

**STUDIES ON VIRUS INFECTING TOMATO
(*Lycopersicon esculentum* Mill.) IN
MARATHWADA REGION**

BY

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2009

CANDIDATE' S DECLARATION

I hereby declare that the dissertation or part thereof

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(Dhimal Y.A.)

**DEDICATED TO ALL THOSE ASPIRANTS
IN THE WORLD OF VIROLOGY**



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Miss. DHUMAL YOGITA ASHOK has satisfactorily prosecuted her course of research for a period of not less than two semesters and that the dissertation entitled “**STUDIES ON VIRUS INFECTING TOMATO (*Lycopersicon esculentum* Mill.) IN MARATHWADA REGION**” submitted by her is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination.

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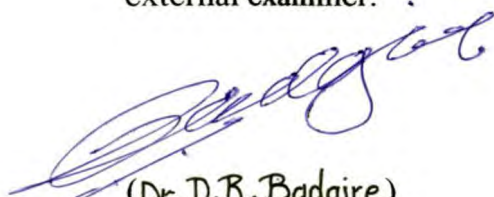
The assistance and help received during the course of this investigation has been fully acknowledged.

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CERTIFICATE-II

This is to certify that the dissertation entitled “**STUDIES ON VIRUS INFECTING TOMATO (*Lycopersicon esculentum* Mill.) IN MARATHWADA REGION**” submitted by **Miss. DHUMAL YOGITA ASHOK** to Marathwada Agricultural University, Parbhani in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **PLANT PATHOLOGY** has been approved by the Student’s Advisory Committee after viva-voce examination in collaboration with the external examiner.



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.... to know even one life has breathed easier because you have lived – that is to have succeeded.

- **Ralph Waldo Emerson**

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Place : Parbhani
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(Ms. Dhumal Y.A.)

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INTRODUCTION



Chapter-I

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an herbaceous plant belonging to the family solanaceae. It is a native of tropical America and is cultivated in about 130 different countries (FAO, 1981).

Tomato is the world's largest vegetable crop. Brazil, China, Cuba, Egypt, India, Indonesia, Russia, Spain etc. are the leading producers of tomato. The area under tomato cultivation is 45,50,719 ha and production is 12,50,15,792 tonnes in the world. The Spanish introduced the tomato to the Asian continent. India ranks 5th in the production with 5,40,000 ha area under tomato cultivation and 76,00,000 tonnes production (FAO, 2005). The area and production of tomato in Maharashtra is 35,000 ha and 9,87,000 tonnes respectively (Anonymous, 2006).

Tomato is mainly cultivated for its fleshy fruit. Carver (1918) gave 115 ways of preparing it for table food. It is used in various processed products like ketchup, sauce, chutney, soup, paste, puree etc. Both green and ripe tomatoes are used for vegetable preparation. It is also consumed as raw fruit. It is an integral part of most of the Indian recipes. It is a rich source of vitamin A and C; minerals like iron and phosphorus. Each 100 gm of ripe tomato can supply about 20 and 40 per cent of the recommended daily allowance of vitamin A and C respectively for adults. It also contains lycopene, beta carotene and dietary fibers. Presence of fructose and citric acid

in tomato are of primary importance in flavour development (Janes, 1994). It also possesses medicinal value as it lowers the risk of cancer (Aykroyd, 1963)

Tomato is most widely grown and commercially important vegetable crop. Amongst the various constraints contributing to low yields and quality, diseases play a major role. The crop is known to be infected by several fungal, bacterial, viral and nematode pathogens. Of these, early blight caused by *Alternaria solani*, bud necrosis caused by tomato spotted wilt virus and leaf curl caused by tomato leaf curl virus are of economic importance in India including Maharashtra.

The viruses infecting tomato are given in Table 1.

Table 1 Viruses infecting tomato

| Taxonomic group | Virus | Key references |
|---------------------------------------|--------------------------|------------------------------------|
| A) Insect transmitted viruses: | | |
| 1) Alfamovirus | Alfa mosaic (AMV) | Martelli and Quacquarelli (1982). |
| 2) Carlavirus | Cowpea mild mottle | Brunt and Phillip (1981). |
| | Potato virus M (PVM) | Misra <i>et al.</i> (1979). |
| | Potato virus S (PVS) | Kuznetsova and Tursumetova (1969). |
| 3) Caulimovirus | Cauliflower mosaic (CMV) | Martelli and Quacquarelli (1982). |

| | | |
|----------------|----------------------------------|---|
| 4) Cucumovirus | Cucumber mosaic (CMV) | Kobatake (1974), Srivastava <i>et al.</i> (1975), Nitzany (1975). |
| | Tomato aspermy (TAV) | Kuti and Moline (1984), Ahmad and Scott (1987). |
| 5) Geminivirus | Tomato golden moaic (TGMV) | Matyis <i>et al.</i> (1976). |
| | Tomato yellow leaf curl (TYLCV)* | Yassin and Nour (1965), Cohen and Nitzany (1966), Nitzany (1975). |
| | Tobacco leaf curl (TLCV)* | Osaki and Inouye (1981), Goodman (1981). |
| | Beet curly top (BCTV)* | Martelli and Quacquarelli (1982). |
| 6) Luteovirus | Beet western yellow (BWYV)* | Duffus (1981). |
| | Potato leaf roll (PLRV)* | Zitter and Everett (1982). |
| | Tomato yellow net (TYNV)* | Sylvester (1954). |
| | Tomato yellow top (TYTV)* | Costa (1949), Braithewaite and Blake (1961), Thomas (1981). |
| 7) Potyvirus | Potato virus Y (PVY) | Nitzany and Wilkinson (1960), Behl <i>et al.</i> (1987). |
| | Tobacco etch (TEV) | Debrot (1976), Fernandez and Gabor Janyi (1978). |
| | Tomato (peru) mosaic (TPMV) | Raymer <i>et al.</i> (1972). |

| | | |
|--|-----------------------------|---|
| | Pepper veinal mottle (PVMV) | Brunt and Kenten (1971), Ladipo and Roberts (1977), Sastry (1982). |
| | Pepper mottle (PeMV) | Nelson and Wheeler (1972), Zitter (1975). |
| | Pepper severe mosaic (PSMV) | Feldman and Gracia (1977). |
| 8) Tymovirus | Eggplant mosaic (EMV) | Martelli and Quacquarelli (1982). |
| 9) Tospovirus | Tomato spotted wilt (TSWV) | Todd <i>et al.</i> (1975), Carbone <i>et al.</i> (1995). |
| B) Contact transmitted viruses: | | |
| 10) Potexvirus | Potato virus X (PVX) | Dubey (1983), Ignash (1987). |
| 11) Tobamovirus | Tobacco mosaic (TMV) | Verma and Kumar (1980), Dubey <i>et al.</i> (1981), Lima Neto <i>et al.</i> (1988). |
| | Tomato mosaic (ToMV) | Martelli and Quacquarelli (1982), Singh and Sastry (1980), Clinton (1909). |
| C) Soil borne viruses: | | |
| 12) Nepovirus | Tomato ring spot (Tom RSV) | Samson and Imde (1942), Brierley <i>et al.</i> (1952). |
| | Tomato black ring (TBRV) | Murant (1981). |
| | Tomato top necrosis (TTNV) | Martelli and Quacquarelli (1982). |
| 13) Tobacco necrosis virus | Tobacco necrosis (TNV) | Gallitelli (1982). |

| | | |
|---|--------------------------------|---|
| 14) Tobravirus | Tobacco rattle (TRV) | Marte <i>et al.</i> (1979), Brown <i>et al.</i> (1989). |
| 15) Tombusvirus | Petunia asteroid mosaic (PAMV) | Martelli and Quacquarelli (1982). |
| | Tomato bushy stunt (TBSV) | Vetten and Koenig (1983). |
| D) Viruses with unknown vectors: | | |
| 16) Ilarvirus | Tobacco streak (TSV) | Martelli and Circulli (1969), Cuperinto <i>et al.</i> (1984). |
| E) Ungrouped viruses: | | |
| | Broad bean wilt (BBWV) | Martelli and Quacquarelli (1982). |
| | Pelargonium zonate spot (PZSV) | Martelli and Circulli (1969). |
| | Tomato white necrosis (TWNV) | Alba <i>et al.</i> (1977). |
| | Tomato pale chlorosis (TPCV) | Cohen and Antigus (1982) |

* = Non-sap transmissible viruses

Recently, tomato spotted wilt disease has become the most destructive disease of tomato. The early symptoms include necrosis of apical bud. The necrosis progresses downward resulting into wilting of that branch. In severe infection whole plant gets wilted. Plants infested early in the season may produce no fruits and those infected after fruit set produce fruit with chlorotic ring spots. Young leaves turn bronze and later develop numerous small dark spots (Allen *et al.* 1989 and McHugh, 1991). Growing tips may show dieback and black oily streaks appear on stem. The losses caused by the tomato spotted wilt virus are dependent on time of infection. Yield losses are 100% in the plants infected upto 47 days after transplanting (Wavle, 2003). Tomato spotted wilt virus is causing yield losses in tomato production of about 20-100 per cent (Wangai *et al.*, 2001a).

Tomato is a short duration crop and gives high yield. It is widely grown and commercially important vegetable crop, hence area under its cultivation is increasing day by day. It is grown throughout the year therefore the host becomes available for multiplication of virus and its vector. This helps the disease to assume epidemic proportions during favourable conditions. Therefore, the present investigation was undertaken to study the tomato spotted wilt disease in tomato.

