

**INVESTIGATIONS ON VIRUS (ES) INFECTING ZUCCHINI
(*Cucurbita pepo* L.) IN WESTERN MAHARASHTRA**

A thesis submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI- 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA STATE (INDIA)**

By

MISS DESHPANDE SHRADHA SUDHIR

Reg. No. 02214

in partial fulfillment of the requirements for the Degree of

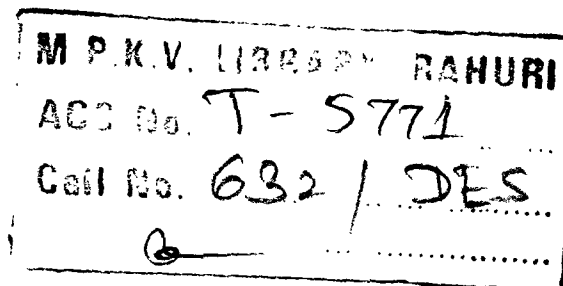
MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

**DEPARTMENT OF PLANT PATHOLOGY AND
AGRICULTURAL MICROBIOLOGY,
COLLEGE OF AGRICULTURE, PUNE-411005
MAHARASHTRA, INDIA**

2004



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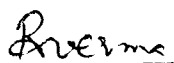
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DEDICATION

Dedicated to my beloved parents,

whose high expectations,

constant inspiration

and everlasting

love form the

base of my

progress.

... SHRADHA

CANDIDATE'S DECLARATION

I hereby declare that the thesis entitled "INVESTIGATIONS ON VIRUS(ES) INFECTING ZUCCHINI (*Cucurbita pepo* L.) IN WESTERN MAHARASHTRA" or part thereof has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma.

Place : Pune

Date : 23 / 8 / 2004


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CERTIFICATE

This is to certify that the thesis entitled, “**INVESTIGATIONS ON VIRUS(ES) INFECTING ZUCCHINI (*Cucurbita pepo* L.) IN WESTERN MAHARASHTRA**”, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY** embodies the results of a piece of *bonafide* research carried out by **MISS DESHPANDE SHRADHA SUDHIR**, under my guidance and supervision and that no part of the thesis has been submitted for any other Degree or Diploma.

The assistance and help rendered during the course of this investigation have been duly acknowledged.

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Maharashtra (India)

CERTIFICATE

This is to certify that the thesis entitled, "**INVESTIGATIONS ON VIRUS (ES) INFECTING ZUCCHINI (*Cucurbita pepo* L.) IN WESTERN MAHARASHTRA**", submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT PATHOLOGY**, embodies the results of a piece of *bonafide* research work carried out by **MISS DESHPANDE SHRADHA SUDHIR**, under the guidance and supervision of **Dr. (Mrs.) RAJ VERMA**, Senior Scientist, IARI, Regional Station, Agricultural College Estate, Shivajinagar Pune-5 and that no part of the thesis has been submitted for any other Degree, Diploma or Publication in any other form.

Place : Pune

Date : / /2004
15 SEP 2004


(D. L. Sale)

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Place : Pune

Date : 23 / 03 / 2004

S. Deshpande
((Shradha S. Deshpande))

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LIST OF ABBREVIATIONS

@	At the rate of
cm	centimeter
cv.	Cultivar
<i>et al.</i>	et alli (and others)
etc	and so forth
Fig.	Figure
hrs.	Hours
Kcal	Kilo-calorie
M	Molar
mg	Milligram
nm	Nanometer
μl	Microlitre
μg	Micro-gram
No.	Number
sp.	Species
var.	Variety
<i>Viz.,</i>	Videlicet (namely)
°C	Degree Celsius
%	Per cent

ABSTRACT

“Investigations on virus (es) infecting zucchini (*Cucurbita pepo* L.) in
Western Maharashtra

by

Miss. Deshpande Shradha Sudhir

B.Sc. (Agri.)

Research Guide : Dr. (Mrs.) Raj Verma

Department : Plant Pathology and Agricultural Microbiology

Survey of zucchini cultivated areas in five districts (i.e. Ahmednagar, Nasik, Pune, Sangli and Satara) of Western Maharashtra was conducted in summer season of 2003-2004 for the incidence of virus diseases and collection of virus isolates. Culture was maintained on zucchini (*Cucurbita pepo* L. var. Sunnyhouse) seedlings for further studies.

The disease symptoms observed on zucchini were veinal chlorosis followed by vein banding, severe mottling, blistering, leaf distortion and filiformity on leaves and malformation and blistering on fruits. Host range of the virus under study was mainly confined to the family Cucurbitaceae. The virus induced local lesions on *Chenopodium amaranticolor* and was found to be transmissible mechanically and also by two aphid species viz., *Aphis gossypii* and *Myzus persicae* in a non-persistent manner. Physical properties i.e. dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* of the virus were 10^{-2} - 10^{-3} , 50-55⁰C and 48 hrs., respectively. In serological tests, the virus reacted positively with antiserum of ZYMV and negatively with other

antisera tested (i.e. CMV, PRSV-P, PVY, TMV, TSWV, SMV and WMV-2). Virions were flexuous rods, measuring 700-750 nm (based on 47 particles). Electron microscopic observations of ultrathin sections made from infected leaves revealed the presence of cytoplasmic cylindrical inclusions consisting of 'pinwheels' and 'scrolls' typical of potyvirus group.

Based on above observations virus infecting zucchini was identified as zucchini yellow mosaic potyvirus belonging to family Potyviridae.

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INTRODUCTION

1. INTRODUCTION

Cucurbita belonging to the family Cucurbitaceae is a genus of annual or perennial, climbing or trailing herbs, comprising of 25 species of which *Cucurbita pepo*, *Cucurbita maxima*, *Curcubita moschata* are of economic importance.

Zucchini (*Cucurbita pepo* L.) is a summer squash that, like all squashes, descended from native related species originating in South America but is believed to have been developed in Italy. Many explorers who came to the America brought back what they considered strange foods. The zucchini eventually found it's way to Italy where it was named Zucchini. Many names have been given to this squash. The French call it courgette, a name that has been adopted by the English. The English also refer to a variety that is slightly longer and plumper as marrow.

Whitaker (1947) and Bohn (1950) defined the form summer squash as "The edible fruit of any species of *Cucurbita*, commonly *Cucurbita pepo*, utilized when immature as table fruit".

Zucchini is a warm season vegetable grown worldwide especially from Mediterranean area to Asia and in USA and is newly introduced to India. Its cultivation is also gaining popularity in Western Maharashtra.

Zucchini plant has the bushy habit rather than the vining habit of the winter squashes. Leaves of zucchini are quite large with more notches per leaf and are characterized by having light greenish gray splotches and streaks. The stems and leaves are hairy. Fruits are cylindrical in shape (size 5 to 16 inch in length and 3 to 4 inch in diameter) also round and intermediate shapes are there. Fruit colour varies from dark green to lighter shades of green both with and without stripes, all the way to tones of yellow. Many are highlighted with various degrees of speckling.

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Nutritionally, zucchini offer valuable antioxidants. Zucchini is good source of Vitamin A, C and potassium and is low in calories (Table 1) making it an excellent choice for dieters. A favourite way to enjoy zucchini is to eat it raw either in salad or as a party dip.

Zucchini like other cucurbits is affected by several diseases *viz.*, downy mildew, powdery mildew, fruit rots and virus diseases. Among these, virus diseases are important ones causing severe losses. About ten viruses have been reported worldwide to occur naturally on this crop. In India zucchini yellow mosaic virus has been reported from Pune (Singh *et al.*, 2003) and from Nainital district in North India (Chalam *et al.*, 2003).

Since very less information is available on viruses affecting zucchini in India, therefore it was felt necessary to carry a detail systemic study.

The present investigations were undertaken to study the virus(es) infecting zucchini in Western Maharashtra with following objectives:

Objectives

1. To survey the important zucchini growing areas in Western Maharashtra for the presence of virus diseases.
2. To collect the virus isolates from different places and to maintain in the glasshouse on indicator hosts.
3. To study the mode of transmission (sap, aphids, whiteflies and thrips).
4. To characterize the virus by conventional virological techniques.
5. To study the host range of different viruses.
6. Serology.
7. Electron microscopy.

Table 1 : Composition of zucchini fruit (per 100g of edible part)

Edible portion (%)	88
Moisture (g)	93.6
Protein (g)	1.3
Fat (g)	0.1
Carbohydrates (g)	1.4
Total dietary fibre (g)	1.3
Energy (Kcal)	11
Sodium (mg)	22
Potassium (mg)	264
Calcium (mg)	21
Phosphorus (mg)	65
Iron (mg)	0.5
Thiamine (mg)	0.08
Riboflavin (mg)	0.12
Niacin (mg)	0.70
Vitamin A (μ g)	6.0
Vitamin C (mg)	11

(Source : [www. google.com](http://www.google.com))

Chapter Opener Page

**REVIEW OF
LITERATURE**

2. REVIEW OF LITERATURE

Zucchini (*Cucurbita pepo* L.) is an important vegetable crop of American origin and is gaining popularity in India. Considerable losses in zucchini have been reported due to infection of several virus diseases. About 10 viruses belonging to poty-, Luteo-, Tospo-, Necro- and Gemini virus groups have been reported on zucchini, infecting naturally in different parts of world (Table 2).

