EPIDEMIOLOGY OF TOBACCO LEAF CURL VIRUS IN INDIA

GIRISHKUMAR B. VALAND

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

1990

EPIDEMIOLOGY OF TOBACCO LEAF CURL VIRUS IN INDIA

GIRISHKUMAR B. VALAND

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

1990

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ
ವಿಶ್ವವಿದ್ಯಾನಿಲಯ ಗ್ರಂಥಾಲಯ
ಗಾ.ಕೃ.ವಿ.ಕೆ, ಬೆಂಗಳೂರು-65.
12 SEP 1990
ಅನುಪ್ರಾಪ್ತಿ ಸಂ. Th. 2497
ವ ಸಂ

EPIDEMIOLOGY OF TOBACCO LEAF CURL VIRUS IN INDIA

GIRISHKUMAR B. VALAND

Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
for the award of the Degree of

Doctor of Philosophy

IN

PLANT PATHOLOGY

BANGALORE FEBRUARY 1990

Department of Plant Pathology

University of Agricultural Sciences

Bangalore

CERTIFICATE

This is to certify that the thesis entitled "EPIDEMIOLOGY OF TOBACCO LEAF CURL VIRUS IN INDIA" submitted by Mr. GIRISHKUMAR B. VALAND, for the degree of DOCTOR of PHILOSOPHY in PLANT PATHOLOGY to the University of Agricultural Sciences, Bangalore is a record of research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

Bangalore,
24 February, 1990

(V. MUNIYAPPA)
Professor of Plant Virology

Approved by:

Chairman : (V. Muniyappa)

Members 1 : (H. R. Reddy)

2. : (C.A. Virakthamath)

3 : (R.V. Ramamohan)

ACKNOWLEDGEMENTS

I wish to place on record my profound sense of gratitude and heartfelt respects to Dr. V. Muniyappa, Professor of Plant Virology, Department of Plant Pathology, University of Agricultural Sciences, Bangalore and Chairman of my advisory committee, for his untiring guidance and creative suggestions throughout the course of investigation.

I am extremely happy to express my deep sense of gratitude to Dr. H.R.Reddy, Professor and Head, Department of Plant Pathology, University of Agricultural Sciences, Bangalore, and member of my advisory committee, for his valuable guidance and help rendered during my research work.

I wish to express sincere thanks to the members of my advisory committee Dr.C.A.Virakthamath, Professor of Entomology, Department of Entomology, and Dr.R.V.Ramamohan, Professor and Head, Department of Physics, University of Agricultural Sciences, Bangalore for their valuable suggestions and constructive criticisms.

I wish to express my gratitude to Dr.K.G.H.Shetty, Former Professor and Head, Department of Plant Pathology, University of Agricultural Sciences, Bangalore for his valuable help and constructive suggestions during the course of study.

I am also indebted to Dr.B.J.Jaisani, Principal; Dr. D.J.Patel, Professor and Head, Nematology; Dr.J.G. Patel, Professor and Head, Plant Pathology, B.A.College of Agriculture; and Dr. R.Lakshminarayana, Project Co-ordinator (Tobacco), Gujarat Agricultural University, Anand Campus for their constant encouragement and keen interest in my study.

I am equally grateful to Dr. M.S.Chari, Director, Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh for his valuable suggestions and help that he offered me during the course of study.

I sincerely express my gratefulness to Professor Dr. B.D.Harrison and Maud Aiton, Scotish Crop Research Institute, Dundee, U.K., for supplying ACMV antiserum and help in ELISA technique.

I can never pay debt to my wife for her assistance, constant encouragement and sharing the family responsibility without which it would have been difficult for me to persue the Ph.D. study.

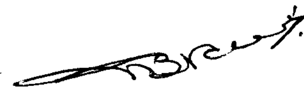
I owe a lot to my parents, other family members and friends for their affection and support to complete this study.

At this juncture, I feel to extend my sincere thanks to all the staff members of Plant Pathology Department, University of Agricultural Sciences, Bangalore for their cooperation during the investigation.

I am equally indebted to the Indian Council of Agricultural Research(ICAR), New Delhi, for having been awarded the Senior fellowship during the tenure of these studies.

I am highly thankful to Mr.Narayanaswamy for his neat typing of this manuscript.

Finally, I acknowledge Gujarat Agricultural University for granting three years deputation, without which it would not have been possible for me to acquire Ph.D., degree and improve my professional competence.



Date: 24-2-1990

(GIRISHKUMAR B. VALAND)

CONTENTS

Chapter	Title		Page
I	INTRODUCTION	..	1
II	REVIEW OF LITERATURE	..	4
III	MATERIALS AND METHODS	..	28
IV	EXPERIMENTAL RESULTS	..	49
4.1	Survey for the incidence of tobacco leaf curl virus	..	49
4.1.1	TobLCV incidence and vector population in relation to weather conditions of major tobacco growing areas	..	53
4.2	TobLCV incidence on different host species under field conditions	..	53
4.2.1	On different tobacco cultivars	..	53
4.2.2	On cultivated and weed host plants	..	57
4.3	Isolates of TobLCV	..	63
4.3.1	Collection of isolates of TobLCV	..	63
4.3.2	Symptoms of isolates of TobLCV under field conditions	..	63
4.3.3	Grouping of TobLCV isolates based on symptoms after transmitting by *B.tabaci* to Samsun and Anand 119	..	63
4.3.4	Distribution of TobLCV groups in different tobacco growing areas	..	78

CONTENTS
(continued)

	Title		Page
4.4	Host range	..	78
4.4.1	Transmission of TobLCV to different species of *Nicotiana*	..	78
4.4.2	Transmission of TobLCV to different tobacco cultivars	..	81
4.4.3	Transmission of TobLCV to plant species	..	81
4.5	Incidence of TobLCV and vector population	..	121
4.5.1	Incidence of TobLCV and whitefly population in relation to planting date	..	121
4.5.2	TobLCV incidence and vector population in relation to weather conditions	..	126
4.5.3	TobLCV incidence in relation to location of tobacco nursery	..	127
4.5.4	TobLCV incidence in relation to stage of crop growth under field conditions	..	127
4.6	Monitoring of vector population	..	130
4.6.1	Yellow pan water traps	..	130
4.6.2	Detergents in yellow pan water traps	..	130
4.7	Management of TobLCV in nursery	..	135
4.7.1	Covering nursery with nylon net	..	135

CONTENTS
(continued)

Chapter	Title		Page
	4.7.2	Effects of barrier crops ..	137
	4.8	Diagnosis of TobLCV in different host plants ..	137
	4.8.1	Enzyme linked immunosorbent assess(ELISA)..	137
	4.8.2	Immunosorbent electron microscopy(ISEM) ..	143
V	DISCUSSION	..	145
VI	SUMMARY	..	159
VII	REFERENCES	..	166

LIST OF TABLES

Table	Title		Page
1	Survey for the TobLCV incidence and whitefly population in tobacco field during 1988-89	..	50
2	Weather data of the different tobacco growing areas during 1988-89	..	54
3	Weather data of the different tobacco growing areas during 1988-89	..	55
4	Weather data of the different tobacco growing areas during 1988-89	..	56
5	TobLCV incidence on tobacco cultivars under field condition	..	58
6	Incidence of TobLCV on cultivated and weed host plants in tobacco growing areas of Karnataka	..	60
7	Incidence of TobLCV on cultivated and weed host plants in tobacco growing areas of Gujarat	..	61
8	Incidence of TobLCV on cultivated and weed host plants in tobacco growing areas of West Bengal and Bihar	..	62
9	List of TobLCV isolates collected from different tobacco growing areas	..	64
10	Transmission of TobLCV isolates (1-45) to *N. tabacum* cvs. Samsun and Anand-119	..	74

LIST OF TABLES
(continued)

Table	Title		Page
11	Grouping of TobLCV isolates on the basis of symptoms exhibited to N. tabacum cvs. Samsun and Anand-119	..	77
12	Distribution of TobLCV group in tobacco growing area	..	80
13	Transmission of TobLCV to different species of Nicotiana	..	82
14	Symptoms of TobLCV on different species of Nicotiana	..	83
15	Transmission of TobLCV to different tobacco cultivars	..	86
16	Symptoms of TobLCV exhibited on different tobacco cultivars	..	88
17	Transmission of TobLCV to cultivated, ornamentals, weeds and forest plants by B. tabaci	..	98
18	Symptoms of TobLCV on cultivated, ornamentals and weed host plants	..	104
19	Incidence of TobLCV and whitefly population in relation to date of planting	..	123
20	Weather data in relation to the whitefly population and the incidence of TobLCV during 1988-89	..	125
21	Incidence of TobLCV and whitefly population in tobacco nursery raised at two locations(a) nursery raised near infected tobacco field, and (b) nursery raised away from infected tobacco field	..	128

LIST OF TABLES
(Continued)

Table	Title		Page
22	Incidence of TobLCV and whitefly population at different stages of crop growth under field conditions	..	129
23	Monitoring of whitefly adults by yellow pan water traps	..	131
24	Monitoring of whitefly adults by yellow pan water traps using different detergents	..	133
25	Effect of nylon net covering for tobacco nursery on the incidence of TobLCV	..	136
26	Effect of barrier crops on the incidence of TobLCV in nursery	..	138
27	Detection of TobLCV by ELISA in different infected host plants; Reactions of ACMV with different TobLCV samples	..	141

LIST OF FIGURES

Figure	Title		Page
1	*B. tabaci* adults feeding on cotton leaf	..	31
2	Tobacco nursery covered with nylon net	..	40
3	Incidence of tobacco leaf curl virus and whitefly population in different states of India	..	51
4	Incidence of tobacco leaf curl virus and whitefly population in Karnataka State	..	52
5	TobLCV isolate Bangalore 1 showing curling and dwarfing of leaves, stem zig zag, vein thickening and enation	..	67
6	TobLCV isolate Anand 5 showing curling, leaves leathery and brittle, greening of veins and enation	..	67
7	TobLCV isolate Guntur 10 showing reduction in leaf size, vein thickening, curling, leathery and brittle leaves and enation	..	68
8	TobLCV isolate Rajahamundry 11 showing crinkling and dwarfed leaves, vein thickening, reduction of inflorescences and stem zig zag	..	68
9	TobLCV isolate East Godavari 13 showing vein thickening, curling, greening of vein and enation	..	68
10	TobLCV isolate Nipani 14 showing downward curling, foliage pale green, vein thickening, zig zag, depressions and enation	..	69

LIST OF FIGURES
(continued)

Figure	Title		Page
11	TobLCV isolate Anand 18 showing foliage pale green, depressions, vein thickening and enation	..	69
12	TobLCV isolate Baroda 21 showing dark green foliage, vein thickening, zig zag, curling and enation	..	69
13	TobLCV isolate Hunsur 22 showing leaf narrowing, foliage pale green, vein thickening and leaves leathery and brittle	..	70
14	TobLCV isolate Shimoga 26 showing vein thickening, zig zag, curling, greening of vein and enation	..	70
15	TobLCV isolate Nipani 29 showing severe vein thickening, depression, curling, enation cup shape and leaf brittle and leathery	..	71
16	TobLCV isolate Dinhata 36 showing reduction of leaves and internode, curling, vein thickening and depression	..	71
17	TobLCV isolate Pusa 38 showing stunting of plant, stem zig zag, vein thickening, depression and cup shape enation	..	71
18	TobLCV infected tobacco cvs. Samsun and Anand-119 leaves showing vein thickening, zig zag, greening of veins and stalked and sessile cup shape enation (Group I)	..	73
19	TobLCV infected tobacco cvs. Samsun and Anand-119 leaves showing thorny enation on marginal veins and vein thickening (Group II)	..	73

LIST OF FIGURES
(Continued)

Figure	Title		Page
20	TobLCV infected tobacco cvs. Samsun and Anand-119 leaves showing tiny protruding enation inbetween the veins and vein thickening (Group III)	..	79
21	TobLCV infected tobacco cvs. Samsun and Anand-119 leaves showing irregular vein thickening, swelling and frilled (boat) shape enation and bright green veins (Group IV)	..	79
22	TobLCV infected _N. benthamiana_ transmitted by _B. tabaci_	..	85
23	TobLCV infected _N. glauca_ transmitted by _B. tabaci_	..	85
24	TobLCV infected _N. glutinosa_ transmitted by _B. tabaci_	..	85
25	TobLCV infected _N. undulata_ transmitted by _B. tabaci_	..	85
26	TobLCV infected _N. tabacum_ cv. Anand-119 transmitted by _B.tabaci_	..	90
27	TobLCV infected _N. tabacum_ cv. FCV-Sp. transmitted by _B. tabaci_	..	90
28	TobLCV infected _N. tabacum_ cv. Florida-22 transmitted by _B.tabaci_	..	90
29	TobLCV infected _N. tabacum_ cv. Godavari transmitted by _B. tabaci_	..	92
30	TobLCV infected _N. tabacum_ cv. Hirac transmitted by _B. tabaci_	..	92
31	TobLCV infected _N. tabacum_ cv. Hicks Sp. transmitted by _B. tabaci_	..	92
32	TobLCV infected _N. tabacum_ cv. Jayasri transmitted by _B. tabaci_	..	94

LIST OF FIGURES
(Continued)

Figure	Title		Page
33	TobLCV infected *N. tabacum* cv. Jayasri (MR) transmitted by *B. tabaci*	..	94
34	TobLCV infected *N. tabacum* cv. Mc Nair-12 transmitted by *B. tabaci*	..	94
35	TobLCV infected *N. tabacum* cv. FCT-7 transmitted by *B.tabaci*	..	94
36	TobLCV infected *N. tabacum* cv. Samsun transmitted by *B.tabaci*	..	96
37	TobLCV infected *N. tabacum* cv. Virginia gold transmitted by *B. tabaci*	..	96
38	*B.tabaci* transmitted TobLCV to *Beta vulgaris*	..	105
39	*B. tabaci* transmitted TobLCV to *Carica papaya*	..	105
40	*B.tabaci* transmitted TobLCV to *Crotalaria juncea*	..	107
41	*B. tabaci* transmitted TobLCV to *Cymopsis tetragonoloba*	..	107
42	*B. tabaci* transmitted TobLCV to *Lycopersicon esculentum*	..	107
43	*B. tabaci* transmitted TobLCV to *Sesamum indicum*	..	108
44	*B. tabaci* transmitted TobLCV to *Phaseolus vulgaris*	..	108
45	*B. tabaci* transmitted TobLCV to *Althaea rosea*	..	108

LIST OF FIGURES
(Continued)

Figure	Title	Page
46	*B. tabaci* transmitted TobLCV to *Callistephus chinensis* ..	110
47	*B. tabaci* transmitted TobLCV to *Dianthus caryophyllus* ..	110
48	*B. tabaci* transmitted TobLCV to *Petunia hybrida* ..	110
49	*B. tabaci* transmitted TobLCV to *Acanthospermum hispidum* ..	112
50	*B. tabaci* transmitted TobLCV to *Acalypha indica* ..	112
51	*B. tabaci* transmitted TobLCV to *Ageratum conyzoides* ..	112
52	*B. tabaci* transmitted TobLCV to *Croton bonplandianum* ..	114
53	*B. tabaci* transmitted TobLCV to *Datura stramonium* ..	114
54	*B. tabaci* transmitted TobLCV to *Eupharbia geniculata* ..	114
55	*B. tabaci* transmitted TobLCV to *Galinsoga puriflora* ..	114
56	*B. tabaci* transmitted TobLCV to *Heliotropium indicum* ..	115
57	*B. tabaci* transmitted TobLCV to *Nicandra physaloides* ..	115

LIST OF FIGURES
(Continued)

Figure	Title		Page
58	*B. tabaci* transmitted TobLCV to *Oxalis corniculata*	..	117
59	*B. tabaci* transmitted TobLCV to *Parthenium hysterophorus*	..	117
60	*B. tabaci* transmitted TobLCV to *Phyllanthus niruri*	..	118
61	*B. tabaci* transmitted TobLCV to *Solanum forvum*	..	118
62	*B. tabaci* transmitted TobLCV to *Stachytarpheta indica*	..	120
63	*B. tabaci* transmitted TobLCV to *Synedrella nodiflora*	..	120
64	TobLCV incidence in relation to whitefly population and weather condition	..	124
65	Monitoring whitefly adults by yellow pan water traps	..	132
66	Monitoring whitefly adults by yellow pan water traps using different detergents	..	134
67	Host preference for oviposition by whitefly	..	139
68	Geminivirus particles trapped from crude leaf extracts of tobacco infected with TobLCV by using Indian cassava mosaic virus antibody in ISEM	..	144

INTRODUCTION

I. INTRODUCTION

Tobacco is said to have been introduced into India in the 17th century. The genus Nicotiana, one of the five largest genera of Solanaceae is represented by about 64 species. Among the species Nicotiana tabacum developed for cigarette, cigar, cheroot, bidi, hookah and snuff are grown in the states of Andhra Pradesh, Karnataka, Tamil Nadu and Gujarat, whereas N. rustica are used for only chewing, hookah and snuff and it's cultivation is restricted to the cooler parts of West Bengal, Bihar, Uttar Pradesh and Punjab (Gopalachari, 1984).

Tobacco is one of the remunerative non-food cash crop of India cultivated in an area of about 323900 hectares with a total yield of 358900 tonnes and an average yield of 1086 kg per ha. Karnataka produces about 27100 tonnes of tobacco over an area of 37800 hectares with an average yield of 719 kg per ha (Anon., 1988, 1989).

India is the world's fourth largest producer and eighth largest exporter of tobacco and accounts for about seven per cent of the area and five per cent of the tobacco production in the world. This narcotic crop fetches around Rs.1500 crores as excise revenue and Rs.150170 crores in foreign exchange (Chari, 1989).

Tobacco is a rich source of several phyto-chemicals viz., nicotine, nicotine acid, nicotine amide, solanesol, organic acid (mallic, oxalic and citric), leaf protein concentrate (LPC), pentosans and non edible oil. Nicotine sulphate is used as insecticide and for pharmaceutical purposes (Chari and Jaisani, 1987).

There are several diseases caused by fungi, bacteria, viruses, nematodes and the root parasite (Orobanche sp) affecting the yield and quality of tobacco both in the nursery and field. Among the virus diseases leaf curl is notorious and destructive in nature and causes over 90 per cent damage (Pal and Tandon, 1937). Loss in tobacco yield by over 50 per cent was noticed in Sudan due to tobacco leaf curl virus (TobLCV) (Yassin and Abu Salih, 1972) and upto 5 per cent from India (Reddy and Nagarajan, 1982). Tobacco leaf curl virus is graft transmissible but not seed or sap. A common vector is the whitefly Bemisia tabaci Gennadius, a member of the family Aleurodidae in the order Hemiptera.

Though the disease has been known for quite a long period in India, the available information on

per cent disease incidence, prevalence of whitefly in the tobacco growing areas, role of the other host plants as source of inoculum, symptomatology and characterization of symptom variations are quite inadequate. Therefore, it was considered that detailed studies with the following objectives would throw more light for effective management of the virus, and vector.

1. Survey of different tobacco growing areas for the incidence of TobLCV
2. Collection of different isolates of TobLCV for comparative studies
3. Incidence of TobLCV on different cultivars, host plants and weeds in and around tobacco fields
4. Determination of host range of TobLCV
5. Spread of disease in relation to vector and weather conditions
6. Management of TobLCV in tobacco nursery
7. Diagnosis of TobLCV in cultivated and weed host plants by ELISA and ISEM

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The literature pertaining to studies on different aspects of tobacco leaf curl virus (TobLCV) disease is presented here.

Many virus diseases affect tobacco crop(<u>Nicotiana</u> spp.) (Lucas, 1965). Among the virus diseases affecting tobacco crop, leaf curl virus (TobLCV) is the most destructive in India. The leaves of diseased plants exhibit severe curling, vein thickening and ruffling (Pruthi, 1945). The natural occurrence of TobLCV was observed in India by Pruthi and Samuel (1937). The disease was recognized as a serious threat to tobacco cultivars as early as 1912, but it's cause was unknown. Storey 1931 from Tanganyika and Thung 1932 in Java independently announced that leaf curl was induced by virus and transmitted by a species of whitefly (<u>Bemisia</u> sp.).

The severity of leaf curl disease of tobacco was reported for the first time in Northern India by Pal and Tandon (1937). Incidence of TobLCV was also reported in India by Pruthi and Samuel (1937, 1939, 1942, 1942), Vasudeva and Sam Raj (1948), Sastry and Nariani (1962) and Dhinghra and Nariani (1962).

Besides India, the tobacco leaf curl virus was reported from **Africa:** Cameroons(Ludwings, 1913), Nyasaland (Hornby, 1933), Rhodesia(Moore, 1933, 1934), Sierra Leone (Deighton, 1940), Sudan (Yassin and Abu Salih, 1972), Tanganiyaka (Storey, 1931), Transvaal (Hill, 1968; Hopkins, 1932); **Asia:** Belgian Congo(Mayne and Ghesquiera, 1934) Ceylone (Gadd and Loos, 1941), China (Osaki et al., 1979; Yan et al., 1983), Iraq (AL-Ani et al., 1987), Japan (Tsumagari, 1967), Java (Thung, 1932; Jensen, 1920; Jochems, 1928), Philippines (Martinez, 1964; Olivares et al., 1972), Pakistan (Munshi and Chaudhari, 1964), Taiwan (Mastumoto and Tateoka, 1940; Yu-Cheng, 1964); **South America:** Brazil (Costa and Carvalho, 1960); Venezuela (Wolf et al., 1949), U.S.S.R.(Khudyana, 1936); **Europe:** Netherland East Indies (Peters and Schwartz, 1912); **West Indies:** Puerto Rico (Bird, 1958). The disease is wide-spread in many tropical areas and has been found in Algeria, Australia, Bangla-desh, Burma, Cuba, Colombia, Denmark, Jamaica, Korea, Madagascar, Panama, Romania, Sumatra, Switzerland and Thailand.

Tomato leaf curl virus is a strain of tobacco leaf curl virus and closely resembles in symptoms. It has been reported from India (Vasudeva and Sam Raj,1948); Sudan (Cowland, 1932; Yassin and Nour, 1965a, 1965b), Sri Lanka (Newton and Peiris, 1953; Shivananthan, 1983);

Egypt (Nour Eldin et al., 1969), Philippines (Retuerma et al., 1971), Somalia (Castellani et al., 1981), Thailand (Thanapase et al., 1983) and Japan (Tomato yellow dwarf virus; Osaki and Inouye, 1978; Kobatake et al., 1981).

2.1 Incidence and loss due to TobLCV

Epiphytotics in certain fields in Rhodesia was noticed by Hopkins (1932). Thilliard (1921) stated that 60 per cent of the crop in Cameroons was affected in 1917. Moore (1933, 1934) noted that, entire tobacco crop was affected with leaf curl virus in East Africa, Rhodesia and the Transvaal. Storey (1935) regarded it as the most destructive tobacco disease in many parts of East Africa. Pal and Tandon (1937) stated that tobacco leaf curl virus is usually present in northern India to the extent of 5 to 10 per cent, but that in some fields virtually renders the crop worthless. In India, the disease is most common in the Gangetic delta, Bihar and Gujarat and sometimes assumes an epidemic from 60 to 70 per cent by tobacco leaf curl virus (Pruthi, 1945). Fifty to seventy per cent incidence was recorded in Pusa(Bihar) by Pruthi and Samuel (1937). Wolf et al. (1949) noticed destructive nature during the dry season and generally

involves 5 to 10 per cent crop loss. In 1975, an outbreak of tobacco leaf curl virus occurred in a late planted tobacco in Japan and 50 to 69 per cent diseased plants were recorded in the field (Yamaguchi et al., 1976). Reddy and Nagarajan (1982) estimated 100 per cent loss in 30 days old seedlings after planting, 40 per cent when plant infected in 60 days of planting and 10 per cent at topping of tobacco. They further attributed 1 to 5 per cent loss due to tobacco leaf curl disease on national average basis. Reduction in yield of tobacco by over 50 per cent was estimated by Yassin and Abu Salih (1972) from Sudan.

2.2 Symptomatology

The most characteristic symptoms of tobacco leaf curl virus are the production of leafy outgrowth (enations) from the veins of lower surface of curled and uneven twisted leaves which are much dwarfed, the greening of the veins and severe stunting of the plant.

On the basis of the symptoms Thung (1932) described three types and designated as (1) common Kroepoek, in which the leaves are much smaller, the tip and leaf margins are rolled downward, the smaller veins are knotted and crooked and the larger veins bear flap like enations (2) curl disease, characterized by curling of the whole leaf margin

downward, the stems are branched, the oldest leaves are thick and brittle, the plant becomes broom like, and (3) transparent kroepoek, the leaf margins are rolled upward and the smaller veins tend to become transparent and the absence of enations. Storey(1932) reported more than one strain of tobacco leaf curl virus and observed considerable variations in the severity of the symptoms. Kerling (1933) described three types of tobacco leaf curl disease as 'A', 'B' and 'C' based on the microscopic observations. These types differed from each other not only externally but also anatomically.

Pal and Tandon (1937) described the tobacco leaf curl virus disease symptoms exhibited on tobacco cv. Pusa H.142 and grouped into five types as follows.

(1) Tobacco leaf curl virus A (Tobacco leaf curl virus 1)

Plant greatly dwarfed, stem zig zag, internode reduced, leaves reduced in size, thickened and curled downward uniformly at the margin, markedly rugose and brittle. Foliage dark green, transient vein clearing present at an early stage, vein thickening and greening of veins irregular. Enation numerous of sessile and stalked cup shape and frilled. Inflorescence greatly reduced.

(2) Tobacco leaf curl B(Tobacco leaf curl virus 2)

Plant greatly dwarfed, stem straight, internode reduced. Leaves reduced in size. Upper leaves rolled, surface much wrinkled, rugose but not brittle. Foliage pale green, secondary veins thickened, twisted, wrinkled and vein clearing transient. Inflorescence reduced and condensed.

(3) Tobacco leaf curl C(Tobacco leaf curl virus 3)

Plant not reduced much, stem straight and internode not reduced. Leaves little or not reduced in size, not thickened. Upper leaves moderately rolled and surface normal or wrinkled but not brittle. Intense vein clearing and persists in young leaves. Greening of veins and enations absent but small green stitch developed on veins. Inflorescences slightly or not at all reduced.

(4) Tobacco leaf curl D(Tobacco leaf curl virus 4)

Plant dwarfed, stem straight, internode reduced, leaves somewhat reduced in size but not thickened and slightly curled at the margin. Upper leaves rolled and lower much wrinkled at the margins. The veins between each pair of secondary veins present a ' gathered '

appearance vein clearing persist but not distinct from type 'C'. Greening and enations absent. Inflorescences reduced and condensed.

(5) Tobacco leaf curl x

Mixture of two or more virus hence symptoms in wider and more variable than in any other of the foregoing.

Plant slightly to greatly reduced. Stem straight, zig zag or spirally twisted. Leaves slightly or much reduced in size, occasionally thickened curled to varying degrees. Leaves sometimes surface wrinkled, puckered or rugose. Foliage colour pale green, dark green or normal green. Vein clearing transient, rarely persistent. Greening and all kinds of enation present. Reduction of inflorescence variable.

Tomato leaf curl virus (TLCV) is a strain of tobacco leaf curl virus and produces similar type of symptoms. Vasudeva and Sam Raj (1948) and Yassin and Nour (1965a) described three types of the symptoms referred as (1) leaf curl (2) tomato vein thickening, and (3) tomato condensed top. Seetharama Reddy(1978) and Seetharama Reddy et al. (1981) described five isolates of the TLCV. Saikia (1985) made detail study on

TLCV symptoms and framed five groups (1) exhibited pale yellowing of terminal leaves with vein clearing followed by upward curling, reduction in leaf size and thickening of veins, group (2) showed vein clearing followed by upward curling of leaves and twisting of petioles. The top leaves become narrow and lower leaflets become stiff and erect followed by leaf rolling, group (3) showed severe leaf curl and symptoms with enations and group (4) downward curling, severe twisting of petioles and internodes with severe reduction in leaf size and (5) severe curling, greening and vein thickening, small cup shape enations and stunting of the plant.

2.3 Insect Transmission

The only known vector of the tobacco leaf curl virus is Bemisia tabaci (Storey, 1931; Thung, 1932; West, 1936; Orlando and Silberschmidt, 1946; Pruthi and Samuel, 1942; Pruthi, 1945; Capoor and Varma, 1950; Costa and Bennett, 1950; Dickson et al., 1954; Loebenstein and Harpaz, 1960; Nariani, 1963; Hill, 1968; Kiriyama, 1972; Olivares et al., 1972; Yassin and Nour, 1965a; Osaki et al., 1979; Valand and Desai, 1980; Al-Ani et al., 1987).

Bemisia tuberculata Bondar and Aleurotrachelus socialis Bondar have been reported as vector of tobacco leaf curl virus in Venezuela (Wolf et al., 1949) and

Trialeurodes natalensis Cobb was supposed to be responsible for spreading this malady in the Transvaal (Mc Clean, 1940).

Pruthi and Samuel (1937) tried to transmit TobLCV by capsid bug Engytatus tennis Reut. but obtained negative results. Thung (1932) also failed to get positive results with this species in Java.

Pruthi and Samuel (1939) successfully transmitted TobLCV to tobacco by vector whitefly (B.gossypiperda Misra and Lamb) and obtained upto 90 per cent infection. They ~~were~~ also made attempts to transmit TobLCV with B.giffardi Kot. at Pusa in 1941 but failed.

Pruthi (1945) conducted a series of tests with the capsid bug Crytopettus crassicornis, the whitefly B. gossypiperda and the aphid Macrosiphum sp. but only B. gossypiperda (M4L) successfully transmitted TobLCV.

