STUDIES ON VIRUS DISEASES OF CHILLI (Capsicum annuum L.)

THESIS SUBMITTED TO THE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
IN PART FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE
(PLANT PATHOLOGY)

BY
T. V. NAGAPADMINI, B.Sc., (Ag.)

DEPARTMENT OF PLANT PATHOLOGY
SRI VENKATESWARA AGRICULTURAL COLLEGE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
TIRUPATI

MAY 1988

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CERTIFICATE

This is to certify that Smt. T.V. Nagapadmini has satisfactorily prosecuted the course of research and the thesis entitled "STUDIES ON VIRUS DISEASES OF CHILLI (*Capsicum annuum* L.)" submitted is the result of original research work and is of sufficiently standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any University.

K. Seethar Reddy

Date: MAJOR ADVISOR

CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON VIRUS DISEASES OF CHILLI (*Capsicum annuum* L.)" submitted in partial fulfilment of the requirements for the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by Smt. T.V. Nagapadmini under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. Published part has been fully acknowledged. All the assistance and help received during the course of the investigation have been duly acknowledged by her.

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DECLARATION

I, T.V. NAGAPADMINI hereby declare that the thesis entitled "STUDIES ON VIRUS DISEASES OF CHILLI (*Capsicum annuum* L.)" is a result of the original research work done by me. It is further declared that the thesis or any part thereby has not been published earlier in any manner.

Date: 19-5-86

T.V. Nagapadmini

T.V. NAGAPADMINI

ABSTRACT

Title of the thesis	:	"Studies on virus diseases of chilli (Capsicum annuum L.)"
Name of the student	:	T.V. Nagapadmini
Guide	:	Dr. K. Seetharama Reddy
Submitted for the award of the degree	:	Master of Science in Agriculture
Faculty	:	Agriculture
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Chilli (Capsicum annuum L.) is one of the popular vegetables extensively cultivated throughout India. Mosaic disease is found to reduce yield of this vegetable crop. A detailed survey of chilli crop made during 1985-86 in various localities in Chittoor, Prakasam and Guntur districts indicated the presence of mosaic disease to an extent ranging from 5.21 to 20.02 per cent. The infected plants exhibited typical mosaic symptoms with mosaic mottling, stunted growth, less number of flowers and reduced fruit setting.

The virus was readily transmitted by mechanical inoculation but not through seeds or soil.

The virus produced systemic symptoms on Capsicum fuitescens L., Nicotiana tabacum L., N. glutinosa L., Datura stramonium L., Datura metal L., Cucumis sativus L., Cucumis melo L., Memordica charantia L., Lycopersicon esculentum Mill., Petunia oleracia L., Amaranthus hypochondriacus and Solanum melongena L., and local lesions on D. metel L., and Chenopodium amaranticolor Costo and Reyn.

The virus under study had its physical properties viz., thermal inactivation point between 90-95 °C, dilution end point between 10^{-6} to 10^{-7} and longevity *in vitro* for 9 days at room temperature (23.5 to 28.5 °C) and 17 days at 10°C.

The virus was purified by using PEG (Poly Ethylene Glycol) method and electronmicroscopy showed virus particles of 300 nm x 18 nm.

A decrease in percentage of infection and severity of symptoms was observed when inoculated at older age of 50, 60 and 70 days. Chilli plants when inoculated at young age of 20, 30 and 40 days, showed increased percentage of infection and severe symptoms. The disease was found to cause considerable reduction in growth characters viz., root length, shoot length, leaf area and fresh and dry weights of plant. There was also a considerable reduction in chlorophyll fractions viz., chlorophyll a, chlorophyll b and total chlorophyll.

All the 20 varieties under test showed susceptibility with varying symptoms of mild mosaic, mottling, vein clearing, leaf distortion and stunting.

On the basis of symptomatology, transmission, host-range, physical properties and electronmicroscopy, the virus under test was identified as common strain of tobacco mosaic virus.

CHAPTER I

INTRODUCTION

Chilli (Capsicum annuum L.) belongs to the genus capsicum and is one of the chief spices of India. In Andhra Pradesh it is cultivated in an area of 1,81,417 hectares with a production of 1,91,511 tonnes (Appendix-I). A major portion of chilli is consumed within the country. Some quantity is also exported as dry pods and as an essential contribute of curry powder and hot pickles to several countries.

Chilli is a common host for several fungal, bacterial, viral and mycoplasma diseases. Any loss caused to this crop directly affect the nutritional habits of the people.

The literature pertaining to the virus diseases of chilli revealed that it is infected naturally by 21 different viruses and artificially by 17 other viruses. Among the 21 viruses reported to occur naturally on chilli, Indian chilli mosaic virus (Mc Rae, 1924; Jha and Raychaudhury, 1956), potato virus Y (Jeyarajan and Ramakrishnan, 1961, 1969; Joshi and Bhargava, 1962); potato virus X (Ramakrishnan, 1959, Rao et al. 1970); tobacco mosaic virus (Kandaswamy et al. and Mathur et al., 1966); cucumber mosaic virus (Anjaneyulu and Appa Rao, 1967); and leaf

curl virus (Hussain, 1932; Vasudeva, 1954) have been reported from India.

Ramakrishnan (1961) reported that 17 viruses were recorded to infect chilli on artificial inoculation. These are potato virus X, tomato bushy stunt virus, tomato ring spot virus, potato aucuba virus, potato leafroll virus, potato stunt virus, broad been vascular wilt virus, carrot mottley dwarf virus, sweet potato mosaic virus, Yam mosaic virus, sunflower mosaic virus, radish stunt virus, Trinidad tomato twisted leaf virus, Trinidad tomato bronze-leaf virus, Trinidad egg plant mosaic virus, tomato necrosis virus and tomato yellow mosaic virus.

Many of the above said viruses produce mosaic symptoms on chilli.

Since no information is available regarding the nature of the virus(es) causing mosaic disease(s) of chilli in Rayalaseema region, detailed studies on disease incidence, transmission, host-range, physical properties, purification and electron-microscopy and varietal reaction were taken up. The results of these investigations are presented in this thesis.

CHAPTER II

REVIEW OF LITERATURE

Chilli (Capsicum annuum L.) is a common host for several fungal, bacterial, viral and mycoplasma diseases. A mosaic disease of chilli was first reported in India by Mc Rae (1923) and Kulkarni (1924) in the Bombay province. Later twentyone viruses were reported to occur naturally on chilli. Among them, Indian Chilli mosaic virus (Mc Rae, 1924); (Jha and Raychaudhuri, 1956); potato virus Y (Jeyarajan and Ramakrishnan, 1961, 1969); (Joshi and Bhargava, 1962); potato virus X (Ramakrishnan, 1959); (Rao et al., 1970); Tobacco mosaic virus (Kandaswamy et al. and Mathur et al., 1966); cucumber mosaic virus (Anjaneyulu and Appa Rao, 1967); and leafcurl virus (Hussain, 1932); (Vasudeva, 1954) had been reported from India.

A detailed review of the five virus diseases which induce mosaic symptoms on chilli, in India is presented below.

2.1 TOBACCO MOSAIC VIRUS

Transmission of tobacco mosaic virus (TMV) to chilli was first reported by Palm (1923) in Indonesia. Palm and Joachens (1924) reported the occurrence of this virus on pepper.

2.1.1 Symptomatology

Holmes (1937) reported four kinds of symptoms on pepper. These were (1) systemic chlorosis, (2) local lesions on leaves and consequent shedding, (3) delayed necrosis combined with systemic infection and (4) systemic chlorosis combined with streaking and some times resulted in the death of the plant. Nakata and Takimoto (1940) reported bright yellow mottling of leaves caused by a ring strain of tobacco mosaic virus. Kovachevsky (1942) reported two types of symptoms, acute and chronic types on pepper plants. Chamberlain (1947) reported tomato streak virus a strain of TMV, which caused mosaic mottling and stunting.

Two strains of TMV were isolated by Mc Kinney (1952) which caused necrotic local lesions and strong light green mosaic mottling. Miller and Thornberry (1958) described a mosaic disease with bright interveinal chlorosis and malformed fruits by a strain of TMV and named it as 'Tomato atypical mosaic virus'.

Murakishi (1960) reported a new TMV strain which caused slightly raised reddish brown streaks on fruits. Fletcher (1963) reported a strain of TMV which caused main vein necrosis of leaves. Greenleaf et al. (1964) isolated a strain of TMV and named it as Samsun latent tobacco mosaic virus (SLTMV), which caused severe leaf mottling, wrinckling and necrotic patterns on stems.

Feldman et al. (1969) reported that economic loss was caused by TMV in capsicum fields. Farrag and Ramakrishnan (1969) reported that TMV caused reduction in chlorophyll content and increased the rate of respiration. Nambiar and Ramakrishnan (1969) observed that the rate of increase in respiration activity was influenced by the age of the plant and intensity of infection.

Demski (1970) conducted inoculation tests at four different stages of growth and reported that the total yield of ripe fruit was reduced principally by early infections.

A leaf curling strain of TMV was reported on C. fruitescens var. grossum characterised by the symptoms of mosaic mottling, blistering, malformation and distortion (Baskerous, 1971). A strain of TMV tentatively named as 'virus producing local lesions on tobacco' (VPLIT) was reported by Adsuar et al. (1971). This virus caused mottling, chlorosis, leaf wrinkling and retarded growth. A strain of TMV was isolated by Feldman and Oremianer (1972) which was named as "pepper unusal strain" producing mosaic mottling, vein clearing, chlorosis and stunting.

Fribourg and Fernondez-North Cote (1972) isolated two strains of TMV from local varieties of capsicum with symptoms of mosaic, stunting and leaf and fruit deformation.

Misra and Singh (1972) reported about morbid anatomy of mosaic infected chilli plants which developed small leaves and disorganised vascular bundles.

Rogozzino et al. (1972) reported tomato strain of TMV, which caused bright yellow mosaic, reduction of leaf size, stem necrosis, defoliation, stunting, deformation, and produced yellow spots on fruits. They also reported tobacco strain with similar symptoms along with light interveinal mosaic.

Sutk et al. (1978) reported a type of necrosis caused by TMV from Yugoslavia.

Aleksic and Marinkovic (1981) isolated TMV from naturally infected chilli plants with symptoms of mosaic mottling.

A strain of tobacco mosaic virus producing small local lesions on chilli was reported by Gebre Selassie and Dumas De Vaulx (1981).

Duggal and Singh (1981) recorded the symptoms of virus affected capsicum plant as reduction in relative growth, net assimilation rates, leaf area ratio and chlorophyll a, chlorophyll b and total chlorophyll.

A strain of tobacco mosaic virus producing an unfamiliar mosaic with severe mosaic mottling on all parts of plants and yellow streaks on fruits was reported by Nagai et al. (1981).

Pop (1981) incubated the pepper cv. Du Danule plants with pepper strain of TMV and observed yellow mosaic on inoculation. Further, he recorded that the disease intensity was high in warmer periods.

Rogozzino and Angelaccio (1981) conducted studies on tomato strain of TMV and reported that the disease was severe on capsicum by producing necrotic spots on stems and fruits and mosaic mottling on leaves.

Sulaiman and Gimw (1981) described typical symptoms developed by TMV as leaf puckering, veinal necrosis, stem lesions and irregular black markings on ripe fruits.

Betti et al. (1983) reported that TMV was infectious on seeds (seed coat and endosperm) of chilli.

Erkan and Yorcan (1983) reported a strain of TMV on pepper which induced symptoms such as wilting, stunting, stem necrosis, defoliation, mosaic and leaf and fruit deformation.

2.1.2 Transmission

Tobacco mosaic virus was easily transmissible through sap. Sap transmission of TMV was reported by several workers viz., Palm (1923), Holmes (1937), Nakata and Takimoto (1940), Doolittle and Thacher (1942), Kovachevsky (1942), Kohler and Rajan (1944), Chamberlain (1947), Zabala and Dellecosta (1947), Mc Kinney (1952), Newton (1953), Miller and Thornberry (1958), Murakishi (1960), Eskarous (1971), Adsuar et al. (1971), Feldman and Oremianer (1972) and Ragozzino et al. (1972).

Lojek and Orlob (1973) reported transmission of TMV by Myzus persicae.

Mc Kinney (1952) reported seed borne nature of TMV on pepper. Demaki (1981) reported seed borne nature of TMV in capsicum plants. Rogozzino and Angelaccio (1981) reported that the mosaic disease of TMV (tomato strain) was transmitted by seeds.

Tosic and Mitrovic (1981) suggested the use of healthy seed stock as the seed infection was important in epidemiology of the tobacco mosaic virus.

2.1.3 Host range

Miller and Thornberry (1958) reported infection of tomato atypical mosaic strain of TMV which produced stem necrotic lesions on C. annuum L. var. California Wonder, only necrotic lesions on Datura stramonium L., D. wrightii, N. glutinosa L. and mosaic mottling on Lycopersicon esculentum Mill. Murakishi (1960) described necrotic pod streak strain of TMV which produced local lesions followed by systemic mottle on N. tabacum L. var. Samsun and only local lesions on N. tabacum L. var. Xanthi and tabasco pepper, while Phaseolus vulgaris L. var. Pinto and Scotia remain uninfected. Greenleaf et al. (1964) observed that the Samsun latent tobacco mosaic virus produced local lesions on N. tabacum L. var. Samsun NN and Xanthi, N. rustica Linn., N. rependa, N. sylvestris Speg. and comes, N. glutinosa L., D. stramonium L., and Chenopodium amaranticolor Costo and Reyn. Feldman and Oremianer (1972) reported the symptoms produced by the pepper unusual strain of TMV on Plantago minor Linn. and on all tested cultures of N. tabacum L. except on turkish tobacco. Verma and Singh (1973) reported a new host for TMV as Sonchus arvensis. Gebre Selassie and Dumas De Vaulx (1981) isolated a new strain of TMV, which produced small local lesions on tomato and Capsicum fruitescens L., Nagai et al. (1981) described the pepper strain of TMV which produced a new mosaic disease on sweet pepper and very small, local lesions on leaves

of N. glutinosa L. Tosic and Videnov (1981) reported an isolate from pepper cv. Niska Sipka, which caused local lesions on N. glutinosa L. Erkan and Yorgan (1983) reported that a strain of TMV induced latent infection on tomato, chlorotic local lesions on Gomphrena, necrotic local lesions on Phaseolus vulgaris L. local and systemic symptoms on Petunia hybrida. The literature on host range of tobacco mosaic virus is presented in Table 1.

2.1.4 Physical properties

The literature on physical properties of various strains of TMV is presented in Table 2.

2.1.5 Purification and Electron microscopy

Hollings et al. (1967), Fischer and Lockhart (1976) purified cucumber mosaic virus from cowpea. Lima and Nelson (1975) purified squash mosaic virus. Kesavamurthy and Govindu (1976) reported a new modified method for purification of plant viruses. Wetter et al. (1984) purified pepper mild mottle virus, a strain of Tobacco virus from infected pepper plants by using rate-zonal density gradient centrifugation in sucrose (10-50%). The yield of virus was 1.3 g/kg tissue.

Table 1. Host range of Tobacco Mosaic Virus

S. No.	Name of the host	Type of reaction	Strain of TMV	Reference
1.	Capsicum annuum L. var. California Wonder	Necrotic lesions on stem	Tomato atypical strain of TMV	Miller and Thornberry (1958)
2.	Capsicum fruitescens L.	Small local lesions	-	Murakishi (1960) Gebre Selassie and Dumas De Vaulx (1981)
		Small local lesions	Pepper strain of TMV	Nagai et al. (1981)
3.	Chenopodium amaranticolor Costo and Reyn.	Local lesions	Necrotic pod streak strain of TMV	Murakishi (1960)
4.	Datura wrightii	Necrotic lesions	-	Miller and Thornberry (1958)
5.	Datura stramonium L.	Necrotic lesions, local lesions	Necrotic pod streak strain of TMV	Murakishi (1960)
		Necrotic lesions, local lesions	-	Miller and Thornberry (1958)
6.	Gomphrena globosa L.	Chlorotic local lesions	-	Erkan and Yorcan (1983)
7.	Lycopersicon esculentum Mill.	Mosaic mottling small local lesions	-	Miller and Thornberry (1958), Gebre selassie and Dumas De Vaula (1981), Erkan and Yorcan (1985).

S. No.	Name of the host	Type of reaction	Strain of TMV	Reference
8.	Nicotiana glutinosa L.	Necrotic lesions, local lesions	-	Miller and Thornberry (1958)
		Necrotic lesions, local lesions	Necrotic pod streak strain of TMV	Murakishi (1960)
		Necrotic lesions, local lesions	Pepper strain of TMV	Nagai et al. (1981)
		Necrotic lesions, local lesions	-	Tosic and Videnov(1981)
9.	Nicotiana rependa	Local lesions	Necrotic pod streak strain of TMV	Murakishi (1960)
10.	Nicotiana rustica L.	Local lesions	Necrotic pod streak strain of TMV	Murakishi (1960)
11.	Nicotiana sylvestris Speg. and Comes.	Local lesions	Necrotic pod streak strain of TMV	Murakishi (1960)
12.	Nicotiana tabacum L. var. Samsun	Local lesions followed by systemic mottling	Necrotic pod streak strain of TMV	Murakishi (1960)
13.	Nicotiana tabacum L. var. Samsun NN	Local lesions	Samsun latent tobacco mosaic virus	Greenleaf et al.(1964)

Table 1 (contd...)

S. No.	Name of the host	Type of reaction	Strain of TMV	Reference
14.	*Nicotiana tabacum* L. var. *Samsum xanthi*	Local lesions	Samsun latent tobacco mosaic virus	Greenleaf *et al.*(1964)
15.	*Nicotiana tabacum* L. var. *Xanthi*	Local lesions	Necrotic pod streak strain of TMV	Murakishi (1960)
16.	*Petunia hybrida* Vilm.	Local lesions and systemic symptoms	-	Erkan and Yorcan(1983)
17.	*Phaseolus vulgaris* L.	Necrotic local lesions	-	Erkan and Yorcan(1983)
18.	*Plantago minor* L.	-	Pepper unusual strain of TMV	Feldman and Oremianer (1972)
19.	*Sonchus arvensis*	-	-	Verma and Singh (1973)

Table 2. Physical properties of various strains of TMV

Sl. No.	Name of the virus/ strain of TMV	TIP °C	DEP	LIV	Reference
1.	Ring strain	90°C	$1:10^{-6}$	-	Nakata and Takimoto (1940)
2.	TMV on pepper	90-95°C	$1:10^{-6}$	3 years at 21-27°C	Doolittle and Beecher (1942)
3.	TMV on pepper	80°C	-	-	Zabala and Dellecosta (1947)
4.	Strain producing necrotic lesions on pepper	88-89°C	10^{-8}	27 months	Mc Kinney (1952)
5.	Seed-borne strain	85-89°C	10^{-8}	18 months	Mc Kinney (1952)
6.	Tomato atypical mosaic virus	72°C	10^{-8}	-	Miller and Thornberry (1958)
7.	A strain of TMV	93°C	-	-	Murakishi (1960)
8.	Samsun latent tomato mosaic strain	35-90°C	10^{-6}-10^{-7}	-	Greenleaf et al. (1964)
9.	Leaf curling strain	-	-	55 days at 23-28°C	Eskarous (1971)
10.	Virus producing leaf lesions on tobacco	85°C	10^{-5}	14 days	Adsuar et al. (1971)
11.	Pepper unusual strain of TMV	85-90°C	10^{-6} x3x 10^{-6}	5 years	Feldman and Oremianer (1972)
12.	Strains of TMV	85-90°C	-	-	Fribourg and Fernondez North-Cote (1972)
13.	Common strain of TMV	90-95°C	10^{-7}-10^{-8}	50 days	Jasnic (1979)
14.	Strain of TMV	90-95°C	10^{-7}-10^{-8}	60 days	Erkan and Yorcan (1983)

TIP : Thermal inactivation point
DEP : Didution end point
LIV : Longevity in vitro

Miller and Thornberry (1958) reported that the tomato atypical mosaic virus contain rod shaped particles measuring 800 x 15 m u. Electranmicrographs developed by Greenleaf et al. (1964) showed typical TMV rods indistinguishable from those of the standard TMV strain. Electronmicrographs of pepper umusual strain of TMV by Feldman and Oremianer (1972) showed numerous, straight, rod shaped particles resembling those of common strain of TMV. Horne et al. (1976) applied negative staining carbon film technique for electronmicroscopic studies of TMV. Hayashi et al. (1975) observed '3' size classes of particles which were, intact particles (300 nm long, sub particles (150-240 nm) and short particles (70-150 nm). Jasnic (1979) reported rod shaped particles of (300 nm). Erkan and Yorcan (1983) reported a strain of TMV with rod shaped particles of 300 x 15 nm in dimensions.

2.1.6 Varietal reaction

Several varieties of pepper were tested against TMV in different parts of the world by various workers. Reaction of several varieties of pepper to various strains of TMV is presented in Table 3.

Table 3. Reaction of chilli varieties to Tobacco Mosaic Virus

S. No.	Name of the variety	Type of reaction	Reference
1.	Anaheim chilli	Necrotic local lesions	Miller and Thornberry (1958)
2.	Banana	Mottling and stem necrotic lesions	Miller and Thornberry (1958)
3.	Cayene long pod	Necrotic local lesions	Miller and Thornberry (1958)
4.	California Wonder	Mottling and stem necrotic lesions	Miller and Thornberry (1958)
5.	Golden California Wonder	Mottling and stem necrotic lesions	Miller and Thornberry (1958)
6.	Harris early giant	Mottling	Miller and Thornberry (1958)
7.	Illinois P_{51-1}	Necrotic local lesions and mottling	Miller and Thornberry (1958)
8.	Large Bell	Mottling and necrotic local lesions	Miller and Thornberry (1958)
9.	Pimiento perfection	Mottling	Miller and Thornberry (1958)
10.	Ruby King	Mosaic mottling	Miller and Thornberry (1958)
11.	World Beater	Mosaic mottling	Miller and Thornberry (1958)
12.	Capsicum annuum P 11	Resistant to TMV	Cook and Anderson (1959)
13.	Yolo Wonder	Resistant to TMV	Fletcher (1963)
14.	Pine tree		
15.	Several pepper varieties	Susceptible to Samsun latent strain of TMV	Greenleaf et al. (1964)
16.	PTSR	Most resistant to TMV	Danko and Michalikova (1965)
17.	PA_3		
18.	Chekuranii		

Table 3 (contd...)

