CHARACTERISATION AND MANAGEMENT OF BITTER GOURD DISTORTION MOSAIC VIRUS

REENY MARY ZACHARIA

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2006

Department of Plant Pathology
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

DECLARATION

I hereby declare that this thesis entitled **"Characterisation and management of bitter gourd distortion mosaic virus"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
14-11-2006

REENY MARY ZACHARIA

CERTIFICATE

Certified that the thesis, entitled **"Characterisation and management of bitter gourd distortion mosaic virus"** is a record of research work done independently by **Smt. Reeny Mary Zacharia** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Dr. Sally K. Mathew
Chairperson, Advisory Committee
Associate Professor (Plant Pathology)
College of Horticulture
Vellanikkara

Vellanikkara
14-11-2006

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Smt. Reeny Mary Zacharia,** a candidate for the degree of **Doctor of Philosophy in Agriculture,** agree that the thesis entitled **"Characterisation and management of bitter gourd distortion mosaic virus"** may be submitted by Smt. Reeny Mary Zacharia, in partial fulfilment of the requirement for the degree.

Dr. Sally K. Mathew
(Chairman, Advisory Committee)
Associate Professor (Plant Pathology)
College of Horticulture
Kerala Agricultural University, Thrissur

Dr. Koshy Abraham
(Member, Advisory Committee)
Associate Professor & Head
Department of Plant Pathology
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur

Dr. A.M. Ranjith
(Member, Advisory Committee)
Associate Professor
Department of Entomology
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur

Dr. D. Girija
(Member, Advisory Committee)
Assistant Professor
Centre for Plant Biotechnology and Molecular Biology
College of Horticulture
Kerala Agricultural University
Vellanikkara, Thrissur

Dr. Babu George
(Member, Advisory Committee)
Associate Professor
Sugarcane Research Station
Thiruvalla

EXTERNAL EXAMINER

19.9.06

Dedicated to

My Mother

Late Mrs. Gracy Issac

ACKNOWLEDGEMENT

It is with great pleasure that I express my sincere gratitude and indebtedness to:

***Dr. Sally K. Mathew**, Associate Professor, Department of Plant Pathology, College of Horticulture, Vellanikkara for her valuable guidance, and constructive criticism throughout the course of the study and research work. Her incisive comments and close scrutiny are of great help in the preparation of the thesis.*

***Dr. Koshy Abraham**, Associate Professor and Head, Department of Plant Pathology, College of Horticulture, Vellanikkara, **Dr. A.M. Ranjith**, Associate Professor, Department of Agricultural Entomology, **Dr. D. Girija**, Assistant Professor, Centre for Plant Biotechnology and Molecular Biology and **Dr. Babu George**, Associate Professor, Sugarcane Research Station, Thiruvalla, esteemed members of my advisory committee for their interest, constructive suggestion and generous help during the course of this investigation and preparation of thesis.*

Dr. M. Krishna Reddy, Senior Scientist and Sri.Devaraj, Senior Research Fellow, Indian Institute of Horticultural Research, Bangalore for their enormous help in purification, electronmicroscopic and serological studies.

Dr. P.R. Chandrasekhar, Associate Professor, Central Instrumentation Lab, College of Veterinary and Animal Sciences, Mannuthy and Sri. Tojo, Research Fellow, College of Horticulture, for their assistance in purification and molecular studies.

Dr. N.R. Nair, Former Professor and Head and Dr. Jessy M. Kuriakose, Associate Professor and Sri. Mohanan, Labourer, Sugarcane Research Station, Thiruvalla for their help and support in conducting field experiments.

Dr. Maicykutty P. Mathew, Associate Professor, Department of Agricultural Entomology, College of Horticulture for identification of insect vectors.

Sri. S. Krishnan, Assistant Professor, Department of Agricultural Statistics and Sri. Eldho Varghese, Post graduate student, College of Horticulture for help in statistical analysis.

Dr. Mani Chellappan, Assistant Professor, Department of Agricultural Entomology, College of Horticulture for his help in photography.

Dr. Umamaheswaran, K. Assistant Professor, College of Agriculture, Vellayani for the help extended at different occasions.

Dr. Achamma Oommen, Professor and Head, Department of Plant Breeding and Genetics and Dr. Sosamma Jacob, Department of Agricultural Entomology, College of Horticulture for their moral support and encouragement .

Smt. Beena and Sri. Jayasankar for their help at various occasions.

My friends Reni, Annie, Lilly and Jayalakshmi for the support extended through out the study.

Kerala Agricultural University for awarding senior research fellowship.

Words fail to express my heartful gratitude and indebtedness to my husband, child and family members, for their patience, wholehearted support, glorious love and prayers which make this endeavour a success.

Help and support of my servant Smt. Maria is thankfully acknowledged.

Sri. Jomon, JMJ Computer Centre, Thottappady for his help in typing.

I bow my head before God Almighty for giving me strength, patience, will power and health to complete this endeavour successfully.

Reeny Mary Zacharia

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

BGDMV	- Bitter gourd distortion mosaic virus
BGMV	- Bitter gourd mosaic virus
CEM	- Citrate EDTA mercapto ethanol
CI	- Coefficient of infection
cm	- Centimetre
CMV	- Cucumber mosaic virus
D	- Daltons
DAC ELISA	- Direct antigen coating ELISA
DAS	- Days after sowing
DNA	- Deoxyribo nucleic acid
EDTA	- Ethylene diamine tetra acetic acid
ELISA	- Enzyme linked immuno sorbent assay
g	- Gram
h	- Hour
ICMV	- Indian cassava mosaic virus
kb	- Kilo base pair
Kd	- Kilo daltons
Kg	- Kilogram
l	- Litre
ml	- Milli litre
min	- Minutes
nm	- Nanometre
NSKE	- Neem seed kernel extract
PAGE	- Polyacrylamide gel electrophoresis
PBS	- Phosphate buffered saline
PBS-T	- PBS Tween
PDI	- Per cent disease incidence
PDS	- Per cent disease severity
PEG	- Polyethylene glycol
PRSV-W	- Papaya ring spot water melon strain
AVP	- Anti Viral Principle
PVP	- Polyvinyl pyrrolidone
PYVMV	- Pumpkin yellow vein mosaic virus
SDS	- Sodium dodecyl sulphate
sec	- Seconds
SLCV	- Squash leaf curl virus
TE	- Tris EDTA
ToLCV	- Tomato leaf curl virus
TYLCV	- Tomato yellow leaf curl virus
U.V.	- Ultra Violet
WCMoV	- Water melon curly mottle virus

Introduction

INTRODUCTION

Vegetables are rich in vitamins, proteins, carbohydrates and minerals and hence constitute an important component in human nutrition. India produces the largest quantity of vegetables next to China. Among the various vegetables, cucurbits are the largest group of summer vegetables grown all over India. Cucurbit vegetables are fair source of thiamine and riboflavin.

Bitter gourd (*Momordica charantia* L.), a unique vegetable having high nutritive and medicinal values is being cultivated in the tropical and subtropical regions of the world. It ranks first among cucurbits in respect of iron and vitamin C content.

Bitter gourd is the leading vegetable crop of Kerala. The highest yield and the maximum returns make it the most preferred vegetable crop of farmers (Nandakumar, 1999). Major factor which seriously impede the production of bitter gourd in Kerala is the occurrence of pests and diseases (Jayapalan and Sushama, 2001).

Among the various diseases affecting the crop, virus diseases are important of which mosaic disease is known to cause serious damage and loss to the crop especially during the summer. Mathew *et al.* (1991) reported almost cent per cent loss in yield in early infected bitter gourd crop from different parts of Kerala during summer season.

Uppal (1933) reported the mosaic disease of bitter gourd for the first time in India. Mathew *et al.* (1991) observed a mosaic disease of bitter gourd for the first time in Kerala.

The earlier studies conducted by Mathew *et al.* (1991) and Purushothaman (1994) have revealed informations on symptomatology, transmission and etiology of

various mosaic diseases occurring on bitter gourd in Kerala. However, the reports on transmission and etiology were contradictory and informations on characterisation and management of the virus were sketchy and lacking. Hence the present study was taken with the following objectives:

- Symptomatology of bitter gourd distortion mosaic virus (BGDMV).
- Studies on transmission of BGDMV
- Host range of BGDMV
- Purification of virus BGDMV
- Characterisation of nucleic acid and protein coat of the virus
- Identification of the virus using electron microscopy and serology
- Studies on disease management aspects.

Review of Literature

2. REVIEW OF LITERATURE

Bitter gourd (*Momordica charantia* L.) is one of the major vegetable crop of Kerala. Among the various diseases affecting the crop, viral diseases which were of minor importance in early days are known to cause serious damage in recent years. Uppal (1933) reported a mosaic disease of bitter gourd for the first time in India. A mosaic disease of bitter gourd was observed for the first time in Kerala by Purushothaman (1994). Dahal *et al.* (1997) reported the occurrence of papaya ring spot poty virus - water melon strain (PRSV-W) on bitter gourd from Nepal. A search on literature revealed that the information on bitter gourd mosaic virus disease are meagre and scanty.

2.1 ECONOMIC IMPORTANCE

Mathew *et al.* (1991) observed almost cent per cent loss in yield in early mosaic infected bitter gourd crop from different parts of Kerala during summer season. Nandakumar (1999) reported 12 per cent incidence of bitter gourd mosaic virus (BGMV) from Thiruvananthapuram district of Kerala.

2.2 SYMPTOMATOLOGY

Symptomatology of the disease has been described by various workers and were found varying. Nagarajan and Ramakrishnan (1971a) observed vein clearing, yellowing, shoe-stringing effect and no severe damage on vines due to BGMV. Giri and Misra (1986) reported a leaf distortion virus disease of bitter gourd showing vein clearing, reduction in leaf size and distortion of leaves, in addition to proliferation of tendrils. Mathew *et al.* (1991) found that mosaic disease was characterised by typical mosaic symptoms of light green and dark green patches, upward curling, crinkling and severe stunting. They also noticed that the internodal length of the vines was very much reduced and early infected seedlings failed to trail on the pandal. The fruits of the diseased plants were smaller in size and number and were also rough and corky in texture. Purushothoman (1994) observed the initial symptom as clearing of vein and veinlets, followed by mild mottling which later showed typical mosaic pattern of dark

green and light green with blisters all over the leaf lamina. In some cases, leaves had a large area of light green patches. The leaves were reduced in size and of filiform shape. Diseased plants remained stunted and produced only a few flowers and fruits. Pandey *et al.* (1998) observed that distortion mosaic disease of bitter gourd was characterised by presence of small and distorted leaves, shortened internodes, reduction in flower buds and deformed fruits. According to Arunachalam (2002), the disease appeared first in newly formed leaves and later spread to other leaves with chlorotic spots which later developed in to typical mosaic pattern of green and yellow patches. In another case, leaves with mosaic symptom of light green and dark green patches were observed. At this stage, leaf margin curl upward and leaf size reduced with severe puckering, crinkling and distortion. Shortening of inter nodes, clustered appearance of distorted leaves, long tendrils, unusual thickening of top end of the vine with numerous hairs, reduced number of flowers and fruits and failure of flower to open were the other symptoms noticed by him.

2.3 TRANSMISSION

2.3.1 Sap transmission

Successful transmission of bitter gourd mosaic through infected sap has already been reported. But certain workers failed to obtain the transmission of the virus through mechanical means. Nagarajan and Ramakrishnan (1971b) observed that bitter gourd mosaic virus was not transmissible to bitter gourd through sap, whereas Giri and Misra (1986) obtained transmission of leaf distortion virus disease of bitter gourd through infected sap. Mathew *et al.* (1991) also failed to obtain the transmission of BGMV through sap. However, Purushothaman (1994) observed the transmission of mosaic virus by mechanical means, in which sap extracted in distilled water and phosphate buffer of pH 7.0 gave maximum infection of 90 per cent and the minimum infection of 55 per cent with tris buffer. Pandey *et al.* (1998) also reported the transmission of BGDMV through sap.

2.3.2 Seed transmission

According to Nagarajan and Ramakrishnan (1971a), Mathew *et al*. (1991) and Purushothaman (1994) bitter gourd mosaic virus was not transmitted through seeds. Giri and Misra (1986) and Pandey *et al.* (1998) reported the seed transmissible nature of a leaf distortion and distortion mosaic virus diseases of bitter gourd.

2.3.3 Insect transmission

With respect to the insect transmission studies conducted by the earlier workers, aphids and whiteflies were reported as vectors of bitter gourd mosaic virus.

Nagarajan and Ramakrishnan (1971b) observed the mosaic disease of bitter gourd transmitted through five species of aphid vectors, viz., *Aphis gossypii, A. malvae, A. nerii, Brevicoryne brassicae* and *Myzus persicae*. Studies on virus-vector relationship showed that the pre-acquisition fasting threshold, acquisition threshold and inoculation threshold were 15 min., 5 sec. and 60 sec. respectively for *M. persicae*; 15 min., 20 sec. and 5 min. respectively for *A. gossypii*; 30 min., 30 sec. and 5 min. respectively for *A. nerii* and *B. brassicae* and 30 min., 20 sec. and 5 min. for *A. malvae*. The optimum number of viruliferous aphids required for transmission of virus was 15 per plant for all the above five aphid vectors tested. Purushothaman (1994) also reported the transmission of mosaic by the aphid vectors, viz., *A. gossypii, A. malvae, A. craccivora* and *M. persicae* of which *A. gossypii* and *A. malvae* were found to be the most efficient ones. He also found that ten viruliferous aphids were required for the successful transmission of the virus and the acquisition and inoculation threshold were 20 min. Tomar *et al.* (2001) studied the virus-vector relationship of bitter gourd mosaic virus with its six aphid vectors. Results of the study indicated that BGMV was transmitted more efficiently by *A. gossypii* and *Dactynotus sonchi* than *A. craccivora, A. malvae, A. nerii* and *Brevicoryne brassicae*. The maximum infection was obtained when 7-15 day old virus culture was used as virus source. The second leaves from the apex and chlorotic part of infected leaves proved to be the best sites for the acquisition of virus by aphids.

Sabitha (1992) and Dutt (2000) reported *Amrasca biguttula biguttula* and *Empoasca motti* as serious pests of bitter gourd. But the role of these jassids in transmitting any virus diseases of bitter gourd have not been reported by any workers.

Giri and Misra (1986) reported white fly as the vector of leaf distortion virus disease of bitter gourd. Mathew *et al.* (1991) noticed 40 per cent transmission of bitter gourd mosaic virus with a single viruliferous whitefly. They also observed that the rate of transmission increased with increase in number of insects and cent per cent transmission was obtained with 10 whiteflies per plant.

2.4 HOST RANGE

According to Nagarajan and Ramakrishnan (1971a), the host range of the virus was confined to members of the cucurbitaceae. They experimentally proved that the bitter gourd mosaic virus could not infect *Solanum tuberosum* and *Citrus aurantifolia.* Mathew *et al.* (1991) found that BGMV was transmissible to cucumber (*Cucumis sativum*) through *Bemisia tabaci* where symptoms appeared 20-25 days after inoculation causing mild mosaic and stunting, but snake gourd and pumpkin failed to produce symptom of BGMV. They also observed that the disease was not transmissible to other common hosts of whitefly transmitted viruses such as *Abelmoschus esculentus, Acalypha indica, Ageratum conyzoides, Lycopersicon esculentum, Manihot esculentus* and *Nicotiana tabacum.*

Purushothaman (1994) reported a number of hosts of BGMV. Systemic infections were observed on *Cucumis melo*, *C. metuliferus*, *Luffa acutangula*, *Citrullus vulgaris, Trichosanthes anguina*, *Musa* sp. cv. Palayankodan, *Antigonon leptopus, Capsicum annum* , *N. glutinosa* . and *Physalis minima*, while *Chenopodium amaranticolor* and *Datura stramonium* produced local lesion symptoms and *D. metel* acted as symptomless carrier of the virus. *C. sativus, Benincasa hispida* and *Lagenaria siceraria* belonging to cucurbitaceae were found to be non hosts of BGMV. Tomer *et al.* (2001) reported natural infection of BGMV, a strain of water melon mosaic virus in weeds such as *Solanum nigrum, Sonchus oleraceus, Nicandra physaloides* and *Blumea eranthea.*

2.5 PURIFICATION OF THE VIRUS

Various protocols have been adopted by different workers for the purification of gemini viruses.

Bock *et al.* (1978) purified cassava latent virus by clarification of leaf extracts with butanol or chloroform mixtures. Cohen *et al.* (1983) purified squash leaf curl virus (SLCV) by differential centrifugation method after clarification of leaf extracts with chloroform in which virus yield reached to 130 µg per 100 g of plant material.

Honda *et al.* (1983) purified a whitefly borne mungbean yellow mosaic virus in Thailand using 0.1 M potassium phosphate buffer (pH 7.8) containing 0.1 per cent thioglycollic acid, 0.01 M sodium diethyl dithiocarbamate and 0.001 M sodium ethylene diamine tetra acetate (2 ml g^{-1} tissue) and the yield of the virus particles was below 1 mg kg^{-1} of the tissue.

Czosnek *et al.* (1988) adopted two methods for the purification of tomato yellow leaf curl virus (TYLCV). In the first method, leaf tissues were extracted in a buffer containing 100 mM trisodium citrate, 18.5 mM ascorbic acid, 60 mM sodium sulphite, 5 mM ethylene diamine tetra acetic acid (EDTA) at pH 8 and one per cent (v/v) 2-mercapto ethanol, followed by an overnight stirring in Triton x-100 and differential centrifugation. In the second method, leaf tissues powdered in liquid nitrogen was stirred overnight in a buffer containing 0.1 M sodium phosphate, at pH 7.0, 2.5 mM EDTA, 10 mM sodium sulphite, 0.1 per cent (v/v) and Triton x-100 (3 ml g^{-1}) and the filtered sap was then clarified after addition of 10 per cent (v/v) cold chloroform and subjected to differential centrifugation.

Brown and Nelson (1989) used 100 mM potassium phosphate buffer, pH 8 containing 200 mM sodium sulphite and 2 mM EDTA for the purification of water melon curly mottle virus (WCMoV), a gemini virus infecting water melon.

Raj *et al.* (1989) used 10-40 per cent sucrose density gradient centrifugation method for the purification of a gemini virus from *Dolichos lab lab.*

2.6 CHARACTERISATION OF VIRUS

2.6.1 Characterisation of nucleic acid

Czosnek *et al.* (1988) purified TYLCV from infected tomato and datura plants. A circular single stranded DNA containing 2800 nucleotides was isolated from the infective virus preparations. According to Brown and Nelson (1989), purified preparation of water melon curly mottle virus (WCMoV) exhibited a typical nucleoprotein absorbance profile with a maximum absorbance at 258 nm; and the ratio of A 280/A 260 was 0.61-0.64 and the DNA isolated from virus particles was resolved by electrophoresis into two circular single stranded DNA bands of approximately 2.6 to 2.7 kb. Raj *et al.*, (1989) in their studies with yellow disease of *Dolichos lab lab.* observed that the ultra violet absorption spectrum of the lower band was typical of nucleoproteins with maximum absorbance at 258 nm and minimum at 242 nm and the A 260/A 280 ratio was 1.46 which was similar to that of several gemini viruses.

Morales *et al.* (1990) conducted partial characterisation of bean dwarf mosaic virus (BDMV) and the dissociated infectious nucleoproteins yielded a single stranded DNA.

2.6.2 Characterisation of viral protein

Brown and Nelson (1989) observed that water melon curly mottle virus isolated from water melon had the viral capsid protein within the doublet of 29100 (± 1550) and 27733 (± 1550) Daltons. Mathew (1988) conducted polyacrylamide gel electrophoresis of Indian cassava mosaic virus (ICMV) and found that its protein consisted of a major component of molecular weight of 34,200 D and a minor component at a position corresponding to molecular weight of 33,400 D. In addition, ICMV possessed an additional component of molecular weight of 33,920 D.

2.7 IDENTIFICATION OF VIRUS

2.7.1 Electron microscopy

Giri and Mishra (1986) observed twinned geminate particles measuring about 19 x 30 nm with leaf distortion virus infected bitter gourd samples. Czosnek *et al.* (1988) detected typically twinned particles of the gemini virus from partially purified preparations of TYLCV infected tomato plants. Mathew (1998) observed the association of three different viruses viz., gemini virus, cucumber mosaic virus and water melon mosaic virus with bitter gourd mosaic disease in Kerala. Pandey *et al.* (1998) observed geminate particles of size 19 x 30 nm associated with the leaf distortion virus disease of. bitter gourd.

2.7.2 Serology

Mathew *et al.* (1991) reported that bitter gourd mosaic virus showed positive results in NASH tests with Indian cassava mosaic virus and showed close analogy to ICMV DNA.

Studies conducted by Purushothaman (1994) showed that bitter gourd mosaic virus was serologically related to the strains of cucumber mosaic virus, pumpkin mosaic virus and snake gourd mosaic virus found in Kerala and was not related to antisera of cucumber green mottle virus, squash mosaic virus and tobacco mosaic virus type strains obtained from Denmark and he identified BGMV as Cucumis virus I.

Muniappa *et al.* (2003) observed that pumpkin yellow vein mosaic virus (PYVMV) was distinct from other begomo viruses and was very much similar to New Delhi isolate of bipartite ToLC virus, based on the comparative phylogeny of complete coat protein gene sequences.

2.8 DISEASE MANAGEMENT STUDIES

Mathew (1998) adopted an integrated approach for the management of mosaic diseases in bitter gourd which include high seed rate, rouging of infected

plants, basal application of thimet 10 g per plant, weekly sprays of five per cent neem oil-soap suspension and fortnightly sprays of 0.03 per cent dimethoate.

2.8.1 Use of botanicals

2.8.1.1 Use of plant extracts

Verma *et al.* (1985a) reported that leaf extracts of *Clerodendron aculeatum* induced local and systemic resistance in several host plants against TMV infection. The inhibitory effects of *Clerodendron fragrans* and *Boerhaavia diffusa* against the natural infection of tobacco mosaic virus in *Vigna radiata* and *V. mungo* were tested by Verma *et al.* (1985 b). They observed that *C. fragrans* four per cent foliar spray given at an interval of three to four days from seedling stage onwards reduced infection up to 60 per cent, while, *B. diffusa* root extract was found to delay symptom expression only. Devi (1990) obtained maximum inhibitory effect of 80-90 per cent against cowpea mosaic virus with the extracts of *Phyllanthus niruri, C. infortunatum* and *Vitex negundo*.

