaranticolor* leaf showing chlorotic local lesions (Isolate Tom-6)

Fig.26 *Datura metel* var. *festuosa* showing mild mosaic, vein clearing and leaf distortion (Isolate Tom-6)

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Table 12. **Longevity *in vitro* of tomato virus isolates (room temperature 22-26°C)**

Treatment	Tom-1	Tom-2	Tom-3	Tom-4	Tom-5	Tom-6	Tom-7
0 h	100	100	80	40	100	100	90
1 h	-	-	-	30	-	-	-
3 h	-	-	-	25	-	-	-
5 h	-	-	-	10	-	-	-
7 h	-	-	-	0	-	-	-
1 day	90	90	60	0	90	90	90
2 days	-	-	40	-	-	-	-
3 days	-	-	30	-	-	-	-
4 days	-	-	10	-	-	-	-
5 days	80	80	0	-	90	70	80
10 days	80	70	-	-	75	70	70
20 days	70	70	-	-	70	60	60
30 days	50	60	-	-	65	40	50
40 days	30	45	-	-	50	35	40
50 days	20	25	-	-	30	20	25
60 days	10	20	-	-	20	10	10
70 days	0	5	-	-	10	0	0
80 days	0	0	-	-	0	0	0

PLATE-VIII

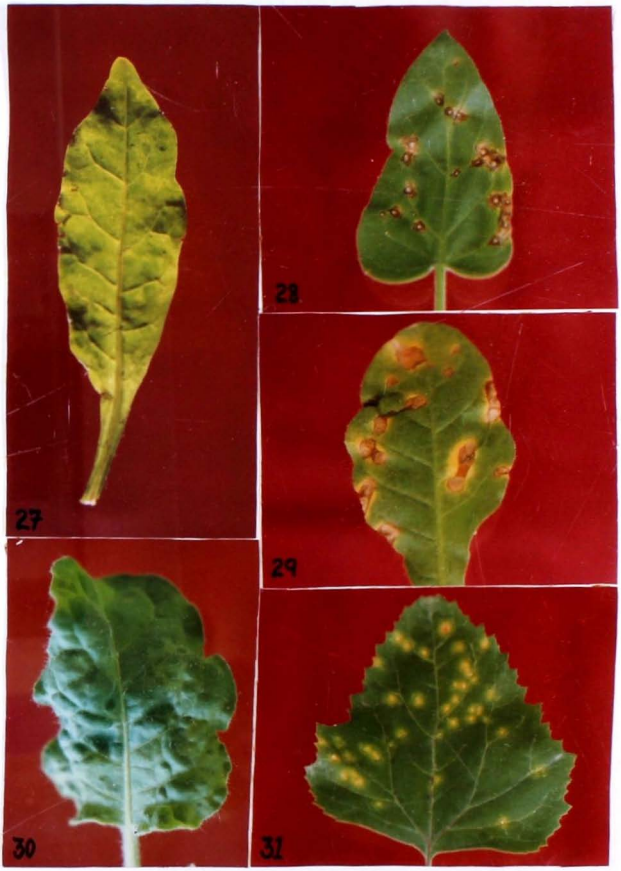
Fig.27 *Nicotiana debneyi* leaf showing mild mosaic (Isolate Tom-6)

Fig.28 *Nicotiana glutinosa* leaf showing necrotic lesions (Isolate Tom-6)

Fig.29 *Nicotiana tabacum* var. white burley leaf exhibiting larger necrotic spots (Isolate Tom-6)

Fig.30 *Nicotiana rustica* leaf exhibiting severe mosaic, blistering and leaf distortion (Isolate Tom-6)

Fig.31 *Chenopodium amaranticolor* leaf showing chlorotic local lesions (Isolate Tom-7)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It is evident from Table 13 that out of the eight antisera used for detection viz., potato virus X (PVX) Tomato aspermy virus (TAV), Tomato mosaic virus (ToMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Tomato black ring virus (TBRV), Tomato ringspot virus (TRSV), and Tomato bushy stunt virus (TBSV), virus isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 reacted positively with the antiserum against ToMV whereas isolate Tom-4 reacted positively with antiserum against TSWV thereby indicating a possible serological relationship between virus isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 with ToMV and Tom-4 with TSWV.

ELECTRON MICROSCOPIC STUDIES

Electron microscopic studies, were carried out by using "Dip Method" for determining the size and shape of the virus particles of different isolates. Only the isolates Tom-3 and Tom-4 were investigated by examining the ultrathin sections of infected host cells to study the presence of specific diagnostic cytoplasmic inclusion bodies. Besides, immunosorbent electron microscopy (ISEM) was employed to study the serological relationship of isolates Tom-1, Tom-2, Tom-3, Tom-5, Tom-6 and Tom-7.

ISOLATES Tom-1, Tom-2, Tom-5, Tom-6 AND Tom-7

PARTICLE MORPHOLOGY

A large number of rod shaped virus particles with a modal length of about 300 nm and diameter of about 18 nm were observed in the negatively stained preparations (Fig. 35).

PLATE-IX

Fig.32 *Datura metel* leaf showing necrosis of top most leaf (Isolate Tom-7)

Fig.33 Necrotic spots on leaf of *Nicandra physaloides* (Isolate Tom-7)

Fig.34 *Nicotiana tabacum* Var. white burley showing chlorotic spots (Isolate Tom-7)

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TRAPPING AND DECORATION

The isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 showed markedly enhanced trapping and decoration of the virions with antisera only to TMV-D (Table 14). Antisera to other TMV strains and cucumber green mottle mosaic virus did not appreciably enhance the trapping of the virus particles. It is apparent from the data presented in Table 14 that trapping index (ratio of virus particles trapped with antiserum to those adsorbed on normal serum coated grids) was maximum with antiserum to TMV-D.

ISOLATE Tom-3

PARTICLE MORPHOLOGY

A large number of flexuous virus particles with a modal length of about 730 nm and diameter of about 11 nm were observed in the negatively stained preparations (Fig. 36).

TRAPPING AND DECORATION

The isolate Tom-3 showed markedly increased trapping of the virions with antiserum to leaf distortion virus of datura followed by trapping with antisera to papaya ringspot and Peru tomato viruses (Figs. 37-41). Trapping of virions did not take place with antisera to PVS and PVM (Table 15).

Decoration behaviour of the isolate with different antisera was studied both in liquid and solid phases. Results presented in Table 16 show that the decoration of virions with antisera to leaf distortion virus of datura was maximum (very good) followed by decoration with antisera to Peru tomato and papaya ringspot viruses. The integrity of the virions was maintained in all the cases.

Table 13. **Serological detection of different tomato virus isolates using direct antigen coating (DAC) ELISA**

Isolate	Antisera used							
	PVY	TAV	ToMV	TSWV	CMV	TBRV	TRSV	TBSV
Tom-1	-	-	+	-	-	-	-	-
Tom-2	-	-	+	-	-	-	-	-
Tom-3	-	-	-	-	-	-	-	-
Tom-4	-	-	-	+	-	-	-	-
Tom-5	-	-	+	-	-	-	-	-
Tom-6	-	-	+	-	-	-	-	-
Tom-7	-	-	+	-	-	-	-	-

- = No reaction

Table 14. Trapping behaviour of isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7

Trapping with antisera to		Trapping Index
U1	3/20 at 21,000	7.5
Tom	1/20 at 21,000	2.5
D	10/20 at 21,000	25.0
Brinjal	2/20 at 21,000	5.0
P11	1/20 at 21,000	2.5
P14	1/20 at 21,000	2.5
Cucumber green mottle mosaic virus	1/20 at 21,000	2.5
Control (normal serum)	0.4/20 at 21,000	

Decoration : Only with TMV-D antiserum

Table 15. Trapping index of Tom-3 isolate with different antisera

Antiserum to virus	No. of virions trapped[a]	Trapping index Virions trapped with antiserum/ Virions trapped with normal serum
Leaf distortion virus of Datura	5.0	10.0
Peru tomato virus	4.5	9.0
Papaya ringspot virus	4.0	8.0
Potato virus Y	2.0	4.0
Potato virus A	1.5	3.0
Tobacco etch virus	3.0	6.0
Zucchini yellow mosaic virus	2.0	4.0
Bean common mosaic virus	2.5	4.0
Bean yellow mosaic virus	2.0	4.0
Turnip mosaic virus	2.5	5.0
Potato virus X	0.5	1.0
Potato virus S	0	-
Potato virus M	0	-
Normal saline	0.5	-

(a) Number of virus particles observed per 20 cm^2 screen area at 21,000 displayed magnification