S. No.	Name of the variety	Type of reaction	Reference
19.	Capsicum annuum	Resistant to the local lesion type of infection due to the presence of 'L' gene	Lee and Smith (1968)
20.	C. fruitescens		
21.	C. chinense		
22.	California Wonder	Systemic infection	Feldman and Oremianer (1972)
23.	Perfection	Systemic infection	
24.	Yolo Wonder	Local lesions	
25.	Yolo-Y	Local lesions	
26.	P-11	Local lesions	
27.	Medican	Local lesions	
28.	New Carolina	Local lesions	
29.	Hot pepper	Local lesions	
30.	Pimson	Local lesions	
31.	Tabasco	Local lesions	
32.	S.C. 46252	Local lesions	
33.	Yolo Wonder	Resistant	Morales et al. (1980)
34.	Yolo-Y	Resistant	
35.	Cv. 136	Resistant	
36.	Florida Giant	Susceptible	
37.	Early Wonder	Susceptible	
38.	Cv. Soroksari	Susceptible, systemic infection	Aleksic and Marinkovic (1981)
39.	A_1-12		
40.	Zeleni rotund		
41.	Cv. Lamuyo	Susceptible, systemic infection	Gebre Selassie & Dumas De Valux (1981)
42.	New Face	Systemic infection	Nagai et al.(1981)
43.	Shin Sakigake	Systemic infection	

2.2 CHILLI MOSAIC VIRUS

2.2.1 Symptomatology

A mosaic disease of chilli was first reported by Mc Rae (1923) and Kulkarni (1924). Kulkarni (1924) reported that this disease caused considerable reduction in yield. Diseased plants exhibited leaf distortion with blistering and pronounced mottling. Further he observed that early infections caused absence of flowers and fruits and late infection caused small and distorted fruit formation. Jha and Raychaudhuri (1956) reported symptoms like mosaic mottling, distortion and filiformity of leaves, slight curling, marginal rolling and stunting of leaves in chilli mosaic affected plants. Mishra and Singh (1973) reportedthat chilli mosaic caused stunted growth and disorganised vascular bundles.

2.2.3 Transmission

Vasudeva (1954) reported that chilli mosaic virus was easily transmitted through sap and aphid Aphis gossypii. Nariani and Shastry (1958) found that the vectors Myzus persicae, A. evonymi and A. gossypii transmitted chilli mosaic virus successfully. Further, the vector-transmission was studied in detail by Nariani and Shastry (1962). Dubey andJoshi (1974) conducted transmission studies on Aphis gossypii causing chilli mosaic.

Khatri and Sekhon (1974) reported that the chilli mosaic virus could be easily transmitted by Aphis gossypii.

2.2.4 Host range

Jha and Raychaudhuri (1956) reported that chilli mosaic was easily transmitted by sap inoculation to Nicotiana tabacum L., N. tabacum L. var. White Burley, N. glutinosa L., Solanum nigrum L., Petunia hybrida Vilm., Cucumis sativus L., C. melo L. var. Utilissimus and Carthamus tinctorius L. They also recorded that potato varieties president and Craigs' Defiance and Datura stramonium L. were symptomless carriers. Verma and Singh (1973) recorded a new host Sonchus arvensis for chilli mosaic virus.

2.2.5 Physical properties

Jha and Raychaudhuri (1956) stated that the Indian chilli mosaic virus had a thermal death point of 55 to 60°C, dilution end point of 1:25000 to 1:30000 and was active for 15-22 days at room temperature.

2.2.6 Varietal reaction

According to Jha (1953) chilli varieties NP_{20} and NP_{23} were resistant to chilli mosaic virus. Anand et al. (1961) screened 132 varieties of chilli belonging to six different species Capsicum annuum, C. fruitescens, C. microcarpum, C. pendulum, C. pubescens and C. sinensis. He found that the varieties Puri Red, Puri Orange, Kondiverum, G_2 and local variety were resistant to chilli. Singh (1973) indicated that chilli varieties viz., Puri Red, Puri Orange, G_2, Kondiverum and Suryamukhi were resistant to mosaic under field conditions. Further he observed that 6 morehybrids viz., A_{127}, C_1, IC 3425, IC 3440, IC 3588 and IC 3779 were free from mosaic.

2.3 CUCUMBER MOSAIC VIRUS

Doolittle (1921) reported that cucumber mosaic virus could infect pepper plants.

2.3.1 Symptomatology

Doolittle and Walker (1923, 1925) described symptoms of CMV affected chilli plants as downward curling of younger leaves along the midrib, mottling, reduction in leaf size, shortening of internodes, compact appearance of plants, narrowed and filiform leaves and warty fruits with dark green

outgrowth. Southern celery mosaic virus, a strain of CMV was reported by Wellman (1934a) from Cuba and Florida. The virus caused stunting, leaf distortion with greyish coloured markings without filiformity and the plants showed starved appearance. The blossom were dropped and malformed flowers are produced. Kovachevsky (1940, 1942) from Bulgaria reported a "Rosette disease" of pepper caused by CMV. He observed distorted and assymetrical leaves, necrotic patches on stems, some times like linear pustules along stems and reduced fruit setting. Doolittle and Zaumeyer (1952, 1953) described a ring spot disease of pepper caused by a strain of CMV, which was characterised by large chlorotic rings and Oak leaf patterns on old leaves and concentric yellow rings on fruits. Simons (1957) described 3 strains of CMV viz., southern cucumber mosaic virus, calico virus and pepper oak leaf virus. Southern cucumber mosaic virus caused vein clearing, local chlorosis, severe stunting and red spider like injury. Oak leaf virus caused vein clearing and necrotic Oak leaf pattern and senescent leaves while the Calico virus caused vein clearing, local necrosis and calico mottle.

Anjaneyulu and Appa Rao (1967) reported a mosaic disease of pepper in India which was characterised by mosaic mottling and various types of leaf distortions mainly exhibiting filiform leaf tip. Further, they stated that the infected plants exhibited marked stunting and reduction in size of leaves and warty swellings on fruits.

Awasthi and Singh (1975) described histopathological symptoms in CMV infected plants. Nitzany (1975) reported that CMV caused growth reduction and death of the plants and also encouraged root pathogens like Pythium and Fusarium. Agrios et al. (1985) observed symptoms like necrotic rings on leaves and oak leaf patterns and systemic infection with narrow leaves and yellowish green mottlings. He also recorded small fruits with wrinkled or bumpy growth and dark depressed spots.

2.3.2 Transmission

Doolittle and Walker (1923) reported that cucumber mosaic virus was mechanically transmitted to chilli plants. Kovachevsky (1940) transmitted the virus by Myzus persicae. Doolittle and Zaumeyer (1953) showed that the ring spot strain of cucumber mosaic virus was mechanically transmissible. Simons (1955) found that the cucumber mosaic virus was transmitted by the three vectors viz., Aphis gossypii, Myzus persicae and Aphis rumicis. Szalay-Marzso and Solymossy (1963) reported the transmission of cucumber mosaic virus by Myzus persicae, Aphis nasturtii and Aphis craccivora. Danko and Praslicka (1966) reported Myzus persicae as a vector transmitting the mosaic caused by cucumber mosaic virus on chillies. Anjaneyulu and Appa Rao (1967) studied the virus-vector relationship and stated that the aphid Aphis gossypii transmitted the CMV to chilli in non-persistant manner.

Horvath and Szirmai (1973) reported that the 'E' isolate of CMV was transmitted by Myzus persicae and also by seeds to wild cucumber. Dubey and Joshi (1974) reported that a single Myzus persicae could transmit the virus in non-persistant manner to Capsicum annuum. Debrot (1984) reported non-persistant transmission of CMV on pepper by Myzus persicae. Raccah et al. (1986) reported that CMV was transmitted by Aphis citricola, Myzus persicae and Macrosiphum euphorbiae under field conditions.

2.3.3 Host range

The host range of cucumber mosaic virus is presented in Table 4.

2.3.4 Physical properties

According to Wellman (1935a) the Southern Celery mosaic strain had a thermal inactivation point of 75°C and a dilution end point of 1:10000 and was active in vitro for 6-8 days at room temperature. The ring spot strain reported by Doolittle and Zaumeyer (1952) had a thermal inactivation point of 70 to 72°C and a dilution end point of 1:10000 and longevity in vitro for 4 days at 18°C (room temperature). Simoms reported (1957b) pepper Calico strain and Oak leaf strain, which had a thermal inactivation point of 65 to 70°C and a dilution end point of 1:10000 to 1:20000. Anjaneyulu and Appa Rao (1967) found that

Table 4. Host range of Cucumber Mosaic Virus

S. No.	Host	Reaction	Reference
1.	*Amaranthus retroflexus* L.	-	Schemelzer and Molnar (1975)
2.	*Apium graveolens* var. duke	-	Simons (1957b)
3.	*Beta vulgaris* L.	Systemic infection and chlorotic lesions	Simons (1957b), Anjaneyulu and Appa Rao (1967)
4.	*Capsicum annuum* L. var. California Wonder	Systemic infection	Simons (1957b)
5.	*Chenopodium amaranticolor* Costo and Reyn.	Necrotic lesions	Anjaneyulu and Appa Rao (1967)
6.	*Commelina communis*	-	Wellman (1934a)
7.	*C. nudiflora*	-	Wellman (1934a)
8.	*Cucumis melo* L.	Systemic infection	Anjaneyulu and Appa Rao (1967)
9.	*Cucumis sativus* L.	Systemic infection	Simons (1957b)
10.	*Geranium dissectum*	-	Horvath and Szirmai (1973)
11.	*G. sibricum*	-	Horvath and Szirmai (1973)
12.	*Luffa acutangula* (L.) Roxb.	Systemic infection	Anjaneyulu and Appa Rao (1967)
13.	*Memordica charantia* L.	Systemic infection	Simons (1957b)
14.	*Nicotiana glutinosa* L.	Systemic infection	Simons (1957b), Anjaneyulu and Appa Rao (1967)

S. No.	Host	Reaction	Reference
15.	*N. tabacum* L.	Systemic infection	Simons (1957b), Anjaneyulu and Appa Rao (1967)
16.	*Ocimum canum* L.	-	Horvath and Szirmai (1973)
17.	*Paulownia fargesepi*	-	Horvath and Szirmai (1973)
18.	*Physalis floridana* L.	-	Anjaneyulu and Appa Rao(1967)
19.	*P. peruviana* L.	Systemic infection	Simons (1957b)
20.	*Solanum gracile*	Systemic infection	Simons (1957b)
21.	*S. melongena* L.	Systemic infection	Simons (1957b)
22.	*S. nigrum* L.	Systemic infection	Anjaneyulu and Appa Rao (1967)
23.	*Tetragonia echinata*	Systemic infection	Horvath and Szirmai (1973)
24.	*Trichosanthes anguina* L.	Systemic infection	Anjaneyulu and Appa Rao (1967)
25.	*Vigna sinensis* L.	Necrotic lesions	Anjaneyulu and Appa Rao (1967), Wellman (1934a)
26.	*Zinnia elegans* L.	Systemic infection	Anjaneyulu and Appa Rao (1967), Simons (1957b)

the cucumber mosaic virus on chilli had a thermal death point of 55 to 60°C and dilution end point of 1:250 to 1:500 and was active for 12-18 hours at room temperature and 36 hours at 10°C. Horvath and Szirmai (1973) recorded that 'E' isolate of CMV had a thermal inactivation point of 68 to 70°C, dilution end point of 10^{-4} to 2×10^{-5} and active for 19-22 days.

2.3.5 Purification

Shohara and Osaki (1974) conducted purification studies of CMV by repeated precipitations with 8 per cent PEG (poly Ethylene Glycol) and 0.2 M NaCl (Sodium chloride) followed by PEG solublit gradient (0-6% PEG stabilized in 35.5% Sucrose) centrifugation. Virus yields were 500-800 mg/kg fresh tissue. Purified preparations were highly infectious and contained uniform virus particles. The sedementation pattern showed a single peak indicating that no aggregation occurred.

2.3.6 Varietal reaction

Doolittle and Zaumeyer (1953) reported that the varieties 'California Wonder' and 'World Beater' were susceptible to CMV infection. Cook and Anderson (1959) reported that the Capsicum annuum var. P_{11} was completely susceptible to CMV. Praslicka (1967) revealed that infection was least on the varieties Moravska and PCR. Szirmai (1970) indicated that the

capsicum hybrid variety 53/B was tolerant to CMV. Saccardo (1973) found that 8 lines out of 53 lines of Capsicum annuum, 10 lines out of 12 of C. fruitescens and one line of C. pendulum, C. microcarpum and C. chinense were resistant to CMV. Moorman and Woodbridge (1983) observed that the variety Yolo Wonder was susceptible to CMV.

2.4 POTATO VIRUS Y

2.4.1 Symptomatology

David and Stormer (1941) recorded potato virus Y on Capsicum annuum plants. They described the symptoms as indefinite flecks on the leaves and a slight swelling of the veins and dark green mosaic mottling with wavy margins. In older plants chlorophyll between veins was completely destroyed with white zones.

Jeyarajan and Ramakrishnan (1961) described symptoms of naturally infected chilli plants by potato virus Y, as vein clearing, mosaic mottling, reduction in size of leaves, stunting and reduction in the number of flower buds and fruits. They added that in artificially infected plants, development of the midrib alone with the complete absence of leaf lamina was the characteristic symptom.

Rana et al. (1971) reported a veinal necrosis strain of PVY on chilli.

Rogozzino et al. (1972) reported two strains of PVY viz., normal strain and necrotic strain, the first one caused typical vein banding, indistinct mosaic, stunting, flower abortion and deformation and chlorotic streaks on fruits and the second one caused veinal necrosis, leaf deformation, necrotic spots on fruits, stem splittling and severe stunting.

Mariappan et al. (1973) described the symptoms developed by a strain of PVY on chilli as characteristic mosaic and blistering on leaves.

Lockhart and Fischer (1974) indicated that PVY caused severe mosaic, systemic mottling, leaf and fruit deformation, stunting and epinasty of the plant with poor fruit setting on chilli.

Osaki et al. (1975) observed green vein banding, inter-veinal chlorosis on leaves and chlorotic stripes on stems and fruits of PVY infected chilli plants.

Giorda and Nome (1975) identified a necrotic isolate of PVY on chilli.

2.4.2 Transmission

Simons (1959) reported that Myzus persicae transmitted PVY in pepper. Laind and Dickson (1963) reported transmission of PVY by Myzus persicae, A. gossypii, Macrosiphum solanifolia, M. pisi and A. spiraecola on pepper. Jeyarajan and Ramakrishnan (1969) reported transmission of PVY by Aphis gossypii. Mariappan et al. (1973) reported the vectors viz., Aphis gossypii, A. craccivora, A. nerii and Myzus persicae for PVY transmission on pepper.

Makkouk and Gumpf (1976) transmitted only one isolate of PVY (PVY-4) by Myzus persicae.

2.4.3 Host range

Jeyarajan and Ramakrishnan (1969) reported that potato virus Y on chilli had a limited host range. Among 18 hosts used in host range studies, the virus was transmitted only to Glyricidia maculata, Nicotiana tobaccum L. and N. glutinosa L. It did not infect Chenopodium amaranticolor Costo and Reyn., Capsicum chinense, C. chacoense and C. pubescens.

2.4.4 Physical properties

Sakimura (1953) reported that PVY on pepper had a thermal inactivation point of 53 to 56 °C, dilution end point of 1:300 to 1:1000 and longevity *in vitro* for 48 to 72 hours at 25-29 °C and 66-82 hours at 0.3 °C.

Jeyarajan and Ramakrishnan (1969) observed that PVY on chilli had a thermal inactivation point of 50-55 °C, dilution and point of 1:100 and was active *in vitro* for 2 days at room temperature and also at 4 °C.

Mariappan *et al*. (1973) reported that the PVY on chilli had a thermal inactivation point of 55 °C, dilution end point of 1:1000 to 1:2000 and was active *in vitro* for 5-8 hours at 23-28 °C.

Khatri and Sekhon (1974) indicated that PVY on chilli had a thermal inactivation point of 55 to 60 °C.

Giorda and Nome (1975) reported that an isolate of PVY on pepper had a thermal inactivation point of 55 °C, dilution end point of 10^{-3} and was active *in vitro* for 19-24 hours at 22 °C.

2.4.5 Electronmicroscopy

Laind et al. (1964) reported that PVY from pepper consisted of flexuous rod shaped particles of 694 mμ in size.

Purcifull et al. (1970) observed PVY as flexuous rods of 730 mμ length.

Nelson and Wheeler (1972) reported that Arizona pepper virus (a strain of PVY) consisted of flexuous rod shaped particles of 722 to ± 22 m u length.

2.4.6 Varietal reaction

Cook and Anderson (1959) reported that the Capsicum annuum strain P_{11} was resistant to PVY. Cook and Anderson (1960) also found that the variety SC 46252 was also resistant to PVY, while the other varieties California Wonder, Florida giant, Improved World Beater and Yolo Wonder were susceptible. Cook (1966) reported that Yolo Y variety showed high degree of resistance to PVY. Nitzany (1966) reported that the variety Yolo Y was resistant to PVY. Van der Meer (1967) isolated a new source of resistance in the variety Magnif Ambato.

Jeyarajan and Ramakrishnan listed (1969) 15 varieties viz., X91-6-5, A158, Warangal, 832, Pandurana, CA 733-1-1-1-1, A 123,

Gollaprolu, CA 766-1-3, A 125, A 126, CA 452-1, A 160, Rosogula and C 60A, which were resistant to PVY.

Nagai (1971) reported that the varieties Agronomico-9 and Agronomico-10 were resistant to PVY.

2.5 POTATO VIRUS X

2.5.1 Symptomatology

Blodgett (1927) reported that when chilli plant was inoculated with PVX, the symptoms appeared 10 days after inoculation as indefinite light spots on leaves. They further observed wrinkling of leaves, distortion and shedding of leaves, necrotic streaks on stem and plants became dwarf.

Van der Meer (1932) described the symptoms developed by PVX on capsicum asleaf wrinkling and necrotic spots on leaf lamina, petioles and stems.

Bohme (1933) observed typical mosaic symptoms by PVX on pepper.

Chester (1936) described symptoms by a latentpotato strain of PVX as a severe systemic necrosis on pepper.

David and Stormer (1941) reported two types of symptoms by PVX on pepper as primary symptoms and secondary symptoms. Primary symptoms were necrotic dark rings on inoculated leaves and secondary symptoms were bleaching and puckering of younger leaves, necrosis and death of the entire apex.

Ramakrishnan (1959) reported the natural occurrence of PVX on chilli variety California Wonder. The chief symptoms mentioned were stunting of plants, narrowed and reduced leaves with mosaic patterns and dark green vein bandings. Fruit set was reduced considerably.

Paulus (1960) reported that PVX produced small necrotic spots on leaves of tabasco pepper.

Varma et al. (1970) observed the symptoms of 3 different strains on C. pendulum when inoculated with PVX. Strain X_1 produced local lesions on inoculated leaves followed by top necrosis of the plant. Strain X_2 induced local lesions on inoculated and uninoculated leaves and necrosis on stem and petioles, while X_3 produced local lesions on inoculated leaves without systemic infection.

Rao et al. (1970) reported a ring strain of PVX on pepper which caused mosaic mottling and yellow chlorotic spots on

leaves which later developed into systemic infection. Fruit setting was reduced.

2.5.2 Host range

Ramakrishnan (1959) observed that potato virus X from chilli caused local necrotic lesions on Gomphrena globosa L. and Chenopodium paganum, C. amaranticolor Costo and Reyn. developed red, ring shaped non-necrotic local lesions. Datura metaloides and D. inoxia exhibited systemic infection with faint chlorotic leaf mottling. Solanum nigrum L., S. pseudocarpum, Physalis paruviana L., Nicotiana tabacum L. var. Samsun and N. glutinosa L. developed neither systemic nor localised symptoms.

Rao et al. (1970) observed that ring spot strain of PVX produced local necrotic lesions with white spot at centre and surrounded by reddish band on Gomphrena globosa L. local lesions with concentric rings on Datura stramonium L., and necrotic local lesions followed by systemic infection on Nicotiana tabacum L. var. White Burley, Harrision special, N.N. strain and N. glutinosa L.

2.5.3 Physical properties

Van der Meer (1932) reported that PVX had a thermal inactivation point of 75°C, a dilution end point of 1:100000 to 1: 1000000 and longevity in vitro of 18 days.

Rao et al. (1970) observed that the ring spot strain of PVX had a thermal inactivation point between 72 to 75°C, dilution end point between 10^{-5} to 10^{-6} and was still active in vitro for 18-20 days at 20-30°C.

2.5.5 Purification and Electron microscopy

Ramakrishnan (1959) reported that purified preparation of PVX under electron microscope, revealed the presence of flexuous rod shaped particles measuring 550-600 m μ in length.

Grama (1975) reported a simple method of purification of PVX. In this method emulcification of the sap of Nicotiana debneyi (the source), precipitation of the cell components at pH 5, precipitation of the virus with 4 per cent polyethylene glycol and subsequent purification on a sephadex G-50 Colemn, are involved. The purity of preparations was confirmed by electron microscopy, sedimentation analysis, UV spectophotometry and chromatography.

CHAPTER III

MATERIALS AND METHODS

3.1 SURVEY, COLLECTION OF VIRUS CULTURE AND ITS MAINTENANCE

The present studies on mosaic disease of chilli were carried out during 1985-86.

Chilli plants, showing characteristic mosaic mottling symptoms, were collected from fields in and around Tirupati and those seedlings collected from Tirupati were maintained in the glasshouse in separate pots. Inoculum from the infected plants was maintained on 30 day old chilli plants of variety X-230. For some experiments the virus was also maintained on 30 day old tobacco plants (local variety), Nicotiana tabacum L., as the virus produced pronounced mosaic mottling symptoms on them. Chilli seedlings were inoculated at regular intervals to have sufficient inoculum whenever the necessity arises. All these plants were kept under insect proof conditions. Nuvacron and Rogor were sprayed at low concentrations (0.2 to 0.5%) at 10 day interval to control the incidence of thrips and aphids on experimental plants.