Russel (1957) concluded that B. inconspicus, Quaint B. costalimai Bondar, B. signata Bondar, B. behiana Bondar, B. longispina Priesner and Hosny, B.gossypiperda var. mosaicivectura Ghesquiere, B. goldingi, B.rhodesiaensis, B. hibisce Takahashi and B. nigeriensis were synonyms of B. tabaci. Only three species, B.tabaci Genn. T.vaporariorum (Westw

and T. abutiloneus were known to transmit plant viruses (Bird, 1981). Of these members, the B. tabaci is by far the most significant vector of plant viruses (Bird and Maramarosch, 1978; Muniyappa, 1980 and Bird, 1981).

Costa (1969) analysed the available evidence and reported Trialeurodes natalensis as a vector of South African tobacco leaf curl and further stated that possibly B. gossypiperda was also involved in transmission.

Nariani (1956) have been recognised B. tabaci as vector of tobacco leaf curl virus and transmitted virus from tobacco to tobacco and papaya to tobacco by vector and proved that the papaya leaf curl is caused by the tobacco leaf curl virus.

Chang (1967) reported that Aleurotuberculatus psidii can transmit tobacco leaf curl virus but further in 1973 he successfully transmitted virus by B. tabaci.

2.4 Testing of Tobacco cultivars to TobLCV

Field tolerance to leaf curl disease was reported in case of N. megalosiphon, N. plumbaginifolia and N. rependa. Among the varieties 'Delcrest' was found the least susceptible during screening of tobacco germplasm and cultivars for leaf

curl incidence at CTRI, Rajahmundry(Anon., 1962). At Anand (Gujarat) N. alata and N.sanderae were found field tolerant to leaf curl disease (Anon., 1961). Patel and Patel (1987) screened 553 lines and cultivars under field condition and identified line 100-26(K-20 x Smyma) x 20 moderately resistant while other cultivars were found moderately susceptible to tobacco leaf curl virus. Reddy and Nagarajan (1982) also reported that there is no variety resistant to tobacco leaf curl disease.

2.5 Host range of TobLCV

The host range is fairly wide and Wolf et al.(1949) recorded a list of 63 susceptible species from 14 families including 45 genera.

Storey (1935) listed soybeans, cassava, wild tomato, Crotalaria usarumoensis and Canavalia sp. as suspected hosts of tobacco leaf curl virus.

Ludwings (1913) noted Colocasia antiquarum as alternate host of tobacco leaf curl virus. Bird (1958) reported Rhynchosia minima DC as a common inoculum source in Puerto Rico.

Transmission of leaf curl virus to tobacco has been reported from the alternate hosts Vernonia indoculyx and

Sida sp. (Storey, 1935), V. cinerea, Ageratum conyzoides L and Synedrella nodiflora Gaertn (Thung, 1934).

The tobacco leaf curl virus was noticed in the field on Acanthospermum hispidum, Ageratum conyzoides, Asystasis caromandeliana, Althea sp., Brassica chinensis, B. juncea, Capsicum annuum, Carica papaya, Datura stramonium, Euphorbia geniculata, E. hirta, Helichrysum monstrosum, Hibiscus esculentus, Lycopersicon esculentum, L. pimpinellifolium, Launia asplenifolia, Nicandra physaloides, Nicotiana glutinosa, N. tabacum, Physalis peruviana, Rhaphanus sativus, Schizanthus sp., Scoparia dulcis, Sesamum indicum, Sida carpinifolia, S. cordifolia, S. rhombifolia, S. veronicaefolia, Solanum melongena, S. nigrum, Stachytarpheta jamaicensis, Synedrella nodiflora, Vigna sinensis var. sesquipedalis, Withania somnifera, Zinnia elegans, Vernonia cinerea, Nicotiana glauca, N. rustica, Malvastrum tricuspidatum, Petunia hybrida, Polanisia viscosa (Pruthi and Samuel, 1939; 1941, 1942; Shepherd, 1940; Mc Clean, 1940; Nariani and Pathanian, 1953; Garga, 1949; Wolf et al., 1949; Phatak and Raychaudhuri, 1967; Hill, 1968; Olivares et al., 1972; Mathur, 1932, 1933; Nariani, 1956; Mishra et al., 1963; Nariani, 1968; Flores, 1961).

Nariani (1956) successfully transmitted leaf curl virus with B. tabaci to Carica papaya and tobacco and

UNIVERSITY OF AGRICULTURAL SCIENCES
UNIVERSITY LIBRARY
GKVK, BANGALORE
T 2497

produced typical curling, crinkling of veins on infected leaves. He indicated that papaya leaf curl is caused by tobacco leaf curl virus (*Nicotiana virus*, 10).

Seth and Dhanraj (1972) noticed a new strain of tobacco leaf curl virus on chilli which produces severe leaf curl symptoms accompanied by enations and cripping effect on *Capsicum annuum*, *Nicotiana tabacum* cv. Harrison special, *N.glutinosa*, *Datura stramonium*, *Petunia hybrida* and *Lycopersicon esculentum*.

Mariappan and Naranswamy (1972) reported the weeds *Acanthospermum hispidum*, *Blainvillea rhomboides* and *Flaveria australasica* harboured the leaf curl disease and the virus. These isolates were inoculated with whitefly (*B.tabaci*) exhibited vein thickening and enations on *A.hispidum*, *B.rhomboides*, *F.australasica*, *Hibiscus rosasinesis*, *Lycopersicon esculentum*, *Nicotiana tabacum* cv. White Burley, *Zinnia elegans*.

Viswanath and Nariani (1981) observed leaf curl disease on spinach (*Spinacea oleracea*) and virus was transmitted by whitefly (*B.tabaci*) to healthy tobacco seedlings and spinach and plants exhibited the leaf curl and enations symptoms.

Tomato leaf curl virus was transmitted to Acanthospermum hispidum, Acalypha indica, Ageratum conyzoides, Althea rosea, Carica papaya, Capsicum annuum, Cassia tora, Centratherum anthelminticum. Datura stramonium, Euphorbia geniculata, Heliotropium sundanicum, Nicotiana tabacum, N. glutinosa, N. rustica, N. sylvestris, Petunia hybrida, Salvia splendens, Sesamum orientale, Solanum dubium, S. seaforthianum, S.tuberosum and Withania somnifera (Vasudeva and Sam Raj, 1948; Varma, 1959; Ramkrishnan et al., 1964; Seetharama Reddy, 1978; Yassin and Dafalla, 1980).

Saikia and Muniyappa (1989) successfully transmitted tomato leaf curl virus (TLCV) with whitefly B.tabaci to Acanthospermum hispidum, Ageratum conyzoides, Bidens pilosa, Capsicum annuum, Centratherum anthelminticum, Datura stramonium, Euphorbia geniculata, Galinosoga purviflora, Lycopersicon esculentum, Nicotiana glutinosa and N.tabacum.

2.6 Incidence of TobLCV vector population in relation to weather condition

Pal and Tandon (1937) studied the incidence and epidemiology of TobLCV in northern India and stated that average incidence of leaf curl in a normal year was 5 to

10 per cent and in an epidemic year (1934-35) nearly the whole of the crop affected badly. They reported that rate of spread of TobLCV was greatest at the end of October and in early November when the seedlings were planted. Thereafter there was decline in incidence until the second week of January. In March however again a distinct rise was observed. They concluded that leaf curl symptoms were rarely noted in nursery stage but the most infection appeared after the transplanting in the field. The condition in the monsoon months was more favourable to the activity of the insect vector.

Pruthi and Samuel (1942) surveyed the whitefly population on tobacco crop in northern parts of India at different times of the year. They stated that population was highest in early autumn to middle of November when the weather was warm (80°F) and relative humidity was about 80 to 90 per cent but decreased from December onwards and again increased in March. They further noticed that the incidence of tobacco leaf curl disease was corresponding with the rise and fall in the population of the whitefly.

Munshi and Choudhry (1964) conducted a trial in Pakistan keeping different dates of transplanting (15th and 30th August, 15th and 30th October) of cigarette

tobacco (cv. Harrisons Special) and severity of leaf curl disease incidence was correlated with the temperature. They derived that as the planting time was advanced towards the winter the infection followed a declining trend, the 15th August transplanted crop showed 81.9 per cent infection whereas 36.4 per cent was in the 15th November transplanted crop. This might be due to high temperature during August prone to high incidence compared to November transplanted crop and the winter condition has checked the activity of whitefly.

Hill (1968) studied the occurrence of B. tabaci in Transvaal (South Africa) and reported that vector population was relatively low during the early part of the September to November but again rapidly increased during December to February and then declined rapidly.

Yamaguchi et al. (1976) reported an outbreak of tobacco leaf curl virus disease incidence in Japan. They noticed that the disease appeared in the first week of July and increased abruptly upto the end of July. They correlated this sudden increase in incidence with the air current and migration of whitefly towards the tobacco field.

Sastry et al. (1978) demonstrated that tomato crop planted during December to May were subjected to low rainfall, low humidity, and high temperature which helped for high population of whitefly and high tomato leaf curl disease incidence ultimately resulted in low yield. Whereas the tomato planted during July to November were subjected to high rainfall, high humidity and low temperature resulting in low whitefly population and low incidence of TLCV with a better yield of tomatoes. Similar studies were also made by Seetharama Reddy (1978).

Saklani and Mathai (1977) reported that October to mid December was the most effective time for transplanting of tomato followed by January to March in Pantnagar (U.P.). Further they attributed that tomato leaf curl disease appeared very early (25 to 45 days) when the crop was planted between 16th March to 16th September and there was delayed appearance (132 to 162 days) of the disease between October to mid December planting dates due to low population of whiteflies in the early stage of the crop. The whitefly population decreased from October onwards with the decreasing trend in maximum temperature.

Nene (1972) also summarized that the development and multiplication of whitefly remains very low due to

low temperature during winter months. The rapid increase in yellow mosaic of pigeon pea disease incidence was noticed from middle of March to September planting with the increasing trend of average maximum temperature and whitefly population.

Saikia and Muniyappa (1989) studied the epidemiology of tomato leaf curl virus (TLCV) in Karnataka and revealed that TLCV incidence and whitefly (*B. tabaci*) population were high in late January to May planted crops and low from late June to early December planted crop. Lowest incidence and vector population were observed from August to November and maximum was from February to May planted crops. They correlated with weather conditions and stated that high temperature, low rainfall and low humidity contributed to the increase in the whitefly population from January to May. The low whitefly population during the months of July to November was related to high rainfall, low temperature, and high humidity. Thus, they obtained a high positive correlation between the per cent TLCV incidence and the whitefly population.

2.7 Monitoring of vector population control

Soil mulching with yellow polythene sheets delayed the spread of TLCV for at least 20 days. In laboratory

and field experiments, B. tabaci was found to be more attracted to yellow polyethylene than to straw or to aluminium or blue colored polyethylene (Cohen, 1981; Cohen and Melamed-Madjar, 1974). A combined treatment of mulching with yellow polyethylene sheet and one per cent sprays of azinophosmethyl starting 20 days after germination was the most effective in preventing the spread of TLCV.

Saikia (1985) reported that yellow pan water (plastic dish of 20 cm diameter) attracted more whiteflies whereas red, blue and green color plates attracted very few numbers. Yellow pan water trap kept at the ground level in tomato field attracted more number of whiteflies than the trap kept at plant height.

Mathew (1988) conducted experiments to monitor the whitefly, a vector of Indian cassava mosaic revealed that yellow pan water trap kept at ground level in cassava field attracted more whiteflies during summer compared to other seasons of the year.

2.8 Management of TobLCV in nursery

A combined treatment of nylon net covering for tomato nursery beds and 2-3 sprays of monocrotophos or

dimethoate or cypermethrin after transplanting in the field was effective in reducing the spread of the leaf curl disease in tomato (Saikia and Muniyappa, 1989).

Saikia and Muniyappa (1989) suggested an integrated approach for the management of TLCV and designed a nylon net (cage) frame (3 m long, 1.2 m wide and 0.45 m height) for covering tomato nursery beds to prevent the entry of vector whiteflies. They further reported that a combined treatment of nylon net covering for nursery for 25 days to 30 days and 3-4 sprays of various insecticides at 10 days interval after transplanting delayed tomato leaf curl virus incidence.

Saikia (1985) reported that TLCV infection in tomato was reduced due to intercropping with avare, bhendi, cucumber, sorghum, sunflower and maize. There was a significant reduction in whitefly population and disease incidence compared to control plots. Cucumber attracted more whiteflies followed by sunflower, bhendi and avare.

2.9 Diagnosis of TobLCV

2.9.1 Enzyme Linked Immunosorbent Assay (ELISA)

Sequeira and Harrison (1982) used Enzyme linked immunosorbent assay (ELISA) to detect cassava latent

virus (CLV). They reported that, CLV was detected readily in the extracts from systemically infected *N. benthamiana* leaves and less readily in extracts from leaves of naturally infected cassava from Angola.

Cohen *et al.* (1983) reported that purified squash leaf curl virus (SLCV) (0.5 g/ml) reacted with the SLCV antisera and also with cassava latent virus in ELISA but not with antisera prepared against beet curly top virus, horse radish curly top virus, bean golden mosaic virus and tomato yellow dwarf virus.

Mathew (1988) successfully used ELISA to detect ICMV in crude sap of infected cassava, cera rubber, *N. benthamiana*, *N. tabacum* cv. Jayasri using ICMV-H antiserum.

2.9.2 Immunosorbent electron microscopy (ISEM)

Immunosorbent electron microscopy (ISEM) using Antibody coated carbon filmed grids was employed to detect several whitefly transmitted gemini viruses in the purified preparation as well as in the crude sap. Sequeira and Harrison (1982) observed that 38 fold increase in number of cassava latent virus (CLV) particles were

trapped in CLV antibody coated grids than those coated with normal serum. They also found that antiserum from bean golden mosaic virus (BGMV) and beet curly top virus (BCTV) gave mean increase of 5.5 fold and 5.5 fold more in number of CLV particles trapped respectively.

Robert et al. (1984) also used homologous and heterologus antibody coated carbon filmed grids in ISEM and found that strong relationship existed among the five whitefly B.tabaci transmitted gemini viruses viz. ACMV, BGMV, EUMV (Euphorbia mosaic virus), SLCV and Tom GMV.

Mathew (1988) used ISEM successfully to detect ICMV particles in the leaf extract of N.benthamiana and Cassava infected with ICMV-H and ICMV-14 using ICMV-H antibody coated grids. He also found that ICMV-H antiserum also detected HYMV in infected french bean, TobLCV in N.tabacum and CYVMV in croton.

In immunosorbent electron microscopy geminate virus particles were observed in the tomato leaf extracts infected by tomato leaf curl virus (Robinson et al., 1987).

2.9.3 Electron microscopy

Although whitefly transmitted plant diseases

have been known for many years (Maramorosch, 1975), little progress was made on etiological studies.

The electron micrographs of the chromium-shadow cast particles from the formolized sap of tobacco leaf curl virus material revealed soft, round, flattened and somewhat indented bodies. Single round images indicated a diameter of 39 nm (Sharp and Wolf, 1951).

Galvez and Castano (1976), Goodman (1977a, 1977b) reported that spherical geminate particles of 15-20 nm in diameter are associated with diseased plants. Bean golden yellow mosaic virus (BGYMV) was the first plant pathogenic virus described with single stranded DNA genome (Goodman *et al.*, 1977).

Kiriyama (1972) partially purified leaf curl virus of tobacco by butanol clarification and differential centrifugation. Negatively stained virus preparations were examined under electron microscope revealed spherical particles approximately 30 nm in diameter. Serological test was also confirmed the presence of tobacco leaf curl virus.

Osaki and Inouye (1978) observed small isometric particles (15-20 nm diameter) often occurring as pair and forming a structure of 15-20 x 25-30 nm, from the purified preparation of yellow dwarf disease of tomato and leaf curl disease of tobacco under the electron microscope.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The present investigations on tobacco leaf curl virus (TobLCV) were carried out during 1987-89. All the field and glasshouse experiments were carried out at the Main Research Station, University of Agricultural Sciences, Hebbal, Bangalore located at 13° North latitude, 77°37' East longitude at an altitude of 899 metres above mean sea level.

3.1 Survey for the TobLCV incidence and whitefly population in tobacco growing areas

Major tobacco growing areas of Andhra Pradesh, Karnataka, Gujarat, West Bengal and Bihar were visited during 1988-89 to assess the incidence of tobacco leaf curl virus disease and prevalence of whitefly population. In each place five to eight fields were selected. Within each field five sectors of 10 m^2 each were examined for diseased and healthy plants. Population of whitefly was assessed by counting the number of adults on 25 tobacco plants in each of the five sectors of the same tobacco field.

TobLCV incidence was recorded on different species and cultivars of tobacco in the major growing

areas of the above mentioned states as described under 3.1. TobLCV incidence was also recorded on other cultivated and weed host plants in or around the tobacco fields.

3.2 Collection and maintenance of tobacco leaf curl virus isolates

Fortyfive isolates of TobLCV were collected during the survey from Karnataka, Andhra Pradesh, Gujarat and Bihar states and serially numbered and named according to the order of collection. The field symptoms of each isolates were recorded separately. The diseased tobacco plants collected were brought in polythene bags to the glasshouse (Hebbal, Bangalore) and planted in pots to maintain them separately for further studies. These isolates were maintained on tobacco cultivar Samsun by periodically inoculating with *Bemisia tabaci*.

3.3 Raising of healthy tobacco seedlings

Seeds of tobacco cultivars Samsun and Anand-119 were sown in nursery pan of 30 cm diameter under insect proof glasshouse condition. Thirty days old seedlings were transplanted to the polythene bags and were used for inoculation with whitefly *B. tabaci*.

3.4 Collection of whiteflies

An aspirator consisting of a glass tube (30 cm length and 0.5 cm diameter) and a polypropelene tube of 40 cm length was used for collection of whiteflies. The non viruliferous *B.tabaci* culture was maintained on cotton (*Gossypium hirsutum* cv. Varalakshmi (Fig.1) under insect proof glasshouse. Whiteflies were collected by turning the leaves slightly upwards and they were sucked into the glass tube and then transferred to polyvinyal chloride (PVC) bottle.

3.5 Rearing cage for whitefly

Wooden cages of size 45 x 45 x 30 cm were constructed and muslin cloth was fixed on three sides and top with an adhesive, Fevicol. The front portion was closed with glass, which can be easily moved on the grooves made in the wooden framework. This frame was kept on the wooden rectangular base (45.5 x 45.5 x 10cm). In each cage healthy cotton plants grown in polythene bags were kept and pure culture of *B.tabaci* was released.

3.6 Cages used for acquisition access

Whiteflies were collected from the colonies reared in the wooden cages with the help of an aspirator

Fig 1 *B. tabaci* adults feeding on cotton leaf

Fig. 1

and released into a round PVC bottle. The tube measured 23 cm long and 6 cm diameter. The bottom portion was removed with the help of a soldering irons and was covered with muslin cloth. A small hole was made in the muslin cloth to release the whiteflies. The TobLCV infected tobacco branch was inserted into the tube and closed with cotton plug. The whiteflies were then released through the hole and allowed 24 h acquisition access period. After the acquisition access period the whiteflies were released to healthy tobacco seedlings at the rate of 10-15 per seedling in small plastic cylindrical cage of size 7.5x2.5cm for inoculation access of 24 h.

3.7 Tobacco leaf curl virus culture

A tobacco plant showing typical symptoms of TobLCV was collected from Bidi Tobacco Research Station, Nipani (Karnataka). The virus was transmitted to the healthy seedlings of *N. tabacum* cv. Samsun by whitefly *B. tabaci*. The culture of TobLCV was maintained by frequently inoculating healthy Samsun seedlings.

3.8 Transmission of TobLCV isolates

Each of 45 TobLCV isolates were transmitted to *N. tabacum* cvs. Samsun and Anand-119 by *B. tabaci*.

whiteflies were given an acquisition access and inoculation access periods of 24 h each. Ten to fifteen whiteflies were releases ~~enclosed~~ on each 35 day old seedlings. After inoculation access period the whiteflies were killed by spraying 0.2 % dimethoate 30 EC. Inoculated plants were maintained in the insect proof glasshouse and sprayed weekly with monocrotophos (0.2%).

3.9 Host range

3.9.1 Transmission of TobLCV to species of *Nicotiana*

The study was undertaken to determine the intensity of TobLCV and to search for resistance in *Nicotiana* species. Thirteen Nicotiana species ~~belonging to Solanaceae~~ were raised through seeds in the insect proof glasshouse. Seeds of *N. clevelandii*, *N. megalosiphon* and *N. nudicaulis* were sown after treating with 60 ppm of gibberellic acid (GA-3) for 12 h. The whiteflies in large numbers were given an acquisition period of 24 h on diseased tobacco culture. Afterwards 10-15 whiteflies were released on each 35 day old test plant in polythene bag. After the inoculation access period the whiteflies were killed by spraying 0.2% dimethoate 30 EC. The inoculated plants were maintained in insect proof glasshouse for symptom production.

3.9.2 Transmission of TobLCV to tobacco cultivars

Seeds of 28 different tobacco cultivars were sown in nursery seed pans filled with manured soil in insect proof glasshouse. Thirty five day old seedlings were transplanted in polythene bags and used for inoculation after 4-5 days. The whiteflies in large numbers were collected and 24 h acquisition access period was given on infected tobacco. At the rate of 10-15 whiteflies were released on the seedlings. After 24 h of inoculation access period the whiteflies were killed by spraying 0.2% dimethoate 30 EC. The inoculated plants were kept in insect proof glasshouse for symptoms expression.

3.9.3 Transmission of TobLCV to cultivated crops, ornamental plants, weeds and forest plants

Healthy seedlings of cultivated plants (35), ornamental plants (13) weeds (29) and forest plants (11) belonging to 24 families were raised from seeds in the insect proof glasshouse. Seedlings at 2-3 leaf stage were used for inoculation. Ten to twenty seedlings of each species were inoculated with TobLCV. The whiteflies were given 24 h acquisition access and 24 h inoculation access. Ten to fifteen whiteflies were

released per seedling. After 24 h of inoculation access period all the whiteflies were killed by spraying 0.2% dimethoate 30 EC and maintained in the insect proof glasshouse for symptom production.

The plants which did not show any symptoms even after 12-15 weeks of inoculation were subjected to back transmission to the healthy seedlings of *N. tabacum* cv. Samsun by *B. tabaci*.

3.10 Incidence of TobICV and vector population

3.10.1 Incidence of TobICV in relation to planting date

The experiment was carried out at Main Research Station, University of Agricultural Sciences, Bangalore during 1988-89. *N. tabacum* cv. FCV Special was transplanted at a spacing of 90 x 75 cm in an area of 4.5x7.5m plot. The disease incidence and whitefly population were recorded weekly for three months. Whitefly population was estimated by direct count method as described under 3.11.1.

3.10.2 TobICV incidence and whitefly population in relation to weather conditions

Weather data was collected for the period of March 1988 to March 1989 from Main Research Station,

University of Agricultural Sciences, Hebbal, Bangalore, where the experiments were conducted.

3.10.3 Incidence of TobLCV and whitefly population in relation to location of tobacco nursery

Nursery trials were conducted at two locations, one near the tobacco field and another at 1000 m away from the tobacco field. Twelve nursery beds of size 1.2 m long, 1.0 m wide, 0.45 m depth were prepared. Seeds of three *N. tabacum* cvs. FCV Special, Anand-119 and CTRI Special were sown at the rate of 0.2 g/m^2 and beds were covered with paddy straw till germination. Disease incidence was recorded 45 days after sowing by counting diseased and healthy seedlings. Whitefly population was counted at weekly intervals on 25 seedlings per bed upto the nursery period.

3.10.4 Incidence of TobLCV and vector population at different growth stages of tobacco under field conditions

Fortyfive day old tobacco cv. FCV special, Anand-119 and CTRI special were transplanted in plots of 4.5x7.5m size and replicated twice. Spacings of 90 x 75 cm was given for each variety. The incidence of TobLCV and whitefly population were recorded 15, 30, 45, 60, 75, 90, 105 and 120 days after transplanting in the field. Seeds of

all the cultures were sown at the rate of 0.2 g/m^2 in nursery bed on 20.1.89 and seedlings transplanted on 11.9.89 in the field. TobLCV disease incidence and whitefly populations were recorded ~~weekly~~ at different growth stages of tobacco after 15 days of transplanting in field. Whitefly population was counted on 25 plants by direct count method.

3.11 Monitoring of vector population

3.11.1 Direct count

Whitefly adults would be dull and reluctant to fly during the early hours of the day when the atmosphere temperature and the intensity of light are low. Even if the tobacco leaf is lifted slightly between 6.00 to 7.30am they will not be active. This habit of the whitefly was taken into account to estimate the population within the crop area and the counts were made between this period. Adult whiteflies were counted on 25 plants per plot. Periodical counts were made on the whitefly adults by holding each leaf from the petiols with two fingers and gently turning it upside down.

3.11.2 Yellow pan water traps

Plastic dishes (30 cm diameter and 7 cm high)

were painted with bright yellow paint. These plates were kept at ground level in the tobacco field and filled with water. Ten traps of yellow pan water were kept in separate plot for 24 h and whiteflies were recorded.

3.11.3 Detergents in yellow pan water traps

Different detergents viz. Nirma powder (Nirma Chemical Works, Ahmedabad); Surf powder (Hindustan Lever Ltd. Bombay) and Teepol liquid (Organic Chemical Industries Ltd., Bombay) were purchased from the market. Yellow plastic dishes (30 cm diameter and 7 cm high) were kept at ground level in the tobacco plot of 4.5 x 7.5 m and filled with 5 per cent Nirma, Surf and Teepol, separately. Yellow pan water traps were also kept as control along with the detergents and two yellow pan traps were maintained for each treatments. The treatments were replicated four times in a randomized block design. Whiteflies were counted after 24 h at weekly intervals in each pan .

3.12 Management of TobLCV in tobacco nursery

3.12.1 Covering nursery bed with nylon net

Nursery trial was conducted to estimate the TobLCV incidence, prevalence of whiteflies in protected and open

tobacco nursery and to get maximum healthy transplantable tobacco seedlings.

Four nursery beds of size 3 m long and 1.2 m wide, 0.45 m depth were prepared. Tobacco cultivar FCV special was sown at the rate of 0.2 g/m^2 in all beds. Nylon nets (40 mesh) of size 3 m long and 1.2 m wide and 0.45 m depth were prepared (stiched with sewing machine). Nylon nets were placed on bamboo frame and then covered on nursery beds (Fig.2), and retained upto transplanting stage (45 days). Whitefly counts were made on 25 seedlings per bed at weekly intervals. TobLCV incidence was estimated counting diseased and healthy seedlings after 45 days of sowing in all the beds.

3.12.2 Effect of barrier crops

A nursery trial was conducted with barrier crops, sunflower and castor to minimise the TobLCV incidence. Three nursery beds of size 3 m long, 1.2 m wide, 0.45 m depth were prepared and tobacco cv Swarna seeds sown at the rate of 0.2 g/m^2. Seeds of barrier crops were also sown at 45 cm distance around individual nursery bed prior to 15 days of tobacco sowing.

Fig. 2. Tobacco nursery covered with nylon net

Fig. 2

Adult whiteflies were recorded on 20 leaves of barrier plants at weekly intervals. Number of eggs were also counted on 20 randomly selected young leaves of barrier plants under stereo binocular microscope 10 days after sowing.

Whiteflies were also recorded on 25 tobacco seedlings in each bed at every week and TobLCV incidence was estimated after 45 days of nursery period counting healthy and diseased seedlings.

3.13 Detection of TobLCV

3.13.1 Enzyme linked immunosorbent assay (ELISA)

Materials

Polystyrene microtiter plate (Nunc, Denmark and also Cooke Microtiter, Dynatech micro ELISA), micro pipette (50 to 200 µl and 20 µl variable), BIO TEK MICROPLATE AUTO READER, EL 309 (USA), rotor-shaker, laboratory glasswares, magnetic stirrer, polythene bags, marker, pestle and mortar, muslin cloth, buffers, coating antibody, ACMV γ globulin and African Cassava mosaic virus antiserum conjugated with alkaline phosphatase, substrate p-nitrophenyl phosphate (Sigma 104).

Buffers and solutions

1. Coating buffer, pH 9.6
   For 1 Litre

Na_2CO_3	1.59 g
$NaHCO_3$	2.93 g

   pH was adjusted using 6N HCl

2. Phosphate buffer saline (PBS)
   For 1 Litre

NaCl	8 g
Na_2HPO_4, 2 H_2O	1.44 g
KH_2PO_4	0.2 g
KCl	0.2 g

3. PBS - Tween - 20 (PBS-T)
   PBS + 0.05% Tween-20

4. PBS - T- Polyvinyl Pyrrolidone (PVP)
   PBS-T + 2% PVP

5. PBS - T - PVP - Ovalbumin (OA)
   PBS-T-PVP + 0.2% OA

6. Extraction buffer, pH 8

   Tris 0.05 M

   EDTA 0.005 M

   pH was corrected with 6 N HCl. Then, 2% Polyvinyl pyrrolidone (PVP) and 0.05% Tween-20 was added.

7. Substrate buffer, pH 9.8

   10% Diethanolamine

   pH was adjusted with 6 N HCl

Dried milk

Sagar skimmed milk powder was again defatted by steeping in diethylether overnight at 4 to 8°C. Next day defatted milk was air dried at room temperature and used for ELISA as a blocking agent.

Antigen preparation

Top 2-3 leaves from TobLCV inoculated plants were harvested in properly labelled polythene bags. Tissues were grounded in extraction buffer (10 ml buffer/1g tissue) with the help of pestle and mortar. Immediately before adding test antigen to the plates homogenates were filtered in petri plates using muslin cloth.