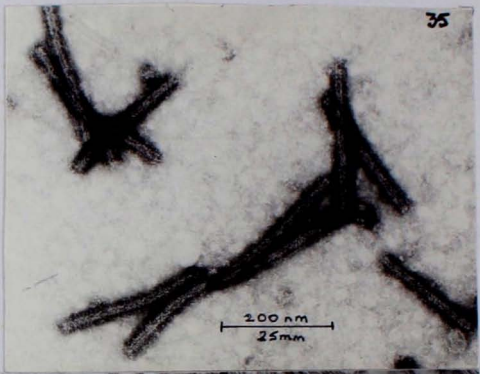
Table 16. **Clumping and decoration behaviour of Tom-3 with different antisera in liquid phase**

Antiserum to virus	Degree of clumping and decoration	Remarks/comments
Leaf distortion virus of Datura	Moderately large clumps and very good decoration	Integrity of virions was maintained
Peru tomato virus	Small clumps and good decoration	Integrity of virions was maintained
Papaya ringspot virus	Small clumps and good decoration	Integrity of virions was maintained
Potato virus Y	No clumps and poor decoration	Integrity of virions was maintained
Potato virus A	No clumps and poor decoration	Integrity of virions was maintained
Tobacco etch virus	Very small clumps with fair decoration	Integrity of virions was maintained
Zucchini yellow mosaic virus	No clumps and poor decoration	Integrity of virions was maintained
Bean common mosaic virus	No clumps and poor decoration	Integrity of virions was maintained
Turnip mosaic virus	No clumps and poor decoration	Integrity of virions was maintained
Potato virus X	No clumps and no decoration	Integrity of virions was maintained
Potato virus S	No clumps and no decoration	Integrity of virions was maintained
Potato virus M	No clumps and no decoration	Integrity of virions was maintained

PLATE-X

Fig.35 Electron micrograph of Isolate Tom-1 showing rigid rod shaped virus particles decorated with TMV-D antiserum

Fig.36 Electron micrograph of Isolate Tom-3 showing flexuous virus particles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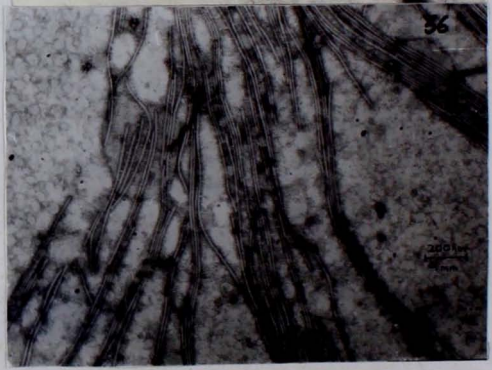
200 nm
25mm


36

Similar results were obtained while studying the decoration behaviour of the isolate in solid phase (Table 17). The decoration of virions was very good with antisera to leaf distortion virus of datura, Peru tomato and papaya ringspot viruses, whereas there was no decoration with PVX, PVS and PVA.

CYTOPATHOLOGY

This isolate produced characteristic cytoplasmic cylindrical inclusions in the infected cells of tomato leaves. They consisted of pinwheels, scrolls and laminated aggregates (Fig. 42).

Isolate Tom-4

Particle morphology

Electron microscopic studies revealed the presence of isometric virions measuring 80 nm in diameter (Fig. 43)

IEM Studies

The IEM studies confirmed the virus as tomato spotted wilt virus.

Cytopathology

In thin section studies, the characteristic TSWV particles were observed to aggregate in the cisternae of endoplasmic reticulum (ER) (Fig. 44).

IDENTIFICATION AND TAXONOMIC GROUPING OF TOMATO VIRUS ISOLATES

Identification of plant viruses is based on many characteristics such as symptomatology, transmission, hostrange, biophysical properties, particle morphology, cytopathology and scerological relationship. However, the first four

Table 17. **Decoration behaviour of Tom-3 with different antisera in Immunosorbent electron microscopy on solid phase[a]**

Antiserum to virus	Degree of decoration[b]	Remarks/comments
Leaf distortion virus of Datura	Very good	Virions not dislodged whether fixed or unfixed[c]
Peru tomato virus	Very good	Virions not dislodged whether fixed or unfixed[c]
Papaya ringspot virus	Very good	Unfixed virions get dislodged, only fixed ones were retained
Potato virus Y	Poor	Unfixed virions get dislodged, only fixed ones were retained
Potato virus A	Poor	Unfixed virions get dislodged, only fixed ones were retained
Tobacco etch virus	Good	Unfixed virions get dislodged, only fixed ones were retained
Zucchini yellow mosaic virus	Poor	Unfixed virions get dislodged, only fixed ones were retained
Bean common mosaic virus	Poor	Unfixed virions get dislodged, only fixed ones were retained
Turnip mosaic virus	Poor	Unfixed virions get dislodged, only fixed ones were retained
Potato virus X	Nil	Dislodged
Potato virus S	Nil	Dislodged
Potato virus M	Nil	Dislodged

(a) Solid phase: Virions were immunosorbed on antiserum coated grids

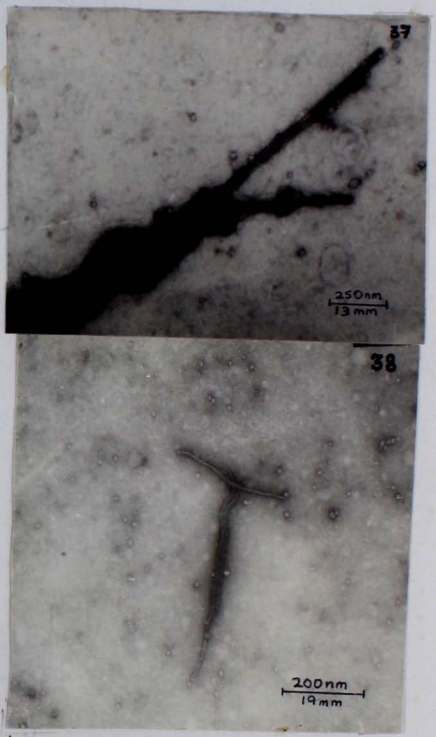
(b) Degree of decoration:
Very good: virions fully covered with antibodies
Good : Virions fairly covered with antibodies
Poor : No perceptible antibody covering

(c) Unfixed : No fixation of virions after trapping
Fixed : Trapped virions were fixed with 3% buffered glutaraldehyde for 1 hour at room temperature prior to treatment with antiserum

PLATE-XI

Fig.37 Electron micrograph of Isolate Tom-3 decorated with antiserum against leaf distortion virus of datura (liquid phase).

Fig.38 Electron micrograh of Isolate Tom-3 showing flexuous virus particles decorated with Peru tomato virus antiserum (liquid phase)


37
250nm
13mm
38
200nm
19mm

parameters mentioned above are considered to be inadequate criteria for the proper identification and assigning of particular virus/viruses to a definite group. Thus, in the present investigations, the identification of different virus isolates was mainly based on particle morphology, cytopathological observations under electron microscope and serology. Thus, based on these parameters the different isolates under investigations were found to belong to three different genera i.e., *Tobamovirus, Potyvirus* and *Tospovirus*. The isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 belong to *Tobamovirus*, isolate Tom-3 to *Potyvirus*, whereas isolate Tom-4 belonged to the genus *Tospovirus*. Edwardson (1974) has proposed a further grouping of different members of the *Potyvirus* into three sub divisions on the basis of type of inclusions produced:

a) Scrolls or tubes (in longitudinal sections) :

Sub-divison I (12 viruses)

b) Laminated aggregates :

Sub-divison II (11 viruses) and

c) Scrolls, tubes and laminated aggregates :

Sub-divison III (17 viruses)

Hence, on the basis of type of inclusions the isolate Tom-3 falls in the sub-division III of genus *Potyvirus*.

EPIDEMIOLOGICAL STUDIES

VIRUS-VECTOR AND ENVIRONMENTAL FACTORS

Epidemiological studies were carried out at the Experimental Farm of the Department of Vegetable Crops at the University Campus, Nauni, Solan in order to

PLATE-XII

Fig.39 Electron micrograph of Isolate Tom-3 showing flexuous virus particle decorated with antiserum against Tobacco etch virus (solid phase).

Fig.40 Electron micrograph of Isolate Tonm-3 showing flexuous virus particles decorated with antiserum against Tobacco etch virus antiserum (liquid phase).

200 mμ
31 mm

200 mμ
3.1 mm

study the role played by different a meteorological factors and vector population in the development of disease. These parameters were temperature (^{o}C), relative humidity (%), cumulative rainfall (mm), wind velocity (Km/hr), total sunshine hours (hrs) and aphid vector population. The observations for these parameters were recorded at weekly interval and are presented in Tables 18 and 19 for cropping seasons of 1996 and 1997, respectively.