The percentage of incidence of disease was calculated in the following way. The area of the chilli crop was divided into separate plots. Total number of plants and diseased

plants were counted separately. The percentage infection was calculated by using the formula.

$$100 \times \frac{\text{Total number of diseased plants}}{\text{Total number of plants in the plots}}$$

The diseased samples collected from different regions were inoculated on healthy chilli plants by mechanical sap transmission and one isolate collected from Chittoor district was established in the glasshouse.

3.1.1 The virus

Chilli mosaic virus was isolated by mechanical inoculation from mosaic affected plants of chilli collected from the farmers fields of Chittoor, Prakasam and Guntur districts during 1985-86 and maintained on chilli cv. X-230 and Nicotiana tabacum in an insect proof glasshouse. The virus inoculum extracted from X-230 (chillies) and N. tabacum was used throughout the course of investigation.

3.1.2 Test plants

For experiments on physical properties of the virus Nicotiana tabacum L. and Chenopodium amaranticolor Costo & Reyn. were taken as test plants. Nursery of both the plants was raised in separate pots of 75 cm diameter for about 30 days. Then the seedlings were transplanted in individual pots of 30 cm diameter.

For studying host range studies, plants belonging to 50 species were sown in different pots and transplanted in separate pots at 30 days age.

For varietal reaction, chilli variety X-230 and several other varieties like X-235, X-190, X-210, X-206, G_3, G_4, G_5, 1077, 1078, 1079, Aparna, Cluster, Santaka, Paprika-50, Warangal, Sindhur, Kiran, S 118, LIC-45, 1081, were also sown in earthen pots separately and transplanted when they were 30 days old.

For purification, several seedlings of 30 day old chilli variety X-230 were maintained in about 50 pots.

3.1.3 Preparation of Phosphate Buffersolution

Phosphate buffer of pH 7.0 was prepared by mixing 39.0 ml of 0.2 M solution of KH_2PO_4 (Potassium dihydrogen phosphate) with 61.0 ml of 0.2 M solution of K_2HPO_4, $12H_2O$ (Potassium monohydrogen phosphate) (Sorenson, 1909). The buffer solution was prepared for every 20 days and kept in a refrigerator (10°C) at low temperature.

3.1.4 The Virus

Standard extract of the virus was prepared by macerating the infected leaves of known weight in a sterilized mortar after adding a few drops of phosphate buffer of pH 7.0 (0.2M). After maceration the same buffer at 1 ml/g fresh tissue was added and the extract was squeezed through double layered muslin cloth. This formed the standard inoculum for studying the various properties of the virus. The standard extract was used in studying some of the physical properties and host range of the virus.

3.1.5 Method of inoculation

Sap inoculations of the virus were done on vigorously growing seedlings of test plants. The upper surface of the leaves was first dusted with carborundum (300 mesh), the leaves were then inoculated with standard extract of the virus by dipping the forefinger in inoculum and gentle rubbing by the same finger with the support of left hand palm from base to tip. Immediately after inoculation the inoculated leaves were washed with tap water to remove excess carborundum. All the apparatus used were sterilised according to requirements for each experiment.

3.1.6 Seed transmission

For seed transmission studies the seeds were extracted from the tagged fruits of the infected plants. The seeds were dried and lots of 100 seeds were sown at the rate of 100 seeds/pot. Seedlings were kept under observation for a period of two months in the insect proof glasshouse. Seeds collected from healthy fruits were sown and kept for control. Data regarding the number of seedlings grown and thenumber showing symptoms were recorded.

3.1.7 Soil transmission

To determine the soil transmission of the virus, seeds were sown in pots, in which diseased plants were grown for about 3 months. Seeds were also sown in another lot of pots filled with sterilized soil to serve as control. Sterilization was done in an autoclave by subjecting the soil to a pressure of 20 lb/inch^2/hour. Plants were allowed to grow for a period of 50-60 days. The number of seedlings grown and the number showing symptoms was recorded.

3.2 HOST RANGE

In the present studies 50 species of plants belonging to different genera distributed in several families were tested. Nurseries of test plants belonging to the families. Amaranthaceae, Solanaceae, Cruciferae, Compositae, Apocyanaceae, Chenopodiaceae, Leguminaceae, Malvaceae and Graminae were raised in 30 cm diameter pots and individual plants were transplanted. Seeds of the test plants belonging to the above families were sown singly in pots and theplants were raised in insect proof cages. Ten plants, each of tobacco and chenopodium were inoculated when they were 6-8 leaf stage, whereas cucurbits and legumes were inoculated when the plants were of 8-10 leaf stage and equal number of uninoculated plants of the same age served as control. The deviations were recorded daily for about 40 days. Back inoculations were done on N. tabacum.

3.3 PHYSICAL PROPERTIES OF THE VIRUS

The physical properties of the virus like thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV) were studied using both systemic and local lesion hosts.

3.3.1 Thermal inactivation point

For this study the infected leaf extract was distributed in small test tubes of uniform size, sealed at one end. 2 ml of standard extract was transferred to each test tube and these were exposed to different temperatures ranging from 40 to 95 °C for 10 minutes in thermostatically controlled water bath. Care was taken to immerse completely the portion of test tube containing sap into the water. Immediately after the exposure the test tubes were cooled under cool, running water. The sap subjected to each temperature was inoculated to a set of tobacco plants N. tabacum and Chenopodium amaranticolor. These plants were kept under observation for 60 days.

3.3.2 Dilution end point

For determining the dilution end point of the virus the infective sap was extracted by grinding 1 gm of the infected leaves in mortar and pestle adding 1 ml buffer. Serial dilutions from 1/10 to 1/100000000 were prepared using buffer as diluent and were inoculated to a set of N. tabacum plants and C. amaranticolor and the number of seedlings in each set showing symptoms were recorded.

3.3.3 Longevity *in vitro*

To determine the longevity *in vitro* of the virus the following method was adopted. Standard extract of the virus was divided into 2 equal parts. One conical flask with standard extract was stored at room temperature (23.5 to 28.5°C), while the other was stored at 10°C in a refrigerator. In both the cases inoculations were made at definite intervals on the test plants, *N. tabacum* and *C. amaranticolor*. Plants inoculated with freshly prepared sap served as control.

3.4 PURIFICATION AND ELECTRON MICROSCOPY

3.4.1 Purification

The virus was purified by the following method adopted from Hollings *et al.* (1968), Lima and Nelson (1975) and Fisher and Lockhart (1976a, b).

Systemically infected leaves, were harvested at 15-20 days after inoculation.

↓

Cooled at 4°C

25-30 grams of infected leaves were mixed in an equal volume of phosphate buffer (pH 7.2, 0.5 M) containing 0.2 per cent 2-mercapto-ethanol.

Squeezed through a double layered muslin cloth and 8.5 per cent n-butanol added to the final volume.

Then this mixture was kept over night at 4°C.

Subjected to the centrifugation at 3000 rpm for 40 minutes.

Resultant pellet was discorded by filtering the supernatent through the filter paper.

4 to 8 per cent poly Ethelene Glycol (6000 molecular weight) and 4 per cent Sodium chloride were added to the resultant supernatent while stirring constantly in cold for 30-40 minutes.

Then centrifuged at 15,000 rpm for 40 minutes.

Resulting pellet was dissolved in 2 ml of 0.1 M phosphate buffer containing 0.5 per cent sodium sulphate.

This suspension was centrifuged at 5000 rpm for 15 minutes.

The resultant supernatant containing virus particles was used for electronmicroscopy.

3.4.2 Electron microscopy

The purified preparation of both healthy and diseased leaves of infected plants were sprayed by means of a nebulizer on the copper grids, previously coated with thin colloidian film. The grids when dried were shadow casted with vapourised palladium on the specimen under vaccum. The specimen grids were then observed with the Hitachy Hu-iie electronmicroscope at various magnifications. The photographs of virus particles were taken and the size of the particles were calculated by the following formula.

$$\text{Actual size of the particle (in microns)} = \frac{\text{Size of particle in electronmicrograph in mm}}{\text{magnification}}$$

3.5 AGE OF CHILLI PLANTS IN RELATION TO SUSCEPTIBILITY

Chilli seeds were sown separately at an interval of 10 days. The seedlings were transplanted in individual pots to get plants of different ages (20 to 70 days old) at the same time. The plants of each different age were inoculated on the same day by sap inoculation. Uninoculated plants of respective ages served as control.

3.6 EFFECT OF DISEASE ON CERTAIN GROWTH CHARACTERS

With a view to determine the effect of infection of the virus on certain growth characters of the host, a set of 15 chilli plants of variety X-230 of 30 days old were inoculated with the virus and a similar set of 15 plants without inoculation served as control. The plants were kept under observation for 30 days after inoculation. Thereafter they were uprooted for recording the data. The growth characters viz., shoot length, root length, leaf area, fresh and dry weights were recorded.

For obtaining the height of the plant, perpendicular length of plant from the ground level to the tip of the terminal bud was taken.

For root length, the length of the main root was measured with the help of a metre scale.

For recording the fresh weight, the fresh plants immediately after uprooting were weighed and the individual weights were recorded for both the healthy and diseased plants.

For dry weight, the plants kept in paper bags were oven dried at 60°C to a constant weight. Both the diseased and healthy plants were weighed.

For measuring the leaf area of healthy and diseased leaves, leaf area meter was used and the readings were recorded.

The results are interpreted based on the percentage loss in each character by using the following formula:

$$\text{Per cent loss} = 100 \left(1 - \frac{a}{b}\right)$$

where,

a = value of diseased material

b = value of healthy material

3.6.2 Estimation of chlorophyll fractions

Chlorophyll a, chlorophyll b and total chlorophyll were estimated following the method of Arnon (1949).

Two hundred mg (milligrams) of fresh leaf material was repeatedly extracted with 80 per cent acetone, till the extract was colourless and filtered through whatman No. 1 filter paper. The filterate was made upto 25 ml in a standard flask and optical density of the extract was read in separate spectronic-20 at 645 and 663 nm (Nanometre) for chlorophyll a and chlorophyll b respectively. The contents are expressed on fresh weight basis as mg/g leaf tissue.

Chlorophyll a	:	O.D x 20.20 x 25
Chlorophyll b	:	O.D x 8.02 x 25
Total Chlorophyll	:	Chlorophyll a + Chlorophyll b

3.7 VARIETAL REACTION

Twenty varieties of chillies obtained from Regional Agricultural Research Station, Lam, Guntur were screened for their reaction to sap inoculation. In each variety 10 seedlings at 4-6 leaf stage (30 days old) were mechanically inoculated. The inoculated plants were observed periodically and the disease symptoms were recorded. The following were the varieties screened, G_3, G_4, G_5, X-235, X-180, X-210, X-206, Santaka, Warangal, Aparna, 1077, 1078, 1079, S 118, Cluster, Sindhur, Kiran, LIC-45, 1081 and Paprika-50.

3.8 STATISTICAL METHODS

The data pertaining to the growth character studies were statistically analysed after angular transmission, wherever necessary, following the method of analysis of variation for RBD as outlined by Snedecor (1962), Panse and Sukhatme (1978).

CHAPTER IV

EXPERIMENTAL RESULTS

4.1 INCIDENCE OF CHILLI MOSAIC DISEASE

During 1985-86, a survey was made in the chilli growing areas around Tirupati as well as Prakasam and Guntur districts of Andhra Pradesh to record the incidence of mosaic disease.

The incidence of mosaic disease was recorded in surrounding villages of Tirupati viz., Narasingapuram, Thondawada, Karakambadi and Perumallapalli and in Bapatla of Prakasam district and Lam of Guntur districts (Table 5). The incidence of disease ranged from 5.21 to 20.02 per cent. The age of plants ranged from 1 to 3 months.

Incidence of mosaic was also recorded in orchard block, S.V.Agricultural College, Tirupati (Table 6). The percentage of incidence ranged from 0.0 to 85.80. The cultivars viz., OT-3, S 118 X (G4 x MP), long pod x small pod, G4 x 960, K_2 x Pant C_1, ms x ms x 206, (ms) x (G4 x MP), Pant C_1, LCA-1079 and LCA-180 were free from mosaic. Minimum incidence was recorded in culture 960 x G4 (4.2%) and maximum (85.8%) in LCA-210.

Table 5. Natural occurrence of chilli mosaic in Chittoor, Prakasam and Guntur districts

S. No.	Name of the village	No. of plots surveyed	Name of the variety	Per cent incidence
1.	Narasingapuram	4	Local	16.54
2.	Thondavada	9	Local	10.71
3.	Karakambadi	12	Local	8.33
4.	Cherlopalli	3	X-235	5.21
5.	Perumallapalli	6	Local	9.27
6.	Bapatla	15	Local	20.02
7.	Lam	5	Sindhur	12.52

Table 6. Screening of chilli cultivars/cultures against mosaic disease under natural field conditions

Serial No.	Name of the culture	Per cent incidence
1.	CT-3	-
2.	S 118 x long pod-2	40.00
3.	960 x long pod-1	10.00
4.	960 x G4	50.00
5.	K_2 x Paprikas	15.00
6.	Long pod x Nixeric stout	12.00
7.	253 x Bomvumirapa	16.23
8.	(LC x 235) x (Cfg x BP)	11.50
9.	960 x G4-2	16.20
10.	197 x Small pod	16.25
11.	235 x 1077	55.00
12.	960 x Bomvumirapa	11.00
13.	235 x long pod	5.00
14.	Leaf calyx x 235	8.00
15.	960 x LIC-45	7.00
16.	960 x Paprikas	60.00
17.	Santaka x Jwala-2	50.00
18.	G4 x 960-5	80.00
19.	G4 x 960 (Safed)	70.00
20.	CT-4	50.00
21.	LCA-235	10.00

Table 6 (contd...)

Serial No.	Name of the culture	Per cent incidence
22.	Long pod x Jawahar	36.36
23.	G4 x MP	50.00
24.	Cfgreen x C. sinense	22.30
25.	Cfwhite x C. sinense	25.00
26.	S 118 x (G4 x MP)	-
27.	Paprika 50 x long pod	60.00
28.	Santaka x Jwala	50.00
29.	Long pod x small pod	-
30.	960 x 1077	66.50
31.	960 x G4	44.40
32.	G4 x 960-1	22.20
33.	G4 x 960-2	33.30
34.	Male sterile lines	22.30
35.	(LP x BP) x (LC x 1068)	5.60
36.	960 x G4	4.20
37.	960 x small pod	6.50
38.	G4 x 960	-
39.	Cfg x Pendulum	16.23
40.	Warangal	9.09
41.	LCA-197	11.20
42.	(LC 1068) x (LP x BP)	11.80

Table 6 (cont...)

Serial No.	Name of the culture	Per cent incidence
43.	K_2 x Pant C_1	-
44.	ms x ms x 206	-
45.	(ms) x (G4 x MP)	-
46.	Pant C_1	-
47.	Jhati Lanka	11.23
48.	LCA-1079	-
49.	C. sinense	28.60
50.	Sindhur	9.10
51.	OT-2	40.00
52.	G4	62.50
53.	LCA-210	85.80
54.	LCA-206	22.30
55.	LCA-200	8.05
56.	LCA-1068	30.00
57.	OT-5	5.80
58.	OT-1	22.30
59.	Simamirapa	21.80
60.	G3	18.00
61.	LCA-180 (Cluster)	-

SYMPTOMATOLOGY

Naturally infected chilli plants showed a distinct mosaic mottling, distortion of leaves and retardation of growth. Occasionally leaves developed large blisters of green tissue (Plate 1). The affected plants were stunted and bushy in appearance with delayed flowering. Eventhough the flowers were produced, more flower shedding was found. Often, the infected plants produced no flowers.

The first symptom of the disease in sap inoculated plants appeared 7-9 days after inoculation during summer and 10-12 days during winter. Symptoms were noticed in the leaves next to inoculated leaves but not in the inoculated leaves. Affected leaves were reduced in size and chlorotic areas appeared on the leaf lamina followed by small irregular darkgreen blotches alternating with the light green areas developing into a typical mosaic pattern (Plate 2). The affected plants were stunted with retarded growth.

SAP-TRANSMISSION

To know the sap-transmission ability of the virus, a set of 10 plants of chilli variety K-230 (30 days old) were artificially inoculated as described under materials and methods and kept under observation for 10 days. Symptoms were observed

PLATE 1A. Naturally infected chilli plant showing mosaic symptoms

B. Naturally infected leaves showing mosaic symptoms

PLATE 1A

PLATE 1B

PLATE 2. Chilli plant showing mosaic symptoms and stunted growth on artificial inoculation

PLATE 2

7 days after inoculation. In various tests at different times of a year, 75-90 per cent of chilli cultivars were infected by sap inoculation.

The symptoms observed on artificial inoculation were mosaic mottling, slight blistering, reduction in leaf lamina and stunted growth.

SEED TRANSMISSION

In order to determine the seed transmission, chilli seeds collected from diseased plants were sown in the insect proof glasshouse and kept under observation. The results (Table 7) indicated that the virus under test was not transmitted by seed. Back inoculation from the seedling plants did not induce any symptoms on chilli plants indicating that the virus was not carried to symptomless carriers.

SOIL TRANSMISSION

To determine the soil transmission of the virus, chilli seeds were sown in two sets of pots separately. One set of pots were filled with sterilised soil. Another set of pots were filled with soil, in which diseased (mosaic) plants were grown previously. In both the cases plants were allowed to grow for about 60 days. The observations were recorded and presented in Table 8. The results indicated that the virus

Table 7. Seed transmission

S. No.	Treatment	Source of seed	Number sown	Number germinated	No. showing symptoms	Back inoculation
1.	Control	From healthy fruit	100	86	-	0
2.	1	From diseased fruit	100	80	-	0
3.	2		100	75	-	0
4.	3		100	73	-	0
5.	4		100	78	-	0
6.	5		100	77	-	0

Table 8. Soil transmission

S. No.	Soil	Total number of seedlings in six pots	No. showing symptoms	Per cent transmission	Back inoculation
1.	Control (Sterilized soil)	120	-	-	-
2.	Diseased soil	115	-	-	-

was not transmitted through soil.

HOST RANGE

In order to determine the host range of the virus under investigation, plant species belonging to different families were mechanically inoculated as described under 'Materials and Methods'. Symptoms produced on individual plant species were recorded and the virus was recovered back on chilli plants by sap inoculations. The results of the host-range studies are presented in Table 9 . The virus was found to be transmissible to the various species of solanaceous plants like Capsicum fruitescens L., Petunia oleracia L., Datura metel L. D. stramonium L., Nicotiana tabacum L., N. glutinosa L., Solanum melongena L. and Lycopersicon esculentum Mill., producing systemic mosaic symptoms (Plate 4 and 6). D. metel L. also produced local lesions (Plate 5).

In cucurbitaceae the virus was found to be transmissible to Cucumis sativus L., C. melo L. and Momordica charantia L. producing systemic mosaic symptoms. In Amaranthaceae, Amaranthus hypochondriacus produced systemic symptoms and Chenopodium amaranticolor Costo and Reyn. produced local lesions (Plate 3). The virus was found to be not transmissible to any one of the members of leguminaceae, Cruciferae, Apocyanaceae, Malvaceae and Compositae. Out of 47 plants used under host-range studies, only 13 species of plants exhibited reaction. Back-inoculations were also carried out to determine whether there were any symptomless carriers.

Table 9. Host range studies of chilli mosaic virus

S. No.	Name of the host	Reaction	Type of reaction	Family
1.	*Capsicum fruitescens* L.	+	Systemic	Solanaceae
2.	*Petunia oleracia* L.	+	Systemic	Solanaceae
3.	*Datura metel* L.	+	Local & systemic	Solanaceae
4.	*D. stramonium* L.	+	Systemic	Solanaceae
5.	*Nicotiana tabacum* L.	+	Systemic	Solanaceae
6.	*N. glutinosa* L.	+	Systemic	Solanaceae
7.	*Solanum melongena* L.	+	Systemic	Solanaceae
8.	*Lycopersicon esculentum* Mill.	+	Systemic	Solanaceae
9.	*Vigna sinensis* Savi.	-	-	Leguminaceae
10.	*Cicer arietinum* L.	-	-	Leguminaceae
11.	*Phaseolus aureus* Roxb.	-	-	Leguminaceae
12.	*P. mungo* var. *radiatus* L.	-	-	Leguminaceae
13.	*P. vulgaris* L.	-	-	Leguminaceae
14.	*Cajanus cajan* Milsp.	-	-	Leguminaceae
15.	*Dolichos biflorus* Roxb.	-	-	Leguminaceae
16.	*Pisum sativum* Roxb.	-	-	Leguminaceae
17.	*Arachis hypogeae* L.	-	-	Leguminaceae
18.	*Glysine max* L.	-	-	Leguminaceae
19.	*Cyamopsis tetragonaloba* L.	-	-	Leguminaceae
20.	*Crotolaria striata* Taub.	-	-	Leguminaceae
21.	*Canavalia ensiformis*	-	-	Leguminaceae
22.	*Visia faba*	-	-	Leguminaceae
23.	*Cucumis sativus* L.	+	Systemic	Cucurbitaceae
24.	*Cucumis melo* L.	+	Systemic	Cucurbitaceae

Table 9 (contd...)

