DISEASE RESISTANCE IN THE MANAGEMENT OF COWPEA APHID-BORNE MOSAIC VIRUS

By

RADHIKA N.S.

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE

MASTER OF SCIENCE IN AGRICULTURE

(PLANT PATHOLOGY)

FACULTY OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI

THIRUVANANTHAPURAM

1999

DECLARATION

*I hereby declare that this thesis entitled "**Disease resistance in the management of cowpea aphid-borne 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 of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.*

Vellayani,

17-8-1999

RADHIKA N. S.

CERTIFICATE

*Certified that this thesis entitled "**Disease resistance in the management of cowpea aphid-borne mosaic virus**" is a record of research work done independently by Ms Radhika, N. S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.*

Vellayani,
[illegible]-2-1999

Dr. K. Umamaheswaran
(Chairman, Advisory Committee)
Assistant Professor
Department of Plant Pathology
College of Agriculture, Vellayani
Thiruvananthapuram

APPROVED BY:

CHAIRMAN

Dr. K. Umamaheswaran

MEMBERS

1. Dr. S. Balakrishnan

2. Dr. P. Rajendran

3. Dr. M. Abdul Vahab

EXTERNAL EXAMINER

(N. Gangadharan Nair)

ACKNOWLEDGEMENT

The author wishes to extend her deep sense of indebtedness and gratitude to,

Dr. K. Umamaheswaran, Assistant Professor of Plant Pathology, Chairman of Advisory Committee for his sincere guidance, wholehearted co-operation, constant encouragement and kind help throughout the period of investigation.

Dr. S. Balakrishnan, Professor and Head of Plant Pathology for his valuable advice and critical evaluation of the manuscript.

Dr. P. Rajendran, Associate Professor of Soil Science and Agricultural Chemistry for his valuable suggestions and co-operation.

Dr. Abdul Vahab, Associate Professor of Horticulture for his valuable suggestions, kind help and co-operation rendered throughout the study.

Dr. George Thottappilly, IITA, Ibadan, Nigeria for his services rendered during the identification of the virus.

Dr. R. P. Pant, Scientist, IARI for preparing the electron micrograph of the virus.

Dr. C. Gokulapalan and Dr. V. K. Girija for their valuable suggestions and encouragement during the investigation.

Dr. Vijayan, Department of Animal Husbandry and Dr. V. A. Celine, Department of Horticulture for their kind help during the investigation.

To all the students, teachers and non-teaching staff of the Department of Plant Pathology for their help and co-operation throughout the study.

C. E. Ajithkumar, Department of Agricultural Statistics for analysing the data.

ARDRA Computers for their timely and neat preparation of the manuscript.

Ranjit, A. for graphical presentation of the data.

To all my friends, especially Romy, Manoj, Suja, Annie, Raakhee, Dournami, Lekshmi, Samasya, Rajkumar and Linu for their selfless help, support and encouragement throughout the study.

To my parents, brother and relatives for their support.

Kerala Agricultural University for awarding the Junior Research Fellowship.

RADHIKA. N. S.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

INTRODUCTION

1. INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a versatile leguminous crop grown in different parts of the world for vegetable, grain and fodder purpose. It provides more than half the amount of plant protein in human diet (Rachie, 1985). It is a good cover crop and rich supplier of nitrogen. In India, cowpea is cultivated in West Bengal, Tamil Nadu, Andhra Pradesh, Kerala and Maharashtra. In Kerala, cowpea is cultivated mainly as a vegetable and grain crop.

Disease and pests are a major constraint in increasing the production of the crop. Among the pathogens, viruses are a threat to the cultivators. More than twenty viruses are reported in cowpea from different parts of the world (Thottappilly and Rossel, 1992). Management of these viruses has been difficult as some of them are seed borne and form the major source of inoculum for further spread in the field through vectors.

Exploitation of host-plant resistance has now emerged as the most effective method of management. For this an adequate knowledge of virus strain causing the disease is essential. Only very little work has been done on the cowpea viruses in Kerala. This study was undertaken with the following objectives,

- Identification and characterisation of the virus causing aphid-borne mosaic disease of cowpea
- Screening the sources of resistance against the virus
- Estimation of biochemical changes of disease resistance.
- Induction of systemic resistance using antiviral chemicals

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Symptomatology

Cowpea aphid-borne mosaic virus (CABMV) was first described from Italy by Lovisolo and Conti (1966) and was referred to as the 'European strain' of the virus. It was reported to cause severe distorting mosaic in cowpea. Bock (1973) described three isolates of CABMV. The first isolate from Kenya, referred to as the 'African strain' induces irregular, angular broken mosaic. Another isolate from East Africa described as 'African vein-banding strain,' induces broad dark green vein-banding. 'African mild strain', the third African isolate, induces very mild mottle with little or no effect on plant growth. Bock and Conti (1974) described the symptoms as dark green vein-banding on leaves, with distortion, blistering and stunting. Fischer and Lockhart (1976) observed that the diseased plants showed mosaic patterns, leaf bumping and distortion and severe stunting which resulted in yield reduction.

Mali and Kulthe (1980) described symptoms on primary leaves as mild mosaic, followed by irregular mosaic or yellow mottling, puckering, slight distortion and arching of trifoliate leaves. Slight stunting of plants was also observed. Patel and Kuwaite (1982) reported that the infected plants showed severe puckering, mottling and defoliation of trifoliates and extensive necrotic streaks in the stem sometimes leading to death of plants. Symptoms due to infection by CABMV include severe mosaic, with the severity dependent on host cultivar and virus strain (Rossel and Thottappilly, 1985, Thottappilly and Rossel, 1985,

Thottappilly and Rossel, 1992). Burke *et al.* (1986) reported mosaic symptoms on seedlings infected with CABMV. Sudhakumari (1993) reported that symptom of CABMV first appeared as mild vein clearing on primary trifoliate leaves six to seven days after sap inoculation. Later, the trifoliates showed mosaic mottling with dark green and light green patches. Umamaheswaran (1996) described that the first visible symptom appeared as vein clearing followed by conspicuous mottling, chlorosis of leaflets, cupping, arching and inward curling of the margin of leaf lets, stunting, shortening of internodes, excessive branching and deformed pods. Kline and Anderson (1997a) found strong mosaic symptom on primary and trifoliate leaves of cowpea seedlings. Gumedzoe *et al.* (1998) reported that two isolates of CABMV from Nigeria induced mild green mosaic and vein banding symptoms on different cowpea lines.