The data set out in Tables 18 and 19 revealed that the disease appeared in the month of May in both the cropping seasons i.e. 1996 and 1997 when mean air temperature was 25.50^{o}C and 24.10^{o}C and relative humidity 32.00 and 38.50 per cent, respectively. The disease assumed serious proportion towards the end of June and remained almost static after mid July during both the years with simultaneous increase in the vector population.

The disease incidence is dependent on various meteorological parameters such as temperature, relative humidity, total sunshine hours, wind velocity, cumulative rainfall and vector population. Therefore, various correlations viz., simple, partial and multiple were worked out to establish their relative contribution for the spread of the disease.

SIMPLE CORRELATION

Simple correlation coefficients worked out between all combinations of disease incidence and vector population with mean air temperature, relative humidity, total sunshine hours, wind velocity and cumulative rainfall are presented in Table 20.

Data presented in Table 20 revealed that simple correlation coefficients of disease incidence with relative humidity and wind velocity were found to be highly

PLATE-XIII

Fig.41 Electron micrograph of Isolate Tom-3 showing flexuous virus particles decorated with antiserum against Papaya ringspot virus (liquid phase).

Fig.42 Electron micrograph of ultrathin section of a leaf cell of tomato infected with Isolate Tom-3 showing cytoplasmic inclusions comprising of laminated aggregates (LA), Scrolls (S) and Pinwheels (PW).

41
250nm
21mm

42

Table 18. Incidence of virus disease(s) of tomato in relation to epidemiological parameters during 1996 cropping season

Date of observation	Temperature (°C)	Relative humidity (%)	Total sunshine hours (hr)	Wind velocity (km/hr)*	Cumulative rainfall (mm)	Vector population*	Incidence (%)
20.05.96	23.25	42.00	52.50	6.0	-	12.50	0.00
27.05.96	25.50	32.00	78.40	7.0	6.80	24.00	0.40
06.06.96	24.45	49.50	63.00	7.0	6.00	32.50	10.21
10.06.96	23.75	57.50	30.80	4.0	25.80	45.00	21.34
17.06.96	23.90	80.50	15.40	4.0	84.60	34.00	30.74
24.06.96	24.20	79.00	25.90	5.0	59.80	43.00	53.10
01.07.96	22.80	73.50	48.30	4.0	15.60	47.00	59.60
08.07.96	24.05	84.50	21.00	3.0	-	55.00	60.00
15.07.96	29.50	80.00	56.00	4.0	13.60	54.00	62.47
22.07.96	25.10	86.50	24.50	4.0	52.40	42.50	66.35
29.07.96	24.00	83.00	14.70	3.0	58.40	40.00	67.72
05.08.96	24.20	85.00	32.90	3.0	34.70	44.50	67.72

** Represents seven days average data*

Table 19. Incidence of virus disease(s) of tomato in relation to epidemiological parameters during 1997 cropping season

Date of observation	Temperature (°C)	Relative humidity (%)	Total sunshine hours (hr)	Wind velocity (km/hr)*	Cumulative rainfall (mm)	Vector population*	Incidence (%)
17.05.97	22.60	39.00	73.50	6.0	-	15.00	0.00
24.05.97	24.10	38.50	73.50	6.0	7.80	25.00	0.68
31.05.97	24.00	51.00	49.00	5.0	1.40	32.50	9.18
07.06.97	22.15	66.50	38.50	4.0	58.80	42.00	18.23
14.06.97	24.50	54.50	39.90	5.0	3.40	45.50	26.83
21.06.97	25.30	57.50	42.00	6.0	25.20	47.00	49.11
28.06.97	25.00	76.00	41.30	4.0	13.40	49.50	54.41
05.07.97	26.65	71.00	60.20	6.0	3.40	45.00	55.09
12.07.97	24.90	78.00	37.80	4.0	24.20	48.00	56.27
19.07.97	25.40	81.50	23.10	4.0	15.00	47.50	59.24
26.07.97	24.55	78.00	21.10	4.0	4.20	50.00	61.85
02.08.97	22.85	91.00	7.00	3.0	27.42	51.50	62.45

** Represents seven days average data*

Table 20. **Simple correlation coefficients between epidemiological parameters and disease incidence**

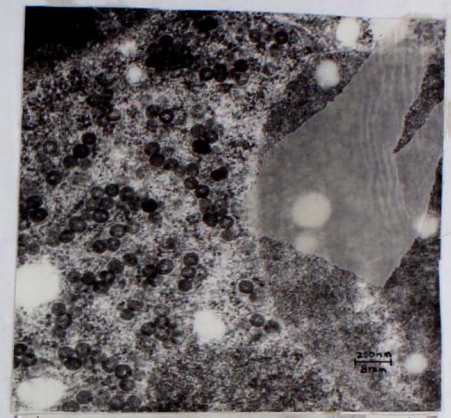
Correlation pair	Correlation coefficient	
	1996	1997
Disease incidence x Temperature	+0.1764	+0.5053
Disease incidence x Relative humidity	+0.9298**	+0.9054**
Disease incidence x Total sunshine hours	-0.5857*	-0.7560**
Disease incidence x Wind velocity	-0.8342**	-0.5701
Disease incidence x Cumulative rainfall	+0.3581	+0.3293
Disease incidence x Vector population	+0.8051**	+0.8929**
Vector population x Temperature	+0.3028	+0.4205
Vector population x Relative humidity	+0.7535**	+0.8590**
Vector population x Total sunshine hours	+0.4190	+0.8112**
Vector population x Wind velocity	-0.7174**	-0.6302*
Vector population x Cumulative rainfall	+0.1101	+0.3293

** Significant at 1% level
* Significant at 5% level

PLATE-XIV

Fig.43 Electron micrograph of Isolate Tom-4 showing isometric particles decorated with Tomato spotted wilt virus antiserum.

Fig.44 Electron micrograph of ultrathin section of a leaf cell of tomato infected with virus Isolate Tom-4 showing cytoplasmic inclusions comprising aggregation of isometric virus particlas.


200nm
8mm

44
200nm
10mm

significant during 1996. However, the correlation between disease incidence and wind velocity was negative. Simple correlation with respect to vector population during the same year with relative humidity, total sunshine hours and wind velocity were also found to be significant. However, a negative correlation was observed between the vector population and wind velocity.

During 1997, the simple correlation coefficients of disease incidence with relative humidity, with total sunshine hours and with vector population, vector population with relative humidity, with total sunshine hours and with wind velocity were found to be significant.

The correlation coefficients between disease incidence and temperature were found to be non-significant but positively correlated during 1996 and 1997 indicating thereby the consistent effect of temperature on disease development.

The correlation coefficients between disease incidence and vector population were highly significant and positive during both the cropping seasons, showing thereby the consistent effect of vector population on disease development i.e. increase in vector population also increases the disease incidence.

Both factors i.e. vector population and disease incidence were significantly but negatively correlated with wind velocity during 1996, however, during 1997, also both these parameters were negatively correlated with wind velocity but the correlation coefficient of vector population with wind velocity was significant.

PARTIAL CORRELATION

Change in one parameter is often influenced/accompanied by simultaneous changes in several other parameters of a disease epidemic. Therefore, valid

conclusions can not be drawn by the study of only simple correlation coefficients among the factors included in the study. In such situations, essentiality of the study of partial correlation coefficients assumes a special significance. The different partial correlation coefficients determined between various meteorological parameters and disease incidence are described in Table 21.

A perusal of the data in Table 21 indicates that the partial correlation of disease incidence with relative humidity was positive and significant during 1996. During the same year correlation coefficients of disease incidence with total sunshine hours and vector population were positive and non-significant. Partial correlation coefficients of vector population with temperature and relative humidity were positive and non-significant. Similarly, during the same year partial correlation coefficients of disease incidence with temperature, wind velocity and cumulative rainfall were negative and non-significant. Correlation coefficients of vector population with total sunshine hours, wind velocity and cumulative rainfall were negative and non-significant.

During the year 1997, none of the correlation coefficients were found to be significant. The partial correlation coefficients of disease incidence with temperature, relative humidity, total sunshine hours, wind velocity and vector population were positive and non-significant. On the contrary, correlation coefficients of vector population were negative and non-significant with wind velocity and cumulative rainfall. Almost all the partial correlation coefficients were non-significant during both the years, thus indicating low consistency of the association in the disease spread during both the years of investigation.