POTYVIRUS GROUP

Watermelon mosaic virus- 2 (WMV-2)

WMV-2 has a wide geographical distribution, probably throughout the world and has been reported from Eastern Transvaal Lowveld (Eulitz, 1977), Iraq (Shawkat and Fegla, 1979), Jordan (Al-Musa and Mansour, 1982), Greece (Avgelis, 1983), Czechoslovakia (Chod *et al.*, 1987), Louisiana (Fernandes *et al.*, 1991) and Yugoslavia (Dukic *et al.*, 2002).

The symptoms of WMV-2 infection on zucchini squash are diffuse mosaic and mottling, followed by appearance of raised green blisters and filiform leaves (Al-Musa and Mansour, 1982).

WMV-2 is transmitted mechanically and by several species of aphids i.e. *Myzus persicae*, *Aphis gossypii*, *A. fabae*, etc. (Al-Musa and Mansour, 1982; Shawkat and fegla, 1979).

Under natural conditions WMV-2 infects mainly cucurbits, but artificially it also infects certain species belonging to other families.

Zucchini yellow fleck virus (ZYFV)

ZYFV on zucchini was first reported from South Italy (Vovlas *et al.*, 1981). Initially virus was considered to be serologically related to WMV-2 (Russo *et al.*, 1979; Antignus *et al.*, 1995) but now regarded as a totally distinct virus, distinguishable from WMV-2 on the basis of

serology, test plant reaction, inclusion bodies, coat protein and genome structure (Lovisolo, 1980; Antignus *et al.*, 1995).

Vovlas *et al.* (1981) reported an isolate of zucchini yellow fleck virus from zucchini squash (*Cucurbita pepo* L.) with pinpoint yellow leaf spots. It infected only Cucurbitaceae and was transmitted to squash by *Myzus persicae*. Physical properties i. e. DEP, TIP and L.IV were between 10^{-4} to 10^{-5} , 55-60⁰C and 25-30 days respectively. Virus particles were flexuous, filamentous 700-800 nm in length.

Martelli *et al.* (1981) described different inclusions induced by ZYFV in leaf tissues of mechanically inoculated zucchini squash. The inclusions described were: vesiculate, upto 300 nm, cylindrical inclusions appearing as tubes and bundles or pinwheels and scrolls.

Zucchini yellow mosaic virus (ZYMV)

ZYMV is one of the most aggressive and destructive viruses of some cucurbits and is distributed throughout the world (Table-2). It was first reported from zucchini squash from Italy and France (Lecoq *et al.*, 1981; Lisa *et al.*, 1981).

The host range of the virus is very wide belonging to the different families such as Chenopodiaceae, Leguminosae, Amaranthaceae, Solanaceae and Umbelliferae (Lovisolo, 1980) whereas host range of the virus isolated from zucchini was restricted mainly to family Cucurbitaceae and a local lesion host i.e. *Chenopodium amaranticolor* (Singh *et al.*, 2003). Virus also produces local lesions on *Chenopodium quinoa* and *Gomphrena globosa* (Vega *et al.*, 1995). Cucumber, muskmelon, watermelon and zucchini squash are natural hosts of ZYMV (Chalam *et al.*, 2003). Experimentally it induces local lesions, severe yellowing, mosaic and often death of melons and zucchini squash (Lovisolo, 1980). The other symptoms include vein clearing, vein

banding, systemic mosaic, blistering and filiform leaves along with fruit distortion and reduction in fruit size (Lecoq *et al.*, 1991; Purcifull *et al.*, 1984; Chalam *et al.*, 2003; Singh *et al.*, 2003).

ZYMV is transmitted by sap inoculation and also by several species of aphids viz. *Aphis citricola* (Purcifull *et al.*, 1984), *Aphis gossypii*, *Myzus persicae* (Lecoq *et al.*, 1981; Lisa *et al.*, 1981), and *Aphis aromoracia*, *A. craccivora*, *Acrythosiphon pisum*, *Hydaphis erysimi* and *Uroleucon* sp. (Adlerz, 1987), in a non-persistent manner. The virus is also known to be seed transmissible in zucchini squash at a very low rate (3.5%) (Schrijnwerkers *et al.*, 1991, Fletcher *et al.*, 2000; Tobias and Polvics, 2003).

Physical properties i.e. dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV) of the virus are 10^{-3} , 50-55°C and 48 hrs., respectively (Singh *et al.*, 2003).

The virions are flexuous rods measuring 750 nm in length. ZYMV induces cytoplasmic inclusions consisting of pinwheels and scrolls (Lisa *et al.* 1981; Vega *et al.* 1995; Singh *et al.* 2003). The virus can be easily detected through ELISA test (Purcifull *et al.* 1984; Vega *et al.* 1995; Singh *et al.* 2003).

Squash yellow leaf curl virus (SqYLCV)

The virus has been reported from Batinah region of the Sultanate of Oman (Zouba *et al.*, 1998). The symptoms of the virus infection were small yellow spots; diffuse veinal yellowing and curling of young leaves. The virus was easily transmitted by mechanical inoculation and by whitefly i.e. *Bemisia tabaci* in a semi-persistent manner. The host range of the virus was restricted to two cucurbit species i. e. *Cucurbita pepo* and *Luffa aegyptiaca*. Virus particles were flexuous filaments measuring 700-750 nm length. The virus induced pinwheel like inclusion bodies and

was serologically related to watermelon mosaic-2 potyvirus but not to zucchini yellow mosaic potyvirus or papaya ringspot potyvirus (watermelon strain).

LUTEOVIRUS GROUP

Cucurbit aphid borne yellows virus (CABYV)

The virus has been reported on zucchini squash from France (Lecoq *et al.*, 1992), United States (Lemaire *et al.*, 1993), Algeria (Balkahla *et al.*, 1994) and Lebanon (Abou-Jawdah *et al.*, 1997).

The symptoms of virus infection are yellowing and thickening of older leaves of melon, cucumber and zucchini. Virus infection reduces the yield of melon and cucumber by decreasing the number of fruits per plant without altering the fruit shape or quality (Lecoq *et al.*, 1992).

CABYV is readily transmitted in a persistent manner by *Myzus persicae* and *Aphis gossypii* but not mechanically (Lecoq *et al.*, 1992; Lemaire *et al.*, 1993; Balkahla *et al.*, 1994).

The virus is common in weeds and in cultivated cucurbits. The host range of the virus includes several cucurbits including melons and marrows (Lemaire *et al.*, 1993)

Virus particles are spherical measuring 25 nm in diameter (Lecoq *et al.*, 1992). DAS-ELISA has been used for identification of this virus by Balkahla *et al.* (1994).

TOSPOVIRUS GROUP

Zucchini lethal chlorosis virus (ZLCV)

The virus has been reported on zucchini (*Cucurbita pepo* cv. Caserta) from Sao Paulo state, Brazil (Rezende *et al.*, 1997). The symptoms of virus infection are chlorosis, systemic necrosis of basal leaves and malformation with narrow and curled leaf blades. The host range of the virus is restricted mainly to the family Cucurbitaceae

(Eezerra *et al.*, 1999). Virus is transmitted mechanically (Rezende *et al.*, 1997) and also by thrips i.e. *Frankliniella zucchini* (Thysanoptera: Thripidae) (Nakahara and Monteiro, 1999). The virus particles are enveloped and spherical having 80-90 nm diameter.

NECROVIRUS GROUP

Tobacco Necrosis virus (TNV)

Roggero and Lisa (1995) isolated tobacco necrosis virus (TNV) from glasshouse zucchini plants in Liguria, Northern Italy. Characteristic symptoms of the virus were yellow spots on young leaves and necrotic symptoms on older leaves, petioles and stems. The virus was identified as serotype D, devoid of satellite particles. Three ELISA procedures were compared for detection of TNV using polyclonal antibodies for serotype D.

GEMINIVIRUS GROUP

Squash leaf curl virus (SqLCV)

Squash leaf curl virus has been reported to occur naturally on zucchini squash (*Cucurbita pepo*) in USA (Cohen *et al.* 1983), California (Dodds *et al.*, 1984) and Saudi Arabia (Al-Shahwan *et al.*, 2002). The disease is characterized by severe stunting and leaf curl symptoms on leaves of *Cucurbita maxima*, *C. moschata* and *C. pepo* (squash) and green mosaic and leaf distortion on *Phaseolus vulgaris*. SqLCV is transmitted mechanically but efficiency is poor (Dodds *et al.*, 1984) and also by whiteflies i.e. *Bemisia tabaci* (Cohen *et al.*, 1983, Al-Shahwan *et al.*, 2002). Virus is circulative in *B. tabaci* with relatively long latent period (Cohen *et al.*, 1983). Virus particles are geminate (22x38 nm) (Cohen *et al.*, 1983).

The salient features of different virus groups with detail account of information available about zucchini viruses have been furnished in Table 3.

Table 2 Geographical distribution of viruses occurring on zucchini (*Cucurbita pepo* L.)