S. No.	Name of the host	Reaction	Type of reaction	Family
25.	*Citrullus vulgaris* Schrad-ex Eckl & Leyh.	-	-	Cucurbitaceae
26.	*Lagenaria cicararia* Standl.	-	-	Cucurbitaceae
27.	*Momordica charantia* L.	+	Systemic	Cucurbitaceae
28.	*Luffa acutangula* (L.) Roxb.	-	-	Cucurbitaceae
29.	*Trichosanthes anguina* L.	-	-	Cucurbitaceae
30.	*Benincasa hispida* (Thumb) Logn.	-	-	Cucurbitaceae
31.	*Cucurbita moschata* Dutch. ex. Poin	-	-	Cucurbitaceae
32.	*Coccinia indica* L.	-	-	Cucurbitaceae
33.	*Brassica juncea* L.	-	-	Cruciferae
34.	*Zinnia elegans* Jacq.	-	-	Compositae
35.	*Tridax procumbens* L.	-	-	Compositae
36.	*Gomphrena globosa* L.	-	-	Compositae
37.	*Hibiscus rosa sinensis* L.	-	-	Malvaceae
38.	*Abelmoschus esculentus* Moench.	-	-	Malvaceae
39.	*Gossypium hirsutum* L.	-	-	Malvaceae
40.	*Amaranthus hypochondriacus*	+	Systemic	Amaranthraceae
41.	*Ricinus communis* L.	-	-	Euphorbiaceae
42.	*Euphorbia hirta* L.	-	-	Euphorbiaceae
43.	*Croton sparciflorus* L.	-	-	Euphorbiaceae
44.	*Vinca rosea* L.	-	-	Apocyanaceae
45.	*Chenopodium amaranticolor* Costo and Reyn.	-	Local lesion	Chenopodiaceae
46.	*Saccharum* sp. L.	-	-	Graminae

PLATE 3A. Chenopodium amaranticolor plant showing local lesions on leaves

B. Leaves of Chenopodium amaranticolor showing local lesions

PLATE 3A

PLATE 3B

PLATE 4. *Datura stramonium* showing systemic symptoms

PLATE 5. *D. metel* leaf showing necrotic local lesions

PLATE 4

PLATE 5

PLATE 6A. Nicotiana tabacum showing mild mosaic symptoms

B. Leaves of Nicotiana tabacum showing mild mosaic symptoms

H : Healthy

I : Infected

PLATE 6A

PLATE 6B

PHYSICAL PROPERTIES

Physical properties often help in the identification of plant viruses. Hence, experiments were carried out to study the physical properties of the virus viz., Thermal inactivation point (TIP),Dilution end point (DEP) and longevity *in vitro*, using *Nicotiana tabacum* and *Chenopodium amaranticolor* as the test plants.

THERMAL INACTIVATION POINT

The standard extract of the virus was prepared and exposed to different temperatures as described under materials and methods. The experiment was carried in '3' series and results are presented (Table 10 and 11).

The results indicated that the virus in the standard extract could withstand heating upto 90°C for 10 minutes, but it was rendered non-infectious when heated to 95°C for the same period. Hence the thermal inactivation point of the virus lies between 90°-95°C. It is also observed that as temperature increased the percentage of infection decreased (Fig. 1 and 2).

Table 10. Determination of thermal inactivation point of chilli mosaic virus ((Assayed on (Nicotiana tabacum L.) at room temperature (23.5°C to 28.5°C))

Test No.	S. No.	Temperature (°C)	No. of plants inoculated	No. of plants infected	Mean incubation period (days)	Per cent infection
I						
	1	Control	5	5	7	100
	2	40	5	4	8	80
	3	50	5	4	8	80
	4	60	5	5	8	100
	5	65	5	3	8	60
	6	70	5	4	8	80
	7	75	5	4	8	80
	8	80	5	3	8	60
	9	85	5	3	9	60
	10	90	5	1	9	20
	11	95	5	-	1	-
II						
	1	Control	5	5	8	100
	2	40	5	5	8	100
	3	50	5	4	8	80
	4	60	5	4	8	80
	5	65	5	3	8	60
	6	70	5	4	8	80
	7	75	5	4	8	80
	8	80	5	2	9	40
	9	85	5	2	9	40
	10	90	5	2	9	40
	11	95	5	-	-	-

Table 10 (contd...)

Test No.	S. No.	Temperature (°C)	No. of plants inoculated	No.of plants infected	Mean incubation period (days)	Per cent infection
III						
	1	Control	5	5	8	100
	2	40	5	4	7	80
	3	50	5	4	8	80
	4	60	5	3	8	60
	5	65	5	5	8	100
	6	70	5	4	8	80
	7	75	5	4	8	80
	8	80	5	3	8	60
	9	85	5	3	8	60
	10	90	5	1	8	20
	11	95	5	-	-	-

FIG:1. THERMAL INACTIVATION POINT OF CHILLI MOSAIC VIRUS ON

Nicotiana tabacum

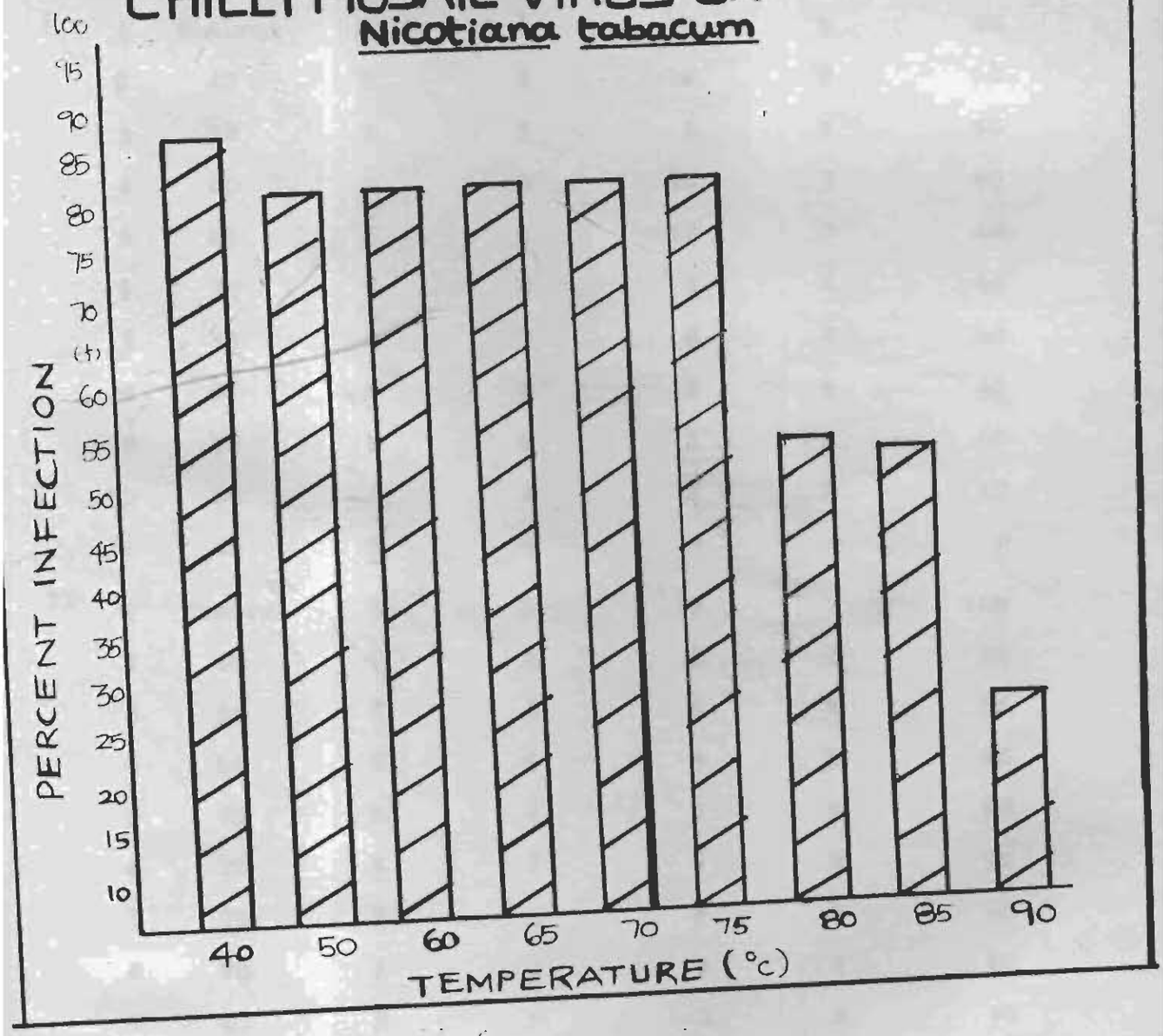


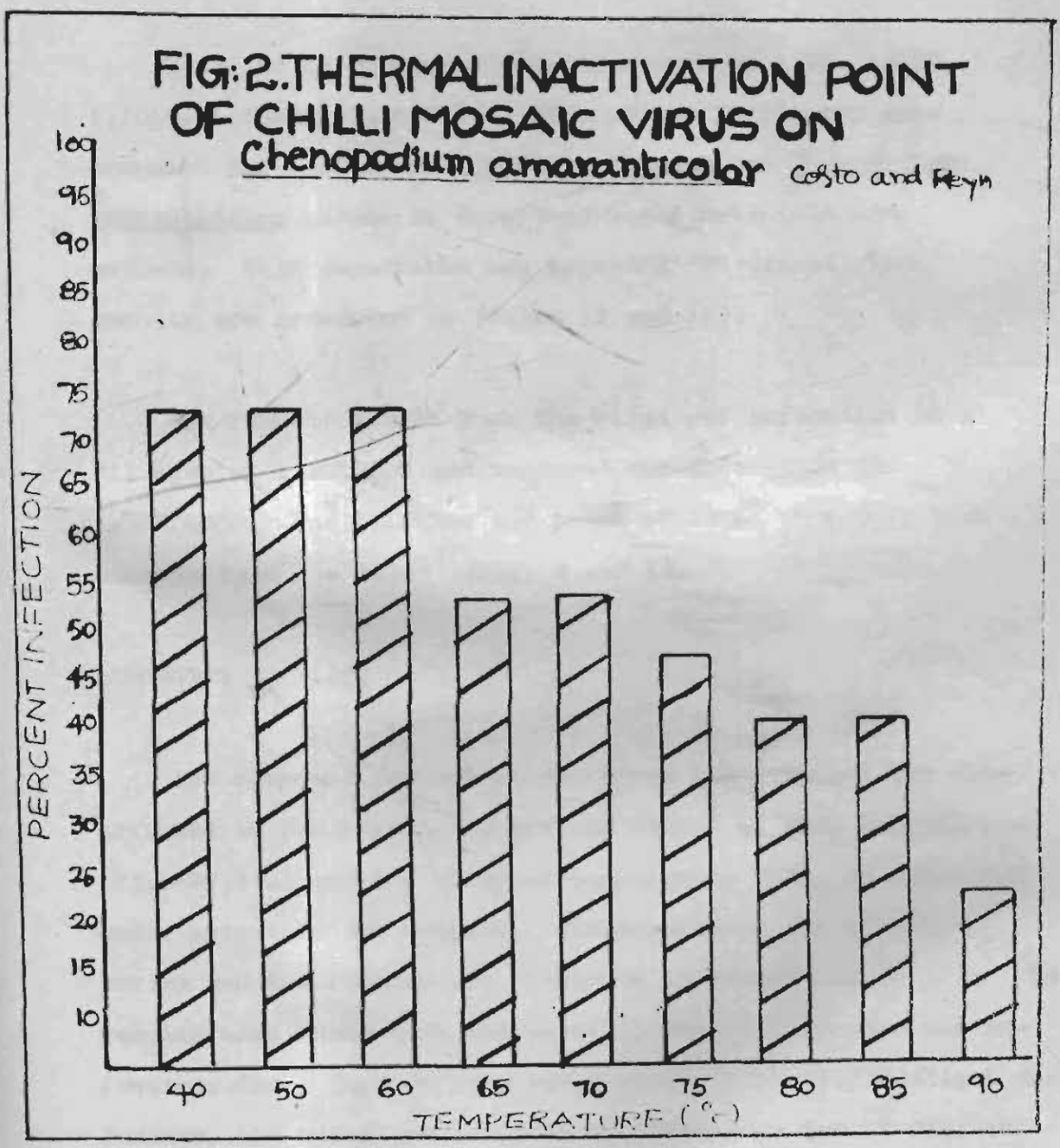
Table 11. Determination of thermal inactivation point of chilli mosaic assayed on (Chenopodium amaranticolor L.) at room temperature (23.5 to 28.5°C)

Test No.	S. No.	Temperature (°C)	No. of plants inoculated	No. of plants infected	No. of lesions/ leaf	Mean incubation period (days)	Per cent infection
I	1	Control	5	4	12	8	80
	2	40	5	3	10	8	60
	3	50	5	3	9	8	60
	4	60	5	3	10	9	60
	5	65	5	2	10	7	40
	6	70	5	2	8	8	40
	7	75	5	3	8	8	60
	8	80	5	2	6	8	40
	9	85	5	2	5	8	40
	10	90	5	2	2	8	40
	11	95	5	-	-	-	-
II	1	Control	5	5	13	7	100
	2	40	5	4	9	8	80
	3	50	5	4	6	8	80
	4	60	5	4	6	8	80
	5	65	5	3	5	8	60
	6	70	5	3	4	8	60
	7	75	5	2	4	8	40
	8	80	5	2	3	8	40
	9	85	5	2	3	8	40
	10	90	5	1	2	8	20
	11	95	5	-	-	-	-

Table 11 (contd...)

Test No.	S. No.	Temperature (°C)	No.of plants inoculated	No. of plants infected	No. of lesions/ leaf	Mean incubation period (days)	Per cent infection
III							
	1	Control	5	4	12	7	80
	2	40	5	4	11	7	80
	3	50	5	4	8	8	80
	4	60	5	4	7	8	80
	5	65	5	3	6	8	60
	6	70	5	3	4	8	60
	7	75	5	2	4	8	40
	8	80	5	2	3	8	40
	9	85	5	2	2	8	40
	10	90	5	1	2	8	20
	11	95	5	-	-	-	-

FIG: 2. THERMAL INACTIVATION POINT OF CHILLI MOSAIC VIRUS ON *Chenopodium amaranticolor* Costo and Keyn



DILUTION END POINT

A series of different dilutions such as 1:10, 1:100, 1:1000, 1:10000, 1:100000, 1:1000000 and 1:10000000 were prepared and indexed on Nicotiana tabacum and Chenopodium amaranticolor plants as described under materials and methods. This experiment was repeated '3' times. The results are presented in (Table 12 and 13).

The results showed that the virus was infectious at a dilution of 1:1000000 and rendered non-infectious at 1:10000000. The dilution end point of virus therefore lies between $1:10^{-6}$ - $1:10^{-7}$ (Fig. 3 and 4).

LONGEVITY in vitro

The standard extract of the virus was prepared and distributed in two conical flasks and stored at room temperature (23.5-28.5°C) and the other at temperature 10°C, as described under materials and methods. The experiment was repeated thrice and the results are presented in (Tables 14 to 17). The results have shown that the virus in standard extract was infectious for 9 days at room temperature (23.5-28.5°C)(Fig.5 and 6). Further, the virus was found to be infectious for 17 days at temperature of 10°C (Fig. 7 and 8).

Table 12. Determination of dilution end point of chilli mosaic virus ((assayed on (Nicotiana tabacum L.))

Test No.	S. No.	Dilutions		No.of plants inoculated	No. of plants infected	Mean incubation period (days)	Per cent infection
I							
	1.	Control		5	5	7	100
	2.	1:10	(10^{-1})	5	4	8	80
	3.	1:100	(10^{-2})	5	4	8	80
	4.	1:1000	(10^{-3})	5	3	8	60
	5.	1:10000	(10^{-4})	5	3	8	60
	6.	1:100000	(10^{-5})	5	3	8	60
	7.	1:1000000	(10^{-6})	5	1	9	20
	8.	1:10000000	(10^{-7})	5	-	-	-
II							
	1.	Control		5	4	7	80
	2.	1:10	(10^{-1})	5	4	7	80
	3.	1:100	(10^{-2})	5	4	8	80
	4.	1:1000	(10^{-3})	5	3	8	60
	5.	1:10000	(10^{-4})	5	3	8	60
	6.	1:100000	(10^{-5})	5	1	8	20
	7.	1:1000000	(10^{-6})	5	1	8	20
	8.	1:10000000	(10^{-7})	5	-	-	-

Table 12 (contd...)

Test No.	S. No.	Dilutions		No.of plants inoculated	No.of plants infected	Mean incubation period (days)	Per cent infection
III							
	1.	Control		5	4	7	80
	2.	1:10	(10^{-1})	5	5	7	100
	3.	1:100	(10^{-2})	5	4	7	80
	4.	1:1000	(10^{-3})	5	4	8	80
	5.	1:10000	(10^{-4})	5	3	8	60
	6.	1:100000	(10^{-5})	5	3	8	60
	7.	1:1000000	(10^{-6})	5	1	9	20
	8.	1:10000000	(10^{-7})	5	-	-	-

FIG:3. DILUTION END POINT OF CHILLI MOSAIC VIRUS
(on Nicotiana tabacum)

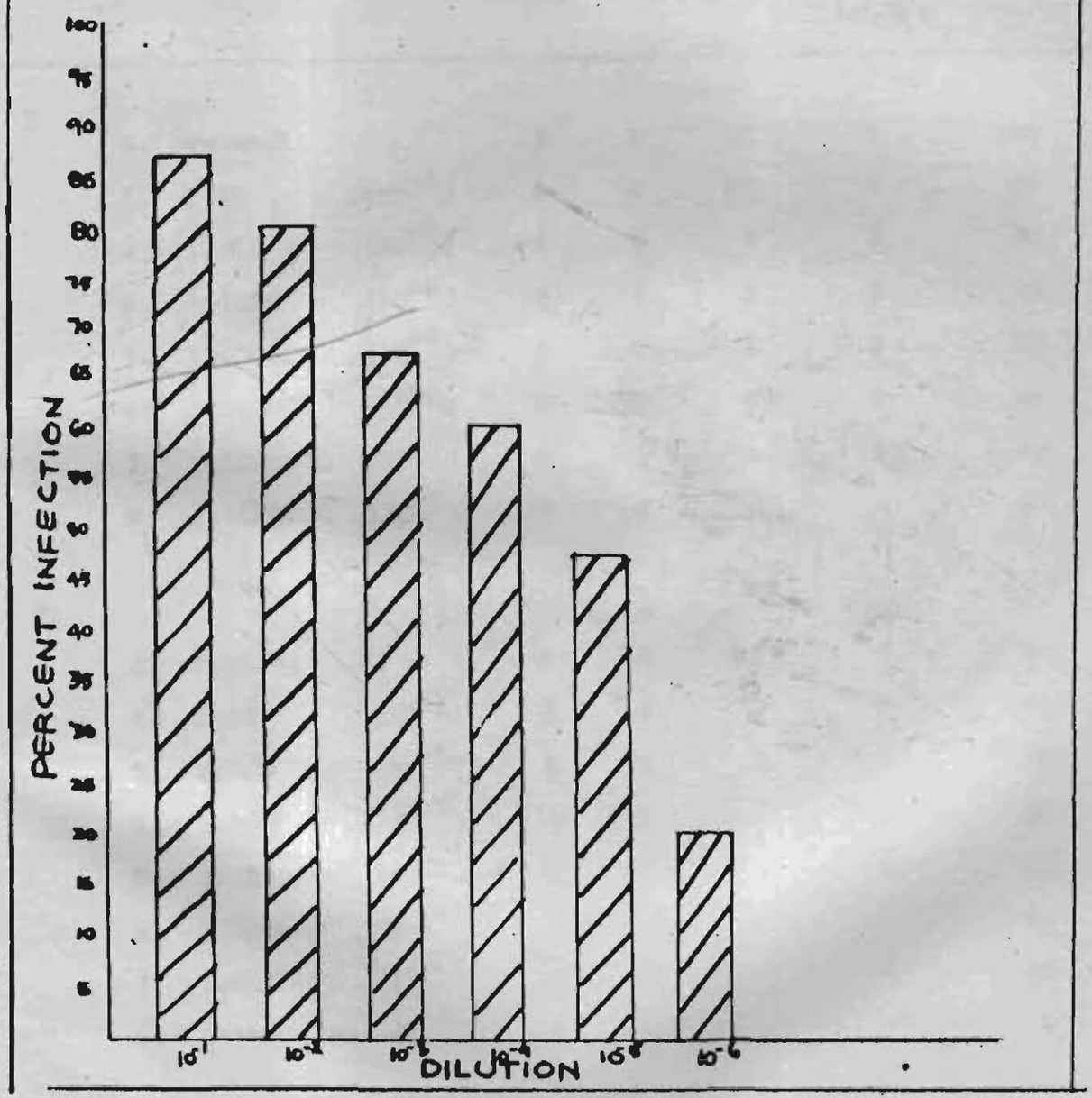


Table 13. Determination of dilution end point of chilli mosaic virus assayed on (Chenopodium amaranticolor)

Test No.	S. No.	Dilutions	No.of plants inocu-lated	No. in-fec-ted	No. lesions per leaf	Mean incu-bation period (days)	Per cent infection
I							
	1.	Control	5	5	10	7	100
	2.	1:10 (10^{-1})	5	4	10	7	80
	3.	1:100 (10^{-2})	5	4	9	8	80
	4.	1:1000 (10^{-3})	5	4	7	8	80
	5.	1:10000 (10^{-4})	5	4	3	8	80
	6.	1:100000 (10^{-5})	5	2	2	8	40
	7.	1:1000000 (10^{-6})	5	1	2	8	20
	8.	1:1000000 (10^{-7})	5	-	-	-	-
II							
	1.	Control	5	4	11	7	80
	2.	1:10 (10^{-1})	5	4	8	7	80
	3.	1:100 (10^{-2})	5	3	7	7	60
	4.	1:1000 (10^{-3})	5	4	4	8	80
	5.	1:10000 (10^{-4})	5	3	3	8	60
	6.	1:100000 (10^{-5})	5	2	2	8	40
	7.	1:1000000 (10^{-6})	5	2	2	8	40
	8.	1:10000000 (10^{-7})	5	-	-	-	-

Table 13 (contd...)