Table 21. Partial correlation coefficients between epidemiological parameters and disease incidence

Correlation pair	Correlation coefficient	
	1996	1997
Disease incidence x Temperature ($r_{12.34567}$)	-0.2744	+0.3125
Disease incidence x Relative humidity ($r_{13.24567}$)	+0.8193*	+0.7227
Disease incidence x Total sunshine hours ($r_{14.23567}$)	+0.5785	+0.4596
Disease incidence x Wind velocity ($r_{15.23467}$)	-0.3948	+0.4505
Disease incidence x Cumulative rainfall ($r_{16.23457}$)	-0.0076	-0.0811
Disease incidence x Vector population ($r_{17.23456}$)	+0.1263	+0.0804
Vector population x Temperature ($r_{72.13456}$)	+0.2519	+0.3713
Vector population x Relative humidity ($r_{73.12456}$)	+0.1927	+0.0964
Vector population x Total sunshine hours ($r_{74.12356}$)	-0.0192	+0.4795
Vector population x Wind velocity ($r_{75.12346}$)	-0.1338	-0.0256
Vector population x Cumulative rainfall ($r_{76.12345}$)	-0.3675	-0.0121

* Significant at 5% level

MULTIPLE CORRELATION

To measure the contribution of linear function of independent variables such as mean air temperature (X_1), relative humidity (X_2), total sunshine hours (X_3), wind velocity (X_4), cumulative rainfall (X_5), and vector population (X_6) on dependent variable (Y); i.e. disease incidence the coefficient of multiple correlation (R^2) was calculated for both the cropping seasons.

The multiple correlation coefficients for 1996 and 1997 are given in Table 22 Multiple correlation coefficients between the disease incidence and group of independent variables were worked out to be 0.9381 and 0.9173 in the year 1996 and 1997, respectively. These results further revealed that 93.81 and 91.73 per cent change in disease incidence was caused by mean air temperature, relative humidity, total sunshine hours, wind velocity, cumulative rainfall and vector population during the course of investigation, whereas, the rest of the variation was due to unexplainable factors of variation (error variation) and the factors not included in the investigation. Thus, the gross effect of the variants account for the development of disease.

MANAGEMENT

A. BY MODIFICATION OF CULTURAL PRACTICES

Field experiments to examine the effect of certain modifications in the cultural practices such as planting of tomato crop at different dates, application of different insecticides and effect of coloured polythene mulches on virus disease(s) incidence and the yield of tomato were conducted at the Experimental Farm of the Department of Mycology and Plant Pathology at the University Campus, Nauni, Solan during the cropping seasons of 1996 and 1997. The data generated from the observations were analysed statistically and are presented in the following paragraphs.

Table 22. Multiple correlation and regression coefficients between disease incidence, temperature, relative humidity, cumulative rainfall, wind velocity, total sunshine hours and vector population

Year	Coefficient of determination (R^2)	Regression equation
		Y=bo+b1X1 +b2X2 +b3X3 +b4X4 +b5X5 +b
1996	0.9381	$Y=-31.038-1.5419X_1+1.4765X_2 +0.6406X_3-4.9654X_4-0.0032X_5 +0.1376X_6$
1997	0.9173	$Y=6.7226-1.6531X_1+1.7035X_2 +0.9519X_3-9.7360X_4+0.0860X_5 -0.7606X_6$

where,

Y = Per cent disease incidence
X_1 = Mean air temperature (°C)
X_2 = Relative humidity (%)
X_3 = Total sunshine hours (hrs)
X_4 = Wind velocity (km/hrs)
X_5 = Cumulative rainfall (mm)
X_6 = Vector population (No.)

i) Effect of different dates of planting

The effect of planting dates on the incidence of virus disease(s) and yield of tomato was studied by planting the crop at different dates i.e. on 15th March, 30th March, 14th April, 29th April and 15th May of 1996 and 1997. The data are presented in Table 23.

From Table 23 it is evident that the incidence of virus disease was less when the crop was planted early. However, the incidence increased significantly with each subsequent planting date in both the years of experimentation. The highest incidence of 58.33 per cent (49.82) and 61.66 per cent (51.76) during 1996 and 1997, respectively, was recorded when the crop was planted on 15th May, the last planting date.

The effect of planting dates on the yield of tomato was however, different. The highest yield of 12.60 kg and 11.70 kg per plot was registered when the planting was done on 14th April in the years 1996 and 1997, respectively. The lowest yield of 8.77 kg and 7.86 kg per plot was obtained when planting was done on 15th May during 1996 and 1997. During both the years of experimentation, the yield per plot obtained by planting the crop on 15th March, 30th March, 29th April and 15th May was found to be statistically at par with each other.

ii) Effect of polythene mulches

The data presented in Table 24 showed that all the mulch treatments significantly reduced the disease incidence in tomato during both the years of experimentation. However, the lowest disease incidence of 40.00 per cent (39.00) and 43.25 per cent (40.12) in both the years was recorded in the plots mulched with yellow polythene. It differed significantly in showing superiority over the white

Table 23. Effect of different dates of planting on virus disease(s) incidence and yield of tomato

Date	1996			1997		
	Disease incidence (%)	Avg. Yield/plot (kg)	Yield/ha (q)	Disease incidence (%)	Avg. Yield/plot (kg)	Yield/ha (q)
15th March	20.00 (26.34)	9.26	290.28	23.25 (28.79)	8.00	250.78
30th March	26.50 (30.87)	9.34	292.78	31.50 (34.08)	8.60	269.59
14th April	38.25 (38.20)	12.60	394.98	41.66 (40.06)	11.70	366.77
29th April	51.50 (45.88)	8.98	281.50	53.33 (46.88)	8.13	254.85
15th May	58.33 (49.82)	8.77	274.92	61.66 (51.76)	7.86	246.39
$CD_{0.05}$	3.70	1.53		3.92	1.78	

Figures in parentheses are arc sine transformed values.

Table 24. Effect of polythene mulches on the incidence of virus disease(s) and yield of tomato

Sr.No.	Treatment	Disease incidence (%)		Average yield (kg/plot)		% increase in yield over control		Average yield (q/ha)	
		1996	1997	1996	1997	1996	1997	1996	1997
1.	Polythene green	51.50 (45.87)	56.50 (48.76)	12.13	11.90	49.75	51.92	380.25	373.04
2.	Polythene yellow	40.00 (39.00)	43.25 (40.12)	15.60	13.95	98.72	72.22	489.02	437.30
3.	Polythene white	45.10 (42.08)	48.30 (44.00)	12.87	12.30	58.88	56.68	403.44	385.57
4.	Polythene black	53.25 (46.88)	58.35 (49.77)	11.70	10.25	44.44	30.57	366.77	321.31
5.	Control (no polythene)	68.00 (55.64)	71.50 (57.76)	8.10	7.85	-	-	253.91	246.08
$CD_{0.05}$		2.39	2.91	1.80	1.62				

Figures in parentheses are arc sine transformed values

polythene mulch. The black mulch which recorded the highest disease incidence of 53.25 per cent (46.88) and 58.35 per cent (49.77) during 1996 and 1997, respectively was also found to be superior over control (68.00 and 71.50%) and statistically at par with green polythene mulch treatment during both the cropping seasons.

The effect of mulches on the yield was significant over control during both the years. The yellow polythene mulch recorded highest yield of 15.60 kg and 13.95 kg per plot and was significantly superior over green, white and black mulch treatments during both the years. During 1996, green, white and black polythene mulch treatments were found statistically at par with each other. However, in 1997, green and white mulch treatments were statistically at par with each other and both were significantly superior over black mulch treatment.

iii) Effect of insecticidal application

Four insecticides namely, Metasystox 25EC, Malathion 50EC, Monocrotophos 36 SL and Rogor 30 EC were tested for their efficacy to control the virus disease of tomato during 1996 and 1997. The observations for disease incidence and yieSld were recorded for each year and data were analysed statistically.

From the observations (Table 25), it is evident that the application of all chemicals reduced the incidence of the disease in both the years of the investigation. Reduction in disease incidence was significant in all the treatments during both the years. Metasystox was adjudged to be the most effective insecticide. It registered 35.00 per cent (36.18) and 40.70 per cent (36.18) infection against 75 per cent (60.10) and 78.00 per cent (62.10) in control during the cropping seasons of 1996 and 1997, respectively. Rogor was significantly superior over

Table 25. Effect of insecticides on the incidence of virus disease(s) and yield of tomato

Sr.No.	Treatment	Disease incidence (%)		Average yield (kg/plot)		% increase in yield over control		Average yield (q/ha)	
		1996	1997	1996	1997	1996	1997	1996	1997
1.	Malathion 50 EC	60.24 (51.80)	67.25 (55.17)	11.30	10.12	39.16	37.87	354.23	317.24
2.	Monocrotophos 36 SL	51.25 (45.72)	55.50 (48.20)	11.95	10.76	47.16	46.59	374.60	337.30
3.	Rogor 30 EC	45.50 (42.41)	48.00 (43.41)	12.20	11.15	50.24	51.90	382.44	349.52
4.	Metasystox 25 EC	35.00 (36.18)	40.70 (36.18)	14.80	13.50	82.26	83.92	463.94	423.82
5.	Control	75.00 (60.10)	78.00 (62.10)	8.12	7.34	-	-	254.54	230.09
$CD_{@05}$		3.20	3.92	1.35	1.23				

Figures in parentheses are arcsine transformed values

monocrotophos in decreasing disease incidence during both the years. Malathion was found to be the least effective in reducing disease incidence, registering 60.24 per cent (51.80) and 67.25 per cent (55.17) virus infection during 1996 and 1997, respectively.