Procedure of ACMV Polyclonal direct-DAS-ELISA

DAS-ELISA was carried out with little modification of the technique as described by Clark and Adams(1977). Detailed procedures are-

1. ELISA plates were coated with ACMV- γ globulin (1 μgm^{-1}) in coating buffer. 100 μl/well was added. Margin row of each side of the plate was left as blank. Plates were incubated covering with lids, at room temperature(27°C) for 3 h.

2. Washing of plates: After 3 h incubation plates were washed with PBS-T. Three quick washes and three washes at 3 min intervals were followed. In the first three quick washes, care was taken so that washing buffer could not spill over. In the next three washes plates were flooded with washing buffer. After sixth wash plates were dried by shaking over a tissue paper pad.

3. Crude leaf extracts of each samples (test antigens) prepared in extraction buffer, were added into wells at the rate of 100 µl/well. Plates were covered with the lids and incubated overnight at 4°C.

4. Plates were emptied and followed by 3 quick and 3 x 3 min washing with PBS-T.

5. Wells were blocked with 5% dried milk(200 µl/well) in PBS-T-PVP. Plates were covered with lids and were incubated for 30 min at room temperature.

6. Milk was shaken off and excess milk was removed keeping the plates upside down on tissue paper (no washing).

7. ACMV polyclonal conjugate (with alkaline phosphatase diluted at the ratio of 1:1000 in PBS-T-PVP-OA was added at the rate of 100 µl/well. Plates were covered with lids and were incubated for 3 h at the room temperature.

8. Plates were washed with PBS-T in the same way.

9. Substrate was freshly prepared dissolving P-nitrophenyl phosphate (0.6 mg/ml) in substrate buffer. 150 µl/well was added. Substrate container was covered with aluminium foil to avoid auto-photo-degradation of p-nitrophenyl phosphate. Plates were then covered with lids and incubated at room temperature on rotor shaker.

10. Absorbance was recorded within 2 to 2½ h at A_{405} nm by biotek microplate auto reader(EL 309).

3.13.2 Immunosorbent electron microscopy technique (ISEM)

Materials

TobLCV infected tissues, plastic capsules, carborundum, comodrill, centrifuge, grids (carbon coated), petridish, toothpick, plastic wells, filter paper, forceps, scissors, pasteure pipette, micro pipette, incubator, parafilm coated slides, Tris EDTA buffer of 0.1 M and pH 8.0; sodium phosphate buffer of 0.07 M/ pH 6.5, Indian cassava mosaic virus antiserum produced at University of Agricultural Sciences, Bangalore, 2% Uranyl acetate, electron microscope and carbon evaporator.

Preparation of carbon coated grids

A clean mica sheet was kept in vaccum evaporator and after getting carbon film coated on it, the film was stripped off in a glass jar containing water. Individual copper grid was first brought underneath the carbon film and then lifted upward along with the film of carbon and was placed on a Whatman filter paper. Coated grids were dried in a desiccator.

ISEM technique

ISEM technique was carried out by the methods described by Roberts and Harrison (1979). Detailed procedure is given below:

Preparation of Sample

1. Small quantity of TobLCV infected leaf sample (200 mg) was placed in a small plastic beam capsule.
2. Pinch of carborundum was then added.
3. Three drops of 0.01 M Tris-EDTA pH 8.0 was then added.
4. Infected leaves were then ground within the capsule with the help of comodrill for 1-2 min.

5. Again four drops of 0.01 M Tris EDTA were added and ground.

6. Then it was centrifuged at 8,000 g for 15 min and supernatant was used for ISEM.

Preparation of ICMV antibody coated grids (AC grids)

1. 10 µl drop of ICMV (Indian cassava mosaic virus) antiserum diluted at 1:1000 in 0.07 M sodium phosphate buffer pH 6.5, were placed on a para-film coated slide kept in a petridish containing a moist filter paper.

2. Then, carbon coated grids were floated on 10 µl ICMV antiserum (carbon film side down) and incubated for one hour at 37°C within a moist petridish.

3. Grids were then washed by floating twice for 10 min in a plastic well containing sodium phosphate buffer of 0.07 M and pH 6.5.

4. Excess buffer was drained briefly by touching a filter paper strips. Then ICMV antibody coated grids (ICMV-ACG) were ready for loading the test sample.

ISEM Procedure

1. 10 µl drops of test sample (leaf extract) was placed on a slide covered with parafilm kept in a petri dish containing a moist filter paper.

2. ICMV-ACG grids were placed on those 10 µl drops of test samples, petri dish was then covered and incubated for 2 h, 4 h and 24 h at 4°C.

3. Control grids were also prepared by placing the ICMV-ACG grids on 10 µl drop of healthy tobacco extract.

4. After the prescribed incubation period grids were removed from the drop of extracts and were washed with 3-4 drops of double distilled water followed by 3-4 drops of 2% uranyl acetate.

5. Excess stain was drained by touching briefly with a filter paper strip. The grids were examined in a transmission electron microscope (JEOL 100 S Model).

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

4.1 Survey for the incidence of tobacco leaf curl virus

Surveys were undertaken to assess the incidence of TobLCV in Andhra Pradesh, Karnataka, Gujarat, West Bengal and Bihar states as described under materials and methods. During the course of survey the percentage of TobLCV incidence and adult whitefly population were recorded and the data are presented in Table 1. The maximum incidence of disease was observed in cigarette and bidi tobacco growing areas of Andhra Pradesh(77.6%) and Gujarat (59.4%), respectively. The data indicated that TobLCV incidence varied with the locations and tobacco cultivars in India (Fig. 3). The whitefly population was also varied in a similar manner. The maximum incidence of disease was found to be 77.6 per cent in Guntur (Andhra Pradesh), followed by East-Godavari District (75.6%) and Rajahmundry (72.2%)(Andhra Pradesh) Baroda (59.4%) and Anand (53.4%) (Gujarat), Nipani (17.1%) (Karnataka), Pusa (11.6%) (Bihar), Dinhata (5.4%) (West Bengal), Hunsur(3.2%) and Shimoga (1.2%) (Karnataka) (Table 1, Fig. 3, 4). More number of white-flies were recorded in Guntur (32 whiteflies/plant),

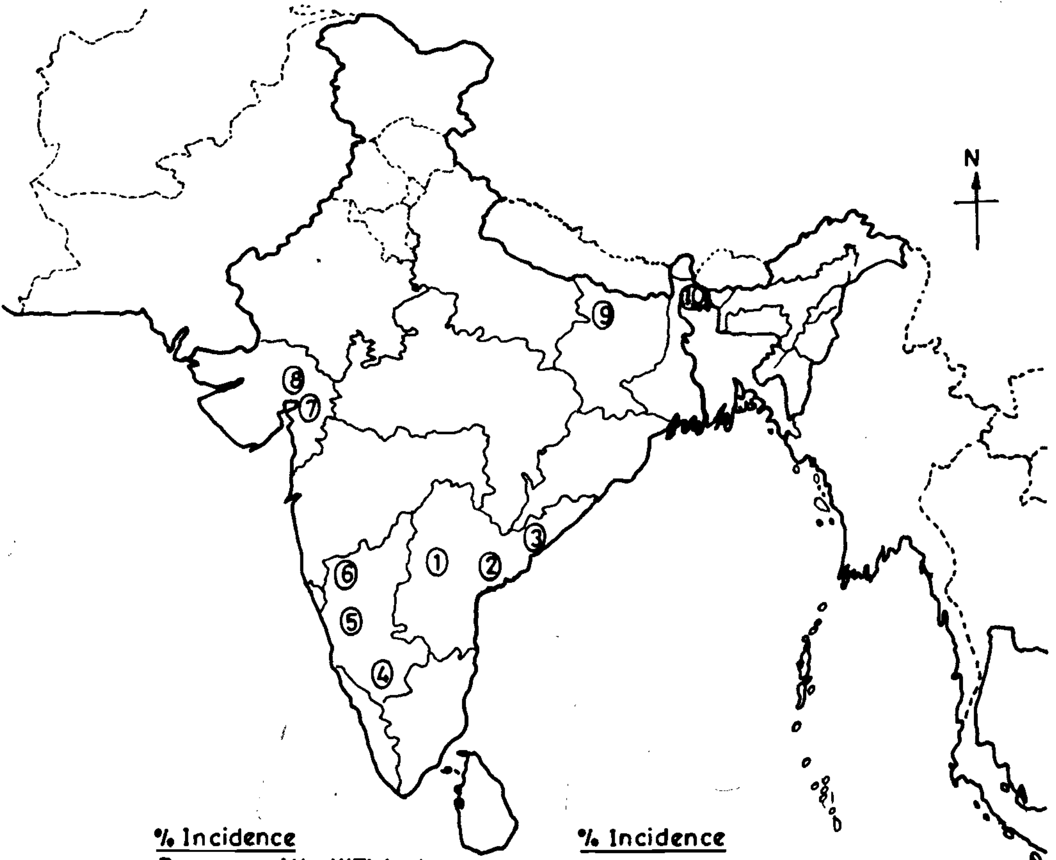
Table 1. Survey for the TobLCV incidence and whitefly population in tobacco fields during 1988-89

Place	State	Month of survey		% TobLCV[a]		Whitefly population[c]
				Mean	(Range[b])	
Guntur	Andhra Pradesh	January	88	77.6	(27-100)	32
Rajahamundry	Andhra Pradesh	January	88	72.2	(22-100)	22
East Godavari Dt.	Andhra Pradesh	January	88	75.6	(24-100)	24
Hunsur	Karnataka	August	88	3.2	(2- 8)	2
Shimoga	Karnataka	September	88	1.2	(0.6-1.5)	1
Nipani	Karnataka	January	88	17.1	(8.2-27)	12
Baroda	Gujarat	April	89	59.4	(57- 62)	20
Anand	Gujarat	April	89	53.4	(38- 72)	18
Pusa	Bihar	February	89	11.6	(6- 22)	8
Dinhata	West Bengal	February	89	5.4	(2- 12)	5

[a] TobLCV incidence recorded in 10 m^2 area each of 5 sectors

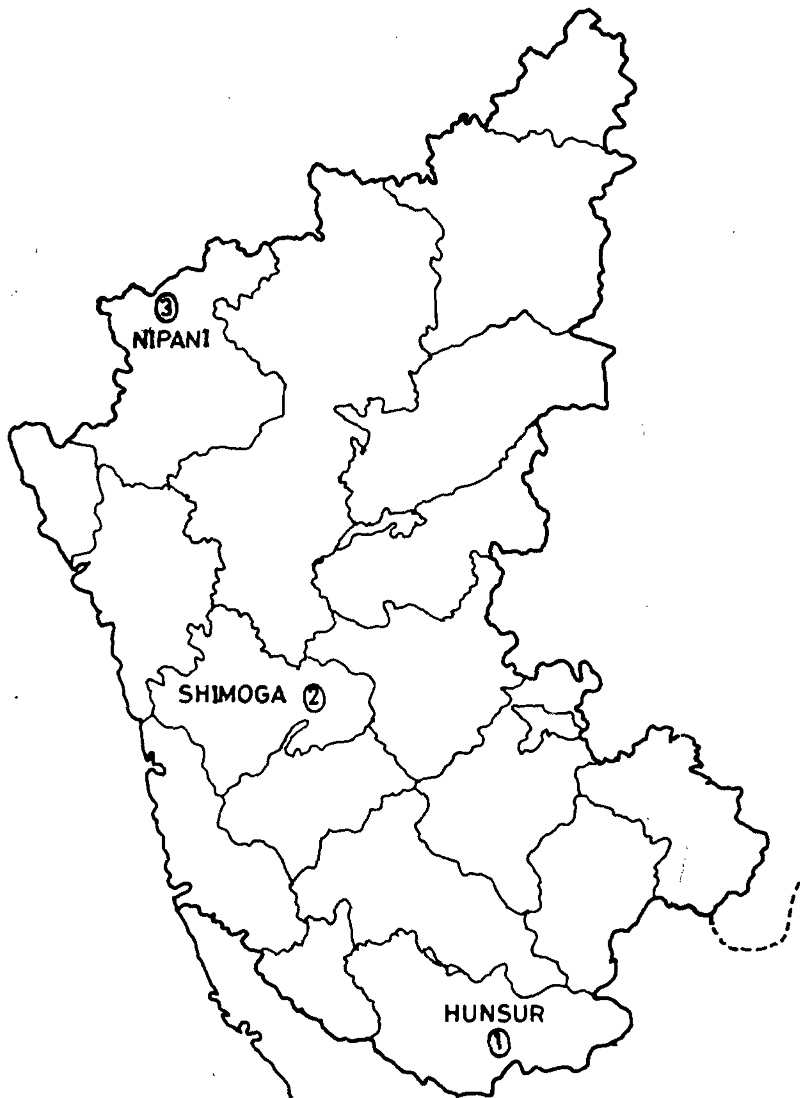
[b] Range of incidence recorded from 5 to 8 fields in each locality

[c] Whitefly population, average of 25 plants



	% Incidence Range	% Incidence AV	WF/plant
1. Guntur	27-100	77·6	32
2. Rajahamunry	22-100	72·2	22
3. East Godavari	24-100	75·6	24
4. Hunsur	2-8	3·2	2
5. Shimoga	0·6-1·5	1·2	1
6. Nipani	8·2-27	17·1	12
7. Baroda	57-62	59·4	20
8. Anand	38-72	53·4	18
9. Pusa	6-22	11·6	8
10. Dinhata	2-12	5·4	5

FIG.3 INCIDENCE OF TOBACCO LEAF CURL VIRUS AND WHITEFLY POPULATIONS IN DIFFERENT STATES OF INDIA



	% Incidence Range	% Incidence AV	WF/plant
1. Hunsur	2-8	3·2	2
2. Shimoga	0·6-1·5	1·2	1
3. Nipani	8·2-27	17·1	12

FIG. 4 INCIDENCE OF TOBACCO LEAF CURL VIRUS AND WHITEFLY POPULATIONS IN KARNATAKA STATE

East Godavari Districts(24/plant) and Rajahmundry(22/plant) of Andhra Pradesh. The minimum vector population was recorded in Shimoga (1/plant) and Hunsur (2/plant) of Karnataka followed by Dinhata of West Bengal(5/plant) (Table 1, Figs. 3,4).

4.1.1 TobLCV incidence and vector population in relation to weather conditions of major tobacco growing areas

The weather data from different tobacco growing areas under survey were collected and presented in Tables 2, 3, 4. The data revealed that Guntur(Andhra Pradesh) and Anand (Gujarat) were the hottest places. The low temperature and high rainfall were noticed in Dinhata (West Bengal) and Pusa area (Bihar). The weather condition was moderate in Shimoga and Hunsur area (Karnataka), whereas the temperature was high at Nipani during crop period.

4.2 TobLCV incidence on different host species under field conditions

4.2.1 On different tobacco cultivars

Different tobacco growing areas of Andhra Pradesh, Karnataka, Gujarat, West Bengal and Bihar States were visited and the per cent TobLCV incidence was recorded

Table 2. Weather Data of the different tobacco growing areas during 1988-89

Month and year	Guntur						Hunsur						Shimoga					
	Temperature °C		R H %		Rainfall	Rainy	Temperature °C		RH %		Rainfall	Rainy	Temperature °C		RH %		Rain-fall	Rainy
	Max.	Min.	0720 h	1420 h	mm	days	Max.	Min.	0720 h	1420 h	days	days	Max.	Min.	0720 h	1420 h	mm	days
1988																		
January	32.0	16.5	85	76	-	-	28.2	13.9	84	38	0.0	0	30.6	14.5	70.4	36.8	-	-
February	33.1	19.6	88	64	-	-	31.9	17.0	80	30	11.2	1	33.7	16.8	72.5	30.3	5.1	1
March	35.8	23.2	88	58	42.6	2	33.9	17.1	84	28	56.4	2	36.6	19.0	71.6	29.5	-	-
April	37.1	25.7	87	59	1.4	1	33.0	20.9	84	40	281.0	6	36.3	18.7	79.7	37.3	37.8	3
May	41.8	28.1	80	61	28.4	3	33.7	20.3	72	45	331.9	4	36.7	21.4	78.4	41.0	20.6	2
June	39.7	25.6	80	60	567.6	5	29.0	18.3	86	61	82.2	3	31.2	21.5	82.7	54.7	16.6	-
July	33.4	27.2	89	74	262.4	15	27.8	18.6	88	71	107.7	8	29.5	21.2	85.2	71.5	122.0	6
August	33.4	25.2	88	77	328.3	17	27.6	19.0	91	70	143.4	11	28.6	20.9	89.0	74.0	129.4	7
September	32.8	25.1	89	78	160.8	7	27.8	19.1	88	66	93.5	8	28.9	20.8	80.3	70.3	85.4	7
October	33.2	24.2	90	79	24.9	4	29.3	18.2	89	52	58.4	6	30.4	19.6	86.5	50.0	9.8	-
November	32.9	21.5	88	76	13.0	1	29.0	16.2	79	42	7.8	1	32.1	16.3	81.4	35.3	-	-
December	29.6	18.1	86	79	39.1	3	28.3	15.0	77	39	1.0	0	29.5	15.2	76.0	39.1	37.2	1
1989																		
January	30.0	16.9	87	68	-	-	29.0	14.2	76	33	0.0	0	30.9	14.4	71.4	33.4	-	-
February	32.7	18.7	87	69	-	-	31.8	14.3	70	23	0.0	0	33.8	15.4	75.3	29.7	-	-

Table 3. Weather data of the different tobacco growing areas during 1988-89

Month and year	Nipani						Anand					
	Temperature °C		R.H. %		Rainfall mm	Rainy days	Temperature °C		R.H. %		Rainfall mm	Rainy days
	Max.	Min.	0720h	1420h			Max.	Min.	0720h	1420h		
1983												
January	30.4	12.8	85	48	-	-	30.1	12.2	78	46	-	-
February	34.0	14.1	74	47	-	-	32.9	14.3	64	26	-	-
March	36.1	16.5	52	23	-	-	35.4	16.3	63	23	-	-
April	36.8	16.6	56	24	-	-	39.9	22.0	39	22	-	-
May	35.2	18.0	53	35	8.0	3	41.1	24.9	68	28		
June	30.7	22.4	82	59	79.0	9	37.9	26.8	73	45	26	2
July	27.4	21.5	90	74	265.4	16	37.4	25.0	88	75	664	7
August	27.0	21.1	91	79	180.0	13	37.9	25.2	87	69	875	9
September	28.8	21.3	90	66	71.6	10	33.9	25.3	84	63	884.3	8
October	31.8	16.5	77	46	7.2	1	34.9	20.9	76	45	-	-
November	30.7	13.0	74	56	-	-	34.1	14.1	72	38	-	-
December	28.6	13.1	69	37	18.6	2	31.0	12.1	73	35	-	-
1989												
January	30.6	12.1	66	27	-	-	29.9	9.1	72	39	4.0	1
February	33.4	12.6	53	15	-	-	30.3	17.4	73	32	-	-

Table 4. Weather data of the different tobacco growing areas during 1988-89

Month and year	Pusa						Dinhata					
	Temperature °C		R.H. %		Rainfall mm	Rainy days	Temperature °C		R.H. %		Rainfall mm	Rainy days
	Max.	Min.	0720h	1420h			Max.	Min.	0720h	1420h		
1988												
January	24.0	8.5	88	45	-	-	24.8	6.1	87	51	4	1
February	26.5	9.5	84	36	12.7	3	27.1	9.7	86	51	121.2	4
March	31.0	13.5	70	32	-	-	29.4	14.0	84	47	88.0	4
April	36.0	20.1	68	38	28.6	3	32.3	18.3	86	57	145.4	7
May	36.7	24.0	72	45	22.1	4	31.2	20.2	88	67	376.2	14
June	34.2	25.0	72	54	203.6	12	31.9	22.5	86	72	387.2	14
July	33.2	25.8	84	66	310.4	19	31.8	22.7	86	77	763.3	19
August	32.1	25.3	83	70	298.0	19	31.0	22.9	91	80	1196.2	24
September	31.9	24.2	79	62	258.7	11	31.4	21.6	91	80	555.6	19
October	33.1	21.5	83	43	35.8	3	31.4	19.5	88	68	69.8	6
November	30.7	13.9	87	35	-	-	28.7	13.4	86	61	37.7	3
December	23.5	8.3	91	40	15.1	1	26.1	10.5	87	52	1.0	1
1989												
January	23.0	6.8	83	36	-	-	23.1	6.1	87	51	7.4	2
February	26.1	9.1	81	37	15.4	1	24.4	7.6	86	47	12.2	1

on tobacco cultivars under field conditions. The data presented in Table 5 indicate that all the tobacco cultivars are found susceptible to TobLCV, irrespective of plants and cultivars. The incidence varied from 1 to 90 per cent depending on the cultivar and the place. The highest incidence was noticed in cigarette tobacco (90%) and bidi tobacco (45%) of Andhra Pradesh and Gujarat respectively (Table 5). The incidence of TobLCV on different cultivars varied from 1 to 10.2 per cent in Karnataka.

4.2.2 On cultivated and weed host plants

Incidence of TobLCV on cultivated and weed host plants of major tobacco growing areas are presented in Tables 6, 7, 8. The data reveal that causal agent survived on cultivated and weed host plants round the year in and around the tobacco field with varying degree of infection. TobLCV infects *Lycopersicon esculentum* and *N. tabacum* wherever these plants were grown. TobLCV was common on *Acanthospermum hispidum*, *Ageratum conyzoides*, *Datura stramonium* and *Parthenium hysterophorus* in many places in India, thus serving as source of infections to tobacco crop. Other weed hosts *Acalypha indica*, *Euphorbia geniculata*, *Nicandra physaloides*, *Phyllanthus niruri*,

Table 5. TobLCV incidence on tobacco cultivars under field conditions

Place	Cultivars	Recorded days after trans-planting	% TobLCV[a]
1	2	3	4
Guntur[b] (Andhra Pradesh)	*Nicotiana tabacum* cv. Candal	52	47.5
	N. tabacum cv. CTRI Sp	52	85.0
	N. tabacum cv. G.8-1	52	82.0
	N. tabacum cv. G.8-3	52	90.0
	N. tabacum cv. G.11-1	52	65.0
	N. tabacum cv. Godavari sp	52	82.0
	N. tabacum cv. Jayasri	52	85.0
	N. tabacum cv. Kankprabha	52	92.0
	N. tabacum cv. Oxford-3	52	57.5
	N. tabacum cv. PCT-5	52	61.0
	N. tabacum cv. 16-103	52	73.0
Hunsur[c] (Karnataka)	*N. tabacum* cv. Bhavya	95	5.0
	N. tabacum cv. Coker-254	95	3.3
	N. tabacum cv. FCN-6101	95	5.0
	N. tabacum cv. FCV-Sp	95	6.6
	N. tabacum cv. NC-95	95	5.0
	N. tabacum cv. Sp.G-28	95	6.6
	N. tabacum cv. Swarna	95	5.0
	N. tabacum cv. VA-145	95	3.3

Table 5 (continued)

Place	Cultivars	Recorded days after trans-planting	% TobLCV[a]
1	2	3	4
Shimoga[d] (Karna-taka)	*N.tabacum* cv. FCV.sp.	90	1.0
Nipani[e]	*N.tabacum* cv. Anand-119	140	10.2
(Karnataka)	*N.tabacum* cv. Anand-2	140	9.1
	N.tabacum cv. GT.5	140	5.4
	N.tabacum cv. PL-5	140	8.4
Anand[f]	*N.tabacum* cv. A.119	210	45.0
(Gujarat)	*N.tabacum* cv. GT.5	210	38.0
Baroda[g] (Gujarat)	*N.tabacum* cv. A.119	210	42.0
Dinhata[h]	*N.tabacum* cv. Chama	110	7.0
(West	*N.tabacum* cv. Podali	110	8.4
Bengal)	*N.rustica* cv. Bitri	100	4.0
	N.rustica cv. Hemti	100	2.0
Pusa[i]	*N.tabacum* cv. Bendi	115	10.0
(Bihar)	*N.tabacum* cv. Sona	115	13.7
	N.tabacum cv. Sona	100	7.0
	N.tabacum cv. Sona	85	3.0

[a]TobLCV incidence in 10 m^2 area

Date of recordings of TobLCV incidence

[b]30.12.87 [c]28.8.88 [d] 2.9.88 [e] 8.1.88 [f]15.4.89

[g]17.4.89 [h]23.2.89 [i]27.2.89

Table 6. Incidence of TobLCV on cultivated and weed host plants in tobacco growing areas of Karnataka state

Host	% TobLCV incidence[a]			
	Bangalore	Hunsur	Shimoga	Nipani
Cultivated Hosts				
Carica papaya	1.0[b]	-	-	1.0
Crotalaria juncea	0	0	-	2.1
Cymopsis tetragonoloba	0	0.5	0	1.8
Lycopersicon esculentum	50.0[b]	20.8[b]	4.0	19.2
Sesamum indicum	2.8	0	8.0	0
Nicotiana tabacum	34.8[b]	5.0[b]	1.5	16.8[b]
Zinnia elegans	4.3[b]	2.4	0	0.5[c]
Weed Hosts				
Acanthospermum hispidum	13.0[b]	3.4	0	3.5[b]
Acalypha indica	0	-	0	4.1
Ageratum conyzoides	14.5[b]	6.2	1.0	3.7[b]
Datura stramonium	18.0[b]	2.2[b]	0	2.6
Euphorbia geniculata	21.0[b]	3.8	0	7.5
Nicandra physaloides	16.0[b]	0	0	0
Parthenium hysterophorus	25.8[b]	1.5	4.0	16.2[b]
Stachytarpheta indica	0	1.0	0	-
Synedrella nodiflora	13.2	0	0	12.6
Oxalis corniculata	4.5[b]	0	0	0

[a]Incidence of TobLCV recorded from 10 m^2 area

[b]TobLCV antigen was detected in plant extracts by ELISA

Not recorded

Table 7. Incidence of TobLCV on cultivated and weed host plants in tobacco growing areas of Gujarat

Host	% TobLCV incidence[a]	
	Anand	Baroda
Cultivated Hosts		
Carica papaya	3.2[b]	2.5
Crotalaria juncea	1.8	0
Cymopsis tetragonoloba	0.6	2.8
Lycopersicon esculentum	38.5[b]	29.0[b]
Sesamum indicum	-	0
Nicotiana tabacum	56.0[b]	58.0[b]
Zinnia elegans	2.2[b]	-
Weed Hosts		
Acanthospermum hispidum	23.8	25.4
Ageratum conyzoides	11.6	10.1
Datura stramonium	2.2	1.2
Euphorbia geniculata	15.5	6.9
Nicandra physaloides	4.6	8.2
Parthenium hysterophorus	32.8	20.9
Phyllanthus niruri	15.3	6.5
Synedrella nodiflora	2.8	3.5
Oxalis corniculata	1.2	-

[a] Incidence of TobLCV recorded from 10 m^2 area

[b] TobLCV antigen was detected in plant extracts by ELISA

\- Not recorded

Table 8. Incidence of TobLCV on cultivated and weed host plants in tobacco growing area of West Bengal and Bihar

Host	% TobLCV incidence[a]	
	Dinhata	Pusa
Cultivated Hosts		
Carica papaya	0	0
Crotalaria juncea	-	0
Cymopsis tetragonoloba	0	-
Lycopersicon esculentum	4.8[b]	6.2[b]
Sesamum indicum	-	-
Nicotiana tabacum	8.4[b]	13.8[b]
Weed Hosts		
Acanthospermum hispidum	2.5[b]	3.8
Ageratum conyzoides	5.0	4.3[b]
Datura stramonium	1.6	-
Parthenium hysterophorus	4.5[b]	3.8[b]
Croton bonplandianum	0	0
Synedrella nodiflora	3.6[b]	-
Oxalis corniculata	4.2	2.5[b]

[a] Incidence of TobLCV recorded from 10 m^2 area

[b] TobLCV antigen was detected in plant extracts by ELISA

\- Not recorded

Stachytarpheta indica, *Synedrella nodiflora* and *Oxalis corniculata* found infected with TobLCV in the field.

4.3 Isolates of TobLCV

4.3.1 Collection of isolates of TobLCV

Altogether 45 isolates of TobLCV were collected during the survey from Andhra Pradesh(7), Karnataka(19), Gujarat (12), West Bengal (4) and Bihar (3) (Table 9).

4.3.2 Symptoms of isolates of TobLCV under field conditions

The symptoms of TobLCV isolates under field condition were characterized by stunting of plant, stem zig zag, reduction in leaf size, severe curling and crinkling of leaves, ruffling of leaves and shortening of internode. Foliage colour dark green to pale yellow, vein thickening and zig zag, greening of veins and depressions on upper leaf surface. Small scattered cup shaped enations on veins. Inflorescence was severely affected. The symptoms of each isolates of TobLCV are described in Table 9.