Sr. No.	Virus	Distribution	Reference
1	POTYVIRUS GROUP Watermelon mosaic virus- 2 (WMV- 2)	Eastern Transvaal Lowveld	Eulitz (1977)
		Iraq	Shawkat and Fegla (1979)
		Jordan	Al- Musa and Mansour (1982)
		Greece	Avgelis (1983)
		Czechoslovakia	Chod <i>et al.</i> (1987)
		Louisiana	Fernandes <i>et al.</i> (1991)
		Yugoslavia	Dukic <i>et al.</i> (2002)
2	Zucchini yellow fleck virus (ZYFV)	Apulia, Southern Italy	Vovlas <i>et al.</i> (1981)

3	Zucchini yellow mosaic virus (ZYMV)	Italy	Lecoq <i>et al.</i> (1981)
		France	Lisa <i>et al.</i> (1981)
		United states	Provvidenti <i>et al.</i> (1983)
		Connecticut, New York Florida and California	Provvidenti <i>et al.</i> (1984)
		Ohio	Nameth (1986)
		Arkansas	Wickizer <i>et al.</i> (1986)
		Pacific Northwest	Crosslin <i>et al.</i> (1988)
		Jordan	Al-Musa (1989)
		Venezuela	Hernandez <i>et al.</i> (1989)
		Ontario	Stobbs and VanSchagen. (1990)
		Spain	Luis-Arteaga (1990)
		Czechoslovakia	Chod and Jokes (1991)
		Greece	Kyriakopoulou and Varveri (1991)
		Louisiana	Fernandes <i>et al.</i> (1991)
Netherlands	Schrijnwerkers <i>et al.</i> (1991)		

		Ankara province	Ertunc (1992)
		Martinique	Lecoq <i>et al.</i> (1992)
		Singapore	Wong and Lee (1992)
		Nepal	Dahal (1992)
		Hungary	Tobias <i>et al.</i> (1996)
		Sudan	Mahgoub <i>et al.</i> (1997)
		Argentina	Gracia (2000)
		India	Singh <i>et al.</i> (2003) Chalam <i>et al.</i> (2003)
		Czech Republic	Svoboda and Polak (2002)
4	Squash yellow leaf curl virus (SqYLCV)	Oman	Zouba <i>et al.</i> (1998)
5	LUTEOVIRUS GROUP Cucurbit aphid borne yellows virus (CABYV)	France	Lecoq <i>et al.</i> (1992)
		United states	Lemaire <i>et al.</i> (1993)
		Algeria	Balkahla <i>et al.</i> (1994)
		Lebanon	Abou-Jawdah <i>et al.</i> (1997)
		Nepal	Dahal <i>et al.</i> (1997)
6	TOSPOVIRUS GROUP Zucchini lethal chlorosis virus (ZLCV)	Brazil	Rezende <i>et al.</i> (1997)
7	NECROVIRUS GROUP Tobacco necrosis virus (TNV)	Leguria, Northen Itali	Roqpero and Lisa (1995)
8	GEMINIVIRUS GROUP Squash leaf curl virus (SqLCV)	U. S. A.	Cohen <i>et al.</i> (1983)
		Soudi Arabia	Al Shahwan <i>et al.</i> (2002)

Table 3 : Comparison of the virus under investigation with those reported by other researchers on zucchini (*Cucurbita pepo* L.)

Sr. No	Name of the virus	Symptoms	Host range	Transmission	Physical properties			Particle morphology	References
					TIP (°c)	DEP	LIV		
	Virus under investigation	Veinal chlorosis, severe mottling, vein banding, blistering and shoestring of leaves. Blistering on fruits along with malformation.	Systematic symptoms on: <i>Benincasa hispida</i> , <i>Citrullus lanatus</i> , <i>Cucumis melo</i> , <i>C. sativus</i> , <i>Cucurbita maxima</i> , <i>C. pepo</i> , <i>Lagenaria siceraria</i> , <i>Luffa acutangula</i> , <i>L. cylindrica</i> , <i>Momordica charantia</i> , <i>Physalis floridana</i> BlackTurtle- 2, <i>Trichosanthes anguina</i> Local lesions on : <i>Chenopodium amaranticolor</i> .	Sap and aphids i.e. <i>Aphis gossypii</i> and <i>Myzus persicae</i>	50-55	10^{-2} - 10^{-3}	48 hrs.	Flexuous filaments, 700-750 nm	

Sr. No	Name of the virus	Symptoms	Host range	Transmission	Physical properties			Particle morphology	References
					TIP (°C)	DEP	LIV		
A 1	POTYVIRUS GROUP Watermelon mosaic virus-2 (WMV-2)	Diffuse mosaic and mottling on the first two true leaves, followed by appearance of raised green blister-like lesions on leaves. Fruits, if set, severely deformed and not marketable.	Systemic symptoms on: cucumber, squash, snake cucumber, muskmelon watermelon, <i>Pisum sativum</i> Local lesions on : <i>Chenopodium amaranticolor</i> , <i>C. quinoa</i> , <i>Phaseolus vulgaris</i> , <i>Gomphrena globosa</i>	Mechanical and by aphids i.e. <i>Aphis fabae</i> , <i>A. gossypii</i> and <i>Myzus persicae</i>	55-60	10 ⁻³ -10 ⁻⁴	2-3 week	Watermelon mosaic virus-2 (WMV-2)	Shawkat and Fegla (1979), Al Musa and Mansour (1982).
2	Zucchini yellow fleck virus (ZYFV)	Yellow pin-points on leaves, becoming completely yellow and necrotic. stunting and fruitfulness with severe infection.	<i>Citrullus lanatus</i> , <i>Cucumis melo</i> , <i>Cucumis sativus</i> , <i>Cucurbita maxima</i> , <i>Echallim elaterium</i> etc. Assay hosts: (Local lesions - L or systemic-S) <i>Citrullus lanatus</i> (L), <i>Cucumis melo</i> (S), <i>Cucumis sativus</i> (S), <i>Cucurbita pepo</i> (L)	Mechanical inoculation, contact between plants and by Aphids i.e. <i>Aphis fabae</i> , <i>Myzus persicae</i>	55-60 ⁰ C	10 ⁻⁴ - 10 ⁻⁵	30 days	Virions are filamentous with a clear modal length of 750 nm	Martelli <i>et al.</i> (1981); Vovlas <i>et al.</i> (1981); Antignus <i>et al.</i> (1995).

Sr. No	Name of the virus	Symptoms	Host range	Transmission	Physical properties			Particle morphology	References
					TIP (°C)	DEP	LIV		
3	Zucchini yellow mosaic virus (ZYMV)	Prominent veinal chlorosis, severe mottling, vein banding, leaf malformation such as blistering and shoe string. Malformation, discolouration from green to yellow and blistering on fruits.	Systemic symptoms on : <i>Citrullus lanatus,</i> <i>Cucumis melo,</i> <i>C. metuliferus,</i> <i>C. sativus,</i> <i>Cucurbita maxima,</i> <i>C. moschata,</i> <i>C. okechobeensis,</i> <i>C. pepo,</i> <i>Gomphrena globosa,</i> <i>Luffa acutangula,</i> <i>Phaseolus vulgaris,</i> Black turtle soup 2. <i>Pisum sativum,</i> <i>Ranunculus sardous,</i> <i>Senecio vulgaris,</i> <i>Trifolium maritimum</i> Local lesions on: <i>Chenopodium</i> <i>Amaranticolor,</i> <i>C. quinoa.</i>	Sap and aphids i.e. <i>Acrythosiphon pisum,</i> <i>Aphis craccivora,</i> <i>A. citricola,</i> <i>A. gossypii,</i> <i>Hydaphis erysimi,</i> <i>Macrosiphum euphorbiae,</i> <i>Myzus persicae,</i> <i>Toxoptera citricidus,</i> <i>Uroleucon</i> spp and also through seed (upto 3.5%)	50-55°C	10 ⁻³	48 hrs.	Filamentous, not enveloped usually flexuous; 750 nm long, 11 nm wide	Lisa <i>et al.</i> (1981); Lecoq <i>et al.</i> (1981); Lecoq <i>et al.</i> (1983); Lesemann <i>et al.</i> (1983); Provvidenti <i>et al.</i> (1984); Purcifull <i>et al.</i> (1984); Fletcher <i>et al.</i> (2000); Fletcher <i>et al.</i> (2000); Svoboda and Polak (2002); Singh <i>et al.</i> (2003); Chalam <i>et al.</i> (2003)

Sr. No	Name of the virus	Symptoms	Host range	Transmission	Physical properties			Particle morphology	References
					TIP (°C)	DEP	LIV		
4	Squash yellow leaf curl virus (SqYLCV)	Leaves show yellow spotting veinal chlorosis, upward curling and brittleness	<i>Cucurbita pepo</i> and <i>Luffa aegyptiaca</i>	Mechanical transmission and by whiteflies i.e. <i>Bemisia tabaci</i>	-	-	-	Flexuous rod shaped particles, measuring 700 – 750 nm in length	Zouba <i>et al.</i> (1998).
5	LUTEOVIRUS GROUP Cucurbit aphid borne yellows virus	Yellowing and thickening of older leaves and reduction in fruit number	Several cucurbits including melons and marrows, cucumber, zucchini squash etc.	Aphid i.e. <i>Aphis gossypii</i> <i>Myzus persicae</i>	-	-	-	Spherical particles (25 nm in dia)	Lecoq <i>et al.</i> (1992); Balkahla <i>et al.</i> (1994); Lemaire <i>et al.</i> (1995).
6	TOSPOVIRUS GROUP Zucchini lethal chlorosis virus (ZLCV)	Chlorosis, systemic necrosis of basal leaves and malformation with narrow and curled leaf blades. reduced plant growth	-	Mechanical transmission	-	-	-	Enveloped, spherical particles (80-90 nm dia)	Rezende <i>et al.</i> (1997).