The effect of insecticidal applications on yield was highly significant over control in both the years. Metasystox recorded the highest yield of 14.80 kg and 13.50 kg per plot which was 82.26 per cent and 83.92 per cent higher over control during 1996 and 1997, respectively. Malathion was the least effective insecticide in increasing the yield of tomato but it was statistically at par with Monocrotophos and Rogor during both the years.

B. SCREENING OF TOMATO GERMPLASM

One hundred tomato in entries (varieties / collections / lines) were evaluated under glasshouse conditions to see their reaction against the virus isolate (Tom-1) producing the most striking symptoms. Similarly, all the above entries were evaluated under field conditions also to see their reaction against the prevalent viral diseases. Reaction of each entry was recorded based on their comparative reactions (susceptibility/resistance). These were categorized into five classes viz., highly resistant (HR), resistant (R), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) as mentioned under Material and Methods. The reactions of these entries are presented in Tables 26 and 27.

It is apparent from Table 26 that out of 100 entries none was found to be either highly resistant or resistant under glasshouse conditions. Eleven lines/varieties viz., Homestead, Pearson, Valiant, LA1511, LA2702, PS921891, EC174045, EC9222, EC17439, EC37319, EC56123 were susceptible and nine lines/varieties viz., Azad Kirti, Pusaruby, BWR-1, M2, EC130163, EC1221710,

Table 26. Reaction of tomato germplasm to virus diseases under glass house conditions

Variety/collection	Under glass house conditions	
	Per cent disease incidence	Reaction
Arka Vikas	50	HS
Azad Kirti	20	MS
Cal Ace	60	HS
Domestes	80	HS
Homestead	30	S
Holarank	60	HS
Hisarlalima	50	HS
Labonita	70	HS
Marthob	40	HS
Marglobe Supreme	80	HS
Money Maker	70	HS
Pant Bahar	40	S
Pusaruby	20	MS
Punjab Kesri	70	HS
Pearson	30	S
Roma	60	HS
Sioux	70	HS
Srinagar	50	HS
Selection-2	60	HS
Selection-4	50	HS

Contd.....

Variety/collection	Under glass house conditions	
	Per cent disease incidence	Reaction
Selection-16	60	HS
Trucker	40	HS
Yala Bing Ru	40	HS
V. Yorker	50	HS
Valiant	30	S
BT-10	70	HS
BWR-1	20	MS
HS-101	70	HS
HT-6	60	HS
LA-1388	40	HS
LA-1511	30	S
LA-1204	90	HS
LA-35273	70	HS
LA-2702	30	S
LA-2670	80	HS
LE-79	40	HS
M-1	80	HS
M-2	20	MS

Contd .....

Variety/collection	Under glass house conditions	
	Per cent disease incidence	Reaction
M-3	60	HS
M-7	50	HS
M-8	40	HS
M-10	70	HS
S9P7M1	60	HS
S9P8M2	80	HS
PNR-7	70	HS
PS921891	30	S
T11564	40	HS
T111	80	Hs
TH11	80	HS
TER1	70	HS
W55	80	HS
LA1204xLA1283	80	HS
LA2912xEC174057	50	HS
Punjab Chhuhara x LA1783	60	HS
Punjab Chhuhara x LA2992	80	HS
Roma x Punjab Chhuhara	60	HS
Roma x EC714057	80	HS
Sioux x EC7662	70	HS
SGP8M2 x EC174057	40	HS

Contd.....

Variety/collection	Under glass house conditions	
	Per cent disease incidence	Reaction
EC12966	90	HS
EC12963	80	HS
EC93497	60	HS
EC95152	50	HS
EC106761	80	HS
EC122963	70	HS
EC130163	20	MS
EC143593	50	HS
EC143590	100	HS
EC141830	50	HS
EC161245	70	HS
EC174045	30	S
EC174076	80	HS
EC174023	40	HS
EC174031	100	HS
EC203595	60	HS
EC212469	70	HS
EC272982	100	HS
EC503077	40	HS
EC1221710	20	MS
EC-9922	30	S
EC-3264	40	HS

Contd.....

Variety/collection	Under glass house conditions	
	Per cent disease incidence	Reaction
EC-4258	20	MS
EC-7728	100	HS
EC-9918	60	HS
EC-9149	80	HS
EC-10049	60	HS
EC-54315	40	HS
EC-54628	20	MS
EC-27051	50	HS
EC-37373	70	HS
EC-12655	100	HS
EC-13423	20	MS
EC-17439	30	S
EC-25870	40	HS
EC-37235	70	HS
EC-37319	30	S
EC-65984	70	HS
EC-95722	60	HS
EC-56123	30	S
EC-41824	60	HS

Per cent infection	Rating
0-5	Highly resistant (HR)
6-10	Resistant (R)
11-20	Moderately susceptible (MS)
21-30	Susceptible (S)
Above 30	Highly susceptible (HS)

Table 27. Reaction of tomato germplasm to virus diseases under field conditions

Variety/collection	Under field conditions	
	Per cent disease incidence	Reaction
Arka Vikas	60	HS
Azad Kirti	40	HS
Cal Ace	50	HS
Domestes	90	HS
Homestead	50	HS
Holarank	40	HS
Hisarlalima	60	HS
Labonita	80	HS
Marthob	50	HS
Marglobe Supreme	70	HS
Money Maker	90	HS
Pant Bahar	30	S
Pusaruby	50	HS
Punjab Kesri	90	HS
Pearson	40	HS
Roma	80	HS
Sioux	50	HS
Srinagar	30	S
Selection-2	90	HS
Selection-4	30	S

Contd.....

Variety/collection	Under field conditions	
	Per cent disease incidence	Reaction
Selection-16	40	HS
Trucker	60	HS
Yala Bing Ru	50	HS
V. Yorker	60	HS
Valiant	50	HS
BT-10	90	HS
BWR-1	40	HS
HS-101	50	HS
HT-6	40	HS
LA-1388	70	HS
LA-1511	50	HS
LA-1204	70	HS
LA-35273	50	HS
LA-2702	30	HS
LA-2670	100	HS
LE-79	70	HS
M-1	100	HS
M-2	30	S

Contd .....

Variety/collection	Under field conditions	
	Per cent disease incidence	Reaction
M-3	80	HS
M-7	70	HS
M-8	20	MS
M-10	50	HS
S9P7M1	40	HS
S9P8M2	100	HS
PN-7	80	HS
PS921891	40	HS
T11564	90	HS
T111	70	Hs
TH11	90	HS
TER1	100	HS
W55	100	HS
LA1204xLA1283	60	HS
LA2912xEC174057	70	HS
Punjab Chhuhara x LA1783	70	HS
Punjab Chhuhara x LA2992	90	HS
Roma x Punjab Chhuhara	60	HS
Roma x EC714057	90	HS
Sioux x EC7662	100	HS
SGP8M2 x EC174057	30	S

Contd.....

Variety/collection	Under field conditions	
	Per cent disease incidence	Reaction
EC12966	90	HS
EC12963	100	HS
EC93497	100	HS
EC95152	70	HS
EC106761	90	HS
EC122963	60	HS
EC130163	50	HS
EC143593	30	S
EC143590	100	HS
EC141830	70	HS
EC161245	90	HS
EC174045	40	HS
EC174076	90	HS
EC174023	60	HS
EC174031	80	HS
EC203595	90	HS
EC212469	70	HS
EC272982	100	HS
EC503077	60	HS
EC1221710	50	HS
EC-9922	50	HS
EC-3264	70	HS

Contd.....

Variety/collection	Under field conditions	
	Per cent disease incidence	Reaction
EC-4258	50	HS
EC-7728	100	HS
EC-9918	80	HS
EC-9149	60	HS
EC-10049	90	HS
EC-54315	50	HS
EC-54628	30	S
EC-27051	70	HS
EC-37373	80	HS
EC-12655	100	HS
EC-13423	40	HS
EC-17439	50	HS
EC-25870	60	HS
EC-37235	90	HS
EC-37319	70	HS
EC-65984	100	HS
EC-95722	90	HS
EC-56123	50	HS
EC-41824	50	HS

Per cent infection	Rating
0-5	Highly resistant (HR)
6-10	Resistant (R)
11-20	Moderately susceptible (MS)
21-30	Susceptible (S)
Above 30	Highly susceptible (HS)

EC4258, EC54628 and EC13423 were recorded tobe moderately susceptible under glasshouse conditon and the rest were found to be highly susceptible.