Test No.	S. No.	Dilutions		No.of plants inoculated	No. infected	No. lesions per leaf	Mean incubation period (days)	Per cent infection
III								
	1.	Control	(10^{-7})	5	5	12	8	100
	2.	1:10	(10^{-1})	5	4	10	7	80
	3.	1:100	(10^{-2})	5	4	8	7	80
	4.	1:1000	(10^{-3})	5	9	5	8	60
	5.	1:10000	(10^{-4})	5	33	3	8	60
	6.	1:100000	(10^{-5})	5	3	1	8	60
	7.	1:1000000	(10^{-6})	5	1	1	8	20
	8.	1:10000000	(10^{-7})	5	-	-	-	-

FIG: 4. DILUTION END POINT OF CHILLI MOSAIC VIRUS
(on chenopodium amaranticolor)

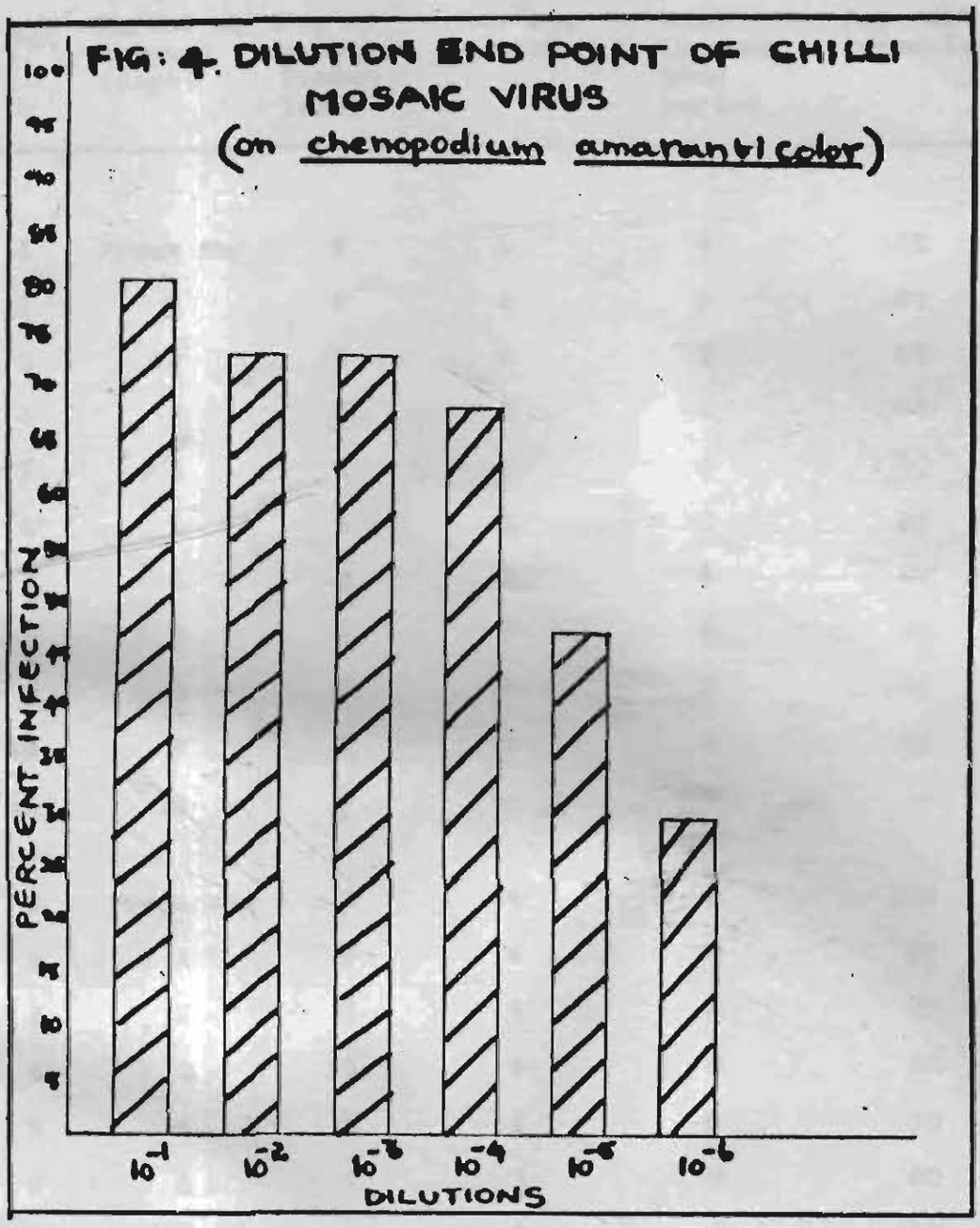


Table 14. Determination of longevity *in vitro* of chilli mosaic virus (assayed on *Nicotiana tabacum* L. at room temperature (23.5 to 28.5°C)

Test No.	S.No.	Period of storage (days)	No. of plants inoculated	No. infected	Mean incubation period	Per cent infection
I						
	1	Fresh sap	5	5	4	100
	2	1	5	4	8	80
	3	2	5	4	8	80
	4	3	5	3	8	60
	5	4	5	4	8	60
	6	5	5	3	8	60
	7	6	5	2	8	40
	8	7	5	2	9	40
	9	8	5	2	9	40
	10	9	5	2	9	40
	11	10	5	-	-	-
II						
	1	Fresh sap	5	4	8	100
	2	1	5	4	8	80
	3	2	5	4	8	80
	4	3	5	4	9	80
	5	4	5	4	9	80
	6	5	5	3	9	60
	7	6	5	3	9	60

Table 14 (contd...)

Test No.	S.No.	Period of storage (days)	No. of plants inocu-lated	No. in-fected	Mean incuba-tion period	Per cent infection
	8	7	5	3	8	60
	9	8	5	3	9	60
	10	9	5	2	9	10
	11	10	5	-	-	-
III						
	1	Fresh sap	5	4	7	80
	2	1	5	4	8	80
	3	2	5	4	8	80
	4	3	5	4	8	80
	5	4	5	3	8	60
	6	5	5	4	8	80
	7	6	5	3	9	60
	8	7	5	3	9	60
	9	8	5	2	9	40
	10	9	5	2	9	10
	11	10	5	-	-	-

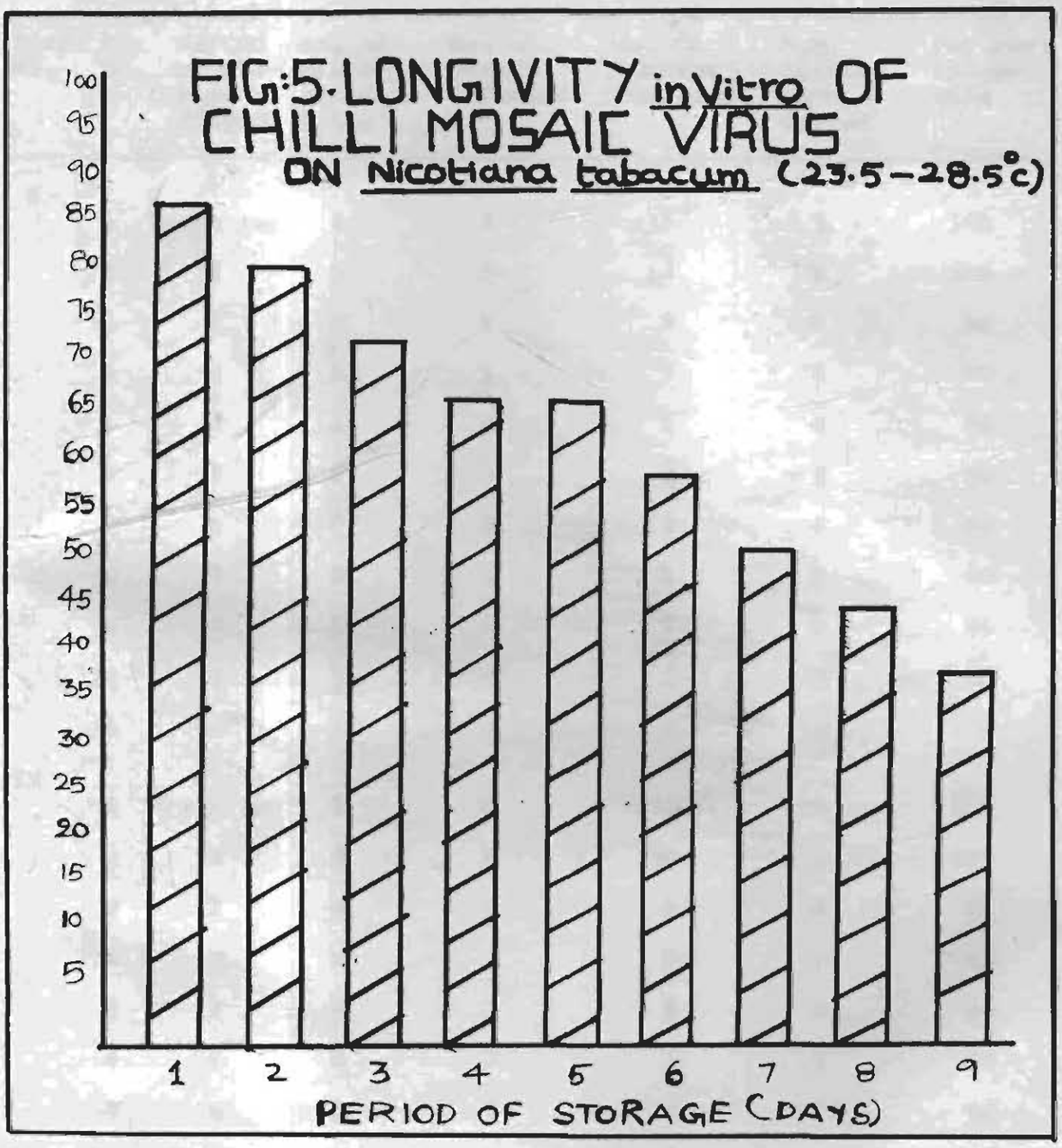


FIG:5.LONGIVITY in Vitro OF CHILLI MOSAIC VIRUS
ON Nicotiana tabacum (23.5–28.5°c)
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
1
2
3
4
5
6
7
8
9
PERIOD OF STORAGE (DAYS)

Table 15. Longevity of the virus *in vitro* (assayed on *Chenopodium amaranticolor* at room temperature (23.5-28.5°C)

Test No.	S. No.	Period of storage (days)	No. of plants inoculated	No. of plants infected	No. of lesions per leaf	Mean infubation period	Per cent infection
I							
	1	Fresh sap	5	4	16	7	100
	2	1	5	5	14	8	100
	3	2	5	5	9	8	80
	4	3	5	4	7	8	80
	5	4	5	4	4	8	80
	6	5	5	4	2	8	80
	7	6	5	3	3	8	60
	8	7	5	3	2	8	60
	9	8	5	2	2	9	40
	10	9	5	2	1	9	40
	11	10	5	-	-	-	-
II							
	1	Fresh sap	5	5	13	8	100
	2	1	5	5	10	8	100
	3	2	5	4	8	8	80
	4	3	5	4	6	8	80
	5	4	5	3	5	8	60
	6	5	5	3	3	8	60
	7	6	5	3	3	8	60
	8	7	5	3	3	8	60
	9	8	5	5	3	9	60
	10	9	5	2	1	9	40
	11	10	5	-	-	-	-

Table 15 (contd...)

Test No.	S. No.	Period of storage (days)	No. of plants inoculated	No. of plants infected	No. of lesions per leaf	Mean incubation period	Per cent infection
III							
	1	Fresh sap	5	4	15	7	80
	2	1	5	4	11	7	80
	3	2	5	4	8	8	80
	4	3	5	4	5	8	80
	5	4	5	3	4	8	60
	6	5	5	3	3	8	60
	7	6	5	3	2	8	60
	8	7	5	2	2	7	40
	9	8	5	2	2	10	40
	10	9	5	1	1	10	20
	11	10	5	-	-	-	-

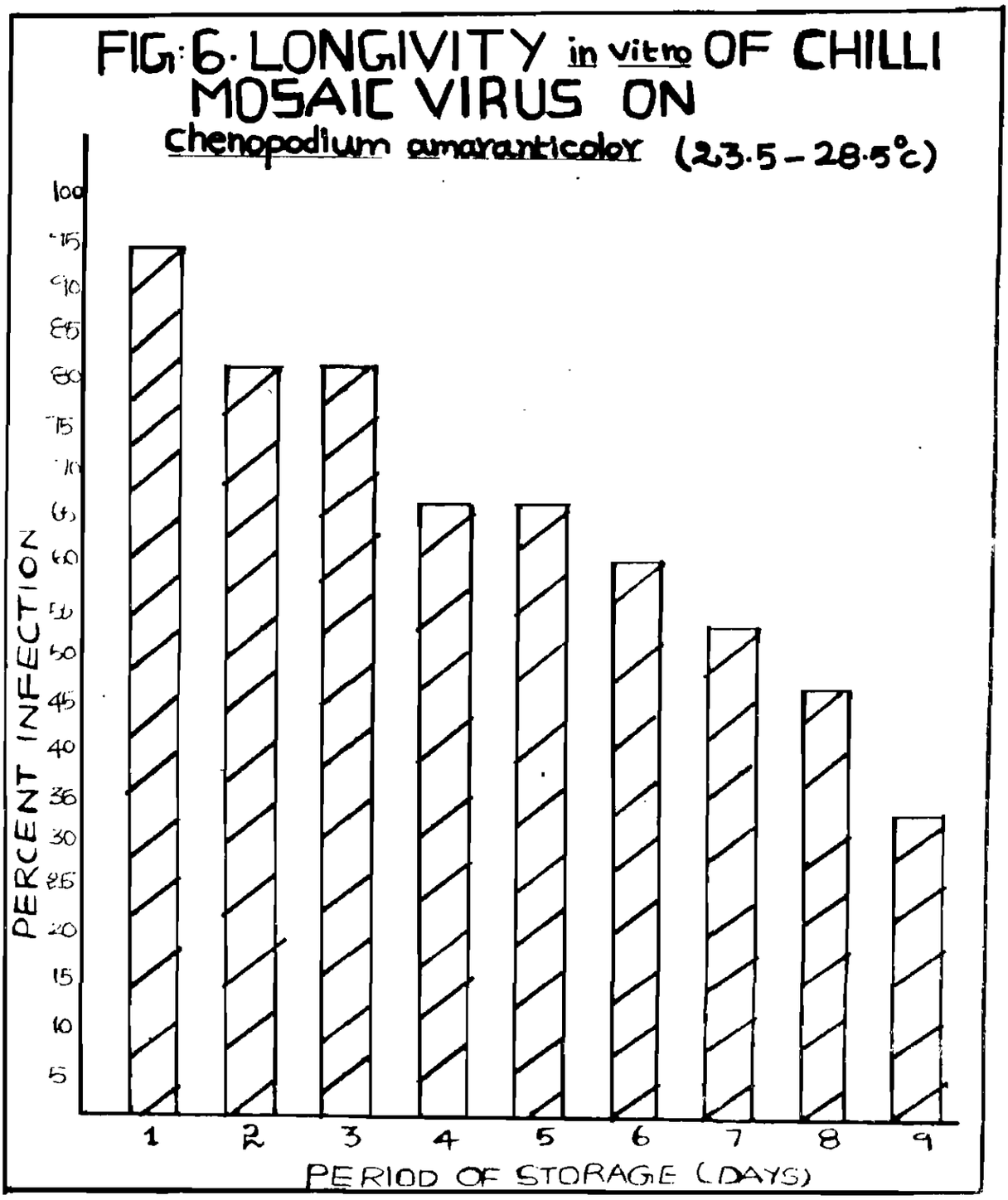


FIG: 6. LONGIVITY in vitro OF CHILLI MOSAIC VIRUS ON
Chenopodium amaranticolor (23.5 - 28.5°c)
PERCENT INFECTION
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
1
2
3
4
5
6
7
8
9
PERIOD OF STORAGE (DAYS)

Table 16. Determination of longevity *in vitro* of chilli mosaic virus (assayed on *Nicotiana tabacum* at 10°C)

Test No.	S.No.	Period of storage (days)	No. of plants inoculated	No. of plants infected	Mean incubation period	Per cent infection
I						
	1	Fresh sap	5	5	8	100
	2	1	5	5	8	100
	3	2	5	5	8	100
	4	3	5	4	8	80
	5	4	5	4	8	80
	6	5	5	4	7	80
	7	6	5	4	8	80
	8	7	5	4	8	80
	9	8	5	4	7	80
	10	9	5	4	7	80
	11	10	5	3	8	60
	12	11	5	3	8	60
	13	12	5	2	8	40
	14	13	5	3	9	60
	15	14	5	3	9	60
	16	15	5	3	9	60
	17	16	5	2	8	40
	18	17	5	2	9	40
	19	18	5	1	9	20
	20	19	5	-	-	-
	21	20	5	-	-	-

Table 16 (contd...)

Test No.	S.No.	Period of storage (days)	No. of plants inoculated	No. of plants infected	Mean incubation period	Per cent infection
II						
	1	Fresh sap	5	5	8	100
	2	1	5	5	8	100
	3	2	5	5	8	100
	4	3	5	4	8	80
	5	4	5	3	8	60
	6	5	5	4	8	80
	7	6	5	4	8	80
	8	7	5	3	8	60
	9	8	5	4	8	80
	10	9	5	3	8	60
	11	10	5	3	9	60
	12	11	5	4	9	80
	13	12	5	4	8	80
	14	13	5	3	9	60
	15	14	5	3	9	60
	16	15	5	2	9	40
	17	16	5	2	10	40
	18	17	5	2	10	40
	19	18	5	-	-	-
	20	19	5	-	-	-
	21	20	5	-	-	-

Table 16 (contd...)

Test No.	S.No.	Period of storage (days)	No. of plants inoculated	No. of plants infected	Mean incubation period	Per cent infection
III						
	1	Fresh sap	5	5	8	100
	2	1	5	5	8	100
	3	2	5	4	8	80
	4	3	5	4	7	80
	5	4	5	4	8	80
	6	5	5	4	8	80
	7	6	5	4	8	80
	8	7	5	5	8	100
	9	8	5	4	9	80
	10	9	5	3	9	60
	11	10	5	3	9	60
	12	11	5	3	9	60
	13	12	5	3	9	60
	14	13	5	3	10	60
	15	14	5	3	10	60
	16	15	5	3	9	60
	17	16	5	2	9	40
	18	17	5	1	10	20
	19	18	5	-	-	-
	20	19	5	-	-	-
	21	20	5	-	-	-

FIG:7. LONGIVITY in Vitro OF CHILLI MOSAIC VIRUS ON Nicotiana tabacum (10°c)

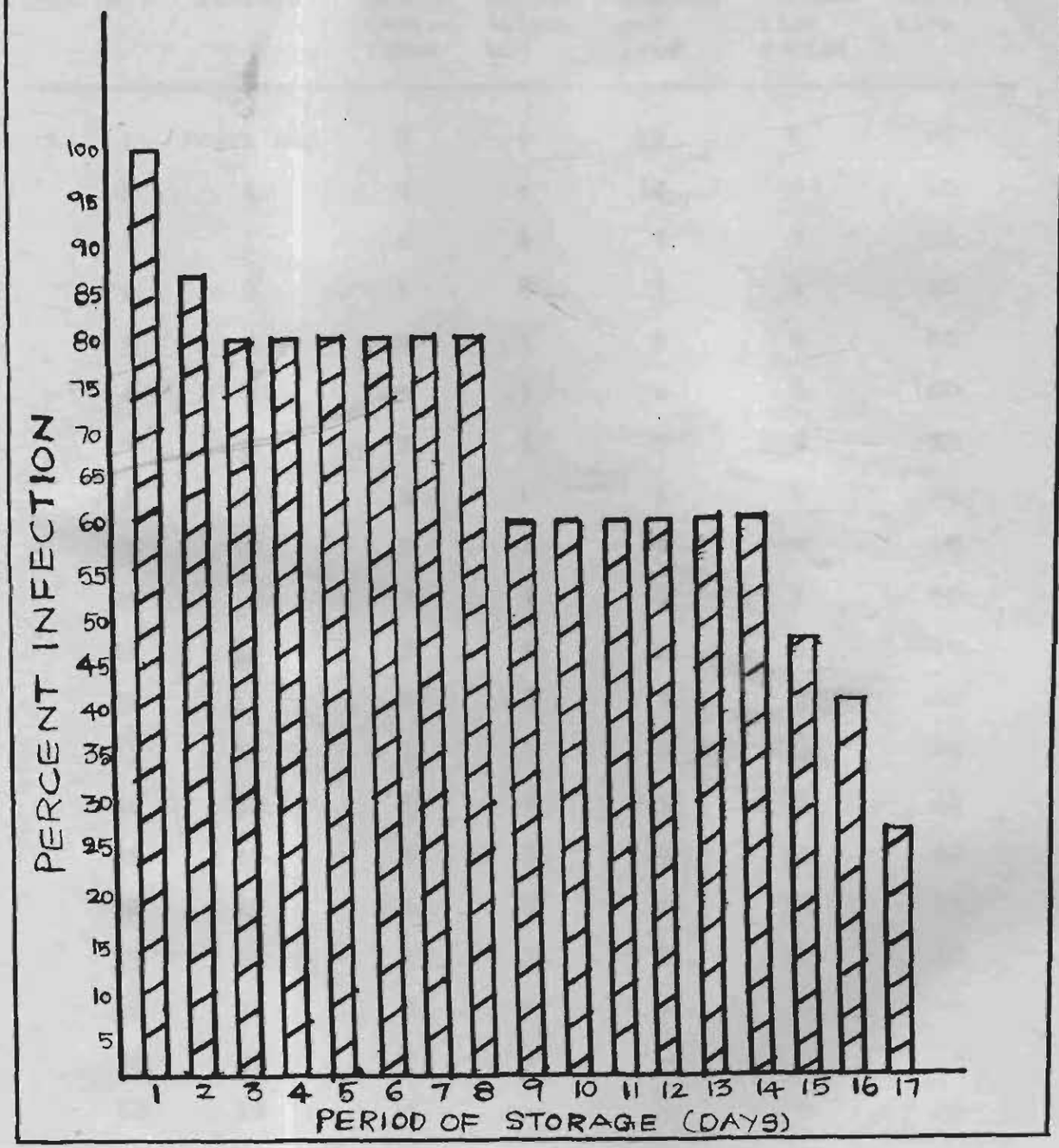


Table 17. Determination of longevity *in vitro* of chilli mosaic virus (assayed on (*Chenopodium amaranticolor*) at 10°C)

Test No.	S. No.	Period of storage	No. of plants inoculated	No. of plants infected	No. of lesions per leaf	Mean incubation period	Per cent infection
I	1	Fresh sap	5	4	13	8	80
	2	1	5	4	10	8	80
	3	2	5	4	9	7	80
	4	3	5	4	7	8	80
	5	4	5	3	6	8	60
	6	5	5	3	5	8	60
	7	6	5	3	5	8	60
	8	7	5	4	5	8	80
	9	8	5	3	4	8	60
	10	9	5	3	3	8	60
	11	10	5	3	3	8	60
	12	11	5	3	3	9	60
	13	12	5	3	3	9	60
	14	13	5	2	3	9	40
	15	14	5	2	2	10	40
	16	15	5	2	2	10	40
	17	16	5	2	2	10	40
	18	17	5	-	-	-	-
	19	18	5	-	-	-	-
	20	19	5	-	-	-	-
	21	20	5	-	-	-	-