Objectives of present investigation are as follows:

1) Transmission studies

- i. Mechanical
- ii. Vector
- iii. Seed
- iv. Dodder
- v. Graft

2) Physical properties

- i. Thermal inactivation point (TIP)
- ii. Dilution end point (DEP)
- iii. Longevity *in vitro* (LIV)

3) Host range

4) Purification of the virus

5) Screening of cultivars for resistance

REVIEW
OF
LITERATURE



Chapter-II

REVIEW OF LITERATURE

Tomato is one of the highly vulnerable crop to many viruses under field conditions. About 29 viruses (Edwardson and Christie, 1997), 4 mycoplasma (Martyn, 1968) and 1 viroid (Chenulu and Giri, 1985) have been reported to cause diseases in tomato. Of these six viruses, tomato mosaic virus (ToMV), tomato leaf curl virus (TLCV), tomato spotted wilt virus (TSWV), tomato bushy stunt virus (TBSV), tomato black ring virus (TBRV), tomato ring spot virus (ToRSV) are economically important (Chenulu and Giri, 1985; Nirmal *et al.*, 1993).

Tomato spotted wilt virus causing the bud necrosis of tomato is reviewed under the following categories for the sake of convenience.

2.1 Historical background

The literature on tomato spotted wilt virus has revealed that the virus and strains of the virus have been variously named. Brittlebank (1919) who reported the disease for the first time named it as “tomato spotted wilt”. Samuel *et al.* (1930) characterized the pathogen and called it as “Tomato Spotted Wilt Virus (TSWV)”. Later on the virus was named as *Lycopersicon virus* (Illingworth, 1931; Linford, 1932; Sakimura, 1940), *Ananas virus-1* (Smith, 1932), *Tomato carcova virus* (Fawcett, 1938), *Lycopersicon virus-3* (Jankowski *et al.*, 1976; Kosciak, 1986), *Tomato Spotted Tospovirus* (Rana *et al.*, 1993;

Arno *et al.*, 1995; Camele *et al.*, 1995; Eckel *et al.*, 1996; Mertelik *et al.*, 1996; Johnson *et al.*, 1996 and Sikora *et al.*, 1998).

2.2 Occurrence and distribution

From the literature it appears that disease has worldwide distribution. Brittlebank (1919) was first to report the incidence of tomato spotted wilt in the state of Victoria, Australia. Samuel *et al.* (1930) were first to characterize the causal agent of the disease as a virus and they named it as tomato spotted wilt virus (TSWV). Smith (1932) from U.K. was first to report incidence of the disease outside Australia. Subsequently, the disease has been recorded from other parts of Europe, South America, North America, Africa and Asia (Iwaki *et al.*, 1984; Reddick *et al.*, 1987; Jaysena *et al.*, 1988; Arno *et al.*, 1995; Camele *et al.*, 1995; Mertelik *et al.*, 1996 and Sikora, 1998).

From India Todd *et al.* (1975) were first to report the occurrence of TSWV on tomato from Nilgiri hills of Tamil Nadu. Subsequently, the disease has been reported from Andhra Pradesh (Prasada Rao *et al.*, 1980), Maharashtra (Nirmal, 1983) and Karnataka (Sastry, 1984).

2.3 Symptomology

The symptoms on tomato leaves produced by TSWV under field conditions reported by Samuel *et al.* (1930), Gardner *et al.* (1935), Costa and Forster (1942) and Smith (1957) can be classified roughly into:

- i) necrotic and pigmented lesions pattern,

- ii) mild surface necrotic ring spots and etch pattern,
- iii) yellows and non necrotic mottle or mosaic greens.

The symptoms on inoculated tomato plants reported by Prasada Rao *et al.* (1980) included chlorotic local spots followed by thickening of veins with few chlorotic spots, downward curling, brownizing with necrotic spots, severe stunting, bleached areas of pale red yellow colour or concentric ring spots on fruits.

Lemmetty (1991) observed symptoms of wilt, terminal dieback, leaf mottling and mosaic in tomato plants grown in green house. Raja and Jain (2005) characterized the disease by bronze or purple coloured leaves, severe necrosis of buds and petioles and yellow concentric rings turning into necrosis of fruits.

2.4.1 Mechanical transmission

The virus was found to be sap transmissible (Saumel and Bald, 1933). Best (1968) suggested for successful mechanical transmission of TSWV that the inoculum should be prepared in buffer solution at optimum pH values containing reducing agents (0.01M) such as cystine and sodium sulphite. TSWV infecting groundnut was successfully mechanical transmitted by preparing inoculum in chilled 0.05 M potassium phosphate buffer (pH 7) containing 0.02 M 2-mercaptoethanol (antioxidant) in cold mortar (Ghanekar *et al.*, 1979, Mali and Patil, 1979, Nirmal, 1983).

2.4.2 Insect transmission

Only nine thrips species were reported as vectors of tospoviruses out of more than 5000 species recorded worldwide (Mound, 1996; Webb *et al.*, 1998). *Thrips tabaci* (Lind) was first reported as a vector of TSWV infecting tomato by Pittman (1927). Since then *T. tabaci* has been reported to be the vector of tomato spotted wilt virus (TSWV) infecting tomato and other hosts (Samuel and Bald, 1931; Smith, 1932; Linford, 1932; Whipple, 1936; Harris, 1939; Sakimura, 1940; Razvyazkine, 1953; Mischke, 1957).

TSWV is transmitted by thrips in persistent manner. *T. tabaci*, *T.setosus*, *T.palmi*, *Frankinella schultzei*, *F.occidentalis*, *Scirtothrips dorsalis* are important species of the vector responsible for the spread of disease.

Tospoviruses are only transmitted by some species of thrips. *F.schultzei* and *F.occidentalis* act as disease vectors (Millan and Gracia, 2001). *T. palmi* is reported as important vector of the TSWV in Indian and other Asian countries (Palmer *et al.*, 1990 and Yeh *et al.*, 1992).

The route of TSWV in the body of its vectors, *F.occidentalis* and *T. tabaci* was studied during their development (Kritzman *et al.*, 2002). Thrips become viruliferous when they acquire TSWV at larval stage (Sakimura, 1962; Ullman *et al.*1992). The virus was propagatively transmitted and viruliferous adult were able to transmit the virus indefinitely (Wijkamp *et al.*, 1993). Larvae generally donot disperse, viruliferous adult thrips are most important in the spread of the virus and represent the most likely source of primary inoculum initiating epidemics in crops (German *et al.*, 1992; Mau *et al.*, 1991and Ullman *et al.*, 1992). In some cases, it was found that the vector retained virus for entire

life, while in others for a short period of time (Sakimura, 1960). Amin (1983) proved non-multiplication of the virus in thrips.

2.4.3 Seed transmission

Although the virus has been reported to be transmitted by seed on a very few occasions, hardly any evidence is reported.

The virus was reported transmissible by seeds (Jones, 1944, Crowley, 1957). However, the virus was not reported to be seed transmissible by Prasada Rao *et al.* (1980).

2.4.4 Dodder transmission

Benett (1944) reported that the virus was transmissible by dodder to the lesser extent.

2.4.5 Graft transmission

In different ways graft can be established (Gardner, 1958, Bos, 1967). Transmission of TSWV infecting groundnut by graft has been reported by Ghanekar *et al.* (1979). Plants can be graft inoculated using infected scions, although this is more reliable method it is more laborious (Solomon-Blackburn and Barker, 2001).

2.5 Physical properties

Physical properties viz., thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) has been reported by various workers. The TIP ranges from 40-46°C, DEP from 10^{-2} to 10^{-3} and LIV from 1 to 5 hrs at room temperature (Is, 1970; Ghanekar *et al.*, 1979; Mali and Patil, 1979; Nirmal, 1983).

2.6 Host range

Amongst plant viruses, TSWV is considered as the most wide spread and to have largest host range. More than 15 families are susceptible to TSWV infection (Smith, 1957; Best, 1968).

In India, the natural hosts of tomato spotted wilt virus (TSWV) included plant species such as *Arachis hypogea* (Nirmal 1983), *Alysicarbus rugosus* (Nirmal, 1983), *Solanum melongena* (Singh and Tripathi, 1991), *Calotropis procera* (Nirmal, 1983; Nirmal and Mali, 1987), *Capsicum annum* (Bidari and Reddy, 1984), *Chenopodium amaranticolor* (Patil and Joi, 1994), Dahlia (Khurana *et al.*, 1990), Mungbean (Nene, 1972 ; Nirmal, 1983) and weeds like *Desmodium dichtomum* (Nirmal, 1983), *Datura metel* (Patil and Joi, 1994). The crop plants viz. *A. hypogea*, *Glycine max*, *Pisum sativum*, *Vigna mungo*, *V. radiata*, *V. unguiculata*, *Lycopersicon esculentum* and *S. melongena* were also found to be natural host of TSWV (Nirmal *et al.*, 1993).

Recently, TSWV host range consist of 1090 plant species belonging in 15 families of monocotyledonous plants, 69 families of dicotyledonous plants and one family of pteridophytes (Parrella *et al.*, 2003).

2.7 Virus purification

The method of partial purification of TSWV is given by Van den Hurk *et al.* (1977). The procedure of partial purification was found to be difficult by Francki and Hatta (1981).

2.8 Screening for resistance

The use of resistant or tolerant varieties provides the most effective and durable way to minimize crop losses due to TSWV infection (Pappu *et al.*, 1998).

Honrao (1986) reported varieties La-Bonita and ATV-1 of tomato are highly resistant to spotted wilt virus.

Jasmine and Seemanthini (1993) reported that the tomato hybrid ARTH-1 and variety CO3 to be intermediate tolerant to tomato spotted wilt disease.

Herrero *et al.* (2000) transferred the nucleocapsid gene of TSWV to tomato. The transgenic line thus developed was named “Burley 21” which performed well against TSWV under field conditions.