Sr. No	Name of the virus	Symptoms	Host range	Transmission	Physical properties			Particle morphology	References
					TIP (°c)	DEP	LIV		
7	GEMINIVIRUS GROUP Squash leaf curl virus (Sq LCV)	Severe stunting and leaf curl	<i>Cucumis sativus</i> , <i>Cucurbita maxima</i> , <i>C. moschata</i> , <i>C. pepo</i> (squash), <i>Medicago sativa</i> , <i>Phaseolus vulgaris</i> .	Mechanical transmission and by whitefly i.e. <i>Bemisia tabaci</i>	-	-	-	-	Cohen <i>et al.</i> (1983); Al – Shahwan <i>et al.</i> (2002).

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**MATERIAL AND
METHODS**

3. MATERIALS AND METHODS

3.1 Survey and collection of zucchini (*Cucurbita pepo* L.) virus isolates

Surveys of zucchini cultivated areas of Western Maharashtra were conducted in summer season of 2003 for the incidence of virus diseases and collection of virus disease samples.

The leaves of zucchini plants exhibiting virus symptoms were collected randomly from commercial fields of zucchini. The virus infected samples collected from different places were brought to the laboratory for further studies. The per cent incidence of the virus disease was calculated as per the following formula:

$$\frac{\text{Total number of virus infected plants}}{\text{Total number of plants cultivated in the field}} \times 100$$

These samples were further tested through ELISA to confirm the identity of the viruses associated with disease symptoms in zucchini.

The virus infected samples collected from the commercial fields were sap inoculated on zucchini seedlings to maintain the culture. Later, the host range of these virus isolates was studied by inoculating on various indicator plants. The plants which did not exhibit the symptoms even after 4-6 weeks of inoculation were indexed back on healthy zucchini plants to know whether they are symptomless carriers. A representative isolate from each group was chosen for the study of transmission, physical properties, serology and electron microscopy in order to identify the virus(es).

3.2 Raising seedlings

Zucchini (*Cucurbita pepo* L. var. Sunnyhouse) plants were used in all the experiments. The seedlings were raised from the seeds in the earthen pots containing mixture of sand, loam and compost (1 : 1: 2) and were watered uniformly and regularly. All the experiments were carried out in an insect proof glasshouse which was regularly sprayed with 0.05% Dimethoate.

3.3 Transmission Studies

3.3.1 Mechanical transmission

Preparation of standard inoculum

Young infected leaves showing prominent virus symptoms were collected, washed thoroughly in running tap water to remove dirt, and blotted dry. The inoculum was prepared by grinding young infected leaves in chilled 0.01 M phosphate buffer (pH 7.0) in mortar and pestle . The buffer was added at the rate of 1 ml. per gm of infected leaf tissue while macerating. The resulting pulp after maceration was squeezed between 2 folds of sterile absorbent cotton. This extract was used as “Standard inoculum” for further studies.

Method of inoculation

All the plants were watered at least half an hour earlier before they were inoculated, so that the leaves may remain turgid at the time of inoculation. Prior to inoculation, 600 mesh carborundum powder was dusted uniformly on the upper surface of the leaves of the test plants. The inoculation was made by gently rubbing the upper surface of the leaves with cotton swab dipped in the inoculum. The leaves were washed immediately with a jet of tap water or from a squeeze bottle to remove excess of inoculum. The inoculated plants were kept for observation in

the glasshouse for 6 to 8 weeks. The control plants were treated similarly using phosphate buffer only.

3.3.2 Insect transmission

3.3.2.1 Aphid transmission

Experiments were conducted to find out the aphid vectors for the transmission of zucchini viruses. The following aphid species were tried :

Aphis craccivora Koch.,
Aphis gossypii Glov.,
Aphis malvae Koch.,
Aphis nerii L.,
Macrosiphum rosae L.,
Macrosiphum pisi Kalt.,
Myzus persicae Sulz.,
Rhopalosiphum maidis Fitch.,
Toxoptera citricidus Kirk.

The colonies of these aphids were maintained on the following plants :

<i>Aphis craccivora</i> Koch.	on	Cowpea (<i>Vigna sinensis</i>)
<i>Aphis gossypii</i> Glov.	on	Okra (<i>Abelmoschus esculentus</i>)
<i>Aphis malvae</i> Koch.	on	<i>Hibiscus rosa-sinensis</i>
<i>Aphis nerii</i> L.	on	<i>Nerium oleander</i>
<i>Myzus persicae</i> Sulz.	on	Cabbage (<i>Brassica oleracea</i>)
<i>Macrosiphum pisi</i> Kalt.	on	<i>Pisum sativum</i>
<i>Macrosiphum rosae</i> L.	on	<i>Sonchus oleraceus</i>
<i>Rhopalosiphum maidis</i> Fitch.	on	Maize (<i>Zea mays</i>)
<i>Toxoptera citricidus</i> Kirk.	on	Kagzi lime (<i>Citrus aurantifolia</i>)

Method of aphid transmission

Aphids from their host plant were gently disturbed by slightly touching their antennae with a camel hair brush No. 1 which made them withdraw their stylets. They were carefully picked up and transferred in a test tube with the moistened tip of a camel hair brush No. 1 .

Prior to each test the aphids were starved for 90 min. by keeping them in a glass tube covered with muslin cloth. For acquisition feeding, aphids were placed on virus infected detached young leaves, in a clean petriplate.

The aphids were watched with the help of hand lens and when they assumed the probing position, the duration of the probe was timed with a stop watch. All the aphids were given 15 min. of acquisition feeding on virus source plant and then transferred to healthy young zucchini plants for inoculation feeding for 1 hour, these plants were sprayed with insecticide dimethoate (0.05%) solution. The plants were observed for 50 days for symptom development.

3.3.2.2 Thrips transmission

Transmission studies were also carried out to ascertain the transmission of the disease by thrips. The following thrips were tried :

Thrips tabaci Lind. from tobacco and onion.

Thrips palmi Kamy from sunflower and watermelon.

Scirtothrips dorsalis Hood from chilli.

The above thrips (both adults and nymphs) were reared on their respective hosts for maintenance of their colonies.

Method of thrips transmission

Nymphs of the above thrips were carefully picked up and transferred to a test tube with the moistened tip of a camel hair brush No. 1.

For acquisition feeding, the nymphs were placed on infected detached young leaves, showing prominent symptoms, separately in a small clean petridish. All the nymphs were given 5 days acquisition access feeding on virus source and then transferred on to healthy young zucchini seedlings for inoculation feeding for 10 days after which they were killed with spray of an insecticide. For each inoculation feeding, 25 nymphs per plant were used. All the experiments were conducted in insect proof cages. The plants were observed for symptom development.

3.3.2.3 Whitefly transmission

Transmission studies with whiteflies were also carried out to find out whether the present virus is transmitted by whiteflies. The colony of whiteflies viz., *Bemisia tabaci* was maintained on *Nicotiana tabacum* cv. White Burley.

Method of whitefly transmission

Whiteflies were collected from tobacco plants in glass globe and covered with black paper and its mouth tied with muslin cloth. A fasting of four hours was given. For acquisition feeding diseased leaves of zucchini were inserted in the glass globe containing whiteflies by keeping the closed end upwards and removing the muslin cloth at open end. After 24 hrs. of acquisition feeding whiteflies were transmitted to healthy zucchini plants for inoculation feeding for 24 hrs. Thereafter plants were sprayed with insecticide dimethoate (0.05%). The plants were observed for symptom development.

3.3.3 Seed transmission

For studying seed transmission of the viruses, fruits from severely infected tagged plants of zucchini were collected and the seeds were collected from these fruits. These seeds were dried and 50 seeds were sown in 25 earthen pots (6" diameter) in an insect proof glasshouse and

the seedlings were kept under observation for about two months. For control, seeds collected from the healthy fruits were sown simultaneously. The number of seeds germinated and the number of plants exhibiting virus symptoms was recorded.

3.4 Host Range

Host range study was undertaken with a view to find out experimental hosts since knowledge of these studies could provide information on possible natural sources of virus infection of zucchini plants under field condition as also aid in identifying symptomless carrier. Fifty-eight plants belonging to ten families were raised in the glasshouse. Plants belonging to Cucurbitaceae family were inoculated when they were at the cotyledonary stage. The test plants were observed daily for 30 days for their reaction to virus isolates. Back inoculations were made on zucchini plants from the sap extracted from leaves. Inoculated plants were also tested serologically for the presence of the virus by using ELISA test.

The following species were used as test hosts:

Amaranthaceae

Amaranthus caudatus L.

A. viridis L.

Celosia argentea L.

Gomphrena globosa L.

Chenopodiaceae

Beta vulgaris L.

Chenopodium album L.

C. amaranticolor Coste & Reyn.

C. murale L.

C. quinoa Wild.

Compositae

Helianthus annuus L.

Zinnia elegans Jacq.

Cruciferae

Brassica campestris L.

B. oleracea var. Botrytis.

B. rapa L.

Cucurbitaceae

Benincasa hispida (Thunb.) Cogn.