Suppression of mosaic infection in *Glycine max* with leaf extract of *C. aculeatum* was reported by Verma *et al.* (1992). Early flowering and fruiting, higher nodulation and better yield were also noticed with this treatment. Patel and Patel (1993) observed the antiviral properties of *C. inerme, Parkinsonia aculeata* and *Ipomea carnea* in suppressing chlorotic mottle mosaic virus in tobacco. Ten per cent extracts of *C. infortunatum* was found to inhibit the production of local lesions by brinjal mosaic virus on *Datura stramonium* (Surendran, 1996).

Louis and Balakrishnan (1996) reported that pre-inoculation application of plant extracts of *Basella alba, Glycyrrhiza glabra, Phyllanthus fraternus, Plumbago rosea* and *Thespesia populnea* reduced pumpkin mosaic virus (PMV) infection by about 80 per cent. Baranwal and Ahmad (1997) observed that foliar spraying coupled with soil application of aqueous solution of 1:8 dilution of *C. aculeatum* powder had better effect in delaying and lowering ToLCV disease incidence.

Jayashree *et al.* (1999) tested the efficacy of ten plant extracts against pumpkin yellow vein mosaic virus (PYVMV) and obtained maximum reduction of 93.3 and 91.7 per cent over control with *Bougainvilla spectabilis* and *Boerhaavia diffusa* respectively. Pun *et al.* (1999) obtained 83.3 per cent and 81.7 per cent reduction of bhindi yellow vein mosaic virus disease with leaf extracts of *Prosopsis chilensis* and *B. spectabilis* respectively.

Saradamma (1989) reported reduction in aphid population in brinjal with two per cent benzene extract of *C. infortunatum.* Lilly and Saradamma (1994) obtained control of epilachna beetle in bitter gourd with acetone and water extracts of *C. infortunatum.*

2.8.1.2 Use of neem seed extract

Cherian and Menon (1944) observed that cold extracts of neem seed kernel was efficient as an insecticide against *A. gossypii* and the toxicity was found to increase by addition of soap. Asari and Nair (1972) reported the effectiveness of neem seed suspension against brinjal aphid. Siddique (1987) and Kathirvel (1988) found that neem seed kernel extract (NSKE) five per cent could control whitefly and fruit borer infestation in bhindi and was comparable to chemical pesticides. Srinivasan and Babu (2001) observed reduction in population of *B. tabaci* in brinjal with three rounds spray of five per cent NSKE.

2.8.1.3 Use of neem seed oil

Srinivasava *et al.* (1986) observed inhibition of CMV in cucumber with crude oil from *Azadirachta indica.* Reghunath and Gokulapalan (1994) reported the effectiveness of neem oil against cowpea aphids and thereby reducing cowpea mosaic virus incidence. Samuel and Mariappan (1996) reported reduction in survival of aphids and transmission of mosaic in chillies with neem seed oil. Jayashree *et al.* (1999) observed that neem oil three per cent was more effective than NSKE (five per cent) against pumpkin yellow vein mosaic virus.

Use of neem oil 2.5 or 5 per cent with garlic (20 g per plant) was found effective in controlling epilachna beetle, jassids, aphids and mites in bitter gourd (KAU, 1996a).

2.8.2 Use of homocopathic drugs

Khurana (1968) observed antiviral properties of *Apis melliphica* 30 potency against tomato leaf curl disease and other viral diseases of chilli. Khurana (1980) also studied the antiviral properties of *Arsenicum album* against several viruses. According to Cheema *et al.* (1991), Thuja 30 potency inhibited cucumber mosaic cucumo virus in one month old *Capsicum annum in vitro*.

2.8.3 Use of chemicals

A search on literature revealed numerous reports on the effectiveness of various chemicals against whiteflies. But due to limitation of the present study to go into that vast literature, the reports pertaining to those chemicals used in the present investigation are furnished here.

2.8.3.1 Acephate

Caetano *et al.* (1987) reported the efficacy of acephate @ 125 g ai ha^{-1} against bean leafhopper *Empoasca kerri* under field condition. Banbote *et al.* (1995) found that spraying acephate 0.05 per cent was highly effective against aphid, leafhopper and whitefly infestation in cotton. Nikholov and Ivanova (1998) tested a number of insecticides against *A. gossypii* in cucumber and found that spraying acephate (Orthene 75 WP) at 0.1 per cent was effective which resulted in 90 per cent mortality of the insect. Reddy and Rao (1998) found that acephate was as effective as monocrotophos in controlling leafhopper infestation in bitter gourd. Dutt (2000) also obtained effective control of leafhopper and aphid in bitter gourd with acephate 0.1 per cent.

2.8.3.2 *Carbosulfan*

Rushtapakornchai *et al.* (1996) reported the effectiveness of carbosulfan five per cent in reducing yellow leaf curl disease in tomato.

2.8.3.3 *Dicofol*

According to Jayaraj *et al.* (1995) and Mohapathra (1996), dicofol 0.05 per cent was very effective against the mite *Polyphagotarsonemus latus* affecting chilli and jute respectively. Effectiveness of dicofol 0.1 per cent in controlling mite attack in chilli had also been reported by Kumar (1999).

2.8.3.4 *Imidacloprid*

Schmeer *et al.* (1990) noticed reduction of barley yellow dwarf virus by controlling *Aphis fabae* with seed treatment of imidacloprid.

According to Verghese (1998), imidacloprid 0.2 -1.6 ml l^{-1} gave cent per cent control of mango leafhoppers, *Idioscopis* spp. within 24 hours of spraying and the efficacy of the chemical extended up to three weeks. Ashok *et al.* (2002) also found good control of cotton jassid, *A. devastans* with imidacloprid treatment @ 20 g ai ha^{-1}. Dharmendar *et al.* (2003) obtained reduction in population of *B. tabaci* in brinjal with imidacloprid (40 ml ai $acre^{-1}$). Karthikeyan (2003) observed that foliar application of imidacloprid (20 g ai ha^{-1}) was consistently effective in reducing sucking pests like leafhoppers and aphids in bitter gourd.

2.8.4 Weather factors

According to Mathew *et al.* (1991), *B. tabaci* population was high in summer season which caused 54 per cent mosaic incidence in bitter gourd. Latha (1992) observed maximum whitefly population during the months of April-May. Rekha (1999) also observed high incidence of bitter gourd mosaic in summer. According to Arunachalam (2002), high incidence of BGDMV occurred during April-May and he also found that maximum number of genotypes expressed mosaic symptoms under maximum temperature of 31-33°C and relative humidity 70-85 per cent.

Materials and Methods

3. MATERIALS AND METHODS

The studies on bitter gourd distortion mosaic virus (BGDMV) were carried out during 1999-2004. Studies on transmission aspects and characterisation of the virus were conducted at College of Horticulture, Vellanikkara, Thrissur. Purification of BGDMV was conducted at College of Veterinary and Animal Sciences, Mannuthy, Thrissur. Studies on electron microscopy and serology were conducted at Indian Institute of Horticultural Research, Hessaraghatta, Karnataka. Field experiments for management studies were laid out at Sugarcane Research Station, Thiruvalla.

3.1 STUDIES ON THE TRANSMISSION OF BGDMV

3.1.1 Sap transmission

Sap transmission of the virus was conducted using different buffers such as 0.1 M potassium phosphate buffer (pH-7.2) (Appendix - I) and 0.1 M tris buffer (pH-7.2). Young bitter gourd leaves showing typical symptoms of BGDMV were collected from the field, washed under tap water to remove dust particles and then dried by rubbing with blotting paper. Fifty grams of the leaf tissue was homogenized separately with 50 ml ice cold buffer solutions using a chilled mortar and pestle. The homogenate was filtered through a thin layer of sterile muslin cloth. Bitter gourd plants at two leaf stage previously kept in shade were used for the inoculation. Carborundum powder (600 mesh) was added to the extract and the test plants were inoculated by gently rubbing on the upper surface of the leaves with cotton dipped in the extract. The leaves were then washed with distilled water to remove the excess inoculum. Thirty plants were inoculated with each buffer. Uninoculated plants served as control. Plants were kept in insect proof net house and observed daily for a period of two months for the development of symptoms.

3.2 SEED TRANSMISSION

Seeds collected during summer season from severly BGDMV infected bitter gourd plants were sown in earthern pots containing potting mixture and 10 pots were kept for each replication. Ninety seeds were sown with three in each pot and were kept in insect proof condition. Germinated seedlings were observed daily for a

period of one month for the development of mosaic symptoms and per cent germination was also recorded.

3.3 INSECT TRANSMISSION

Experiments on insect transmission were conducted using aphid (*Aphis gossypii* Glover), jassid (*Empoasca motti* Pruthi) and whitefly (*Bemisia tabaci* Genn.) during different seasons.

3.3.1 Rearing of insects

Pure culture of aphids and jassids for transmission studies were reared on healthy bitter gourd plants raised in earthern pots kept in insect proof cages. While for whiteflies, brinjal plants were used for rearing. Old plants were changed periodically with healthy young plants for the proper maintenance of the insect cultures.

3.3.2 Aphid transmission

Moistened camel hair brush (number zero) was used for the transfer of aphids. The aphids were disturbed with the brush by gently touching the antennae or cornicles without injuring the stylets. After ensuring the withdrawal of their stylets from plant tissue, a gentle tap was given on the plant, subsequently, the moving aphids were picked up with the moistened brush and put into a Petri plate. After ensuring adequate number of aphids, the Petri plate was covered with black paper to provide dark condition to avoid movement and the aphids were subjected to preacquisition fasting period for 30 min. These aphids were then released on young bitter gourd leaves infected with BGDMV for the acquisition of the virus. After an acquisition access period (AAP) of 30 min., viruliferous aphids were released on 30 potted bitter gourd plants of two leaf stage @ 10 per plant and covered with plastic cages having opening at the top and sides covered with muslin cloth. After 24 h of inoculation access period (IAP), the aphids were removed and the plants were sprayed with quinalphos (0.05%) and inoculated plants were kept in insect proof net house for the development of symptoms for a period of two months. Control plants were also kept for each experiment.

3.3.3 Jassid transmission

An aspirator consisting of a glass tube (10 cm length and 0.5 cm diameter) connected to a rubber tube (30 cm length and 0.5 cm diameter) was used for the transmission of jassids. Before connecting to the rubber tube, one end of the glass tube was covered with a muslin cloth for avoiding the entry of jassid into the rubber tube. Jassids were collected from the lower surface of leaves using the aspirator and then released into a source plant kept in a mylar cage (150 cm length and 30 cm diameter). After an acquisition access period of 24 h, they were then released into healthy bitter gourd plants kept in mylar cages for completion of incubation periods (IP). Jassids were given different incubation period of one to four weeks. After the required incubation period viruliferous insects were transferred serially to healthy plants of vining stage for a period of 30 days @ one per plant. Ten plants were used for each experiment. After the required inoculation access period of 48 h, insects were killed by spraying 0.05 per cent acephate and the inoculated plants were kept in insect proof net house for a period of two months for the symptom appearance. Control plants were also kept for each experiment.

3.3.4 Whitefly transmission

Whiteflies were collected from the rearing cages with the help of a test tube and the transmission studies were conducted using a specially designed plastic micro cage of 6 cm in length and 4.5 cm in diameter (Plate 1). From the bottom portion, an area of one centimetre diameter was removed using a soldering rod and muslin cloth was fixed to the opened portion which helped in avoiding the accumulation of excess moisture inside the cage. A longitudinal slit of 3 cm length was made at the mouth of the cage and a rectangular slit made on the side of the lid covering the bottle. A small hole (0.5 cm) was made in the middle portion of the cage to release the whiteflies. One leaf of the infected plant was introduced into this transmission cage through the rectangular slit at the mouth of the cage. The lid of the cage was immediately screwed

Plastic microcage

Transmission study using microcage

Plate 1. Whitefly transmission studies

on. The opened portion of the slit was plugged with cotton. Whiteflies were given preacquisition fasting for 30 min. and were then released into the cage @ 10 per plant through the hole at the middle of the cage and the hole was plugged with cotton. The cage was covered with a black cloth. The cage was kept in position by tying it to two bamboo sticks (Plate 1). After the acquisition access feeding period of 12 h, cage along with whiteflies were removed carefully and fixed on the leaf of the test plant of two leaf stage for inoculation feeding. After 12 h of inoculation access period, the cage was removed and the inoculated plants were sprayed with 0.05 per cent dimethoate. Plants were labelled and kept in insect proof net house for symptom appearance. Observations were taken for a period of two months. Control plants were also kept for each experiment.

3.4 STUDY ON SYMPTOMATOLOGY

Symptomatology was studied by observing the development of symptoms on naturally infected as well as artificially inoculated bitter gourd plants at different stages of plant growth. Symptoms developed on leaves, flowers as well as on fruits were observed for studying the symptomatology of the disease.

Similarly, the symptoms were also observed on plants artificially inoculated through whitefly *B. tabaci* Genn. under insect proof conditions.

3.5 MAINTENANCE OF BGDMV CULTURE

The culture of BGDMV was maintained in bitter gourd plants by periodical transmission through whitefly under insect proof condition. These plants were used as virus source for various transmission studies.

3.6 STUDIES ON VIRUS-VECTOR RELATIONSHIP

Based on the transmission studies, whitefly *B. tabaci* was found to be the vector of BGDMV and the virus-vector relationship was studied in detail.

3.6.1 Effect of different durations of acquisition feeding period on the transmission of BGDMV

The whiteflies were given acquisition feeding periods of 15, 20, 30 and 60 min. after a preacquisition fasting of 30 min. After the required acquisition feeding period, whiteflies were released on bitter gourd plants @ 10 per plant. After 12 h of inoculation access feeding, insects were killed by spraying 0.05 per cent dimethoate and the plants were kept for observation for two months. Fifteen plants were kept for each experiment. Per cent transmission and time taken for symptom expression were recorded. Uninoculated plants were also kept as control.

3.6.2 Effect of different durations of inoculation feeding period on the transmission of BGDMV

Nonviruliferous whiteflies were given an acquisition feeding period of 12 h after starvation of 30 min. Ten whiteflies were released to each test plant and were given inoculation feeding periods of 15, 20, 30 and 60 min. Fifteen seedlings were kept for each experiment. Per cent transmission and incubation period in host were recorded. Uninoculated plants served as control.

3.6.3 Effect of number of infective *B. tabaci* on the transmission of BGDMV

In order to determine the effect of number of infective *B. tabaci* on the transmission of BGDMV, young seedlings were inoculated with viruliferous whiteflies, which had been given 12 h of acquisition access period. Whiteflies were released singly and in groups of two, three, five, seven and ten to the test plants with 12 plants in each treatment. Whiteflies were removed after 12 h of access feeding and inoculated plants were kept under observation and number of plants infected and the time taken for symptom appearance were recorded.

3.7 STUDIES ON HOST RANGE OF BGDMV

Different cucurbitaceous vegetables such as ash gourd (*Benincasa hispida),* , bottle gourd (*Lagenaria sicenaria*), Coccinia (*Coccinia grandis*), Cucumber

(*Cucumis sativus*), pumpkin (*Cucurbita moschata)*, snake gourd (*Trichosanthes cucumerina*) and water melon (*Citrullus lanatus*) were artificially inoculated with whiteflies as mentioned earlier in 3.3.4 to find out the host range of BGDMV.

3.8 PURIFICATION OF THE VIRUS

Two methods were tried to standardise the purification method of BGDMV.

3.8.1 Method I

Method as suggested by Czosnek *et al* (1988) was tried.. Five hundred grams of infected leaves were frozen in liquid nitrogen, crushed to fine powder and homogenized in a warring blender after adding ice cold buffer @ 2 ml g^{-1} tissue, containing 100 mM sodium citrate, 18.5 mM ascorbic acid, 600 mM sodium sulphite, 5 mM ethylene diamine tetra acetic acid (EDTA) at pH 8.0 and 1 per cent (v/v) 2 mercapto ethanol (buffer A). The homogenate was made to 2.5 per cent with Triton x-100, stirred overnight, squeezed through four layers of sterilized cheese cloth and then clarified by centrifugation at 8000 g for 10 min. The supernatant was centrifuged for three hours at 90,000g in a SW_{28} Beckmann rotor. Pellets were suspended in buffer containing 10 mM trisodium citrate, 1 mM EDTA, pH 8.0 and 0.1 per cent 2 mercapto ethanol (CEM buffer) and subjected to centrifugation at 8000 g for 10 min. Pellets were suspended in 10 per cent w/v sucrose in CEM (1 ml of buffer per equivalent of 100 g of leaves), homogenized and clarified by centrifugation at 4000 g for 10 m. The supernatant was subjected to sucrose density gradient centrifugation (10-40 per cent w/v sucrose gradient in CEM) at 90,000 g for 1.5 h in SW28 rotor. Gradients were analysed and fractions showing similar absorbance values were pooled and ultra centrifugation at 90,000g for 3 h was conducted for pelleting of virus particles. Pellets resuspended in CEM buffer and stored at -20°C were used for further studies like electron microscopy, serology and characterisation of the virus.

3.8.2 Method II

Standard protocol of Honda *et al.* (1983) was used with slight modification in which butanol was also tried for initial clarification. Three hundred grams of infected leaves of bitter gourd plants were frozen and then were crushed to a fine powder in liquid nitrogen in an ice cold pestle and mortar and homogenized in 0.1 M potassium phosphate buffer at pH 7.8 containing 0.1 per cent thioglycollic acid, 0.01 M sodium ethylene diamine tetra acetate (2 ml g^{-1} of tissue). The extract was clarified by adding one half volume of chloroform and stirred at 4°C for 30 min. Emulsion was broken by centrifugation at 5000 g for 10 min. and the aqueous phase was recovered. Polyethylene glycol (PEG molecular weight 6000) and sodium chloride were added to the aqueous phase to give a final concentration of 6 per cent and 0.2 M, respectively. After stirring at 4°C for two hours, the mixture was centrifuged at 15,000 g for 30 min. and the precipitate was dissolved in 0.1 M potassium phosphate buffer at pH 7.8 and clarified by centrifugation (9000 g for 10 min.) before being subjected to ultra centrifugation at 90,000 g for three hours. The pellets resuspended in potassium phosphate containing six per cent PEG and 0.2 M sodium chloride were concentrated by ultra centrifugation as before. The resuspended pellets were subjected to sucrose density gradient centrifugation in a swinging rotor at 90,000 g for three hours using 10-40 per cent linear sucrose gradients. Gradients were analysed and fractions showing similar absorbance values were pooled and concentrated by ultracentrifugation as before. The pellets precipitated were resuspended in 0.1 M potassium phosphate buffer and stored at -20°C for further use.

3.9 DETERMINATION OF CONCENTRATION OF THE PURIFIED VIRUS

To determine the concentration of virus particles in the purified preparation ultra violet (UV) absorption study was conducted using a spectrophotometer. The purified virus preparation was diluted to 1:10, 1:50, 1:100 and 1:500 concentrations with 0.1 M potassium phosphate buffer. The UV absorption of 1:10 dilution was taken from 220 to 320 nm and in the case of other dilutions the UV absorption at 260 and

280 nm were recorded. The concentration of the purified virus in one milliliter was calculated by using the formula A_{260} x dilution factor / 7.7, assuming an extinction coefficient of 7.7 for gemini virus and A_{260} / A_{280} ratio was also calculated (Honda *et al.*, 1983)

3.10 STUDIES ON CHARACTERISATION OF VIRUS

3.10.1 Extraction of DNA

DNA extraction was done as per the method by Maniatis *et al.* (1982). Viral DNA was extracted from concentrated viral preparation by the phenol-chloroform method, following a two hour incubation in 0.2 per cent (w/v) sodium dodecyl sulphate (SDS) and 50 µg ml^{-1} proteinase K in a microfuge tube. After incubation, equal volume of phenol - chloroform (v/v) was added. Centrifugation at 10,000 rpm for 10 min. and the aqueous phase removed to a fresh microfuge tube. Equal volume of chloroform:isoamyl alcohol (24:1 v/v) was added and after centrifugation at 10,000 rpm for 10 min. the aqueous phase was removed. The previous step was repeated. The aqueous phase mixed with isopropanol (0.6 ml/ml) and kept at -20°C for half an hour for precipitation of DNA. DNA was pelleted by centrifugation at 10,000 rpm for 10 min. Pellet was airdried, dissolved in 20 µl TE buffer and stored at -20°C.

3.10.1.2 Gel electrophoresis

Electrophoresis of nucleic acid was performed in non denaturing horizontal gel containing one per cent agarose and 0.5 µg ml^{-1} ethidium bromide in Tris acetic acid buffer (Maniatis *et al.*, 1982). Lambda DNA (EcoR1/Hind III digest) was used as molecular marker.

3.10.2 Estimation of viral protein

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) methods described by both Laemmli (1970) and Bock *et al.* (1977) were tried with slight modifications. In the present study coomassie brilliant blue was used for staining the gel instead of silver staining.

3.10.2.1 Materials used

1. Antigen: Purified BGDMV was used to run PAGE
2. 2x sample buffer (Appendix - II)
3. Acrylamide stock

   Acrylamide : 30 g

   Bisacrylamide : 0.8 g

   Made upto 100 ml with distilled water., filtered and stored in a dark bottle at 4°C.
4. 2x stacking gel buffer

   Trisbase (0.25 m) : 3 g

   Distilled water. : 90 ml

   pH adjusted to 6.8 with HCl

   10 per cent SDS : 4 ml

   Made upto 100 ml with distilled water.
5. 4x resolving gel buffer

   Trisbase (1.5 m) : 16.95 g

   Distilled water : 90 ml

   pH adjusted to 8.8 with hydrochloric acid.

   10 per cent SDS : 4 ml

   Made upto 100 ml with distilled water.
6. 10 x reservoir buffer stock

   Trisbase (0.25 m) : 30.25 g

   Glycine : 144 g

   Distilled water. : 850 ml

   pH : 8.3

   10 per cent SDS : 100 ml

   Made upto 1000 ml with distilled water
7. Coomassie brilliant blue stain

   Coomassie brilliant blue (R-250) : 0.2 g

   Methanol : 50 ml

   Acetic Acid : 10 ml

   Distilled water. : 40 ml

   The stain was filtered through Whatman No.1 filter paper before use.