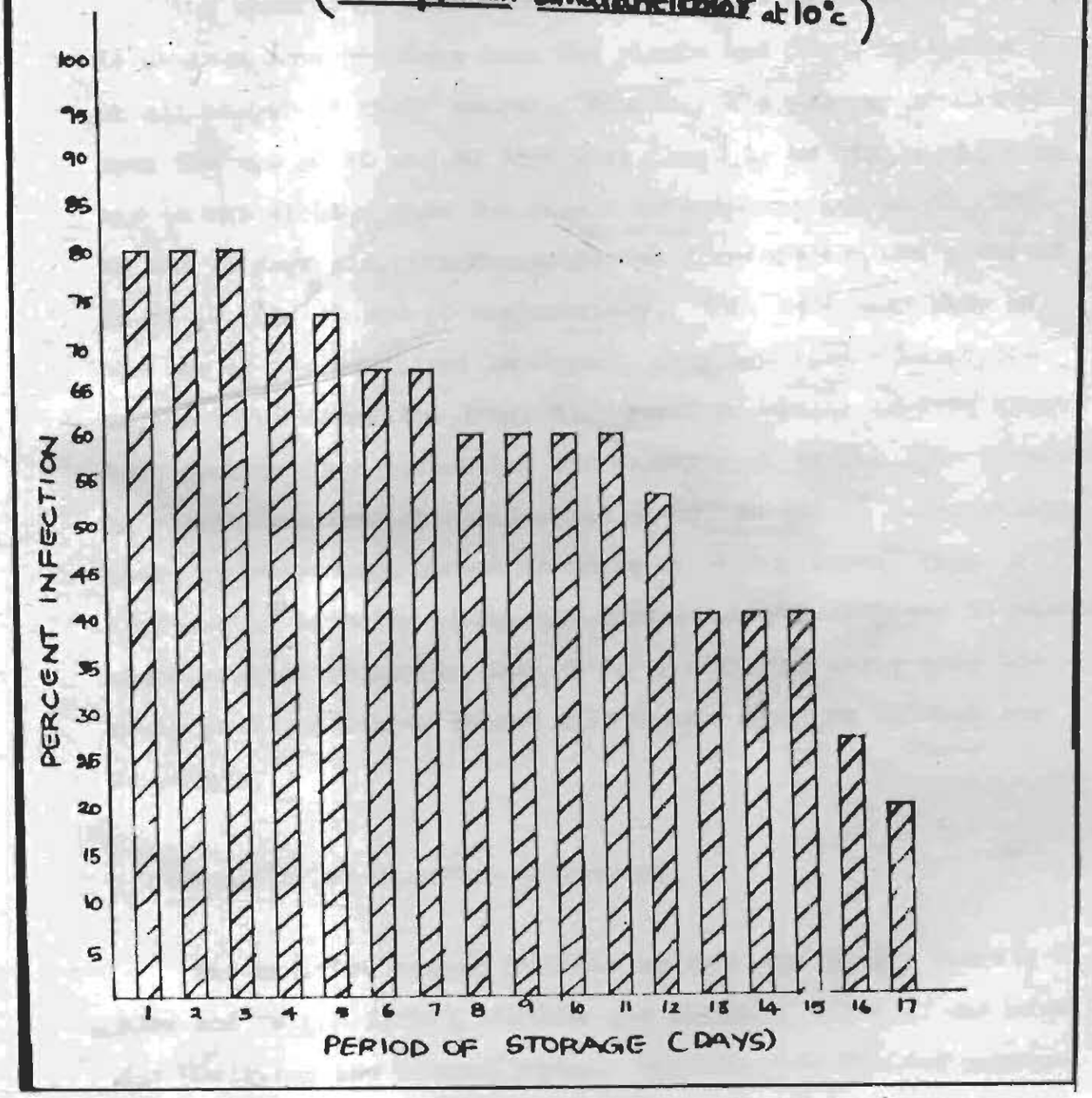
Table 17 (contd...)

Test No.	S. No.	Period of storage	No.of plants inocu-lated	No.of plants infect-ed	No. of lesions per leaf	Mean incuba-tion period	Per cent infec-tion
XI	1	Fresh sap	5	5	16	7	100
	2	1	5	4	13	8	80
	3	2	5	4	11	8	80
	4	3	5	4	8	8	80
	5	4	5	4	7	8	80
	6	5	5	4	6	8	80
	7	6	5	3	7	8	60
	8	7	5	3	5	9	60
	9	8	5	3	5	9	60
	10	9	5	3	4	9	60
	11	10	5	3	4	9	60
	12	11	5	3	3	9	60
	13	12	5	3	3	9	60
	14	13	5	2	3	10	40
	15	14	5	2	2	10	40
	16	15	5	2	2	10	40
	17	16	5	1	2	10	20
	18	17	5	-	-	-	-
	19	18	5	-	-	-	-
	20	19	5	-	-	-	-
	21	20	5	-	-	-	-

Table 17 (contd...)

Test No.	S. No.	Period of storage	No. of plants inocu-lated	No. of plants infected	No.of lesions per leaf	Mean incuba-tion period	Per cent infec-tion
III	1	Fresh sap	5	5	17	7	100
	2	1	5	4	14	7	80
	3	2	5	4	12	8	80
	4	3	5	4	10	8	80
	5	4	5	4	9	8	80
	6	5	5	4	9	8	80
	7	6	5	4	7	8	80
	8	7	5	3	5	8	60
	9	8	5	3	4	8	60
	10	9	5	3	4	8	60
	11	10	5	3	3	9	60
	12	11	5	3	3	9	60
	13	12	5	2	4	9	40
	14	13	5	2	3	10	40
	15	14	5	2	3	10	40
	16	15	5	2	2	10	40
	17	16	5	1	2	10	20
	18	17	5	-	-	-	-
	19	18	5	-	-	-	-
	20	19	5	-	-	-	-
	21	20	5	-	-	-	-

FIG: 8. LONGIEVITY *in vitro* OF CHILLI MOSAIC VIRUS (*chenopodium amaranticolor* at 10°c)



AGE OF CHILLI PLANTS AND ITS SUSCEPTIBILITY TO CHILLI MOSAIC VIRUS

The results of this study are presented in Table 18. It is obvious from the data that the plants had taken infection at all stages of their growth. However, the younger seedlings upto the age of 20 and 30 days were found to be highly vulnerable to the virus. When the plants were inoculated at 40, 50, 60 and 70 days old, the percentage of transmission was observed to be 45, 25, 15 and 10 respectively. This indicated that as the age of the seedlings increased, they are less susceptible to the virus infection (Fig. 9). Further, severe disease symptoms such as leaf distortion and stunting of plants were observed in plants infected at earlier age of 20, 30 and 40 days, while these symptoms were absent in those of higher ages. Thus, a decrease in severity of symptom expression was observed in plants when infected at higher ages of 60 and 70 days where only the newly produced leaves showed mild mosaic symptoms without any stunting.

PURIFICATION AND ELECTRON MICROSCOPY

The modified method followed by Hollings et al. (1968); Lima and Nelson (1975), Fischer and Lockhart (1976); was used for purifying the present virus. Purification yielded concentrated preparations of the virus which was used for electron-microscopy. Electron microscopy revealed the presence of virus particles of typical rigid rods, measuring 300 x 18 nm (Plate 7).

Table 18. Age of chilli plants and its susceptibility to chilli mosaic virus

S. No.	Age (days)	Test I	Test II	Mean incubation period		Mean per cent infection	Symptoms
				Test I	Test II		
1.	80	8/10	8/10	8	7	80	VC,SM,St,Ld
2.	30	8/10	8/10	8	8	80	SM,Ld, St
3.	40	4/10	5/10	8	8	45	SM,Ld,St
4.	50	3/10	2/10	9	10	25	MM
5.	60	1/10	2/10	9	9	15	MM
6.	70	1/10	1/10	11	10	10	MM

MM : Mild Mosaic
SM : Severe Mosaic
St : Stunting
Ld : Leaf distortion
VC : Vein Clearing

FIG: 9 AGE OF CHILLI PLANTS IN RELATION TO SUSCEPTIBLITY TO CHILLI MOSAIC VIRUS

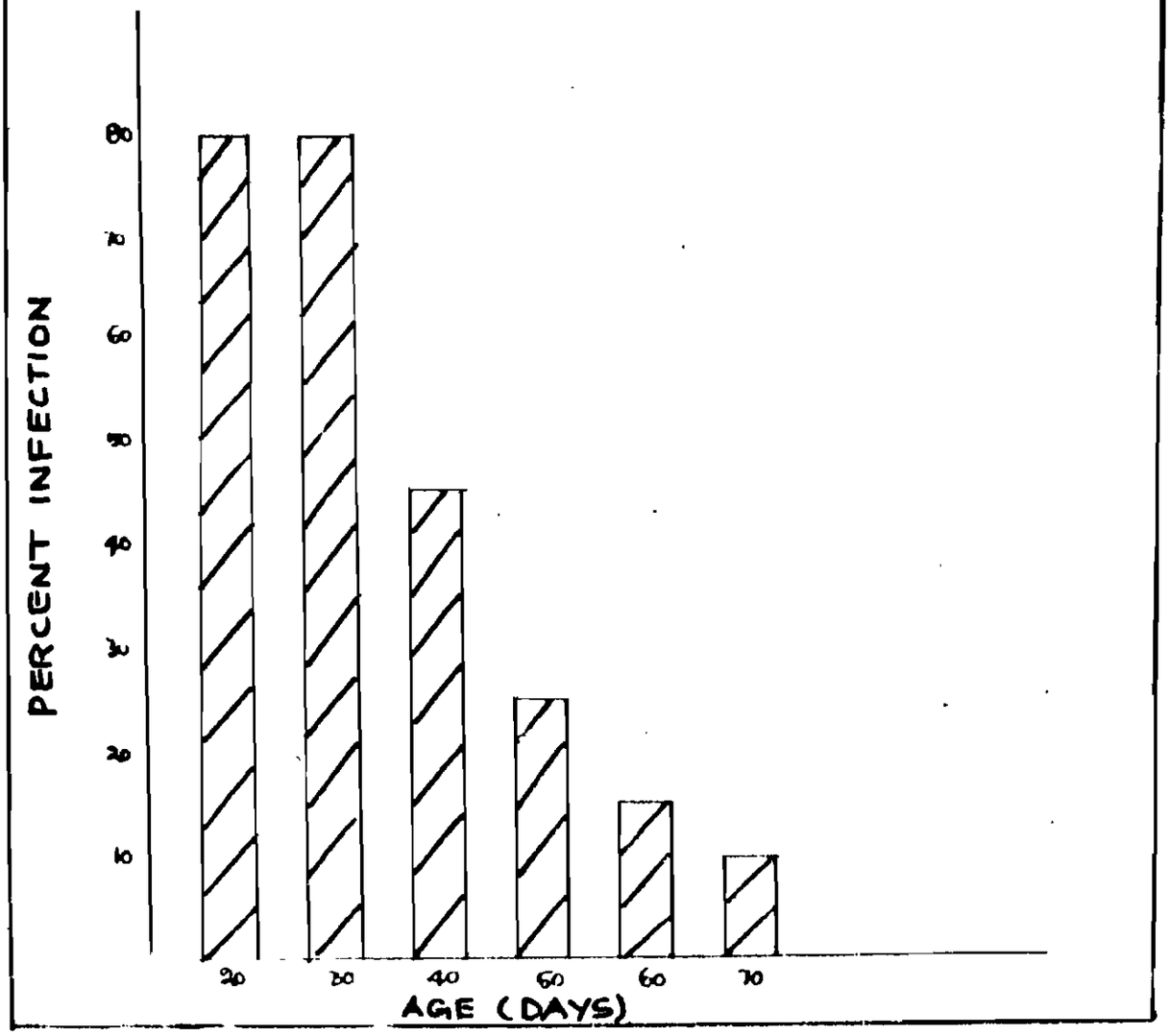


PLATE 7A. Electron micrograph of partially purified preparation of mosaic virus (on chilli) showing rigid rods (50,000 magnification) (300 x 18 nm)

B. Electron micrograph of partially purified preparation of mosaic virus (on chilli) showing rigid rods (70,000 magnification) (300 x 18 nm)

PLATE 7A

PLATE 7B

EFFECT OF DISEASE ON GROWTH CHARACTERS

To determine the effect of virus infection on certain growth characters, viz., root length, shoot length, leaf area, fresh weight and dry weight, five experiments were conducted. For this purpose a set of chilli plants of variety X-230 were grown and 30 days old plants were inoculated on the same day. A similar set of plants, uninoculated, served as control.

To know the effect of disease on root length, the length of the main root was measured by means of a metre scale and readings were recorded in a table for comparison. From the data (Table 19) significant reduction in root length was observed due to infection of the virus. The per cent reduction in root length varied from 22.72 to 60.22 (Fig. 10).

In case of shoot length, perpendicular length of the plant from ground level to the tip was taken from both the healthy and diseased plants. The results (Table 20) indicated that shoot length of the virus affected plants was considerably reduced (Fig. 11). The percent reduction varied from 1.15 to 15.15.

Fresh leaves of both healthy and diseased plants were kept in a leaf area meter which measured leaf areas. From the results (Table 21) it is evident that leaf area was significantly

reduced in virus affected plants and the percentage of reduction varied from 4.3 to 66.14 (Fig. 12).

Fresh plants of both healthy and mosaic affected plants were weighed in balance for recording fresh weights of the leaves. The percentage of reduction in virus affected plants varied from 28.00 to 68.12 (Table 22 and Fig. 13).

For dry weight, both healthy and diseased plants in paper bags were oven dried at 60°C to a constant weight. There was considerable reduction in dry weight of diseased plants than the healthy plants and reduction ranged from 16.21 to 60.00 per cent (Table 23 and Fig. 14).

EFFECT OF MOSAIC DISEASE ON CHLOROPHYLL FRACTIONS

For this experiment ten plants of chilli (30 days old) were inoculated with the virus and similar number of plants were kept without inoculation to serve as control. 15-20 days after inoculation, the newly expanding leaves of both healthy and diseased plants were detached and analysed for chlorophyll fractions viz., chlorophyll a, chlorophyll b, and total chlorophyll. The results (Table 24) indicated that there was a considerable reduction in chlorophyll fractions. The percentage of reduction ranged from 3 to 26, 0.3 to 9.5 and 0.4 to 25.3 in case of chlorophyll a, chlorophyll b and total chlorophyll, respectively.

Table 19. Effect of mosaic on root length

S.No.	Root length (cm)		Per cent decrease over healthy
	Healthy	Diseased	
1.	43	28.0	34.88
2.	44	17.5	60.22
3.	29	12.0	58.62
4.	31	17.5	43.54
5.	33	16.0	51.51
6.	37	17.0	54.05
7.	26	19.0	26.92
8.	29	13.0	55.17
9.	37	27.0	27.02
10.	35	24.0	31.42
11.	30	22.0	26.67
12.	28	18.0	35.71
13.	33	25.5	22.72
14.	34	23.0	32.55
15.	37	26.5	28.37
Mean	33.73	20.40	39.50
t value : 5.28			

Significant at 5% and 1% level of probability

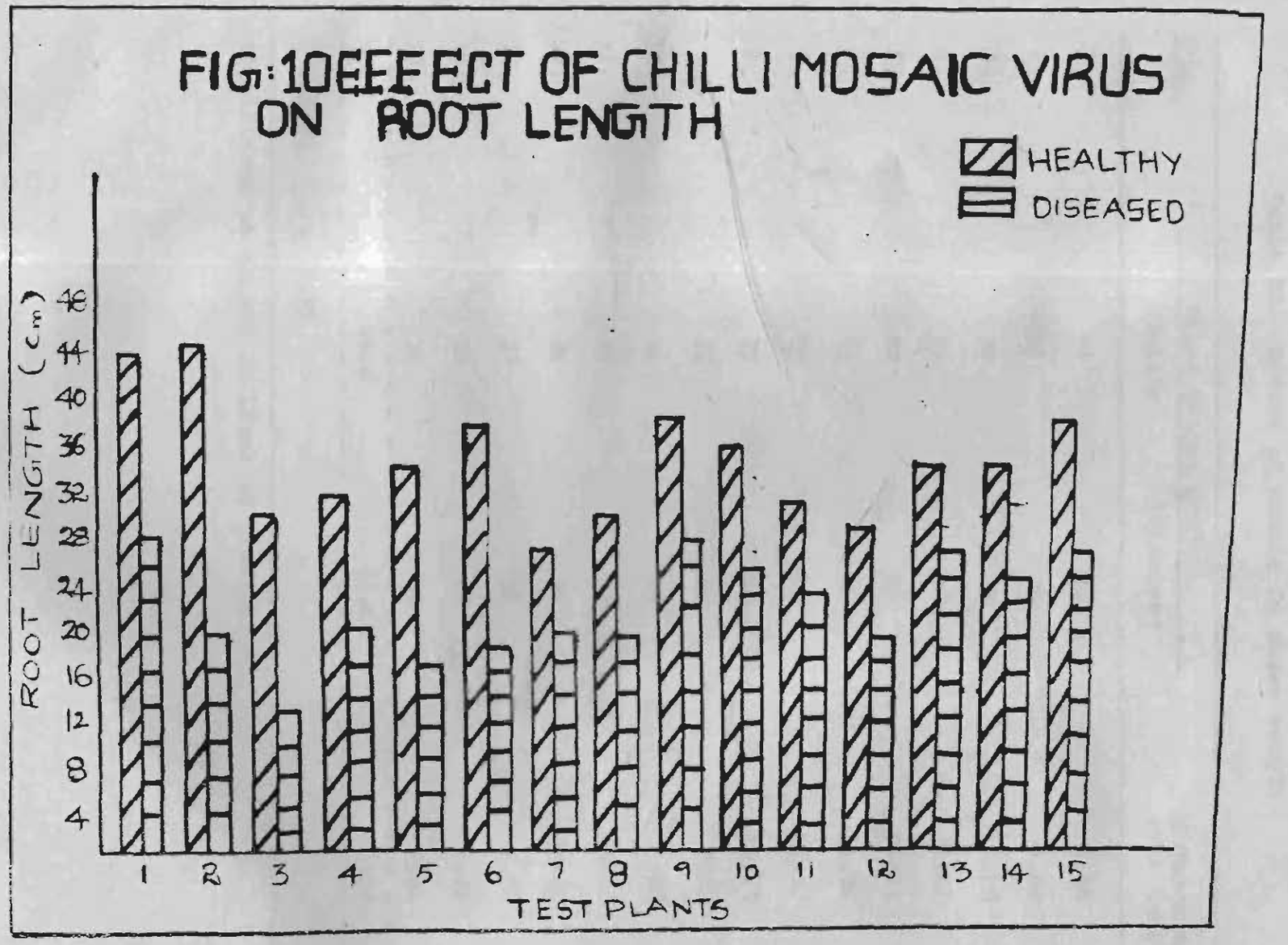


FIG:10EFFECT OF CHILLI MOSAIC VIRUS ON ROOT LENGTH
HEALTHY
DISEASED
ROOT LENGTH (cm)
4
8
12
16
20
24
28
32
36
40
44
48
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TEST PLANTS

Table 20. Effect of mosaic on shoot length

S.No.	Plant height (cm)		% decrease over healthy
	Healthy	Diseased	
1.	44	42	4.60
2.	32	30	6.60
3.	33	29	12.20
4.	34	31	8.82
5.	33	28	15.15
6.	34	30	11.76
7.	30	26	13.33
8.	35	28	10.71
9.	34	32	5.88
10.	32	30	6.60
11.	36	33	8.35
12.	35	34	2.08
13.	37	32	13.70
14.	36	35	1.15
15.	38	36	5.26
Mean	34.4	31.53	8.34
t value : 1.97			

Significant at 5% and 1% level of probability

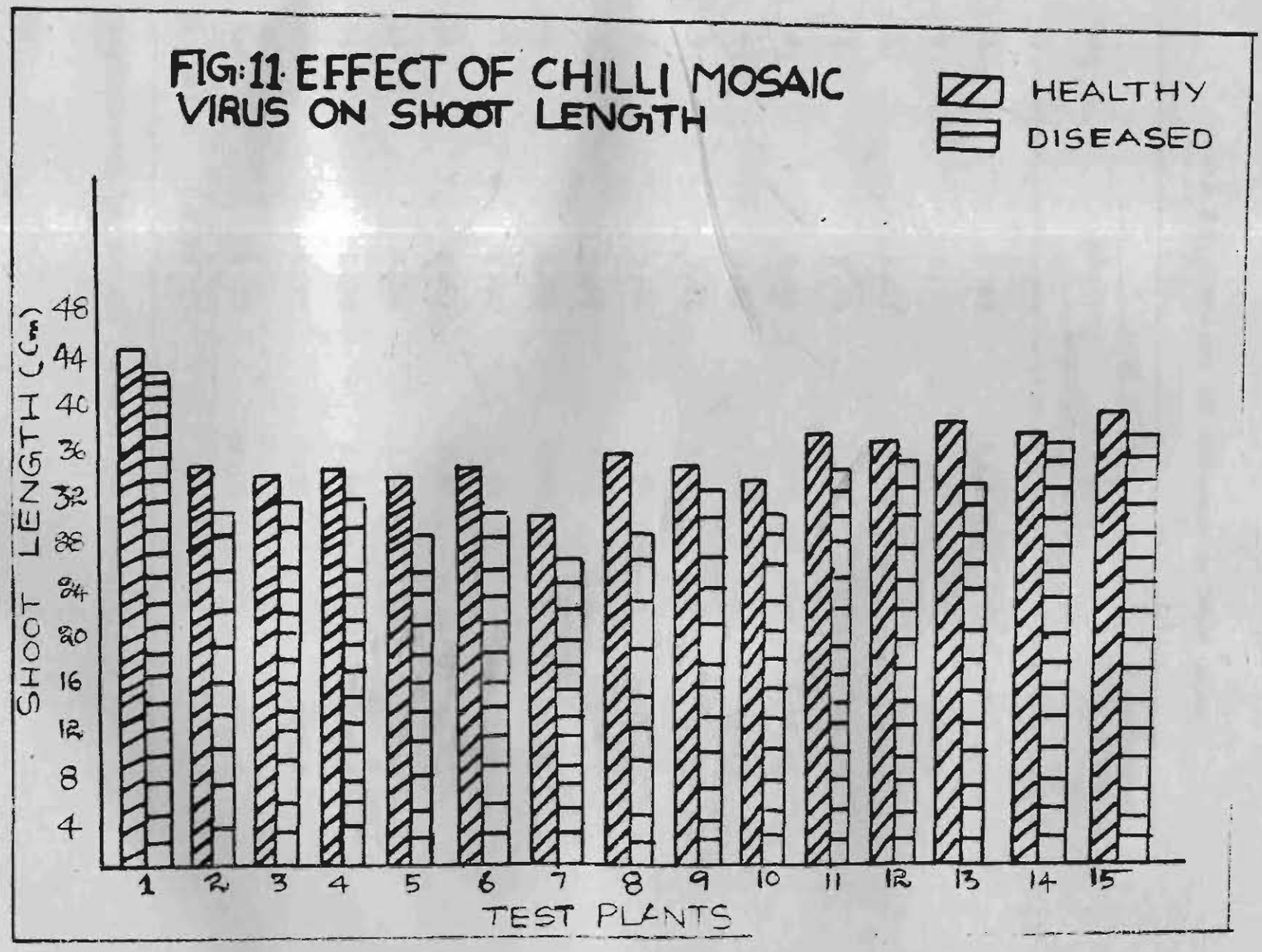


FIG:11 EFFECT OF CHILLI MOSAIC VIRUS ON SHOOT LENGTH
HEALTHY
DISEASED
SHOOT LENGTH (cm)
48
44
40
36
32
28
24
20
16
12
8
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TEST PLANTS