4.3.3 Grouping of TobLCV isolates based on symptoms after transmitting by *B. tabaci* to Samsun and Anand 119

Transmission of 45 TobLCV isolates were performed

Table 9. List of TobLCV isolates collected from different tobacco growing areas

TobLCV isolate No.	Place	Date of collection	Symptoms observed in field
1	2	3	4
BNG. 1	Bangalore (Karnataka)	31. 1.88	E,C,DC,VT,RL,SZ (Fig. 5)
BNG. 2	Bangalore (Karnataka)	31. 1.88	VT, C, E, D, RL
BNG. 3	Bangalore (Karnataka)	31. 1.88	C,E,RI, RL,DC, VT
BNG. 4	Bangalore (Karnataka)	31. 1.88	RL, VT, VZ, UC, E
AND. 5	Anand (Gujarat)	15.12.88	RL, DC, VZ, C, E (Fig. 6)
AND. 6	Anand (Gujarat)	15.12.88	RL. DC, VZ, C, E
GNT. 7	Guntur (Andhra Pradesh)	1. 1.88	SP, RL,VT, E, LLB
GNT. 8	Guntur (Andhra Pradesh)	1. 1.88	GV, VT, RI, RL, E
GNT. 9	Guntur (Andhra Pradesh)	1. 1.88	SZ,FPG,VT,DC,D,C,E
GNT.10	Guntur (Andhra Pradesh)	1. 1.88	LLB,RL,UC,VT,RL,E (Fig. 7)
RAJ.11	Rajahamundry (Andhra Pradesh)	2. 1.88	GV,RL,VT,RI,E,SZ (Fig. 8)
RAJ.12	Rajahamundry (Andhra Pradesh)	2. 1.88	VT,FPG,DC,VZ, D, E
EDG.13	East Godavari (Andhra Pradesh)	2. 1.88	VT,UC,RL, GV, E (Fig. 9)
NIP.14	Nipani (Karnataka)	9. 1.88	DC,RL,RI,GV,C,E,FPG (Fig.10)
NIP.15	Nipani (Karnataka)	9. 1.88	GV,DC,VZ,VT,C,E

Table 9 (continued)

1	2	3	4
NIP.16	Nipani (Karnataka)	9. 1.88	UC,VT,VZ,GV, LLB
AND.17	Anand(Gujarat)	10. 8.88	UC,GV,VZ,LLB,E,D
AND.18	Anand(Gujarat)	10. 8.88	DC,GV,VZ,LLB,E,D,FPG (Fig.11)
AND.19	Anand(Gujarat)	11. 8.88	SZ,LLB,VT,VZ,DC,C,E
AND.20	Anand(Gujarat)	11. 8.88	VT,VZ,RL,LLB, GV
BRD.21	Baroda(Gujarat)	12. 8.88	SZ,DC,VZ,VT,E,C, D (Fig.12)
HUN.22	Hunsur (Karnataka)	29. 8.88	FPG,LLB, VT, VZ, D (Fig.13)
HUN.23	Hunsur (Karnataka)	29. 8.88	VT,VZ,GV, LLB, E
HUN.24	Hunsur (Karnataka)	30. 8.88	VT,VZ,UC, FPG, E
SHI.25	Shimoga (Karnataka)	1. 9.88	SP,RL,VT,VZ,DC,C,E
SHI.26	Shimoga (Karnataka)	1. 9.88	DC,C,VT,VZ,DC,C, E (Fig.14)
SHI.27	Shimoga (Karnataka)	1. 9.88	UC, GV, VT, E, D
NIP.28	Nipani (Karnataka)	5. 9.88	SP,VT,VZ,DC,GV,RI,ECS
NIP.29	Nipani (Karnataka)	5. 9.88	VT,SP,DC,LLB,ECS,SZ (Fig.15)
NIP.30	Nipani (Karnataka)	5. 9.88	UC, VT, LLB, D, E
NIP.31	Nipani (Karnataka)	6. 9.88	VT,DC,ECS,LLB, C,D
NIP.32	Nipani (Karnataka)	6. 9.88	SP,VT,VZ,UC,E,D

Table 9 (continued)

1	2	3	4
NIP.33	Nipani (Karnataka)	6.9.88	RI, RL, VT, DC, E
DIN.34	Dinhata (West Bengal)	22.2.88	FPG, LLB, VT, RL, E, D
DIN.35	Dinhata (West Bengal)	22.2.88	SP, VT, VZ, ECS, C, GV
DIN.36	Dinhata (West Bengal)	22.2.88	VT, RL, RI, LLB, D, E (Fig.16)
DIN.37	Dinhata (West Bengal)	23.2.89	UC, VT, VZ, RL, SP, E
PUS.38	Pusa (Bihar)	26.2.89	SP, VT, VZ, RL, GV, ECS, SZ (Fig.17)
PUS.39	Pusa (Bihar)	26.2.89	VT, RL, GV, SP, ECS, D, C
PUS.40	Pusa (Bihar)	26.2.89	VT, RL, LLB, E, UC
AND.41	Anand (Gujarat)	14.4.89	SP, VT, VZ, ECS, GV, C
AND.42	Anand (Gujarat)	14.4.89	RL, VT, DC, VZ, E
AND.43	Anand (Gujarat)	16.4.89	RI, RL, VT, UC, E, D
AND.44	Anand (Gujarat)	16.4.89	SP, VT, DC, ECS, D, C
BRD.45	Baroda (Gujarat)	17.4.89	VT, VZ, RL, GV, LLB, E

C= Crinkling (crumpling); D= Depression; E= Enation,
UC=Upward curling DC= Downward curling;
GV=Greening of veins, R= Reduction of internode,
RL=Reduction in leaf size; SP=Stunting of plant;
SZ=Stem zig zag; VT= Vein thickening;
VZ=Vein zig zag; ECS= Enation cup shape;
FPG=Foliage pale green; LLB= Leaves leathery and brittle

Fig.5. TobLCV isolate Bangalore 1 showing curling and dwarfing of leaves, stem zig zag, vein thickening and enation

Fig.6. TobLCV isolate Anand 5 showing curling, leaves leathery and brittle, greening of veins and enation

Fig. 5

Fig. 6

Fig.7 TobLCV isolate Guntur 10 showing reduction in leaf size, vein thickening, curling, leathery and brittle leaves and enation

Fig.8 TobLCV isolate Rajahamundry 11 showing crinkling and dwarfed leaves, vein thickening, reduction of inflorescences and stem zig zag.

Fig.9 TobLCV isolate East Godavari 13 showing vein thickening, curling, greening of vein and enation.

Fig. 7

Fig. 8

Fig. 9

Fig.10 TobLCV isolate Nipani 14 showing downward curling, foliage pale green, vein thickening, zig zag, depressions and enation

Fig.11 TobLCV isolate Anand 18 showing foliage pale green, depressions, vein thickening and enation

Fig.12 TobLCV isolate Baroda 21 showing dark green foliage, vein thickening, zig zag, curling and enation

Fig. 10

Fig. 11

Fig. 12

Fig.13 Tob LCV isolate Hunsur 22 showing leaf narrowing, foliage pale green, vein thickening and leaves leathery and brittle

Fig.14 TobLCV isolate Shimoga 26 showing vein thickening, zig zag, curling, greening of vein and enation

Fig. 13

Fig. 14

Fig.15 TobLCV isolate Nipani 29 showing severe vein thickening, depression, curling, enation cup shape and leaf brittle and leathery

Fig.16 TobLCV isolate Dinhata 36 showing reduction of leaves and internode, curling, vein thickening and depression

Fig.17 TobLCV isolate Pusa 38 showing stunting of plant, stem zig zag, vein thickening, depression and cup shape enation

Fig. 15

Fig. 16

Fig. 17

by B. tabaci to N. tabacum cvs. Samsun and Anand-119 as described in materials and methods. According to the variation in symptoms expression on tobacco hosts, they were grouped into four and designated as TobLCV group I, II, III and IV. The symptoms of each group are described below.

TobLCV group-I: Plant height reduced, curling and uneven ruffling of leaves followed by shortening of internode. The leaves remain leathery and brittle. Greening and vein thickening resulting to depression on the upper surface of leaves. Numerous prominent leafy outgrowths (enations) developed in the form of stalked and sessile cup shape along the veins. Inflorescences reduced greatly (Figs.18 a,b; Tables 10, 11).

TobLCV group II: Terminal young leaves remain pale green in colour. Reduction of leaf size and shortening of internode were the initial symptoms. The typical symptoms were, the formation of thorny (spiny) erect enations on the marginal veins of leaves. The pit like depressions developed on the upper surface of the leaves. Rarely small pin head enations occurred on the secondary veins (Figs.19 a,b; Tables 10, 11).

Fig.18 TobLCV infected tobacco cvs.(a) Samsun and (b) Anand-119 leaves showing vein thickening, zig zag, greening of veins and stalked and sessile cup shape enation (Group I)

Fig.19 TobLCV infected tobacco cvs.(a) Samsun and (b) Anand-119 leaves showing thorny enation on marginal veins and vein thickening (Group II)

Fig. 18 a

Fig. 18 b

Fig. 19 a

Fig. 19 b

Fig 18a

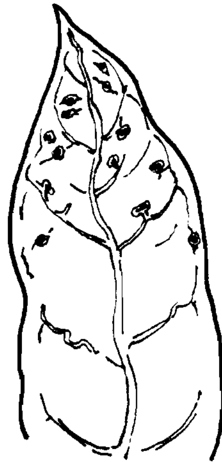


Fig 18b

Fig 19a

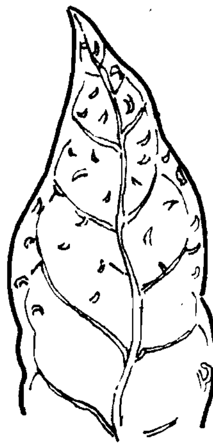


Fig 19b

Table 10. Transmission of TobLCV isolates(1-45) to *N.tabacum* cvs. Samsun and Anand-119

Sl. No.	Isolate number	Place	Symptoms exhibited in *N.tabacum* cvs. Samsun and Anand-119	Symptoms group
1	2	3	4	5
1	BNG. 1	Bangalore	RL, VT, SP, GV, ECS, LLB, C	I
2	BNG. 2	Bangalore	SI, RL, VT, DM, ET, FPG	II
3	BNG. 3	Bangalore	SP, VT, GV, SI, ECS, C	I
4	BNG. 4	Bangalore	RL, GV, IVS, IVT, EFS	IV
5	AND. 5	Anand	SP, VT, GV, ECS, C	I
6	AND. 6	Anand	RL, SP, VT, LLB, ECS, C	I
7	GNT. 7	Guntur	GV, RL, IVS, IVT, EFS	IV
8	GNT. 8	Guntur	VT, IR, LN, ETP, GV, LLB	III
9	GNT. 9	Guntur	SP, RL, VT, GV, ECS, C	I
10	GNT.10	Guntur	SP, VT, ECS, RL, LLB, C	I
11	RAJ.11	Rajahamundry	RL, VT, IR, GV, LN, ETP	III
12	RAJ.12	Rajahamundry	SI, DM, VT, ET, FPG	II
13	EDG.13	East Godavari	SI, RL, VT, DM, ET, FPG	II
14	NIP.14	Nipani	SP, RL, GV, VT, IR, ECS	I
15	NIP.15	Nipani	RL, SI, ECS, LLB, IR, C	I
16	NIP.16	Nipani	IVT, RL, GV, EFS, IVS, LLB	IV
17	NIP.17	Nipani	VT, DM, RL, FPG, ET	II

Table 10 (continued)

1	2	3	4	5
18	NIP.18	Nipani	RL, ET, VT, DM, FPG	II
19	NIP.19	Nipani	VT, ECS, SP, IR, GV, C	I
20	AND.20	Anand	GV, RL, IVT, IVS, EFS, C	IV
21	BRD.21	Baroda	SP, VT, RL, GV, ECS, SI, C	I
22	HUN.22	Hunsur	LN, ETP, VT, GV, RL, LLB	III
23	HUN.23	Hunsur	GV, RL, EFS, IVS, IVT	IV
24	HUN.24	Hunsur	EFS, GV, IVS, IVT,LLB	IV
25	SHI.26	Shimoga	VZ, VT, GV, SP, ECS, LLB, C	I
26	SHI.26	Shimoga	RL, VT, GV, SI, ECS, LLB	I
27	SHI.27	Shimoga	LN, ETP, GV, RL, LLB	III
28	NIP.28	Nipani	SP, RL, GV, ECS, SI, C	I
29	NIP.29	Nipani	SP, IR, VT, RL, GV, IR, ECS	I
30	NIP.30	Nipani	GV, IVT, IVS, EFS, LLB	IV
31	NIP.31	Nipani	VT, SP, IR, ECS, SI, LLB, C	I
32	NIP.32	Nipani	LN, VT, RL, GV, IR, ETP	III
33	NIP.33	Nipani	SP, GV, VT, RL, SI, ECS, IR, C	I
34	DIN.34	Dinhata	SI, VT, DM, ET, FPG, C	II
35	DIN.35	Dinhata	SP, VT, IR, GV, ECS, LLB, C	I
36	DIN.36	Dinhata	RL, IVT, IVS, GV, EFS, LLB	IV
37	DINH.37	Dinhata	LN, VT, GV, RL, ETP, IR	III

Table 10 (continued)

1	2	3	4	5
38	PUS.38	Pusa	SP, VT, IR, ECS, GV, LLB, C	I
39	PUS.39	Pusa	SP, RL, VT, IR, ECS, C, SI	I
40	PUS.40	Pusa	GV, IVS, IVT, EFS, LLB, RL	IV
41	AND.41	Anand	SP, SI, ECS, LLB, IR, SI, C	I
42	AND.42	Anand	RL, GV, VT, FPG, LLB, D	II
43	AND.43	Anand	GV, VT, LN, ETP, IR, RL	III
44	AND.44	Anand	VT, GV, RL, ECS, SB, LLB, C	I
45	BRD.45	Baroda	IVT, IVS, EFS, LLB, GV, RL, D	IV

C = Curling; D = Depression; DM = Depression on marginal vein;
ET = Thorny enation; GV = Greening of veins; IR = Inflorescences reduced;
LN = Leaf narrowing; RL = Reduction in leaf size;
SI = Shortening of internode; SP = Stunging of plant; VT = Vein thickening;
VZ = Vein zig zag; ECS = Enation cup shape; EFS = Enation frilled shape;
FPG= Foliage pale green; ETP = Enation tiny protruding
IVT= Irregular vein thickening; IVS = Irregular vein swelling;
LLB= *Leaf leathery and brittle*

Table 11. Grouping of TobLCV isolates on the basis of symptoms exhibited by N. tabacum cvs. Samsun and Anand-119

TobLCV Group	Symptoms	No.of isolates	Isolate numbers
I	RL, VT, SP, SI, GV, IR, ECS, LLB, C	21	1, 3, 5, 6, 9, 10, 14, 15, 19, 21, 25, 26, 28, 29, 31, 33, 35, 38, 39, 41, 44
II	SI, VT, RL, DM, ET, FPG	7	2, 12, 13, 17, 18, 34, 42
III	VT, RL, GV, LN, IR, ETP, LLB	7	8, 11, 22, 27, 32, 37, 43
IV	RL, GV, IVS, IVT, EFS, LLB	10	4, 7, 16, 20, 23, 24, 30, 36, 40, 45

Tob LCV group III: Reduction in plant height followed by narrowing and reduction in leaf size were the initial symptoms. The infected leaves remain brittle and leathery. Veins become zig zag and thick. The characteristic symptoms were small protruding enation, developed inbetween veins consistantly. Inflorescences and capsule size were reduced (Fig.20a, b; Tables 10, 11).

TobLCV group IV: The symptoms were similar to group III except the irregular thickening and swelling of veins and veinlets and enation developed in the form of frilled (boat) shape, on the bright green veins. (Fig.21 a,b; Tables 10,11).

4.3.4 Distribution of TobLCV groups in different tobacco growing areas

Distribution of TobLCV groups in tobacco growing areas of India (Table 12) indicate that all the four symptoms variations were prevailing in almost all the places. Isolates of TobLCV collected from Bihar state showed only two(groups I, IV) symptoms variation. Overall distribution of TobLCV groups (I, II, III and IV) were found consistant irrespective to tobacco cultivars and agroclimatic conditions.

4.4 Host range

4.4.1 Transmission of TobLCV to different species of Nicotiana

TobLCV was inoculated to 13 *Nicotiana* species by

Fig. 20 TobLCV infected tobacco cvs.(a) Samsun and (b) Anand-119 leaves showing tiny protruding enation inbetween the veins and vein thickening (Group III)

Fig. 21 TobLCV infected tobacco cvs.(a) Samsun and (b) Anand-119 leaves showing irregular vein thickening, swelling and frilled (boat) shape enation and bright green veins (Group IV)

Fig. 20 a

Fig. 20 b

Fig. 21 a

Fig. 21 b

Fig 20a

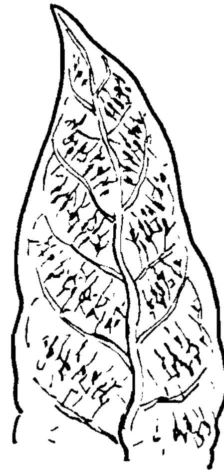


Fig 20b

Fig 21a

Fig 21b

Table 12. Distribution of TobLCV groups in tobacco growing areas

Place	Type of tobacco cultivation	TobLCV groups
Guntur, Rajahamundry East Godavari (Andhra Pradesh	Cigarette (VFC)	I, II, III, IV
Anand, Baroda (Gujarat)	Bidi	I, II, III, IV
Bangalore, Hunsur, Shimoga, Nipani (Karnataka)	Cigarette (VFC) and Bidi	I, II, III, IV
Dinhata (West Bengal)	Hookah and Chewing	I, II, III, IV
Pusa (Bihar)	Hookah and Chewing	I, IV

B. tabaci in the glasshouse. The results presented in Table 13 indicate that all the species were infected and exhibited typical severe to mild leaf curl symptoms in 12-35 days. *Nicotiana ampelsia* and *Nicotiana megalosiphon* were found free from TobLCV infection. Among the species, *Nicotiana glauca* showed typical vein thickening and enation after long incubation period of 35-45 days. Symptoms of *Nicotiana* species are described in Table 14.

4.4.2 Transmission of TobLCV to different tobacco cultivars

TobLCV was transmitted to 28 *Nicotiana tabacum* cultivars in glasshouse by *B.tabaci* (Table 15). The results indicate that all the inoculated tobacco cultivars were succumbed to TobLCV. The minimum incidence of disease was 40 per cent in *N. tabacum* cv. Candel, whereas all other cultivars showed above 50 per cent incidence. The symptoms exhibited by the tobacco cultivars are described in Table 16.

4.4.3 Transmission of TobLCV to host species

In order to determine the host range of TobLCV, different plant species belonging to different families viz., Amaranthaceae, Annonaceae, Apiceae, Asteraceae, Brassicaceae, Caricaceae, Caryophyllaceae, Chenopodiaceae,

Table 13. Transmission of TobLCV to different species of *Nicotiana*

Sl. No.	Inoculated *Nicotiana* species	Infected[a,b] / Inoculated	% trans-mission	Incubation period in plant (days)
1	*Nicotiana ampelsia*	0/18	0	-
2	*N. benthamiana*	11/15	73.3	12-15
3	*N. clevelandii*	3/14	21.4	18-20
4	*N. corymbosa*	12/15	80.0	14-18
5	*N. excelsior*	12/28	42.8	20-25
6	*N. glauca*	10/14	71.0	35-45
7	*N. glutinosa*	13/15	86.6	14-20
8	*N. megalosiphon*	0/20	0	-
9	*N. nudicaulis*	4/15	26.6	15-20
10	*N. occidentalis*	2/12	16.6	18-20
11	*N. rosulata*	6/10	60.0	25-28
12	*N. suaveolens*	6/18	33.3	20-25
13	*N. undulata*	7/10	70.0	15-18

[a] 10-15 whiteflies released per plant

[b] AA, and IA 24 h each

Table 14. Symptoms of TobLCV on different species of *Nicotiana*

Nicotiana spp. inoculated	Symptons	Incubation period in plant (days)
Nicotiana benthamiana	Infected leaves crippled, curled and became leathery, leaf size reduced greatly and reduction of internode on stunted plant gave bushy appearance. Vein thickening and small enations were produced on twisted veins (Fig.22)	12-15
Nicotiana clevelandii	Upward curling and crinkling of leaves followed by greening and thickening of veins were the characteristic symptoms after 18 days of inoculation	18-20
Nicotiana corymbosa	Downward curling of leaves and reduction of leaf size followed by vein thickening. Infected leaves remain brittle and leathery.	14-18
Nicotiana exalsior	Infected leaves showed downward curling, depression on upper surface followed by vein zig zag and thickening after 20 days of inoculation. Plant remain stunted.	20-25
Nicotiana glauca	Inoculated plants by *B.tabaci* showed mild vein thickening, downward curling after 35 days of incubation period. The characteristic green enation appeared after 42 days of inoculation (Fig.23)	35-45
N. glutinosa	Vein clearing and thickening were the initial symptoms followed by crinkling, curling and bushy growth of plant.	14-20

Table 14. (continued)

1	2	3
Nicotiana nudicaulis	Mild symptoms appeared in the form of vein thickening, slight zig zag and reduction in leaf size. Depression developed on upper surface of infected leaves.	15-20
Nicotiana occidentalis	Narrowing of leaves, vein thickening and depressions of the upper surface of leaves appeared after 18 days of inoculation.	18-20
Nicotiana rosulata	Vein thickening and zig zag followed by greening of veins. Leaves became leathery and brittle on infected plant.	25-28
Nicotiana suaveolens	Typical symptoms appeared in the form of upward curling, greening of vein followed by vein thickening and reduction in leaf size. Enation developed on infected leaf veins.	15-18
Nicotiana undulata	Severe symptoms developed on infected plant in the form of downward curling and vein thickening. Leaves became brittle and leathery. (Fig.25)	15-18

Fig. 22 TobLCV infected *N. benthamiana* transmitted by *B. tabaci*

Fig. 23 TobLCV infected *N. glauca* transmitted by *B. tabaci*

Fig. 24 TobLCV infected *N. glutinosa* transmitted by *B. tabaci*

Fig. 25 TobLCV infected *N. undulata* transmitted by *B. tabaci*

Fig. 22

Fig. 23

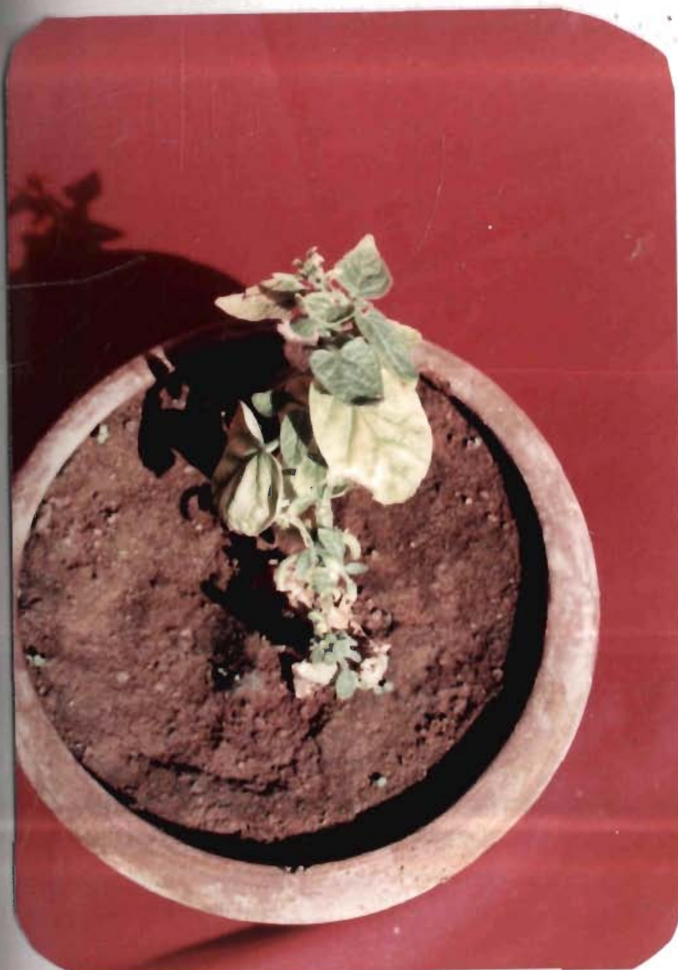


Fig. 24

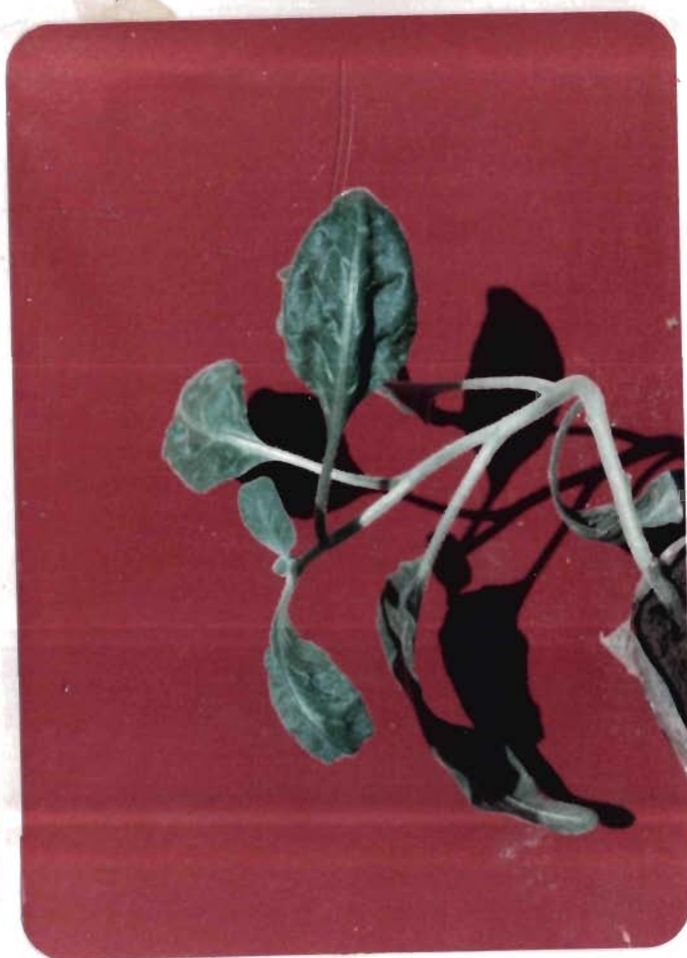


Fig. 25

Table 15. Transmission of TobLCV to different tobacco cultivars

Sl. No.	Inoculated tobacco cultivars	Infected[a,b] / Inoculated	% Trans-mission	Incubation period in plant (days)
1	2	3	4	5
1	*Nicotiana tabacum* cv. Anand-2	12/15	80.0	18-20
2	*N. tabacum* cv. Anand-119	15/15	100.0	14-16
3	*N. tabacum* cv. Anand-145	12/14	85.4	20-25
4	*N. tabacum* cv. Candel	8/20	40.0	15-18
5	*N. tabacum* cv. CTRI Sp.	15/15	100.0	13-15
6	*N. tabacum* cv. Delcrest	5/20	25.0	25-28
7	*N. tabacum* cv. FCV Sp.	15/15	100.0	14-18
8	*N. tabacum* cv. Florida-22	10/10	100.0	18-20
9	*N. tabacum* cv. Godavari	16/18	88.8	20-24
10	*N. tabacum* cv. GT-4	13/15	86.6	16-20
11	*N. tabacum* cv. GT-5	12/15	80.0	18-20
12	*N. tabacum* cv. GT-6	10/15	66.6	20-25
13.	*N. tabacum* cv. Hirac	8/13	61.5	20-24
14.	*N. tabacum* cv. Hicks(M)	10/10	100.0	20-24
15.	*N. tabacum* cv. Hicks-SP.	16/16	100.0	12-16
16.	*N. tabacum* cv. HR-10-64	13/15	86.6	15-18
17.	*N. tabacum* cv. Jayasri	20/20	100.0	12-15
18.	*N. tabacum* cv. Jayasri(MR)	17/20	85.0	18-20

Table 15 (continued)

1	2	3	4	5
19	*N. tabacum* cv. Mc Nair-12	10/15	71.4	16-20
20	*N. tabacum* cv. MDS-7	12/12	100.0	20-25
21	*N. tabacum* cv. Oxford-3	10/15	66.6	25-30
22	*N. tabacum* cv. PCT-7	14/14	100.0	13-15
23	*N. tabacum* cv. Samsun	15/15	100.0	12-16
24	*N. tabacum* cv. Swarna	15/15	100.0	15-20
25	*N. tabacum* Virginia gold	15/18	83.3	16-20
26	*N. tabacum* cv.Yellow gold	15/18	83.3	20-25
27	*N. tabacum* cv. White Burley	18/18	100.0	20-25
28	*N. tabacum* cv. 16-103	15/15	100.0	16-20

[a]10-15 whiteflies inoculated per plant

[b]AA, and IA 24 h each

Table 16. Symptoms of TobLCV exhibited on different tobacco cultivars

Tobacco cultivars inoculated	Symptoms	Incubation period in plants (days)
1	2	3
Nicotiana tabacum cv. Anand-2	Initial symptoms appeared in the form of downward curling, vein zig zag, thickening and greening of veins. Entire network became dark green and thick. Leafy outgrowth (enation) developed on veins in the advanced stage of disease.	18-20
N. tabacum cv. Anand-119	Severe curling, and uneven twisting of leaves followed by vein thickening and greening were the typical symptoms. Foliage became dark green colour, narrowing of leaves and profuse enations developed on infected leathery leaves (Fig.26)	14-16
N. tabacum cv. Anand-115	Symptoms appeared in the form of downward curling from leaf margin, vein thickening and inward banding of leaf from midrib. Enation developed in the advanced stage of disease.	20-25
N. tabacum cv. Candel	Mild symptoms developed on infected leaf after 15 days of inoculation in the form of vein thickening, downward curling and depressions on upper leaf surface	15-18

Table 16(Continued)