2.2 Transmission

2.2.1 Mechanical transmission

Bock (1973) reported mechanical transmission of CABMV using 0.06 M phosphate buffer, pH 7.7. This was done by rubbing the sap on to carborundum powder dusted leaves. Kaiser and Mossahebi (1975) extracted sap from infected leaves in mortar and pestle using 0.01 M phosphate buffer (pH 7.0). Sap was applied with the thumb and forefinger on carborundum (320 mesh) dusted leaves of young indicator plants. Sharma and Varma (1975) reported that the virus was sap transmissible and extraction was done using 0.05 M phosphate buffer (pH

7.0). Fischer and Lockhart (1976) conducted mechanical transmission using 0.05 M phosphate buffer (pH 7.1). Ladipo and Allen (1979) reported that the virus was mechanically transmitted to the local lesion host and systemic host. Mali and Kulthe (1980) conducted mechanical transmission by extracting sap from infected cowpea leaves using cold pestle and mortar with chilled 0.1 M Tris buffer (pH 7.0). Test plants were inoculated at primary leaf stage using cotton swab and carborundum as abrasive. Patel and Kuwaite (1982) used 0.01 M phosphate buffer (pH 7.0) for mechanical transmission with carborundum powder as the abrasive.

Chang and Kuo (1983) reported the mechanical transmission of CABMV to nine species of Leguminosae family. Tsuchizaki *et al.* (1984) reported that blackeye cowpea mosaic virus (BlCMV) from asparagus bean (*Vigna sesquipedalis*) was sap transmitted.

Mechanical transmission of the virus CABMV was also reported by Atiri *et al.* (1984), Thottappilly and Rossel (1992) , Kannan and Doraiswamy (1994), Nain *et al.* (1994), Bashir and Hampton (1996 a) and Kline and Anderson (1997a).

2.2.2 Seed transmission

Bock and Conti (1974) reported that the extent of seed transmission of CABMV ranged from 0 to 3 per cent. Sharma and Varma (1975) reported 31 per cent seed transmission in cv. Pusa Phalguni, 22 per cent in cv. Pusa Dophasali and

15 per cent in cv. Pusa Barsati. Under field condition, seed transmission varied from 1.1 to 39.8 per cent (Kaiser and Mossahebi, 1975). They also found that there was no correlation between yield reduction and per cent seed transmission. Fischer and Lockhart (1976) found that seed transmission of a strain of CABMV from Morocco was low.

Mali and Kulthe (1980) obtained transmission of the virus through seeds of the variety CM 11. They recorded 41.6 per cent transmission. Seed transmissible nature of the virus was not dependent on the virus strain and cultivar (Aboul-Ata *et al.*, 1982). Mali *et al.* (1988) observed that seed transmission ranged from 0 to 22.6 per cent for BlCMV and 0 to 18.5 per cent for CABMV. Mali *et al.* (1989) reported 3.1 to 20 per cent seed transmission of the virus CABMV. Fiddan and Yorganci (1990) reported seed transmission of the virus in seeds collected from farmers field and artificially inoculated plants. Seed transmission of the virus, CABMV in Senegal was reported by Ndiaye *et al.* (1993).

Gillaspie *et al.* (1993) reported 0.4 to 50 per cent seed transmission of BlCMV from four of the seven cowpea seed lots tested. Bashir and Hampton (1994) reported that RN-27C, an asolate of CABMV was transmitted at a rate of 55 per cent in cowpea genotype 48-57. Nain *et al.* (1994) found that there was 42.10 per cent seed transmission and that an inverse relationship existed between per cent seed transmission and age of plant at inoculation.

Presence of virus in seeds collected from CABMV infected cowpea cultivars was detected by ELISA and symptoms were observed on the plants

grown from the seeds (Konate and Neya, 1996).

2.2.3 Insect transmission

A large number of aphid species are reported to be the vectors of CABMV.

Bock and Conti (1974) reported *Aphis craccivora* as the vector of CABMV. Transmission of the virus occurred in a non-persistent manner by *A. craccivora* (Ndiaye *et al.*, 1993). Bashir and Hampton (1994) also observed a non-persistent aphid transmission by *A. craccivora* and the per centage of transmission ranged between 18 to 57.

Fischer and Lockhart (1976) obtained successful non-persistent transmission of a strain of CABMV in Morocco using *Myzus persicae*. Chang and Kuo (1983) reported transmission of CABMV in a non-persistent manner by *M. persicae* in asparagus bean (*V. sesquipedalis*). Murphy *et al.* (1987) successfully obtained non-persistent transmission of BlCMV by *M. persicae*. The symptoms of virus infection appeared 10-15 days after the release of the vector.

Mali and Kulthe (1980) reported non-persistent transmission of CABMV by the cotton aphid, *Aphis gossypii*. Successful transmission of CABMV and BlCMV was obtained by Mali *et al.* (1988) using *A. gossypii* and *M. persicae*.

Kaiser and Mossahebi (1975) reported transmission of CABMV by *A. craccivora* and *Acyrthosiphon pisum* and *Aphis seshamae*. CABMV was reported to be transmitted in a non-persistent manner by *A. craccivora*, *Aphis*

fabae, *A. gossypii* and *M. persicae* (Atiri, 1984; Atiri *et al.*, 1984; Atiri *et al.*, 1986). Thottappilly and Rossel (1992) found that *A. craccivora*, *A. gossypii*, *Aphis spiraecola*, *Aphis medicaginis*, *Macrosiphum euphorbiae*, *M. persicae*, *Rhopalosiphum maidis* and *Cerataphis palmae* transmitted CABMV in a non-persistent manner. Roberts *et al.* (1993) reported the ability of adult apterous *A. craccivora*, *A. gossypii* and *Aphis citricola* to transmit CABMV. Nain *et al.* (1994) found that *A. gossypii*, *A. craccivora* and *M. persicae* could transmit CABMV. Eighty eight per cent transmission was obtained with *A. craccivora*. Umamaheswaran (1996) obtained 93.33 per cent transmission with *A. craccivora*, 30 per cent with *M. persicae* and 26.67 per cent with *A. gossypii*.

2.3 Physical properties of the virus

Physical properties of the virus include dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV).

Lovisolo and Conti (1966) and Bock (1973) studied the physical properties of CABMV. In cowpea sap, the thermal inactivation point was between 57 and 60^0 C, the dilution end point was between 10^{-3} and 10^{-4} and longevity *in vitro* was 1-3 days at 20^0 C. They found that frozen leaves retained infectivity for at least seven weeks. Kaiser and Mossahebi (1975) elucidated the physical properties of CABMV. The virus was inactivated at a dilution range of 10^{-4} - 10^{-5} and temperature range of 55-60^0 C. The virus retained its infectivity for seven days at 20^0 C. Fischer and Lockhart (1976) reported the physical properties of CABMV

as, DEP at 10^{-4} - 10^{-5}, TIP at 50-55^{0} C and LIV for four to eight days at 22 to 25^{0}C. Mali and Kulthe (1980) reported that CABMV was inactivated at a temperature range of 60-65^{0} C and at a dilution of 10^{-4}. Virus lost its activity after 64 h.

Patel and Kuwaite (1982) reported different physical properties for two isolates of CABMV. One isolate had TIP at 70 - 75^{0} C, DEP at 10^{-6} - 10^{-7} and LIV for six to seven days. The TIP of second isolate ranged between 65-70^{0} C, DEP and LIV was in the range of 10^{-5} - 10^{-6} and four to five days respectively. Boswell and Gibbs (1983) reported that BlCMV was characterised by a TIP at 65^{0} C, LIV for 2 days and DEP at 10^{-4}. The physical properties of CABMV was identified as TIP at 60^{0} C, LIV for 72 days, and DEP at 10^{-3}.