Vijaya *et al.* (2003) reported HTY-1 variety of tomato to be tolerant to tomato spotted wilt disease.

MATERIALS

&

METHODS



Chapter-III

MATERIALS AND METHODS

3.1 Source of inoculum

The leaves showing chlorotic spots, leaf narrowing and downward curling symptoms from naturally infected tomato plants from field were taken as primary inoculum. The virus was transmitted by mechanical means to greenhouse grown tomato (*Lycopersicon esculentum*). Subsequently virus was maintained and multiplied on tomato and was used as a source of inoculum in all the studies undertaken in present investigations.

3.2 Transmission studies

3.2.1 Mechanical transmission

For mechanical transmission test, sap was extracted by triturating symptomatic leaves of tomato with a chilled mortar and pestle in a chilled 0.05M potassium phosphate buffer, pH 7.0 containing 0.02M 2-mercaptoethanol. Test plants were inoculated by conventional leaf rub method with a cotton swab. Carborundum (800 mesh) was used as an abrasive. Immediately after virus inoculation, the leaves of test plants were rinsed with tap water. Test plants used for mechanical transmission were raised from healthy seeds in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture. The test plants were maintained in an

insect-free screenhouse for 3 to 4 weeks and observations were recorded periodically with respect to symptom development and incubation period.

3.2.2 Vector transmission

Three species of *Scirtothrips dorsalis*, *Thrips palmi*, *T. flavus* and *Frankinella schultzei* were collected from infected tomato plants early in the morning and released on tomato seedlings raised in an insect proof screen house and covered with lantern globe to study their ability to transmit virus. On each plant 15 to 20 thrips were released. After completion of 12 days inoculation feeding, they were killed by spraying 0.02% Metasystox. Test plants were observed for development of symptoms and those showing symptoms were assayed on cowpea cv. Pusa komal for confirming the presence of the virus.

3.2.3 Seed transmission

For seed transmission, the seeds were collected from virus infected tomato plants. Seeds were sown in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture and maintained in an insect-free screen house. Observations were recorded regularly and percentage transmission was recorded. The plants showing symptoms were back indexed on cowpea cv. Pusa komal to reveal the presence of virus under study.

3.2.4 Dodder transmission

For dodder (*Cuscuta spp*) transmission of tomato spotted wilt virus, the virus free dodder culture was raised by sowing seeds collected from dodder and grown on healthy seedling of tomato host plants in earthen pots. The stems of dodder seedlings after having attained the length of 2 to 4 cm were placed on tomato seedlings with one end of the seedling in the soil and the other in contact with petiole or stem. Subsequently, when the dodder culture were found well established and had produced abundant number of new vines, they were used for transmission of the tomato spotted wilt virus from tomato to tomato.

For effective dodder transmission of the tomato spotted wilt virus, the virus free dodder was first established on virus infected plants of tomato. For this purpose, pieces of dodder collected from virus free culture were wrapped around petioles and stems of infected test host plants and were allowed to establish. After having ensured the establishment of dodder on infected plants, vigorously growing, uninjured tips of dodder vines were trailed and secured by wrapping on to healthy test plants. Later, all the dodder inoculated plants were maintained in an insect free screen house. Observations with respect to incubation period, symptoms and percentage transmission of the virus on dodder inoculated plants were recorded.

3.2.5 Graft transmission

For graft transmission the virus isolate maintained on tomato was used. The test was conducted on 10 healthy and actively growing tomato

plants ranging from one to one and a half months in age grown in earthen pots from healthy seeds. The union between healthy and virus infected tomato plants established by approach grafting as per the procedure outlined by Bos (1967). For this purpose, the cortical tissue (4-5 cm in length and 0.5-1 cm in width) of both healthy and infected test host plants are carefully sliced with a sterile razor blade in order to expose the cambium in an equal pattern. The healthy and test host plants, were then tied together with polythene tape in order to secure the cut portions in a place and to ensure maximum contact of cambium infected plants with that of healthy ones. Later, the grafted plants were maintained in an insect free screen house and regularly observed for symptom development and per cent infectivity was recorded.

3.3 Physical properties

The studies on physical properties viz., thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) were carried out as per procedure outlined by Bos *et al.* (1960) using tomato infected leaves as source and *cowpea cv. Pusa komal* as an assay host.

3.3.1 Thermal inactivation point (TIP)

Standard leaf extract of infected leaves of tomato was prepared in 1:1 ratio of the leaf tissue to buffer. Aliquotes of 2 ml standard extract were pippered into each glass test tube and exposed for 10 minutes to different heat treatments from 35°C to 90°C at an interval of 5°C in a thermostatic waterbath. After heat treatment, sap was cooled by running tap water on outside of the

tubes. A set of 10 uniform plants of an assay host was used for each treatment. All the treated extracts were then inoculated on cowpea cv. Pusa komal by conventional leaf rub method, using carborundum 800mesh as an abrasive. Similarly a set of 10 plants of assay hosts was inoculated with untreated extract which served as control. Later on the leaves of assay hosts were washed with tap water and maintained in an insect-free screenhouse. Local lesions were recorded and from local lesions number of local lesions per leaf was counted.

3.3.2 Dilution end point (DEP)

Standard leaf extract prepared from infected leaves of tomato was diluted in 10 fold (X^{-10}) series upto 10^{-12} dilution. A set of 10 uniform plants of assay hosts was inoculated separately, with each dilution starting with the highest dilution and ending up with the crude standard leaf extract, which served as control. Immediately, after inoculation the assay plants were washed and maintained in an insect-free screenhouse for observations. Number of local lesions per leaf was counted.

3.3.3 Longevity *in vitro* (LIV)

In order to know the longevity of the crude sap an experiment of aging *in vitro* was carried out. The standard extract prepared from infected leaves of tomato was kept at room temperature ($28 \pm 2^\circ\text{C}$) in conical flask with rubber stopper.

Sap stored in this condition was inoculated by conventional leaf rub method on each set of 10 uniform plants of assay hosts with an interval of 0.5 hours. Subsequently the inoculated leaves were washed and pots were maintained in an insect-free screenhouse. Local lesions were counted and an average local lesion per leaf was carried out.

3.4 Host range

For host range studies, the plant species belonging to the different families viz. Amaranthaceae, Apocynaceae, Caesalpiaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Graminae, Leguminosae and Solanaceae were raised from healthy seeds in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture. Six plants of each host species were inoculated with the sap extracted from virus infected tomato plants by conventional leaf rub method. All the leguminous plants were sap inoculated with infective sap on primary leaves before the emergence of trifoliate leaves. All other plants were inoculated on the first leaf or the fully expanded leaves. The inoculated host plants were kept for observation for 3 to 6 weeks along with the uninoculated control plants. The plants not showing symptoms after the period of 3 to 6 weeks were back indexed on cowpea cv. Pusa komal for the detection of latent infection if any. The host range experiment was conducted during July, 2008 to February, 2009 and temperature range was minimum of 16.5°C and maximum of 33.5°C and relative humidity was 70-85 per cent.



The list of test plants species included in host range is given as below:

| | | | |
|-----|----------------------------------|---|-----------------|
| 1. | <i>Calotropis procera</i> | - | Asclepiadiaceae |
| 2. | <i>Vinca rosea</i> | - | Apocynaceae |
| 3. | <i>Cassia tora</i> | - | Caesalpiaceae |
| 4. | <i>Beta vulgaris</i> | - | Chenopodiaceae |
| 5. | <i>Chenopodium amaranticolor</i> | - | Chenopodiaceae |
| 6. | <i>C. murale</i> | - | Chenopodiaceae |
| 7. | <i>C. quinoa</i> | - | Chenopodiaceae |
| 8. | <i>Aster alpines</i> | - | Compositae |
| 9. | <i>Helianthus annuus</i> | - | Compositae |
| 10. | <i>Tagetes minuta</i> | - | Compositae |
| 11. | <i>Citrullus lanatus</i> | - | Cucurbitaceae |
| 12. | <i>Cucumis sativus</i> | - | Cucurbitaceae |
| 13. | <i>Arachis hypogaea</i> | - | Leguminosae |
| 14. | <i>Glycine max</i> | - | Leguminosae |
| 15. | <i>Phaseolous vulgaris</i> | - | Leguminosae |
| 16. | <i>Pisum sativum</i> | - | Leguminosae |
| 17. | <i>Vigna mungo</i> | - | Leguminosae |
| 18. | <i>V. radiata</i> | - | Leguminosae |
| 19. | <i>V. unguicalata</i> | - | Leguminoceae |
| 20. | <i>Capsicum annum</i> | - | Solanaceae |

| | | | |
|-----|--------------------------------|---|------------|
| 21. | <i>Lycopersicon esculentum</i> | - | Solanaceae |
| 22. | <i>Nicotiana glutinosa</i> | - | Solanaceae |
| 23. | <i>Nicotiana tabacum</i> | - | Solanaceae |
| 24. | <i>Petunia hybrida</i> | - | Solanaceae |
| 25. | <i>Solanum melongena</i> | - | Solanaceae |

3.5 Purification of virus

The purification of virus was done as outlined by Van den Hurk *et al.* (1997). Plant material infected with TSWV was grinded in 0.01M tris buffer (pH- 8.0) containing 0.01M sodium sulphite and 0.1% cystine hydrochloride. The extract was centrifuged for 10 min at 10,000g. The pellet was resuspended in the buffer. After clarification for 10 min at 10,000g, the suspension was spun for 30 min at 25,000g. The pellet was resuspended in buffer and inoculated on assay host cowpea cv. Pusa komal by doing serial dilutions. The infectivity of the virus was tested.