Table 21. Effect of disease on leaf area

S. No.	Leaf area (sq.cm)		% decrease over control
	Healthy	Diseased	
1.	8.07	5.25	24.94
2.	7.19	4.88	32.12
3.	5.55	4.14	25.38
4.	5.10	2.48	51.37
5.	4.46	3.76	46.05
6.	3.85	2.84	26.23
7.	3.49	3.34	4.30
8.	5.54	3.56	35.74
9.	6.97	2.36	66.14
10.	5.05	2.76	45.34
11.	4.90	3.45	28.57
12.	5.53	2.98	46.11
13.	7.50	4.56	59.20
14.	6.20	4.89	21.12
15.	6.97	3.76	46.05
Mean	86.37	55.01	55.86
t value	: 4.25		

Significant at 5% and 1% level of probability

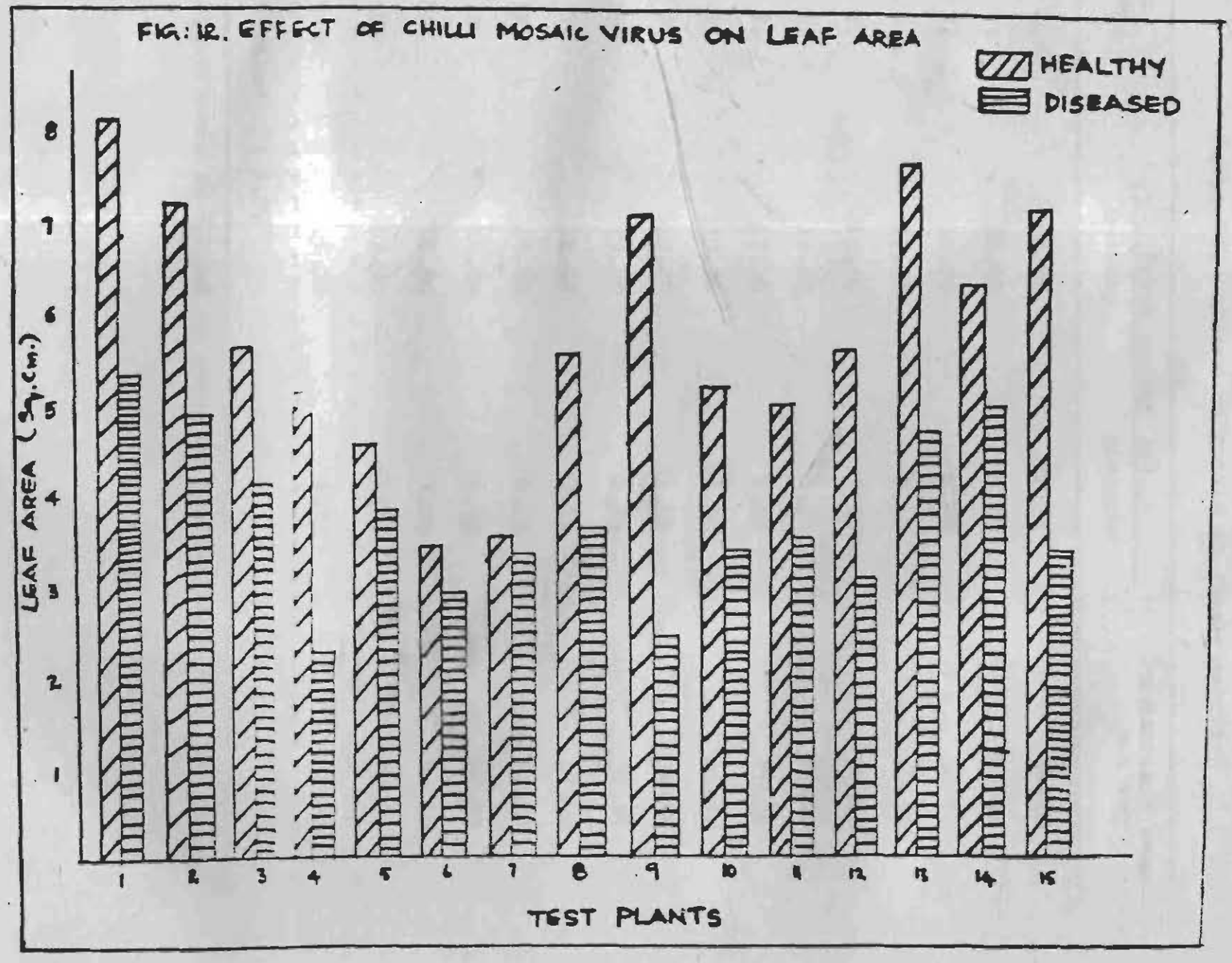


FIG: 12. EFFECT OF CHILLI MOSAIC VIRUS ON LEAF AREA
HEALTHY
DISEASED
LEAF AREA (Sq. Cm.)
0
1
2
3
4
5
6
7
8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
TEST PLANTS

Table 22. Effect of mosaic on fresh weight

S. No.	Fresh weight (g)		% decrease over healthy
	Healthy	Diseased	
1.	20.00	10.78	46.10
2.	18.00	7.80	56.66
3.	10.00	6.93	30.70
4.	16.00	5.10	68.12
5.	10.00	7.20	28.00
6.	14.00	6.52	53.42
7.	17.20	8.50	50.58
8.	19.16	10.50	45.19
9.	18.69	11.25	39.80
10.	13.80	8.56	37.97
11.	15.60	9.75	37.50
12.	19.50	8.00	58.97
13.	18.90	11.00	41.97
14.	15.80	7.90	50.63
15.	19.30	11.20	41.96
Mean	16.39	8.72	45.44

t value : 8.90

Significant at 5% and 1% level of probability

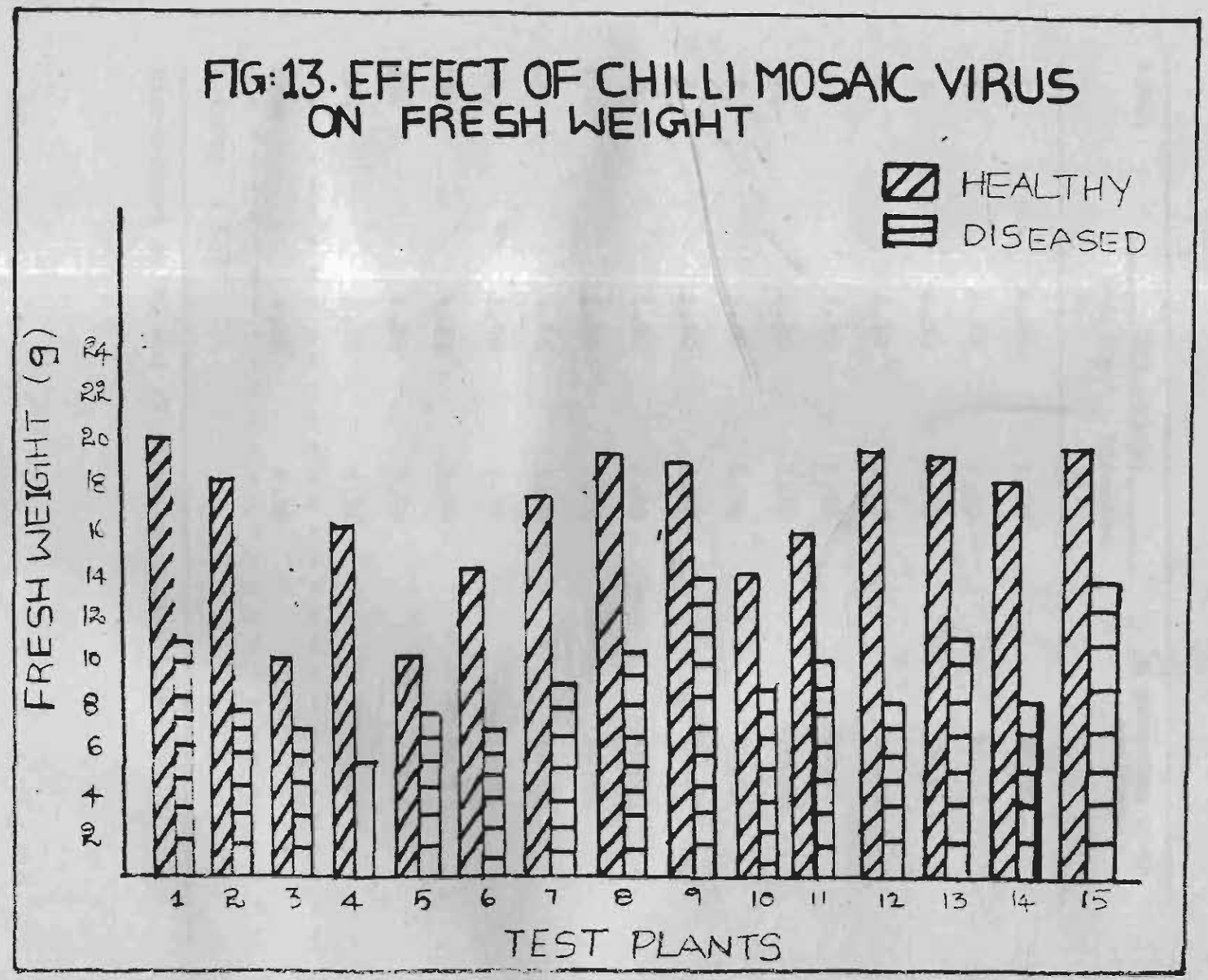


FIG:13. EFFECT OF CHILLI MOSAIC VIRUS ON FRESH WEIGHT
HEALTHY
DISEASED
FRESH WEIGHT (g)
2
4
6
8
10
12
14
16
18
20
22
24
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TEST PLANTS

Table 23. Effect of mosaic on dry weight

S.No.	Dry weight		% decrease over healthy
	Healthy (g)	Diseased (g)	
1.	4.34	2.20	49.30
2.	4.56	3.40	25.43
3.	4.80	2.50	47.91
4.	3.70	3.10	16.21
5.	3.50	2.80	20.00
6.	5.00	2.50	50.00
7.	3.30	2.70	18.18
8.	2.90	1.90	34.48
9.	4.40	2.60	40.90
10.	2.80	1.70	39.28
11.	4.50	1.80	60.00
12.	3.70	1.50	59.48
13.	5.20	2.90	44.23
14.	5.50	3.00	45.45
15.	6.00	4.20	30.00
Mean	4.28	2.58	

t value : 5.7

Significant at 5% and 1% level of probability

FIG:14. EFFECT OF CHILLI MOSAIC VIRUS ON DRY WEIGHT

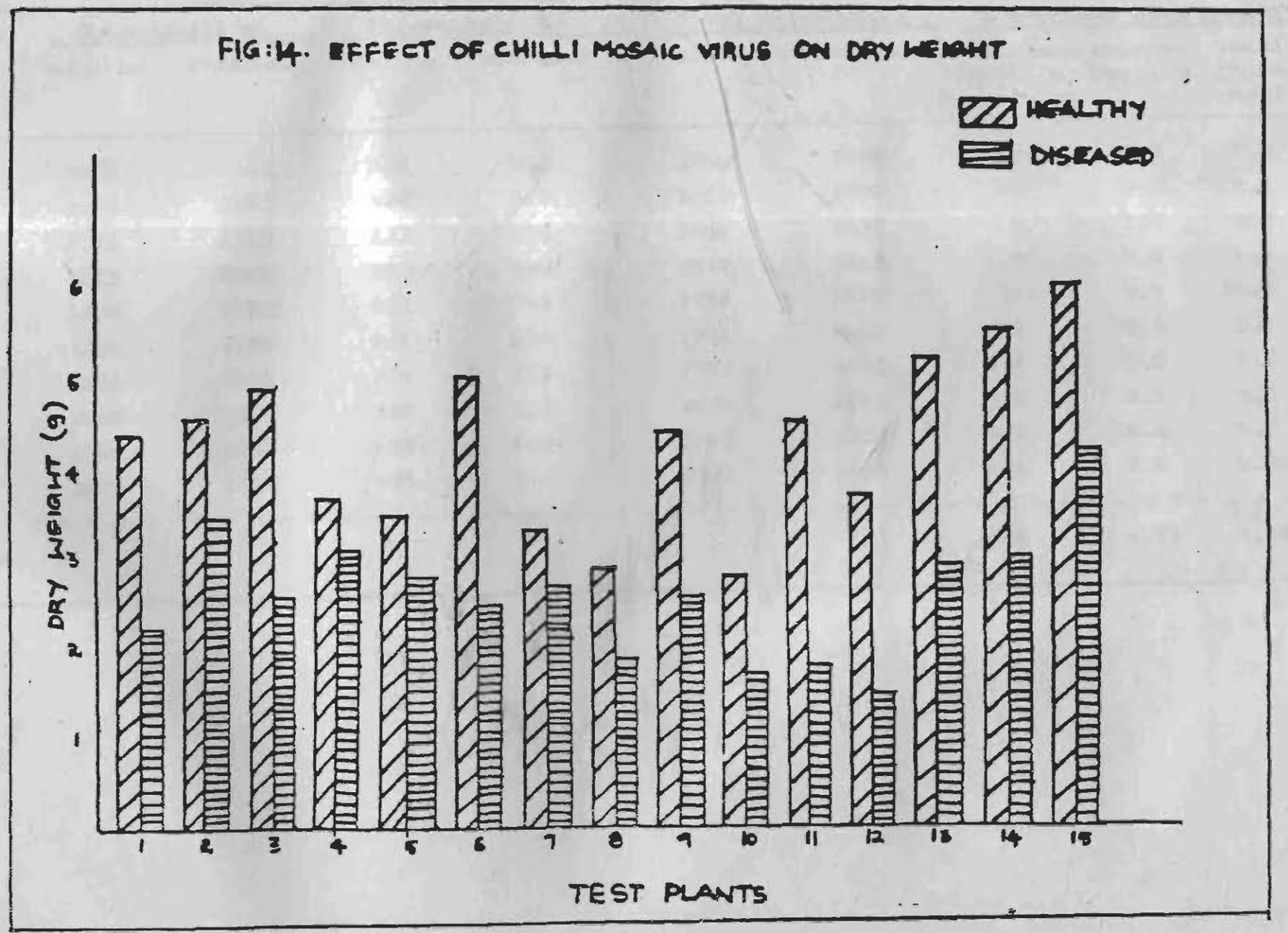


Table 24. Effect of mosaic disease on chlorophyll fraction

S.No.	Chlorophyll a		Chlorophyll b		Total chlorophyll		% decrease over healthy		
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Chloro-phyll a	Chloro-phyll b	Total chloro-phyll
1.	1208	1195	552	550	1760	1745	10	0.3	0.8
2.	1035	1020	584	562	1619	1582	14	3.0	2.2
3.	1125	1115	521	515	1646	1630	8	1.0	0.4
4.	1015	1005	560	548	1575	1553	9	2.0	1.3
5.	1134	1120	621	562	2254	1682	12	9.5	25.3
6.	1156	1125	605	570	1761	1695	26	6.0	3.2
7.	1085	1025	603	586	1783	1681	4.2	8.0	5.7
8.	1058	1018	516	505	1574	1523	3.0	2.1	3.2
9.	1144	1095	639	586	1783	1681	4.2	8.0	5.7
10.	1185	1130	598	554	1783	1684	4.6	7.4	5.55
Mean							9.5	4.73	5.34

REACTION OF VARIETIES OF CHILLI TO THE VIRUS

Twenty varieties of chilli, obtained from Regional Agricultural Research Station, Lam, Guntur were screened for their reaction by sap inoculation. Inoculated plants were observed periodically and symptoms were recorded. The results (Table 25) revealed that all the cultivars under test were susceptible to mosaic. The cultivars X-235, X-180, X-210 showed symptoms like mild mosaic, stunting, vein clearing, blisters and distortion (Plate 8 and 10). The cultivar G3 showed mild mosaic and curling, while the cultivars X-206 Aparna, Paprika-50, Kiran, S 118 and LIC-45 showed mild mosaic and vein clearing (Plate 11). The cultivar G4 showed mild mosaic and distortion (Plate 9), whereas the cultivars 1079 exhibited mild mosaic and stunting and the other varieties cultivars like G5, 1077, 1078, Cluster, Santaka, Warangal and 1081 showed only mild mosaic symptoms.

Table 25. Reaction of varieties of chilli to the virus

Name of the variety	No. infected / No. inoculated	Mean per cent infection	Mean incubation period	Type of reaction
X-235	9/10	90	7	MM, St, VC, Bl, dt.
X-180	7/10	70	8	MM, St, VC, Bl, dt.
X-210	5/10	50	8	MM, dt, St, VC
X-206	3/10	30	7	MM, VC
G3	9/10	70	7	MM, Slight curling
G4	3/10	30	8	MM, dt.
G5	4/10	40	8	MM
1077	3/10	30	8	MM
1078	4/10	40	7	MM
1079	3/10	30	7	MM, St.
Aparna	4/10	40	7	MM, VC
Cluster	6/10	60	8	MM
Santaka	5/10	50	7	MM
Paprika-50	6/10	60	8	MM, VC
Warangal	3/10	30	8	MM
Sindhur	3/10	30	8	MM
Kiran	4/10	40	8	MM, VC
S 118	8/10	80	8	MM, VC
WC-45	5/10	50	8	MM, VC
1081	2/10	20	8	MM

MM : Mild mosaic
St : Stunting
VC : Vein clearing
Bl : Blistering
dt : distortion

PLATE 8. The Cv. X-235 showing mosaic symptoms and stunted growth

H : Healthy

PLATE 8

PLATE 9A. Chilli cultivar C_4 showing mild mosaic symptoms

B. Leaves showing mild mosaic symptom
H : Healthy
I : Infested

PLATE 9A

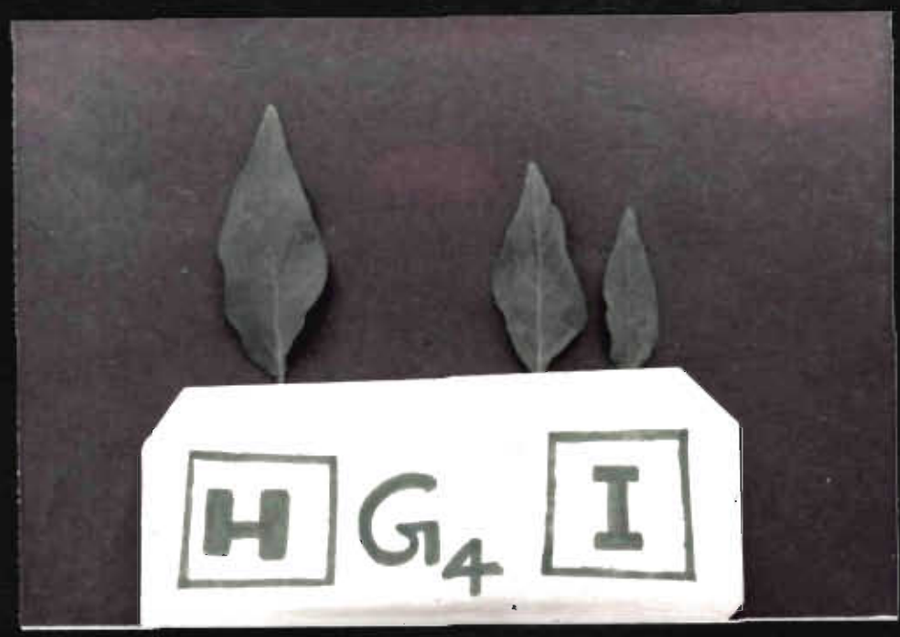
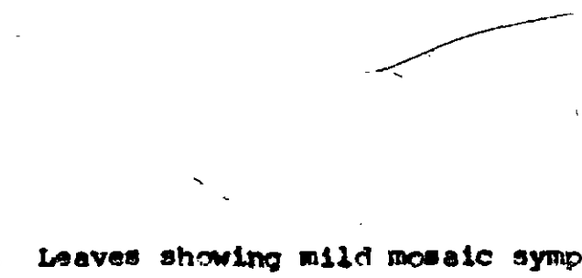