CABMV infecting asparagus bean (*V. sesquipedalis*) lost its infectivity at 55 - 60^{0} C, 10^{-4} - 10^{-5} dilution and storage for 7 - 8 days at 20^{0} C (Chang and KUO, 1983). Tsuchizaki *et al.* (1984) studied the physical properties of BlCMV from asparagus bean and found that TIP ranged between 54 - 65^{0} C, DEP ranged between 10^{-4} - 10^{-5} and LIV for one to three days at 20^{0} C. Murphy *et al.* (1987) assayed the physical properties of BlCMV and reported the TIP as between 60 to 65^{0} C, DEP in the range of 10^{-4} to 10^{-5} and LIV for 24 hours.

2.4 Virus-vector relationship

Many authors have reported the non-persistent nature of transmission of the virus, CABMV by aphid vectors (Kaiser and Mossahebi, 1975; Fischer and

Lockhart, 1976, Mali and Kulthe, 1980; Chang and Kuo, 1983; Atiri, 1984 and Thottappilly, 1984; Murphy *et al.*, 1987; Ndiaye *et al.*, 1993; Nain *et al.*, 1994).

Govindaswamy *et al.* (1970) reported that a single aphid could transmit the virus causing mosaic in cowpea to healthy plants. Aphids could acquire and inoculate the virus with in a second. Kaiser and Mossahebi (1975) studied the virus-vector relationship of CABMV with aphid vectors. Single *A. craccivora* and *A. pisum* could transmit the virus with a short acquisition feeding period. Transmission was rated as 18 per cent and 50 per cent, respectively.

Mali and Kulthe (1980) reported that *A. gossypii* could transmit CABMV with a brief acquisition access period of one minute and inoculation access period of four hours. Atiri *et al* (1984) studied virus - vector relationship in cowpea lines and reported that CABMV was acquired and transmitted by its vector, *A. craccivora*, during brief probes.

Nain *et al.* (1994) could successfully transmit CABMV using viruliferous aphid nymphs of *A. craccivora, A. gossypii* and *M. persicae*. The nymphs were given one hour fasting, five minutes acquisition access and 24 h inoculation access for effective transmission of the virus. Umamaheswaran (1996) found that *A. craccivora* required a short acquisition access of five minutes to acquire CABMV. Viruliferous aphids could transmit the virus to healthy plants with one minute inoculation access period. Pre-acquisition fasting for one hour resulted in an increase in per cent infection. Even a single aphid could effectively transmit the virus.

2.5 Serodiagnosis and electron microscopy

2.5.1. Ouchterlony agar double diffusion

Fischer and Lockhart (1976) performed Ouchterlony test to prove that an isolate of CABMV was not related to bean common mosaic virus. Double immunodiffusion tests were conducted to prove the relationship of BlCMV with other potyviruses and found that BlCMV was serologically related to but distinct from other poty viruses tested (Lima *et al.*, 1979).

Lima and Purcifull (1980) used double immunodiffusion tests to detect BlCMV in hypocotyls of infected cowpea seedlings and soybean mosaic virus in soybean hypocotyls. Taiwo and Gonsalves (1982) studied the relationship between two isolates of BlCMV and four isolates of CABMV using double diffusion methods and found that the antisera of isolates of CABMV group did not react with isolates of BlCMV group.

Dijkstra *et al.* (1987) compared two potyvirus isolates, one from germplasm of yard long bean and another one from soybean plants with two isolates of BlCMV from U.S.A and Moroccan isolate of CABMV. All the viruses were grouped as BlCMV. Mali *et al.* (1989) used SDS - immunodiffusion test for the serodiagnosis of six seed-borne cowpea viruses in India and the presence of BlCMV, CABMV, cucumber mosaic virus and southern bean mosaic virus were confirmed.

Zhao *et al.* (1991) found that BlCMV from the plant Alyce - clover (*Alysicarpus vaginalis* (L.) DC.) was serologically related to BlCMV isolates of

cowpea from Florida and South Carolina but was less related to CABMV, peanut poty virus and nine other poty viruses. Nain *et al.* (1994) used SDS-double diffusion test to identify eight viruses causing mosaic symptom on cowpea in different parts of North India. Bashir and Hampton (1995) distinguished BlCMV from CABMV isolates in seed lots of cowpea by immunodiffusion method. Kline and Anderson (1997a) used gel diffusion assays to detect the presence of CABMV on cowpea in United States.

2.5.2 Enzyme linked immunosorbent assay (ELISA)

ELISA test was conducted to compare the isolates of CABMV and BlCMV (Taiwo and Gonsalves, 1982). Dijkstra *et al.* (1987) used ELISA to differentiate BlCMV and CABMV isolates from yard long bean. Zhao *et al.* (1991) conducted ELISA to prove that BlCMV from Alyce-clover is related to BlCMV isolates of cowpea from Florida and South Carolina. Huguenot *et al.* (1992) conducted ELISA and confirmed that BlCMV and CABMV are two different poty viruses. Gumodzoe (1993) reported the use of immunosorbent assays for the identification of virus diseases of cowpea in Togo. Ndiaye *et al.* (1993) used direct antigen coating (DAC) and double antibody sandwich (DAS) ELISA for the detection of seven seed-borne viruses in cowpea seeds. Bashir and Hampton (1995) conducted DAS and DAC ELISA to identify CABMV from infected cowpea plants. Anderson *et al.* (1996) used ELISA for the evaluation of cowpea lines for resistance against BlCMV. Konate and Neya (1996) proved the reliability of ELISA technique for selecting CABMV free stocks of cowpea seeds. Kline and

Anderson (1997a) detected the presence of CABMV from cowpea grown commercially in United States using ELISA.

2.5.3 Electron microscopy

Electron micrographs of negatively stained leaf dip preparations revealed that CABMV particles were flexuous rods of about 750 nm in length (Bock, 1973; Kaiser and Mossahebi, 1975; Lima and Purcifull, 1980; Patel and Kuwaite, 1982; Taiwo *et al.*, 1982; Bashir and Hampton, 1995).

2.6 Varietal screening

Ladipo and Allen (1979) screened cowpea germplasm and categorized 52 lines as immune, six lines as tolerant and all the remaining lines as susceptible to a Nigerian isolate of CABMV. Five resistant lines were identified by Taiwo *et al.* (1982) from among 58 cowpea cultivars. Collins *et al.* (1985) inoculated 16 cowpea cultivars with major cowpea viruses and rated their susceptibility and reported that Brown Crowder, Magnolia Blackeye, Mississippi Silver, Magnolia Purple, and Worthmore had promising levels of tolerance to BlCMV.