3.6 Screening of cultivars / hybrids of tomato for resistance

The screening of 13 varieties was done in pot culture. Test seedlings were grown in a pot and 25 days old seedling were transplanted in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture and maintained in an insect-free screen house.

Five luxuriantly growing seedlings were maintained in each pot. Three pots per variety were used for screening, which were inoculated mechanically 30 days after transplanting by adopting the procedure outlined by Holmes (1948) and kept under observations for 45 days.

RESULTS



Chapter-IV

RESULTS

Observations on tomato crop grown in the field of Vegetable Research Station, Parbhani indicated that some tomato plants were affected by spotted wilt disease that consisted variety of symptoms. Initially symptoms on leaves were chlorotic or necrotic spots with slight downward curling of leaves, forming inverted cup like structure. Later on these develop into necrotic concentric ring spots. In few cases, these symptoms were associated with thickening of dorsal veins of leaves. Later on necrosis extended to petiole, stems and buds.

Infection occurred at seedling stage resulted in dieback like symptoms in plants (Fig.1). Plants infected at early stages remained severely stunted and showed proliferation of auxiliary buds imparting bushy appearance to the plants. Some of the infected plants showed bronzing and purplish discolouration of leaves (Fig.2). Plants infected early beared no flowers and fruits. However, late infected plants produced few fruits, some of which exhibited pale red, yellow or bleached areas sometimes even exhibiting distinct concentric rings.

The inoculums taken from this type of symptoms, when inoculated on cowpea cv. Pusa komal produced local lesions.

Lycopersicon esculentum and *Vigna unguiculata* were used as maintenance and propagation host.



(Fig.1) Dieback like symptoms in tomato plant due to virus infection



(Fig.2) Bronzing and purplish discoloration of leaves infected by the virus

4.1 Transmission

4.1.1 Mechanical transmission

The results of sap inoculation indicated that tomato spotted wilt virus was transmissible by mechanical means using chilled 0.05M potassium phosphate buffer, pH 7.0 containing 0.02M 2-mercaptoethanol from tomato to tomato and to other hosts producing symptoms within 7 to 8 days after virus inoculation.

The virus was also found to be transmissible from tomato to cowpea and back from cowpea to tomato. However, tomato to cowpea transmission was easier as compared to tomato to tomato. Success in mechanical transmission was obtained when inoculum was prepared by grinding young tomato leaves showing chlorotic symptoms in cold mortar and pestle along with chilled buffer.

Cowpea cv. Pusa komal produced chlorotic or necrotic local lesions on primary inoculated leaves 4 to 7 days after virus inoculation. Since it produced local lesions consistently, it was used as an assay host in all the studies.

The systemic symptoms produced by virus upon mechanical inoculation on tomato were similar to those produced by plants infected naturally. Leaf mottling of tomato plants was also observed on plants kept in the greenhouse.

4.1.2 Insect transmission

The results on thrips transmission of virus causing tomato spotted wilt in tomato are presented in Table 2 and graphically in Fig. 3.

It is revealed from the table that three thrips species viz., *Thrips palmi*, *Scirtothrips dorsalis* and *Franklinella schultzei* were found to transmit from tomato to tomato. *T. palmi* showed highest transmission (55.00 per cent) followed by *S. dorsalis* (40.00 per cent) and *F. schultzei* (15.00 percent) in transmitting the virus. *T. flavus* could not transmit the virus from tomato to tomato.

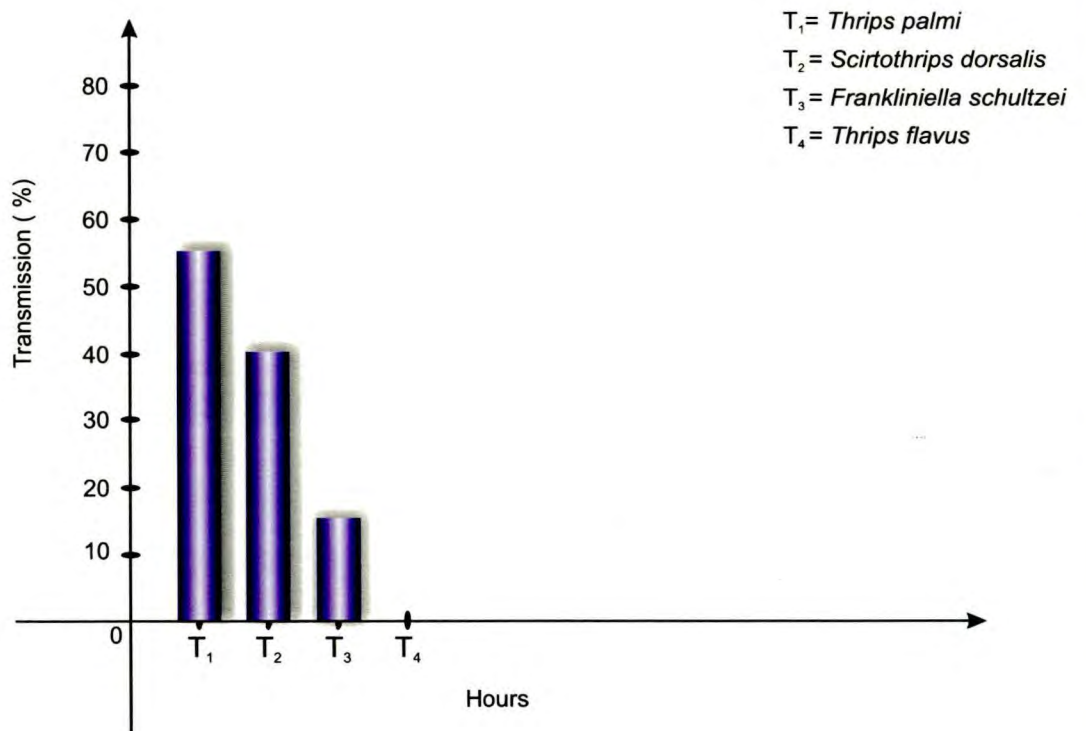
Symptoms produced on tomato plants upon thrips inoculation were identical with those that were produced on tomato plants upon mechanical sap inoculation and natural infection in field.

Table 2 Thrips transmission of tomato spotted wilt virus from tomato to tomato

| Sr. No. | Thrips species | No. of thrips per plant | Transmission (per cent) |
|---------|--------------------------------|-------------------------|-------------------------|
| 1. | <i>Thrips palmi</i> | 25 | 55.00 (11/20)* |
| 2. | <i>Scirtothrips dorsalis</i> | 25 | 40.00 (8/20) |
| 3. | <i>Frankliniella schultzei</i> | 25 | 15.00 (3/20) |
| 4. | <i>Thrips flavus</i> | 25 | 0.00 (0/20) |

Where,

* = Percentage transmission followed by (in parentheses) number of plants infected upon number of plants inoculated by thrips species.



(Fig.3) Thrips transmission of the virus causing tomato spotted wilt disease in tomato.

4.1.3 Seed transmission

Seedling of Vaishali, Pusa Ruby, CO1, CO3, Bhagyashree were raised from the seeds collected from fruits of infected plants. No symptoms were exhibited of the spotted wilt and when the samples were back indexed on cowpea. Cv. Pusa komal no virus was recovered. These results indicated that the virus was not seed borne in tomato.

4.1.4 Dodder transmission

The dodder (*Cuscuta reflexa*) could not be colonized on tomato plants due to non-preference host. Hence virus under study is not transmissible by dodder.

4.1.5 Graft transmission

The results on graft transmission indicated that the virus was transmissible by approach grafting from diseased to healthy plants of tomato. Grafting was successful to the extent of 50 to 60 percent.

4.2 Physical properties

The results on physical properties which are thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) are given in Table 3.

4.2.1 Thermal inactivation point (TIP)

The results on TIP are displayed in Table.3 and presented graphically (Fig. 4). It was observed from the table and graph that the virus was inactivated between 45-50°C. For the TIP, all the inoculated plants of assay host showed the symptoms at 45°C but not at 50°C. It is also evident from the table and graph that the infectivity decreased progressively as the temperature were increased.

4.2.2 Dilution end point (DEP)

The results on DEP are displayed in Table.3 and presented graphically (Fig.5). It was revealed from the table and graph that, all the inoculated plants of assay host became infected upto 10^{-2} dilution but not at 10^{-3} dilution. Number of local lesions decreased as dilutions were increased.

4.2.3 Longevity *in vitro* (LIV)

The results on LIV are displayed in Table.3 and also presented graphically (Fig. 6). The data presented in the table and graph indicated that the virus was viable upto 4.5 hours but not upto 5 hours at room temperature ($28\pm 2^{\circ}\text{C}$). However, the percentage infectivity decreased progressively as the aging of the sap was increased at room temperature.