Citrullus lanatus Thunb.

Cucumis melo L.

C. sativus L. cvs. Himangi, Poona Khira

Cucurbita maxima Duch.

C. pepo L var. Sunnyhouse

Lagenaria siceraria Standl.

Luffa acutangula Roxb.

L. cylindrica L.

Momordica charantia L.

Trichosanthes anguina

Labiatae

Osimum basilicum L.

O. sanctum L.

Leguminosae

Arachis hypogea L.

Cajanus cajan (L.) Millsp.

Cicer arietinum L. cv. Chaffa
Crotolaria juncea L.
Cyamopsis tetragonoloba (L.) Taub.
Glycine max (L.) Merr. cv. Bragg.
Phaseolus aureus Roxb.
P. lanatus L.
P. mungo Roxb.
Pisum sativum L. var. Bonneville
Vigna unguiculata (L.) Walp cv. 'Black Eye'

Malvaceae

Abelmoschus esculentus (L.) Moench.

Poaceae

Zea mays L.

Solanaceae

Capsicum annum L.
Datura metel L.
D. stramonium L.
Lycopersicon esculentum Mill. var. Pusa Ruby
Nicotiana benthamiana
N. clevelandii A. Gray
N. glutinosa L.
N. rustica L.
N. sylvestris Speg.
N. tabacum L. var. White Burley
N. tabacum L. var. Samsun
N. tabacum L. var. Xanthi
Petunia hybrida Villm.



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Physalis floridana Black Turtle 2.

P. peruviana L.

Solanum melongena L.

S. nigrum L.

S. tuberosum L.

3.5 Physical Properties

All the glasswares required for determining the physical properties of virus isolate were sterilized before use.

The physical properties were studied with the crude extract. For this purpose, zucchini plants showing severe virus symptoms were selected and extract was prepared by macerating young leaves in a mortar without adding any diluents. The macerate was squeezed through a muslin cloth and resulting extract was used for these studies.

3.5.1 Thermal Inactivation Point (TIP)

For determination of thermal inactivation point 1ml undiluted leaf extract obtained from infected young leaves of zucchini was added in each 5 ml. thin wall tubes of uniform size and subjected to different temperatures ranging from 40⁰C-95⁰C for 10 min. in thermo-statistically controlled water-bath. Care was exercised to see that the portion of the tube containing sap was completely immersed in water. After 10 min. tubes were taken out of water bath and cooled under running tap water. The sap thus subjected to each treatment was inoculated on a set of 10 zucchini plants. Undiluted sap, which served as control was inoculated on another set. The symptoms were recorded thereafter.

3.5.2 Dilution End Point (DEP)

For determining dilution end point, the infective sap was extracted by grinding 1 g. of the infected leaves in a mortar and pestle without adding any buffer. Serial dilutions of the extracted sap from 1: 10 to

1 : 1,00,00,000 were made by using sterile distilled water as a diluent. Each dilution was inoculated on a set of 10 zucchini plants. Undiluted sap was inoculated on another set to serve as control. The symptoms were recorded after 5 to 7 days following inoculation.

3.5.3 Longevity *in vitro* (LIV)

The undiluted sap was transferred to a rubber stoppered glass tube and was kept at room temperature (25 ± 1). Samples of stored sap was taken out at periodical intervals and inoculated on a set of 10 zucchini plants. Freshly extracted sap served as control. The plants were observed for symptoms every day.

3.6 Serological Studies

This study was conducted to find out the possible relationship of the viruses under investigation with the different available standard antisera. The antisera used for serological studies were as follows :

Cucumber Mosaic Virus (CMV)

Papaya Ringspot Virus P (PRSV- P)

Potato Virus Y (PVY)

Tobacco Mosaic Virus (TMV)

Tomato Spotted Wilt Virus (TSWV)

Watermelon Mosaic Virus 2 (WMV-2)

Watermelon Silver Mottle Virus (WSMV)

Zucchini Yellow Mosaic Virus (ZYMV)

Enzyme Linked Immunosorbent Assay (ELISA)

All the serological tests were done using ELISA technique. Direct Antigen Coating (DAC) indirect ELISA was performed as per the procedure of Chakraborty and Jain (1996) and included the following steps. The various buffers used in ELISA were prepared as follows :

A. Phosphate Buffer Saline (PBS)

Sodium hydrogen phosphate (Na_2HPO_4)	2.38 g
Potassium dihydrogen phosphate (KH_2PO_4)	0.40 g
Sodium chloride (NaCl)	16.00 g
Potassium chloride (KCl)	0.40 g
Distilled water	2.0 lit.
pH	7.40

B. Wash Buffer (PBS-T)

PBS	1.00 lit
Tween-20	0.5 lit

C. Extraction Buffer

PBS-T + PVP-40 (2%)

D. Coating buffer (Carbonate buffer)

Sodium carbonate (Na_2CO_3)	1.59 g
Sodium hydrogen carbonate (NaHCO_3)	2.93 g
Distilled water	1.0 lit.
pH	9.60

E. Enzyme conjugate buffer

PBS	100.0 ml
Polyvinyl pyrrolidone (PVP) (40000 Mw.)	2.0 g
Ovalbumin (Crystalline)	0.2 g

F. Substrate buffer

Diethanol amine	97.0 ml
Distilled water	800 ml

pH was adjusted to 9.8 using 1M HCl and the volume was made up to one litre. This was preserved in a brown bottle at room temperature. P-nitrophenyl phosphate (PNPP) was used as substrate. PNPP was

dissolved in substrate buffer just before use @ 0.6 mg/ml. It was stored in dark by covering the vessel with aluminum foil.

Procedure of DAC-ELISA

1. Crude plant extract was prepared with extraction buffer (1g of leaf sample per 9 ml buffer) and diluted with coating buffer using diution of 1:10. 200 µl of the extract was dispensed into each well of microtitre plate using a micropipette and the plates were incubated overnight at 4⁰C.
2. The contents of the plates were poured off and washed with PBS-T four times allowing 3 min. for each wash.
3. The antisera was used at 1:200 dilution and 200 µl was added to each well. The plates were incubated at 37⁰C for 2 hrs. and were washed with PBS-T as in step-2.
4. Alkaline phosphatase (ALP) labelled antirabbit IgG (Sigma Co., st. Louis, Missoure, USA.) was diluted in enzyme conjugate buffer to a concentration of 1 : 10000 dilution and 200 µl was added in each well. The plates were incubated at 37⁰C for 2 hrs. and washed with PBS-T as in step-2.
5. Substrate was then dispensed to each well of microtitre plate @ 200 µl per well and incubated at 37⁰C for 15 min. followed by 15 min at room temperature. Colour reaction of each well was recorded and the results were quantitatively recorded on ELISA reader (EL_x 808₁₀) at 405 nm.

3.7 Electron Microscopy

Leaf dip preparations were made by grinding small pieces of diseased young leaves of each virus isolate previously fixed in 3 per cent

gluteraldehyde for 1 hr. A drop of sap was placed on formvar- coated copper grids for one minute, rinsed three times with sterile distilled water, stained with (2%) uranyl acetate (UA) or phosphotungstic acid and examined under transmission electron microscope (TEM) for the presence of virus particles.

For ultra thin sectioning, 1 cm bits of infected leaves (midrib and lamina) and thin petioles of each virus isolate under study were fixed for 3 hrs. in 3 per cent gluteraldehyde in 0.01 M cacodylate buffer (pH 7.2). The samples were washed 3 times in phosphate buffer and then subjected for dehydration in 20, 40, 60, 80 and 100% ethanol for 30 min. in each grade. The samples were embedded in Spurr's embedding medium (Spurr, 1967) and kept for polymerization for 8 hrs. at 70⁰C. Ultra thin sections (60-80 nm) were cut with a diamond knife mounted on LICA ultracut ultramicrotome. Sections were placed on 200 mesh copper grids, doubly stained with 2% uranyl acetate followed by lead citrate, rinsed with distilled water and examined under transmission electron microscope (JEOL-100-CX II) for the presence of inclusion bodies.

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RESULTS

4. RESULTS

4.1 survey and incidence of zucchini viruses in Western Maharashtra

Survey conducted during 2003-2004 in zucchini growing areas of Western Maharashtra *viz.*, Ahmednagar, Nasik, Pune, Sangali and Satara revealed the presence of only one virus causing variety of symptoms. Details of survey are presented in Table 4.

Table 4: Per cent incidence and symptoms of zucchini virus in Western Maharashtra

Place	Area (acre)	Total number of plants	Number of plants infected	% incidence	Symptoms
Rastapur (Ahmednagar)	0.5	3500	1750	50	Vein banding, mosaic, leaf distortion
Gangapur (Nasik)	0.7	5100	1550	30	Vein clearing, vein banding, mosaic
Aundh and Shivajinagar (Pune)	0.25	1700	1450	85	Vein banding, mosaic, mottling, blistering, filiformity and blistering on fruits
Karad (Satara)	0.25	5500	3800	69	Vein banding, mosaic, blistering, leaf distortion, filiformity and blistering on fruits
Islampur (Sangali)	1.0	7500	7500	100	Vein banding, mosaic mottling, filiformity and reduced size of fruits

4.2 Transmission Studies

4.2.1 Mechanical transmission

The results of mechanical transmission indicated that the virus was transmitted by sap from zucchini to zucchini and to other herbaceous hosts.