8. Destaining solution

First destaining solution (for rapid destaining)

Methanol	: 250 ml
Acetic acid	: 50 ml
Distilled water.	: 200 ml

Second destaining solution (for rehydration and storage)

Methanol	: 25 ml
Acetic acid	: 50 ml
Distilled water.	: 425 ml

9. Molecular weight markers (sigma)

Carbonic anhydrase	: 29,000
Ovalbumin	: 45,000
Bovine serum albumin	: 66,000

3.10.2.2 Methodology

1. PAGE plates were first cleaned in water, followed by 10 per cent SDS, water, ethanol and water and then air dried.
2. Prepared the stopping gel by making 3.5 ml of acrylamide, 1.25 ml of resolving gel buffer, 5 ml of distilled water, 35 µl of TEMED (N, N, N, N - tetra methyl diamine) and 1.3 ml of 1.5 per cent ammonium persulphate.
3. The cleaned plates were attached to each other and the bottom (2 cm) of the plates were then sealed by pouring the stopping gel and allowed to polymerize.
4. Mixed 20 ml of 30 per cent acrylamide stock with 15 ml of 4x resolving gel buffer and 100 µl of 1.5 per cent ammonium persulphate and 30 µl of TEMED. The volume was made up to 60 ml with distilled water to give 10 per cent gel.
5. The resolving gel was then poured into the prepared plates without making any air bubbles. Then 2x stacking gel buffer was gently layered on top to form a flat surface and the gel was allowed to polymerize.
6. After one hour the upper unpolymerized liquid was poured out. A top stacking gel was prepared by mixing 1 ml of 30 per cent acrylamide stock, 4 ml of distilled water, 5 ml of 2x stacking gel buffer, 10 µl of 1.5 per cent ammonium per

sulphate and 5 µl TEMED. Enough of this stacking gel mixture was poured to fill the upper 3 cm of the gel. Then the sample position forming cob was inserted and the stacking gel was allowed to polymerize.

7. The gel was then attached to the electrophoresis unit (LKB). Then 500 ml of 1x reservoir buffer stock was added to both the top and bottom chambers of the unit and the leak, if any was checked.
8. The sample wells were cleaned by pipetting the reservoir buffer in and out of each.
9. 10, 20 and 30 µl of virus samples were separately mixed with equal quantity of 2x sample buffer. The samples were then heated to 95°C for 3 min. in a steaming water bath and applied on the gel with a microsyringe. Similarly treated mixture of molecular weight markers were also included in the run.
10. The gel was then run at a constant voltage of 100V until the dye front is near the stopping gel (6 hours).
11. The gel was carefully removed from the glass plates and stained overnight in comassie brilliant blue and destained in first destaining solution. The second destaining solution was used to destain and rehydrate the gel.

Proteins bands obtained were visualised on an alpha imager (Alpha Innotech Corporation, Germany).

3.11 STUDIES ON IDENTIFICATION OF VIRUS

3.11.1 Electron Microscopy

Electron microscopic studies were conducted at Indian Institute of Horticultural Research, Bangalore.

Carbon coated copper grids were floated on drops (50 µl) of the purified virus preparation for five min. at room temperature and then washed with 30 drops of deionised distilled water and stained with two per cent aqueous uranyl acetate. Stained grids were examined using a JEOL 100S electron microscope operated at 60 KV and at 75,000 magnification to observe the shape and size of the virus particles.

3.11.1.1 Immunosorbent electron microscopy

Immunosorbent electron microscopic study was conducted following the protocol suggested by Miller and Martin (1988).

3.11.1.1.1 Tissue extraction

Using a cork borer (0.5 cm) a piece of infected leaf tissue was punched out and was placed on a glass slide. Five drops of phosphate buffer 0.1 M of pH 7 were placed on leaf tissue and the tissue was squashed with flat end of a glass rod. Slide was tilted to collect the clear liquid at one side. It was then collected with a Pasteur pipette and diluted with buffer to light green colour.

3.11.1.1.2 Methodology

One drop of antiserum (diluted to 10^{-3} with phosphate buffer) was taken on a paraffin membrane and the grid was placed on the drop for five min. After that the grid was washed with 20 drops of phosphate buffer and was drained. Grid was floated again on a drop of extracted plant sap for one hour and washed with 40 drops of double distilled water. Grid was then stained with five drops of uranyl acetate, drained with filter paper and dried. The grid was observed under electron microscope as in 3.11.1.

3.11.2 Serology

For the serological studies DAC ELISA method suggested by Barbara and Clark (1982) was adopted using purified samples of both healthy and infected plants. The dilution of the antigens were 1:10 and 1:100. Antisera of three isolates of tomato leaf curl virus (546/1, 546/2 and 546/3), two isolates of Indian cassava mosaic virus (0424/1 and SCR-60) and one isolate of squash leaf curl virus (0614/1) obtained from DSMZ, Germany were used for the study. Two replications were maintained for all samples.

3.11.2.1 Solutions used

1. Phosphate buffer saline (PBS) pH 7.4(Appendix - III)
2. Coating buffer: Sodium carbonate buffer, pH 9.6 (Appendix - III)
3. Wash buffer: PBS - Tween (PBS-T)
   Added 0.5 ml tween 20 to one litre PBS
4. Sample buffer: PBS-T containing two per cent poly vinyl pyrrolidone (PVP) Molecular weight - 15,000
5. Conjugate buffer: Added 20 g PVP and 2 g egg oval bumine to one litre PBS-T
6. Substrate buffer - Diluted 97 ml Diethanol amine in 800 ml distilled water, pH adjusted to 9.8 using 6 N hydrochloric acid and made upto one litre
7. Blocking buffer : PBS-T combining 2 per cent PVP and 4 per cent skimmed milk powder

3.11.2.2 Methodology

1. Added BGDMV antigen 200 µl in sample buffer to ELISA microtitre plate well and incubate at 37°C for 3 h.
2. Washed the plates in PBS-T three times. Finally removed the remaining drops of washing buffer by gently tapping plates upside down on a paper.
3. Added 200 µl of blocking solution to each well and incubated at 37°C for 1 h. to block the unoccupied sites.
4. Washed plates with PBS-T as before
5. Added 200 µl of antisera diluted (10^{-3}) in PBS-T to each well. Incubated at 37°C for 3 h.
6. Washed plates with PBS-T as before
7. Added 200 µl per well anti- first animal immunoglobulin (antirabbit IgG in goat) conjugated enzyme antibody in PBS-T and incubated at 37°C for 2 h.
8. Washed the plates with PBS-T.
9. Added 20 µl of P-nitrophenyl phosphate to each well and incubated at room temperature for 15 min. in dark.
10. Reaction was stopped by adding 50 µl of 3 M sodium hydroxide to each well.

11. Evaluated by measuring absorbance at 405 nm in an ELISA reader (Lab System Multiskan MS).

3.12 STUDIES ON MANAGEMENT OF BGDMV

For the disease management studies two field experiments were laid out during March to June, 2001 and January to April, 2002 at Sugarcane Research Station, Thiruvalla (Plate 2).

Experimental details were as follows:

Design	- RBD
Replication	- 3
Treatments	- 15
Number of plants per treatment	- 12
Plot size	- 3.2 m x 3.2 m
Spacing	- 2 m x 2 m
Season	- March to June 2001 and January to April 2002
Variety	- Priyanka

3.12.1 Preparation of land

Land was prepared thoroughly and mounds of size 45 cm in diameter and 50 cm in height were taken at a spacing of 2 m x 2 m. Well dried farmyard manure and fertilizer were applied as per the Package of Practices Recommendation of KAU (1996b).

Five seeds were sown in each mound. After germination, only three healthy and vigorous seedlings were retained in a mound and excess seedlings were thinned out. There were four mounds for each treatment. Plants were trailed on bamboo poles.

First two treatments T_1 and T_2 were given before sowing and T_{14} at 14 and 34 days after sowing (DAS). In case of other treatments, four foliar sprays were given at 15 day interval from 15 DAS.

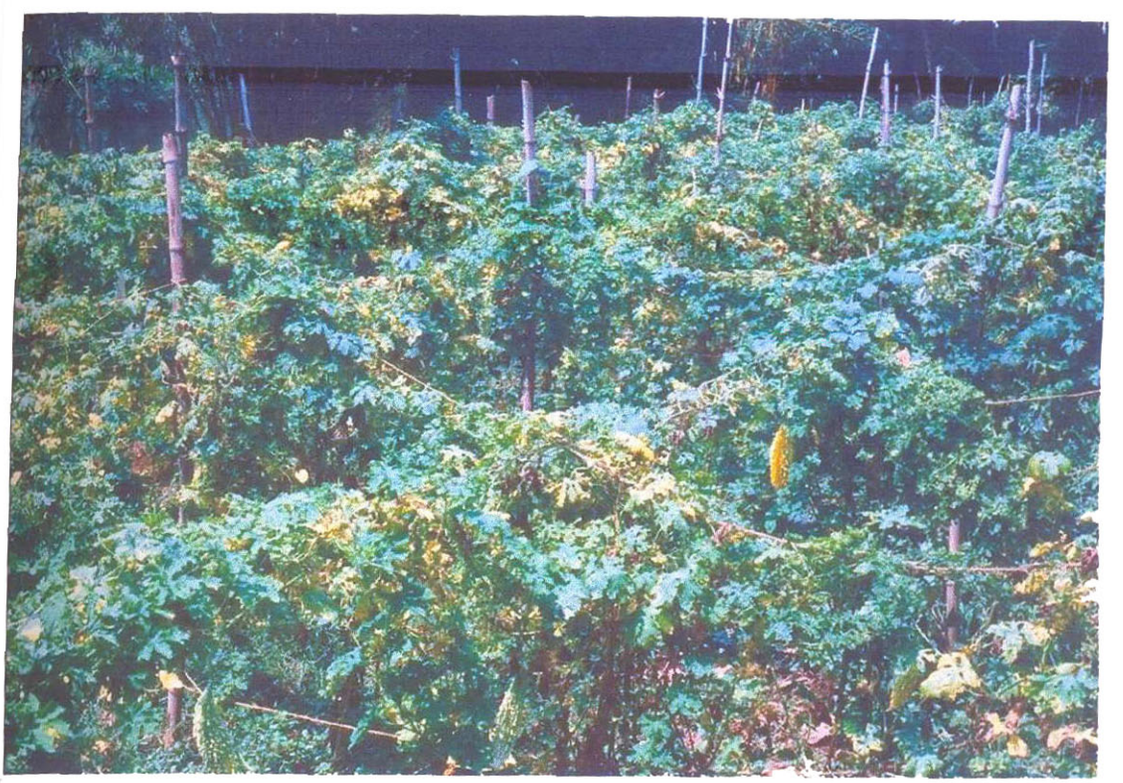
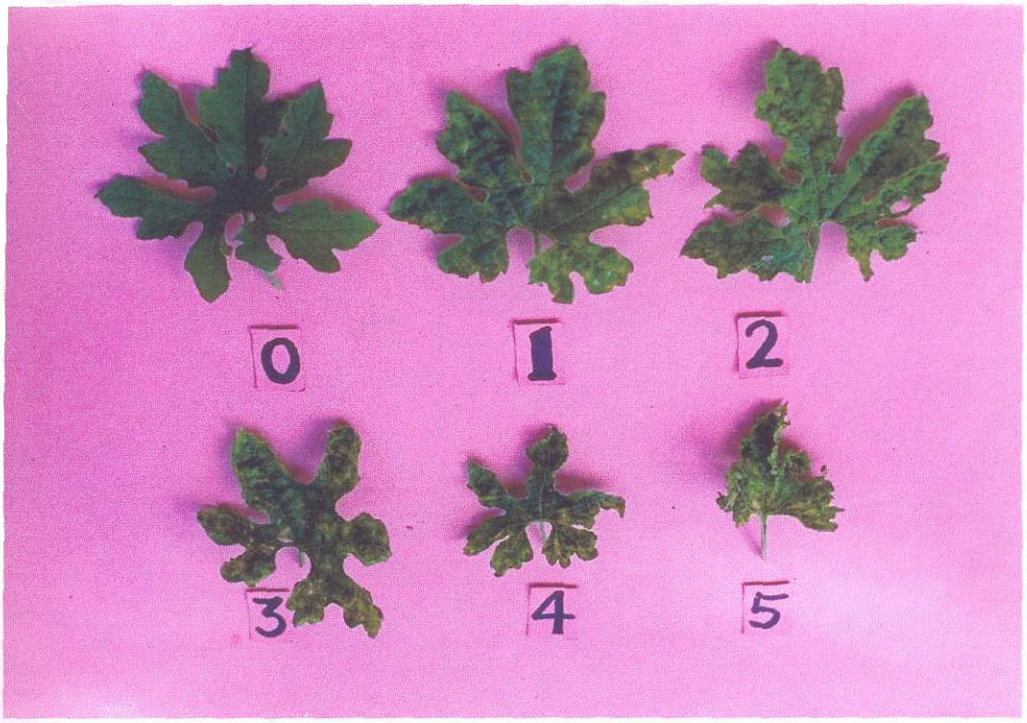


Plate 2. A field view of experimental plot



0 - No symptom
1 - Minute chlorotic specks or patches on leaf
2 - Wide area of mosaic symptom on leaf without distortion
3 - Distortion and reduction about 25 per cent of the normal leaf area
4 - Distortion and reduction about 25 to 75 per cent of the normal leaf area
5 - Distortion and reduction about more than 75 per cent of the normal leaf area

Plate 3. Score chart of BGDMV infection

3.12.2 Treatments

Treatments adopted for the field experiments are given in Table 1.

Table 1. Details of treatments

Treat-ments	Treatment details	Concentration	Method of application
T_1	Carbosulfan	50 g kg^{-1} seed	Seed treatment
T_2	Phorate	10 g per pit	Soil application
T_3	Acephate	0.05 per cent	Foliar spraying
T_4	Dicofol	0.05 per cent	Foliar spraying
T_5	Neem oil - garlic emulsion	2 per cent	Foliar spraying
T_6	Neem seed kernel extract	5 per cent	Foliar spraying
T_7	*Thespesia populnea fresh* leaf extract	10 per cent	Foliar spraying
T_8	*Clerodendron infortunatum fresh* leaf extract	10 per cent	Foliar spraying
T_9	*Apis melliphica*	30 potency	Foliar spraying
T_{10}	*Arsenicum album*	30 potency	Foliar spraying
T_{11}	Infected plant extract	10^{-3} dilution with water	Foliar spraying
T_{12}	Coconut toddy	1:3 dilution with water	Foliar spraying
T_{13}	Coconut vinegar	1 per cent	Foliar spraying
T_{14}	Imidacloprid	0.025 per cent	Foliar spraying
T_{15}	Control		

3.12.3. Disease incidence and severity of BGDMV

Incidence and severity of typical distortation mosaic disease were recorded after each spraying. Number of plants infected in each plot were recorded and the per cent disease incidence (PDI) was calculated using the formula:

$$PDI = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

To record the disease severity, five plants were selected randomly from each treatment and five leaves were tagged from each plant. Severity was recorded using 0-5 scale score chart as suggested by Arunachalam (2002) (Plate 3).

0 - No symptom

1 - Minute chlorotic specks or patches on leaf

2 - Wide area of mosaic symptom on leaf without distortion

3 - Distortion and reduction about 25 per cent of the normal leaf area

4 - Distortion and reduction about 25 to 75 per cent of the normal leaf area

5 - Distortion and reduction about more than 75 per cent of the normal leaf area

Based on the scoring, per cent disease severity (PDS) was calculated using the formula:

$$PDS = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed x Maximum disease category}} \times 100$$

Based on PDI and PDS, the coefficient of infection (C.I) was calculated according to Datar and Mayee (1981).

$$\text{Coefficient of infection (C.I)} = \frac{\text{PDI x PDS}}{100}$$

3.12.4 Effect of treatments on pest incidence

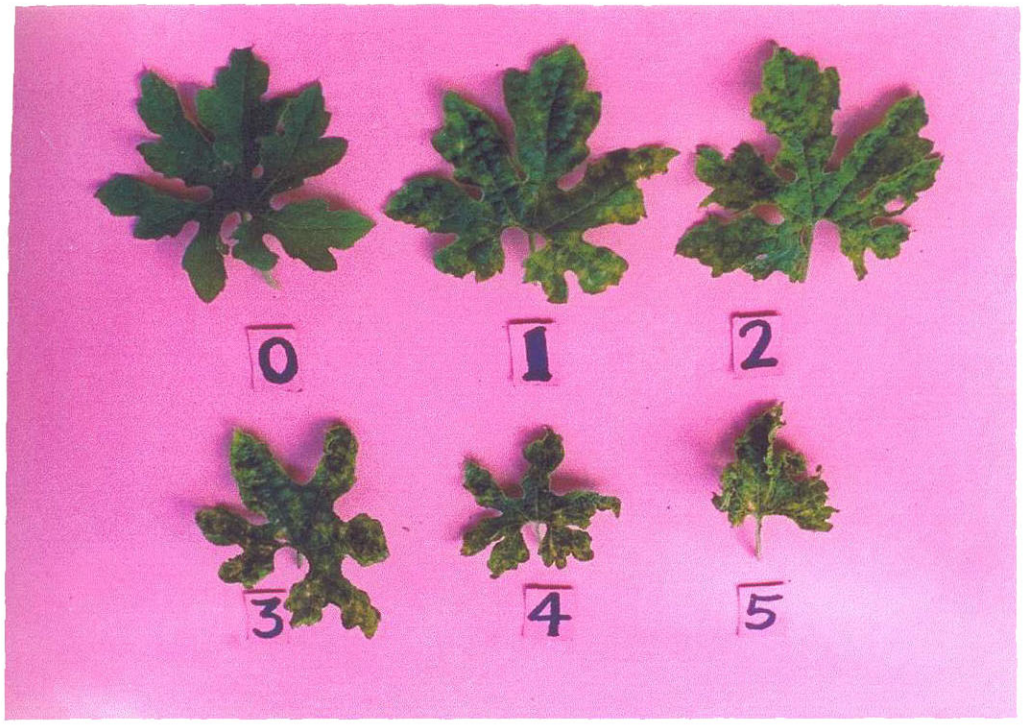
Observations on the population of whitefly, *Bemisia tabaci* were recorded before spraying and 15 day after each spray. Whiteflies were counted from top four leaves. The population of insect was expressed as mean number of insects per plant.

3.12.5 Yield

The fruit yield from different treatments were recorded separately and compared.

3.13 METEOROLOGICAL OBSERVATIONS

Maximum and minimum temperature, relative humidity, total rainfall and number of rainy days were recorded during the cropping periods. Correlations of



0 - No symptom
1 - Minute chlorotic specks or patches on leaf
2 - Wide area of mosaic symptom on leaf without distortion
3 - Distortion and reduction about 25 per cent of the normal leaf area
4 - Distortion and reduction about 25 to 75 per cent of the normal leaf area
5 - Distortion and reduction about more than 75 per cent of the normal leaf area

Plate 3. Score chart of BGDMV infection

weather parameters with disease incidence, disease severity and coefficient of infection were worked out using MSTAT package.

3.14 STATISTICAL ANALYSIS

Data was analysed following analysis of variance for randomized block design (Gomez and Gomez, 1984). Multiple comparison among treatment means where the F test was significant was done with Duncan's Multiple Range Test using MSTAT package. The data was transformed if necessary and statistically analysed.

Results

4. RESULTS

The results of studies on the various aspects of bitter gourd distortion mosaic virus (BGDMV) are presented in this chapter.

4.1 STUDIES ON TRANSMISSION OF BGDMV

4.1.1 Sap transmission

None of the plants inoculated with the infected plant sap prepared either in buffer solutions of 0.1 M potassium phosphate or Tris phosphate produced symptoms of BGDMV which indicated that BGDMV was not sap transmissible.

4.1.2 Seed transmission

It was observed that none of the germinated seedlings produced any symptom of BGDMV upto a period of one month which showed that BGDMV was not transmitted through seeds. Mean germination percentage of seeds from infected plants was 45 per cent, whereas seeds from healthy plants showed 85 per cent germination (Table 2). The seedlings raised from infected seeds were pale, lanky and non vigorous.

4.1.3 Studies on insect transmission

Transmission studies using aphids, jassids and whiteflies were conducted as per the methods described under the chapter Materials and Methods, inorder to identify the vector of BGDMV and the result is presented in Table 3

4.1.3.1 Aphid transmission

In the transmission studies using aphid, *Aphis gossypii* @ 10 per plant, the inoculated plants did not show any symptom of BGDMV for a period of two months, which showed that *A. gossypii* was not able to transmit this virus.

4.1.3.2 Jassid transmission

Jassid, *Empoasca motti* was used for this study. None of the inoculated plants produced any symptoms of mosaic disease which indicated that jassid, *E. motti* was also a non vector of BGDMV.

4.1.3.3 Whitefly transmission

In the transmission studies using *B. tabaci,* it was observed that all the plants inoculated showed typical symptoms of BGDMV giving cent per cent transmission, which proved whitefly to be the actual vector of BGDMV.

4.2 SYMPTOMATOLOGY OF BGDMV

Symptoms of BGDMV was studied under both natural and artificial conditions.

4.2.1 Symptoms observed under natural condition

Four types of mosaic symptoms were observed on bitter gourd plants infected under natural conditions which were as follows.

4.2.1.1 Type 1

Initial symptoms occurred as mild mottling with alternate light green and yellow patches. Symptoms were prominent on young leaves. Neither distortion nor reduction of leaf size were noticed. Blisters were observed on leaf lamina. Internodal length was also normal. Flowering was slightly reduced but the fruits were of normal size and shape (Plate 4).

4.2.1.2 Type 2

In this case, veins and veinlets of the leaves were thick and prominent and showed vein clearing symptoms. Symptoms were prominent on matured leaves, eventhough all leaves in the vines were infected. Leaves were brittle and slightly distorted. Flower and fruit formations were slightly reduced. Fruits were of smaller size (Plate 4).

Table 2. Transmission of BGDMV through seeds

Sl. No.	Seed sample	Per cent germination				Per cent transmission			
		R_1	R_2	R_3	Mean	R_1	R_2	R_3	Mean
1	Seeds from infected plants	45	30	60	45	0	0	0	0
2	Seeds from healthy plants	80	85	90	85	0	0	0	0

Table 3. Transmission of BGDMV through different insect vectors

Sl. No.	Insect vector	Number of plants inoculated	Number of plants infected	Per cent transmission	Incubation period in plant (days)
1	Aphid *Aphis gossypii*	30	0	0	-
2	Jassid *Empoasca motti*	30	0	0	-
3	Whitefly *Bemisia tabaci*	30	30	100	14

4.2.1.3 Type 3

Mosaic disease was characterised by the presence of light green and dark green patches which was first observed on middle aged leaves, subsequently all leaves were found infected. Newly produced leaves showed light green and dark green mosaic pattern with upward curling and crinkling. Internodal length was increased and the tender vines were more pubescent. Both male and female flower production were reduced. Fruits were small and distorted (Plate 4).

4.2.1.4 Type 4

Younger leaves showed light and dark green mosaic areas. The internodal length was highly reduced, leaf size was reduced and leaves were clustered around the nodes, giving a bushy appearance to the plant (Plate 4). Vines were flattened towards the top portion of the plants. Infected plants rarely produced flowers and if flowered, the fruits were mostly aborted.