Lima *et al.* (1986) screened 248 cultivars for resistance to cowpea severe mosaic and CABMV. They were mechanically inoculated and classified based on symptomatology and serology as immune, highly resistant, resistant, susceptible or highly susceptible. Sreelakha (1987) screened ten lines of cowpea varieties of which the variety C-152 was found to be highly susceptible and the variety CG-

104 was tolerant to the disease.

Improved cowpea varieties have been developed at International Institute of Tropical Agriculture by combining multiple resistance to viruses. Cowpea varieties IT82 D-889, IT83S-818, IT83 D-442 and IT85F - 867-5 are resistant to cowpea mosaic comovirus (CPMV), CABMV, cowpea golden mosaic Gemini virus (CGMV), cucumber mosaic cucumo virus and southern bean mosaic virus (SBMV) (Singh *et al.*, 1987 ; Thottappilly *et al.*, 1988 ; Singh *et al.*, 1997).

Quindere and Barreto (1988) evaluated 81 genotypes of cowpea and classified seven as resistant to cowpea severe mosaic and CABMV. The variety CNC XII - OD was found to be resistant to virus and other pathogens and was also a good yielder. Gumodzoe (1993) reported the lines TVx 1850-01E, IT82 E-16, IT838-819, IT81D-1006, 58146, IT 82D704, IT82D 786 and TVx 323601 G resistant to one of the major viruses affecting cowpea such as CABMV, BICMV, BCMV, cowpea mottle virus, cowpea mosaic comovirus, southern bean mosaic sobemo virus, cowpea mild mottle carla virus, and cowpea strain of tobacco mosaic tobamo virus. Ndiaye *et al.* (1993) evaluated 35 genotypes and found TVu 401, TVu-498 P2, TVu-1000, TVu-1016-1, TVu -1582 resistant to all isolates of poty viruses affecting cowpea. Sudhakumari (1993) screened 59 varieties and identified V-317 and V-276 as resistant to CABMV. Miller and scheuring (1994) released Texas Pinkeye Purple Hull cowpea belonging to *V. unguiculata*. This was found to be immune to two American isolates of CABMV. Ponte *et al.* (1994) reported the reaction of cowpea cultivar 'Pampo' to three

viruses and showed that the variety was highly susceptible to cowpea severe mosaic and CABMV. Resistance was observed against cucumber mosaic comovirus. Bashir *et al.* (1995) screened 50 cowpea genotypes for identifying varieties resistant to BlCMV and found ten genotypes resistant to BlCMV. Ndiaye *et* al. (1995) released a *V. unguiculata* cv. Mouride with resistance to cowpea storage weevil, *Xanthomonas campestris* pv. *Vignicola* and CABMV. Bashir and Hampton (1996) tested 51 lines of cowpea by mechanical inoculation against seven diverse isolates of BlCMV. Five genotypes, IT 8082049, Big Boy, Corona, Serido, and Tennessee Cream were immune to all the seven isolates. Three genotypes TVu-2657, TVu-2740 and TVu-3435 were immune to six isolates. Kline and Anderson (1997b) released UARK-M2, progeny of single plant selections of symptomless *V. unguiculata* cv. Coronet from the commercial fields where high incidence of BlCMV was noticed. The variety was high yielding and resistant.

2.7 Biochemical changes of host-pathogen interaction

Khatri and Chenulu (1969) reported that reducing sugar content was not appreciably affected by cowpea mosaic virus in resistant and susceptible cowpea cultivars. Ramiah (1978) found that there was decreased synthesis of total carbohydrates in infected leaves of susceptible cowpea. He also observed that the trifoliate leaves showed reduction in the level of carbohydrates commencing from 10^{th} day after inoculation. Singh and Singh (1984) observed that the virus

infection decreased total sugar and starch in cowpea cultivars infected with southern bean mosaic virus. They found that both southern bean mosaic virus and cowpea mosaic virus reduced the carbohydrate fraction (total, reducing and non reducing sugars and starch) in cowpea cv. Pusa Dofasli. Sastry and Nayudu (1988) recorded a higher quantity of carbohydrate in hypersensitive cowpea cultivars infected with tobacco ring spot NEPO virus and suggested that the infected area acts as a metabolic sink. Mayoral *et al.* (1989) reported that the carbohydrate level was much reduced in infected leaf tissues.

Singh and Suhag (1982) observed reduction in chlorophyll content of mung and urd bean leaves infected with yellow mosaic virus, mung urd mosaic virus 1 and mung urd mosaic virus 2. Kaur *et al.* (1991) found that infection of yellow mosaic virus in soybean cultivars reduced the chlorophyll content. Wani *et al.* (1991) recorded reduction in total chlorophyll in sorghum leaves infected with maize mosaic virus.

Padma *et al.* (1976) reported that cowpea mosaic virus infected seeds contained a higher percentage of proteins, than healthy seeds. Singh *et al.* (1978) found that southern bean mosaic virus infection resulted in higher total nitrogen, total protein, nitrate and nitrite nitrogen than in healthy leaves of cowpea. Singh and Singh (1981) while investigating the changes in nitrogenous constituents of cowpea fruits due to cowpea mosaic virus found that there was an increase in total nitrogen, protein and nitrate nitrogen. Singh and Suhag (1982) reported reduction in protein content of mung and urd bean leaves infected with yellow

mosaic virus, mung urd mosaic virus 1 and mung urd mosaic virus 2. Further studies have also confirmed that virus infection increased the protein content in cowpea (Singh and Singh, 1984., Singh and Singh, 1987., Yadav, 1988., Yadav and Sharma, 1988., Mayoral *et al.*, 1989., Patil and Sayyd, 1991)

Ramiah (1978) found that there was no difference in phenol contents between healthy and inoculated leaves of MS 9804 and Co-1 He found that in variety Co-2 the inoculated leaves had higher content of phenolics than that of healthy leaves 40 days after inoculation. Ando *et al.* (1984) reported that fungitoxic phenolic compounds were released from cucumber mosaic virus infected cowpea protoplast. Rathi *et al.* (1986) assayed total phenol and other biochemical parameters in pigeonpea cultivars resistant and susceptible to sterility mosaic virus and reported that there was not much difference between varieties with respect to total phenol content. Kato *et al.* (1993) extracted and characterised two phenolic compounds from cowpea leaves infected with cucumber mosaic virus. Sohal and Bajaj (1993) reported increase in total phenols in both resistant and susceptible varieties of mung bean infected with yellow mosaic virus.

2.8 Defence related enzymes

Khatri and Chenulu (1970) studied the changes in the peroxidase enzyme activity in leaves of resistant and susceptible cowpea varieties and observed that peroxidase activity increased in both resistant and susceptible varieties, but was

higher in susceptible variety. Batra and Kuhn (1975) found that when primary leaves of hypersensitive soybean plants were infected with cowpea chlorotic mottle virus, the enzymes polyphenol oxidase and peroxidase increased 2-3 times. They found that the increase was concomitant with the development of acquired resistance. Wagih and Coutts (1982) reported that tobacco necrosis virus infected cowpea and cucumber showed alterations on soluble protein accompanied by an increase in the amounts of extractable peroxidase and polyphenol oxidase activity. Rathi *et al.* (1986) assayed peroxidase, polyphenol oxidase and isozymes of peroxidase in pigeonpea cultivars resistant and susceptible to sterility mosaic disease and noted less difference between two varieties with respect to peroxidase. Polyphenol oxidase activity increased in susceptible cultivar following infection. Resistance was characterised by the presence of specific isoperoxidase and proteins.