4.2.2 Insect transmission

In the present study tests, on transmission of virus was carried out by aphids (*viz.*, *Aphis craccivora*, *A. gossypii*, *A. nerii*, *A. malvae*, *Macrosiphum rosae*, *Macrosiphum pisi*, *Myzus Persicae*, *Rhopalosiphum maidis* and *Toxopetera citricidus*), whiteflies (*Bemisia tabaci*) and thrips (*viz.*, *Thrips tabaci*, *T. palmi* and *Scirtothrips dorsalis*). Out of these insect tested, only aphids (i.e. *Aphis gossypii* and *Myzus persicae*) were able to transmit the virus from zucchini to zucchini, non-persistently.

4.2.3 Seed transmission

The experiment conducted on seed transmission revealed that the present virus isolate was not seed transmissible.

4.3 Host Range

In host range studies, fifty-eight plant species belonging to ten families *viz.* *Amaranthaceae*, *Chenopodiaceae*, *Compositae*, *Cruciferae*, *Cucurbitaceae*, *Labiatae*, *Leguminosae*, *Malvaceae*, *Poaceae* and *Solanaceae* were used. The reaction of plants to virus isolated from zucchini is presented in Table 5.

Table 5 : Host range of the virus under investigation

Species	Symptoms *
Amaranthaceae	
<i>Amaranthus caudatus</i> L.	-
<i>Amaranthus viridis</i> L.	-
<i>Celosia argentea</i> L.	-
<i>Gomphrena globosa</i> L.	-
Chenopodiaceae	
<i>Beta vulgaris</i> L.	-
<i>Chenopodium album</i> L.	-
<i>Chenopodium amaranticolor</i> Coste & Reyn.	CLL, NLL

<i>Chenopodium murale</i> L.	-
<i>Chenopodium quinoa</i> Wild.	-
Compositae	
<i>Helianthus annuus</i> L.	-
<i>Zinnia elegans</i> Jacq.	-
Cruciferae	
<i>Brassica campestris</i> L.	-
<i>Brassica oleracea</i> var. Botrytis.	-
<i>Brassica rapa</i> L.	-
Cucurbitaceae	
<i>Benincasa hispida</i> (Thunb.) Cogn.	CS, M
<i>Citrullus lanatus</i> Thunb.	VC, VB, M
<i>Cucumis melo</i> L.	VC, M, BL, LD
<i>C. sativus</i> L. cv. Himangi, Poona Kheera	CS, M, VB
<i>Cucurbita maxima</i> Duch.	VC, VB, M, LD, BL
<i>Cucurbita pepo</i> L. var. Sunnyhouse	VC, M, MT, BL, LD
	Shoestring
<i>Lagenaria siceraria</i> Standl.	CS, M
<i>Luffa acutangula</i> Roxb.	M
<i>Luffa cylindrica</i> L.	VC, M
<i>Momordica charantia</i> L.	VC, M, LD
<i>Trichosanthes anguina</i>	M
Labiatae	
<i>Osimum basilicum</i> L.	-
<i>Osimum sanctum</i> L.	-
Leguminosae	
<i>Arachis hypogea</i> L.	-
<i>Cajanus cajan</i> (L.) Millsp.	-

<i>Cicer arietinum</i> L. cv. Chaffa	-
<i>Crotolaria juncea</i> L.	-
<i>Cyamopsis tetragonoloba</i> (L.) Taub.	-
<i>Glycine max</i> (L.) Merr. cv. Bragg.	-
<i>Phaseolus vulgaris</i> L. Pinto.	-
<i>Phaseolus aureus</i> Roxb.	-
<i>Phaseolus lunatus</i> L.	-
<i>Phaseolus mungo</i> Roxb.	-
<i>Pisum sativum</i> L. var. Bonneville	-
<i>Vigna unguiculata</i> (L.) Walp cv. 'Black Eye'	-
Malvaceae	
<i>Abelmoschus esculentus</i> (L.) Moench.	-
Poaceae	
<i>Zea mays</i> L.	-
Solanaceae	
<i>Capsicum annuum</i> L.	-
<i>Datura metel</i> L.	-
<i>Datura stramonium</i> L.	-
<i>Lycopersicon esculentum</i> Mill. var. Pusa	-
Ruby	-
<i>Nicotiana benthamiana</i>	-
<i>Nicotiana clevelandii</i> A. Gray	-
<i>Nicotiana glutinosa</i> L.	-
<i>Nicotiana rustica</i> L.	-
<i>Nicotiana sylvestris</i> Speg	-
<i>Nicotiana tabacum</i> L. var. White Burley	-
<i>Nicotiana tabacum</i> L. var. Samsun	-

<i>Nicotiana tabacum</i> L. var. Xanthi	-
<i>Petunia hybrida</i> Villm.	-
<i>Physalis floridana</i> Black Turtle-2	VB, M
<i>Physalis peruviana</i> L.	-
<i>Solanum melongena</i> L.	-
<i>Solanum nigrum</i> L.	-
<i>Solanum tuberosum</i> L.	-

*

-	= No symptoms	Bl	= Blistering
CLL	= Chlorotic local lesions	CS	= Chlorotic spots
LD	= Leaf distortion	M	= Mosaic
Mt	= Mottling	NLL	= Necrotic local lesions
VB	= Vein banding	VC	= Vein clearing

4.4 Symptomatology

4.4.1 Field symptoms

Field symptoms of the disease observed in zucchini were severe mosaic (Fig. 1), vein banding (Fig. 2, 3), leaf distortion, filiformity and malformation and blistering on fruits (Fig. 4A,4B).

4.4.2 Symptoms of disease in experimental plants

Symptoms on zucchini (*Cucurbita pepo* L.)

The first visible symptoms on inoculated leaves appeared 6 to 8 days after inoculation. The virus exhibited prominent veinal chlorosis, followed by severe mosaic mottling (Fig. 5), vein banding, blistering and shoestring of leaves (Fig. 6A, 6B and 7) similar to those observed in the field. Malformation, discolouration from green to yellow and blistering on fruits of infected plants were also observed.

Symptoms on pumpkin (*Cucurbita maxima* Duch.)

The symptoms on this host were similar to those on zucchini. The inoculated leaves showed vein clearing followed by vein banding (Fig. 8), seven days after inoculation. Systemic symptoms appeared in the form of mosaic, distortion, followed by dark green blisters (Fig. 9) scattered over the entire leaf lamina.

Symptoms on cucumber (*Cucumis sativus* L.)

The symptoms observed on inoculated leaves were chlorotic spots mosaic and vein banding (Fig. 10).

Symptoms on muskmelon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* Thunb.)

Systemic symptoms in muskmelon consisted of vein clearing, mild mosaic, blistering (Fig. 11) and severe distortion along with stunting of



Fig. 1 Zucchini var. sunnyhouse grown in field showing severe mosaic symptoms due to Zucchini yellow mosaic potyvirus (ZYMV)



Fig. 2 Zucchini leaf infected with ZYMV showing severe mosaic and vein banding symptoms



Fig. 3 Field grown zucchini plant showing mosaic and vein banding symptoms due to ZYMV



Fig. 4-A Field grown zucchini plant showing leaf distortion and filiformity due to ZYMV



Fig 4-B A closer view of field grown zucchini plant infected with ZYMV showing leaf distortion, filiformity along with malformation and blistering on fruits.

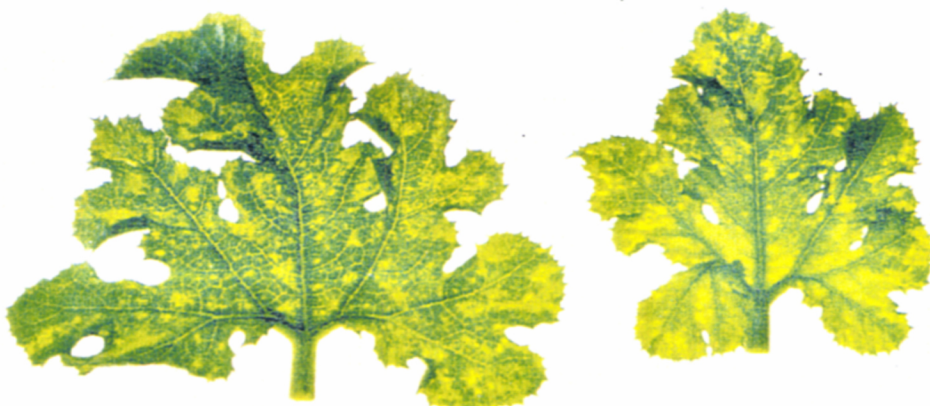


Fig 5. Zucchini var. sunnyhouse leaves showing chlorotic spots and mosaic symptoms after mechanical inoculation with ZYMV



Fig 6-A. Zucchini plants showing vein banding, blistering and shoestring symptoms after mechanical inoculation with ZYMV under glasshouse conditions



Fig. 6-B. Closer view of ZYMV inoculated zucchini plant showing blistering, leaf distortion and shoestring symptoms



Fig. 7. Zucchini leaves mechanically inoculated with ZYMV showing blistering and shoestring symptoms