4.2.2 Symptoms observed under artificial conditions

In artificially inoculated plants, symptoms initiated as light green and dark green mosaic pattern on inoculated leaves in about 14 days after inoculation which later spread to younger leaves. Size of the leaf lamina was reduced and tip of the lobes became pointed. Number of leaf lobes were not reduced. Leaves were rough and brittle. Blisters were produced on the under surface of the leaves. Flowering started 35 days after sowing and flowers were less in number. Length of the vines were not reduced and internodal length was also normal. Vines were neither pubescent nor flattened (Plate 5).

4.3 STUDIES ON VIRUS - VECTOR RELATIONSHIP

Based on the results of transmission studies, whitefly *B. tabaci* was found to be the vector of BGDMV and the various aspects of virus - vector relationship was studied on detail.

Initial stage Later stage Type II

Type I

On vine On fruit Type IV

Type III

Plate 4. Symptoms of BGDMV under natural condition

Diseased Healthy

Plate 5. Symptoms of BGDMV under artificial condition

4.3.1 Effect of different durations of acquisition feeding period on transmission of BGDMV

Whiteflies were given acquisition feeding periods of 15, 20, 30 and 60 min. after a preacquisition fasting of 30 min. The whiteflies were then allowed 12 h of inoculation access feeding period on test plants. Result of the experiment is presented in Table 4 and Fig. 1. It was observed that a minimum acquisition feeding period of 15 min. was required for the acquisition of BGDMV from the infected plants as evident from the 40 per cent transmission obtained. However, the per cent of transmission increased from 40 to 73 with increase in acquisition feeding period from 15 to 60 min. It was also noticed that the incubation period of BGDMV in host decreased with increase in acquisition feeding period.

4.3.2 Effect of different durations of inoculation feeding period on the transmission of BGDMV

Viruliferous insects were given inoculation feeding periods of 15, 20, 30 and 60 min. on healthy test plants. From the data presented in Table 5 and Fig. 2, it was observed that a minimum inoculation feeding period of 15 min. was enough for the successful transmission of BGDMV as evidenced from 46 per cent transmission obtained. However the per cent transmission increased from 46 to 80 per cent with increase in inoculation feeding period from 15 to 60 min. It was noted that the incubation period of virus in host plant also decreased with increase in inoculation feeding period.

4.3.3 Effect of number of infective *Bemisia tabaci* on the transmission of BGDMV

In order to determine the effect on the number of *B. tabaci* on the transmission of BGDMV, infective whiteflies were released on healthy test plants singly and in groups of 2, 3, 5, 7 and 10 for the inoculation of virus. From Table 6 and Fig. 3, it was noticed that even a single viruliferous whitefly was capable of transmitting BGDMV as it could render 33 per cent transmission. A steady increase in transmission was observed with increase in number, where, cent per cent transmission

Table 4. Effect of different durations of acquisition feeding period on the transmission of BGDMV

Sl. No.	Acquisition feeding period (minutes)	Number of plants inoculated	Number of plants infected	Per cent transmission	Incubation period in plant (days)
1	15	15	6	40	41
2	20	15	7	46	34
3	30	15	9	60	22
4	60	15	11	73	18

Inoculation feeding period - 12 hr
Number of white flies used - 10 per plant

Table 5. Effect of different durations of inoculation feeding period on the transmission of BGDMV

Sl. No.	Inoculation feeding period (minutes)	Number of plants inoculated	Number of plants infected	Per cent transmission	Incubation period in plant (days)
1	15	15	7	46	24
2	20	15	9	60	21
3	30	15	10	66	18
4	60	15	12	80	17

Acquisition feeding period - 12 hr
Number of white flies used - 10 per plant

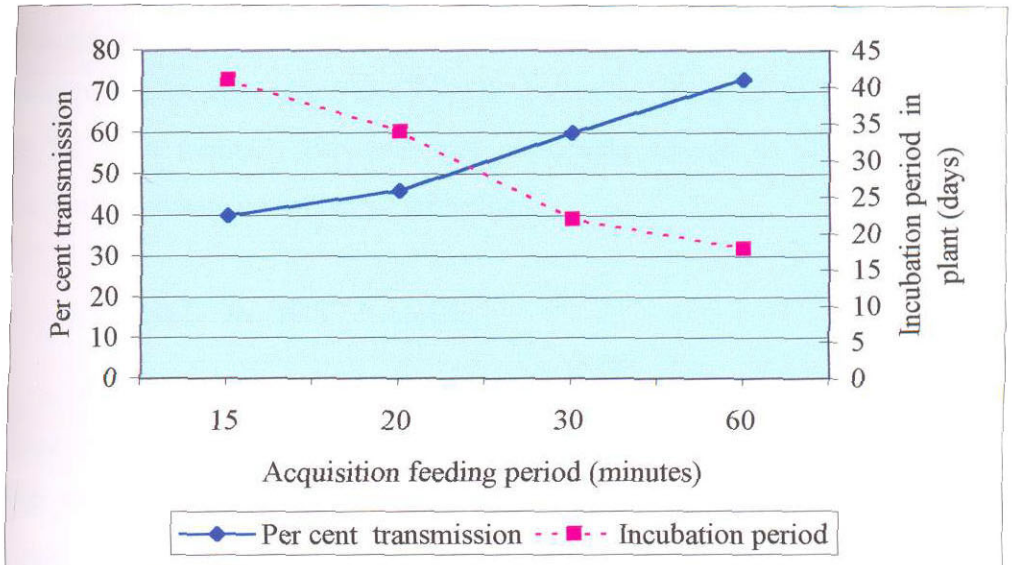


Fig. 1. Effect of different durations of acquisition feeding period on the transmission of BGDMV

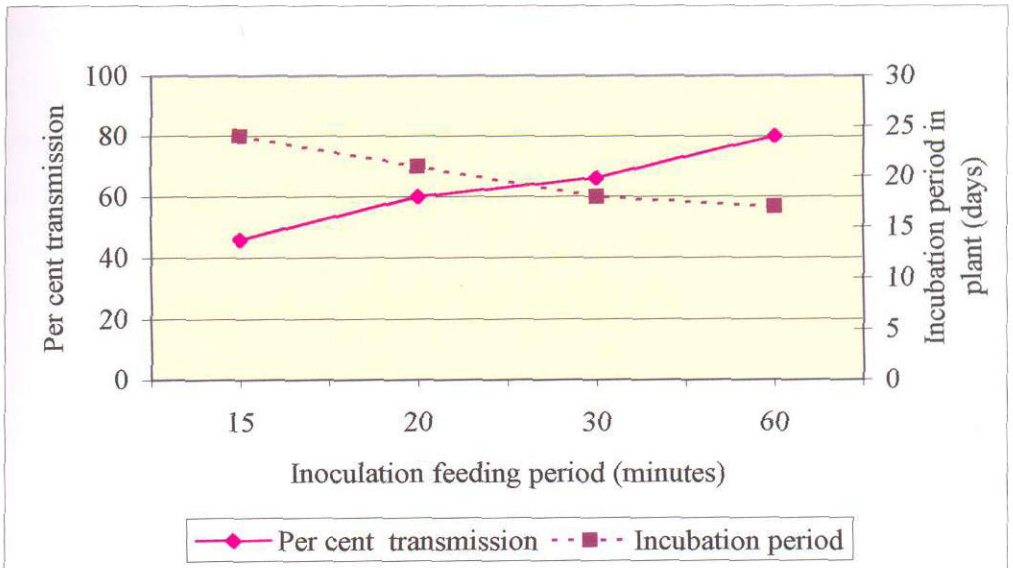


Fig. 2. Effect of different durations of inoculation feeding period on the transmission of BGDMV

was obtained when 10 whiteflies were used. A positive trend was observed on the incubation period in host plant also, which decreased from 25 to 14 days with the increase in insect number. However any noticeable change in the symptoms was observed with increase in number of whiteflies.

4.4 STUDIES ON HOST RANGE

In order to find out the host range of BGDMV, different cucurbitaceous vegetables such as ash gourd, bottle gourd, coccinia, cucumber, pumpkin, snake gourd and water melon were inoculated using viruliferous whiteflies. It was found that none of the inoculated plants showed any symptoms of BGDMV, which revealed that the above cucurbitaceous plants were not collateral hosts of BGDMV.

4.5 PURIFICATION OF BGDMV

Method for purification of BGDMV was standardised using the procedure of Honda *et al.* (1983). Chloroform was found to be better than butanol for the initial clarification as it yielded more geminate particles under electron microscopic studies. Purified sample obtained after the final ultra centrifugation was stored at -20°C and used for characterisation, electron microscopy and serological studies of the virus.

4.5.1 Determination of concentration of BGDMV purified preparation

In a test to determine the concentration of virus particle, the virus sample was serially diluted with 0.1 M potassium phosphate buffer to 1:10, 1:50, 1:100 and 1:500 concentration and UV absorption at 260 nm and 280 nm were noted. The purified virus preparation gave an average A260/A280 value of 1.62 (Table 7). Average yield of the virus was 0.102 mg ml^{-1}/100 g plant tissue.

4.6 STUDIES ON CHARACTERISATION OF VIRUS

UV absorption of purified virus sample at 10^{-1} dilution was measured at 220 to 320 nm (Table 8). Ultra violet absorption spectra of BGDMV resembled to that of a typical nucleoprotein with a peak at 241 nm (Fig. 4).

Table 6. Effect of number of infective *Bemisia tabaci* on the transmission of BGDMV

Sl. No.	Number of insects	Number of plants inoculated	Number of plants infected	Per cent transmission	Incubation period in plant (days)
1	1	12	4	33	25
2	2	12	5	42	24
3	3	12	6	50	20
4	5	12	7	58	19
5	7	12	9	75	19
6	10	12	12	100	14

Acquisition feeding period - 12 hr
Inoculation feeding period - 12 hr

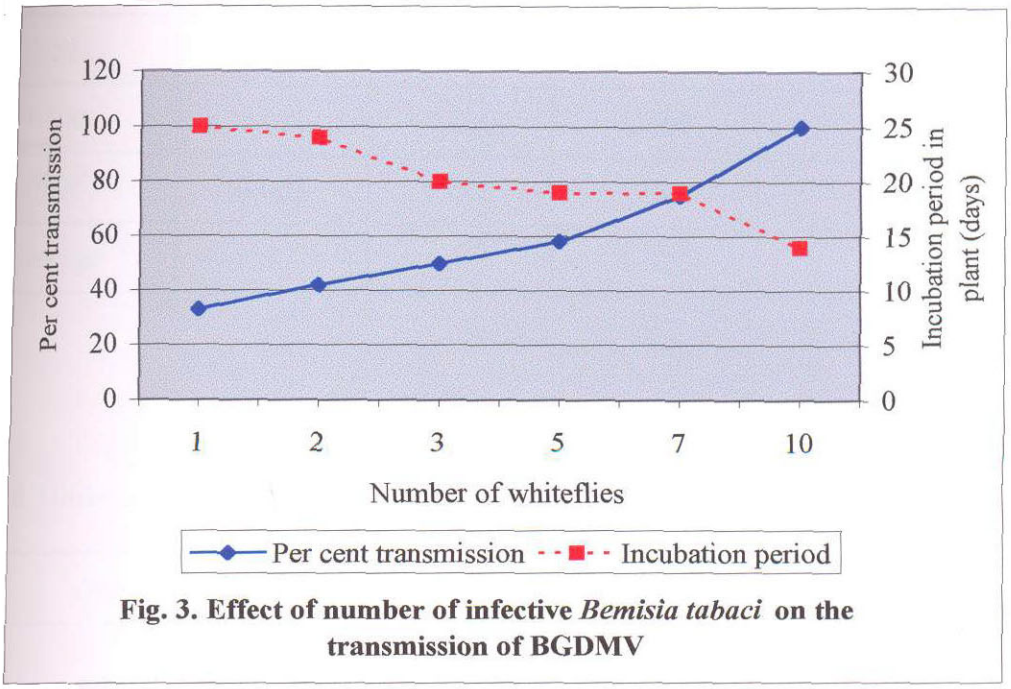


Fig. 3. Effect of number of infective *Bemisia tabaci* on the transmission of BGDMV

Table 7. Determination of concentration of BGDMV in purified samples

Dilutions of purified virus samples	Ultraviolet absorption at		A 260/A 280	Concentration of virus mg ml^{-1}/100 g plant tissue
	260 nm	280 nm		
1:10	1.064	0.481	2.200	0.304
1:50	0.020	0.012	1.670	0.029
1:100	0.011	0.010	1.100	0.030
1:500	0.003	0.002	1.500	0.043
Average	-	-	1.620	0.102

Table 8. Ultraviolet absorption of purified preparation of BGDMV at 10^{-1} dilution

Wavelength (nm)	Absorbance value
220	0.024
240	1.498
260	1.064
280	0.481
300	0.147
320	0.053

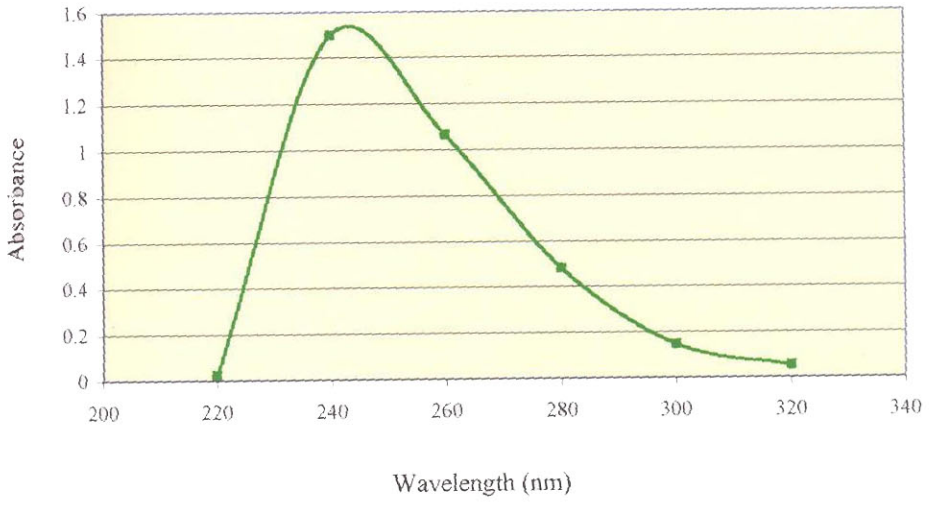


Fig. 4. Ultraviolet absorption of purified preparation of BGDMV at 10^{-1} dilution

Characterisation of BGDMV was attempted by characterisation of DNA and protein coat by adopting standard protocols.

4.6.1 Characterisation of Nucleic acid

Characterisation of nucleic acid isolated from purified virus sample by phenol-chloroform method did not show any characteristic band of virus particles. So characters of the nucleic acid could not be studied.

4.6.2 Characterisation of viral protein

Characterisation of protein by SDS PAGE using purified preparation did not reveal any band characteristic of viral protein. However, on SDS PAGE using 15 per cent polyacrylamide gel, a total of seven bands ranging from molecular weight 14610 D to 86400 D were observed in infected sample. Healthy sample yielded five bands. Two additional bands of 16417 D and 24970 D were observed in infected sample (Plate 6).

4.7 STUDIES ON IDENTIFICATION OF VIRUS

4.7.1 Electron microscopy

Electron microscopy using the purified preparation of BGDMV from artificially inoculated plant samples revealed the presence of geminate particles consisting of monomer particles measured 18-20 nm diameter and dimers 30 nm. Monomers and dimers were observed in the ratio 40:3, while trimers and tetramers were totally absent in the purified samples (Plate 7).

In the case of purified preparation of BGDMV obtained from field samples, two types of virus particles, of which one was of geminate nature of the 30 nm size and the other was of flexuous rod shaped particle measuring 750 nm in length resembling to poty virus were observed (Plate 7).

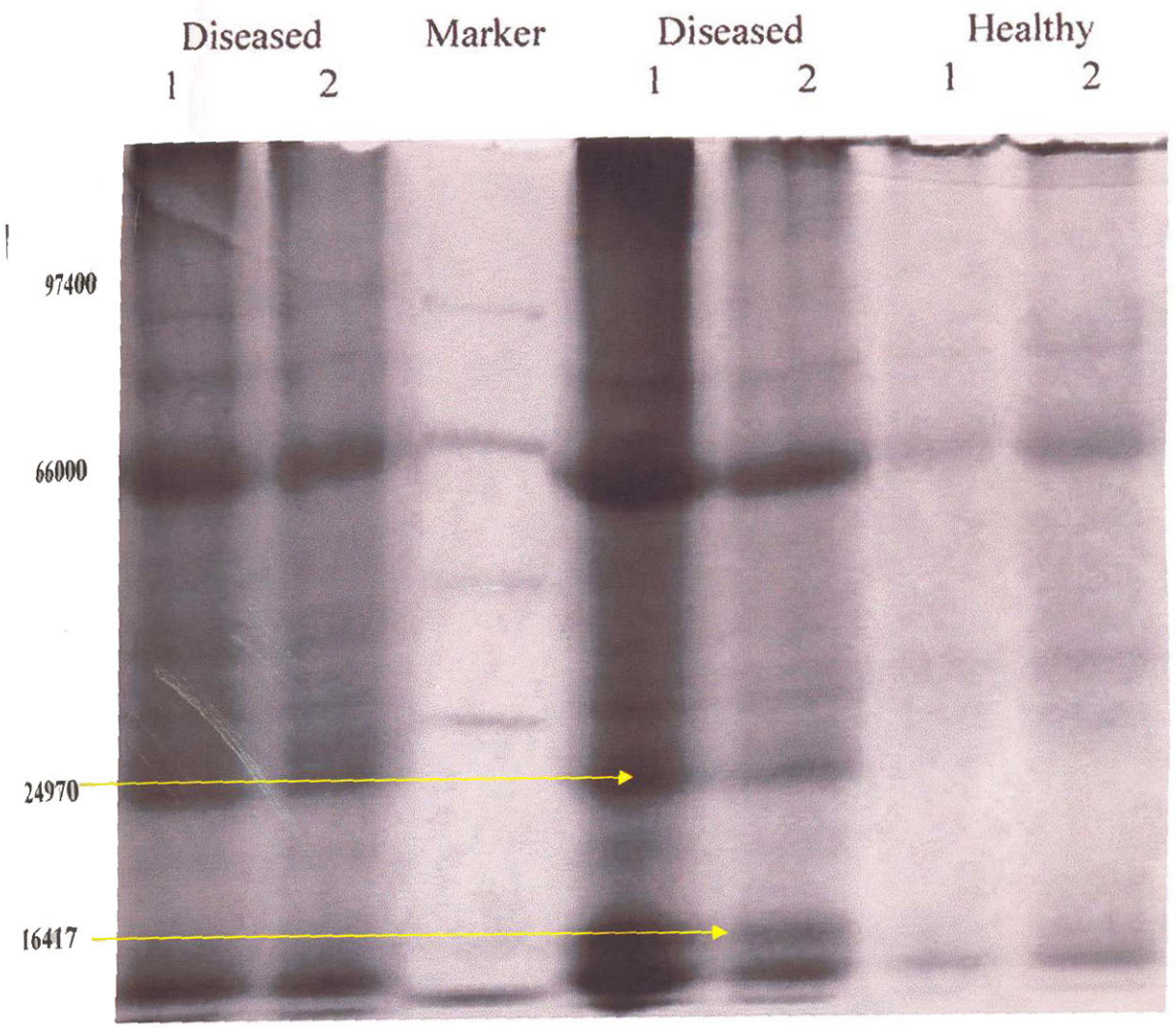
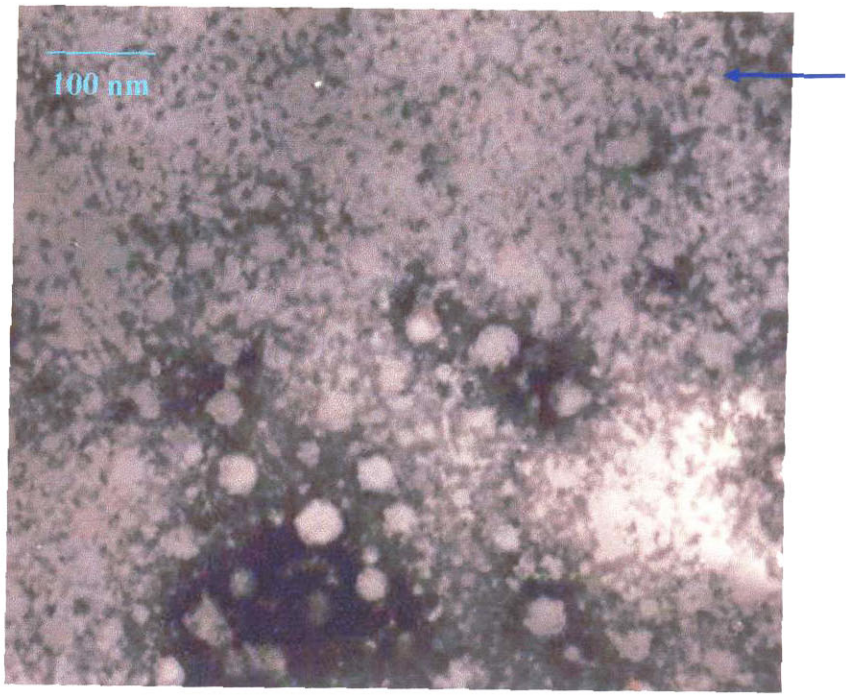
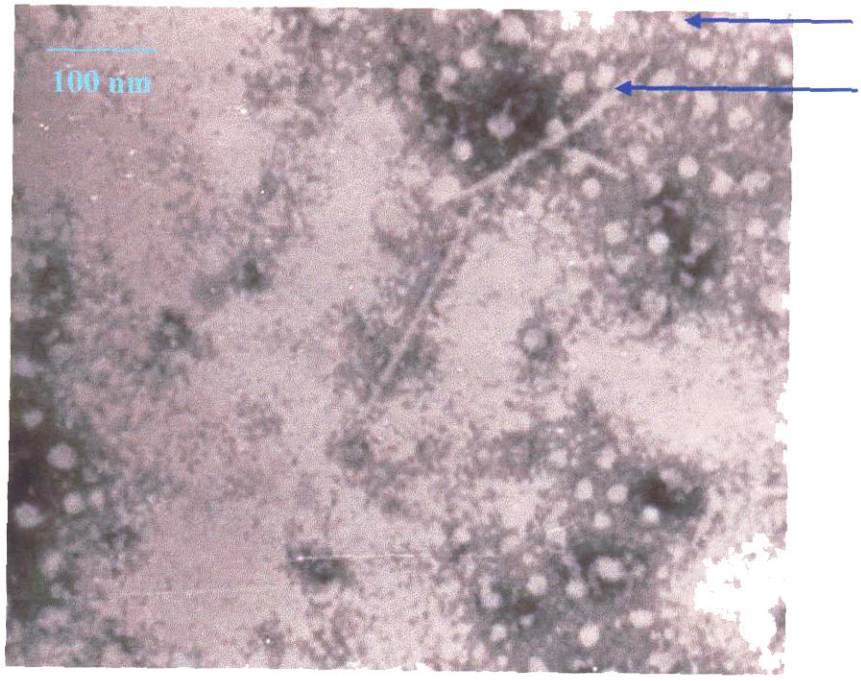


Plate 6. SDS PAGE at 15 per cent agarose gel



Purified virus sample (X 75000)

Size of: **Monomers - 18 to 20 nm**
Geminate particles - 18 to 20 x 30 nm



Field sample (X 75000)

Size of: **Monomers - 18 to 20 nm**
Geminate particles - 18 to 20 x 30 nm
Road shaped particles - 750 nm

Plate 7. Electronmicroscopic observations of BGDMV

4.7.2 Serology

In serological studies using DAC-ELISA method, two dilutions of antigens viz., 10^{-1} and 10^{-2} were used. Positive reaction was observed only with 10^{-1} dilution. Antigen of BGDMV at 10^{-1} dilution was found reacted with the monoclonal antibodies of squash leaf curl virus (SLCV - 0614/1) and one isolate of tomato yellow leaf curl virus (TYLCV - 546/1) which were reported to be gemini viruses (Table 9 and Fig. 5). Thus, this result also indicated the geminate nature of BGDMV. High positive reaction clearly explained the close analogy of BGDMV with squash leaf curl virus. However, purified preparations of BGDMV did not react with the antisera of other gemini viruses like, Indian cassava mosaic virus isolates (0424/1 and SCR - 60) and two isolates of tomato yellow leaf curl virus (546/2 and 546/3).