Sohal and Bajaj (1993) reported increase in polyphenol oxidase activity in resistant variety of mung bean infected with yellow mosaic virus. The activity decreased in susceptible variety. Phenylalanine ammonia-lyase activity decreased in both varieties.

2.9 Induction of systemic resistance

Application of chemicals to plants for the management of viral diseases is gaining importance. These chemicals induce the formation of some new substances in plants that impart resistance to plants against the virus. Studies

undertaken in this field reveal several promising antiphytoviral substances.

Prakash and Joshi (1979) reported maximum inhibition of cowpea banding mosaic virus in cowpea by 92 per cent when six sprays of gallic acid was given after virus inoculation. Maximum increase in incubation period was obtained when roots were dipped in salicylic acid for 24h prior to virus inoculation. Coutts and Wagih (1983) have observed the development of resistance against tobacco necrosis virus (TNV) in cowpea and tobacco plants by spraying polyacrylic acid four days before virus inoculation.

In *Nicotiana tabacum* cv. Samsun and *N. tabacum* cv. Samsun NN, treatment with aspirin induced PR-proteins and reduced the amount of tobacco mosaic virus (TMV) (White *et al.*, 1983). Caner *et al.* (1985) reported the antiviral activity of chemicals like polyacrylic acid, acyl clover, aspirin, distamycin A, EHNA, foscarnet and a pyrazino-pyrazine derivative for the control of bean golden mosaic virus on *Phaseolus lunatus* L. White *et al.* (1986) reported that the chemicals salicylic acid, manganese chloride ($MnCl_2$) and barium chloride ($BaCl_2$) induced resistance in Xanthi-nc tobacco leaves to TMV infection. Van Huijsduijnen *et al.* (1986) found that salicylic acid inhibited the systemic multiplication of alfalfa mosaic virus (AMV) in Samsun NN tobacco. Conti *et al.* (1988) found that a very low concentration of 0.01 per cent equivalent to 50 μM of acetyl salicylic acid (ASA) was effective in inducing resistance in *Datura* against TMV. The effect was greater when ASA was applied after the inoculation of virus.

Dawson (1984) reported that 9 - (2, 3 - Dihydroxy propyl) adenine (DHPA) inhibited TMV and cowpea chlorotic mottle virus (CCMV). Antiphytoviral activity of ribavirin, 2,4-dioxohexahydro - 1, 3, 5 triazine (DHT) compounds against tobacco viruses has been reported by Schuster (1986). Schuster and Holy (1988) found that DHPA and 3-(adinin - 9 yl)-2 hydroxy propanoic acid 2-methyl propylester (AHPA-MP) markedly inhibited the replication of potato virus x (PVX) in *N. tabacum* Samsun.

Rao *et al.* (1988) reported the induction of resistance by 100 µg ml^{-1} 12-tungstozincic (II) acid solution (TZA) in hypersensitive *C. amaranticolor* and non-hypersensitive, cowpea and *Vigna radiata* against mung bean severe mosaic and cowpea mosaic como virus. Bauer *et al.* (1993) observed that the chemical 1-(K-carboxyalkyl)-4,5-dimethyl imidazol-3-oxide was capable of restricting the activity of red clover mottle virus (RCMV) and alfalfa mosaic virus (AMV) in systematically infected host plants.

Hayati and Varma (1985) reported that 0.02 per cent validamycin, 0.01 per cent abomycin A, 0.2 per cent guanidine hydrochloride, 0.2 per cent guanidine carbonate, 0.1 per cent 8-azaguanine and 0.2 per cent 8-azaadenine suppressed symptom expression of tomato leaf curl virus (TLCV) on tomato. Kovalenko *et al.* (1993) reported the induction of resistance with mannan sulphates in hypersensitive host plants against TMV infection.

Many plants possess inhibitory substances against virus infection. Some

antiviral principles (AVPs) of plant origin have been known to induce resistance in plants against viruses.

Mariappan and Saxena (1983) found that custard apple seed oil and neem seed oil reduced the transmission of rice tungro virus. Infection by potato virus x in *C. amaranticolor* has been limited by the application of aqueous flower extracts of *Argemone mexicana, Azadiracta indica, Euphorbia mili, Jasminum sambac, Lantana indica, Nerium indicum* and *Vinca rosea*. The resistance developed in the host varied from 79.2 to 99.2 per cent (Rao *et al.*, 1985).

Neem seed oil and neem leaf extract have been reported to inhibit lesion production by mehanically transmitted viruses when mixed with the inoculum or when applied to test plants (Verma, 1974; Chowdhuri and Saha, 1985; Zaidi *et al*, 1988). Pre-inoculation application of neem oil reduced rice tungro virus infection in rice varieties with different levels of resistance (Aiyanathan and Narayanasamy, 1998).

Leaf and bark extracts of *A. indica* inhibited infection of cowpea mosaic virus (Singh *et al.*, 1988). Sreelakha and Balakrishnan (1988) found that cowpea aphid-borne mosaic virus infection could be effectively reduced by pre-inoculation spraying with leaf extracts of *Baugainvillea* sp. and *Eupatorium odoratum*. Mallika Devi (1990) observed that extracts of *A. indica, Boerhaavia diffusa, Calotropis gigantea, Curcuma longa, Phyllanthus niruri* and *Vitex negundo* caused inhibition of cowpea aphid-borne virus. Pre-treatment with neem oil reduced local lesion production by tobacco mosaic virus on *Nicotiana glutinosa*,

N. tabacum, var. Samsun NN, *C. amaranticolor* and *Datura stramonium*. (Roychoudhary and Jain, 1993). Reghunath and Gokulapalan (1996) reported that neem oil emulsion was capable of reducing the symptoms of viral diseases in cowpea.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 ISOLATION AND CHARACTERIZATION OF THE VIRUS

3.1.1 Symptomatology

Seeds of cowpea (*Vigna unguiculata* (L.) Walp) were obtained from Instructional Farm, College of Agriculture, Vellayani. They were sown in pots containing potting mixture of sand, soil and cowdung in the ratio of 1 : 1 : 1. Leaves showing severe mosaic symptoms were collected from field and the culture of the virus was maintained by repeated transfers on cowpea cultivar Sharika in insect proof glasshouse by mechanical inoculation using 0.01 M phosphate buffer pH 7.0 at periodic intervals of 4-6 weeks at 24-32^{0}C. Symptomatology was studied by observing the development of symptoms in naturally infected as well as artificially inoculated cowpea plants.