Table 3 Physical properties of the virus under study

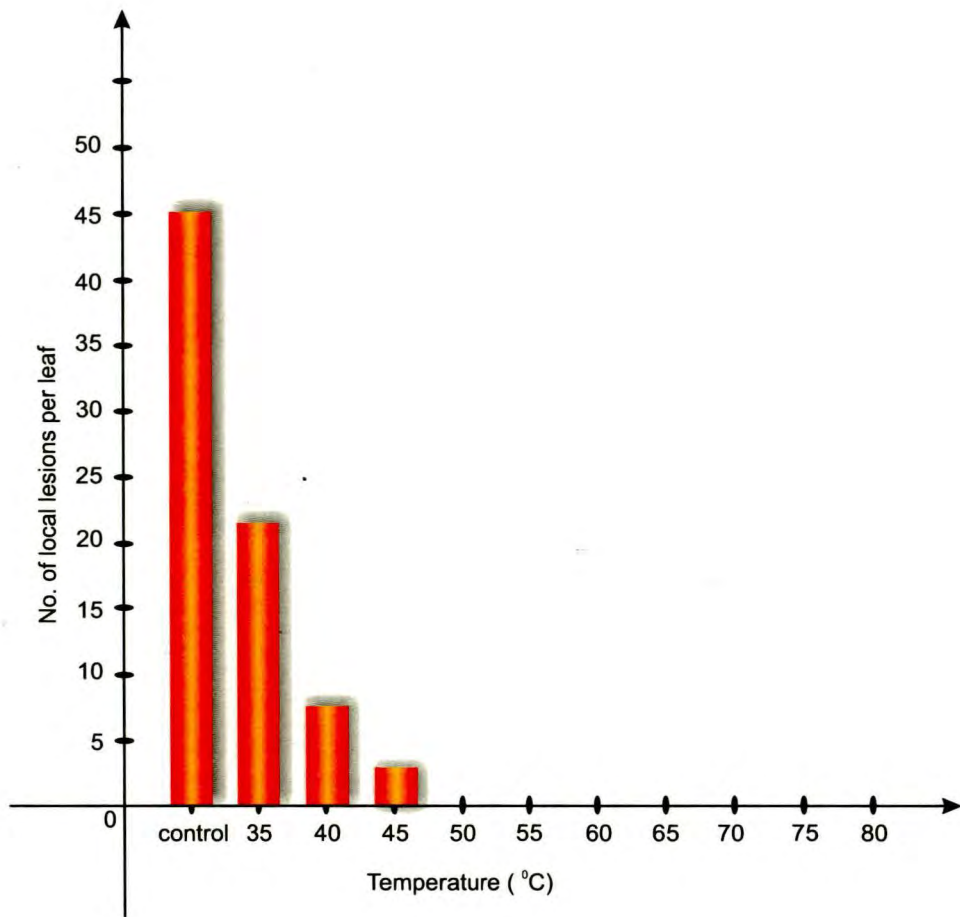
| Sample No. | Treatments* | | | | | |
|------------|-------------|----------------------------|-------------------|----------------------------|-------------|----------------------------|
| | TIP (°C) | No. of local lesions/ leaf | DEP | No. of local lesions/ leaf | LIV (hours) | No. of local lesions/ leaf |
| 1. | Control | 45 | Control | 45 | Control | 45 |
| 2. | 35 | 22 | 10 ⁻¹ | 20 | 0.5 | 29 |
| 3. | 40 | 7 | 10 ⁻² | 3 | 1.0 | 21 |
| 4. | 45 | 3 | 10 ⁻³ | 0 | 1.5 | 18 |
| 5. | 50 | 0 | 10 ⁻⁴ | 0 | 2.0 | 12 |
| 6. | 55 | 0 | 10 ⁻⁵ | 0 | 2.5 | 9 |
| 7. | 60 | 0 | 10 ⁻⁶ | 0 | 3.0 | 7 |
| 8. | 65 | 0 | 10 ⁻⁷ | 0 | 3.5 | 4 |
| 9. | 70 | 0 | 10 ⁻⁸ | 0 | 4.0 | 2 |
| 10. | 75 | 0 | 10 ⁻⁹ | 0 | 4.5 | 1 |
| 11. | 80 | 0 | 10 ⁻¹⁰ | 0 | 5.0 | 0 |

Where,

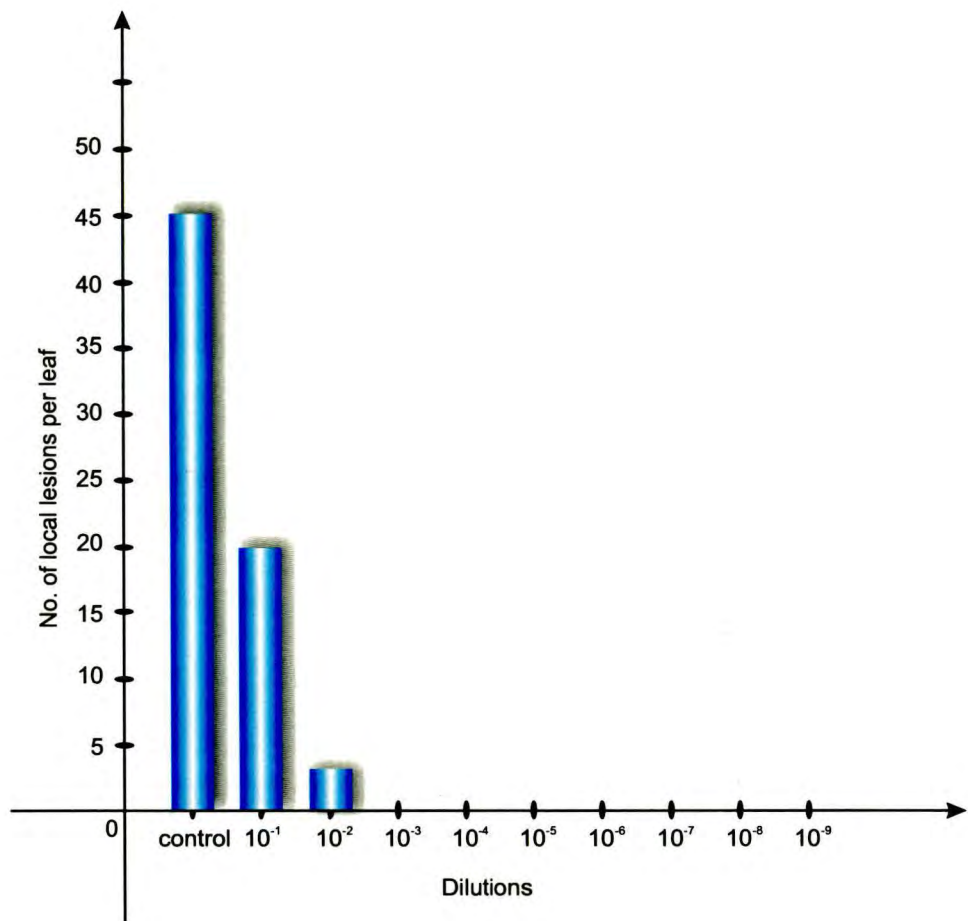
* = TIP = Thermal inactivation point

DEP = Dilution end point

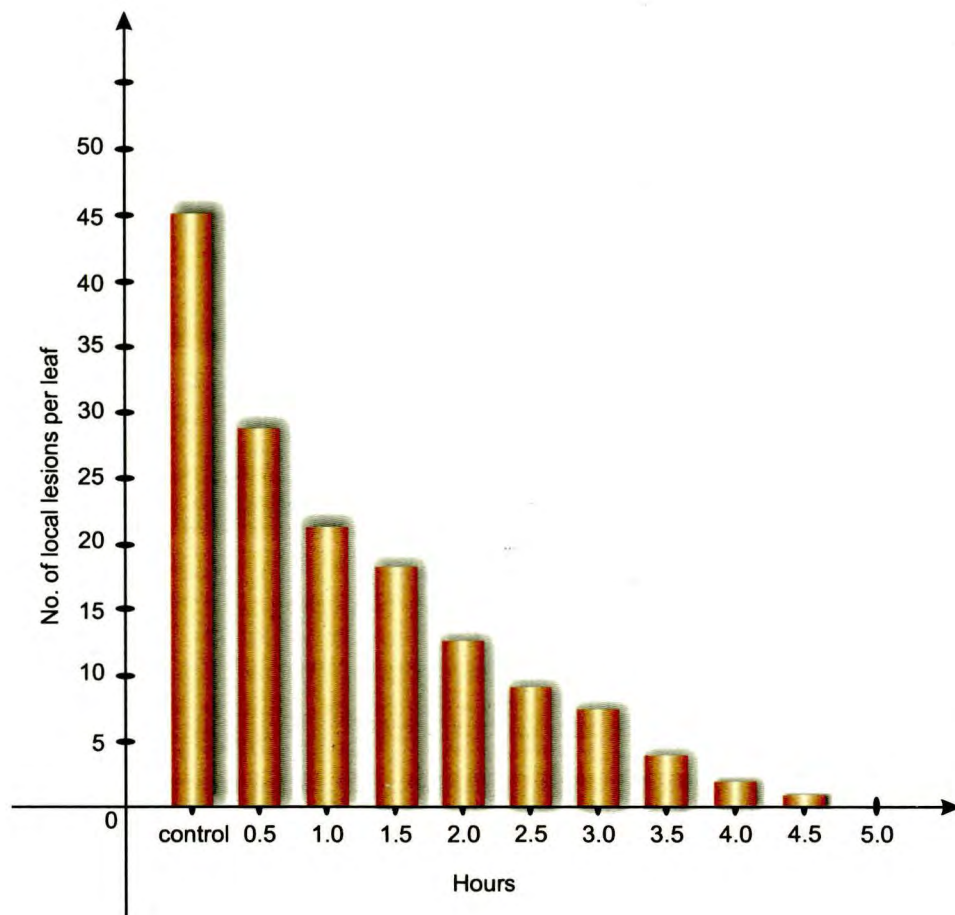
LIV = Longevity *in vitro*



(Fig.4) Thermal inactivation point (TIP) of the virus causing tomato spotted wilt disease in tomato.



(Fig.5) Dilutions end point (DEP) of the virus causing tomato spotted wilt disease in tomato.



(Fig.6) Longevity *in-vitro* (LIV) of the virus causing tomato spotted wilt disease in tomato.

4.3 Host range

The results on host range and reactions of the virus are displayed in Table 3 and described as under. All the test plant species i.e. 25 belonging to 8 families viz., Asclepiadaceae, Apocynaceae, Caesalpinaceae, Chenopodiaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Leguminaceae and Solanaceae. Among these 9 were local, 2 were systemic and 14 were both local and systemic hosts.