Similarly, none of the entries were found to be highly resistant or resistant under field conditions (Table 27). Out of the 100 varieties/lines/ collections tested, 7 varieties/lines/collections viz. Pant Bahar, Sringar, Selection 4, M2, SPG8M2 x EC174057, EC143593, EC54628 were found susceptible and 1 line viz.; M8 was found to be moderately susceptible under field conditions. Ninety two lines were found to be highly susceptible.

DISCUSSION

Tomato (*Lycopersicon esculentum* Mill.) has been reported to be infected by about 36 viruses and their strains belonging to 17 genera under natural conditions in different parts of the world (Chenulu and Giri, 1985).

The viral disease (s) of tomato occurring under natural conditions in different localities of Solan, Shimla, Sirmour, Kullu and Mandi districts of Himachal Pradesh have been studied in detail with respect to incidence of viral diseases in different localities and the identification of the tomato virus isolates collected from different places by taking into account the various established identification criteria. Studies were also conducted on the epidemiology and the management of the disease(s) through modification of cultural practices, insecticidal sprays and varietal screening. The results of these investigations are discussed hereunder.

The incidence of the virus disease(s) in different localities varied from 10 to 80 per cent and 13 to 72 per cent in the years 1996 and 1997, respectively. The symptomatology and transmission studies of different isolates ruled out the possible association of any fungal/bacterial pathogen, indicating thereby the viral etiology of the disease(s). The various isolates representing different locations have been studied for their identification keeping in view the guidelines laid down by Hamilton *et al.* (1981) and Black (1982). Thus, during the course of present investigations efforts were made to identify the isolates by employing as many parameters as could be possible as mentioned by above workers. The criteria included

symptomatology, host range, transmission, biophysical properties, particle morphology, cytopathology and serological relationships.

Different tomato virus isolates (T_1 to T_{21}) were initially collected from different tomato growing areas of the state and were maintained under glasshouse conditions. After careful comparision of the symptoms produced by these isolates and also the reaction on certain indicator plants, these were grouped into seven isolates for further studies on the identification. These isolates not only produced different types of symptoms in host plants, but also differed in their biophysical properties, host range and in their ability to be transmitted by certain aphid vectors.

All the isolates viz. Tom-1, Tom-2, Tom-3, Tom-4, Tom-5, Tom-6 and Tom-7 were found to be sap transmissible. None of these were transmitted by aphids except the isolate Tom-3 which was transmitted by the aphid *Myzus persicae* and *Aphis craccivora*. However, the aphid *Brevicoryne brassicae* failed to transmit this isolate.

In symptomatology, isolate Tom-1 resembles rosette strain of TMV (Price and Fenne, 1951)as both caused extreme malformation of foliage accompanied by severe stunting of plants. Similarly, Isolate Tom-2 resembles common strain of ToMV reported by Rast (1975). The symptomatology of Tom-5 and Tom-7 isolates did not resemble with any of the strains of ToMV reported in literature indicating thereby that on the basis of symptoms these isolates may be distinct from the already reported tomato strains. Isolate Tom-6 induced similar type of symptoms on tomato as those produced by Dahlemese strain of ToMV (Melchers *et al.*, 1940). The symptoms induced by the isolate Tom-3 resembled with those induced in *Datura* by a *Potyvirus* (Prasanna *et al.*, 1996) which caused formation of filiform leaves in Datura. Both these viruses produce similar symptoms on *Nicotiana*

tabacum var. white burley and *Nicotiana rustica*; and had almost similar biophysical properties.

All these isolates also differed in their biophysical properties. Isolates Tom-1, Tom-2, Tom-6 were inactivated at a temperature of 85°C, Tom-5 and Tom-7 at 80°C, whereas Tom-3 got inactivated at 60°C and Tom-4 at 45°C, respectively. Similarly, the dilution end point of isolates Tom-2, Tom-3, Tom-4, Tom-5, Tom-6 and Tom-7 was found to be between 10^{-3} and 10^{-4}, whereas, isolate Tom-1 was rendered innocuous at 10^{-5} dilution. The longevity *in vitro* of isolates Tom-1, Tom-6 and Tom-7 was between 60 and 70 days whereas isolates Tom-2 and Tom-5 had longevity *in vitro* between 70 and 80 days when stored at room temperature (22-26°C). Isolate Tom-3 remained infective for 4 days whereas longevity in vitro of isolate Tom-4 ranged between 5 to 7 hours only. The isolates Tom-2, Tom-5 and Tom-7 resemble TMV strain reported by Prasada and Reddy (1971) in respect of having almost similar biophysical properties particularly dilution end point. This strain was found to withstand a dilution of 10^{-3}. However, isolate Tom-1 differed from this strain in having a high dilution end point (infectious at 10^{-4} dilution). All the above isolates (Tom-2, Tom-5, Tom-7) seem to resemble a strain of TMV reported on plantago by Holmes (1941) in respect of thermal inactivation point. The strain from plantago was inactivated at 93°C. On the basis of symptomatology and dilution end point (10^{-3}-10^{-4}), the virus resembled the one reported by Prasada *et al.* (1980) from Andhra Pradesh. However, it differed from it in respect of TIP (40-45°C) and LIV (5-7 hrs). The above workers reported the TIP and LIV of the virus as 45-50°C and 1-2 hours, respectively. The two viruses also resembled in their host range (*Chenopodium quinoa, C. amaranticolor, Nicotiana glutinosa and N. tabacum* Var. white burley.

The host range of all the isolates except Tom-3 was found to be confined to members of the families chenopodiaceae, and Solanaceae only indicating thereby that they had rather restricted host range. Isolate Tom-3 did not infect any plant species belonging to family Chenopodiaceae. Lovisolo and Conti (1969) also did not find any two tomato virus isolates identical when they compared 60 isolates (obtained from different localities) on host range and symptom basis. Similarly, in a similar analogy Parmar (1991) collected twenty two bell pepper virus isolates from different localities in Himachal Pradesh but did not find any two isolates to be identical in their host range.

The DAC ELISA studies revealed the presence of TMV in isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 and TSWV in isolate Tom-4.

Electron microscopic studies of Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 isolates confirmed the presence of TMV particles (300 x 18nm). All these isolates contained rigid rod shaped virus particles. In IEM studies also these isolates were decorated only with antiserum against strain D of TMV. Trapping index was also found to be the highest with antiserum against the same strain. Thesefore, these isolates are serologically closely related to strain D of TMV.

Isolate Tom-3 consisted of flexuous virus particles measuring 730 x 11nm. The virus was confirmed as belonging to *Potyvirus* genus on the basis of shape and size of virus particle and type of inclusions produced. The trapping and decoration of virions was very good with antisera against leaf distortion virus of Datura, Peru tomato and Papaya ringspot viruses indicating thereby its close serological relationship with these viruses.

Immuno electron microscopical studies of isolates Tom-4 confirmed the identity of the virus as tomato spotted wilt virus (TSWV). The particle diameter was 80nm.

Hence, based on different identification criteria the seven isolates were identified as TMV (Isolates Tom-I, Tom-2, Tom-5, Tom-6 and Tom-7); *Potyvirus* (Isolate Tom-3) and TSWV (Isolate, Tom-4) and were placed in *Tobamovirus*, *Potyvirus* and *Tospovirus* genera.

However, these isolates could not be precisely characterized upto specific strains of the above mentioned viruses due to non-availability of sophisticated instrumental facilities to work out the intrinsic properties at molecular level.

EPIDEMIOLOGY

Plant virus epidemiology is a complex phenomenon as vector populations which are actively and intimately involved in the spread of plant virus diseases are also to be taken into account besides populations of different individuals (pathogen and host) and their varying interaction over a wide range of environmental factors.

During the present investigations, an attempt has been made to find out the role played by different environmental components (temperature, relative humidity, cumulative rainfall, wind velocity, total sunshine hours) and vector population in the development of tomato virus disease(s) under field conditions.

VIRUS VECTOR AND ENVIRONMENTAL FACTORS

The results demonstrated that the disease appeared in the month of May in both the cropping seasons of the years 1996 and 1997 with 0.40 and 0.60 per cent incidence, respectively. The prevailing mean air temperature was 25.50 and 24.10 °

C, alongwith the relative humidity of 32.00 and 38.50 per cent. The vector population (aphids) also started building up.