3.1.2 Transmission

3.1.2.1 Mechanical transmission

Culture of the virus was collected from field and maintained in the insect proof glasshouse.

Sap transmission was conducted using different buffers for extraction. The buffers used were 0.1 M citrate buffer (pH 6.2), 0.1 M citrate-phosphate buffer (pH 7.0), 0.01 M phosphate buffer pH 7.0, 0.1M tris buffer (pH 7.2), and 0.1 M borate buffer (pH 7.6). In all sap inoculation studies 600 mesh carborundum powder was used as abrasive.

Sap was extracted from young leaves showing severe mosaic symptom. One part of the leaf tissue was homogenised with one part of cold buffer using a chilled mortar and pestle. The homogenate was strained through a thin layer of cotton and maintained in an ice box and immediately used for inoculation.

Inoculation was done on the leaves of the local lesion host, *Chenopodium amaranticolor*. Plants at 8-15 leaf stage were chosen. Leaves at the middle portion (fourth to eighth leaf) of the plant were inoculated. Prior to inoculation, leaves were uniformly dusted with carborundum powder. Test plants were inoculated with a cotton swab or forefinger moistened with the inoculum, by gently rubbing on the upper surface of the fully opened leaves. The excess sap on the surface was rinsed off after ten seconds using distilled water.

The buffer which gave the maximum number of local lesions on *C. amaranticolor* was chosen for extraction of sap to inoculate on test plants. 0.01 M phosphate buffer (pH 7.0) was used for all transmission studies. The procedure for extraction and inoculation of sap on cowpea plants was as mentioned above. The plants were kept in glasshouse condition for 6-7 days for the development of symptoms.

3.1.2.2 Seed transmission

Seed transmission studies were conducted using 135 seeds collected from artificially inoculated and infected plants. They were sown in pots kept in an insect proof glasshouse and examined for the development of symptoms in the primary and first trifoliate true leaves.

3.1.2.3 Insect transmission

Insect transmission studies were conducted using three species of aphids viz., *Aphis craccivora* Koch, *Aphis gossypii* and *Myzus persicae*. Colonies of aphids were established in cowpea (*V. unguiculata*) from apterous single clonal line of the species. Cowpea plants, each infested with a single aphid, were maintained in separate cages in glasshouse for the multiplication of the aphids. The old plants inside the cages were replaced from time to time with younger ones.

Pre-acquisition fasting of one hour and an acquisition access period of ten minutes were given to ten non-viruliferous aphids. These aphids were carefully released to ten plants using a moistened camel hair brush and allowed to feed for 24 hours. The aphids were killed using 0.1 per cent quinalphos.

3.1.3 Physical properties of the virus

3.1.3.1 Dilution end point (DEP)

Infected cowpea leaves of known weight were homogenised in chilled mortar and pestle by adding 1 ml of 0.01 M phosphate buffer (pH 7.0) for every one gram of the leaf sample. The homogenate was strained through a thin layer of cotton. Serial dilutions of the sap, viz., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were made as follows. Six test tubes were kept in a row in a test tube rack. Nine ml of buffer was dispensed into each of five test tubes starting from the second test tube by using a clean pipette. The prepared extract was poured into the first test tube. One ml of the sap from this tube was transferred to the second tube with nine ml buffer to get a dilution of 10^{-1}. It was thoroughly mixed and one ml of 10^{-1}

dilution was transferred to the next test tube to obtain a dilution of 10^{-2}. This process was continued till a dilution of 10^{-5} was obtained. The dilutions were inoculated on fully opened leaves of *C. amaranticolor*. Five replications were maintained for each treatment. The inoculated leaves were labelled and kept under insect proof conditions and observed for the development of local lesions. Leaves treated with buffer were kept as control.

3.1.3.2 Thermal inactivation point (TIP)

Sap was extracted from infected leaves of cowpea as mentioned in the above experiment. Three ml of sap was pipetted into a thin walled glass test tube. Care was taken not to smear the upper part of the test tube. This was placed in a water bath with thermostat arrangement. The level of water was maintained three cm above the level of sap in the tubes. Three ml aliquots of the sap were treated for ten minutes each at 35, 40, 45, 50, 55, 60, 65 and 70^0 C. After each treatment, the tubes were removed and cooled immediately. Control was kept at room temperature (28 ± 4^0 C). The samples were inoculated on five fully opened leaves of *C. amaranticolor*. Observations on number of local lesions produced were recorded.

3.1.3.3 Longevity *in vitro* (LIV)

Infected leaves were ground with mortar and pestle and the homogenate was filtered through a thin layer of cotton. One ml each of this sap was pipetted into 18 test tubes and closed with cotton. Nine tubes were kept at room

temperature (28 ± 4^0 C) and nine in a refrigerator (8^{0}C). One tube each containing the sap of each treatment was taken after specific periods viz., 2,4, 6, 8, 12, 24, 48 and 72 h and inoculated on the leaves of *C. amaranticolor*. Inoculation immediately after extraction was treated as control. The plants were observed for the development of local lesions.

3.1.4 Virus-vector relationship

Cowpea leaves showing symptoms of severe mosaic were collected from field and culture of the virus was maintained in insect-proof glasshouse by repeated transfers to healthy plants by mechanical inoculation. Virus free aphid colonies were maintained on healthy cowpea plants as mentioned in 3.1.2.3. Adult apterous aphids were used for inoculation trials. After the required inoculation access period, the aphids were killed by spraying 0.1 per cent quinalphos.

3.1.4.1 Acquisition access period

This experiment was conducted using *A. craccivora*. A large number of non viruliferous aphids were collected and given a pre-acquisition starvation of one hour. Batches of ten aphids were given acquisition access period of 1, 5, 10 and 15 minutes on diseased source before transferring them to ten healthy cowpea plants. The aphids were allowed to remain for 24 hours on test plants before they were killed.

3.1.4.2 Inoculation access period

Non viruliferous aphids were given one hour pre-acquisition starvation and an acquisition access period of 10 minutes. Batches of ten viruliferous aphids were then transferred to ten healthy cowpea plants. Each batch was given separate inoculation access periods of 1, 5, 10 and 15 minutes.

3.1.4.3 Effect of pre and post-acquisition fasting

3.1.4.3.1 Pre-acquisition fasting

Batches of ten aphids were starved for 30 minutes, 1h, 1½h and 2h respectively. They were provided an acquisition access period of ten minutes and released on healthy cowpea plants and given inoculation access for a period of 24 h.

3.1.4.3.2 Post-acquisition fasting

A large number of non-viruliferous aphids were collected from healthy cowpea plants and starved for 1h after which they were given an acquisition access of ten minutes. Batches of ten viruliferous aphids were given post-acquisition fasting of 30 min, 1h, 1½h and 2h. Each batch was released on ten healthy cowpea plants and allowed to feed for 24h.

3.1.4.4 Minimum number of aphids

Single aphid as well as aphids in groups of 3 and 10 were collected from non-viruliferous colony and starved for one hour. They were given an acquisition

access period of 10 minutes before they were introduced on healthy cowpea plants. On the healthy plants, they were allowed to feed for 24 h.