1	2	3
N. tabacum cv. CTRI Sp.	The characteristic symptoms exhibited in the form of curling, vein thickening, twisting of midrib and greening of veins. Small scattered protruding enation appeared on secondary veins.	13-15
N. tabacum cv. Delcrest	Stunting of infected seedling, reduction of leaf size and internode formed bushy growth of apical portion. Vein thickening and curling were the prominent symptoms.	25-28
N. tabacum cv. FCV Sp.	Clearing of veins and wrinkling of upper surface of leaves were the initial symptoms followed by thickening of veins and veinlets. Tender leaves reducing in size, curled, uneven twisting, greening, vein thickening and enations were the characteristic symptoms. (Fig.27)	14-28
N. tabacum cv. Florida-22	Downward curling, vein thickening and reduction in size of leaves followed by veinal depressions were the chief symptoms. Foliage was pale yellow and became leathery (Fig.28)	18-20
N. tabacum cv. Godavari	Tender leaves reduced greatly on infected plant, vein thickening and narrowing of leaves were the characteristic symptoms. Lower leaves became pale yellow, Enation developed on veins. (Fig.29)	20-24
N. tabacum cv. GT-4	Uneven twisting, curling and wrinkling of leaves were the distinguished symptoms of infected plant. Greening and thickening of veins followed by formation of cup like enations after 18 days of inoculation.	16-20

Fig.26 TobLCV infected *N.tabacum* cv.Anand-119 transmitted by *B.tabaci*

Fig.27 TobLCV infected *N.tabacum* cv. FCV-Sp. transmitted by *B.tabaci*

Fig.28 TobLCV infected *N.tabacum* cv. Florida-22 transmitted by *B. tabaci*

Fig. 26

Fig. 27

Fig. 28

Table 16 (continued)

1	2	3
N. tabacum cv. GT.5 and GT.6	In both the cases curling appeared on newly emerged leaves after 20 days of inoculation. Uneven twisting of leaf from midrib, vein thickening and dark green enations were the typical symptoms.	18-20
N. tabacum cv. Hirac	Rolling of leaf tip and downward curling followed by uneven twisting of midrib and vein thickening were the initial symptoms. Foliage became pale yellow and green enation developed on veins. (Fig.30)	20-24
N. tabacum cv. Hicks(M)	Severe vein thickening, curling of leaves, rolling of tips and inward folding of leaves from midrib were the initial symptoms. Vein became zig zag and foliage colour dark green. Enation developed on twisted veins.	20-24
N. tabacum cv. Hicks Sp.	Tender leaves became narrow, lemina portion protruding to the upper side leaving midrib down. Veins and midrib thickened severely and became zig zag. Foliage was dark green and produced green enation on veins.(Fig.31)	12-16
N. tabacum cv. HR-10-64	Vein thickening and depressions were the mild symptoms on infected plant leaves followed by curling and narrowing of leaves.	15-18
N. tabacum cv. Jayasri	Tender leaves dwarfed, became dark green in colour, and curled downward after 12 days of inoculation. Lower leaves became pale yellow. (Fig.32)	12-15

Fig.29 TobLCV infected *N. tabacum* cv. Godavari transmitted by *B.tabaci*

Fig.30 TobLCV infected *N. tabacum* cv. Hirac transmitted by *B.tabaci*

Fig.31 TobLCV infected *N.tabacum* cv. Hicks sp. transmitted by *B.tabaci*

Fig. 29

Fig. 30

Fig. 31

Table 16 (continued)

1	2	3
N. tabacum cv. Jayasri(MR)	Newly emerged leaves reduced in size, dark green in colour and bending downward were the initial symptoms. Vein thickening and greening appeared as the diseased advanced. (Fig.33)	12-15
N. tabacum cv. Mc Nair-12	Mild symptoms appeared in the form of vein thickening and curling half leaf from the tip. (Fig.34)	16-20
N. tabacum cv. MDS-7	Vein thickening, zig zag and greening appeared after 20 days of inoculation. Foliage became pale green and enation developed on veins.	20-25
N. tabacum cv. Oxford-3	Vein thickening was conspicuous, Curling and rolling of tender leaves appeared within 25-30 days of inoculation.	25-30
N. tabacum cv. PCT-7	Bending and twisting of parallel veins resulting in folding of lamina. Swelling of veins and prominent leafy enation developed on veins. (Fig.35)	13-15
N. tabacum cv. Samsun	Curling, wrinkling, shorting of internode, reduction of leafsize, stem zig zag and depressions on the leaf surface were appeared on severely infected plant. Small cup like and tiny protruding enations were accompanied by greening of veins. (Fig.36)	12-16
N. tabacum cv. Swarna	Initial symptoms appeared in the form of mild vein clearing, curling and uneven twisting of leaf lamina from mid rib followed by vein thickening and enations.	15-20

Fig.32 TobLCV infected _N.tabacum_ cv. Jayasri transmitted by _B.tabaci_

Fig.33 TobLCV infected _N.tabacum_ cv. Jayasri(MR) transmitted by _B.tabaci_

Fig.34 TobLCV infected _N.tabacum_ cv. Mc Nair-12 transmitted by _B.tabaci_

Fig.35 TobLCV infected _N.tabacum_ cv. PCT-7 transmitted by _B.tabaci_

Fig. 32

Fig. 33

Fig. 34

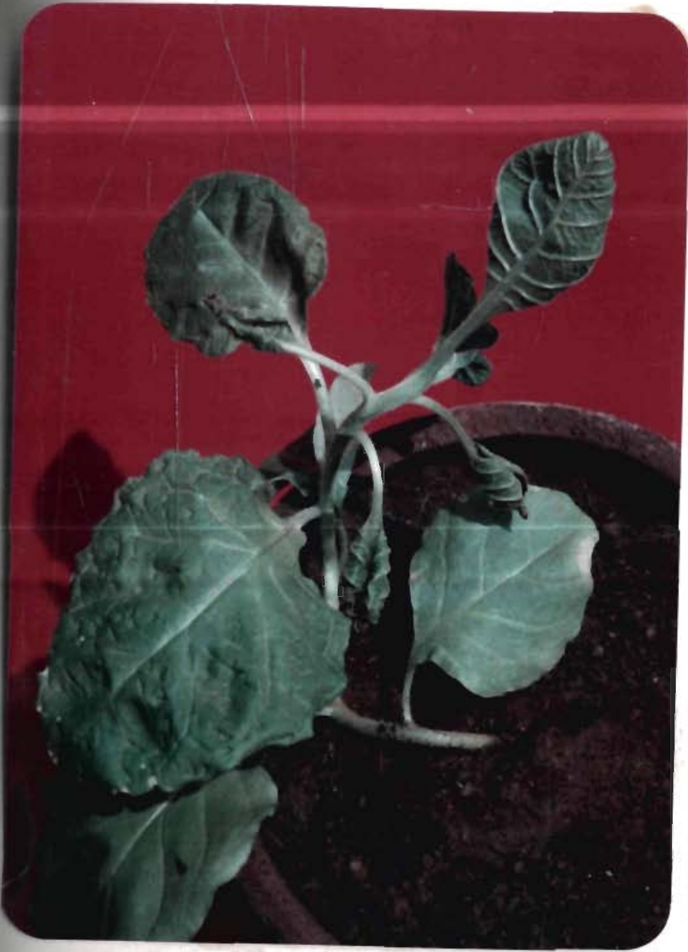
Fig. 35

Table 16 (continued)

1	2	3
N. tabacum cv. Virginia gold	Narrowing of leaves, with and rolling of tip downward were the typical symptoms. Infected leaves remain folded. Enations developed on green veins. Fig.37)	16-20
N. tabacum cv. Yellow gold	Whole the lamina was much curled in appearance. In the advanced stage leaves rolled along the margin. Vein thickening and greening followed by enation on veins were the typical symptoms.	20-25
N. tabacum cv. 16-103	Mild symptoms appeared in the form of reduction of leaves, depressions on upper surface of leaves followed by vein thickening and small scattered enations on veins.	16-20
N. tabacum cv. White Burley	Symptoms appeared after inoculation of 20 days. Curling, folding of leaves, and reduction in size were the initial symptoms. Enation developed on veins and corresponding depressions were also present.	20-25

Fig.36 TobLCV infected *N.tabacum* cv. Samsun transmitted by *B.tabaci* showing severe curling and depressions (a) vein thickening, greening and enations (b) prominent cup shape enation

Fig.37 TobLCV infected *N.tabacum* cv. Virginia gold transmitted by *B.tabaci*



cucurbitaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Malvaceae, Minoceae, Myrtaceae, Nactaginaceae, Oxalidaceae, Pedaliaceae, ~~Pepilinoceae~~, Poaceae, Solanaceae and Verbenaceae were inoculated with TobLCV by *B.tabaci* as described under materials and methods.

A total of 88 different types of host plants of cultivated planting (35), ornamental plants (13), weeds (29) and forest plants (11) were tested against TobLCV in glasshouse (Tables, 17, 18).

The data showed that cultivated hosts *Beta vulgaris* (Fig.38), *Carica papaya* (Fig.39), *Capsicum annuum*, *Crotalaria juncea* (Fig.40), *Cymopsis tetragonoloba* (Fig.41), *Hellianthus annuus*, *Lycopersicon esculentum* (Fig.42), *Sesamum indicum* (Fig.43), *Phaseolus vulgaris* (Fig.44); the ornamental host plants *Althaea rosea* (Fig.45), *Callistephus chinensis*(Fig.46), *Dahalia* sp., *Dianthus caryophyllus* (Fig.47), *Petunia hybrida* (Fig.48), *Salvia officinalis*, *Tagetes erecta* and *Zinnia elegans* and weed hosts (*Acanthospermum hispidum* (Fig.49), *Acalypha indica* (Fig.50), *Ageratum conyzoides* (Fig.51), *Bidens pilosa*, *Centella asiatica*, *Croton bonplandianum* (Fig.52), *Datura stramonium* (Fig.53), *Euphorbia geniculata* (Fig.54), *Galinsoga purviflora* (Fig.55), *Heliotropium indicum* (Fig.56), *Nicandra physalodes* (Fig.57), *Oxalis*

Table 17. Transmission of TobLCV to cultivated, ornamentals, weeds and forest plants by *B. tabaci*

Sl. No.	Plant species inoculated	Family	Infected / Inoculated	% transmission	Incubation period in plant (days)
1	2	3	4	5	6
CULTIVATED PLANTS					
1.	*Abelmoschus esculentus*	Malvaceae	0/10	0	-
2.	*Arachis hypogaea*	Fabaceae	0/15	0	-
3.	*Beta vulgaris*	Chenopodiaceae	27/37	72.0	30-35
4.	*Brassica oleraceae*				
	cv. Knol Rabi	Brassicaceae	0/16	0	-
	cv. Express	Brassicaceae	0/20	0	-
5.	*Cajanus cajan*				
	cv. V. 16	Fabaceae	0/16	0	-
	cv. TT.B 7	Fabaceae	0/18	0	-
	cv. TVX	Fabaceae	0/25	0	-
	cv. HY-36	Fabaceae	0/15	0	-
	cv. DL-82	Fabaceae	0/18	0	-
6.	*Capsicum annuum*	Solanaceae	10/20	50.0	15-20
7.	*Carica papaya*	Caricaceae	3/20	15.0	55-60
8.	*Citrullus vulgaris*	Cucurbitaceae	0/20	0	-
9.	*Crotalaria juncea*	Fabaceae	18/30	60.0	20-25

Table 17 (continued)

1	2	3	4	5	6
10.	*Cymopsis tetragonoloba*	Fabaceae	22/30	73.0	26-30
11.	*Cucumis sativus*	Cucurbitaceae	0/25	0	-
12.	*Daucus carrota*	Apiaceae	0/30	0	-
13.	*Eleusine coracana* cv. Indalf-5	Poaceae	0/20	0	-
14.	*Gossypium hirsutum*	Malvaceae	0/25	0	-
15.	*Helianthus annuus* cv. EC. 68415	Asteraceae	11/15	73.3	23-30
	cv. KBSH-1	Asteraceae	9/15	60.0	25-30
16.	*Glycine max*	Fabaceae	0/15	0	-
17.	*Lagenaria vulgaris*	Cucurbitaceae	0/18	0	-
18.	*Luffa acutangula*	Cucurbitaceae	0/15	0	-
19.	*Lycopersicon esculentum*	Solanaceae	33/33	100.0	15-20
20.	*Macrotyloma uniflorum*				
	cv. IC. 11095	Fabaceae	0/20	0	-
	cv. Maccintosh	Fabaceae	0/18	0	-
	cv. BGM.1	Fabaceae	0/14	0	-
21.	*Manihot esculenta*	Euphorbiaceae	0/18	0	-
22.	*Manihot glasiovii*	Euphorbiaceae	0/24	0	-
23.	*Phaseolus aureus*				
	cv. P. 16	Fabaceae	0/10	0	-
	cv. Pusa Baisakhi	Fabaceae	0/15	0	-
24.	*Vigna mungo*	Fabaceae	0/30	0	-
25.	*Phaseolus vulgaris* Top crop	Fabaceae	15/15	100.00	18-20

Table 17 (continued)

1	2	3	4	5	6
26.	*Psidium guajava*	Myrtaceae	0/15	0	-
27.	*Raphanus satinum*	Brassicaceae	0/16	0	-
28.	*Ricinis communis*	Eupharbiaceae	0/18	0	-
29.	*Sesamum indicum*	Pedaliaceae	15/18	83.3	20-25
30.	*Spinacea oleracea*	Chenopodiaceae	0/20	0	-
31.	*Solanum melongena*	Solanaceae	0/14	0	-
32.	*Trichosanthus anguina*	Cucurbitaceae	0/10	0	-
33.	*Triticum vulgare*				
	cv. Bajaj yellow	Poaceae	0/18	0	-
	cv. Local	Poaceae	0/30	0	-
34.	*Vigna unguiculata*				
	cv. C. 152	Fabaceae	0/18	0	-
	cv. KBC-1	Fabaceae	0/16	0	-
	cv. Lolitha	Fabaceae	0/25	0	-
35.	*Zea mays*				
	cv. M-400	Poaceae	0/20	0	-
	cv. G.25	Poaceae	0/16	0	-
	cv. Ganga	Poaceae	0/30	0	-
ORNAMENTALS					
36.	*Althea rosea*	Malvaceae	10/14	71.4	15-20
37.	*Callistephus chinensis*	Asteraceae	14/14	100.0	15-20

Table 17 (continued)

1	2	3	4	5	6
38.	*Celosia argentea*	Amaranthaceae	0/18	0	-
39.	*Cosmos* sp.	Apiaceae	0/14	0	-
40.	*Dahalia* sp.	Apiaceae	15/20	75.0	22-25
41.	*Dianthus caryophyllus*	Caryophyllaceae	6/10	60.0	16-20
42.	*Impatiens balsamina*	Balsaminaceae	0/30	0	-
43.	*Mirabilis jalapa*	Nactaginaceae	0/16	0	-
44.	*Petunia hybrida*	Solanaceae	14/15	93.0	23-30
45.	*Salvia officinallis*	Laminaceae	4/16	25.0	36-40
46.	*Tagetes erecta*	Asteraceae	4/10	25.0	35-40
47.	*Zinnia elegans*	Asteraceae	14/15	93.3	21-25
48.	*Zinnia* sp.	Asteraceae	12/12	100.0	18-20
WEEDS					
49.	*Acalypha indica*	Euphorbiaceae	10/12	83.3	15-20
50.	*Acanthospermum hispidum*	Asteraceae	20/20	100.0	15-20
51.	*Ageratum conyzoides*	Asteraceae	15/15	100.0	20-25
52.	*Alternanthera triandra*	Amaranthaceae	0/17	0	-
53.	*Amaranthus viridis*	Amaranthaceae	0/20	0	-
54.	*Bidens pilosa*	Asteraceae	24/24	100.0	15-18
55.	*Centella asistica*	Apiaceae	15/16	93.7	15-25

Table 17 (continued)

1	2	3	4	5	6
56.	Chenopodium album	Chenopodiaceae	0/19	0	-
57.	Croton bonplandianum	Euphoribiaceae	9/10	90.0	23-25
58.	Datura metal	Solanaceae	0/19	0	-
59.	Datura stramonium	Solanaceae	18/18	100.0	15-20
60.	Eclipta prostratra	Asteraceae	0/16	0	-
61.	Euphorbia geniculata	Euphorbiaceae	19/20	95.0	24-30
62.	Euphorbia prunifolia	Euphorbiaceae	0/18	0	-
63.	Euphorbia hirta	Euphorbiaceae	0/14	0	-
64.	Galinsoga purviflora	Asteraceae	25/25	100.0	12-18
65.	Heliotroprium indicum	Boraginaceae	5/18	27.7	23-30
66.	Leucas aspera	Laminaceae	0/15	0	-
67.	Nicandra physalodes	Solanaceae	16/20	80.0	16-22
68.	Oxalis corniculata	Oxalidaceae	12/12	100.0	14-20
69.	Parthenium hysterophorus	Asteraceae	15/15	100.0	15-20
70.	Phyllanthus niruri	Euphorbiaceae	9/15	60.0	42-50
71.	Physalis minima	Solanaceae	0/15	0	-
72.	Solanum nigrum	Solanaceae	0/18	0	-
73.	Solanum torvum	Solanaceae	30/35	85.7	14-20

Table 17 (continued)

1	2	3	4	5	6
74.	Sonchus brachyotis	Asteraceae	15/15	100.0	16-20
75.	Stachytarphata indica	~~Solanaceae~~ Vernonenieae	4/10	40.0	38-40
76.	Synedrella nodiflora	Asteraceae	12/15	80.0	38-40
77.	Tridax procumbens	Asteraceae	0/14	0	-
[illegible].	FOREST PLANTS				
78.	Acacia auriculiformis	Mimoceae	0/20	0	-
79.	Albizia lebbeck	Mimoceae	0/16	0	-
80.	Cassia fistula	Fabaceae	0/15	0	-
81.	Casuarina equisitifolia	Casuarinaceae	0/15	00	-
82.	Dalbergia sissoo	Fabaceae	0/18	0	-
83.	Eucalyptus sp.	Myrtaceae	0/15	0	-
84.	Enterolobium saman	Fabaceae	0/12	0	-
85.	Poinsettia pulcharrima	Euphorbiaceae	0/12	0	-
86.	Polyalthia longifolia	Annonaceae	0/15	0	-
87.	Pongamia pinnata	Popilionaceae	0/10	0	-
88.	Solanum macranthus	Solanaceae	0/16	0	-

Table 18. Symptoms of TobLCV on cultivated, ornamental and weed host plants

Host inoculated	Symptoms	Incubation period in host(days)
1	2	3
CULTIVATED PLANTS		
1. Beta vulgaris	The mild curling of the leaves were observed after 30-35 days after inoculation followed by vein thickening and prominent green sessile cup like enations(Fig.38)	30-35
2. Capsicum annuum	Initial symptoms appeared in the form of mild vein clearing and faint yellowing of leaves followed by moderate vein thickening and downward curling from the leaf margin.	18-20
3. Carica papaya	The characteristic symptoms were observed after long incubation period of 55 to 60 days in the form of crinkling and puckering of leaves. Leaf size reduced drastically and thickening of veins and midrib was the typical symptoms.(Fig.39)	55-60
4. Crotalaria juncea	Typical symptoms were noticed in the form of twisting of leaves, downward curling, vein thickening and greening of veins. (Fig.40)	20-25
5. Cymopsis tetragonoloba	The symptoms appeared in the form of mild curling of leaves, vein thickening and greening of veins followed by dark green sessile enations. Leaves become leathery and dark green colour. (Fig.41)	26-30

Fig.38 *B.tabaci* transmitted TobLCV to *Beta vulgaris*

Fig.39 *B.tabaci* transmitted TobLCV to *Carica papaya*

Fig. 38

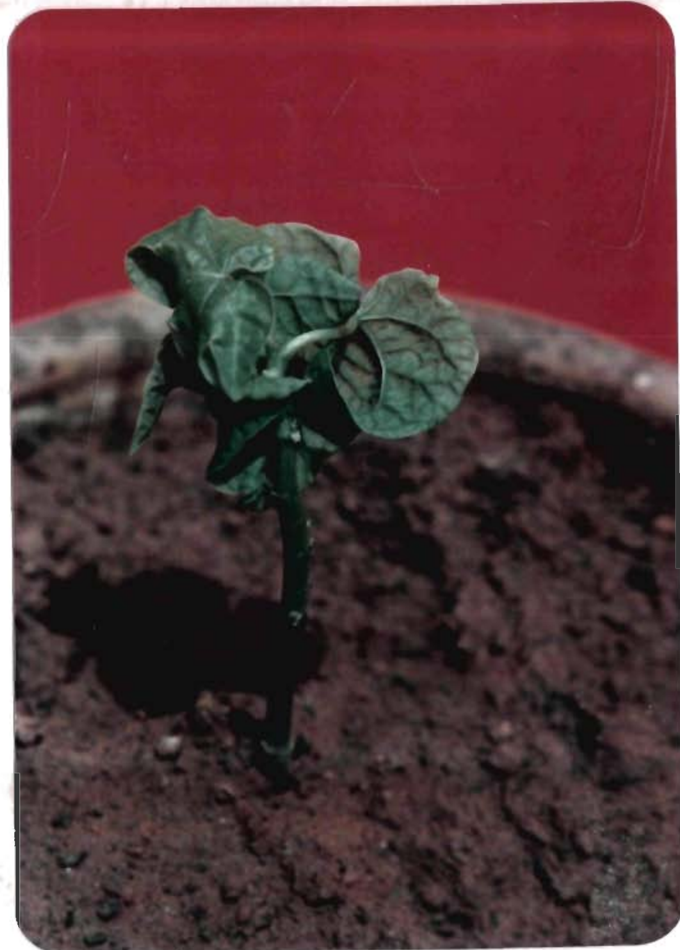


Fig. 39

Table 18 (continued)

1	2	3
6. Hellianthus annuus	The symptoms appeared in the form of twisting and rolling of the leaves followed by thickening of veins. Leaf color become dark green, Reduction in leaf size and brittle were the chief symptoms.	23-30
7. Lycopersicon esculentum	The symptoms were characterized by severe curling, twisting of petiols, shortening of inter-nodes and reduction in the leaf size. Thickening and greening of veins was the typical symptoms. Infected plant remain stunted and bushy in appearance, Enations produced on veins. (Fig.42)	15-20
8. Phaseolus vulgaris	Crinkling and curling of tender leaves were the initial symptoms after 18 days of inoculation. Severe vein thickening, leathery and brittle leaves and reduction in leaf size were observed in advanced stages. (Fig.43)	18-20
9. Sesamum indicum	The initial symptoms were mild vein clearing and downward curl-ing from the leaf margin. As the disease advanced severe vein thickening and greening of veins were the main distinguished symptom. Infected leaves remain narrow, reduced in size and dark green in colour. (Fig.44)	20-25
ORNAMENTAL **PLANTS**		
10. Althaea rosea	The characteristic symptoms were the thickening of veins and down-ward curling of leaves. Prominent enations were noticed between 15-20 days after inoculation and leaves become leathery and brittle. (Fig.45)	15-20

Fig.40 *B.tabaci* transmitted TobLCV to *Crotalaria juncea*

Fig.41 *B.tabaci* transmitted TobLCV to *Cymopsis tetragonoloba*

Fig.42 *B.tabaci* transmitted TobLCV to *Lycopersicon esculentum*

Fig. 40

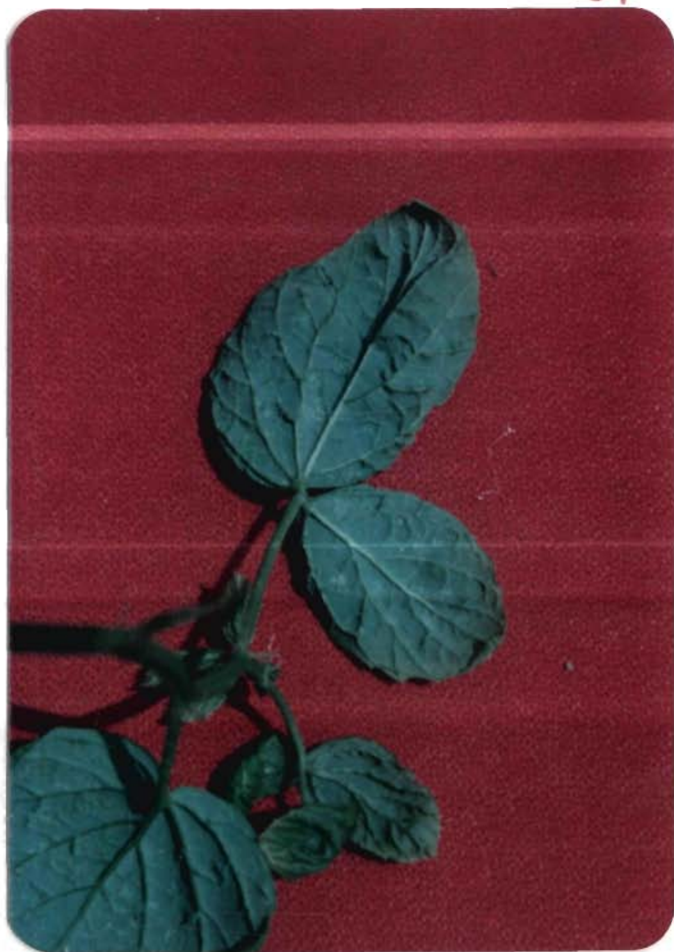


Fig. 41

Fig. 42

Fig.43 *B. tabaci* transmitted TobLCV to *Sesamum indicum*

Fig.44 *B.tabaci* transmitted TobLCV to *Phaseolus vulgaris*

Fig.45 *B.tabaci* transmitted TobLCV to *Althaea rosea*

Fig. 43

Fig. 44

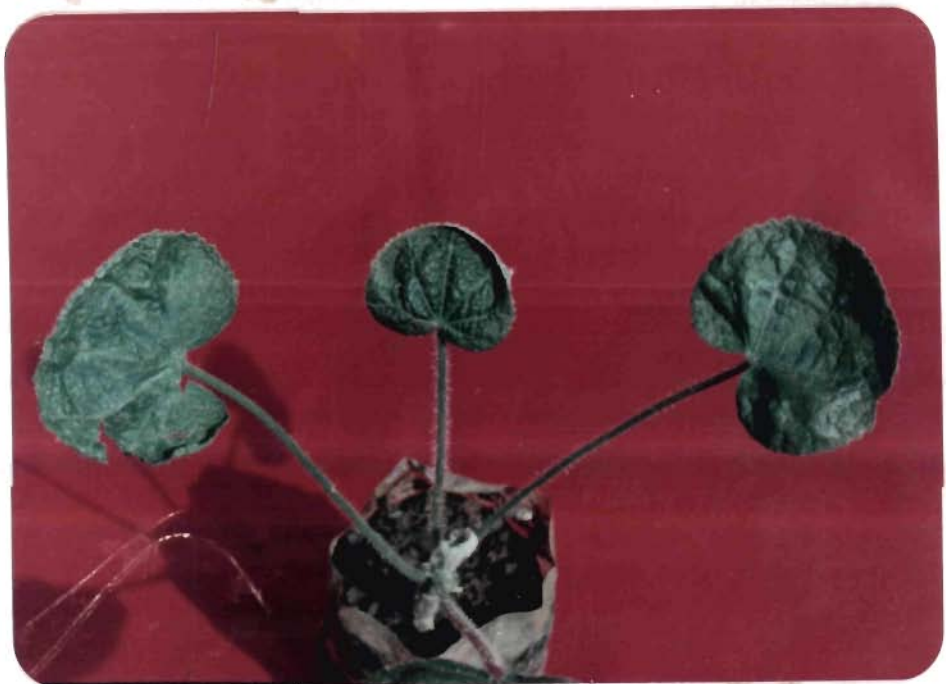


Fig. 45

Table 18 (continued)

1	2	3
11. Callistephus chinensis	The typical prominent vein thickening was observed 15-20 days after inoculation followed by slight curling of leaves. Leaf size reduced and dark green enation observed on veins. (Fig.46)	15-20
12. Dahalia sp.	The infected plant developed slight curling of leaves followed by thickening of veins and downward curling of leaves. The leaves were reduced in size.	22-25
13. Dianthus caryophyllus	The initial symptoms appeared in the form of prominent vein thicknening and curling of leaves followed by uneven twisting and rolling of leaves. The infected plant remained stunted with reduced leaf size.(Fig.47)	16-20
14. Petunia hybrida	Leaves of infected plant showed curling downward and veins become thick and zig zag. Depressions on the upper surface of the leaves were the typical symptoms. Petiols of infected plant become zig zag and remain stunted. (Fig.48)	23-30
15. Salvia officinalis	The initial symptoms were rolling of leaves from tips and curling. Vein thickening and uneven twisting of leaves appeared in the advanced stage of disease after 40 days of inoculation.	36-40
16. Tagetes erecta	The curling of leaves were noticed between 35-38 days after inoculation. The young leaves exhibited curling from the margin and thickening of veins were the typical symptoms. Leaves become pale yellow and reduced in size.	