The simple, partial and multiple correlation studies indicated that the disease incidence had a strong and positive correlation with the vector population and temperature during the course of studies, which indicates that the disease increased with an increase in the vector population as well as an increase in temperature.

The correlation of disease incidence with vector population has also been demonstrated by various workers. Watson (1936, 1938, 1940) and Watson and Roberts (1939) correlated the widespread occurrence of PVY in tomato with the prevalence of the vector, *Myzus persicae*. They also reported transmission of the virus by *Aphis fabae, Macrosiphum euphorbiae, Myzus certus, Phorodon humuli, Rhopalosiphum insertum and R. padi.* Butter and Rataul (1977) correlated spread of tomato leaf curl virus in tomato with the whitefly vector i.e. *Bemisia tabaci* populations on the crop. Similarly, Basky (1983) found a positive correlation between total number of aphids i.e. *Myzus persicae* caught during late May to early August and the incidence of CMV. McKeen (1954) correlated the widespread occurrence of tobacco etch virus disease of sweet pepper in Ontario with the prevalence of the vector, *M. persicae*. It was postulated by the above authors that the epidemic in 1950 might have resulted from migration of winged adult aphids from the etch infested tobacco crops in the nearby fields. Sharma (1988) also observed a direct correlation of the incidence of CMV and potyvirus isolates with vector populations in bell pepper.

The temperature is often a very important determining factor in the incidence of many virus diseases. It not only determines the ease of infection but also affects the multiplication of virus and symptom expression. The relationship might be

assigned to the evidence of adaptation of the virus to the weather conditions as influenced by temperature (Singh *et al.*, 1984).

Temperature was observed to be positively correlated with the disease incidence in the present studies. Chowfla *et al.* (1989) reported a highly significant positive correlation between severity of shoe-string disease of tomato caused by potato virus Y and temperature in Himachal Pradesh, India.

The role of temperature as a determining factor of disease development has also been reported in the findings of Nagaich *et al.* (1969) and Bindra *et al.* (1974) who found that the incidence of PVY and potato leaf roll virus (PLRV) increased with an optimum of 26^{o}C which was also congenial for the aphid population.

The population density, flight behaviour and feeding activity of vectors are also determined by temperature alongwith the rainfall (Bos, 1982). Berg (1984) studied the effect of temperature on *Aphis craccivora* populations build up and found that the intrinsic rate of natural increase arose from zero at 5^{o}C to a maximum at 30^{o}C and then declined rapidly to zero at 35^{o}C indicating thereby, a strong relationship between temperature and vector populations. The results obtained under the present studies also showed a positive correlation of vector population with temperature when all other factors were kept constant during both the years of experimentation.

The significant positive correlation of relative humidity with disease incidence during the course of present studies indicated that relative humidity itself is not independent but is controlled by some other environmental factors. The effect of humidity during both the cropping seasons might be on the activity of vectors rather than on the virus. Nitzany (1975) in Israel noticed that tomato leaf curl virus (TLCV) outbreaks were always in the months with a mean relative humidity of more than 60

per cent. However, the incidence of mosaic disease of bell pepper was observed to be negatively correlated with relative humidity during the cropping seasons of 1986 and 1987 (Sharma, 1988).

The rainfall, in general, has a negative effect on the vector population and disease incidence (Singh *et al.*, 1984; Sharma, 1988; Andotra, 1990 and Parmar, 1991). The correlation of cumulative rainfall with disease incidence when all other factors were kept constant was observed to be negative during the years 1996 and 1997.

The other important environmental factors such as wind may be an important factor not only in assisting or inhibiting spread of viruses by air borne vectors but also in determining the predominant direction of spread. The observations recorded in the present studies showed a negative correlation of disease incidence with wind velocity during 1996 while it showed a positive correlation during 1997 cropping seasons when all other factors remained constant. The probable reason for this discrepancy could be the prevailing direction of wind.

Ossianilsson (1966) described an outbreak of bean yellow mosaic virus in Scandinavia that appeared to be due to a large scale invasion of swarms of winged aphids.

The correlation of disease incidence and total sunshine hours in the present investigations was observed to be positive when all other factors remained constant. Yagamuchi (1976) and Matsuka and Miller (1978) observed that the population of green spruce aphids, *Elatobium abietinum* is influenced by crowding, host quality and temperature, the dominant factor being the increase in daylength in spring. The critical daylength is 17 hours beyond which the proportion developing into alate declines. Increased alate production in response to change in daylength

has also been reported for *M. persicae*. The present investigations also showed a positive correlation of total sunshine hours with vector population (*M. persicae*) during the two years of experimentation. Kotwal (1981) also observed that *Brevicoryne brassicae* reached their peak at the accumulation of 688 degree-days ($5^{o}C$ base). It might be possible to predict the aphid population based on degree days in the field to determine if they were close to the economic threshold.

MANAGEMENT

A. BY MODIFICATION OF CULTURAL PRACTICES

i) Effect of different dates of planting

The spread of virus diseases by air borne vectors can be avoided or minimized by altering the sowing or transplanting dates of the crop. The optimum time of planting will depend on the normal migration period of the vector, so late plantings will avoid early vector flights and vice-versa (Walkey, 1985). In the present investigations, early grown tomato crops showed less incidence of virus disease(s) in comparison to the later planting dates in both the years (Table 23). However, the results on yield varied significantly. The maximum yield of 394.98 q/ha and 366.77 q/ha was found in tomato crop grown by the second week of April (14th April) during 1996 and 1997, respectively. The crop transplanted during the second week of May (15th May) resulted in maximum disease incidence and reduced yields in both the years.

Elenkov (1959), Aleksic *et al.* (1969) and Martinov (1969) also recorded significant reduction in the incidence of Stolbur virus infection in the early grown pepper crops with substantial yield reductions. In Southern Turkey, TYLCV incidence could be considerably reduced by planting the fall crop a few weeks later, at the beginning of October, instead of the usual planting in August/September when whitefly populations are highest (Abak *et al.*, 1991). Similarly, in the Jordan

valley, disease incidence is lower when the fall crop is planted later (Kasrawi, 1991).

However, the reduced fruit yield in the present studies inspite of low incidence in early planted crop might be due to the hot and dry weather conditions prevailing in the early part of the crop growth period. Further more, the best time to plant will depend on the time of migration of the vector. If it is alate migration, early planting may allow the plants to become quite large before they are infected. In the north western India seed potato are raised during autumn (October to December) when the aphid vector, *M. persicae* populations were negligible (Pushkarnath, 1976). In the present studies, aphid vectors (*M. persicae and A. craccivora*) populations also reached its peak at a time when the plants were quite old to resist the attack.

ii) Effect of polythene mulches

Yellow, green, white and black polythene mulches were used to see their effect on the virus disease incidence and yield of tomato during the cropping seasons of 1996 and 1997. The use of polythene mulches was found to be quite effective in reducing the disease incidence as well as in enhancing yield of tomato significantly in both the cropping seasons. Reflected yellow light induces alighting behaviour and favours settling. Aphids land on the yellow surface and probe on it. This leads to loss of virus particle on the surface. The use of yellow polythene mulch resulted in lowest disease incidence with significant increase in yield. Similar findings were also reported by Kring and Schuster (1992) in field experiments conducted at Florida (USA) by using aluminium painted mulch.

Soil mulching with yellow polythene sheets delays spread of tomato yellow leaf curl virus on tomato for atleast 20 days (Cohen and Melamed Madjar, 1978).

Cohen and Marco (1973) also recorded reduced spread of CMV and PVY in Israel by placing the sticky yellow polythene sheets outside a field of pepper. Similar observations in respect of aluminium foil and plastic sheets have also been reported in case of aphid borne CMV and PVY infections (Loebenstein *et al.*, 1975).

Iii) Effect of insecticidal sprays

In the present studies, the use of metasystox, a systemic insecticide resulted in highly significant control of disease by reducing the incidence (35.00 and 40.70 per cent) as well as increase in fruit yield (82.26 and 83.92 per cent increase over control). Szalay and Solymossy (1962) were also able to reduce infection of CMV in *C. annuum* in Hungry by 33 per cent after spraying with 0.1 per cent metasystox at 10 days interval in 1958 and 1959. Similarly, the incidence of CMV in tomato had also been reported to be reduced by spraying a number of insecticides viz. endosulfan 35EC, methomyl 18 L, aldicarb 15 G and carbofuran 34 (Xuan *et al.*, 1988).