Table 4 Host range of the virus causing tomato spotted wilt in tomato.

| Sr. No. | Host | Local | IP (days) | Systemic | IP (days) | Remarks |
|------------|----------------------------------|-------------|-----------|----------|-----------|---------|
| I | Asclepiadiaceae | | | | | |
| 1. | <i>Calotropis procera</i> | NLL | 7-8 | NRS/ CRS | 14-15 | LH, SH |
| II | Apocynaceae | | | | | |
| 1. | <i>Vinca rosea</i> | CLL | 12-13 | CS/ NS | 16-17 | LH, SH |
| III | Caesalpinaceae | | | | | |
| 1. | <i>Cassia tora</i> | NLRL | 6-8 | NRS, TN | 10-12 | LH, SH |
| IV | Chenopodiaceae | | | | | |
| 1. | <i>Beta vulgaris</i> | NLL | 16-18 | - | - | LH |
| 2. | <i>Chenopodium amaranticolor</i> | NLRL | 2-4 | - | - | LH |
| 3. | <i>C. murale</i> | NLRL | 4-5 | - | - | LH |
| 4. | <i>C. quinoa</i> | NLRL | 3-4 | - | - | LH |
| V | Compositae | | | | | |
| 1. | <i>Aster alpines</i> | CLL/ NLL | 10-11 | NS,Y | 15-16 | LH, SH |

| | | | | | | |
|-------------|--------------------------------|------------------|-------|----------------------------------|-------|--------|
| 2. | <i>Helianthus annuus</i> | CCLL/ NLRL,Ld | 6 | - | - | LH |
| 3. | <i>Tagetes minuta</i> | YB | 15 | CRS, Mt, Y, VN, Stn | 19-20 | LH, SH |
| VI | Cucurbitaceae | | | | | |
| 1. | <i>Citrullus lanatus</i> | NL | 10-18 | - | - | LH |
| 2. | <i>Cucumis sativus</i> | CLL | 5-6 | - | - | LH |
| VII | Leguminosae | | | | | |
| 1. | <i>Arachis hypogea</i> | CLRL/ NLL | 15 | CRS/ NRS, Stn, BN, Ld | 7-8 | SH |
| 2. | <i>Glycine max</i> | CCLL | 6-7 | CRS, M, Mt | 12-13 | LH, SH |
| 3. | <i>Phaseolus vulgaris</i> | CCLL | 6-7 | CRS | 16-18 | LH, SH |
| 4. | <i>Pisum sativum</i> | NLRL | 6-7 | NRS, Ld | 10-12 | LH, SH |
| 5. | <i>Vigna mungo</i> | CCLL, NLRL,VN | 6 | NRS, VN, Lc, Ld | 12-13 | LH, SH |
| 6. | <i>V. radiate</i> | CCLL, VN, Vc | 6 | Y, M, Def, VN, Vd, Lc, Stn | 10-11 | LH, SH |
| 7. | <i>V. unguiculata</i> | CCLL/ NLRL | 4-7 | CRS/ NRS, LP, Ld, Ep | 8-9 | LH, SH |
| VIII | Solanaceae | | | | | |
| 1. | <i>Capsicum annum</i> | CCLL | 7-8 | CRS, NRS, Ld, LP, Def, TN | 9-11 | LH, SH |
| 2. | <i>Lycopersicon esculentum</i> | CCLL, NLRL | 15-16 | Br, NS, VN, Ld, TN, M | 20-21 | LH, SH |
| 3. | <i>Nicotiana glutinosa</i> | NLRL, Ld | 5-6 | NRS, Stn, NRS,TN,Vy | 8-9 | LH, SH |

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| | | | | | | |
|----|--------------------------|------|-----|------|-------|----|
| 4. | <i>Nicotiana tabacum</i> | CCLL | 4-5 | - | - | LH |
| 5. | <i>Petunia hybrid</i> | NLL | 2-3 | - | - | LH |
| 6. | <i>Solanum melongena</i> | - | - | M, W | 10-18 | SH |

Where,

BN = Bud necrosis

Br = Bronzing of leaves

CLL = Chlorotic local lesions

CLRL = Chlorotic local ring lesions

CRS = Chlorotic ring spots

CS = Chlorotic spots

D = Defoliation

Ep = Epinasty

Lc = Leaf crinkling

Ld = Leaf dry

LH = Local host

M = Mosaic

Mt = Mottling

NH = Non host

NLL = Necrotic local lesions

NLRL = Necrotic local ring lesions

NRS = Necrotic ring spots

NS = Necrotic spots

SH = Systemic host

Stn = Stunting

TN = Tip necrosis

VN = Veinal necrosis

Vy = Vein yellowing

Y = Yellowing of leaves

YB = Yellow blotch

W = Wilting.

I. Asclepiadiaceae

1. *Calotropis procera*

This host reacted both locally and systemically to the virus inoculation. On inoculated leaves virus produced chlorotic or necrotic concentric ring spots. The systemic symptoms also induced chlorotic and necrotic ring spots.

II. Apocynaceae

1. *Vinca rosea*

This host reacted both locally and systemically to the virus inoculation. On inoculated leaves virus produced chlorotic concentric ring spots with dark necrotic margins. In severe cases, defoliation of inoculated leaves occurred. The systemic symptoms were chlorotic (Fig.7) followed by drying of leaves and defoliation.

III. Caesalpinaceae

1. *Cassia tora*

The host produced necrotic concentric ring spots 6 days after inoculation. The incubation period for systemic symptoms was 10 to 12 days. Concentric ring spots followed by tip necrosis and stunting of plants was observed.



(Fig.7) Chlorosis of leaves in *Vinca roseae*.

IV. Chenopodiaceae

1. *Beta vulgaris*

This host reacted only locally to virus inoculation. On inoculated leaves necrotic local lesions were produced after 16 days.

2. *Chenopodium amaranticolor*

The virus produced local lesions 2 to 4 days after inoculation. It was found to be local lesion host of the virus. Initially pinpoint symptoms appeared which later developed into necrotic concentric ring spots.

3. *Chenopodium murale*

The host reacted locally to the virus inoculation. Necrotic lesions with whitish centre and red margins were produced after 4 to 5 days of incubation period.

4. *Chenopodium quinoa*

Necrotic local lesions with water soaked haloes were produced after incubation of 3 to 4 days. No systemic symptoms were produced.

V. Compositae

1. *Aster alpines*

The virus produced small chlorotic lesions on inoculated leaves. Systemic symptoms were necrotic spots and yellowing of the leaves.

2. *Helianthus annuus*

The virus produced necrotic local lesions with ash coloured centre and yellowish margin (Fig. 8). The incubation period was 6 days. When local lesions were produced, they coalesced and produced yellowish patches with necrotic areas (Fig. 9).

3. *Tagetes minuta*

This host was found to react both locally and systemically to virus inoculation. On inoculated leaves, virus produced yellow blotches. Initially on inoculated leaves, chlorotic or necrotic spots were produced which later became evident as necrotic concentric ring spots. Slight yellowing of leaves, veinal necrosis and stunting was also observed along with mottling.

VI. Cucurbitaceae

1. *Citrullus lanatus*

Brown spots along with rings appeared 10 to 12 days after inoculation. These spots gradually enlarged and coalesce leading to leaf necrosis.

2. *Cucumis sativus*

The host reacted only locally to the virus inoculation. The local symptoms produced were chlorotic lesions.



(Fig.8) Leaf of *Helianthus annuus* showing necrotic local lesions.



(Fig.9) Coalescion of local lesions and formation of yellowish patches with necrotic areas on leaf of *Helianthus annuus*.

VII Leguminosae

1. *Arachis hypogea*

Initially on inoculated leaves chlorotic specks (Fig. 10) were produced which later developed into chlorotic or necrotic spots after 15 days of incubation period. Systemic symptoms included chlorotic or concentric ring spots, necrosis of terminal buds, severe stunting and proliferation of axillary buds. Inward curling of leaves was also observed (Fig. 11).

2. *Glycine max*

The host reacted both locally and systemically to the virus inoculation. The local symptoms were chlorotic spots (Fig. 12), produced after 6 to 7 days on inoculation. Necrotic spots also developed. Mosaic and mottling symptoms were produced systemically (Fig. 13). Bronzing of leaf veins was noticed in some cases (Fig. 14).

3. *Phaseolus vulgaris*

The host reacted both locally and systemically to the virus inoculation. Within 6 to 7 days local symptoms were produced which showed chlorotic concentric broken rings. Ring spot like symptoms were also developed on uninoculated leaves.

4. *Pisum sativum*

The virus produced concentric necrotic rings within 6 to 8 days after inoculation. In severe cases, drying of leaves was also noticed. Ring spot type symptoms were produced systemically.



(Fig.10) Chlorotic specks on leaf of *Arachis hypogaea*.



(Fig.11) Inward curling of leaves of *Arachis hypogaea*.



(Fig.12)
Leaves of *Glycine max*
showing chlorotic spots.



(Fig.13)
Mosaic on leaves
of *Glycine max*.



(Fig.14)
Bronzing of leaf veins
in *Glycine max*.

5. *Vigna mungo*

The host reacted both locally and systemically to the virus inoculated. The local symptoms were necrotic concentric ring spots and necrosis of vein developed after 6 days of inoculation. Systemic symptoms were necrotic concentric ring spots (Fig. 15) with veinal necrosis and crinkling of leaves was observed.

6. *Vigna radiata*

Both local and systemic symptoms were produced on inoculation of the virus. The local symptoms were chlorotic concentric ring spots with few chlorotic spots later turning to necrotic spots. The necrosis of vein was also noticed. The systemic symptoms were slight yellowing of leaves followed by mosaic, veinal necrosis, vein distortion, curling of leaves, defoliation and stunting of plants.