Immunosorbent electron microscopic study with Indian cassava mosaic antisera also did not give any visual reaction of BGDMV antigen which also indicated that BGDMV was not serologically related to Indian cassava mosaic virus.

4.8 STUDIES ON MANAGEMENT OF BGDMV

4.8.1 Effect of different treatments on disease incidence

From the results presented in tables 10 and 11, it was observed that none of the treatments could prevent the incidence of BGDMV in both seasons.

During the first season, it was observed from Table 10 that no significant difference could be noticed between the treatments after the various sprays, except for 15 days after third spray. It was also noticed that none of the treatments could reduce the disease incidence even after the four sprays. However in case of 3rd spray the plots sprayed with *Arsenicum album* (T_{10}) showed the lowest disease incidence (72.83 per cent). This was on par with T_4 (Dicofol 0.05 per cent), T_3 (Acephate 0.05 per cent), and T_8 *Clerodendron infortunatum* leaf extract 10 per cent.

Table 9. Serological reaction of BGDMV to different antisera

Sl. No.	Monoclonal antibody	Absorbance at 405 nm							
		Dilution of different antigens							
		10^{-1}				10^{-2}			
		Buffer	Healthy	BGDMV inoculated		Buffer	Healthy	BGDMV infected	
				R_1	R_2			R_1	R_2
1	SLCV 0614/1	0.155	0.111	1.076***	1.091***	0.125	0.127	0.278	0.206
2	TYLC 546/1	0.132	0.133	0.955**	0.847**	0.124	0.125	0.203	0.223
3	TYLC 546/2	0.166	0.146	0.194	0.213	0.162	0.167	0.168	0.174
4	TYLC 546/3	0.222	0.151	0.160	0.176	0.222	0.176	0.154	0.143
5	ICMV 0424/1	0.117	0.150	0.179	0.199	0.162	0.142	0.123	0.126
6	ICMV SCR-60	0.137	0.309	0.153	0.166	0.129	0.133	0.125	0.126

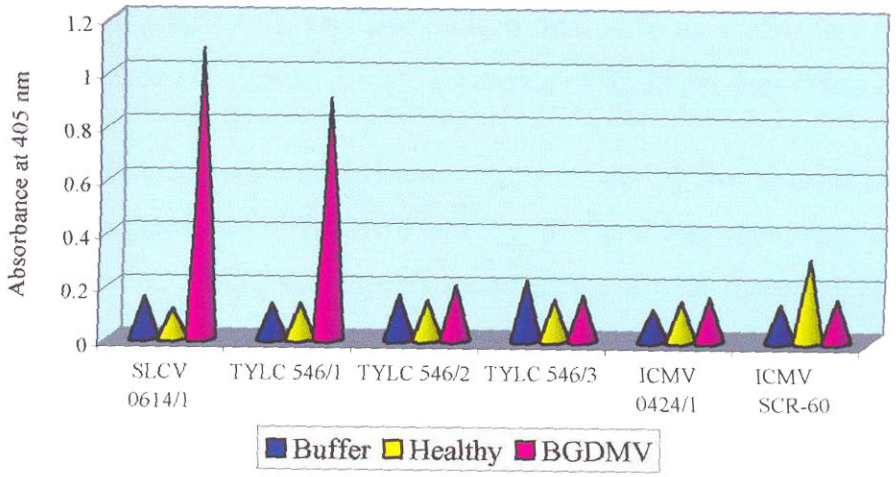
** Positive reaction

*** High positive reaction

SLCV - Squash leaf curl virus

TYLCV - Tomato yellow leaf curl virus

ICMV - Indian Cassava Mosaic Virus



SLCV - Squash leaf curl virus; TYLCV - Tomato yellow leaf curl virus; ICMV - Indian Cassava Mosaic Virus

Fig. 5. Reaction of BGDMV at 10^{-1} dilution to different antisera

During the second season, it is seen from the Table 11 that no significant difference could be noticed among treatments after first and second sprays. It was also observed that the disease incidence was comparatively low in all treatments at 15 days after first spray, but, increased subsequently after each spray. While at 15 days after third and fourth spray, treatments differed significantly. At 15 days after third spray, T_{14} (Imidacloprid) recorded the lowest disease incidence of 33.32 per cent and was on par with T_4 (Dicofol 0.05 per cent), T_5 (Neem oil - Garlic emulsion 2 per cent) and T_9 (*Apis melliphica* 30 potency). It was also noticed that, at 15 days after last spray, T_{14} (Imidacloprid) recorded the lowest disease incidence of 58.33 per cent (Plate 8).

Overall performance of different treatments during two seasons at 15 days after last spray are presented in Table 16 and Fig. 6. There was significant difference among treatments and T_{14} (Imidacloprid) recorded the lowest disease incidence of 76.38 per cent and was on par with T_9 (*Apis melliphica* 30 potency) and T_{13} (Coconut vinegar 1 per cent).

During the first season at 15 days after last spray, mean per cent disease incidence for the treatments ranged between 91.67 and 100. For the second season, it ranged between 58.33 and 91.64.

Disease incidence was less during the second season when compared to that of the first season.

4.8.2 Effect of different treatments on disease severity

Effect of different treatments on mean per cent disease severity during the first season is presented in Table 12. Even after four sprays per cent disease severity progressed in all the treatments through out the season. No significant difference among treatments was observed.

From the Table 13, it was observed that during the second season there was no significant difference among treatments at 15 days after first spray. Per cent disease severity showed a progressive trend throughout the season irrespective of the treatments. Significant difference among treatments was noticed at 15 days after

Table 10. Effect of different treatments on disease incidence during first season (March to June, 2001)

Treatments	Mean per cent disease incidence			
	15 days after first spray	15 days after second spray	15 days after third spray	15 days after fourth spray
T_1 - Carbosulfan 50 g kg^{-1} seed	45.47 [a] (0.739)	83.93 [a] (1.182)	100.00 [d] (1.426)	100.00 [a] (1.426)
T_2 - Phorate 10 g per pit	37.98 [a] (0.660)	96.00 [a] (1.363)	99.13 [d] (1.420)	100.00 [a] (1.426)
T_3 - Acephate 0.05 per cent	56.83 [a] (0.864)	80.03 [a] (1.109)	82.80 [abc] (1.143)	100.00 [a] (1.426)
T_4 - Dicofol 0.05 per cent	44.43 [a] (0.729)	75.00 [a] (1.065)	80.57 [abc] (1.128)	94.47 [a] (1.328)
T_5 - Neem oil - garlic emulsion 2 per cent	52.77 [a] (0.816)	94.23 [a] (1.324)	97.23 [bcd] (1.377)	97.23 [a] (1.377)
T_6 - Neem seed kernel extract 5 per cent	25.00 [a] (0.506)	91.63 [a] (1.284)	97.23 [bcd] (1.377)	100.00 [a] (1.426)
T_7 - *Thespesia populnea* fresh leaf extract 10 per cent	30.57 [a] (0.579)	94.43 [a] (1.334)	94.43 [bcd] (1.334)	94.43 [a] (1.334)
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	40.00 [a] (0.678)	72.23 [a] (1.076)	86.13 [abcd] (1.220)	91.67 [a] (1.300)
T_9 - *Apis melliphica* 30 potency	34.87 [a] (0.625)	77.03 [a] (1.085)	91.17 [bcd] (1.278)	93.93 [a] (1.327)
T_{10} - *Arsenicum album* 30 potency	44.43 [a] (0.729)	51.23 [a] (0.798)	72.83 [a] (1.026)	96.67 [a] (1.367)
T_{11} - Infected plant extract 10^{-3} dilution	60.16 [a] (0.894)	97.23 [a] (1.377)	97.23 [cd] (1.377)	100.00 [a] (1.426)
T_{12} - Coconut toddy 1:3 dilution	22.23 [a] (0.450)	86.62 [a] (1.213)	93.33 [bcd] (1.320)	93.33 [a] (1.320)
T_{13} - Coconut vinegar 1 per cent	33.33 [a] (0.572)	87.70 [a] (1.228)	94.43 [bcd] (1.334)	97.77 [a] (1.228)
T_{14} - Imidacloprid 0.025 per cent	41.67 [a] (0.608)	97.23 [a] (1.377)	97.23 [cd] (1.377)	97.23 [a] (1.377)
T_{15} - Control	75.00 [a] (1.134)	91.67 [a] (1.30)	100.00 [d] (1.426)	100.00 [a] (1.426)

Arc sine transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT

Table 11. Effect of different treatments on disease incidence during second season (January to April, 2002)

Treatments	Mean per cent disease incidence			
	15 days after first spray	15 days after second spray	15 days after third spray	15 days after fourth spray
T_1 - Carbosulfan 50 g kg^{-1} seed	0.00 [a] (0.145)	29.16 [a] (0.554)	72.19 [d] (1.02)	83.23 [cd] (1.158)
T_2 - Phorate 10 g per pit	2.78 [a] (0.194)	41.65 [a] (0.698)	66.65 [d] (0.966)	91.63 [d] (1.277)
T_3 - Acephate 0.05 per cent	0.00 [a] (0.145)	36.10 [a] (0.635)	72.21 [d] (1.055)	87.50 [d] (1.30)
T_4 - Dicofol 0.05 per cent	2.78 [a] (0.194)	33.34 [a] (0.572)	58.32 [bcd] (0.879)	80.54 [cd] (1.143)
T_5 - Neem oil - garlic emulsion 2 per cent	2.78 [a] (0.194)	41.66 [a] (0.698)	55.66 [b] (0.731)	80.54 [cd] (1.143)
T_6 - Neem seed kernel extract 5 per cent	2.78 [a] (0.194)	38.87 [a] (0.664)	61.10 [d] (0.942)	80.54 [cd] (1.143)
T_7 - *Thespesia populnea* fresh leaf extract 10 per cent	2.78 [a] (0.194)	36.11 [a] (0.641)	66.64 [cd] (0.893)	80.54 [cd] (1.143)
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	2.78 [a] (0.194)	38.87 [a] (0.650)	63.86 [d] (0.955)	87.47 [cd] (1.235)
T_9 - *Apis melliphica* 30 potency	0.00 [a] (0.145)	41.66 [a] (0.698)	58.31 [bcd] (0.879)	74.99 [bc] (1.020)
T_{10} - *Arsenicum album* 30 potency	0.00 [a] (0.145)	44.43 [a] (0.726)	69.42 [d] (1.006)	86.10 [cd] (1.208)
T_{11} - Infected plant extract 10^{-3} dilution	8.33 [a] (0.271)	52.76 [a] (0.828)	63.86 [d] (0.949)	86.09 [cd] (1.219)
T_{12} - Coconut toddy 1:3 dilution	0.00 [a] (0.145)	58.32 [a] (0.879)	77.75 [d] (1.085)	91.64 [d] (1.284)
T_{13} - Coconut vinegar 1 per cent	0.00 [a] (0.145)	30.54 [a] (0.564)	61.00 [cd] (0.901)	82.32 [cd] (1.177)
T_{14} - Imidacloprid 0.025 per cent	0.00 [a] (0.145)	19.44 [a] (0.443)	33.32 [bc] (0.615)	58.33 [a] (0.842)
T_{15} - Control	8.33 [a] (0.271)	55.53 [a] (0.865)	72.21 [d] (1.055)	88.87 [d] (1.334)

Arc sine transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT

Control plot (T_0)

Imidacloprid treated plot (T_{14})

Plate 8. Effect of treatments on BGDMV infection

fourth spray and T_{14} (Imidacloprid) recorded the lowest disease severity of 26 per cent. It was on par with T_1 (Carbosulfan), T_2 (Phorate), T_4 (Dicofol), T_5 (Neem oil garlic emulsion), T_{10} (*Arsenicum album*), T_{11} (infected plant extract) and T_{13} (Coconut vinegar).

Overall performance of different treatments during two seasons at 15 days after last spray is presented in Table 16 and Fig. 6. There was significant difference among treatments and the imidacloprid treatment (T_{14}) recorded the lowest disease severity of 37.84 per cent. This was on par with coconut vinegar treatment (T_{13}) which recorded a mean per cent severity of 42.13.

During the first season at 15 days after last spray, mean per cent disease severity for the treatments ranged between 49.67 and 76.90. For the second season, it ranged between 26 and 62.67. Disease severity was less during the second season when compared to that of the first season.

4.8.3 Effect of different treatments on coefficient of infection

Results presented in Tables 14 and 15 showed that none of the treatments could prevent the infection of BGDMV. Coefficient of infection progressively increased with age of the crop inspite of all treatments.

In the first season, there was no significant difference among treatments at 15 days after each sprays.

In the second season (Table 15), no significant difference could be noticed among the treatments after first three sprays. At 15 days after the fourth spray, treatments differed significantly and imidacloprid treatment (T_{14}) showed the lowest coefficient of infection of 14.59 per cent. This was on par with T_{13} - coconut vinegar.

Overall performance of different treatments during two seasons at 15 days after last spray is presented in Table 16 and Fig. 6. There was significant difference between treatments and T_{14} and T_{13} were found to be superior over all treatments and recorded the mean coefficient of infection of 35.84 and 36.31 respectively. While control plots recorded the highest coefficient of infection of 67.73 per cent.

Table 12. Effect of different treatments on disease severity during first season (March to June, 2001)

Treatments	Mean per cent disease severity			
	15 days after first spray	15 days after second spray	15 days after third spray	15 days after fourth spray
T_1 - Carbosulfan 50 g kg^{-1} seed	29.10[a] (5.338)	50.40[a]	61.77[a]	72.27[a]
T_2 - Phorate 10 g per pit	22.67[a] (4.745)	46.57[a]	54.13[a]	64.67[a]
T_3 - Acephate 0.05 per cent	31.67[a] (5.541)	38.07[a]	60.27[a]	63.33[a]
T_4 - Dicofol 0.05 per cent	29.60[a] (5.161)	32.07[a]	55.73[a]	63.67[a]
T_5 - Neem oil - garlic emulsion 2 per cent	28.43[a] (5.186)	44.53[a]	59.27[a]	76.90[a]
T_6 - Neem seed kernel extract 5 per cent	17.77[a] (3.297)	38.57[a]	49.93[a]	64.67[a]
T_7 - *Thespesia populnea* fresh leaf extract 10 per cent	24.67[a] (4.954)	49.33[a]	56.33[a]	58.07[a]
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	19.67[a] (3.618)	43.30[a]	54.87[a]	59.43[a]
T_9 - *Apis melliphica* 30 potency	16.00[a] (3.248)	58.33[a]	58.53[a]	59.10[a]
T_{10} - *Arsenicum album* 30 potency	15.60[a] (3.877)	28.67[a]	48.33[a]	75.33[a]
T_{11} - Infected plant extract 10^{-3} dilution	26.33[a] (3.833)	47.07[a]	56.33[a]	61.33[a]
T_{12} - Coconut toddy 1:3 dilution	12.00[a] (2.824)	34.67[a]	46.90[a]	49.67[a]
T_{13} - Coconut vinegar 1 per cent	10.00[a] (2.545)	36.20[a]	48.73[a]	51.83[a]
T_{14} - Imidacloprid 0.025 per cent	25.33[a] (4.926)	37.47[a]	52.00[a]	52.90[a]
T_{15} - Control	25.37[a] (5.015)	55.50[a]	63.27[a]	74.50[a]

$\sqrt{x + 0.5}$ transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT

Table 13. Effect of different treatments on disease severity during second season (January to April, 2002)

Treatments	Mean per cent disease severity			
	15 days after first spray	15 days after second spray	15 days after third spray	15 days after fourth spray
T_1 - Carbosulfan 50 g kg^{-1} seed	0.00[a] (0.318)	22.77[a]	30.56[a]	35.67[ab]
T_2 - Phorate 10 g per pit	2.67[a] (0.513)	28.00[a]	42.78[a]	43.00[abc]
T_3 - Acephate 0.05 per cent	0.00[a] (0.318)	22.22[a]	43.33[a]	62.67[cd]
T_4 - Dicofol 0.05 per cent	4.00[a] (0.572)	28.67[a]	34.44[a]	44.56[abc]
T_5 - Neem oil - garlic emulsion 2 per cent	6.67[a] (0.646)	25.89[a]	30.44[a]	46.00[abcd]
T_6 - Neem seed kernel extract 5 per cent	4.00[a] (0.572)	25.67[a]	42.22[a]	50.56[bcd]
T_7 - *Thespesia populnea* fresh leaf extract 10 per cent	4.00[a] (0.572)	28.45[a]	33.78[a]	48.89[bcd]
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	5.33[a] (0.613)	37.33[a]	42.55[a]	56.89[bcd]
T_9 - *Apis melliphica* 30 potency	0.00[a] (0.318)	28.56[a]	35.89[a]	53.33[bcd]
T_{10} - *Arsenicum album* 30 potency	0.00[a] (0.318)	32.33[a]	35.00[a]	41.33[abc]
T_{11} - Infected plant extract 10^{-3} dilution	9.33[a] (0.694)	27.67[a]	35.67[a]	44.89[abc]
T_{12} - Coconut toddy 1:3 dilution	0.00[a] (0.318)	27.33[a]	28.00[a]	54.33[bcd]
T_{13} - Coconut vinegar 1 per cent	0.00[a] (0.318)	13.78[a]	28.78[a]	35.52[ab]
T_{14} - Imidacloprid 0.025 per cent	0.00[a] (0.318)	16.00[a]	19.55[a]	26.00[a]
T_{15} - Control	9.33[a] (0.694)	45.78[a]	46.00[a]	67.58[b]

Logarithmic transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT

Table 14. Effect of different treatments on coefficient of infection during first season (March to June, 2001)

Treatments	Mean per cent coefficient of infection			
	15 days after first spray	15 days after second spray	15 days after third spray	15 days after fourth spray
T_1 - Carbosulfan 50 g kg^{-1} seed	27.69[a] (5.109)	44.33[a] (6.479)	61.77[a] (7.82)	74.07[a]
T_2 - Phorate 10 g per pit	9.14[a] (3.02)	44.66[a] (6.654)	53.59[a] (7.31)	64.67[a]
T_3 - Acephate 0.05 per cent	19.83[a] (4.45)	30.72[a] (5.464)	49.88[a] (7.054)	63.33[a]
T_4 - Dicofol 0.05 per cent	14.09[a] (3.477)	23.38[a] (4.821)	46.32[a] (6.684)	61.18[a]
T_5 - Neem oil - garlic emulsion 2 per cent	15.69[a] (3.752)	41.94[a] (6.464)	56.17[a] (7.458)	74.65[a]
T_6 - Neem seed kernel extract 5 per cent	6.74[a] (2.59)	35.80[a] (5.938)	48.20[a] (6.924)	64.67[a]
T_7 - *Thespesia populnea* fresh leaf extract 10 per cent	7.83[a] (2.724)	46.83[a] (6.822)	53.30[a] (7.287)	55.67[a]
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	9.42[a] (3.06)	61.18[a] (7.634)	42.89[a] (6.193)	54.70[a]
T_9 - *Apis melliphica* 30 potency	7.14[a] (2.146)	44.83[a] (6.623)	60.46[a] (7.774)	63.40[a]
T_{10} - *Arsenicum album* 30 potency	4.49[a] (2.103)	14.98[a] (3.826)	34.97[a] (7.246)	53.49[a]
T_{11} - Infected plant extract 10^{-3} dilution	20.07[a] (4.08)	46.13[a] (6.715)	55.10[a] (7.391)	61.33[a]
T_{12} - Coconut toddy 1:3 dilution	4.22[a] (2.05)	31.73[a] (5.376)	43.57[a] (6.654)	46.57[a]
T_{13} - Coconut vinegar 1 per cent	6.11[a] (2.47)	43.10[a] (6.539)	53.30[a] (7.287)	50.61[a]
T_{14} - Imidacloprid 0.025 per cent	11.67[a] (3.235)	55.74[a] (7.465)	60.28[a] (7.751)	51.44[a]
T_{15} - Control	18.30[a] (4.259)	52.50[a] (7.187)	63.30[a] (7.93)	74.50[a]

$\sqrt{x} + 0.5$ transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT

Table 15. Effect of different treatments on coefficient of infection during second season (January to April, 2002)

Treatments	Mean per cent coefficient of infection			
	15 days after first spray	15 days after second spray	15 days after third spray	15 days after fourth spray
T_1 - Carbosulfan 50 g kg^{-1} seed	0.00 [a] (0.707)	6.99 [a] (2.72)	22.14 [a]	29.73 [bc]
T_2 - Phorate 10 g per pit	0.22 [a] (0.832)	13.33 [a] (3.71)	26.30 [a]	39.40 [bc]
T_3 - Acephate 0.05 per cent	0.00 [a] (0.707)	8.30 [a] (2.92)	32.52 [a]	57.63 [cd]
T_4 - Dicofol 0.05 per cent	0.33 [a] (0.880)	15.81 [a] (4.04)	21.29 [a]	37.17 [bc]
T_5 - Neem oil - garlic emulsion 2 per cent	0.55 [a] (0.961)	12.63 [a] (3.62)	36.25 [a]	37.64 [bc]
T_6 - Neem seed kernel extract 5 per cent	0.33 [a] (0.880)	15.79 [a] (4.02)	26.22 [a]	41.56 [bc]
T_7 - *Thespesia populnea* fresh leaf extract 10 per cent	0.33 [a] (0.880)	11.40 [a] (3.45)	21.40 [a]	42.29 [bc]
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	0.33 [a] (0.880)	17.18 [a] (4.20)	28.43 [a]	50.35 [bcd]
T_9 - *Apis melliphica* 30 potency	0.00 [a] (0.707)	15.71 [a] (4.02)	22.68 [a]	39.99 [bcd]
T_{10} - *Arsenicum album* 30 potency	0.00 [a] (0.707)	17.16 [a] (4.18)	22.21 [a]	36.07 [bc]
T_{11} - Infected plant extract 10^{-3} dilution	2.33 [a] (1.384)	14.59 [a] (3.86)	24.25 [a]	38.35 [bc]
T_{12} - Coconut toddy 1:3 dilution	0.00 [a] (0.707)	22.17 [a] (4.05)	29.55 [a]	50.55 [bcd]
T_{13} - Coconut vinegar 1 per cent	0.00 [a] (0.707)	5.25 [a] (2.18)	18.86 [a]	29.36 [ab]
T_{14} - Imidacloprid 0.025 per cent	0.00 [a] (0.707)	6.13 [a] (2.38)	10.81 [a]	14.59 [a]
T_{15} - Control	2.33 [a] (1.384)	27.20 [a] (5.16)	34.18 [a]	64.29 [d]

$\sqrt{x} + 0.5$ transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT

During the first season at 15 days after last spray, mean per cent coefficient of infection for the treatments ranged between 46.57 and 74.65. For the second season, it ranged between 14.59 and 57.63. Disease severity was less during the second season when compared to that of the first season.