Fig.46 *B.tabaci* transmitted TobLCV to *Callistephus chinensis*

Fig.47 *B.tabaci* transmitted TobLCV to *Dianthus caryophyllus*

Fig.48 *B.tabaci* transmitted TobLCV to *Petunia hybrida*

Fig. 46

Fig. 47

Fig. 48

Fig.49 *B.tabaci* transmitted TobLCV to *Acanthospermum hispidum*

Fig.50 *B.tabaci* transmitted TobLCV to *Acalypha indica*

Fig.51 *B.tabaci* transmitted TobLCV to *Ageratum conyzoides*

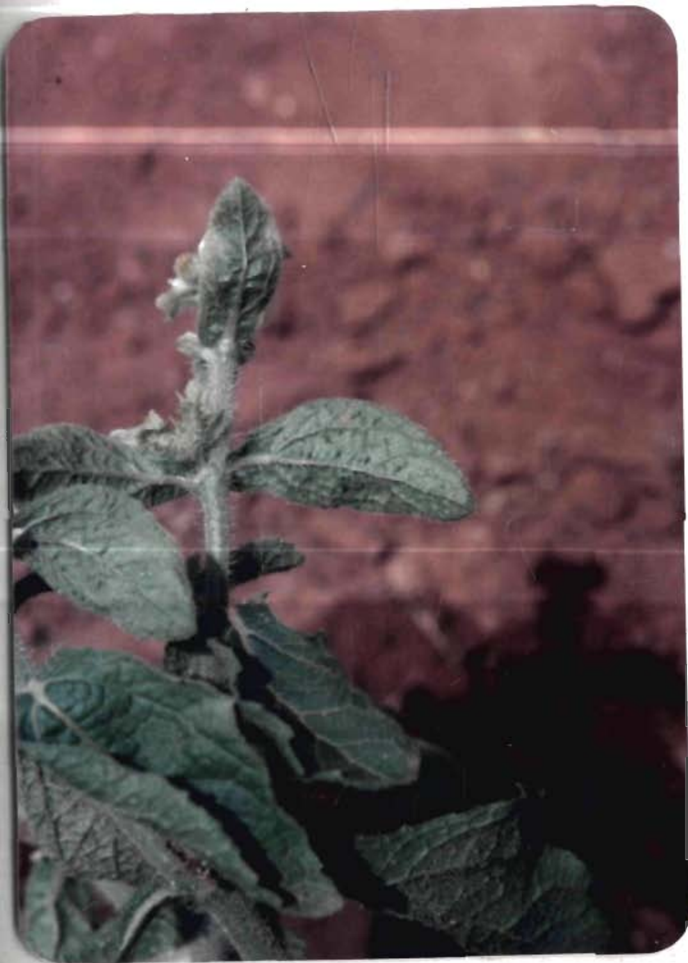


Fig. 49

Fig. 50

Fig. 51

Table 18 (continued)

1	2	3
17. Zinnia elegans	The symptoms appeared in the form of upward curling of leaves and thickening of veins. The infected plants produced elongated narrow and bright green colour leaves. Enation developed on veins.	21-25
WEEDS		
18. Acanthospermum hispidum	The characteristic symptoms were the thickening of veins and curling of leaves downward. Prominent enations were noticed 15-20 days after inoculation and leaves became leathery and brittle. (Fig.49)	15-20
19. Acalypha indica	The symptoms appeared in the form of ruffling and downward curling of leaves followed by vein thickening. Plant remained stunted and leaves reduced in size. Leaf colour became pale green in colour. (Fig.50)	15-20
20. Ageratum conyzoides	The typical symptoms after inoculation by whiteflies were bright yellowing, slight curling of leaves and vinal clearing. Infected leaves become pale yellow and leathery. (Fig.51)	20-25
21. Bidens pilosa	The symptoms appeared in the form of mild curling and vein clearing on terminal leaves followed by thickening of veins.	15-18
22. Centella asiatica	The curling of leaves and vein thickening and greening were the typical symptoms. Small spiny enations were also noticed on veins after 22 days of inoculation. Leaf size was reduced.	19-25

Table 18 (continued)

1	2	3
23. Croton bonplandianum	The initial symptoms were the non-persistent vein clearing on tender leaves and curling. Vein thickening and crinkling of the leaves were observed. Leaf size was reduced and became pale green colour. (Fig.52)	23-25
24. Datura stramonium	Clearing of veins and veinlets in young leaves were observed in 15-18 days of inoculation. As the disease advanced vein thickening and downward curling and crinkling of leaves became prominent. Enation was developed on veins. (Fig.53)	15-20
25. Euphorbia geniculata	The typical symptoms appeared after inoculation as prominent vein clearing on tender leaves. This was followed by severe curling and rolling of leaves. The leaves of infected plants remained in hanging position. (Fig.54)	24-30
26. Galinsoga parviflora	The symptoms appeared in the form of vein thickening followed by curling and twisting of petiols on infected plant. Leaf size was reduced and veins remains zig zag. (Fig.55)	12-18
27. Heliotropium indicum	The leaves developed slight curled followed by prominent vein thickening. Small enations were developed on veins. (Fig.56)	23-30
28. Nicandra physalodes	Vein clearing of young leaves were observed in 16-20 days after inoculation. Severe vein thickening was the typical symptoms followed by protruding of vein with the corresponding forming veinal depression on the upper surface. Leaves became leathery and pale green in colour. Fig.57)	16-22

Fig.52 *B.tabaci* transmitted TobLCV to *Croton bonplandianum*

Fig.53 *B.tabaci* transmitted TobLCV to *Datura stramonium*

Fig.54 *B.tabaci* transmitted TobLCV to *Euphorbia geniculata*

Fig.55 *B.tabaci* transmitted TobLCV to *Galinsoga parviflora*

Fig. 52

Fig. 53

Fig. 54

Fig. 55

Fig.56 *B.tabaci* transmitted TobLCV to *Heliotropium indicum*

Fig.57 *B.tabaci* transmitted TobLCV to *Nicandra physaloides*

Fig. 56

Fig. 57

Table 18 (continued)

1	2	3
29. Oxalis corniculata	The initial symptoms appeared in the form of downward curling and crinkling of leaves followed by thickening and zig zag of veins. The typical green colour spiny (thorny) enations were noticed on the veins after 18-20 days of inoculation. (Fig.58)	14-20
30. Parthenium hysterophorus	The characteristic symptoms appeared in the form of thickening and greening of veins followed by pit like depressions on the upper surface of the leaves. Severely infected plant remain stunted. (Fig.59)	15-20
31. Phyllanthus niruri	The initial symptoms were vein clearing and yellow mosaic pattern on tender leaves. Apical portion of plant remained zig zag, vein became thick and plant growth was remain stunted. (Fig.60)	42-50
32. Solanum torvum	The symptoms appeared in the form of vein thickening followed by protruding of veinal depressions on the upper surface of leaves and cupping of leaves was the chief symptom. (Fig.61)	14-20
33. Sonchus brachyotis	Vein clearing was observed in leaves after 16-20 days of inoculation which became faint, yellow in the advanced stage.	16-20
34. Stachytarpheta indica	The initial symptoms were appeared in the form of vein thickening, uneven twisting and wrinkling of leaves. Petiols became zig zag and leaves remained brittle and leathery on the severely infected plant. (Fig.62)	37-40

Fig.58 *B. tabaci* transmitted TobLCV to *Oxalis corniculata*

Fig.59 *B. tabaci* transmitted TobLCV to *Parthenium hysterophorus*

Fig. 58

Fig. 58

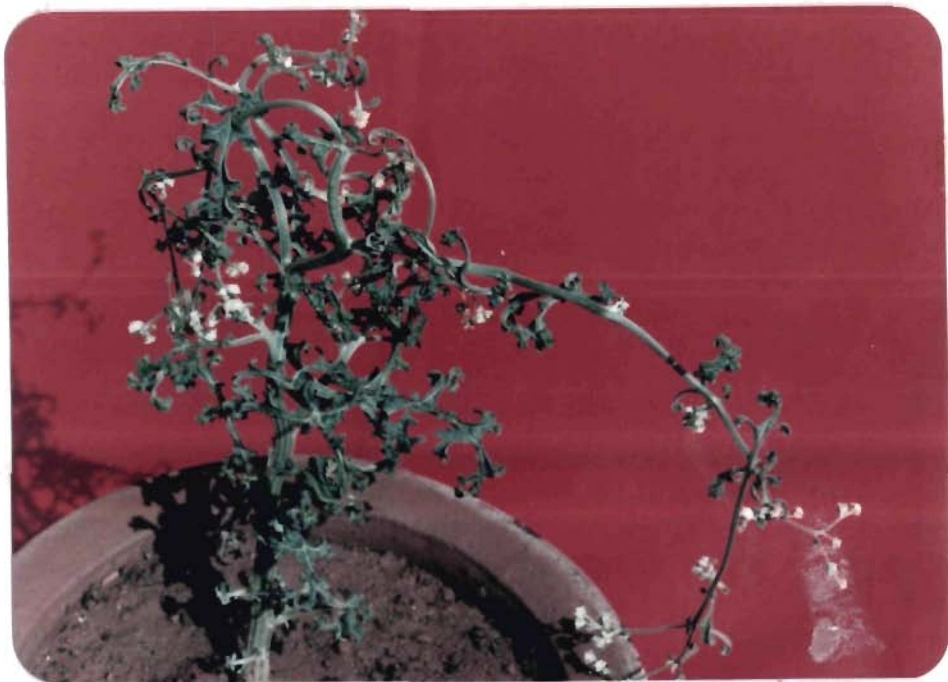


Fig. 59

Fig.60 *B.tabaci* transmitted TobLCV to *Phyllanthus niruri*

Fig.61 *B.tabaci* transmitted TobLCV to *Solanum torvum*

Fig.60

Fig.61

Table 18 (continued)

1	2	3
35. <u>Synedrella nodiflora</u>	The symptoms appeared in the form of uneven twisting of leaves from midrib, thickening of veins, downward curling of leaves in about 40 days after inoculation. As the disease advanced, leaves became brittle and leathery and dark green in colour. (Fig.63)	38-40

Fig. 62

Fig. 63

corniculata (Fig.58), Parthenium hysterophorus (Fig.59), Phyllanthus niruri (Fig.60), Solanum torvum (Fig.61),. Sonchus brachyotis, Stachytarpheta indica (Fig.62) and Synedrella nodiflora (Fig.63) were infected with TobLCV.

Of 88 hosts inoculated 35 plant species were found susceptible to TobLCV and virus produced typical permanent vein clearing symptoms in Ageratum conyzoides (Fig.51) and Euphorbia geniculata (Fig.54), mild faint vein clearing on Capsicum annuum, Bidens pilosa and Sonchus brachyotis, thorny erect enation on veins in Oxalis corniculata(Fig.58), prominent small green outgrowth (enation), were also observed in Althaea rosea (Fig.38), Cymopsis tetragonoloba (Fig.42) and Beta vulgaris (Fig.39). The curling and crinkling of leaves, vein thickening, reduction in leaf size, greening of veins, leathery and brittle leaves symptoms produced on different host plants are described in Table 18.

4.5 Incidence of TobLCV and vector population

4.5.1 Incidence of TobLCV and whitefly population in relation to planting date

The aim of this experiment was to find out the incidence of disease in relation to planting date and

vector *B. tabaci*. The different weather factors which have direct effect on whitefly population viz., temperature, relative humidity and rainfall have also been studied.

The results presented in Table 19 and Fig.64 indicates that the disease incidence in plots planted during 9th March, 8th April, 20th May and 12th June 1988, 14th February and 14th March 1989 was 90.5, 100.0, 98.2, 100.0, 100.0 and 88.2 per cent respectively in which the total number of whiteflies were 510, 582, 485, 328, 487 and 456 respectively.

A sudden decrease in the incidence was noticed in the plots planted during 12th July, 12th August, 10th September, 14th October, 22nd November and 1st December. The percentage of disease incidence was 73.5, 57.4, 52.2, 41.0, 46.5 and 44.4 respectively and vector population was 305, 213, 95, 66, 84 and 196, respectively during these periods. The planting done during 16th January, 14th February and 14th March showed a gradual increase in disease incidence and whitefly population.

It was interesting to note from the results that minimum whitefly population was recorded in September to December planted crops. The maximum TobLCV incidence was observed in March, April, May, June 1988 and February

Table 19. Incidence of TobLCV and whitefly population in relation to date of planting

Planting date	Observations recorded during the period	% TobLCV incidence during the period of observation	Whitefly[a] population during the period of observation
9. 3. 88	16. 3.88-21. 6.88	90.5	510
8. 4.88	15. 4.88-20. 7.88	100.0	582
20. 5.88	27. 5.88- 1. 8.88	98.2	485
12. 6.88	19. 6.88-24. 9.88	100.0	328
12. 7.88	19. 7.88-25.10.88	73.5	305
12. 8.88	19. 8.88-25.11.88	57.4	213
10. 9.88	17. 9.88-22.12.88	52.2	95
14.10.88	21.10.88-26.1. 89	41.0	66
22.11.88	29.11.88- 5. 3.89	46.5	84
1.12.88	7.12.88- 2. 4.89	44.4	196
16. 1.89	23. 1.89-30. 4.89	62.8	238
14. 2.89	21. 2.89-25. 5.89	100.0	487
14.3. 89	21. 3.89-25. 6.89	88.2	456

[a]Cumulative whitefly numbers per 20 plants recorded at weekly intervals for 12 weeks

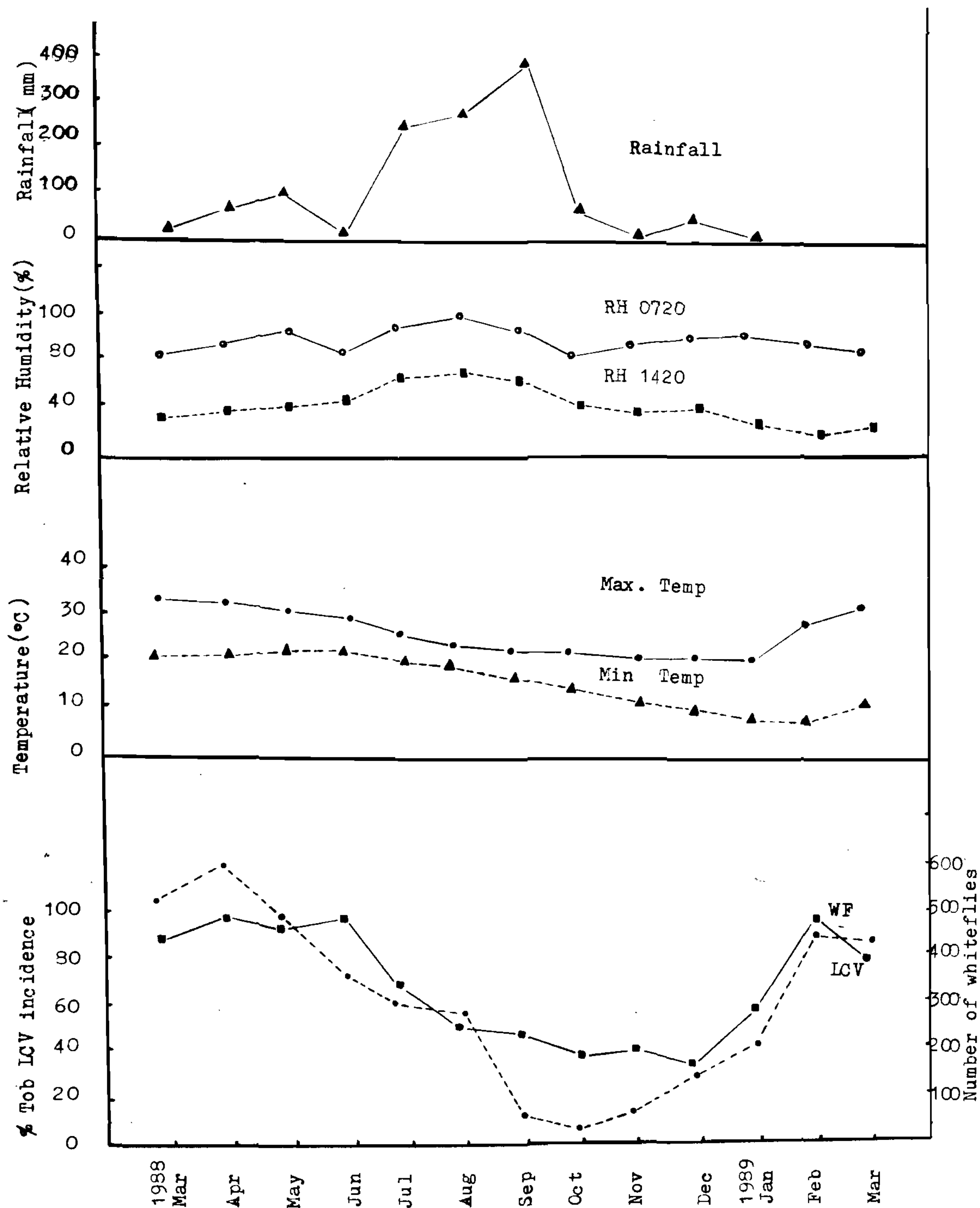


Fig 64 Tob LCV incidence in relation to whitefly population and weather conditions

Table 20. Weather data in relation to the whitefly population and the incidence of TobLCV during 1988-89

Month	Temperature(°C)		Relative humidity %		Rain-fall (mm)	Rainy days
	Max.	Min.	0720 h	1420 h		
1988						
March	33.3	20.1	81	31	29.5	2
April	33.3	21.0	84	39	72.9	7
May	32.8	21.8	87	48	117.9	6
June	30.9	20.9	80	49	2.5	2
July	28.4	20.1	92	66	276.9	17
August	28.1	20.1	95	69	278.3	16
September	28.1	19.5	94	68	442.4	18
October	28.0	17.7	86	50	68.6	6
November	27.5	15.5	90	45	10.0	2
December	26.0	14.9	92	47	33.9	3
1989						
January	26.9	13.9	93	39	-	-
February	30.5	12.3	84	22	-	-
March	31.5	17.3	81	36	-	-

and March 1989 planted crops. The whitefly population in these plots was very high during the crop growth.

4.5.2 TobLCV incidence and vector population in relation to weather conditions

Temperature: It was observed that vector population and maximum temperature have positive relation during tobacco growth at different planting dates. The decrease in temperature was associated with a regular decrease in whitefly population. The fall in maximum temperature below 30°C results in low population of vector and temperature above 30°C after February favours increased activity of whitefly (Table 20, Fig.64).

Relative humidity: The results presented in Table 19, Fig.64 showed that there was a negative relation between the vector population with relative humidity. High humidity is not congenial for the multiplication of whiteflies. The maximum whitefly population was recorded from March to June 1988 and February to March 1989 planted crops during which the relative humidity was comparatively low.

Rainfall: Rainfall showed direct effect on the population of whitefly (Fig.64). The number of whitefly declined when there was a heavy rainfall from July to October 1988.

It can be stated from the foregoing results that the maximum and minimum temperature, relative humidity, and rainfall play an important role in building up of whitefly population in nature.

4.5.3 TobLCV incidence in relation to location of tobacco nursery

The results presented in Table 21 indicate that TobLCV incidence and whitefly populations were higher in tobacco nursery raised near infected tobacco field, whereas lower in case of nursery raised 1000 M away from infected tobacco field.

4.5.4 TobLCV incidence in relation to stage of crop growth under field conditions

A field trial results (Table 22) indicate that per cent TobLCV incidence was significantly increased 15 days after planting under field conditions in all three cultivars, and as the crop growth advanced the disease incidence stabilized after 75 days of planting. There were no significant differences in per cent disease of TobLCV in three cultivars viz., FCV Special, Anand-119 and CTRI Special.

The whitefly population in the field decreased after 90 days of planting in all the three cultivars. The reason

Table 21. Incidence of TobLCV and whitefly population in tobacco nursery raised at two locations (a) nursery raised near infected tobacco field, and (b) nursery raised away from infected tobacco field

Cultivar[a]	Nursery raised near infected tobacco		Nursery raised away from infected tobacco	
	% TobLCV[b]	Whitefly[c] numbers	% TobLCV[b]	Whitefly[c] numbers
FCV. Sp	19.0	341	4.5	90
Anand-119	11.8	281	3.8	61
CTRI. Sp	11.4	279	2.4	68

[a] Sowing date 20.12.88

[b] Incidence recorded 45 days after sowing

[c] Weekly counts of whiteflies on 25 seedlings for 5 weeks (cumulative counts of 5 weeks)

Table 22. Incidence of TobLCV and whitefly population at different stages of crop growth under field conditions

Age of[a] the crop after planting (days)	Tobacco cultivar					
	FCV.Sp		Anand-119		CTRI.Sp	
	% TobLCV[b]	WF[cd]	% TobLCV	WF	% TobLCV	WF
15	2.8	30.0	0.8	30.5	2.5	31.2
30	17.0	32.5	8.8	32.5	15.6	32.0
45	24.6	31.5	15.4	23.8	25.1	24.0
60	34.9	38.2	20.8	28.2	35.5	37.8
75	38.3	38.8	29.7	30.8	36.4	29.5
90	39.0	27.5	32.6	22.8	39.4	16.0
105	44.5	20.8	36.7	16.0	40.2	15.2
120	45.4	12.8	40.9	12.2	42.5	9.5

[a]Planting date 11.3.89

[b]TobLCV incidence average of 2 replications

[c]Whitefly count on 25 plants

[d]Whitefly count average of 2 replications

may be increased nicotine content and gumy substances in the tobacco leaves which obstructed adult whitefly from feeding and settlement.

4.6 Monitoring of vector population

4.6.1 Yellow pan water traps

Yellow pan water traps were used for monitoring whitefly adults as described in materials and methods. Results presented in Table 23 and Fig.65 indicate that yellow pan water attracted more number of adult white-flies immediately in the second week after transplanting of tobacco in the field. As the crop growth advanced the whitefly population also decreased. At the end of crop period there were less number of whiteflies trapped in yellow water pans.

4.6.2 Detergents in yellow pan water traps

The main objective of this experiment was to improve monitoring method using different detergent in yellow pan water traps. The results (Table 24, Fig.66) indicate that trapping ability of whitefly adults was higher in Nirma (5%) detergent solution compared to rest of the treatments. There were no differences in trapping ability

Table 23. Monitoring of whitefly adults by yellow pan water traps

Weeks after planting	Whitefly adults[b] per trap
2[a]	2.1
3	2.0
4	2.2
5	2.3
6	2.8
7	3.7
8	3.8
9	4.5
10	2.8
11	1.6
12	1.0
13	0.8
14	0.9

[a] Planting date 16.6.88

[b] Average of 10 yellow pan water traps

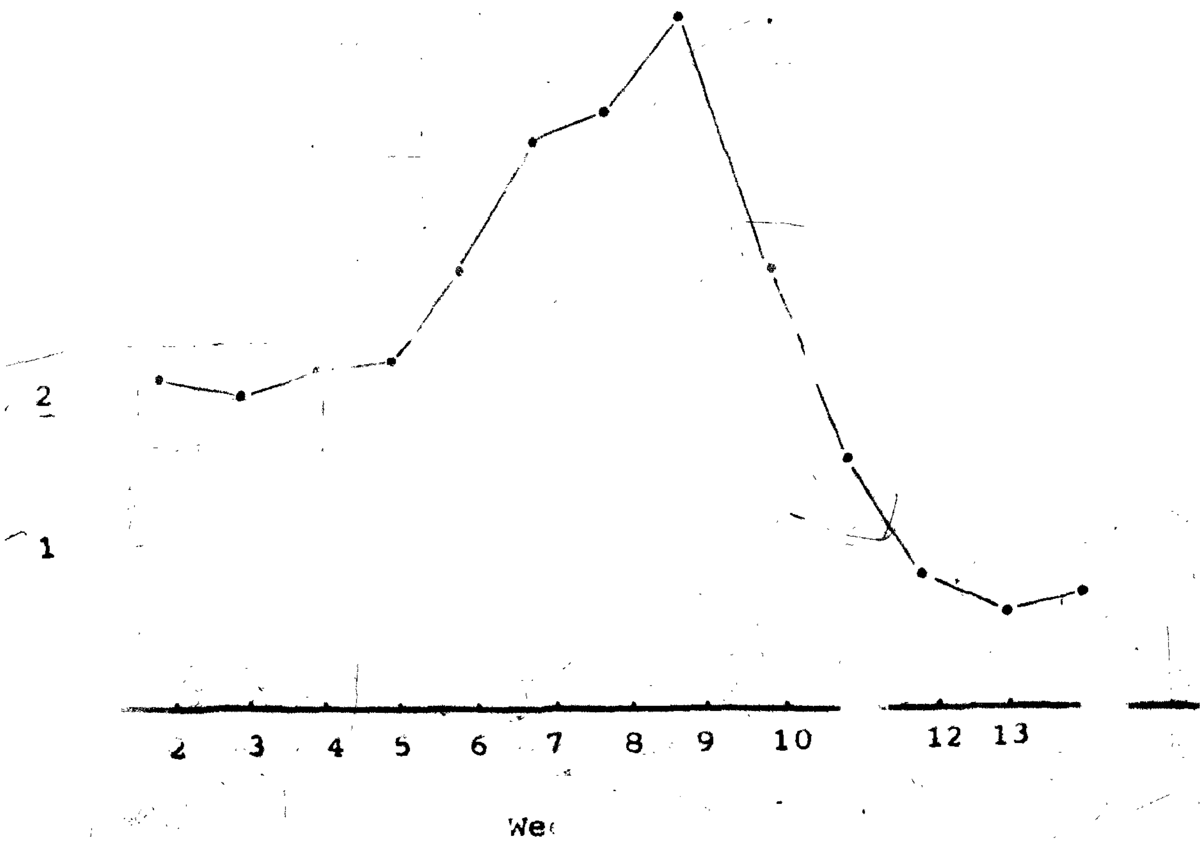


Fig.65 Monitoring of w. te adults by yellow

Table 24. Monitoring of whitefly adults by yellow pan water traps using different detergents

Weeks after transplanting	Whitefly adults trapped in yellow pan water traps				Mean
	Nirma (5%)	Teepol (5%)	Surf (5%)	Water (control)	
2	4.25	1.75	1.50	1.75	2.30
3	4.50	3.00	2.75	2.75	3.25
4	5.75	4.25	2.50	3.50	4.00
5	8.75	4.50	2.50	4.75	5.12
6	10.75	5.00	3.50	4.50	5.93
7	12.50	8.25	6.25	5.75	8.18
8	12.75	9.25	5.25	8.25	8.57
9	11.50	7.75	5.25	9.50	8.50
10	9.00	5.50	4.00	6.50	6.25
11	7.50	3.75	2.25	2.50	4.00
12	4.00	1.50	2.00	1.75	2.31
Mean	8.29	4.95	3.43	4.68	

	Cal F.	S.Em.	CD(5%)
Weekly interval (A)	55.61	0.357	0.990
Treatment (B)	2.24	0.216	0.597
A x B	8.03	0.715	1.981

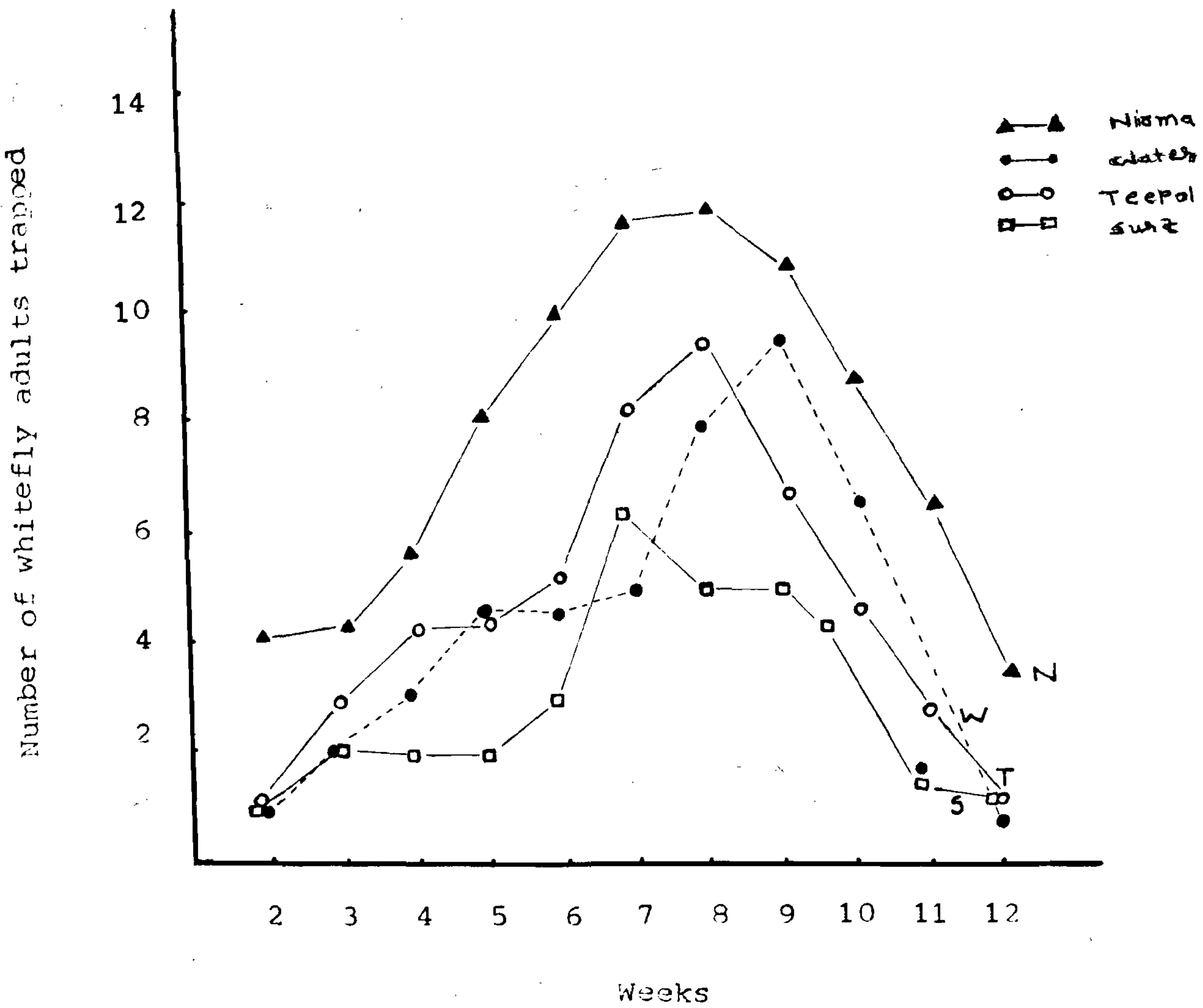


Fig.66 Monitoring of whitefly adults by yellow pan trap using different detergents

of the Teepol and yellow pan water traps, whereas Surf trapped least number of whiteflies. With regards to the age of the crop, significant differences were noticed in the different treatments for trapping ability. More number of whiteflies were trapped in the pans after third week of transplanting and gradually increased upto the eighth week. Then number of whitefly adults trapping declined as the tobacco growth advanced towards the maturity of crop. Interaction effect differ significantly.