7. *Vigna unguiculata*

The virus produced chlorotic concentric ring spots on inoculated leaves (Fig. 16). Sometimes both chlorotic concentric and necrotic concentric ring spots were produced. The systemic symptoms were chlorotic ring spots, leaf distortion (Fig. 17), epinasty, drying of leaves and in severe case top wilting. The veins became bronze in colour with purplish tinge (Fig. 18). In some cases cuppling and curling of leaves was noticed (Fig. 19).



(Fig.15) Necrotic ring spots on leaf of *Vigna mungo*.



(Fig.16) Chlorotic spots on inoculated leaves of *Vigna unguiculata*.



(Fig.17) Leaf distortion in *Vigna unguiculata*.



(Fig.18)
Veinal bronzing in leaf
of *Vigna unguiculata*.



(Fig.19) Cuppling and curling of leaves of *Vigna unguiculata*.

VII. Solanaceae

1. *Capsicum annum*

The host reacted systemically and locally to the virus under study.

The virus induced locally chlorotic ring spots with concentric rings after 7-8 days of inoculation (Fig. 20). Later on they became necrotic and defoliation occurred. Systemic symptoms produced ring spots. Leaf distortion, tip necrosis and defoliation were also observed.

2. *Lycopersicon esculentum*

The host produced both local and systemic symptoms. On inoculated leaves, chlorotic concentric ring spots developed (Fig. 21) which later became necrotic. The systemic symptoms were thickening of dorsal vein, veinal necrosis, drying of leaves, necrotic concentric ring spots, stunting and occasionally top wilting. Bronzing of leaves occurred rarely. Mottling and mosaic symptoms also appeared on leaves (Fig. 22). Sometimes deformation of leaves was also evident (Fig. 23). On few fruits concentric ring spots were produced (Fig. 24).

3. *Nicotiana glutinosa*

The host reacted locally and systemically to virus inoculation. On inoculated leaves initially small necrotic spots appeared and developed into concentric ring spots. The systemic symptoms were small necrotic spots which were later evident as concentric ring spots. Withering of leaves, tip necrosis and severe necrosis were also evident.



(Fig.20) Chlorotic ringspots with concentric rings on leaves of *Capsicum annum*.



(Fig.21) Chlorotic concentric ringspots on leaves of *Lycopersicon esculentum*.



(Fig.22)
Mosaic and mottling
symptoms on leaves of
Lycopersicon esculentum.



(Fig.23)
Leaf deformation in leaves
of *Lycopersicon esculentum*.



(Fig.24)
Concentric ring spots induced
by the virus on tomato fruit.

4. *Nicotiana tabacum*

The host reacted only locally. Initially chlorotic lesions (Fig. 25) were developed which later turned to necrotic concentric rings (Fig. 26).

5. *Petunia hybrida*

The host reacted only locally. On inoculated leaves, necrotic local lesions with dark margins were produced.

6. *Solanum melongena*

Light green to yellow mosaic patterns were observed in the interveinal region 10 to 18 days after inoculation. Severe wilting was observed and later on the plants collapsed.

4.4 Virus purification

Results on partial purification of the virus were found difficult. Systemically infected leaves were used as source for the purification of the virus. Infectivity assay was done on cowpea cv. Pusa komal. The virus was infectious to cowpea, assay host upto the dilution of 10^{-1} to 10^{-2} , based on the original weight of tissue.

4.5 Screening of cultivars of tomato for resistance

The results of the reaction of tomato cultivars on mechanical inoculation in green house conditions are given in Table. 5. From the data it



(Fig.25) Chlorotic lesions on leaves of *Nicotiana tabacum*.



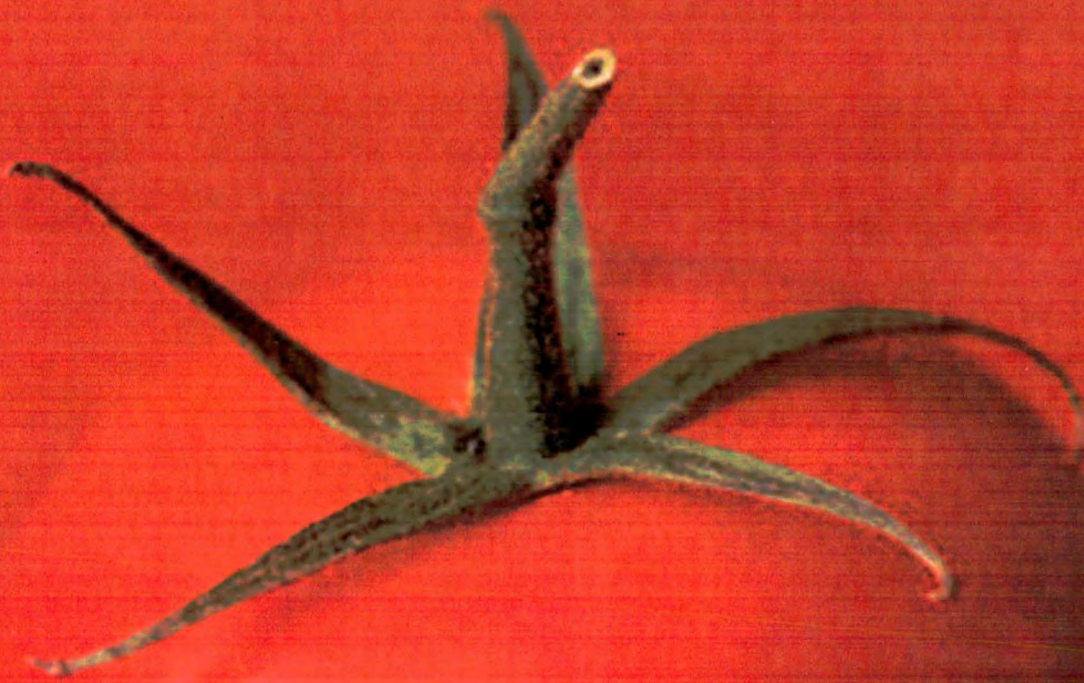
(Fig.26) Necrotic concentric rings on leaf of *Nicotiana tabacum*.

was evident that all the cultivars were susceptible to the virus. However, differences were apparent to disease level. The disease incidence ranged from 26.6 to 83.3 percent. ATV-1 recorded highest incidence of disease (83.3 percent) where as the PI-134417 recorded the lowest incidence (26.6 percent) and treated as moderately resistant.

Table 5 Reactions of tomato cultivars to tomato spotted wilt virus.

| Sr. No | Cultivars | Incidence (%) |
|--------|----------------|---------------|
| 1. | ATV-1 | 83.30 |
| 2. | Bhagyashree | 46.60 |
| 3. | CO1 | 70.00 |
| 4. | CO3 | 66.66 |
| 5. | La bonita | 73.33 |
| 6. | PI-134417 | 23.60 |
| 7. | Naveen | 76.60 |
| 8. | Princess | 53.30 |
| 9. | Pusa Ruby | 53.00 |
| 10. | Pusa selection | 63.33 |
| 11. | Roma | 60.00 |
| 12. | Roopali | 56.60 |
| 13. | Vaishali | 50.00 |

DISCUSSION



Chapter-V

DISCUSSION

The tomato (*Lycopersicon esculentum* Mill.) has been reported to be infected spontaneously by 29 viruses belonging to 15 groups (Edwardson and Christie, 1997). The tomato spotted wilt disease of tomato is identified to be caused by tomato spotted wilt virus (TSWV), based on the symptomatology, transmission, physical properties, host range and reaction of diagnostic hosts. The symptomatology of the present disease, both under natural and artificially inoculated conditions were similar to spotted wilt reported from other countries and India. The findings of present investigation are more or less similar to that of earlier workers Samuel *et al.* (1930), Gardner *et al.* (1935), Costa and Forster (1942), Chamberlain and Taylor (1938), Smith (1975), Prasada Rao *et al.* (1980), Nirmal (1983), Camele *et al.*, 1995, Mertelik *et al.* (1996) and Sikora, (1998).

The virus was found to be mechanically transmissible. The ability to transmit through mechanical means increased when reducing agent was incorporated in extraction buffer, similar results have been reported by Best (1968), Ghanekar *et al.* (1979) and Nirmal (1983). Symptoms observed on tomato such as downward curling, thickening of vein with chlorotic spots, bronzing of veins and severe necrosis was observed. Bleached areas or concentric ring spots on fruits were also observed. These symptoms were similar to those of TSWV noticed by Prasada *et al.* (1980) and Raja and Jain (2005). Symptoms of wilt, terminal die back, leaf mottling and leaf mosaic in

tomato plants grown in glass house were similar to the findings of Lemmetty (1991). Symptoms observed on tomato plants in the form of necrotic lesions on the inoculated leaves are local symptoms. These lesions are circular and well delimited typical of a hypersensitive response without showing any negative effect on growth and development. Mosaic symptoms are systemic symptoms, which can evolve into stunting and general necrosis of the plant (Moury *et al.*, 1997).

TSWV infecting tomato and other host has been reported to be transmitted by thrips species which are *Thrips tabaci*, *T. sestosus*, *Frankliniella schultzei*, *F. occidentalis*, *F. fusca*, *Scirtothrips dorsalis* by Mound (1996) and Webb *et al.* (1998). However virus under investigation was found to be transmissible from tomato to tomato by *T. palmi*, *S. dorsalis* and *F. schultzei* which had resemblance to the findings of Nirmal *et al.* (1993). *Thrips palmi* was found to be efficient vector of TSWV as reported by Yeh *et al.* (1992).