4.8.4 Yield of bitter gourd

Effect of different treatments on yield of bitter gourd was also recorded during two cropping seasons and the results are presented in Table 17. There was no significant difference between treatments in yield during both seasons.

Effect of different treatments on mean yield of bitter gourd during both seasons is also presented in Table 17 and Fig. 7. There was significant difference between treatments. Imidacloprid treated plots (T_{14}) recorded the highest yield of 2.20 kg $plot^{-1}$ and was on par with T_7.

4.8.5 Incidence of pests on bitter gourd

Effect of different treatments on the natural incidence of whitefly (*Bemisia tabaci*) was also observed in two seasons and the results are presented in Tables 18 and 19.

During the first season at 15 days after second spray, treatments differed significantly. T_1, T_2, T_3, T_4, T_{13} and T_{14} were found effective in reducing whitefly population.

During the second season also at 15 days after second spray, there was significant difference between treatments. All treatments except T_1, T_6 and T_{11} were effective in reducing white fly population. Control plots recorded the highest incidence of white flies.

Table 16. Effect of different treatments on disease incidence, disease severity and coefficient of infection of BGDMV

Treatments	15 days after last spray		
	Mean per cent disease incidence	Mean per cent disease severity	Mean per cent coefficient of infection
T_1 - Carbosulfan @ 50 g kg^{-1} seed	91.65^{bcd}	55.97^{cd}	51.90^{bc}
T_2 - Phorate 10 g per pit	95.81^{bcd}	53.83^{bcd}	50.37^{bc}
T_3 - Acephate 0.05 per cent	95.83^{cd}	63.00^{cd}	58.82^{c}
T_4 - Dicofol 0.05 per cent	87.50^{bcd}	54.11^{bcd}	52.49^{bc}
T_5 - Neem oil - garlic emulsion 2 per cent	88.88^{bcd}	60.46^{cd}	56.15^{bc}
T_6 - Neem seed kernel extract 5 per cent	90.27^{bcd}	56.90^{cd}	56.45^{bc}
T_7 - *Thespesia populnea* leaf extract 10 per cent	87.49^{bcd}	52.15^{bc}	52.15^{bc}
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	90.26^{bcd}	56.95^{cd}	52.52^{bc}
T_9 - *Apis melliphica* 30 potency	83.06^{ab}	56.22^{cd}	56.99^{bc}
T_{10} - *Arsenicum album* 30 potency	93.05^{bcd}	51.66^{bc}	50.03^{bc}
T_{11} - Infected plant extract 10^{-3} dilution	93.04^{bcd}	53.11^{bc}	48.17^{ab}
T_{12} - Coconut toddy 1:3 dilution	92.49^{bcd}	50.61^{bc}	47.56^{b}
T_{13} - Coconut vinegar 1 per cent	85.54^{abc}	42.13^{ab}	36.31^{a}
T_{14} - Imidacloprid 0.025 per cent	76.38^{a}	37.84^{a}	35.84^{a}
T_{15} - Control	97.21^{d}	71.00^{d}	67.73^{d}

Figures followed by same letters do not differ significantly according to DMRT

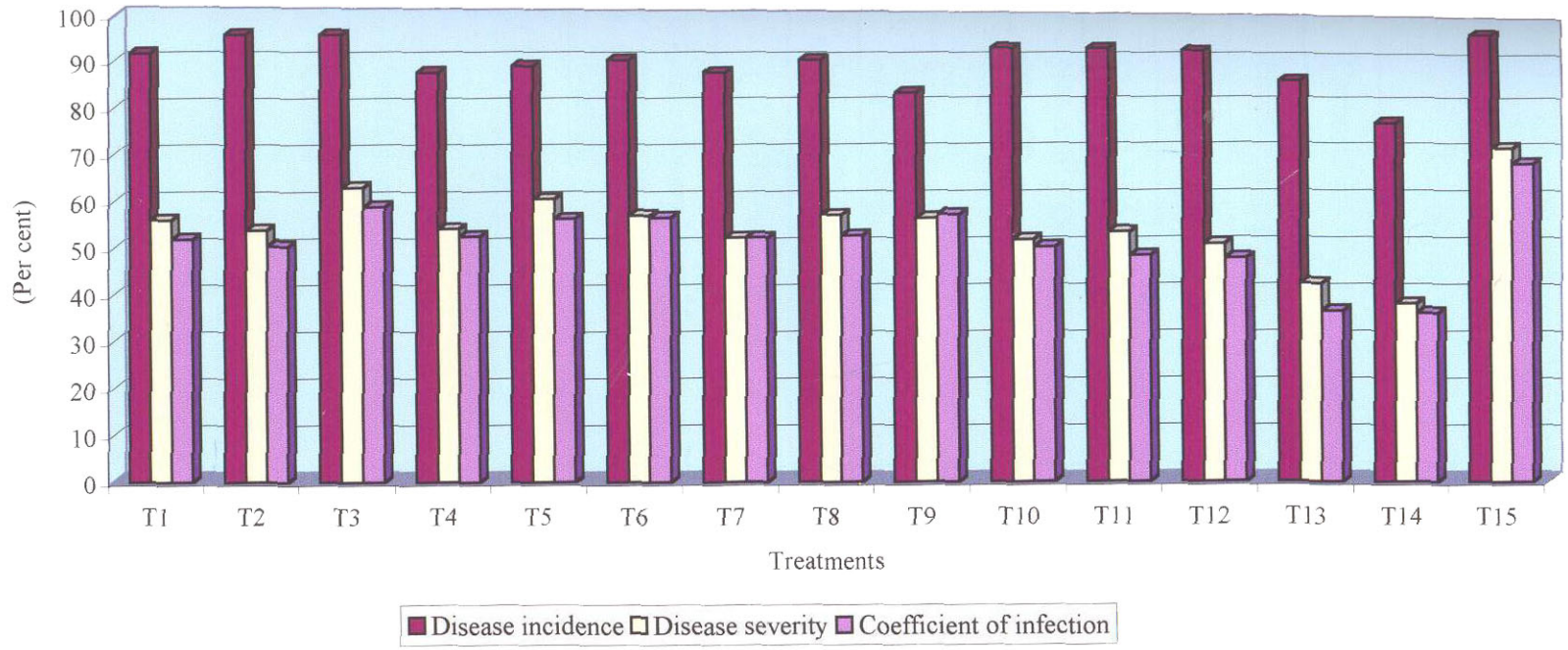


Fig. 6. Effect of different treatments on disease incidence, disease severity and coefficient of infection of BGDMV (15 days after last spray)

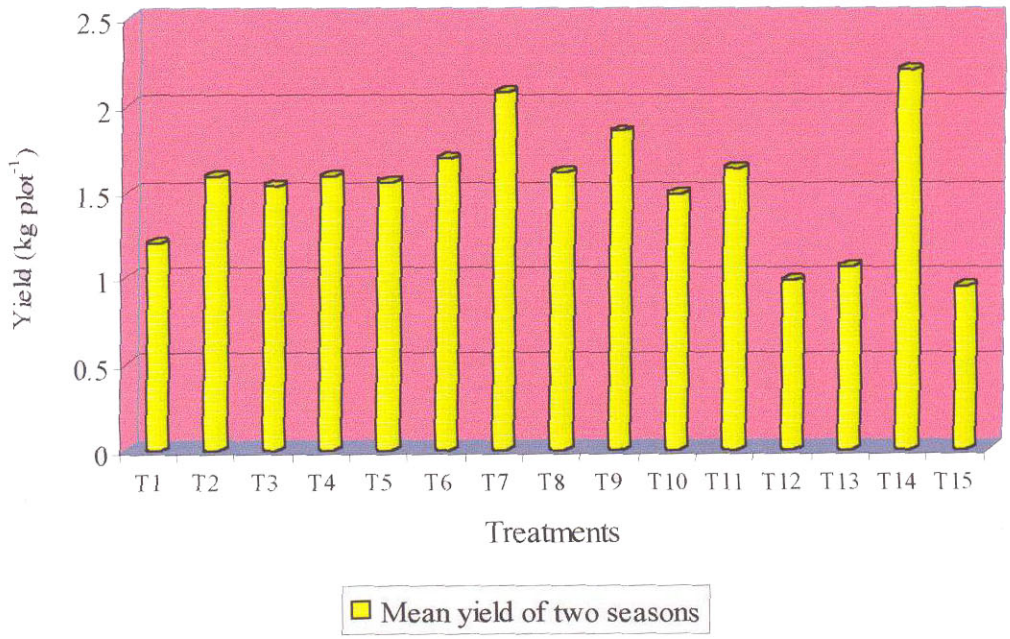
T_1 - Carbosulfan 50 g kg^{-1} seed	T_6 - Neem seed kernel extract 5 per cent	T_{11} - Infected plant extract 10^{-3} dilution
T_2 - Phorate 10 g per pit	T_7 - *Thespesia populnea* leaf extract 10 per cent	T_{12} - Coconut toddy 1:3 dilution
T_3 - Acephate 0.05 per cent	T_8 - *Clerodendron infortunatum* leaf extract 10 per cent	T_{13} - Coconut vinegar 1 per cent
T_4 - Dicofol 0.05 per cent	T_9 - *Apis melliphica* 30 potency	T_{14} - Imidacloprid 2.5 per cent
T_5 - Neem oil - garlic emulsion 2 per cent	T_{10} - *Arsenicum album* 30 potency	T_{15} - Control

Table 17. Effect of different treatments on yield of bitter gourd (Plot size - 10.24 m^2)

Treatments	Yield (kg $plot^{-1}$)		
	March to June, 2001	January to April, 2002	Mean yield for two seasons
T_1 - Carbosulfan 50 g kg^{-1} seed	0.93 [a] (0.96)	1.36 [a] (1.17)	1.20[bc]
T_2 - Phorate 10 g per pit	2.03 [a] (1.42)	1.15 [a] (1.07)	1.59[abc]
T_3 - Acephate 0.05 per cent	1.51 [a] (1.23)	1.55 [a] (1.24)	1.53[abc]
T_4 - Dicofol 0.05 per cent	1.39 [a] (1.18)	1.78 [a] (1.33)	1.59[abc]
T_5 - Neem oil - garlic emulsion 2 per cent	1.66 [a] (1.29)	1.46 [a] (1.21)	1.56[abc]
T_6 - Neem seed kernel extract 5 per cent	1.79 [a] (1.34)	1.60 [a] (1.26)	1.69[abc]
T_7 - *Thespesia populnea* leaf extract 10 per cent	2.15 [a] (1.47)	1.83 [a] (1.35)	2.07[a]
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	1.43 [a] (1.19)	1.79 [a] (1.34)	1.61[abc]
T_9 - *Apis melliphica* 30 potency	1.22 [a] (1.10)	2.58 [a] (1.60)	1.85[ab]
T_{10} - *Arsenicum album* 30 potency	1.22 [a] (1.28)	1.74 [a] (1.32)	1.49[abc]
T_{11} - Infected plant extract 10^{-3} dilution	1.66 [a] (0.95)	1.34 [a] (1.15)	1.63[abc]
T_{12} - Coconut toddy 1:3 dilution	0.96 [a] (1.06)	0.99 [a] (0.99)	0.98[c]
T_{13} - Coconut vinegar 1 per cent	1.12 [a] (1.48)	1.00 [a] (1.00)	1.06[bc]
T_{14} - Imidacloprid 0.025 per cent	2.19 [a] (1.00)	2.26 [a] (1.50)	2.20[a]
T_{15} - Control	1.00 [a] (1.00)	0.89 [a] (0.94)	0.94[c]

$\sqrt{x+1}$ transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT



T_1 - Carbosulfan 50 g kg^{-1} seed
T_2 - Phorate 10 g per pit
T_3 - Acephate 0.05 per cent
T_4 - *Dicofol* 0.05 per cent
T_5 - Neem oil - garlic emulsion 2 per cent
T_6 - Neem seed kernel extract 5 per cent
T_7 - *Thespesia populnea* leaf extract 10 per cent
T_8 - *Clerodendron infortunatum* leaf extract 10 per cent
T_9 - *Apis melliphica* 30 potency
T_{10} - *Arsenicum album* 30 potency
T_{11} - Infected plant extract 10^{-3} dilution
T_{12} - Coconut toddy 1:3 dilution
T_{13} - Coconut vinegar 1 per cent
T_{14} - Imidacloprid 2.5 per cent
T_{15} - Control

Fig. 7. Effect of different treatments on yield of bitter gourd (Plot size - 10.24 sq.m)

Table 18. Effect of different treatments on whitefly population during first season (March to June, 2001)

Treatments	Mean whitefly population (4 leaves plant^{-1})				
	Before spray	15 days after first spray	15 days after second spray	15 days after third spray	15 days after fourth spray
T_1 - Carbosulfan 50 g kg^{-1} seed	0.33 ^{ab} (1.13)	0.33 ^{a} (1.13)	0 ^{a} (1.00)	0.33 ^{a} (1.13)	0.33 ^{a} (1.13)
T_2 - Phorate 10 g per pit	1.67 ^{cd} (1.62)	0 ^{a} (1.00)	0 ^{a} (1.00)	0 ^{a} (1.00)	0 ^{a} (1.00)
T_3 - Acephate 0.05 per cent	0.33 ^{ab} (1.13)	0.67 ^{a} (1.27)	0 ^{a} (1.00)	0 ^{a} (1.00)	0 ^{a} (1.00)
T_4 - Dicofol 0.05 per cent	1.33 ^{bcd} (1.51)	0 ^{a} (1.00)	0 ^{a} (1.00)	0.33 ^{a} (1.13)	0 ^{a} (1.00)
T_5 - Neem oil - garlic emulsion 2 per cent	2.00 ^{cd} (1.71)	0.67 ^{a} (1.27)	1.00 ^{d} (1.37)	0 ^{a} (1.00)	0 ^{a} (1.00)
T_6 - Neem seed kernel extract 5 per cent	1.00 ^{cd} (1.38)	0.67 ^{a} (1.27)	0.67 ^{c} (1.27)	0 ^{a} (1.00)	0 ^{a} (1.00)
T_7 - *Thespesia populnea* leaf extract 10 per cent	0.67 ^{cd} (1.67)	0.33 ^{a} (1.13)	1.00 ^{d} (1.37)	1.00 ^{a} (1.37)	0.67 ^{a} (1.27)
T_8 - *Clerodendron infortunatum* leaf extract 10 per cent	0 ^{a} (1.00)	0.67 ^{a} (1.27)	0.33 ^{b} (1.13)	0.33 ^{a} (1.13)	0.33 ^{a} (1.13)
T_9 - *Apis melliphica* 30 potency	0.67 ^{cd} (1.27)	1.00 ^{a} (1.37)	1.00 ^{d} (1.37)	1.00 ^{a} (1.37)	0.33 ^{a} (1.13)
T_{10} - *Arsenicum album* 30 potency	0 ^{a} (1.00)	0 ^{a} (1.00)	1.00 ^{d} (1.37)	0.67 ^{a} (1.27)	0.67 ^{a} (1.27)
T_{11} - Infected plant extract 10^{-3} dilution	0 ^{a} (1.00)	0.67 ^{a} (1.27)	0.33 ^{b} (1.13)	0 ^{a} (1.00)	0.33 ^{a} (1.13)
T_{12} - Coconut toddy 1:3 dilution	0 ^{a} (1.00)	0.33 ^{a} (1.13)	0.67 ^{c} (1.27)	0 ^{a} (1.00)	0 ^{a} (1.00)
T_{13} - Coconut vinegar 1 per cent	0.67 ^{cd} (1.27)	0 ^{a} (1.00)	0 ^{a} (1.00)	0 ^{a} (1.00)	0.67 ^{a} (1.27)
T_{14} - Imidacloprid 0.025 per cent	1.00 ^{bc} (1.4)	0 ^{a} (1.00)	0 ^{a} (1.00)	1.33 ^{a} (1.47)	0 ^{a} (1.00)
T_{15} - Control	2.67 ^{d} (1.91)	0.67 ^{a} (1.27)	0.67 ^{c} (1.27)	0.33 ^{a} (1.13)	0.33 ^{a} (1.13)

$\sqrt{x+1}$ transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT

Table 19. Effect of different treatments on whitefly population during second season (January to April, 2002)

Treatments	Mean whitefly population (4 leaves plant^{-1})				
	Before spray	15 days after first spray	15 days after second spray	15 days after third spray	15 days after fourth spray
T_1 - Carbosulfan 50 g kg^{-1} seed	0^{a} (1.00)	1.67^{e} (1.57)	0.33^{b} (1.13)	0.33^{a} (1.13)	0.33^{a} (1.13)
T_2 - Phorate 10 g per pit	1.00^{c} (1.37)	1.33^{de} (1.50)	0^{a} (1.00)	0^{a} (1.00)	0.33^{a} (1.13)
T_3 - Acephate 0.05 per cent	0.67^{b} (1.27)	1.67^{e} (1.57)	0^{a} (1.00)	0^{a} (1.00)	0.33^{a} (1.13)
T_4 - Dicofol 0.05 per cent	0^{a} (1.00)	0.67^{bc} (1.27)	0^{a} (1.00)	0.33^{a} (1.13)	0.33^{a} (1.13)
T_5 - Neem oil - garlic emulsion 2 per cent	1.00^{c} (1.37)	0.67^{bc} (1.27)	0^{a} (1.00)	0^{a} (1.00)	0.33^{a} (1.13)
T_6 - Neem seed kernel extract 5 per cent	0.67^{b} (1.27)	1.00^{cd} (1.37)	0.33^{b} (1.13)	0^{a} (1.00)	0^{a} (1.00)
T_7 - *Thespesia populnea* fresh leaf extract 10 per cent	0.67^{b} (1.27)	0.33^{ab} (1.13)	0^{a} (1.00)	0^{a} (1.00)	0^{a} (1.00)
T_8 - *Clerodendron infortunatum* fresh leaf extract 10 per cent	1.00^{c} (1.37)	0^{a} (1.00)	0^{a} (1.13)	0.33^{a} (1.13)	0.33^{a} (1.13)
T_9 - *Apis melliphica* 30 potency	0.67^{b} (1.27)	0.67^{bc} (1.27)	0^{a} (1.13)	0^{a} (1.00)	0^{a} (1.00)
T_{10} - *Arsenicum album* 30 potency	0.67^{b} (1.27)	1.00^{cd} (1.37)	0^{a} (1.13)	0^{a} (1.00)	0^{a} (1.00)
T_{11} - Infected plant extract 10^{-3} dilution	1.00^{c} (1.37)	0.67^{bc} (1.27)	0.33^{b} (1.13)	0^{a} (1.00)	0^{a} (1.00)
T_{12} - Coconut toddy 1:3 dilution	0.67^{b} (1.27)	0^{a} (1.00)	0^{a} (1.13)	0.67^{a} (1.27)	0^{a} (1.00)
T_{13} - Coconut vinegar 1 per cent	1.00^{c} (1.37)	0^{a} (1.00)	0^{a} (1.13)	0.33^{a} (1.13)	0^{a} (1.00)
T_{14} - Imidacloprid 0.025 per cent	0.33^{b} (1.13)	1.67^{e} (1.47)	0^{a} (1.130	0^{a} (1.00)	0^{a} (1.00)
T_{15} - Control	2.60^{d} (1.90)	2.67^{f} (1.93)	1.67^{c} (1.47)	0^{a} (1.00)	0.33^{a} (1.13)

$\sqrt{x+1}$ transformed values are given in parentheses

Figures followed by same letters do not differ significantly according to DMRT

4.8.6 Effect of weather parameters on BGDMV infection

Correlation between mean per cent disease severity, disease incidence and coefficient of infection with weather factors was studied for March to June, 2001 and January to April, 2002.

4.8.6.1 During first season: March to June, 2001

Correlation between mean per cent disease incidence, disease severity and coefficient of infection of BGDMV with weather parameters during first season (Table 20) is presented in Table 21.

Mean per cent disease incidence was significantly and negatively correlated with maximum temperature (r = -0.377) and positively correlated with relative humidity afternoon (r = 0.286) and with total rainfall (r = 0.287).

High significant negative correlation (r = -0.6880) was obtained between per cent disease severity and the maximum temperature. But it was significantly and positively correlated with relative humidity forenoon (r = 0.288), relative humidity afternoon (r = 0.487), total rainfall (r = 0.584) and with number of rainy days (r = 0.382).

There was significant negative correlation between coefficient of infection and maximum temperature (r = -0.510) while it was significantly and positively correlated with relative humidity afternoon (r = 0.364), total rainfall (r = 0.453) and number of rainy days (r = 0.296).

4.8.6.2 During second season: January to April, 2002

Correlation between mean per cent disease incidence, disease severity and coefficient of infection of BGDMV with weather parameters during second season (Table 22) is presented in Table 23.