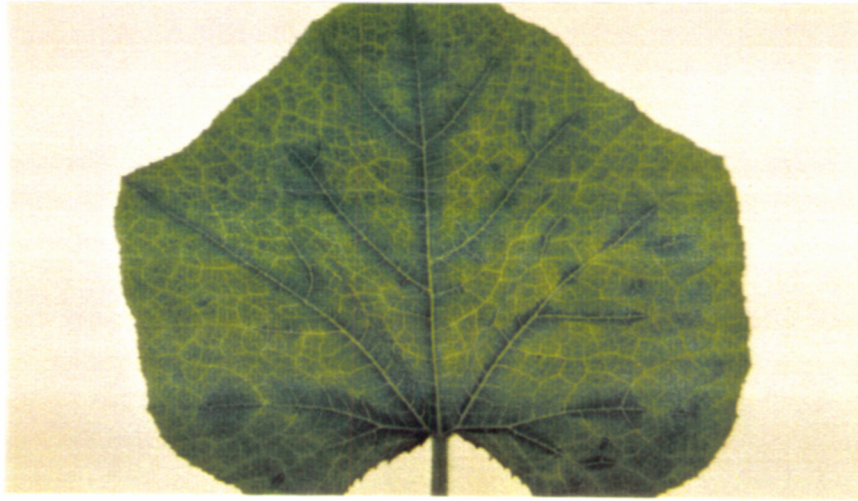


Fig. 8. Pumpkin (*Cucurbita maxima* Duch.) leaf showing vein-banding symptoms after mechanical inoculation with ZYMV



Fig. 9. Pumpkin (*Cucurbita maxima* Duch.) leaf showing mosaic, distortion and blistering symptoms after mechanical inoculation with ZYMV

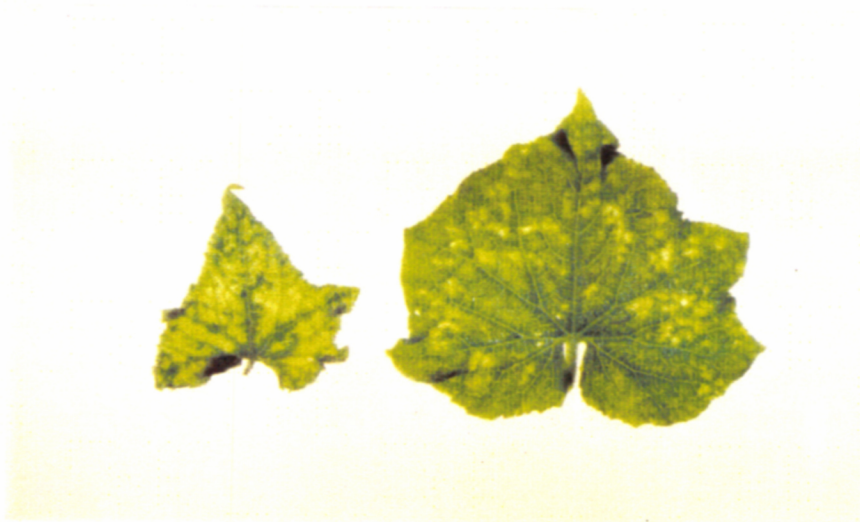


Fig 10. Cucumber (*Cucumis sativus* L.) leaves mechanically inoculated with ZYMV showing chlorotic spots, mosaic and vein banding symptoms

vine. While watermelon showed vein clearing, vein banding and mosaic (Fig. 12).

Symptoms on bottle gourd (*Lagenaria siceraria* Standl.)

The symptoms observed on inoculated leaves of bottle gourd were chlorotic spots and mosaic (Fig. 13).

Symptoms on bitter gourd (*Momordica charantia* L.)

The inoculated leaves of bitter gourd showed vein clearing, mosaic and leaf distortion (Fig. 14).

Symptoms on sponge gourd (*Luffa cylindrica* L.)

Systemic symptoms on sponge gourd consisted of vein clearing and mosaic (Fig. 15).

Symptoms on ridge gourd (*Luffa acutangula* Roxb.)

Mosaic symptom was observed on inoculated leaves of ridge gourd after 15 days of inoculation (Fig. 16).

Symptoms on *Chenopodium amaranticolor* Coste. and Reyn.

The primary symptoms of the virus on inoculated leaves appeared 6 to 8 days after inoculation as small chlorotic lesions of 2 to 3 mm diameter. These lesions coalesced and later turned necrotic within 3 to 4 days (Fig. 17). The young uninoculated leaves didn't show any systemic symptoms.

4.5 Physical Properties

4.5.1 Thermal inactivation point (TIP)

Table 6 indicated that the virus remain infective after heating at 50⁰C for 10 minutes but not at 55⁰C. Percentage infection decreased gradually as crude extract was subjected to successive higher temperatures.



Fig. 11. Muskmelon (*Cucumis melo* L.) leaf showing vein clearing, mosaic and blistering after mechanical inoculation with ZYMV



Fig 12. Watermelon (*Citrullus lanatus* Thunb.) leaf showing vein banding and mosaic symptoms due to inoculation with ZYMV



Fig 13. Bottle gourd (*Lagenaria siceraria* Standl.) leaf showing chlorotic spots and mosaic symptoms due to inoculation with ZYMV

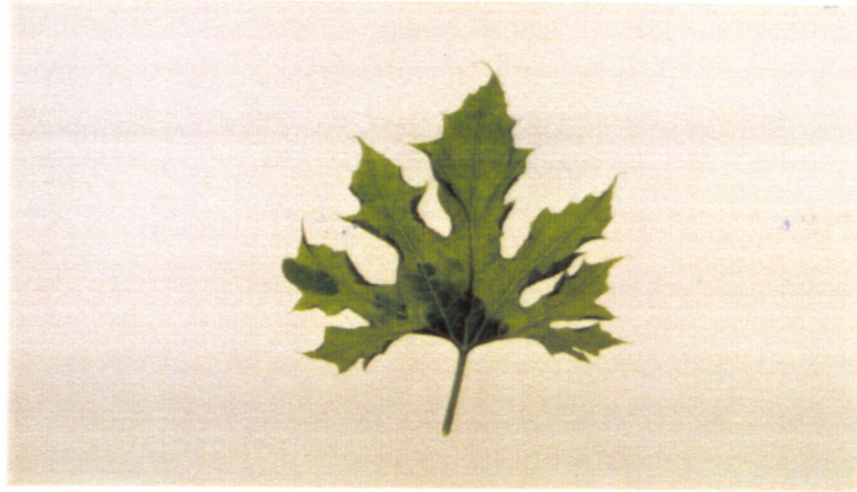


Fig. 14. Bittergourd (*Momordica charantia* L.) leaf showing mosaic symptoms due to inoculation with ZYMV

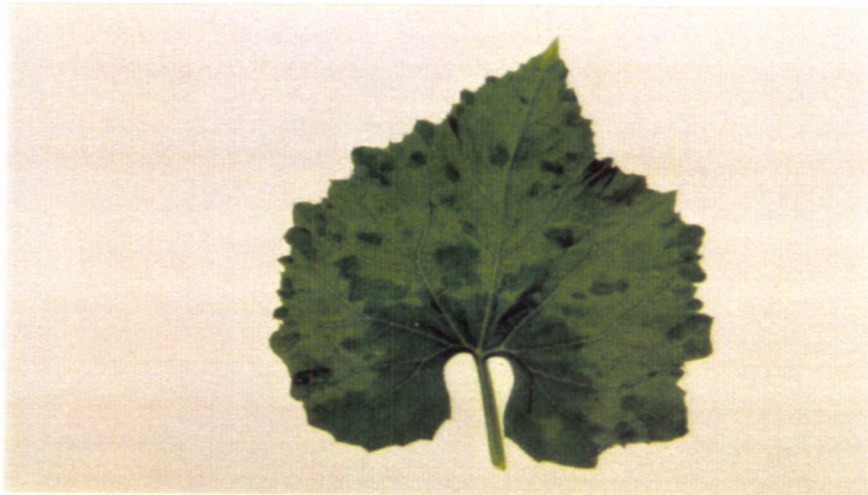


Fig. 15. Spongegourd (*Luffa cylindrica* L.) leaf showing mosaic symptoms due to inoculation with ZYMV



Fig. 16. Mosaic symptoms on ZYMV inoculated leaf of ridgegourd (*Luffa acutangula* Roxb.)



Fig. 17. Chlorotic and necrotic lesions on ZYMV inoculated leaf of *Chenopodium amaranticolor* Coste. and Reyn.

Table : 6 Thermal inactivation point (TIP) of the virus under investigation

Sr. No.	Temperature (°C)	No. of plants inoculated	No. of plants infected	Percentage of infection
1	Unheated sap	10	10	100
2	40	10	6	60
3	45	10	3	30
4	50	10	1	10
5	55	10	0	0
6	60	10	0	0
7	65	10	0	0
8	70	10	0	0

4.5.2 Dilution end point

The dilution end point was found between 1:100 to 1:1000. The percentage infection decreased gradually with increase in dilution of the infective sap. The details have been presented in Table 7.

Table : 7 Dilution end point (DEP) of the virus under investigation

Sr. No.	Dilution	No. of plants inoculated	No. of plants infected	Percentage of infection
1	Crude sap	10	10	100
2	1: 10	10	6	60
3	1: 100	10	2	20
4	1: 1000	10	0	0
5	1: 10000	10	0	0

4.5.3 Longevity *in vitro* (LIV)

The longevity *in vitro* of the virus was found to be 48 hours at room temperature. Beyond this period the infective sap lost its infectivity. The details have been presented in Table 8.