3.1.5 Serology

3.1.5.1 Preparation of antigen

The virus was clarified as described by Khurana *et al.* (1987) with slight modification. The culture of the virus was maintained in cowpea (*V. unguiculata*) plants and systemically infected leaves were used as virus source. Freshly harvested leaves were ground in chilled mortar and pestle with 1 : 2 (w/v) 0.1 M phosphate buffer (pH 7.2) containing 15 mM each of sodium diethyl dithiocarbamate (DIECA) and disodium ethylene diamine tetra acetate (EDTA). The homogenate was strained through a thin layer of cotton. The filtrate was clarified by centrifugation at 5000 rpm for 15 minutes in a refrigerated high speed centrifuge (Hettich EBA 12 R). The supernatant was clarified by mixing with 1 : 1(v/v) mixture of chloroform and N-butanol. It was kept in a shaker for 30 minutes. This was recentrifuged at 5000 rpm for 15 minutes. The aqueous phase was collected and the virus was precipitated by the addition of 4 per cent poly ethylene glycol (PEG 6000) and 0.2 M sodium chloride (NaCl), initially by stirring for 15 minutes and then incubating at 4^0C for 75-90 minutes. The precipitate was pelleted at 10 000 rpm in a refrigerated table top microcentrifuge at 4^0 C (Hettich MIKRO 24-48R) for 30 minutes. This was resuspended in 0.01 M phosphate buffer (pH 7.0) containing 5 m M EDTA and 0.5 M urea and clarified by centrifugation at 5000 rpm for five minutes. The final preparation was dialyzed overnight against one

litre of 0.01 M phosphate buffer at 4^0C. This was concentrated by keeping in a sucrose pack.

The concentrated virus preparation was used as antigen source for immunization of rabbits.

3.1.5.2 Preparation of antiserum

New Zealand white female rabbits (9-12 months old) were used for production of antiserum. The purified virus preparation was emulsified with 1 : 1 (v/v) Freund's incomplete adjuvant (Difco). The rabbits were immunised by four intramuscular injections at weekly intervals. The rabbits were bled through marginal ear vein 15 days after the last injection. Blood was collected in 15 ml tubes and allowed to coagulate. The coagulated blood clot was loosened with the help of a sterilized glass rod and samples were kept overnight at 4^0C. The clear serum was decanted and centrifuged at 5000 rpm for 30 minutes at 4^0C. Purified serum was pipetted out using a micropipette and dispensed into one ml vials. Sodium azide was added to the clarified serum to prevent microbial contamination. Vials were sealed, labelled and stored under refrigerated conditions.

3.1.5.3 Serodiagnosis and electron microscopy

3.1.5.3.1 Ouchterlony agar double-diffusion tests

Serological petri dishes were used for conducting this test. The petri dishes were coated with a thin layer (0.1 mm) of 0.8 per cent agarose containing

0.85 per cent sodium chloride, 0.5 per cent sodium dodecyl sulphate (SDS) and 1.0 per cent sodium azide and allowed to solidify. Then the plates were placed over a marked paper, showing the position of wells, at six equidistant position at 60^0 angle around the centre point and 0.5 mm distance between the outer edge. Tubes of 0.5 mm diameter were placed in each position without disturbing the lower layer. Above this layer, melted agarose was again added to a thickness of 3 mm. After proper solidification, tubes were gently pulled out to form wells on the agarose gel. Each well was labelled from 1 to 7. The central well (No. 1) of each plate was loaded with 30 µl of undiluted antiserum using a micropipette. The surrounding wells, 2, 4, 6 and 7 received 30 µl each of extracts from diseased plants, well 3 received healthy plant sap and well 5 received extraction buffer.

The petri dishes were incubated under a humid glass jar with least disturbance. They were examined periodically for the appearance of characteristic precipitin bands. Three replications were maintained.

3.1.5.3.2 Enzyme linked immunosorbent assay (ELISA)

ELISA for the detection of cowpea viruses was done using the polyclonal and monoclonal antibodies received from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Double antibody sandwich ELISA (DAS-ELISA) and Direct Antigen Coated ELISA (DAC-ELISA) were conducted. Antigen was obtained from the virus culture maintained in the glasshouse through mechanical transmission from diseased to healthy plants. The serodiagnosis of virus was carried out following the procedure described by Huguenot *et al.* (1992).

3.1.5.3.2.1 DAS - ELISA

100 µl each of purified IgG (polyclonal antibodies) raised specific to CABMV and BlCMV at 1 : 500 dilution in coating buffer was dispensed into wells of Nune immunological plates. Treatments were replicated three times. The plates were incubated at 37^0 C for 2 h. After incubation the wells were washed with phosphate buffered saline-tween (PBS-T) three times each for a duration of three minutes. The plates were tapped on a blotting paper to remove the buffer. Blocking was done with 200 µl of 1 per cent bovine serum albumin (BSA) dissolved in PBS-T. This was incubated for 30 minutes at 37^0C. After incubation blocking agent was removed and 100 µl of test samples prepared in PBS-T at different dilutions (1 : 5, 1 : 10 and 1 : 100) were dispensed into respective wells. Suitable control wells were maintained using extracts from healthy tissues. The treatments were replicated six times each. The plates with the antigen were incubated at 37^0C for 2 h. After incubation and washing with PBS-T, monoclonal antibodies were added. 10G5, the monoclonal antibody specific to BlCMV and 5H5, the monoclonal antibody specific to CABMV at a dilution of 1 : 10000 in PBS-T were added and incubated overnight at 4^0C. This was followed by washing with PBS-T. After this 100 µl of alkaline phosphatase conjugated antimouse immunoglobulin diluted in PBS-T (1 : 10 v/v) was added. Plates were incubated for 2h at 37^0C. Wells were washed with PBS-T as before. The substrate p - nitrophenyl phosphate (p-NPP) in diethanolamine buffer (1 mg per ml) was added to each well (100 µl per well) and incubated for 1 h at 37^0C. Reaction was stopped by adding

50 µl of 4 per cent sodium hydroxide (NaOH). The absorbance was measured at 405 nm in an ELISA reader (MULTISKAN MS).

3.1.5.3.2.2 DAC - ELISA

The infected leaf samples were ground in PBS-T (1 : 5 w/v). The homogenate was centrifuged at 5000 rpm for 10 minutes at 4^0C. Antigen was dispensed at the rate of 100 µl into Nune immunological plates. The treatments were replicated thrice. After incubation for 2 h at 37^0C, the wells were washed with PBS-T as in DAS-ELISA (3.1.5.3.2.1). Blocking was done with 200 µl of 1 per cent BSA for 30 minutes at 37^0C. Monoclonal antibodies 10G5 and 16G5 which are specific to BICMV and monoclonal antibodies 5H5, 7D9, and 6C10 specific to CABMV were used as detecting antibodies. The treatments were replicated twice. The antibodies at 1 : 10000 dilution in PBS-T were added and incubated overnight at 4^0C. The plates were washed with PBS-T and then treated with 100 µl of alkaline phosphatase conjugated antimouse immunoglobulin diluted in PBS-T (1 : 10 v/v) and incubated for 2h at 37^0C. Wells were washed with PBS-T as before. The substrate p-nitrophenyl phosphate (p-NPP) in diethanolamine buffer (1 mg per ml) was added to each well (100 µl per well and incubated for 1 h at 37^0C. Reaction was stopped by adding 50 µl of 4 per cent NaOH. The absorbance was measured at 405 nm, in an ELISA reader.