There was high significant and positive correlation between mean per cent disease severity and maximum temperature (r = 0.712). The same relationship was

Table 20. Weather parameters during first season (March to June, 2001)

Week	Period	Mean temperature (°C)		Mean relative humidity (%)		Total rainfall (mm)	Number of rainy days
		Maximum	Minimum	Forenoon	Afternoon		
I	March 28 - April 3	34.68	26.18	76.14	51.57	0	0
II	April 4 - April 10	33.35	24.46	86.71	59.57	29.80	4.00
III	April 11 - April 17	32.47	23.61	86.00	62.71	10.60	3.00
IV	April 18 - April 24	33.42	25.00	80.57	60.28	39.60	3.00
V	April 25 - May 1	33.86	25.25	86.71	71.57	37.00	3.00
VI	May 2 - May 8	33.14	25.00	79.43	63.00	35.00	2.00
VII	May 9 - May 15	33.21	25.64	79.71	61.85	0	0
VIII	May 16 - May 22	32.93	25.64	86.42	61.71	0	0
IX	May 23 - May 30	30.84	23.85	91.57	70.00	122.20	6.00
X	May 31 - June 6	31.46	23.85	88.57	69.14	86.20	5.00
XI	June 7 - June 13	29.22	22.64	85.20	68.85	115.40	7.00

Table 21. Correlation between disease incidence, disease severity, coefficient of infection of BGDMV and weather parameters during first season (March to June, 2001)

Parameter	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (F.N.) (%)	Relative humidity (A.N.) (%)	Total rainfall (mm)	No. of rainy days
Disease incidence	-0.377**	0.177	0.083	0.286*	0.287*	0.138
Disease severity	-0.688**	0.062	0.288*	0.487**	0.584**	0.382**
Co-efficient of infection	-0.510**	0.051	0.228	0.364**	0.453**	0.296*

* - Significant at 5% level

** - Significant at 1% level

also noticed with relative humidity forenoon (r = 0.778). Negative significant correlation was obtained with relative humidity afternoon (r = -0.322).

There was high significant positive correlation between per cent disease incidence and maximum temperature (r = 0.724) and with minimum temperature (r = 0.393). It also showed a significant positive correlation with relative humidity forenoon (r = 0.862). But disease incidence was significantly and negatively correlated with relative humidity afternoon (r = -0.349).

Coefficient of infection and maximum temperature were positively and significantly correlated (r = 0.588). It also showed highly significant positive correlation with relative humidity (r = 0.730). Coefficient of infection and minimum temperature were significantly and positively correlated (r = 0.400).

Table 22. Weather parameters during second season (January to April, 2002)

Week	Period	Mean temperature (°C)		Mean relative humidity (%)		Total rainfall (mm)	Number of rainy days
		Maximum	Minimum	Forenoon	Afternoon		
I	January 6 - Jan. 12	33.89	22.58	84.42	51.85	0	0
II	Jan. 13 - Jan. 19	32.25	20.25	78.50	53.50	0	0
III	Jan. 20 - Jan. 26	33.00	22.50	88.25	61.50	0	0
IV	Jan. 27 - Feb. 2	31.00	22.50	90.00	68.50	69.50	5.00
V	Feb. 3 - Feb. 9	33.40	22.08	84.44	52.33	0	0
VI	Feb. 10 - Feb. 16	34.00	19.50	92.50	63.50	2.20	1.00
VII	Feb. 17 - Feb. 23	34.50	20.50	89.40	52.50	3.20	1.00
VIII	Feb. 24 - March 2	34.00	22.00	91.30	48.50	0	0
IX	March 3 - March 9	34.50	23.50	92.00	49.00	0	0
X	March 10 - March 16	35.00	22.50	83.00	50.50	13.60	1.00
XI	March 17 - March 23	34.50	23.00	84.00	51.50	0	0

Table 23. Correlation between disease incidence, disease severity, coefficient of infection of BGDMV and weather parameters during second season (January to April, 2002)

Parameter	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (F.N.) (%)	Relative humidity (A.N.) (%)	Total rainfall (mm)	No. of rainy days
Disease incidence	0.724**	0.393**	0.862**	-0.349*	-0.175	-0.042
Disease severity	0.719**	0.309*	0.778**	-0.322*	-0.171	0.026
Co-efficient of infection	0.588**	0.400**	0.730**	-0.240	-0.113	-0.021

* - Significant at 5% level

** - Significant at 1% level

Discussion

5. DISCUSSION

Bitter gourd, *Momordica charantia* L. is the most important cucurbitaceous vegetable in Kerala. Among the various diseases affecting the crop, 'Distortion Mosaic' disease incited by virus is known to cause serious damage taking a heavy toll of the crop. It is named as Leaf distortion virus (Giri and Misra, 1986), Bitter gourd Mosaic Virus (BGMV) (Mathew *et al.*, 1991) and Distortion mosaic virus (Pandey *et al.*, 1998 and Arunachalam, 2002). Studies conducted by these research workers have provided some useful informations on certain aspects. But on the whole, work on BGDMV has been rather meager and sketchy.

Moreover, the earlier studies conducted on bitter gourd mosaic disease with regard to the transmission and etiology are found contradictory and the informations on the management of this disease are lacking. Hence, the present study is a serious attempt to enter into certain aspects of the disease with emphasis on transmission, etiology, characterisation of virus, identification and also the role of botanicals, homoeopathic drugs and insecticides on the management of this devastating disease of bitter gourd.

Transmission is an important experimental tool to establish the etiology of viral diseases. With this view, an attempt was made to understand the mode of transmission of the disease through various means such as sap, seed and insect vectors.

Transmission studies conducted using the infected sap prepared in different buffers did not produce any infection of BGDMV and the virus was found to be non transmissible through sap. This supports the fact that all whitefly transmitted viruses are non transmissible through sap. Nagarajan and Ramakrishnan (1971a) and Mathew *et al.* (1991) also failed to transmit the bitter gourd mosaic virus through infected sap prepared in various concentrations and types of phosphate buffers. However, contradictory to these results, Purushothaman (1994) and Pandey *et al.* (1998) reported the transmission of bitter gourd mosaic and distortion mosaic virus through infected sap.

The next attempt for the transmission of virus through seed also showed negative result as none of the seedlings raised from the seeds from infected plants produced mosaic symptoms. However, the seeds from infected plants were small, discoloured and were morphologically unfit for seed purpose. Moreover, the germination per cent was very low i.e. 45 per cent, whereas healthy seeds showed 85 per cent germination. The seedlings raised from infected seeds were pale, lanky and non vigorous. Thus the present study indicate that eventhough the virus is not transmitted through seeds, use of seeds from infected plants for cultivation is not advisable as it reduces germination and may not produce vigorous seedlings. Similar to these findings, Nagarajan and Ramakrishnan (1971a), Mathew *et al.* (1991) also observed the non transmission of bitter gourd mosaic virus through seeds. Earlier reports also provided the information that whitefly transmitted viruses are neither sap nor seed transmissible which is also supportive to the present findings. Whereas, Giri and Misra (1986) and Pandey *et al.* (1998) in their studies observed transmission of leaf distortion virus and bitter gourd distortion mosaic virus of bitter gourd through seeds.

As the transmission through sap and seed showed negative indications, next investigation was made to find out the role of insects in the transmission of this virus. Eventhough aphid, *Aphis gossypii* and jassid, *Empoasca motti* failed to transmit this mosaic virus, successful transmission could be obtained through whitefly, *Bemisia tabaci*, indicating the actual vector of 'BGDMV'. Giri and Misra (1986) and Mathew *et al.* (1991) also observed successful transmission of mosaic and leaf distortion disease of bitter gourd through *B. tabaci,* which also confirm the present findings. Whereas, Purushothaman (1994) reported the transmission of mosaic through the aphid vectors, *A. gossypii, A. malvae, A. craccivora* and *Myzus persicae*. Tomer *et al.* (2001) also observed similar findings. The reason attributes for these results may be due to the presence of other type of virus particles which was confirmed later as CMV.

Study on symptomatology is an important criteria for the early detection and identification of virus diseases. Symptomatology of the bitter gourd mosaic

disease has been described by various workers (Nagarajan and Ramakrishnan, 1971a; Giri and Misra, 1986; Mathew *et al.*, 1991; Purushothaman, 1994; Pandey *et al.*, 1998 and Arunachalam, 2002). However, the type of symptoms described are found varying with different workers. In the present study, different type of symptoms were observed on the infected plants under natural conditions. Mosaic, upward curling, crinkling, distortion of leaves, severe stunting, reduction in number of flowers and formation of deformed fruits were the common symptoms observed as similar to those observed by Mathew *et al.* (1991) and Arunachalam (2002). In addition to the above symptoms, pubescence of vines, brittleness of leaves, clustering of leaves around the nodes, flattening of top portion of vines were also noticed in the present study. Clearing of veins and veinlets and vein banding symptoms were also observed in some cases. The variation in symptoms may be due to the mixed infection of viruses.

Under artificial condition also, almost similar type of symptoms were observed while in certain cases slight differences were noticed. Tip of the lobes of the leaves became pointed, whereas the number of leaf lobes were not reduced. Pubescence and flattening of veins and clustering of leaves around the nodes were also not observed on artificial inoculation. The severity of the symptoms was also less as compared to those observed under field conditions.

As the earlier study on the transmission of virus indicated *B. tabaci* as the vector of BGDMV, the next importance was given to study the virus vector relationship which is very essential for the proper management of the disease. The first approach was on the basic aspects of transmission such as acquisition feeding, inoculation feeding, effect on the number of insects on transmission of virus and the incubation period of the virus in the host.

During these basic studies, it was found that 15 min. was the minimum period required by *B. tabaci* to acquire BGDMV from the source plant and per cent of infective insects increased with an increase in the length of acquisition feeding. Mathew *et al.* (1991) observed 20 min. as the acquisition threshhold period. It is

interesting to note that, increase in acquisition feeding period had a positive effect in reducing the incubation period of virus in the host.

In the experiment on inoculation threshold, the minimum period required to transmit the virus to healthy plant was 15 min. and the per cent of transmission increased with an increase of inoculation feeding period. Increasing inoculation feeding period also showed a positive effect on incubation period of the virus in the host as observed in the case of above study.

The third factor that was taken into consideration was the numerical effect of viruliferous whiteflies on transmission of BGDMV and it was found that even a single viruliferous insect was capable of transmitting the virus. The data suggested that the probability of infection by a single insect is independent of transmission by other insects present on the same plant which is in accordance with the theory of independent action in infection by microorganisms (Meynell, 1957). However it was found that the per cent of infected plants increased with an increase in the number of insects per plant and had a pronounced effect on the incubation period of the virus in the host. Cent per cent transmission of BGDMV was obtained with 10 whiteflies per plant. This was in confirmation with the finding of Mathew *et al.* (1991) with mosaic disease of bitter gourd. While Muniappa *et al.* (2003) observed 100 per cent transmission of pumpkin yellow vein mosaic virus with five or more whiteflies per test plant.

In the general transmission studies, symptoms were expressed by host plants within 12 to 18 days of inoculation. This is in confirmation with the earlier studies of Mathew *et al.* (1991) who also reported that the incubation period of virus in the host plant varied from 8 to 12 days for the expression of symptoms.

Collateral hosts play an important role in the perpetuation of the pathogen and the vectors. The knowledge on this aspect is very useful for the successful management of virus diseases. Therefore, commonly known cucurbitaceous crops such as ash gourd, bottle gourd, coccinia, cucumber, pumpkin, snake gourd and

watermelon were tested to find out whether these plants could be infected by BGDMV and it was observed that all the crops tested in this study did not take up infection on artificial inoculation indicating that these hosts are not playing any role in the perpetuation of this virus. These findings are in confirmation with early investigation of Mathew *et al.* (1991) who observed pumpkin and snake gourd as non hosts of BGDMV. However, they observed cucumber as a host of bitter gourd mosaic virus, while Purushothaman (1994) reported snake gourd and pumpkin as the collateral hosts of this virus which may be attributed due to the mixed infection of viruses as evident from the symptomatology and mode of transmission reported by him.

To standardise the purification of BGDMV, the method suggested by Honda *et al.* (1983) was adopted. Chloroform was superior to butanol for the initial clarification of plant extract since it preserved the geminate particles better than butanol in electron microscopic studies. Similar result was obtained with Indian Cassava Mosaic Virus by Mathew (1988).

Ultra violet absorption spectra of BGDMV resembled that of a typical nucleo protein with a peak at 241 nm. A 260/A280 ratio of the virus was 1.6 which resembled, A260/A280 ratio of 1.5 in squash leaf curl virus as reported by Cohen *et al.* (1983). Average yield of the virus was 0.102 mg ml^{-1} per 100 g plant tissue. Similarly low yield of ICMV (0.2 ml/100 g tissue) was obtained by Mathew (1988) from Cassava.

Electron microscopic studies for the identification of BGDMV revealed the presence of geminate particles of size 18-20 nm as monomers and dimers of size 18-20 nm x 30 nm. It is in confirmation with the findings of Giri and Misra (1986) and Pandey *et al.* (1998) who observed twinned geminate particles measuring about 19 x 30 nm size with leaf distortion as well as in distortion mosaic virus diseases of bitter gourd.

Flexuous rod shaped particles 750 nm long resembling poty virus were also observed along with geminate particles in the infected samples taken from natural conditions. While Mathew *et al.* (1998) observed particles of gemini virus, cucumber mosaic virus and water melon mosaic virus in bitter gourd mosaic infected field samples.

Recalling back the observation made on symptomatology under field conditions it may be concluded that variation and severity on symptom is due to mixed infection of viruses.

The immunosorbent electron microscopic studies using antiserum of Indian cassava mosaic virus did not show any positive reaction which clearly indicated that BGDMV was not related to Indian cassava mosaic virus.

Serological relationships of BGDMV with other gemini viruses was established using ELISA which is a very sensitive test for detection and identification of virus. DAC-ELISA was preferred over DAS-ELISA in the present study based on the reports of Mariappan and Mathikumar (1992) that DAC-ELISA was found stable in the detection of a virus even at a high dilution of 10^{-3}. However, in the present study, positive reaction was obtained only at 10^{-1} dilution of BGDMV and not with 10^{-2} dilution due to the very low concentration of virus in the infected sample.

In the present study, BGDMV antigen was tested against six monoclonal antibodies of gemini viruses. High positive reaction of BGDMV was obtained only with the antiserum of squash leaf curl virus (SLCV). Positive reaction was also observed with one isolate of tomato yellow leaf curl virus. With squash leaf curl virus antiserum, high absorbance values of 1.076 and 1.091 were obtained and with one isolate of tomato yellow leaf curl virus absorbance values were 0.955 and 0.847. This result showed that BGDMV was serologically more related to squash leaf curl virus which is also a gemini virus, indicating again the geminate nature of BGDMV and also supporting the findings obtained in electron microscopic studies.

Purushothaman (1994) reported that bitter gourd mosaic virus in Kerala was serologically related to cucumber mosaic virus, pumpkin mosaic virus and snakegourd mosaic viruses and was not serologically related to squash mosaic virus. In this context, it seemed worthwhile to mention that, these findings were also supportive to his earlier observations on type of symptom and transmission of virus through sap and aphids. It also indicates that the mosaic virus reported by Purushothaman (1994) is different from the distortion mosaic virus of the present study.

Characterisation of nucleic acid of BGDMV obtained from purified virus preparation did not produce any band typical for viral nucleic acid which might be due to very low concentration of virus particles in the purified preparation. Polston *et al.* (1998) also observed low concentration of virus particles in the purified preparations of whitefly-borne viruses as they are located in phloem and phloem associated parenchyma cells.

Characterisation of viral coat protein did not give any conclusive results due to low concentration of virus in the purified preparation. However, SDS - PAGE analysis of field samples revealed two bands corresponding to 16,419 D and 24,970 D which were absent in healthy samples. Hence these could be induced by infection of the plant by BGDMV. According to Uritani (1971) the increased protein content in virus infected plant might be due to the production of new PR proteins.

The above experimental results prove that the causal agent of bitter gourd distortion mosaic disease is a virus belonging to genus Begomo virus of family Geminiviridae.

An appropriate method for the better management of diseases is an integrated approach by the way of use of resistant varieties supplemented with cultural, chemical and biological methods. Earlier studies conducted by Purushothaman (1994) and Arunachalam (2002) showed that most of the bitter gourd varieties grown in Kerala including the varieties released from Kerala Agricultural

University were susceptible to BGDMV. So the control of the disease by plant protection measures is found to be an another alternative method. Plant disease management aims at prevention or reduction in the incidence on severity of the disease.

Among the various methods for the control of plant virus disease, use of chemicals offers comparatively better management of the disease. However, the constant use of chemicals has led to development of resistant biotypes of vectors, phytotoxicity and environmental pollution. Consequently, effects are underway in finding alternatives to chemical insecticides. Studies conducted on the use of botanicals and homoeopathic drugs have opened a new avenue for the management of plant viral diseases. Besides being safe and non phytotoxic, certain selected plant extracts and homoeopathic drugs are known to be effective against various viral diseases and insect vectors. So in the present investigation, an attempt was made to find out the effect of certain botanicals and homoeopathic drugs along with certain insecticides, which are reported to be effective against various virus diseases and insect vectors such as aphids, jassids and whiteflies.

From the field experiments conducted in two seasons, it was found that none of the treatments was found effective in preventing the occurrence of the distortion mosaic disease. Disease incidence and disease severity showed a progressive trend with the age of the plant irrespective of various treatments.

Considering the overall performance of various treatments during the two seasons, it was found that treatment of imidacloprid 0.025 per cent was effective in reducing disease incidence and disease severity. Schmeer *et al.* (1990) also obtained reduction in barley yellow dwarf virus disease with the use of imidacloprid..

In the present study, application of coconut vinegar one per cent also showed some effectiveness in reducing disease incidence and severity of BGDMV. Similarly, Narayanaswamy and Ramiah (1983) observed that, leaf extracts of *Cocos nucifera* was effective against tomato spotted wilt virus. Presence of potent inhibitors

of plant viruses also termed as antiviral principles (AVPs) in extracts of a variety of plant species have been recognized by Smookler (1971) and Pun *et al.* (1999). This is also confirmatory with the results of the present study.

Use of homeopathic drugs in the integrated disease management is a novel approach for combating virus diseases as they are ecofriendly and safe due to their non phytotoxic and non mutagenic chemical nature. In the present study also treatment with *Apis melliphica* 30 potency and *Arsenicum album* 30 potency showed reduction in BGDMV and also increase in yield. Similarly, was effective in reducing disease incidence and severity and increase in yield. Khurana (1980) also observed antiviral properties of *Arsenicum album.*

Any treatment applied in disease management will be effective when it reduces the infection and at the same time gives maximum yield. In the present study, maximum yield was also recorded from imidacloprid treated plots. Hence, to conclude that while considering the overall performance of various treatments during the two seasons, treatment of imidacloprid 0.025 per cent is preferred for the management of bitter gourd distortion mosaic disease.

During the second season, all the treatments were effective in reducing whitefly population than control. This may be the reason for the decreased incidence of BGDMV when compared to that of the first season. Eventhough whitefly population was low in treatment plots, the incidence of BGDMV may be due to the conducive weather factors prevailed during the cropping season.

Studies on weather parameters showed that influence of mean maximum temperature, mean relative humidity on disease incidence varied with two seasons. Relative humidity in the forenoon showed positive correlation with disease incidence and severity in both seasons, and hence found to be the most influencing weather parameter in BGDMV infection. Similarly Arunachalam (2002) also reported that maximum number of genotypes expressed mosaic symptoms under maximum temperature of 31-33°C and relative humidity of 70-85 per cent. From the results of

studies of two seasons, heavy crop loss due to BGDMV was observed during summer season.

Summing up the findings so far, it may be concluded that based on various aspects studied, BGDMV of the present study is similar to the viruses reported by Giri and Misra (1986), Mathew *et al.* (1991) and Pandey *et al.* (1998) which is identified as a virus belonging to genus Begomo virus of family Geminiviride.

Summary

6. SUMMARY

Bitter gourd (*Momordica charantia* L.) is the most important curcubitaceous vegetable in Kerala. The damages caused by the attack of pests and diseases are the major constraints for the bitter gourd cultivation. Among the various diseases affecting the crop, mosaic disease caused by virus is known to cause severe loss especially during summer season.

In transmission studies, BGDMV was found to be not transmitted through sap and seed. Germination percentage of seeds from infected plants ranged from 30 to 60 per cent whereas seeds from healthy plants showed more than 85 per cent germination. The seedlings raised from infected seeds were pale, lanky and non vigorous.

In case of insect transmission studies, aphid, *Aphis gossypii* Glover and jassid, *Empoasca motti* Pruthi were found to be non-vector of BGDMV, whereas the whitefly, *Bemisia tabaci* Genn. was found to transmit the virus and was identified as the vector of BGDMV.

Symptoms of the disease were found to be almost similar under field and artificial conditions. Common symptoms observed were light green and dark green mosaic pattern, curling, crinkling and distortion of leaves, stunting, reduction in number of flowers and fruits and reduction in size of fruits.

Under artificial condition, symptoms initiated as light green and dark green mosaic pattern on inoculated leaves which later spread to younger leaves. Size of the leaf lamina was reduced and tip of the lobes became pointed. Leaves were rough and brittle and blisters were produced on the under surface. Length of the vines were not reduced and vines were neither pubescent nor flattened.

Studies on virus-vector relationship showed that acquisition threshold period and inoculation threshold period were 15 min. Increase in acquisition feeding period from 15 to 60 min. increased per cent transmission and decreased incubation

period in the host. The above trend was also noticed with increase in inoculation feeding period from 15 to 60 min. Likewise, increasing the number of viruliferous whiteflies from 1 to 10 to inoculate a plant was found to have effect on transmission as well as on the incubation period of the virus in the plant. Per cent transmission of BGDMV increased and incubation period decreased with increase in number of infective whiteflies.

Host range studies revealed that ash gourd, bottle gourd, cucumber, pumpkin and water melon were not collateral hosts of BGDMV.

Purified preparation of BGDMV gave a characteristic ultraviolet absorption spectra of nucleoprotein with a peak at 241 nm. Average A_{260}/A_{280} value of purified preparation of BGDMV was 1.62 and concentration of the virus was 0.132 mg ml^{-1}/ 100 g plant tissue.

Characterisation of nucleic acid and protein coat of BGDMV did not give conclusive results and may be due to the low concentration of virus particles in the purified preparation. However, SDS-PAGE of BGDMV infected sample gave two additional bands at 16,419 and 24,970 D and it may be virus induced proteins.