PLATE 9B

PLATE 10A. Chilli cultivar X-180 showing mild mosaic symptoms



B. Leaves showing mild mosaic symptoms

H : Healthy

I : Infected

PLATE 10A

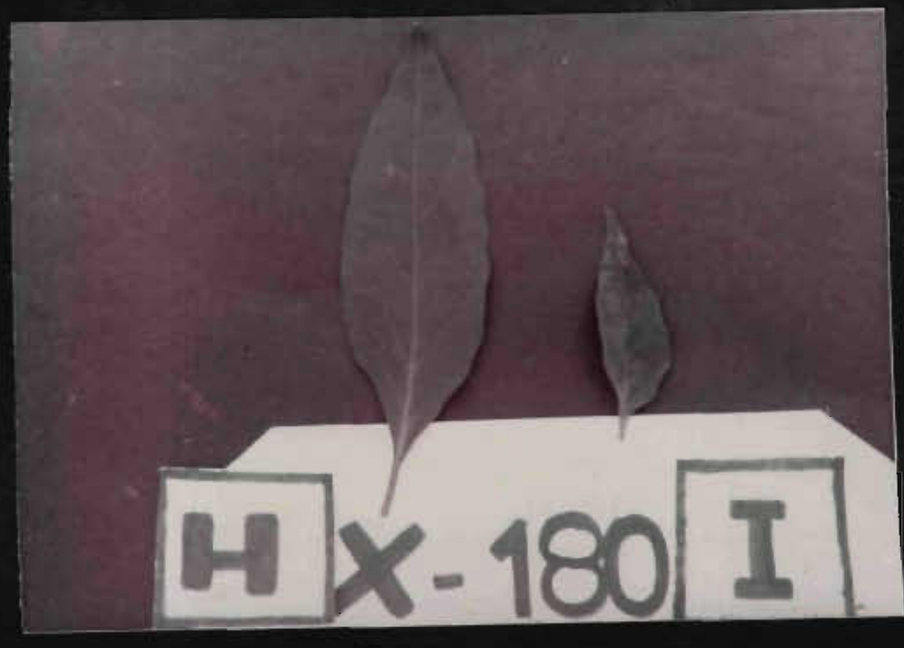


PLATE 10B

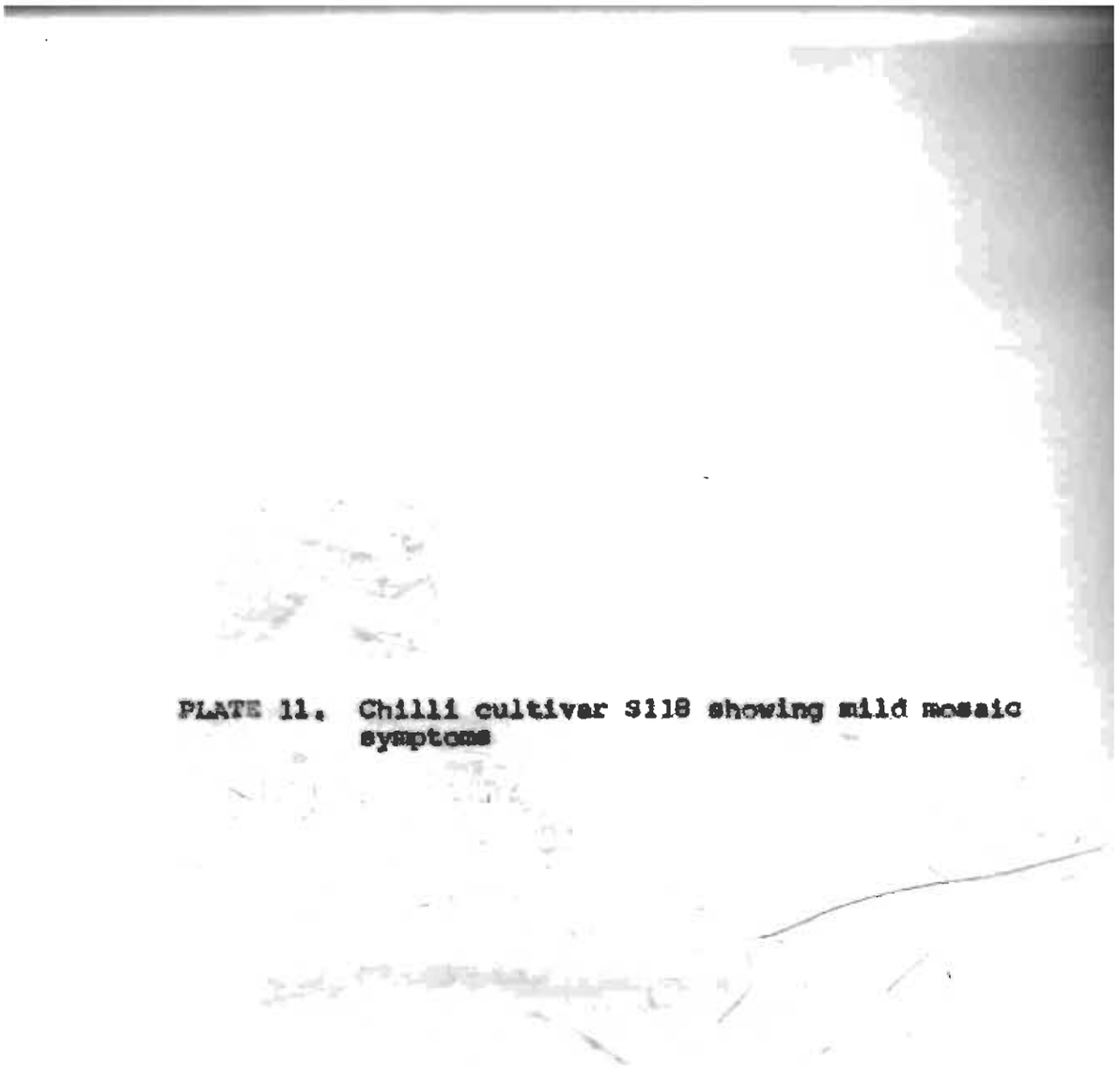


PLATE 11. Chilli cultivar S118 showing mild mosaic symptoms

PLATE 11

CHAPTER V

DISCUSSION

Investigations on chilli mosaic disease reported herein include survey, collection and maintenance of virus cultures, symptomatology, modes of transmission, host-range, varietal screening, purification and electron microscopy and effect of disease on growth characters like root length, shoot length, leaf area, fresh weight, dry weight and chlorophyll fractions.

Survey for occurrence of chilli mosaic virus under field conditions revealed that the percentage of incidence ranged from 5.21 to 16.54 in Chittoor district and from 12.52 to 20.02 in Guntur and Prakasam districts (Table 5).

The incidence of mosaic varied from 0.00 to 85.8 per cent in S.V.Agricultural College Orchard, Tirupati (Table 6). Out of 61 cultivars in different trials, only 10 cultivars viz., OT-3, S 118 x (G_4 x MP), long pod x small pod, G_4 x 960, K_2 x Pant C_1, ms x ms x 206, ms x (G_4 x MP), Pant C_1, LCA 1079 and LCA-180 were free from disease. The culture 960 x G_4 showed lowest incidence of 4.2 per cent, while the culture LCA-210 recorded maximum incidence of 85.8 per cent. However, there were no earlier reports on survey of the disease in Chittoor district of Andhra Pradesh.

Under field conditions, chilli plants infected with mosaic exhibited distinct mosaic mottling, distortion of leaves and retardation of growth (Plate 1). Flowering was delayed and some times no flowers were produced. Flowers were also shed before fruit formation.

Artificially inoculated plants showed chlorosis followed by typical mosaic symptoms like dark green areas alternating with light green areas (Plate 2). Plants were stunted with retarded growth. The symptoms on chilli plants due to mosaic were similar to those described by Kulkarni (1924), Holmes (1937), Kovachevsky (1942), Chamberlain (1947), Jha and Raychaudhuri (1956), Adsuar et al. (1975), Ragozzino et al. (1972), Feldman and Oremianer (1972), Mishra and Singh (1973), Aleksic and Marinkovic (1981), Nagai et al. (1968), Pop (1981), and Erkan and Yorcan (1983). However the virus under study differs in symptomatology from other viruses reported on chilli such as ring strain of tobacco mosaic virus (Nakata and Takimoto, 1940) in not producing bright yellow mottling, strains of tobacco mosaic virus (Mc Kinney, 1952) in not producing necrotic local lesions, tomato atypical mosaic virus (Miller and Thornberry, 1958) in not producing interveinal chlorosis only, a new strain of tobacco mosaic virus (Murakishi, 1960) in not producing raised reddish brown streaks on fruits, a strain of tobacco mosaic virus (Fletcher, 1963) in not producing main vein necrosis, samsun latent tobacco mosaic virus (Greenleaf et al., 1964) in not producing necrotic patterns

and upper stem necrosis, tobacco leaf curling viruses (Dhanraj and Seth, 1971) in failing to produce enations, tomato strain of TMV (Rogozzino et al. 1972) in not producing stem necrosis and yellow spots on fruits and a strain of TMV (Erkan and Yorcan, 1983) in not producing wilting symptoms.

Study on the present virus proved it to be only sap-transmissible. Sap-transmission of the TMV waspreviously reported by Palm (1923), Holmes (1937), Nakata and Takimoto (1940), Doolittle and Thacher (1942), Kovachevsky (1942), Kohler and Rajan (1944), Chamberlain (1947), Zabala and Dellecosta (1947), Mc Kinney (1952), Newton (1953), Miller and Thornberry (1958), Murakishi (1960), Fletcher (1963), Greenleaf et al. (1964), Escarous (1971), Adusar et al.(1971), Feldman and Oremianer (1972) and Rogozzino et al. (1972).

The present virus was not transmitted through seed (Table 7). But seed transmission was reported by Mc Kinney (1952) on pepper variety SC 46252, and Damski (1981), Tosic and Mitrovic (1981), Rogozzino and Angelaccio (1981) and Betti (1983).

Studies on host-range indicated that the virus under study produced systemic symptoms on Capsicum fruitescens L., Petunia olerecea L., Datura stramonium (Plate 4), D. metal (Plate 5), Nicotiana tabacum L. (Plate 6), N. glutinosa L., Lycopersicon esculentum Mill., Cucumis sativus L., C. melo L., and

Momordica charantia L. and local lesions on Chenopodium amaranticolor Costo and Reyn. and C. melo L. (Plate 3). Since all the extensively cultivated hosts tested only the members belonging to solanaceae, cucurbitaceae, chenopodiaceae and Amaranthaceae were reacted. Out of 47 plant species tested, the virus had a restricted host range limited only to 13 plant species belonging to 4 families.

Jha and Raychaudhuri (1956) also stated that the chilli mosaic found in India was transmissible by sap inoculation to Nicotiana tabacum L., N. tabacum var. White Burley, N. glutinosa, Solanum nigrum, Petunia hybrida, Cucumis sativus, C. melo var. utilissimus and Carthamus tinctorius. Miller and Thornberry (1958) reported that tomato atypical mosaic virus produced local lesions on inoculated leaves of Datura stramonium, D. wrightii, N. glutinosa, N. rependa, Solanum nigrum and mosaic mottling on Lycopersicon esculentum. Greenleaf et al. (1964) observed that samsun latent tobacco mosaic virus produced local lesions on Chenopodium amaranticolor, N. tabacum, N. glutinosa and Datura stramonium. Feldman and Oremianer (1972) reported that the pepper unusual strain of TMV produced local lesions on all tested cultures of N. tabacum except turkish tobacco.

Studies on physical properties showed that the virus under study possessed the thermal inactivation point of 90°C to 95°C (Table 10 and 11), dilution end point of 1:1000000 to 1:10000000 (Table 12 and 13) and longevity in vitro of 9 days at room

temperature (23.5 to 28.5°C) (Table 14 and 15) and 17 days at *frigidaire* temperature of 10°C (Tables 16 and 17).

The thermal inactivation point of the present virus coincided with TIP of TMV on pepper reported by Doolittle and Beecher (1942), a strain of TMV reported by Jasnic (1979) and a strain of tobacco mosaic virus reported by Erkan and Yorcan (1985).

Dilution end point of the present virus coincided with that of TMV on pepper reported by Doolittle and Beecher (1942), ring strain of TMV reported by Nakata and Takimoto (1940), samsun latent tobacco strain reported by Greenleaf et al. (1964), pepper unusual strain of TMV reported by Feldman and Oremianer (1972) and a strain of TMV reported by Erkan and Yorcan (1983).

Doolittle and Beecher (1942) reported that 'LIV' of TMV on pepper was 3 years at 21-27°C. Feldman and Oremianer (1972) observed LIV of pepper unusual strain of TMV for 5 years. Adsuar et al. (1972) observed LIV of virus producing local lesions on tobacco for 14 days. But the present virus exhibited LIV only for 9 days at room temperature (23.5 to 28.5°C) and 17 days at 10°C. The physical properties of the present virus differed from those reported by earlier workers viz., Mckinney (1952), Murakishi (1960), Sakarous (1971), Jha and Raychaudhuri (1956) and Ganguli (1960).

The modified method adopted by Hollings et al. (1963), Lima and Nelson (1975), Fischer and Lockhart (1976) was adopted for purification of the chilli mosaic virus.

In Electron-microscopy, copper grids showed the presence of rigid rods measuring 300 nm x 18 nm which resembled the typical TMV rods (plate 7). According to Greenleaf et al. (1964) electron micrographs of Samsun latent strain exhibited typical TMV rods indistinguishable from those of the standard TMV strain. Miller and Thornberry (1958) reported that tomato atypical mosaic virus had rod shaped particles measuring 300 x 15 nm.

Feldman and Oremianer (1972) reported that electron-micrographs of pepper unusual strain of TMV showed numerous straight rod shaped particles resembling those of common TMV. Hayashi et al. (1975) observed three size classes of particles which were intact particles of 300 nm long, subparticles of 150-240 nm and short particles of 70-150 nm. Jasnic (1979) reported rod shaped particles of 300 nm. Erkan and Yorgan (1983) reported a strain of TMV with rod shaped particles of 300 x 15 nm in dimensions. Thus the present virus under investigation was identified as tobacco mosaic virus.

Study regarding age of chilli plants in relation to susceptibility revealed that the susceptibility decreased with increase in age (Fig. 9). Plants between the ages of 20-30 days were highly susceptible and exhibited highest infection and showed decrease with increase in age and severity of symptoms also decreased (Table 18). Nambiar and Ramakrishnan (1969) reported that the respiratory rate in virus infected chilli plants was higher and was influenced by age of the plant. Danko (1970) conducted inoculation tests at 4 different stages of plant growth and reported that the total yield of ripe fruit was reduced principally by early infections.

Studies regarding the effect of mosaic on chilli plant revealed that the disease affected the growth characters viz., root length, shoot length, fresh weight and dry weight of shoot system, leaf area and chlorophyll fractions to a considerable extent. The per cent decrease in root length, shoot length, fresh and dry weights and leaf area was 29.51, 8.34, 46.79, 39.71 and 38.43 respectively. Reduction in root length and development may be due to the failure of infected leaves to synthesize enough food material to maintain all the roots or due to the interruptions to the normal flow of nutrients to the roots. John (1959) reported poor root system and reduction in root length in field bean infected by DSMV (Dolichos enation mosaic virus) and Kausalya et al. (1967, 1970) reported that in case of rosette andmosaic diseases of groundnut also root length was reduced. Sekhar (1975) stated that Sathgudi plants infected

with citrus mosaic exhibited poor and shortened root system.

Reduction in shoot length of groundnut infected by groundnut mosaic virus was reported by Kausalya et al. (1970). Joshi and Dubey (1976) also reported reduction in length of chilli plant infected with cucumber mosaic virus. There are reports available which showed disturbed auxin metabolism in virus infected plants (Grieve, 1936; Smith et al., 1968).

Changes in dry weight content of virus infected plants had been reported primarily in systemic infections as in the present virus. Owen (1957) observed a decrease in dry weight of tobacco leaves infected with TMV when compared with healthy due to decreased assimilary rate. On the other hand accumulation of dry matter in leaves of barley infected with barley yellow dwarf virus (Jensen, 1968) and in Luffa aegytiaca infected with CMV (Mandahar and Garg, 1972) was reported. Duggal and Singh (1981) also reported that in chilli plants affected with PVX, the net assimilatory rate was reduced.

In the present study, decrease in chlorophyll fractions i.e., chlorophyll a (9.5%), chlorophyll b (4.73%) and total chlorophyll (5.34%) over healthy, was observed (Table 24). The decreased chlorophyll content of the virus infected leaves of chilli had been attributed to the higher chlorophyllase activity in that tissue (Ramakrishnan, 1969). Farrag and Ramakri-

shnan (1971) reported that TMV caused chlorosis and reduction in chlorophyll and also reduced the mean size of the chloroplast. Jha (1967) biochemically studied the chlorotic leaves of chilli infected with the virus and the chlorosis was due to loss of chlorophyll a only. Duggal and Singh (1981) reported that in chilli plants affected with PVX the chlorophyll a, chlorophyll b and total chlorophyll were reduced. Reduction in chlorophyll content was also reported by Horvath and Balla (1982) in pepper leaves infected by viruses.

The present virus caused reduction in leaf area. Duggal and Singh (1981) observed reduction in leaf area ratio in chilli plants infected with PVX. Bakerous (1971) reported malformation of leaves which caused reduction of leaf area. Rogozzino et al. (1972) observed reduction in size of leaves in pepper affected by tomato strain of TMV.

In the present investigation, all cultivars (20) were susceptible to TMV which were screened under glasshouse conditions, by mechanical sap-inoculation. From the results it was clear that the susceptibility varied with the cultivar (Table 25). The incidence varied from 29 to 90 per cent. Out of twenty cultivars, eight cultivars viz., G5, 1077, 1078, cluster, Santaka, Warangal, Sindhur and 1081 exhibited only mild mottling while three cultivars viz., X-235, X-180 and X-210 showed mild mosaic, stunting, vein clearing, blistering and distortion. Six cultivars viz., X-206,

Aparna, Papnika-50, Kiran, S 118, LIC-45 exhibited mild mosaic and vein clearing. One cultivar 1079 showed mild mosaic and stunting and the remaining cultivar G_3 showed mild mosaic and slight curling. In the present study (Table 6) it was observed that ten cultivars viz., OT-3, S 118 x (G_4 x MP), Long pod x small pod, G_4 x 960, K_2 x Pant C_1, ms x ms x 206, (ms) x (G_4xMP), Pant C_1, LCA-1079, and LCA-180 were free from infection under natural field conditions.

Reaction of several varieties of pepper to TMV was studied by Holmes (1934, 1937). Miller and Thornberry studied reaction of pepper varieties to tomato atypical strain of TMV. Cook and Anderson (1954) reported that a strain of C. annuum P_{11} was resistant to TMV. Jha (1953) reported that chilli varieties NP-20 and NP-23 were more resistant to chilli mosaic virus than other 52 varieties tested. Anand et al. (1961) found that chilli varieties namely puri red, puri orange, Kondiverum, G_2 and a local variety were resistant against mosaic virus. Singh (1973) reported that chilli varieties viz., Puri red, Puri orange, G_2, Kondiverum and Suryamukhi were resistant to mosaic disease. Further he reported that the hybrids viz., A_{127}, C_1 IC 3425, IC 3440, IC 3779 were not infected by mosaic virus. Lee and Smith (1968) found local lesion type of resistance to TMV in C. fruitescens, C. annuum and C. chinense. Feldman and Cremianer (1972) observed that the unusual strain of TMV produced systemic symptoms on California Wonder and perfection and local lesions on Yolo Wonder, Yolo-Y, P_1, Mexican, New Carolina,

hot pepper, Pimson, Tabasco and S.C. 46252. Aleksic and Marinkovic (1981) observed systemic infection in chilli cultivar Soroksari and A_{12}. Gebre Selassie and Dumas De Valux (1981) recorded systemic infection in Zelemi rotund and Lamuso. Nagai et al. (1981) reported systemic infection in New Face and Shinsakigake.

CHAPTER VI

SUMMARY

Chilli (Capsicum annuum L.) like other commercial vegetable crops was prone to a number viral diseases. Twenty one viruses infect chilli naturally and 17 other viruses can be transmitted by artificial inoculation.

A survey made during 1985-86 in and around Tirupati and Guntur district revealed the incidence of mosaic disease to an extent of 5.21 to 20.02 per cent involving both local and cultivated varieties. In S.V. Agricultural College Orchard, Tirupati, the disease incidence ranged from 0.00 to 85.8 per cent in various cultures of chilli, grown for various varietal trials. The disease was characterised by mosaic mottling, vein clearing, slight blistering, leaf distortion, stunting and reduced flowering and fruiting.

Transmission studies revealed that the virus under study was transmissible by sap-inoculation. Seed-transmission and soil transmission tests gave negative results.

Results of the host range studies revealed that out of 46 species of plants belonging to 11 families tested, it could infect only 13 species belonging to four families. These

include Capsicum fruitescens L., Nicotiana tabacum L., N. glutinosa L., Solanum melongena L., Datura stramonium L., Datura metel L., Petunia oleracia L., and Lycopersicon esculentum Mill. of solanaceae which exhibited systemic infection except D. stramonium L., which exhibited necrotic, concentric local lesions. Chenopodium amaranticolor Costo and Reyn. of chenopodiaceae exhibited local lesion type of infection with concentric chlorotic lesions. Amaranthus hypochondriacus of Amaranthiaceae showed systemic infection. Cucumis sativus L., Cucumis melo L. and Memordica charantia L. of cucurbitaceae developed systemic infection.

The present virus exhibited thermal inactivation point of 90-95°C, dilution end point of 10^{-6} to 10^{-7} and longevity in vitro of 9 days at room temperature (23.5 to 28.5°C) and 17 days at 10°C.

Twenty days and thirty days old plants showed maximum per cent infection than 50, 60 and 70 day old plants. Decrease in symptom expression was also recorded in plants when infected at older age.

Poly Ethylene Glycol method was adopted for purification of the virus. Purifiedpreparations under electronmicroscope by the use of coppergrids showed virus particles of 300 nm x 18 nm in length and width.

Root length, shoot length, leaf area, fresh and dry weights of the infected plant were significantly reduced and the percentage of decrease compared to control was 39.51, 8.34, 38.43, 46.79 and 39.71 respectively.

There was a considerable reduction in chlorophyll fractions also. The percentage of reduction was 9.5, 4.73 and 5.34 in chlorophyll a, chlorophyll b and total chlorophyll respectively.

In varietal screening all 20 varieties under test became susceptible to the virus on artificial inoculation. They exhibited mosaic symptoms which coincided with the symptoms of typical mosaic infection on chilli.

Based on the results of symptomatology, transmission, host range, physical properties and purification and electronmicroscopy, the present virus under study was identified as common strain of TMV.

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*Originals not seen

APPENDIX I

AREA UNDER CROPS IN ANDHRA PRADESH

(Season and Crop Report 1982-83)

Crop	Year	Area in acres	Area in ha.	Average yield per ha (kg)	Production in tonnes
Chilli	1978-79	3,68,589	1,47,138	1,378	2,02,716
	1979-80	4,17,607	1,68,999	1,043	1,76,170
	1980-81	4,00,340	1,62,012	920	1,49,098
	1981-82	3,88,302	1,57,140	1,048	1,64,713
	1982-83	4,48,289	1,81,417	1,056	1,91,571

VITA

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