B) Screening of germplasm

One hundred germplasm entries (varieties / collections / lines) of tomato were evaluated both under glasshouse as well as under field conditions. None of the lines were found to be resistant under both conditions. However, eight entries viz., Pantbahar, Srinagar, Selection-4, LA-2702, M-2, SPG8M2 x EC174057, EC-143593, EC-54628 were found to be susceptible and one line M-8 was found to be moderately susceptible under field conditions. Under glasshouse conditions Homestead, Pearson, Valiant, LA-1511, LA-2702, PS921891, EC-174045, EC-9222, EC-17439, EC-37319 and EC-56123 were found to be susceptible whereas, Azad Kirti, Pusaruby, BWR-1, M-2, EC-130163, EC-1221710, EC-4258, EC-54628 and EC-13423 were found to be moderately susceptible. All other varieties/collections/lines were found to be highly susceptible both under field and

glasshouse conditions. Behl and Chowfla (1989) tested 115 cultivars/lines of tomato against PVY in Himachal Pradesh. None of the lines was immune to the disease. However, tomato lines HC-238 and S-12 were recorded to be highly resistant. Similarly, Silva *et al.* (1986) screened 31 lines/cultivars of *Lycopersicon* species for resistance against TMV under glasshouse conditions and found the lines viz. LA-1221 and LA-2088 to be resistant.

SUMMARY

The present investigations were undertaken to identify the different viruses, causing serious disease(s) in tomato under natural conditions in different tomato growing regions of the state such as Solan, Shimla, Sirmour, Mandi and Kullu. An attempt was also made to study the epidemiology of viral disease(s) of tomato besides working out suitable management practices.

The incidence of viral diseases ranged between 10 to 80 per cent and 13 to 72 per cent during the cropping seasons of 1996 and 1997, respectively. The symptomatology and serial transmission tests proved the viral etiology of tomato diseases.

The symptoms evoked by various isolates were in the form of mosaic, mottling, blistering, leaf rolling, curling of leaves, leaf bronzing, shoe-stringing etc.

All the isolates collected from different localities were found to be sap transmissible,except for isolate Tom-3 which was also transmitted by the aphids *Myzus persicae and Aphis craccivora.*

The host range of different isolates was more or less restricted to the families Chenopodiaceae, Solanaceae and Leguminosae.

The biophysical properties i.e. TIP of isolates Tom-1, Tom-2 and Tom-6 ranged between 80-85^{o}C, while that of isolates Tom-5 and Tom-7 lied between 75-

80°C, and that of isolate Tom-3 between 55-60°C, while the TIP of isolate Tom-4 was between 40-45°C. The DEP of all the isolates except for isolate Tom-1 ranged between 1:1000 and 1:10000. The DEP of isolate Tom-1 ranged between 1:10000 and 1:100000. Similarly, the longevity *in vitro* of isolates Tom-1, Tom-6 and Tom-7 was between 60 and 70 days, of Isolates Tom-2 and Tom-5 between 70 and 80 days and that of isolate Tom-3 between 96 and 120 hours. Isolate Tom-4 had the lowest longevity *in vitro* lying between 5 and 7 hours.

Electron microscopic studies revealed that the isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 consisted of rigid rod shaped particles (300 x 18 nm), whereas the isolate Tom-3 had flexuous particles (730 x 11 nm) and isolate Tom-4 had isometric particles (80 nm diameter).

The cytopathological studies indicated that the isolate Tom-3 induced cylindrical cytoplasmic inclusions in the infected host cells consisting of pinwheel, scrolls and laminated aggregates typical of the genus *Potyvirus*. The isometric particles of the isolate Tom-4 aggregated in the citernae of endoplasmic reticulum.

Immunosorbent electron microscopy (ISEM) showed markedly enhanced trapping of virions (10/20 at 21,000) of isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 with TMV-D strain antiserum. Isolate Tom-3 exhibited enhanced trapping of the virions with antiserum against leaf distortion virus of datura (5.0) followed by trapping with antisera against Peru tomato virus (4.5) and papaya ringspot virus (4.0). Alkaline phosphatase (ALP) based direct antigen coating (DAC) form of ELISA also substantiated the results of ISEM.

Hence, based on different identification parameters particularly the particle morphology, cytopathology and serological relationship the isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 were identified as belonging to the genus *Tobamovirus*

and further serologically characterized to be closely related to strain-D of TMV. Similarly, isolate Tom-3 was identified as belonging to the genus *Potyvirus* and showing, close serological relationship with leaf distortion virus of datura, Peru tomato and papaya ring spot viruses. The tomato isolate Tom-4 was identified as tomato spotted wilt virus.

Epidemiology of tomato virus disease(s) was studied in respect of mean air temperature, average relative humidity, cumulative rainfall, average wind velocity, total sunshine hours and vector population. Multiple correlation coefficients between the disease incidence and group of independent variables were worked out to be 0.9381 and 0.9173 during 1996 and 1997 cropping seasons indicating thereby that the interaction between plants and virus was greatly influenced by these factors.

Early planted tomato showed less disease incidence (20.00 and 23.25 per cent during 1996 and 1997 cropping seasons) than the ones planted on later dates. However, maximum yield per hectare (394.98 q/ha and 366.77 g/ha during 1996 and 1997 cropping seasons) was found in the tomato crop transplanted on 14th of April.

The use of polythene mulches (yellow, white, green and black) not only reduced the disease incidence but also increased the yield (98.72 and 72.22 per cent in yield over control) significantly. Insecticidal application of metasystox 25 EC and rogor 30 EC were also found effective in reducing the incidence of virus disease(s) and increasing the yield of tomato (82.26 and 83.92 per cent increase over control).

Out of hundred tomato collections/lines tested, none of the entries were found to be resistant under glass house and field conditions.

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APPENDIX-I

ELISA BUFFERS

1. Extraction/coating buffer (carbonate buffer, pH 9.6).

Na_2CO_3	:	1.59 g
$NaHCO_3$	:	2.93 g
Distilled water	:	to make 1 litre

2. Stock buffer (PBS-Phosphate buffer saline, pH 7.4)

NaCl	:	8.0 g
Na_2HPO_4	:	1.50 g
KH_2PO_4	:	0.20 g
KCl	:	0.20 g
Distilled water	:	to make 1 litre

3. Wash buffer (PBS-Tween, PBS-T) Add 0.5 ml Tween-20 to 1 litre PBS

4. Enzyme conjugate diluent/buffer (PBS-TPO)

Polyvinyl pyrrolidone (PVP, MW44000):		20.0 g
Egg ovalbumin	:	2.0 g
		Add to 1 litre PBS-T

7. Antibody diluent/buffer : Same as in Sr. No. 4

8. Substrate buffer (Diethanolamine, pH 9.8)

Diethanolamine	:	20 ml
Distilled water	:	160 ml
		Adjust pH with 1 N NaCl

9. Fixing solution (to stop reaction)

NaOH	:	120.0 g
Distilled water	:	to make 1 litre

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THESIS ABSTRACT

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Title of Thesis	:	**Identification and Management of Viruses Infecting Tomato in Himachal Pradesh**
No. of pages in thesis	:	107 + (xxii)
No. of words in abstract	:	254

ABSTRACT

Virus disease(s) infecting tomato and causing serious losses were prevalent in different tomato growing localities of Solan, Shimla, Sirmour, Mandi and Kullu districts of Himachal Pradesh. Total 21 isolates collected were further grouped into 7 on the basis of their reaction on indicator plants. These isolates were sap transmissible. Only the isolate Tom-3 was aphid transmissible. The host range of these isolates was restricted to families *Chenopodiaceae, Solanaceae,* and *Leguminosae*. The TIP of different isolates was: Tom-1, Tom-2, Tom-6 (80-85^{o}C); Tom-5 and Tom-7 (75-80^{o}C); Tom-3 (55-60^{o}C) and Tom-4 (40-45^{o}C). The DEP of all the isolates except for Tom-1 ranged between 10^{-3} and 10^{-4} (Tom-1 : 10^{-4}-10^{-5}). Similarly, LIV of different isolates was: Tom-1, Tom-6, Tom-7 (60-70 days); Tom-2, Tom-5 (70-80 days); Tom-3 (90-120 hours) and Tom-4 (5-7 hours). ELISA and ISEM studies revealed isolates Tom-1, Tom-2, Tom-5, Tom-6 and Tom-7 as TMV; Tom-3 as a *Potyvirus* and Tom-4 as TSWV. Isolate Tom-3 induced formation of pinwheels, scrolls and laminated aggregates in the infected host cells. Multiple correlation coefficients between the disease incidence and group of independent variables were 0.9381 and 0.9173 during 1996 and 1997. The maximum yield was found in the tomato crop transplanted on 14th April during both the years. The yellow polythene mulch significantly reduced the disease incidence and increased the yield as compared to white, green and black mulches. Metasystox 25EC and Rogor 30EC were effective in reducing the tomato virus disease incidence and increasing the yield. None of the tomato germplasm entries were resistant under glasshouse and field conditions.

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