Electrons microscopic observations of purified preparation of BGDMV gave monomers of size 18 nm diameter and geminate particles of size 18-20 x 30 nm. Monomers and dimers were present in the ratio 40:3, while trimers and tetramers were totally absent. In the case of purified preparation of BGDMV obtained from field samples, presence of flexuous rod shaped particles measuring 750 nm in length resembling poty virus were observed along with geminate particles, indicating a mixed infection of both viruses.

Results of serological studies using DAC-ELISA revealed the close relationship of BGDMV with squash leaf curl.

Immunosorbent electron microscopic observations using Indian cassava mosaic virus antiserum did not give a positive reaction, thus ruled out the relationship between BGDMV and Indian cassava mosaic virus.

The above experimental results, prove that the causal agent of bitter gourd distortion mosaic disease is a virus belonging to genus Begomo virus of family Geminiviridae.

In the management studies, it was found that none of the treatments was found effective in preventing BGDMV infection. Disease incidence and severity and coefficient of infection showed a progressive trend with growth of the plant.

Considering the overall performance of various treatments during the two seasons, it was found that treatment of imidacloprid 0.025 per cent was found effective in reducing BGDMV infection.

In the present study, application of coconut vinegar one per cent reduced disease incidence and severity of BGDMV.

In the present study also treatment with *Apis melliphica* 30 potency and *Arsenicum album* 30 potency showed reduction in BGDMV and also increase in yield.

During the second season, all the treatments were effective in reducing whitefly population than control.

Studies on weather parameters showed that influence of mean maximum temperature and mean relative humidity on disease incidence varied with two seasons. Relative humidity in the forenoon showed positive correlation with disease incidence and disease severity in both seasons, and hence found to be the most influencing weather parameter in BGDMV infection.

References

REFERENCES

Arunachalam, P. 2002. Breeding for resistance to Distortion Mosaic Virus in bitter gourd. Ph.D. Thesis, Kerala Agricultural University, Thrissur, 127 p.

Asari, P.A.R. and Nair, M.R.G.K. 1972. On the control of brinjal pests using deterrents. *Agric. Res. J. Kerala* 10(2): 133-135

Ashok, M.P., Dhavan, G. and Simwat, G.S. 2002. Field evaluation of thiamethoxam for control of cotton jassid *Amrasca biguttula biguttula* (Ishida) on upland cotton. *Pestology* 26 (1): 15-19

Banbote, B.G., Gawande, R.B. and Satpute, S.Y. 1995. Efficacy of different synthetic herbal insecticides against the pest of cotton. *PKV. Res. J.* 19(2): 101-104

Baranwal, V.K. and Ahmad, N. 1997. Effect of *Clerodendron aculeatum* leaf extract on tomato leaf curl disease. *Indian Phytopath.* 50: 297-299

Barbara, D.J. and Clark, M.E. 1982. A single indirect ELISA using F'$(ab)_2$ fragments of immunoglobulin. *J. Gen. Virol.* 58: 315-322

Bock, K.R., Guthrie, E.J. and Meredith, G. 1977. RNA and protein components of maize streak and cassava latent viruses. *Ann. Appl. Biol.* 85: 305-308

Bock, K.R., Guthrie, E.J. and Meredith, G. 1978. Distribution, host range, properties and purification of cassava latent virus, a gemini virus. *Ann. Appl. Biol.* 90: 361-367

Brown, J.K. and Nelson, M.R. 1989. Characterisation of watermelon curly mottle virus, a gemini virus distinct from squash leaf curl virus. *Ann. Appl. Biol.* 115: 243-252

* Caetano, W., Bertoldo, N., Carlesi, L.R., Heineck, M.A. and Eick, V.L. 1987. Trial of insecticides for the control of bean leafhopper *Empoasca krameri* (Homoptera:Cicadellidae) in bean fields [In Spanish]. *Agron. sulriograndense* 23(1): 103-108

Cheema, S.S., Chahal, A.S., Bansal, R.D. and Kapur, S.P. 1991. Efficacy of various byproducts and chemicals against cucumber mosaic virus on sweet pepper under screen house conditions. *Indian J. Virol.* 7: 169-175

Cherian, M.C. and Menon, G.E.R. 1944. Preliminary trials with oil emulsion for the control of insect pests. *Med. Agric. J.* 1: 10-11

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

Czosnek, H., Ber, R., Antighus, Y., Cohen, S., Navot, N. and Zamir, D. 1988. Isolation of tomato yellow leaf curl virus, a gemini virus. *Phytopathology*, 78: 508-512

Dahal, G., Lecoz, H. and Albrechtsen, S.E. 1997. Occurrence of papaya ring spot poty virus and cucurbit viruses in Nepal. *Ann. Appl. Biol.* 130(3): 491-502

Datar, V.V. and Mayee, C.D. 1981. Assessment of losses in tomato fields due to early blight. *Indian Phytopath.* 34: 191-195

Devi, M.S. 1990. Inhibitory effects of plant extracts on the incidence of cowpea mosaic virus. M.Sc. (Ag.) Thesis, Kerala Agricultural University, Thrissur, 93p.

Dharmendar, S., Jaglan, R.S., Chauhan, R., Singh, D. and Chauhan, R. 2003. Field studies on the efficacy of insecticides against brinjal whitefly (*Bemisia tabaci* Genn.). *Ann. Biol.* 19: 109-112

Dutt, S.M. 2000. Biopesticides for IPM in bitter gourd. M.Sc. (Ag.) Thesis, Kerala Agricultural University, 105 p.

Giri, B.K. and Misra, M.D. 1986. A whitefly transmitted virus disease of bitter gourd. *Proceedings of National Seminar on Whitefly Transmitted Plant Virus Diseases*. 25-27 June, 1986, I.A.R.I., New Delhi, 16 p.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research.* John Wiley and Sons, Singapore, 156 p.

Honda, Y., Iwaki, M. and Saito, Y. 1983. Mechanical transmission, purification and some properties of whitefly-borne mung bean yellow mosaic virus in Thailand. *Pl. Dis.* 67: 801-804

Jayapalan, M. and Sushama, N.P.K. 2001. Constraints in the cultivation of bitter gourd (*Momordica charantia* L.). *J. Trop. Agric.* 39: 91

Jayaraj, S., Natarajan, S. and Kandaswamy, D.S. 1995. Resurgence of yellow mite in semi-dry chilli. *S. Indian Hort.* 43(3-4): 117-119

Jayashree, K., Pun, K.B. and Doraiswamy, S. 1999. Effect of plant extracts and derivatives, buttermilk and virus inhibitory chemicals on pumpkin yellow vein mosaic virus transmission. *Indian Phytopath.* 52: 357-361

Karthikeyan, R. 2003. Bioefficacy of newer insecticides against leafhopper, *Empoasca motti* Pruthi in bitter gourd, M.Sc. (Ag.) Thesis, Kerala Agricultural University, Thrissur, 102 p.

Kathirvel, M. 1988. Studies on the management of pests and nematodes of bhendi with botanicals and insecticides. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, 122 p.

KAU, 1996a. KHDP (RCD). *Report on On-farm Identification of Problems of Vegetables Grown in KHDP Pilot Project Areas,* Thiruvananthapuram 30 p.

KAU, 1996b. *Package of Practices Recommendations 'Crops'.* Directorate of Extension, Kerala Agricultural University, Trichur, 195 p.

Khurana, S.M.P. 1968. A study of virus diseases of Papaya (*Carica papaya* L.) in Gorakhpur. Ph.D. Thesis, University of Gorakhpur, Uttar Pradesh, 140 p.

Khurana, S.M.P. 1980. Chemotherapeutic potential of homoeopathic drugs against plant viruses. *Proceedings of 225th Hahnemannian Birthday and Annual Function*, 11th April,1980, HMAT, Lucknow, pp.29-35

Kumar, S.T. 1999. Ecofriendly management of important sucking pests of chilli (*Capsicum annuum*). M.Sc. (Ag.) Thesis, Kerala Agricultural University, Thrissur 105 p.

Laemmli, U.K. 1970. Isolation and characterisation of ribosomal nucleic acid. *Biochem. J.* 96: 266-269

Latha, P. 1992. Selection for mosaic resistance in pumpkin (*Cucurbita moschata* Poir.). M.Sc. (Ag.) Thesis, Kerala Agricultural University, Thrissur, 104 p.

Lily, B. and Saradamma, K. 1994. Antifeedant action of different extracts of *Clerodendron infortunatum* on the adults and grubs of epilachna beetle *Henosepilachna vigintioctopunctata*. F. 15th Annual Session. *Acad. Environ. Biol.* Nov. 7-9, 1994, Trivandrum, *Abstract*: 5: 5

Louis, V. and Balakrishnan, S. 1996. Effect of application of selected medicinal plants extracts on the incidence of pumpkin mosaic. *Indian Phytopath.* 49: 373-377

Maniatis, T., Fritsch, E.F. and Sambrook. 1982. *Molecular Cloning - A Laboratory Manual*, Cold Spring Harbor, New York, 430 p.

Mariappan, V. and Mathikumar, N. 1992. Immunological detection of bean yellow mosaic virus in seeds of *Vicia faba*. L. *Indian J. Virol.* 8(2): 179-183

Mathew, A.V. 1988. Studies on Indias Cassava mosaic virus disease. Ph.D. Thesis, University of Agricultural Sciences, Bangalore, 237 p.

Mathew, A.V. 1998. *Final Report on Studies on Mosaic Disease of Bitter Gourd.* Kerala Agricultural University, Thrissur . 13 p.

Mathew, A.V., Mathew, J. and Mathai, G. 1991. A whitefly transmitted mosaic disease of bitter gourd. *Indian Phytopath.* 44: 497-499

Meynell, G.G. 1957. Inherently low precision of infectivity titrations using quantal response. *Biometrics* 13: 149-167

Miller, S.A. and Martin, R.R. 1988. Molecular diagnosis of plant diseases. *A. Rev. Phytopath.* 26: 400-432

Mohapatra, L.N. 1996. Chemical control of yellow mite. *Polyphagotarsonemus latus* (Banks) on jute. *Indian J. Pl. Protection* 24: 15-17

Morales, F., Niessen, A., Ramirez, B. and Castano, M. 1990. Isolation and partial characterization of a gemini virus causing bean dwarf mosaic. *Phytopathology* 80: 96-101

Muniyappa, V., Maruthi, M.N., Babitha, C.R., Coluin, J., Briddon, R.W. and Rangaswamy, K.T. 2003. Characterisation of pumpkin yellow vein mosaic virus from India. *Ann. Appl. Biol.* 142: 323-331

Nagarajan, K. and Ramakrishnan, K. 1971a. Studies on cucurbit viruses in Madras state I. A new virus on bitter gourd (*Momordica charantia* L.). *Proceedings of Indian Academy of Sciences, B.* 73: 31-35

Nagarajan, K. and Ramakrishnan, K. 1971b. Studies on cucurbit viruses in Madras state II. Vector-virus relationships of the bitter gourd mosaic virus. *Proceedings of Indian Academy of Sciences, B.* 73: 84-95

Nandakumar, C. 1999. Monitoring and management of pest complex of bitter gourd (*Momordica charantia* L.). Ph.D. Thesis, Kerala Agricultural University, Thrissur, 180 p.

Narayanaswamy, P. and Ramiah, M. 1983. Screening of non-hosts of tomato spotted wilt virus for the presence of anti-viral principles. *Proceedings of National Seminar on Management of Diseases of Oil Seeds Crops*, Madurai. pp. 15-17

* Nikolov, N. and Ivanova, I. 1998. Comparative evaluation of the aphidicidal action of some insecticides on greenhouse cucumber [in Spanish] *Rastneiv dni Nauki* 35(4): 314-317

Pandey, P.K., Chakraborthy, S. and Ram, D. 1998. Response of bitter gourd varieties against distortion mosaic virus. *National Symposium On Emerging Scenario in Vegetable Research and Development*, December 12-14, Lucknow, 182 p.

Patel, H.R. and Patel, B.N. 1993. Inhibition of chlorotic mottle virus infection by plant extracts and other substances. *Indian J.Mycol. Pl. Pathol.* 23: 191-193

Polston, J.E., Bois, D.A.G., Poliakoff, F. and Urbino, C. 1998. Occurrence of a strain of potato yellow mosaic gemini virus infecting tomato in the eastern Carribean. *Pl. Dis.* 82: 126

Pun, K.B., Doraiswamy, S. and Jayarajan, R. 1999. Screening of plant species for the presence of antiviral principles against okra yellow vein mosaic virus. *Indian Phytopath.* 52(3): 221-223

Purushothaman, S.M. 1994. Investigation on mosaic disease of bitter gourd. M.Sc. (Ag.) Thesis, Kerala Agricultural University, Thrissur 130 p.

Raj, S.K., Aslam, M., Srivastava, K.M. and Singh, B.P. 1989. Association of gemini virus - like particles with yellow mosaic disease of *Dolichos lablab* L. *Curr. Sci.* 58(14): 813-814

Reddy, R.V. and Rao, B.S. 1998. Evaluation of certain insecticides against the foliage insect pests of bitter gourd (*Momordica charantia* Linn.). *J. Res. ANGRAU* 26(4): 41-43

Reghunath, P. and Gokulapalan, C. 1994. Management of American serpentine leaf miner *Liriomyza trifolii*, pea aphid *Aphis craccivora* and cowpea mosaic. 15th *Annual Session of Acad Environ. Biol.* 7-9 Nov. 1994, Trivandrum *Abstract*: 124: 115

Rekha, C.R. 1999. Nutritional management of bitter gourd in relation to pest and disease management. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 88 p.

* Rushtapakornchai, W., Petchwichit, P., Winai, R. and Pakuipa, P. 1996. *Kaen-Kaset-Khon-Kaen* [In Spanish]. *Agric. J.* 24(4): 184-189

Sabitha, R. 1992. Relative susceptibility of population of *Amrasca biguttula biguttula* (Ishida) infesting bitter gourd collected from different locations to insecticides. M.Sc.(Ag) thesis, Kerala Agricultural University, 120p

Samuel, A.L. and Mariappan, U. 1996. Effect of plant derivatives on the transmission of potato virus infecting chilli and its aphid vectors. *Neem and environment.* (Eds. Singh, R.P., Chari, M.S., Raheja, A.K. and Kraus, W.), Lucknow, pp. 777-781

Saradamma, K. 1989. Biological activity of different plant extracts with particular reference to their insecticidal, hormonal and antifeeding action. Ph.D. Thesis, Kerala Agricultural University, Thrissur, 140 p.

Schmeer, H.E., Blutt, D.J., Meredith, R. and Heatherihgton, P.J. 1990. Field evaluation of imidacloprid as an insecticidal seed treatment in sugarbeet and cereals. *Pests and Dis.* 1: 29-36

Siddique, S.A. 1987. A proposed pest management progressive including neem treatments combating potato pests in Sudan. *Natural Pesticide from the Neem Tree and Other Tropical Plants* (Eds. Schmutterer, H. and Ascher, R.R.S.), West Germany, pp. 449-459

Smookler, M.M. 1971. Properties of inhibition of plant virus infection occurring in the leaves of species of Chenopodiaceae. *Ann. Appl. Biol.* 69: 157-158

Srinivasan, G. and Babu, P.C.S. 2001. Field evaluation of neem products against whitefly, *Bemisia tabaci* Genn on brinjal. *Ann. Pl. Protection Sci.* 9: 19-21

Srinivasava, K.M., Rana, N.S., Dwadarh Shreni, V.C. and Singh, B.P. 1986. Mechanism of inhibition of CMV by crude oil from margosa and *Azadirachta indica Indian Phytopath.* 39: 20-25

Surendran, M. 1996. Transmission, physical properties and host range of brinjal mosaic virus. M.Sc. (Ag.) Thesis, Kerala Agricultural University, Thrissur 100 p.

Tomar, S.P.S., Jitendra, M. and Mohan, J. 2001. Bitter gourd mosaic virus in weed hosts. *J. Living World.* 8: 24-27

Uppal, B.N. 1933. India: disease in the Bombay Presidency. *Int. Bull. Pl. Protection.* 7: 103-104

Uritani, J. 1971. Protein changes in diseased plants. Annu. *Rev. Phytopath.* 9: 211-231

*Verghese, A. 1998. Effect of imidacloprid on mango hoppers, *Idioscopses* spp. (Homoptera:Cicadellidae). *Pest Mgt. Hort. Ecosystem* 4: 70-74

Verma, A., Singh, R.B. and Verma, H.N. 1992. Prevention of soyabean mosaic virus infection in *Glycine max* plants by plant extracts. *Bioved.* 3: 225-228

Verma, H.N., Chowdhury, B. and Prasad, V. 1985. *Clerodendron aculeatum* leaf extract induced virus inhibitory agent (VIA) from treated and non treated leaves of healthy *Nicotiana* spp. *Indian Phytopath.* 38: 172-175

Verma, H.N., Rastogi, P., Prasad, V. and Srivastava, R. 1985. Possible control of natural virus infection on *Vigna radiata* and *Vigna mungo* by plant extracts. *Indian J. Pl. Path.* 3: 21-24

* Originals not seen

Appendices

APPENDIX - I

Potassium phosphate buffer 0.1 M, pH 7.2

Solution A - One Molar dipotassium hydrogen phosphate (K_2HPO_4).
Molecular weight (M.W.) = 174.18 g. Dissolved 174.18 g K_2HPO_4 in one litre distilled water.

Solution B - One Molar dihydrogen potassium phosphate (KH_2PO_4).
M.W. = 136.09 g. Dissolved 136.09 g K_2HPO_4 in one litre distilled water.

Mixed 71.7 ml of solution A and 28.3 ml of solution B and diluted to 1000 ml with distilled water.

APPENDIX - II

SDS PAGE

2x sample buffer

Tris base (0.125 M)	-	0.15 g
Distilled water	-	4 ml

pH was adjusted to 6.8 with hydrochloric acid

10 per cent SDS	-	4 ml
10 per cent Glycerol	-	4 ml
2 mercapto ethanol	-	0.4 ml
Bromophenol blue	-	20 mg

Made the volume to 10 ml with distilled water

APPENDIX - III

DAC-ELISA

1) Phosphate buffered saline (PBS - pH 7.4)

Sodium chloride	-	8.0 g
Potassium dihydrogen phosphate	-	0.2 g
Disodium hydrogen phosphate	-	1.1 g
Potassium chloride	-	0.2 g
Sodium azide	-	0.2 g
Water	-	1000 ml
Tween 20 (0.05%)	-	0.5 ml

2) Coating buffer (pH 9.6)

Sodium carbonate	-	1.59 g
Sodium bicarbonate	-	2.93 g
Sodium azide	-	0.2 g
Water	-	1000 ml

CHARACTERISATION AND MANAGEMENT OF BITTER GOURD DISTORTION MOSAIC VIRUS

REENY MARY ZACHARIA

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2006

Department of Plant Pathology
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

ABSTRACT

Studies on 'Characterisation and management of bitter gourd distortion mosaic virus (BGDMV)' conducted at College of Horticulture, Vellanikkara and Sugarcane Research Station, Thiruvalla during 1999-2004 dealt with various aspects especially on transmission, etiology, characterisation, identification of virus and management.

Transmission studies proved that BGDMV was not transmitted through sap and seed. Germination percentage of seeds from infected plants was 45 per cent whereas seeds from healthy plants showed 85 per cent germination. The seedlings raised from infected seeds were pale, lanky and non vigorous.

Studies on insect transmission revealed that whitefly, *Bemisia tabaci* Genn. was the vector of BGDMV. Minimum acquisition and inoculation feeding period for *B. tabaci* for transmission of BGDMV were found to be 15 min.

Under natural conditions, common symptoms observed were light green and dark green mosaic patterns, curling, crinkling and distortion of leaves, reduction in number of flowers and fruits and reduction in size of fruits. Under artificial inoculation, symptoms initiated as light green and dark green mosaic patches in inoculated leaves, which later spread to young leaves and resulted in brittleness, pointed leaf lobes and crinkling. Average incubation period of BGDMV in bitter gourd plant was 14 days and the incubation period was found to decrease with increase in acquisition feeding period, inoculation feeding period and increase in number of viruliferous insects. A single viruliferous whitefly was capable of transmission upto 33 per cent and cent per cent transmission was obtained with increase in number of whiteflies to 10.

Host range studies revealed that ash gourd, bottle gourd, coccinia, cucumber, pumpkin, snake gourd and water melon were not collateral hosts for the perpetuation of the virus.

Purified preparations of BGDMV gave a characteristic ultraviolet absorption spectra of nucleoprotein with a peak at 241 nm. Average A_{260}/A_{280} value of purified preparation of BGDMV was 1.62 and average concentration of the virus was 0.132 mg ml^{-1}/100 g plant tissue.

Characterisation of nucleic acid and viral proteins did not show any specific band of virus particles due to low concentration of particles in the purified preparation. Hence nucleic acid and protein coat could not be characterized. However SDS-PAGE of BGDMV infected field sample gave two additional bands at 16,419 and 24,970 D which could be proteins induced in host plant by virus infection.

Electron microscopic observation of purified preparation of BGDMV gave monomers of size 18 nm diameter and geminate particles of size 18-20 x 30 nm. Infected samples from the field showed flexuous rod shaped particles of 750 nm resembling poty virus, in addition to geminate particles of size 18-20 x 30 nm, indicating a mixed infection of gemini and poty viruses.

Immunosorbent electron microscopic observations using Indian cassava mosaic virus antiserum did not give a positive reaction, thus ruled out the relationship between BGDMV and Indian cassava mosaic virus.

Serological tests using DAC-ELISA with 10^{-1} dilution of BGDMV showed that the virus was closely related to squash leaf curl virus.

Based on transmission, electron microscopy and serological studies it was proved that the causal agent of bitter gourd distortion mosaic disease is a virus belonging to genus Begomo virus of family Geminiviridae.

In the management studies, it was found that none of the treatments was found effective in preventing BGDMV infection. Disease incidence and severity and coefficient of infection showed a progressive trend with growth of the plant.

Considering the overall performance of various treatments during the two seasons, it was found that treatment of imidacloprid 0.025 per cent was very effective in reducing disease incidence, disease severity and coefficient of infection.

In the present study, application of coconut vinegar one per cent was also found to be effective in reducing disease incidence and severity of BGDMV.

In the present study also treatment with *Apis melliphica* 30 potency and *Arsenicum album* 30 potency showed reduction in BGDMV and also increase in yield.

During the second season, all the treatments were effective in reducing whitefly population than control.

Studies on weather parameters showed that influence of mean maximum temperature, mean relative humidity on disease incidence varied with two seasons. Relative humidity in the forenoon showed positive correlation with disease incidence and disease severity in both seasons, and hence found to be the most influencing weather parameter in BGDMV infection.