The virus was reported to be seed borne in Cineraria and tomato (Jones, 1944, Crowley, 1957). However, it was not found to be seed borne in tomato in the present studies and similar findings have been reported by Prasada Rao *et al.* (1980). The virus was not dodder transmissible and Bennett (1944) revealed same conclusions. It was found to be graft transmissible on the lines of Solomon-Blackburn and Barker (2001).

TSWV has been reported to have wide experimental host range infecting 1090 plant species belonging to 85 families by Parella *et al.* (2003).

The present virus was studied for experimental range of 25 plant species belonging to 10 different families. Out of which 15 reacted as local lesion and systemic host, 9 as local lesion host and 1 as systemic host. *Beta vulgaris*, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Helianthus annuus*, *Citrullus lanatus*, *Cucumis sativus*, *Nicotiana tabacum*, and *Petunia hybrida* were found to be local lesion host. The virus had wide host range which included vegetable crops and even ornamentals having high economic value. The reactions of the host range studied were similar to those reported by Honrao (1986). Tomato plants were found to be naturally infected by the virus (Nirmal *et al.*, 1993).

The virus under study had TIP 45-50°C, LIV 4.5-5 hours and DEP 10^{-2} to 10^{-3} . The physical properties of the present virus fall in general range of physical properties reported for various isolates of TSWV (Ghanekar *et al.*, 1979, Mali and Patil, 1979 and Prasada Rao *et al.*, 1980).

The method of partial purification of TSWV was carried out as per the outline given by Van den Hurk *et al.* (1977). The partially purified preparation was used for infectivity test on assay host. The recovery of virus was found to be difficult as reported by Francki and Hatta (1981). The virus has LIV of few hours and therefore the difficulty was faced.

Screening of varieties was done to test the level of tolerance of varieties. All the recommended varieties showed susceptibility to TSWV with some differences.

It was found that the local selection PI-134417 showed resistance to the disease on the same lines as Honrao (1986). The PI-134417 was also

reported to be resistant by Maluf *et al.* (1991). Abscission of inoculated leaves after they became necrotic observed in the variety have been attributed by Torre *et al.* (2002) to low systemic movement of the virus in the plant.

SUMMARY



Chapter-VI

SUMMARY

Tomato spotted wilt virus (TSWV) belongs to the genus *Tospovirus* of the family *Bunyaviridae*. This is the only genus of the family *Bunyaviridae* that comprises of plant infecting viruses. Tomato spotted wilt disease has become the most destructive disease of vegetable and ornamental plants. The losses caused are in the range of 20-80 per cent depending upon the severity of the disease incidence. The tomato spotted wilt disease of tomato which is prevalent in Marathwada region is identified to be caused by TSWV based on symptomatology on tomato, transmission studies, physical properties, host range and reaction of diagnostic host.

The virus was sap transmissible and can be transmitted by mechanical means. The success of mechanical transmission was found to be dependent upon the nature of tissues used and the use of reducing agent in extraction buffer. The thrips transmitted the virus in persistent manner in the range of 15- 55 per cent. Dry conditions favour thrips population. The virus was also graft transmissible. The virus was not reported to be dodder or seed transmissible.

The virus was inactivated at 45-50⁰ C temperature and dilutions of 10⁻² and 10⁻³. The virus was viable upto 4.5 hours but not at 5 hours at room temperature.

The TSWV has been economically important constraint for several crops over the past several decades in several parts of the world. The disease cycle has proven to be extremely difficult to break because of the wide and often overlapping host range of both virus and the thrips vector. The virus infected all the 25 plants belonging to 8 different families. Of these 15 reacted as local lesion and systemic host, 9 as only local lesion host and 1 as systemic host only. Cowpea cv. Pusa komal was used as assay host it reacted both locally and systemically.

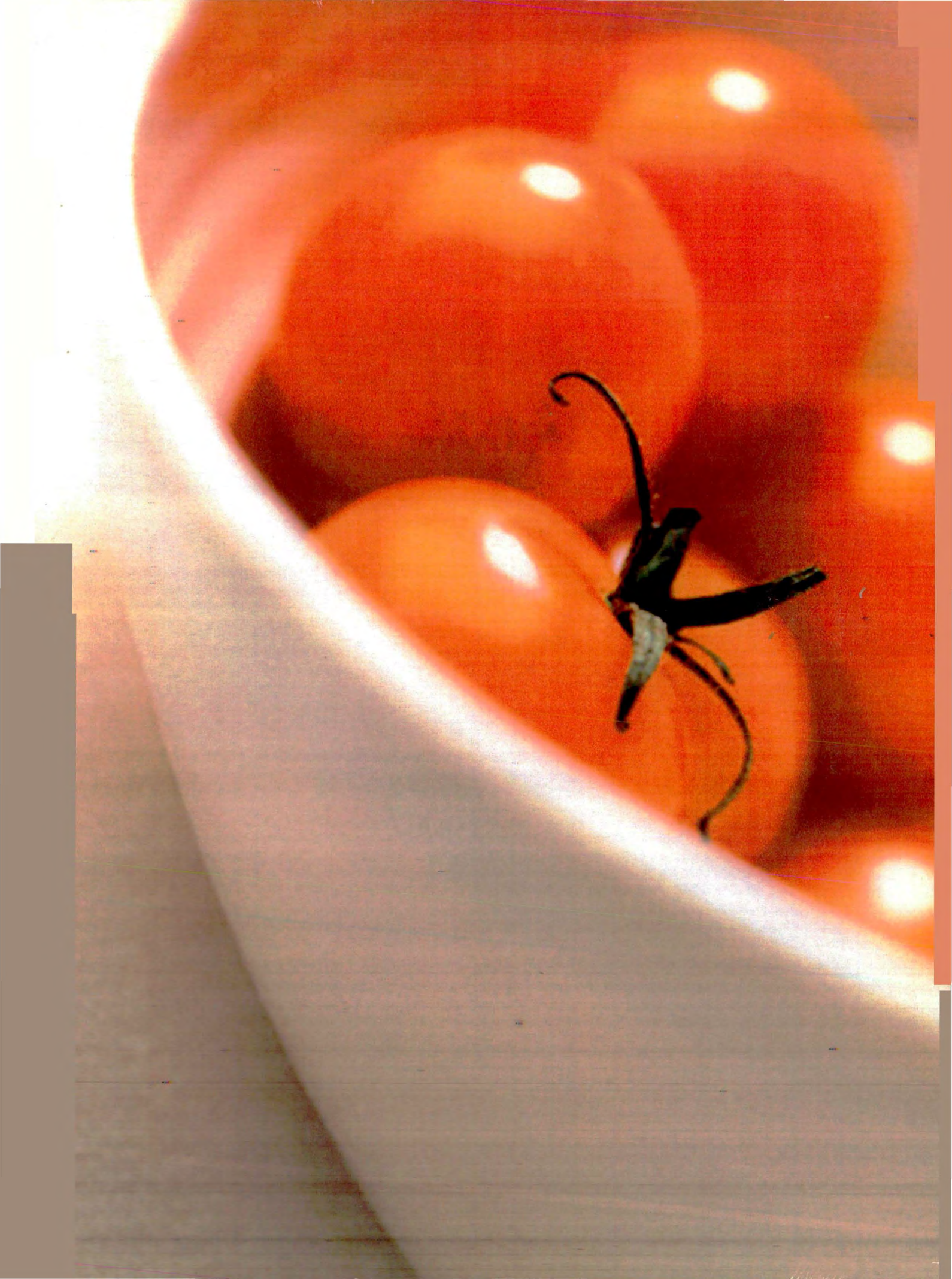
Partially purified preparation was used for infectivity test on cowpea cv. Pusa komal. The virus can be recovered between 10^{-1} and 10^{-2} dilutions. The purification of virus is difficult as TSWV is inactivated in-vitro within 4-5 hours at room temperature.

The use of resistant varieties is most effective and durable technique to decrease the crop losses caused by TSWV. All the 13 test varieties were found to be susceptible to the virus on inoculation. Only PI-134417 recorded the lowest incidence of the disease. Introgression of the identified resistance genes into cultivated tomatoes must continue to develop new resistant varieties.

Hence an integrated management programme is needed to be framed out giving more emphasis on the use of TSWV resistant cultivars with desirable agronomical traits. Continuous evolution of transgenic varieties should be given more importance to save the farmers from the enormous loss caused by tomato spotted wilt disease.

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THESIS ABSTRACT

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ABSTRACT

Tomato is a native of tropical America and is cultivated about 130 different countries. Tomato is one of the important vegetable crop and India ranks fifth in production. The area under cultivation is 35,000 ha with the production of 9,87,000 tonnes in Maharashtra. Tomato is the world's largest vegetable crop and having high processing value. The crop is vulnerable to several diseases including more than 29 viral diseases. The crop is known to be infected by several fungal, bacterial, viral and nematode pathogens. Early blight, TSW disease and tomato leaf curl disease are of economic importance in India including Maharashtra.

Hence, the study was carried on tomato spotted wilt disease of tomato which is prevalent in Marathwada region based on symptomatology, transmission studies, physical properties, host range and reaction of diagnostic host. Screening of cultivars resistant to TSWV was also done.

From the results it can be concluded that virus is sap transmissible. The insect vectors thrips transmit the virus in persistent manner. However, the virus is not seed or dodder transmissible. The TIP is 45-50°C, DEP is 10^{-2} to 10^{-3} and LIV is 4.5 to 5 hours. It is an unstable virus and hence its purification becomes difficult. It also has wide host range. The variety PI-134417 recorded the lowest incidence followed by cultivar Bhagyashree and found to be tolerant to the incidence of TSWV.