4.7 Management of TobLCV in nursery

4.7.1 Covering nursery with nylon net

The results presented in Table 25 reveal that the tobacco nursery beds covered with nylon net frame (3 m long, 1.20 cm wide and 0.45 m depth) for about 45 days protected tobacco seedlings by preventing the entry of viruliferous whiteflies. There was no infection in the nylon net covered nursery. In uncovered nursery 5.4 per cent TobLCV incidence was recorded after 45 days of nursery period, whereas 62, 98 and 146 whiteflies were recorded on 15,30 and 45 days after sowing respectively.

It is clear from the results that nylon net covering can be incorporated in the integrated pest and disease management programme.

Table 25. Effect of nylon net covering for tobacco nursery on the incidence of TobLCV

Treatment	Whitefly numbers[c] in nursery			% TobLCV[d] in nursery
	days			
	15	30	45	
Nylon net[a,b] covering for tobacco nursery	0	0	0	0
Control (no covering)	62	98	146	5.4

[a]Sowing date 10.5.88

[b]Tobacco cultivar FCV Sp.

[c]Weekly counts of whiteflies on 25 seedlings for 5 weeks (cumulative counts of 5 weeks)

[d]Incidence recorded 45 days after sowing

4.7.2 Effects of barrier crops

The results presented in Table 26 indicate that barrier crop, sunflower and castor had prevented the landing of adult whiteflies on nursery tobacco and reduced TobLCV incidence in tobacco nursery. The castor leaves had attracted more number of adult whiteflies and the least in tobacco nursery bed compared to sunflower. Unprotected tobacco nursery bed revealed 11.2 per cent disease incidence and a maximum 90, 167 and 183 whiteflies at 15, 30 and 45 days after sowing, respectively.

The number of eggs laid by whitefly was higher on castor leaves at 10 days after sowing and gradually increased upto 40 days, whereas less on sunflower leaves. The maximum number of whitefly eggs on leaves of barrier crops were noticed after 30 days of sowing (Fig.67).

4.8 Diagnosis of TobLCV in different host plants

4.8.1 Enzyme linked immunosorbent assay

African cassava mosaic virus (ACMV) antibody (Poly-clonal) conjugated with alkaline phosphatase was used to detect TobLCV in crude sap of tobacco and other host species. p-nitrophenyl phosphate (0.6 mg/ml)

Table 26. Effect of barrier crops on the incidence of TobLCV in nursery

Barrier crop[a] around tobacco nursery	Whitefly numbers[d]															% TobLCV[a] incidence
	Barrier crop						Barrier crop						Control tobacco nursery			
	On sunflower			Tobacco[b] nursery			On castor			Tobacco nursery						
	15[c]	30	45	15	30	45	15	30	45	15	30	45	15	30	45	
Sunflower	134	96	36	52	69	85	-	-	-	-	-	-	-	-	-	8.8
Castor	-	-	-	-	-	-	317	452	194	50	32	58	-	-	-	4.6
No barrier crop	-	-	-	-	-	-	-	-	-	-	-	-	90	167	183	11.2

[a] Barrier crop sowing date 28.5.88

[b] Tobacco cv. Swarna Sowing date 11.6.88

[c] Days after tobacco sowing

[d] Whitefly counts on 25 seedlings for 5 weeks (cumulative counts of 5 weeks)

[e] Incidence recorded 45 days after sowing

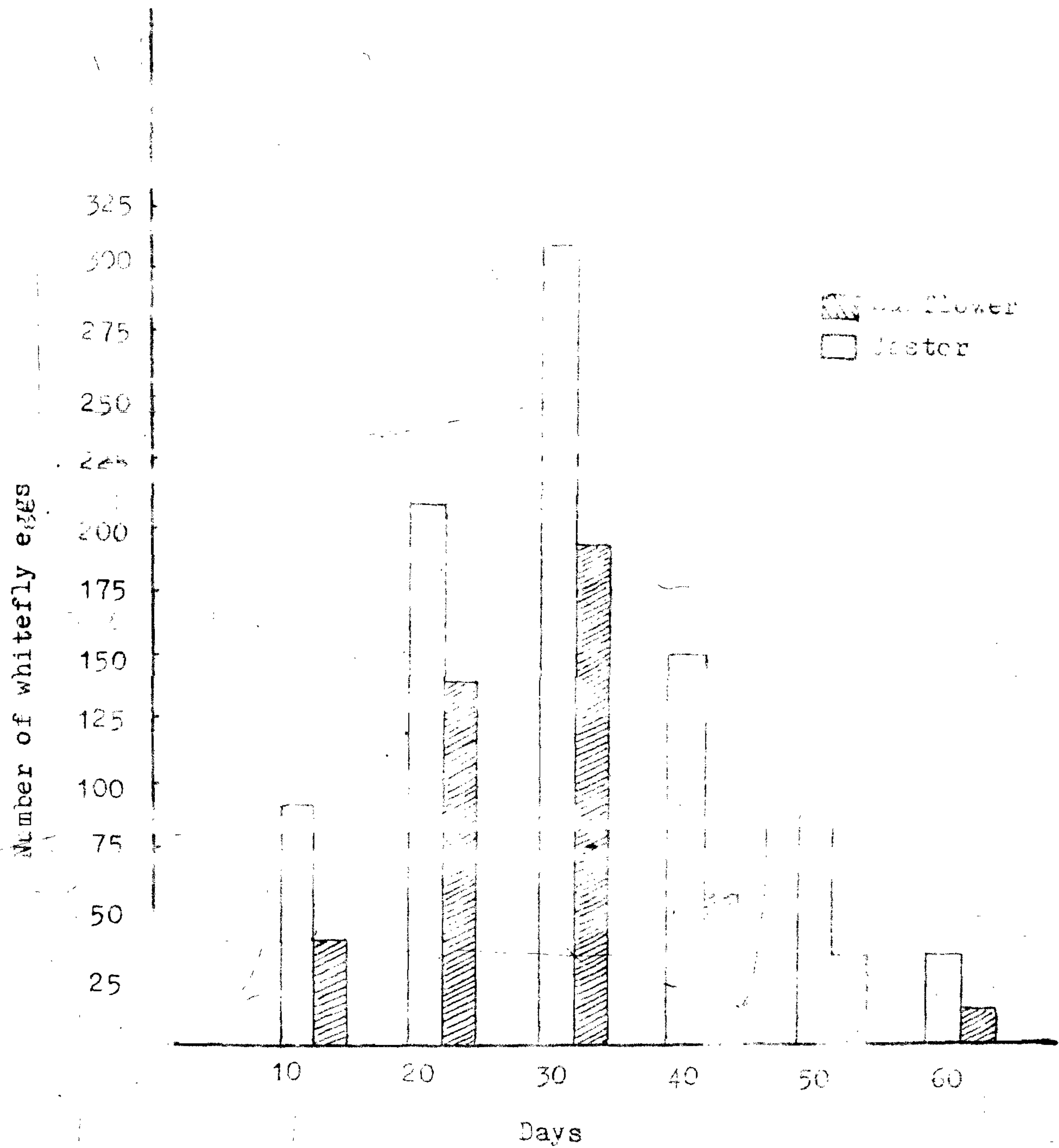


Fig.6 Host preference for the oviposition by whitefly

in 10 per cent diethanol amine, was used as substrate, 30 minutes after adding substrate yellow colour started developing, indicated presence of TobLCV, whereas known healthy samples did not produce any colour upto certain period (Autophoto degradation of p-nitrophenyl phosphate brings extra yellow colour). Two hour after adding substrate, plates were read by biotek microplate auto reader. Absorbance values at A_{405}nm were obtained from 10 replications of each sample are given in Table 27.

ACMV conjugate could efficiently detect TobLCV in several hosts where average A_{405} ranged from 1.01 to 2.50 in N.tabacum cvs. Samsun, Anand-119, White Burley, N. banthamiana, Euphorbia geniculata, Beta vulgaris, Lycopersicon esculentum, Oxalis corniculata, Petunia hybrida, Zinnia elegans, Phaseolus vulgaris and Croton bonplandianum. Moderate to weak reaction was obtained in N.glutinosa, Ageratum conyzoides, Datura stramonium, Nicandra physalodes, Parthenium hysterophorus, Sonchus brachyotis, Synedrella nodiflora, Althaea rosea, Dianthus caryophyllus and Acanthospermum hispidum where average absorbance ranged from 0.90 to 0.91. Healthy samples of above plants showed absorbance values ranging from 0.90 to 0.91. Healthy samples of above plants showed absorbance values ranging from 0.09 to 0.18.

Table 27. Detection of TobLCV by ELISA in different infected host plants. Reaction of ACMV with different TobLCV samples

Host	A_{405} readings [a,b,c,d]	
	Diseased samples	Healthy samples
1	2	3
N. tabacum cv. Samsun	1.83	0.12
N. tabacum cv. Anand-119	1.28	0.13
N. tabacum cv. White Burley	1.37	0.15
N. glutinosa	0.68	0.11
N. benthamiana	2.50	0.13
Euphorbia geniculata	1.57	0.17
Beta vulgaris	1.01	0.09
Lycopersicon esculentum	1.28	0.18
Ageratum conyzoides	0.91	0.10
Datura stramonium	0.57	0.12
Nicandra physaloides	0.68	0.09
Oxalis corniculata	1.01	0.14
Sonchus brachyotis	0.68	0.15
Synedrella nodiflora	0.57	0.11
Petunia hybrida	1.24	0.10
Zinnia elegans	1.92	0.10
Althaea rosea	0.50	0.18

Table 27(continued)

1	2	3
Dicanthus caryophyllus	0.68	0.18
Acanthospermum hispidum	0.50	0.15
Phaseolus vulgaris	1.98	0.17
Croton bonplandianum	1.91	0.17

[a] Absorbance values at A_{405} from 10 duplicating wells

[b] Samples were prepared in extraction buffer (10 ml/g of leaves)

[c] African cassava mosaic antibodies conjugated with alkaline phosphatase

[d] Plates were read 2 h after adding substrate (0.6 mg/ml) by bio tek micro plate auto reader(EL 309)

4.8.2 Immunosorbent electron microscopy (ISEM)

Typical geminate particles (15-18 x 30 nm) were detected in the leaf extract of tobacco infected with TobLCV in the immunosorbent electron microscopy technique by using Indian cassava mosaic antiserum (Fig.68).

Fig.68 Geminivirus particles trapped from crude leaf extracts of tobacco infected with TobLCV by using Indian cassava mosaic virus antibody in ISEM

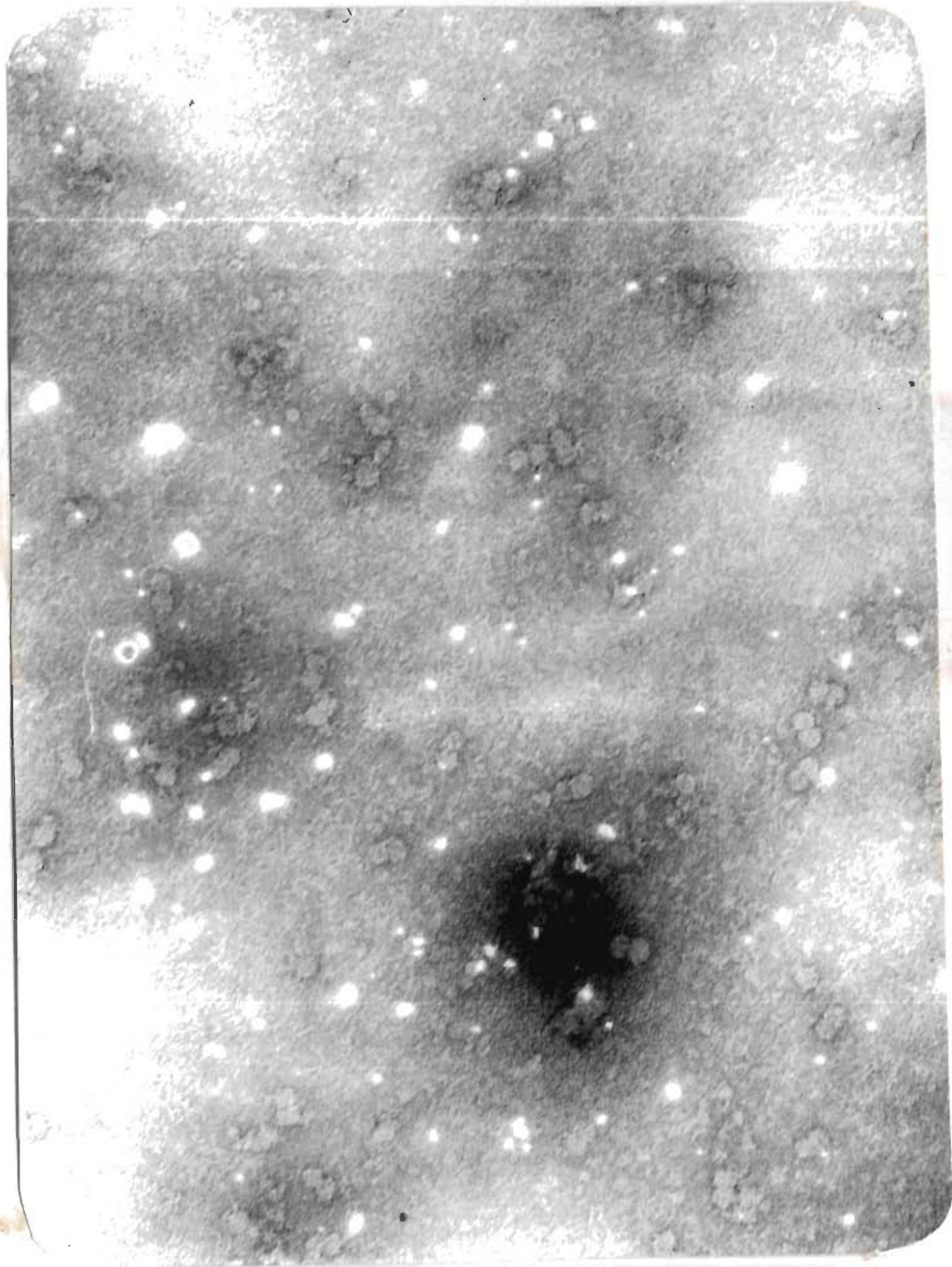


Fig. 68

DISCUSSION

V. DISCUSSION

Tobacco is the foremost remunerative non-food commercially important cash crop and has occupied a significant position in the Indian economy. Among the virus diseases of tobacco, leaf curl has the destructive nature and cause over 90 per cent damage during epidemic condition (Pal and Tandon, 1937). Reduction in yield of tobacco by over 5 per cent was attributed by Reddy and Nagarajan (1982) in India and 50 per cent in Sudan(Yassin and Abu Salih, 1972). The diseased plant exhibits severe vein thickening, curling, crinkling, ruffling and green outgrowth and such diseased leaves cannot be flue cured. Whitefly *Bemisia tabaci* is known to spread disease in nature. TobLCV has occurred regularly in India but in recent years outbreak of whitefly due to indiscriminate use of synthetic pyrethroides on cotton in Andhra Pradesh, Gujarat and Maharashtra, caused serious damage to tobacco crop.

For understanding a widely occurring disease, the present investigations were undertaken with the following aspects: Survey for the incidence of TobLCV and vector population in tobacco growing areas, symptomatology and characterization, host range of TobLCV, spread of the disease in nursery and field, management

of vector and virus by cultural practices and diagnosis of TobLCV by sensitive immunological techniques.

TobLCV and vector whiteflies were found to occur throughout the tobacco growing areas. Survey was conducted during 1988 and 1989 to estimate the incidence of TobLCV in major tobacco growing states of Andhra Pradesh, Karnataka, Gujarat, West Bengal and Bihar. TobLCV incidence was found to vary from 1.2 to 77.6 per cent in different agroclimatic conditions of India. The population dynamics of B.tabaci also varied in a similar manner. The maximum incidence of disease and whitefly population was observed in cigarette and bidi tobacco growing areas of Andhra Pradesh (77.6%, 32 whiteflies/plant) and Gujarat (59.4%, 20 whiteflies/plant) respectively. The minimum incidence of TobLCV was recorded in Shimoga (1.2%, 1 whitefly/plant) and Hunsur (3.2%, 2 whiteflies/plant) of Karnataka followed by West Bengal (5.4%, 5 whiteflies/plant) and Bihar (11.6%, 8 whiteflies/plant).

The incidence of TobLCV and vector populations in tobacco growing areas when correlated with the weather conditions clearly indicated that Andhra Pradesh and Gujarat states are the hot spots of the disease. The higher disease incidence and maximum vector populations

are constantly present due to high temperature, in Andhra Pradesh, Gujarat and Nipani area of Karnataka. Pal and Tandon (1937) observed the disease incidence 5 to 10 per cent in normal period and 70 per cent during an epidemic year. Similar trend of TobLCV was also recorded by Pruthi and Samuel (1937) in North Bihar. Monga and Tripathi(1988) reported 7 and 14 per cent incidence on different tobacco cultivars from Dinhata (West Bengal).

Sequential plantings from March 1988 to March 1989 indicated that the TobLCV incidence was shown to be high in the months of February to June and then onwards gradually decreased following minimum in the months of October to December (Table 19, Fig.64). Similar results on seasonal variation of whitefly population have been obtained by Pal and Tandon (1937) and Pruthi and Samuel (1942) Munshi and Choudhry (1964) derived that as the planting period advanced towards the winter the infection also declined trend. The similar trend of increasing and decreasing of whitefly population in yellow mosaic disease of pigeonpea was noticed by Nene (1972).

Different tobacco growing areas surveyed to estimate disease incidence on <u>Nicotiana</u> species and cultivars, indicated that all the species and cultivars

were found susceptible to TobLCV with varying degree of infection in field conditions. The incidence varied from 1 to 90 per cent depending on cultivars and place. Field tolerance against TobLCV was recorded in *N.megalosiphon*, *N.plumbaginifolia* and *N. repanda* (Anonymous, 1961). Field screening of 553 lines and cultivars revealed only line 100-26(K-20x Smyma) x 20 moderately resistant to TobLCV (Patel and Patel, 1987).

Survey for the alternate host of TobLCV in and around tobacco field in different states of India revealed that causal agent infected cultivated hosts *Lycopersicon esculentum* and *N.tabacum*, and the weed plants *Acanthospermum hispidum*, *Ageratum conyzoides*, *Datura stramonium* and *Parthenium hysterophorus* in many places of India which might be serving as source of infection to the tobacco crop. Pruthi and Samuel (1942) reported 101 host plants belonging to 23 families to be the reservoir hosts of tobacco leaf curl virus. Wolf *et al*. (1949) also recorded fairly wide hosts of 63 plant species from 14 families including 45 genera. *Ageratum conyzoides*, *Synedrella nodiflora* and *Vernonia cinerea* were reported as sources of inoculum by Thung (1934), *A. conyzoides* by Gadd and Loos (1941) and *A.hispidum* by Mariappan and Narayanaswami (1972).

TobLCV infected plant exhibits curling, stunting of plant, reduction of internode length and leaf size, vein thickening, greening and swelling of veins, green stitch or cup like enations on veins, reduction of infloresecneces and shrivelled capsules symptoms in the field. These symptoms were similar to those described by Pal and Tandon (1937), Pruthi and Samuel (1937), Thung (1932), Flores (1961) and Reddy and Nagarajan (1982).

In the present study, a total 45 isolates of TobLCV were collected during survey from Andhra Pradesh, Karnataka, Gujarat, West Bengal and Bihar and transmitted through B.tabaci on tobacco cultivars Samsun and Anand-119. Variation in leaf curl symptoms exhibited by cultivars designated as TobLCV group I, II, III and IV (Tables 10, 11; Figs. 18, 19, 20, 21). Their resemblance with the earlier reports are discussed herein.

TobLCV group I: Plant height reduced, curling and uneven ruffling of leaves, shortening of internode, greening and vein thickening, depressions on upper surface, leafy outgrowth (enations) developed in the form of stalked and sessile cup shape and inflorescences reduced greatly on infected plants. The similar type of symptoms was described in group A and B by Pal and Tandon (1937) and Thung (1932) as common kreopock.

TobLCV group II: Terminal leaves pale green colour, reduction of leaf size and internode, formation of thorny (spiny) erect enations, pit like depression on upper surface of leaves developed on infected plant. This is the first report of distinctive thorny enation on TobLCV infected tobacco leaves. TobLCV group III: Reduction in plant height, leaf narrowing, leaves brittle and leathery, vein zig zag and thick and small protruding enation developed in between veins consistantly. This group resembled with group 'C' described by Pal and Tandon (1937) but differs in pattern of enations. TobLCV group IV: Symptoms of this group similar to group III except the irregular thickening and swelling of veins and frilled (boat) shape enation developed on bright green veins. Symptoms of this are mild as described by Pal and Tandon (1937) in type 'D' except the enation pattern.

All the TobLCV group I, II, III and IV are present in Andhra Pradesh, Karnataka, Gujarat and West Bengal. The Bihar isolates showed only two symptom variants (group I, IV). Overall distributions are found consistant in all the places, irrespective of cultivars and agro-climatic conditions.

The transmission study of TobLCV by B.tabaci to different Nicotiana species and cultivars in the glasshouse, indicated that all the species and cultivars of tobacco were infected and exhibited typical severe to mild leaf curl symptoms. N.ampelsia and N. megalosiphon were found free from infection. Field tolerance against TobLCV was noticed in case of N. megalosiphon (Anon., 1962). Reddy and Nagarajan(1982) reported that there are no cultivars resistant to tobacco leaf curl virus. Patel and Patel (1987) have identified only one line 100-26 (K-20 x Smyma) x 20 moderately resistant to disease.

Of the 88 plant species inoculated by B.tabaci 35 species were infected with TobLCV. The general symptoms produced by TobLCV in different inoculated plants were leaf curl and enations. Only Ageratum conyzoides and Euphorbia geniculata produced prominent vein clearing and yellowing, Oxalis corniculata showed erect thorny enation on veins.

The host range study showed that TobLCV could infect several agriculturally important cultivated plants viz. Beta vulgaris, Carica papaya, Capsicum annuum, Crotalaria juncea, Cymopsis tetragonoloba, Helianthus annuus, Lycopersicon esculentum, Sesamum indicum, Phaseolus

vulgaris, the ornamental plants Althaea rosea, Callistephus chinensis, Dahalia sp., Dianthus caryophyllus, Petunia hybrida, Salvia officinalis, Tagets erecta and Zinnia elegans. In addition, weed host plants Acanthospermum hispidum, Acalypha indica, Ageratum conyzoides, Bidens pilosa, Centella asiatica, Croton bonplandianum, Datura stramonium, Euphorbia geniculata, Galinsoga parviflora, Heliotropium indicum, Nicandra physalodes, Oxalis corniculata, Parthenium hysterophorus, Phyllanthus niruri, Solanum torvum, Sonchus brachyotis, Stachytarpheta indica and Synedrella nodiflora were found susceptible to TobLCV. The natural occurrence of TobLCV on several cultivated, ornamentals and weeds host plants were recorded by Pruthi and Samuel (1939, 1942), Shepherd (1940), Mc Clean (1940), Nariani and Pathanian (1953), Garga (1949), Wolf et al. (1949), Phatak and Raychaudhuri (1967), Hill (1968), Olivares et al. (1972), Mathur (1932, 1933), Nariani(1956, 1968), ~~Nariani (1968)~~, Mishra et al. (1963) and Flores (1961). Mandal (1989) inoculated croton yellow vein mosaic virus (CYVMV) by B.tabaci to different tobacco cultivars, cultivated plants, ornamentals and weed hosts, exhibited leaf curl and enation symptoms similar to TobLCV symptoms. The differences between CYVMV and TobLCV was the yellow vein mosaic produced in Ageratum conyzoides when inoculated with TobLCV whereas distinct leaf curl and enation exhibited

by CYVMV on ~~the same host~~. TobLCV could infect *Euphorbia geniculata* but CYVMV failed to produce any symptoms.

The incidence of TobLCV was observed in the nursery and field conditions. The high incidence of TobLCV was observed in tobacco nursery raised near infected tobacco whereas low incidence was recorded in nursery raised away (1000 m) from infected fields.

Tobacco cultivars FCV Special, Anand-119 and CTRI Special seedlings planted in field showed significant increase in incidence of TobLCV after 15 days of transplanting. As the crop growth advanced the disease incidence also increased and stabilized after 75 days of transplanting. The whitefly population was more in field after transplanting but gradually decreased after 90 days of planting in all the cultivars. Similar trend was observed in tomato leaf curl virus by Datar (1984), Saklani and Mathai (1977).

To study the spread of the disease in relation to vector activity, a monitoring device that accurately measures the number of whiteflies alighting on plant foliage is very essential. To evolve reliable monitoring of whiteflies, different methods viz. direct count, yellow pan water traps and different detergents were used. The

whitefly population trends shown by these methods were almost similar, showing a slow increase of the population in the early stage of crop growth, and as the growth of crop increased the population also increased and there was slow decrease at the end of crop growth. However the actual whitefly numbers differed in different methods. Yellow pan water traps were convenient and the counts represent the population of the whiteflies in the field. This is in conformation with the earlier findings of whitefly population reported by Saikia and Muniyappa (1989) and Mathew (1988) for tomato leaf curl virus and Indian cassava mosaic virus respectively.

The water pan traps with different detergents viz. Nirma, Teepol and Surf at the rate of 5 per cent along with yellow pan water trap showed significant differences. The Nirma solution attracted significantly more number of whitefly adults than the rest of the treatment. In all the treatments more number of white-flies were trapped after third week of transplanting and number increased in seventh week, but then after trapping ability decreased as the tobacco growth advanced toward maturity.

In the light of ecological and epidemiological studies as described in sections, it was considered that the only suitable remedy or the better management of this disease would be by raising healthy seedlings in the nursery.

The nursery beds covered with nylon net frame (3 m long, 1.20 m wide and .45 m width) for about 45 days protected tobacco cv. FCV special seedlings by preventing the entry of viruliferous whiteflies compared to uncovered nursery. The results are in conformity with those reported by Saikia and Muniyappa(1989). The combined treatment of nylon net covering for nursery bed and 2-3 sprays of monocrotophos or dimethoate or cypermethrin after transplanting in the field was effective in reducing the spread of tomato leaf curl virus.

In another attempt, the barrier crops, castor and sunflower when sown around tobacco nursery reduced the TobLCV incidence by preventing the feeding of adult whiteflies in tobacco nursery. The castor leaves attracted more number of adult whiteflies and reduced the TobLCV incidence by about 50 per cent. The castor was found most preferred host and the number of eggs laid by whitefly on leaves were more compared to sunflower. Saikia (1985)

also summarized that avare, bhendi, cucumber, maize and sorghum when used either as intercrop or border crop along with tomato had considerable effect in reducing the tomato leaf curl virus in field. TLCV incidence was comparatively less in the plots planted along with sun-flower.

Direct form of double antibody sandwitch enzyme linked immunosorbent assay (DAS-ELISA) as described by Clark and Adams (1977) was employed with little modification (described in section 3.13.1) for the detection of TobLCV. African cassava mosaic virus(ACMV) antiserum conjugated with alkaline phosphatase could detect TobLCV efficiently in crude diseased leaf sap of tobacco and several other infected host plants.

TobLCV inoculated plant species by B.tabaci in glasshouse were tested by ELISA. Absorbance values of different plants varied from 0.50 to 2.50 (Table 28). Mandal (1989) standardized DAS-ELISA for the detection of croton yellow vein mosaic virus and detected the antigen in 22 plant species inoculated by B.tabaci.

TobLCV particles were detected in crude diseased leaf extracts of tobacco by immunosorbent electron microscopy (ISEM) using Indian cassava mosaic virus

antiserum. Typical geminate particles of 15-18 x 30 nm was observed (Fig.66). Roberts et al.(1984) also used ISEM for detecting geminivirus particles in hostplants by using ACMV antiserum and other antisera against whitefly borne viruses. Mandal (1989) also observed geminate particles in crude leaf extract of CYVMV infected plants in immunosorbent electron microscopy by using ISMV antiserum.

Conclusions: Tobacco leaf curl is one of the most destructive disease in the tobacco growing areas of Andhra Pradesh and Gujarat. It is also present in Karnataka, West Bengal and Bihar with varying degree of infection. The higher disease incidence and vector *B.tabaci* populations were clearly correlated with the high temperature, medium or no rainfall and low relative humidity. The tropical conditions favoured in building up of the whitefly populations and relatively TobLCV incidence also shot up in Andhra Pradesh and Gujarat in the recent years. The survey for incidence of TobLCV in tobacco cultivars and other host species revealed that cultivated hosts, ornamentals and weed host plants serve as sources of TobLCV in the field and thereby infection is carried round the year to main tobacco crop. There are no resistant cultivars as such but some species and lines showed tolerance to TobLCV.

Keeping in view the spread of disease and vector, tobacco nursery management programme was implemented and nylon net covering for 45 days in nursery resulted disease free transplantable seedlings. In another effort castor as barrier crop sown around nursery reduced the incidence of TobLCV by obstructing and attracting adult whiteflies. Along with the cultural practices (nylon net covering, barrier crops) other integrated pest management can be used.