Table : 8 Longevity *in vitro* (LIV) of the virus under investigation

Sr. No.	Storage period (hr.)	No. of plants inoculated	No. of plants infected	Percentage of infection
1	0	10	10	100
2	2	10	9	90
3	4	10	7	70
4	8	10	5	50
5	12	10	4	40
6	20	10	3	30
7	28	10	3	30
8	36	10	2	20
9	40	10	2	20
10	44	10	1	10
11	48	10	1	10
12	52	10	0	0
13	56	10	0	0
14	60	10	0	0
15	62	10	0	0

4.6 Serology and Electron Microscopy

The virus under investigation was tested serologically using ELISA test. The virus isolate reacted positively to antiserum of ZYMV but

negatively to others i.e. CMV, PRSV-P, PVY, TMV, TSWV, WSMV and WMV-2.

This indicated that virus belongs to potyvirus group (Family Potyviridae), which caused a variety of symptoms i.e. vein banding, mosaic, blistering, shoestrings and blistering and reduction in fruit size. This was further confirmed by electron microscopy of virus particles which showed flexuous filamentous particles measuring 700-750 nm (based on 47 particles) in size (Fig. 18).

Electron microscopy of ultrathin sections of infected leaves of zucchini revealed the presence of cytoplasmic cylindrical inclusions consisting of “pinwheels” and “scrolls” (Fig. 19) typical of potyvirus.



Fig. 18. Electron micrograph of flexuous, filamentous virus particles of ZYMV (700 - 750 nm)



Fig. 19 Typical pinwheel like inclusion bodies of ultra thin section of ZYMV infected zucchini leaves observed under electron microscope.

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DISCUSSION

5. DISCUSSION

Systemic studies were carried out on the virus infecting zucchini (*Cucurbita pepo* L.) in Western Maharashtra on various aspects viz. symptomatology, host range, various modes of transmission, physical properties, serology and electron microscopy to establish the identity of the virus under investigation. More than 25 viruses, including at least 7 potyviruses are known to infect cucurbits naturally (Lovisolo, 1980). About 10 viruses, have been reported worldwide to occur naturally on zucchini. Out of these only one i.e. zucchini yellow mosaic potyvirus has been reported from India (Singh *et al.*, 2003; Chalam *et al.*, 2003).

The symptoms produced by virus under study were vein clearing, vein banding, mosaic, severe mottling, blistering and shoestrings of leaves. Blistering on fruits along with malformation was also observed in zucchini. These symptoms are similar to those reported by earlier workers (Lecoq *et al.*, 1981; Chalam *et al.*, 2003; Singh *et al.*, 2003).

Host range of the virus was mainly confined to the family Cucurbitaceae. The virus under study induced mosaic on *Luffa acutangula* and *Trichosanthes anguina*; chlorotic spots and mosaic on *Benincasa hispida* and *Lagenaria siceraria*; chlorotic spots, vein banding and mosaic on *Cucumis sativus*; vein clearing and mosaic on *Luffa cylindrica*; vein clearing, vein banding, and mosaic on *Citrullus lanatus*; vein clearing, mosaic and leaf distortion on *Momordica charantia*; vein clearing, mosaic, blistering and leaf distortion on *Cucumis melo*; vein banding, mosaic, blistering and leaf distortion on *Cucurbita maxima*, whereas vein clearing, mosaic mottling, blistering, leaf distortion and shoestring on *Cucurbita pepo* L. *Physalis floridana* Black turtle 2 develop vein banding and mosaic. The virus induced local lesions on *Chenopodium amaranticolor*. This host range of the virus

under study is similar to the reports of ZYMV (Prieto *et al.*, 2001; Singh *et al.*, 2003).

Some strains of ZYMV have been reported to produce systemic infection without visible symptoms in *Pisum sativum* (Lesemann *et al.*, 1983). But the present isolate did not infect this species and hence resembles Chilean isolate of ZYMV described by Prieto *et al.* (2001).

This indicates that there is little variation in the host range among ZYMV isolates all over the world and these reactions could be useful in differentiating among the potyviruses that infect cucurbits.

The virus under study was transmitted by sap and aphid species *viz.* *Aphis gossypii* and *Myzus persicae*. However, transmission of ZYMV by other species of aphid *viz.*, *Aphis citricola* (Purcifull *et al.*, 1984), *Aphis gossypii*, *Myzus persicae* (Lecoq *et al.*, 1981; Lisa *et al.*, 1981), *Aphis aromoracia*, *A. craccivora*, *Acrythosiphon pisum*, *Hydaphis erysimi* and *Uroleucon* sp. (Adlerz, 1987), in non-persistent manner has also been reported. Seed transmission is rare in ZYMV and has been reported by Schrijnwerkers *et al.*, 1991; Fletcher *et al.*, 2000 and Tobias and Polvics, 2003. But present isolate was not seed transmissible.

The physical properties of virus under study were as follows: TIP was between 50-55⁰C, the DEP was between 10⁻² to 10⁻³ and LIV was 48 hrs at room temperature. These properties of the virus are similar to zucchini yellow mosaic virus reported on zucchini (Singh *et al.*, 2003).

In serological tests, the virus under study reacted positively against the antiserum of zucchini yellow mosaic potyvirus (ZYMV), but negatively against the other antisera tested i.e CMV, PRSV-P, PVY, TMV, TSWV, WSMV and WMV-2. Similar results have also been reported by Purcifull *et al.*, 1984; Vega *et al.*, 1995; Singh *et al.*, 2003.

Electron microscopy of leaf dip and purified preparation showed flexuous filamentous virus particles measuring 700-750 nm (based on 47

particles). Virus induced cytoplasmic cylindrical inclusions, typical of potyviruses. The particle morphology of the virus resembled with zucchini yellow mosaic potyvirus (Vega *et al.*, 1995; Prieto *et al.*, 2001; Singh *et al.*, 2003).

Based on the symptomatology, transmission, host range, serology and electron microscopy the virus has been identified as zucchini yellow mosaic potyvirus belongs to the family potyviridae.

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**SUMMARY AND
CONCLUSION**

6. SUMMARY AND CONCLUSION

Investigations on the survey and incidence, symptomatology, transmission, host range, physical properties, serology and electron microscopy of virus causing zucchini mosaic in five districts (i.e. Ahmednagar, Nasik, Pune, Sangli and Satara) of Western Maharashtra were made. The results of the present investigations are summarized below:

1. Survey conducted during 2003-2004 in above mentioned districts of Western Maharashtra for the presence of zucchini (*Cucurbita pepo*) virus (es) revealed that zucchini crop was severely affected by mosaic virus and incidence of infection ranged between 30-100 per cent. The symptoms of the disease caused by virus showed veinal chlorosis followed by vein banding, severe mottling, blistering, severe distortion of leaves and malformation and blistering on fruits of infected plant.
2. Virus was transmitted successfully by mechanical sap inoculation and by two species of aphids viz., *Aphis gossypii* and *Myzus persicae* in a non-persistent manner.
3. The virus under study was found to have limited host range and mainly confined to the members of family Cucurbitaceae. The virus under study induced mosaic on *Luffa acutangula* and *Trichosanthes anguina*; chlorotic spots and mosaic on *Benincasa hispida* and *Lagenaria siceraria*; chlorotic spots, vein banding and mosaic on *Cucumis sativus*; vein clearing and mosaic on *Luffa cylindrica*; vein clearing, vein banding, and mosaic on *Citrullus lanatus*; vein clearing, mosaic and leaf distortion on *Momordica charantia*; vein clearing, mosaic, blistering and leaf distortion on *Cucumis melo*; vein banding, mosaic, blistering and leaf distortion on *Cucurbita maxima*, whereas vein clearing, mosaic mottling, blistering, leaf distortion and

shoestring on *Cucubita pepo* L. *Physalis floridana* Black turtle 2 develop vein banding and mosaic. The virus induced local lesions on *Chenopodium amaranticolor*.

4. Studies on physical properties of the virus indicated that the virus under investigation lost its infectivity at temperature between 50-55⁰C. The dilution end point of the virus was found to be 10⁻² to 10⁻³ and the longevity *in vitro* was 48 hrs. at room temperature.
5. The virus was found closely related to zucchini yellow mosaic potyvirus. This was further confirmed by electron microscopy of virus particles.
6. Virus particles were flexuous rods, measuring 700-750 nm (based on 47 particles). The ultrathin sections of leaves infected with virus revealed the presence of cytoplasmic cylindrical inclusions consisting of pinwheels and scrolls typical of potyvirus group.

Based on these results, the virus infecting zucchini was identified as : Zucchini yellow mosaic potyvirus belonging to family Potyviridae.

The virus must have introduced to India through importation of infected plants or seed, since ZYMV in zucchini squash is seed transmissible though to a very less extent or through long distance migration of viruliferous aphids.

Control of this virus disease will probably be most effectively obtained by the development of resistant cultivars and by cross-protection through mild strain. Also strict quarantine measures are required as virus is also reported to be seed transmissible. Good agronomic practices will also help to some extent in control of disease.

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VITA

8. VITA

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of

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in

PLANT PATHOLOGY

2004

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