Another useful contribution was the standardization of ELISA and ISEM techniques for the diagnosis of TobLCV in different cultivars, cultivated plants and weeds both in field and also in laboratory conditions.

SUMMARY

VI. SUMMARY

1. Survey conducted to assess the incidence of tobacco leaf curl virus (TobLCV) in tobacco growing areas of Andhra Pradesh,Karnataka, Gujarat, West Bengal and Bihar revealed that the incidence varied from 1.2 to 77.6 per cent. The maximum incidence of 77.6 per cent was observed in Andhra Pradesh following Gujarat (59.4%), Karnataka (17.1%), Bihar (11.6%) and West Bengal (5.4%).

2. Whitefly population on tobacco varied under different agroclimatic conditions of India. The more number of white-flies were observed in tobacco growing areas of Andhra Pradesh and Gujarat when compared to other places.

3. Alternate hosts of TobLCV in tobacco growing area surveyed revealed that the causal agent survived on cultivated and weed host plants in and around the tobacco fields. Lycopersicon esculentum, N.tabacum, Sesamum indicum, Carica papaya were naturally infected with tobacco leaf virus in many places in India. Several weeds eg. Acanthospermum hispidum, Ageratum conyzoides, Datura stramonium, Euphorbia geniculata, Stachytarpheta indica, Parthenium hysterophorus, Synedrella nodiflora, Sonchus brachyotis and Oxalis corniculata infected with TobLCV were observed in all tobacco growing areas in India.

4. Incidence of TobLCV on different *Nicotiana* species and cultivars in different tobacco growing areas varied from 1 to 90 per cent. All the cultivars and species were susceptible to TobLCV in field.

5. Forty-five isolates of TobLCV were collected from Andhra Pradesh, Karnataka, Gujarat, West Bengal and Bihar. All the isolates were transmitted to *N.tabacum* cvs. Samsun and Anand-119 by *B.tabaci* and symptom variation was recorded.

6. The symptom variation of 45 isolates of TobLCV on Samsun and Anand-119 were classified into four groups and designated as groups I, II, III and IV. **Group I:** Plant height reduced, curling, ruffling of leaves and shortening of internode, greening and thickening of vein, enation sessile and cup shaped and inflorescences reduced greatly. **Group II:** Terminal leaves pale green colour, reduction of leaf size and internode, thorny erect enations and pit like depressions on leaves, **Group III:** Leaf narrowing, zig zag vein, leaves leathery and brittle and small protruding enation inbetween veins formed consistantly; **Group IV:** Similar to group III except irregular thickening and swelling of veins and frilled (boat) shape enation developed on bright green veins.

7. The four TobLCV groups were present in major tobacco growing areas of Andhra Pradesh, Karnataka, Gujarat and West Bengal. In Bihar only two virus groups (I, IV) were observed.

8. TobLCV was transmitted to 13 different _Nicotiana_ species and 28 tobacco cultivars in the glasshouse. The infected species were _N. benthamiana_, _N. clevelandii_, _N. corymbosa_, _N. execlsior_, _N.glauca_, _N. glutinosa_, _N. nudicualis_, _N.occidentalis_, _N.rosulata_, _N.suaveolens_ and _N.undulata_. _N. ampelsia_ and _N.megalosiphon_ were not infected. The incubation period in the plants varied from 10 to 45 days.

9. A total of 88 different plant species of cultivated, ornamentals, weeds and forest plants were inoculated by _B.tabaci_ in the glasshouse, of which 35 different host plants, eg. cultivated (10), ornamentals (9) and weed plants(18) were found infected with TobLCV. The following species have infected: _Beta vulgaris_, _Carica papaya_, _Capsicum annuum_, _Crotalaria juncea_, _Gymopsis tetragonoloba_, _Helianthus annuus_, _Lycopersicon esculentum_, _Sesamum indicum_, _Phaseolus vulgaris_ (cultivated plants); _Althaea rosea_, _Callistephus chinensis_, _Dahalia_ sp., _Dianthus caryophyllus_, _Petunia hybrida_, _Salvia officinalis_,

Tagetes erecta and Zinnia elegans (ornamental plants); Acanthospermum hispidum, Acalypha indica, Ageratum conyzoides, Bidens pilosa, Centella asiatica, Croton bonplandianum, Datura stramonium, Euphorbia geniculata, Galinsoga purviflora, Heliotropium indicum, Nicandra physaloides, Oxalis corniculata, Parthenium hysterophorus, Phyllanthus niruri, Solanum torvum, Sonchus brachyotis, Stachytarpheta indica and Synedrella nodiflora (weed hosts). The per cent incidence (15.0 to 100%) and incubation period (12 to 60 days) varied from species to species.

10. TobLCV produced typical prominent vein clearing symptoms in Ageratum conyzoides and Euphorbia geniculata, thorny enations on Oxalis corniculata, small enations on Althaea rosea, Cymopsis tetragonoloba and Beta vulgaris.

11. Incidence of TobLCV and vector population study in the field revealed that season had profound effect on both virus and vector. In general per cent incidence of TobLCV and whitefly populations were high in February to June planted crops and low from August to December planted tobacco crops. Lowest incidence and whitefly populations were noticed from October to December.

12. High temperature, low or no rainfall and low relative humidity contributed to the increase in the whitefly population from March to June. The whitefly population during the months of July to October was low. The vector population was positively correlated with maximum temperature, no or low rainfall and relative humidity.

13. The spread of TobLCV and vector estimated in the field condition indicated that incidence of TobLCV increased after 15 days of transplanting and stabilized after 75 days. Whitefly population in the field declined as the tobacco growth advanced.

14. The nursery raised near infected tobacco field showed high incidence of TobLCV and whitefly populations in all the cultivars compared to nursery raised away(1000m) from infected field. The disease spread quickly through vector from source to main crop.

15. Yellow pan water attracted more number of white-flies immediately after second week of planting of tobacco and further fluctuated as the crop growth advanced.

16. Among the detergents, the attraction ability indicated that Nirma 5 per cent solution in yellow pan water attracted significantly more number of adult white-flies compared to other detergents and yellow pan water traps.

17. Tobacco nursery beds covered with nylon-net resulted disease free transplantable seedlings and prevented entry of the whitefly for about 45 days of nursery period.

18. Barrier crop castor sown around tobacco nursery attracted more number of whiteflies and reduced the incidence of TobLCV compared to sunflower.

19. The whiteflies preferred castor leaves and laid more number of eggs after 10 days of sowing than on sunflower.

20. TobLCV was detected by ELISA in 22 infected plant species eg. Beta vulgaris, Lycopersicon esculentum and Phaseolus vulgaris (cultivated plants); N.tabacum cvs. Samsun, Anand-119, White Burley, N.glutinosa and N. banthamiana; (tobacco plants); Petunia hybrida, Zinnia elegans, Althaea rosea and Dianthus caryophyllus (ornamental plants); Euphorbia geniculata, Ageratum conyzoides, Datura stramonium, Nicandra physaloides, Oxalis corniculata, Parthenium hysterophorus, Sonchus brachyotis, Synedrella nodiflora, Acanthospermum hispidum and Croton bonplandianum (weed hosts). Absorbance values (A_{405}) for all those

infected plant species varied from 0.50 to 2.50 representing relative concentration of TobLCV in different hosts. Whereas values for the corresponding known healthy hosts varied from 0.09 to 0.18.

21. TobLCV was detected by immunosorbent electron microscopy (ISEM) technique using Indian cassava mosaic virus antiserum. Typical geminate virus particles of 15-18 x 30 nm was observed in the transmission electron microscope.

REFERENCES

VII. REFERENCES

Anonymous,, 1961, Breeding for resistant to leaf curl. Annual Report; Bidi Tobacco Research Station, Anand, Gujarat. p. 49.

Anonymous., 1962, Testing of Nicotiana sp. for their relative resistance to leaf curl. Annual Report; Central Tobacco Research Instt., Rajahamundry. pp. 212-213.

Anonymous., 1988, World tobacco production. Indian Tob. J., 20: 16-17.

Anonymous., 1989, All India final estimate of tobacco. Agril. Situation India; 63: 1092-1093.

AL-Ani, R.A., Samir, S.H. and Jarjees, M.M., 1987, Identification and control of tobacco leaf curl virus. Arab. J. Pl. Prot., 5: 70-73.

Bird, J., 1958, Infectious chlorosis of Sida carpinifolia in Puerto Rico. Agr. Exp. Sta., Uni. Puerto Rico Tech. Paper 26: 1-23.

Bird, J., 1981, Relationship between whiteflies and white-fly borne viruses. In. International Workshop on pathogens transmitted by whiteflies. AAB/FBAP/ISPP., Oxford.

Bird, J. and Maramorosch, K., 1978, Viruses and diseases associated with whiteflies. Adv. virus. Res., 22: 55-109.

Castellani, E., Nour, A.M. and Mohamed, M.I., 1981, (Tomato leaf curl in Somalia) L'arricciamento fogliare del pomodaro in Somalia. Annali della Facolta di Scienze. Agrarie. della. Universita degli Studi. Ti. Torino; 12: 145-161.

Chang, Y.C., 1967, Studies on the whitefly (Aleurotuberculatus psidii (Singh) in transmission of tobacco leaf curl. Taiwan Tob. Wine Monop. Bur. Tob. Res. Inst. Annu. Rep., 232 pp.

Chari, M.S., 1989, Tobacco, Need to sustain export market. In. The Hindu, Survey of Indian Agriculture. 271 pp.

Chari, M.S. and Jaisani, B.G., 1987, Substitution of tobacco with alternate crops and diversified uses of tobacco. In. VII National Symposium on tobacco and golden jubilee celebrations of CTRI, Guntur (A.P.); pp. 4-7.

Clark, M.F. and Adams, A.N., 1977, Characteristics of microplate method of enzyme linked Immunosorbent assay for the detection of plant virus. J. Gen. Virology, 34: 476-483.

Clark, M.F. and Bar-Joseph, M., 1984, Enzyme linked immunosorbent assay in plant virology. Meth. Virology., 7: 51-85.

Cohen, S., 1981, Control of whitefly vectors of viruses by colour mulches. In. Harris, K.F. and Maramorosch, K. (Eds.). Pathogens, vectors and plant diseases: Approaches to control. Academic Press Inc. New York., pp. 45-56.

Cohen, S. and Melamed-Madjar, V., 1974, Prevention by soil mulching of the spread of tomato yellow leaf curl virus transmitted by Bemisia tabaci (Gennadius) (Homoptera:Aleyrodidae) in Israel. Bull. Ent. Res., 64: 193-197.

Cohen, S., Duffus, J.E., Larsen, R.C., Liv, H.Y. and Flock, R.A., 1983, Purification, serology and vector virus relationship of squash leaf curl virus, a whitefly transmitted gemini virus. Phytopathology. 73: 1669-1673.

Capoor, S.P. and Varma, P.M., 1950, Yellow vein mosaic of Hibiscus esculentus L. Indian J. agric. Sci., 20: 217-230.

Costa, A.S., 1969, Whiteflies as virus vector. In Maramorosch, K. Ed. Viruses, vectors and vegetation, Wiley (Interscience), New York., pp. 95-119.

Costa, A.S. and Bennett, C.W., 1950, Whitefly (B.tabaci) transmitted mosaic of Euphorbia prunifolia. Phytopathology. 40:266-283.

Costa, A.S. and Carvalho, A.M.B., 1960, Comparative studies between Abutilon and Euphorbia mosaic virus. Phytopathol. Z., 38: 129-152.

*Cowland, J.W., 1932, Rep. Gez. Ent. Sect. Rep. In Gezira Agric. Res. Serv. Sudan.

Datar, V.V., 1984, Seasonal incidence of leaf curl (Tobacco leaf curl virus) in three varieties of tomato. Indian Phytopath., 37: 540-543.

Deighton, F.C., 1940, Tobacco leaf curl in Sierra Leone. Afr. Agric. Conf. 1: 7-8.

Dhingra, K.L. and Nariani, T.K., 1962, Tobacco yellow net virus. Indian J. Microbiol., 2: 21-24.

Dickson, R.C., Johnson, M. McD. and Laird, E.F., 1954, Leaf crumple, a virus disease of cotton. Phytopathology. 44: 479-480.

Flores, A.B., 1961, Varieties of tobacco infected with leaf curl disease and transmission of its virus to healthy tobacco seedlings. Araneta J. agric., 8: 100-109.

Gadd, C.H. and Loos, C.A., 1941, A virus disease of Ageratum conyzoides and tobacco, Trop. Agric., 96: 255-264.

*Galvez, G.E. and Castano, M.S., 1976, Purification of the whitefly transmitted bean golden mosaic virus. Turrialba. 26: 205-207.

Garga, R.P., 1949, Schizanthus sp. A host of tobacco leaf curl virus. Curr. Sci., 7: 260-261.

Goodman, R.M., 1977a, Infectious DNA from a whitefly transmitted virus of Phaseolus vulgaris Nature. 266: 54-55.

Goodman, R.M., 1977b, Single stranded DNA genone in a whitefly transmitted plant virus. Virology. 83: 171-179.

Goodman, R.M., Bird, J. and Thongmeearkom, P., 1977, An unusual virus like particle associated with golden yellow mosaic of beans. Phytopathology. 67: 37-42.

Gopalachari, N.C., 1984, Tobacco. ICAR Pub. New Delhi, pp. 265-267.

Hill, B.G., 1968, Occurrence of Bemisia tabaci in the field and its relation to the leaf curl disease of tobacco. S.Afr. agric. Sci., 11: 583-594.

Hopkins, J.C.F., 1932, Further notes on leaf curl of tobacco in Southern Rhodesia. Rhodesia agr. J., 29: 680-686.

Hornby, A.J.W., 1933, Report of the Asst. Director and Agricultural Chemist. A. Rept. Dept. agric. Nyasaland., pp. 36-47.

Jensen, H., 1920, Ziekten van de Tabak in des Vorstenland, Meded. Proefstat. Vorstenland. Tabak. 40: 1-147.

Jochems, Sc. J., 1928, Eeen nienwe Virusziekte Van Deli-Tabak de Rotterdam B-Ziekte. Bull. Deli. Proefst. Medan. 26: 5-26.

Kerling, L.C.P., 1933, The anatomy of the Kroepoek-diseased leaf of Nicotiana tabacum and Zinnia elegans. Phytopathology. 23: 175-190.

Khudyana, I.P., 1936, State Institute for tobacco investigations, Krasnodar, Bull. No. 130.

Kiriyama, K., 1972, Inoculation, purification and serology of tobacco leaf curl virus. Ann. Phytopath. Soc. Japan., 38: 323-332.

Kobatake, H., Osaki, T. and Inouye, T., 1981, Ecology and control of yellow dwarf disease of tomato caused by tobacco leaf curl virus. Proc. Kansai Pl. Prot. Soc., 23: 8-14.

Loebenstein, G. and Harpaz, I., 1960, Virus diseases of sweet potatoes in Israel. Phytopathology. 50: 100-104.

Lucas, G.B., 1965, Diseases of tobacco. Biological consulting Associates Raleigh. North Carolina. Third Ed. 621 pp.

*Ludwings, K., 1913, Über die kroepoek-kranheit des Tabaks in Kamerun. Ber. Deutch. Bot. Ges., 31: 536-543.

Mandal, B., 1989, Studies on croton yellow vein mosaic virus. M.Sc. thesis., Uni. Agric. Sci., Bangalore, India. 134 pp.

Maramorosch, K., 1975, Etiology of whitefly borne viruses. Tropical diseases of Legumes. (Ed.) Bird, J. and Maramorosch, K., Acad. Press, New York, pp. 71-77.

Mariappan, V. and Narayanaswamy, P., 1972, Acanthospermum hispidum DC., a new host of tobacco leaf curl virus. Madras agric. J., 59: 355-357.

*Mastumoto, T. and Tateoka, R., 1940, Virus diseases of tobacco in Formosa. Trans. Nat. Hist. Soc. Formosa. 30: 197-198.

Mathur, R.N., 1932, Leaf curl of cotton in garden Zinnias in North India. Nature. 129: 797.

Mathur, R.N., 1933, Leaf curl in Zinnia elegans at Dehra Dun. Indian J. agric. Sci., 3: 89-96.

Martinez, A.L., 1964, Leaf curl of tobacco in the Philippines and its transmission by white-flies (Bemisia sp.). Philipp. J. agric., 26: 27-31.

Mayne, H. and Ghesquiere, J., 1934, Hemioteris nuisibles Vegetaux du Congo Belge. Ann. Gambl., 40: 41.

Mc Clean, A.P.D., 1940, Some leaf curl diseases in South Africa. Dept. Agr. and For., S.Afr. Sci. Bul., 225: 1-70.

Mathew, A.V., 1988, Studies on Indian Cassava mosaic virus disease. Ph.D. Thesis., Uni. Agril. Sci., Bangalore , India. 237 pp.

Mishra, M.D., Raychaudhuri, S.P. and Ashrafi, J., 1963, Virus causing leaf curl of chilli (Capsicum annuum L.). Indian J. Microbiol., 3: 73-76.

Monga, D. and Tripathi, S.N., 1988, Factors affecting the incidence of leaf curl in jati tobacco (Nicotiana tabacum L.). Tob.Res., 14: 21-24.

Moore, E.S., 1933, Important to tobacco growers. The crinkly dwarf menace of tobacco. Farming. S.Afr., 8: 276.

Moore, E.S., 1934, The leaf curl or crinkly dwarf disease of tobacco. Farming. S. Afr., 9: 241-242.

Munshi, Z. and Choudhry, A.H., 1964, Some correlation studies between temperature and 'Leaf curl' incidence in Cigarette tobacco. Pak. J. Sci., 16: 48-52.

Muniyappa, V., 1980, Whiteflies., In. Harris, K.F. and Maramorosch, K., Eds., Vectors of Plant Pathogens. pp. 39-85.

Nariani, T.K., 1956, Leaf curl of papaya. Indian Phytopath., 9: 151-155.

Nariani, T.K., 1963, Effect of gibberellic acid on tobacco leaf curl affected plants. Indian Phytopath., 16: 101-102.

Nariani, T.K., 1968, Enation leaf curl of tomato. Pl. Dis. Reptr., 32: 595-596.

Nariani, T.K. and Pathanian, P.S., 1953, Physalis peruviana L. a new host of tobacco leaf curl virus. Indian Phytopath., 6: 143-145.

Nene, Y.L., 1972, A study of viral disease of pulse crop in Uttar Pradesh. Res. Bull. No.4, G.B.Pant Uni. of Agril. and Tech., Pantnagar, U.P., pp. 144.

Newton, W. and Pieris, J.W.L., 1953, Virus diseases of plants in Ceylon. FAO Pl. Prot. Bull., 2: 17-21.

Nour-Eldin, F., Mazyad, H. and Hassan, M.S., 1969, Tomato leaf curl virus disease. Agric. Res. Rev. Cairo., 47: 49-54.

Olivares, F.M., Cruz, Jr., Dela, A.A. and Salisi, L.B., 1972, Progress report on whitefly transmission of tobacco leaf curl virus to vegetable and weeds. Philippines Phytopath., 8: 21-29.

*Orlando, A. and Silberschmidt, K., 1946, Estudos sobre a disseminaceo natural do virus da clorose infecciosa das mulvaceas (Abutilon virus 1 Baur) e a Suarelacao com O inseto-vector Bemisia tabaci (Genn) (Homoptera-Aleyrodidae). Arq. Inst. Biol. Sao Paulo. 17: 1-36.

Osaki, T. and Inouye, T., 1978, Resemblence in morphology and intranuclear appearance of viruses isolated from yellow dwarf diseases and leaf curl diseased tobacco. Ann. Phytopath. Japan., 44: 167-178.

Osaki, T., Kobatake, H. and Inouye, T., 1979, Yellow vein mosaic of honeysuckle (Lonicera japonica, Thumb) a disease caused by tobacco leaf curl virus in Japan. Annls. Phytopath. Soc. Japan. 45: 62-69.

Pal, B.P. and Tandon, R.K., 1937, Types of tobacco leaf curl in Northern India. Indian J. agric. Sci., 7: 363-393.

Patel, B.N. and Patel, R.C., 1987, Evaluation of tobacco germplasm for leaf curl infection. Tob. Res., 13: 85-87.

Pathak, H.C. and Raychaudhari, S.P., 1967, Withania somnifera an additional host of leaf curl virus. Sci. Cult., 33: 234-235.

Peters, L. and Schwartz, M., 1912, Krankheiten und Beshädingen des Tabaks. MIT. BIOL. ANST. LAND-UND FORSTWIRTSCH. Berlin. 198 pp.

Pruthi, H.S. and Samuel, C.K., 1937, Entomological investigation on the leaf curl disease of tobacco in North Bihar. I. Transmission experiments with some suspected insect vectors. II. An alternative host of the virus and the insect transmitter. Indian J. agric. Sci., 7: 659-670.

Pruthi, H.S. and Samuel, C.K., 1939, Entomological investigation on the leaf curl disease of tobacco in Northern India. III. The transmission of leaf curl by the white-fly, Bemisia gossypiperda, to tobacco, Sannhemp, and a new alternate host of the leaf curl virus. Indian J. agric. Sci., 9: 223-275.

Pruthi, H.S. and Samuel, C.K., 1941, Entomological investigations on the leaf curl disease of tobacco in Northern India. IV. Transmission of the disease by whitefly (Bemisia gossypiperda) from some new alternate hosts. Indian J. agric. Sci., 11: 387-409.

Pruthi, H.S. and Samuel, C.K., 1942, Entomological investigations on the leaf curl disease of tobacco in Northern India. V. Biology and population of the whitefly vector (Bemisia tabaci (Genn)) in relation to the incidence of the disease. Indian J. agric. Sci., 12: 35-37.

Pruthi, H.S., 1945, Leaf curl disease of tobacco in India. Indian Fmg., 5: 220-223.

Ramkrishnan, K., Janaki, J.P. and Sellammal, S., 1964, The tomato leaf curl virus. Madras agric. J., 51: 94.

Reddy, T.S.N. and Nagarajan, K., 1982, Leaf curl disease on tobacco, Tob. News., 5: 5-6.

Retuerma, M.L., Pableo, G.O. and Price, W.C., 1971, Preliminary study of the transmission of Philippine tomato leaf curl virus by Bemisia tabaci. Philippines Phytopath., 7: 29-34.

Robinson, D.J., Roberts, I.M., Harrison, B.D. and Muniyappa, V., 1987, Whitefly transmitted gemini viruses from India (Abstract) In: VIIth International Congress of Virology, August 9-14, Edmonton, Canada.

Roberts, I.M., Robinson, D.T. and Harrison, B.D., 1984, Serological relationships and genome homologies among gemini virus. J. Gen. Virology, 65: 1723-1730.

Roberts, I.M. and Harrison, B.T., 1979, Detection of potato leaf roll and potato mop-top viruses by immunosorbent electron microscopy. Ann. Appl. Biol., 93: 289-297.

Russell, L.M., 1957, Synonyms of Bemisia tabaci(Gennadius) (Homoptera-Aleyrodidae). Bull. Brooklyn.Entomol. Soc., 52: 122-123.

Saikia, A.K., 1985, Studies on epidemiology and control of tomato leaf curl virus. Ph.D. Thesis. Univ. Agric. Sci., Bangalore, India. 229 pp.

Saikia, A.K. and Muniyappa, V., 1989, Epidemiology and control of tomato leaf curl virus in Southern India. Trop. Agric.(Trinidad).,66: 350-354.

Saklani, U.D. and Mathai, P.J., 1977, Effect of date of planting on leaf curl disease of tomato. Indian J. Horti., 34: 64-68.

Sastry, K.S.M.and Nariani, T.K., 1962, Effect of host plant nutrition on growth and susceptibility of tobacco plants to infection with leaf curl virus. Indian J. agric. Sci., 32: 288-293.

Sastry, K.S.M. and Singh, S.J. and Sastry, K.S., 1978, Studies on epidemiology of tomato leaf curl virus. Indian J. Hort., 35: 269-277.

Seetharama Reddy, K., 1978, Studies on leaf curl virus disease of tomato (Lycopersicon esculentum Mill.) Ph.D, thesis., Univ. Agric. Sci., Bangalore, 134 pp.

Seetharama Reddy, K., Yaraguntaiah, R.C. and Sastry, K.S., 1981, Strains of leaf curl virus of tomato in India. Z. Pflanzenkr. Pflanzenschutz., 88: 400-404.

Seth, M.L. and Dhan Raj, K.S., 1972, New strain of tobacco leaf curl causing enations in chilli (C. annuum). Phytopathol. Z., 74: 365-370.

Sequeira, J.C. and Harrison, B.D., 1982, Serological studies on Cassava latent virus. Ann. Appl. Biol., 101: 33-42.

Sharp, D.G. and Wolf, F.A., 1951, The virus of tobacco leaf curl II. Phytopathology. 41: 94-98.

Shivanathan, P., 1983, The epidemiology of three diseases caused by whitefly-borne pathogens. In. Plant virus Epidemiology (Ed.) Plumb, R.T. and Thresh, J.M. Black Well Scientific Pub. Oxford, pp. 323-330.

Shepherd, E.F.S., 1940, Tomato leaf curl. Proc. Third W. Afr. agric. Conf., 1: 87-89.

Storey, H.H., 1931, A new virus diseases of the tobacco plant. Nature. 128: 187-188.

Storey, H.H., 1932, Leaf curl of tobacco in Southern Rhodesia. Rhodesian agr. J., 29: 186-192.

Storey, H.H., 1935, Virus diseases of East African plants. II. Leaf curl disease of tobacco. E. Afr. Agric. J., 1: 148-153.

Thanapase, W., Polpol, P., Sutabutra, T. and Attathom, S., 1983, Causal agent and some important characters of tomato leaf curl disease. Kasetsart J. Natural Sciences., 17: 80-89.

*Thilliard, R., 1921, La Culture du tabac Sumatra au Cameroun (Cameroons). L' Agronomic Coloniale., 6: 135-194.

Thung, T.H., 1932, De Krul-en Kroepock-Zeikten Van tabak ende Oorzaken van hare Verbreiding. Meded. Proefst. Vorstenlandsche Tabak., 72: 1-54.

Thung, T.H., 1934, Bestrijding der Krulen Kreopoek-Ziekten Van Tabak, Meded. Profestat. Vorstenlandsche Tabak., 78: 18.

Tsumagari, H., 1967, An aggregate occurrence of tobacco leaf curl in Japan. Ann. Phytopath. Soc. Japan., 33: 311-312.

Valand, G.B. and Desai, M.V., 1980, Studies on transmission of different types of tobacco leaf curl virus. Gujarat agric. Univ. Res. J., 5: 20-24.

Varma, P.M., 1959, Leaf curl disease of tomato. ICAR Seminar. Dist. Hort. Plants.

Vasudeva, R.S. and Sam Raj, J., 1948, A leaf curl disease of tomato. Phytopathology. 38: 364-369.

Viswanath, S.M. and Nariani, T.K., 1981, Spinach leaf curl. Curr. Sci., 50: 326-327.

West, J., 1936, Leaf curl of tobacco in Southern Nigeria. Trop. Agric.. (Trinidad)., 13: 242-244.

*Wolf, F.A., Whitecombe, W.H. and Mooney, W.C., 1949, Leaf curl of tobacco in Venezuela. J. ELISHA. MITCHELL SCIENT. Soc., 65: 38-47.

Yamaguchi, Y., Fukudome, N., Tanaka, Y. and Yoshida, S., 1976, Case of mass outbreak of tobacco leaf curl in Japan. Kagoshima. Jpn. Trob. Exp. Stn. Bull., 20, pp. 145-157.

Yan, D.Y., Wang, Z.F., Pel, M.V., Xie, D.Z. and Wang, X.F., 1983, A gemini virus tobacco leaf curl virus in China. Acta Phytopath. Sinicia., 13: 57-59.

Yassin, A.M. and Nour, M.A., 1965a, Tomato leaf curl diseases in the Sudan and their relation to tobacco leaf curl. Ann. appl. Biol., 56: 207-217.

Yassin, A.M. and Nour, M.A., 1965b, Tomato leaf curl disease, its effect on yield and varietal susceptibility. Sudan agric. J., 1: 3-7.

Yassin, A.M. and Abu Salih, H.S., 1972, Leaf curl of tomato. Tech. Bull. Agril. Res. Corpn., 3: 33.

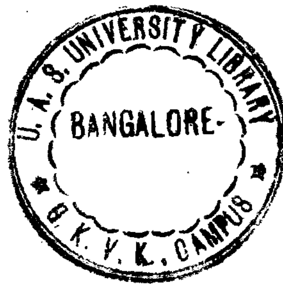
Yassin, A.M. and Defalla, G.A., 1980, Leaf curl epidemic: A threat to tomato production in the Sudan. In. Proceeding of the second International Conference on the Impacts of viral diseases on the development of African and Middle East countries, Nairobi.

*Yu-Cheng, C., 1964, Preliminary study on the insect vector of tobacco leaf curl. Taiwan Tab and Wine Monop. Bur. Tob. Res. Inst. Annu. Rep., pp. 127-134.

---

* Original not seen

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ
ವಿಶ್ವವಿದ್ಯಾನಿಲಯ ಗ್ರಂಥಾಲಯ
ಗಾ.ಕೃ.ವಿ.ಕೇ., ಬೆಂಗಳೂರು-65.
12 SEP 1990
ಅನುಪ್ರವೃತ್ತಿ ಸಂ. Th. 2497


U. A. S. UNIVERSITY LIBRARY
BANGALORE-
B. K. V. K. CAMPUS