3.1.5.3.3 Electron microscopy

Electron microscopic studies were carried out using crude tissue extract from severely infected leaf samples. Tissue homogenate was prepared by grinding 50-200 mg leaf sample with 50-200 µl of 0.01 M phosphate buffer (pH 7.0) using in a mortar and pestle. Fifty microlitre of the tissue homogenate was placed on a parafilm and a carbon coated copper grid was floated over the drop with its film side down on it. This was incubated in a humid petri dish at room temperature for 30 minutes. After incubation the grid was washed with 30 drops of distilled water and the excess water was drained with a strip of filter paper. The grid was immediately floated on a drop of two per cent aqueous uranyl acetate for 30 seconds. Excess stain was drained from the grid, air dried and examined under JEOL-JEM 100 SX transmission electron microscope.

3.2 DISEASE RESISTANCE

3.2.1 Screening the sources of virus resistance

Sixty five varieties were screened for their resistance to cowpea aphid-borne mosaic virus. The seeds of different varieties were obtained from Kerala Horticultural Development Programme (KHDP), Tamil Nadu Agricultural University (TNAU), National Bureau of Plant Genetic Resources (NBPGR) and from farmers field. The seeds of the varieties were sown in pots filled with potting mixture containing soil, sand and cowdung in the ratio of 1 : 1 : 1. The seedlings in the primary leaf stage were mechanically inoculated with the sap

extracted from diseased leaves. The varieties were screened based on the 0 - 5 scale developed by Bos (1982) as mentioned below.

0 = no symptoms

1 = slight vein clearing, very light mottling of light and dark green colour in younger leaves - Resistant (R)

2 = mottling of leaves with light and dark green colour - Medium resistant (MR)

3 = blisters and raised surface on the leaves - Medium susceptible (MS)

4 = distortion of leaves - susceptible (S)

5 = stunting of the plant with negligible or no flowering and fruiting - Highly susceptible (HS)

Based on the rating, vulnerability index (VI) was calculated using the following equation.

$$VI = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5) \times 100}{n_t \quad (n_c - 1)}$$

VI = vulnerability index

$n_0, n_1, \ldots\ldots n_5$ = number of plants in the category 0, 1, 2, 3, 4, 5

n_t = total number of plants

n_c = total number of categories

3.2.1.1 Incorporation of virus resistance

The most popular variety of cowpea, Sharika is high yielding and quite promising. However, this is susceptible to the cowpea aphid-borne mosaic virus. With the objective of incorporation of resistance, this variety was crossed with two resistant lines, Co-6 and Co-Selection obtained from Tamil Nadu Agricultural University, Coimbatore. Sufficient quantity of F_1 and F_2 generations were developed from both the crosses. The parents, F_1 and F_2 were evaluated for resistance and other characters. The seggregating F_2 population was grouped into vegetable types, grain types, resistant and susceptible types. It was also grouped into lines seggregating jointly for virus resistance and vegetable quality. From this, lines possessing vegetable quality and virus resistance were identified for further studies.

3.2.2 Biochemical changes of host-pathogen interaction

Biochemical analysis of resistant and susceptible varieties were carried out. The variety Co-6, obtained from TNAU which showed resistance to the virus was selected as the resistant variety for analysis. The variety Sharika, from College of Agriculture, Vellayani was used as the susceptible variety. The seeds of both the varieties were sown on the same day in pots kept in glasshouse. The seedlings of both varieties were sap inoculated on the same day at the primary leaf stage. Samples were taken one day, five days, ten days and fifteen days after inoculation.

Biochemical analysis was done to estimate changes in total carbohydrate, chlorophyll, protein, phenol and activities of defence related enzymes like peroxidase, polyphenol oxidase and phenyl alanine ammonialyase.

3.2.2.1 Estimation of total carbohydrate

Total carbohydrate content was estimated by Anthrone method (Hedge and Hofreiter, 1962). Samples of 100 mg each were weighed out and hydrolysed with five ml of 2.5 N hydrochloric acid (HCl) at 100^0 C in a water bath. The hydrolysate was neutralised with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged at 5000 rpm for 15 minutes. From the supernatant 0.5 ml aliquot was taken and made up to one ml by adding distilled water. To this four ml anthrone reagent was added and heated for eight minutes at 100^0 C in a water bath. This was cooled rapidly and absorbance was measured at 630 nm in a spectrophotometer (Systronics UV-VIS Spectrophotometer 118). Amount of carbohydrate present was calculated from standard graph prepared using glucose and expressed in terms of milligrams of glucose equivalent per gram of leaf tissue on fresh weight basis.

3.2.2.2 Estimation of chlorophyll

Chlorophyll was estimated by the method described by Arnon (1949). One gram of leaf sample was finely cut and ground in a mortar with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 5000 rpm for five minutes and the supernatant was transferred to a 100 ml volumetric flask. The above

procedure was continued till the residue became colourless. The final volume in volumetric flask was made up to 100 ml. Absorbance of the solution at 645, and 663 nm was read in a spectrophotometer against the solvent (80 per cent acetone) as blank. The chlorophyll content was calculated using the following equations and expressed as milligrams chlorophyll per gram tissue.

$$\text{Total chlorophyll} = 20.2\,(A645) + 8.02\,(A663) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll a} = 12.7\,(A663) - 2.69\,(A645) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9\,(A645) - 4.68\,(A663) \times \frac{V}{1000 \times W}$$

3.2.2.3 Estimation of protein

Total soluble protein content was estimated as per the procedure described by Bradford (1976). One gram of leaf sample was homogenised in 10 ml, 0.1 M sodium acetate buffer (pH 4.7) and centrifuged at 5000 g for 15 minutes at 4^0C. The supernatant was saved for estimation of soluble protein. The reaction mixture consisted of 0.5 ml enzyme extract, 0.5 ml distilled water and 5 ml of diluted (5 times) dye solution. The absorbance was read at 595 nm in a spectrophotometer against reagent blank. Bovine serum albumin was used as the protein standard. The protein content was expressed as microgram albumin equivalent of soluble protein per gram on fresh weight basis.

3.2.2.4 Estimation of phenol

The phenol content was estimated following the procedure described by Bray and Thorpe (1954). One gram leaf sample was ground in 10 ml of 80 per cent ethanol. The homogenate was, centrifuged at 10000 rpm for 20 min, supernatant was saved and residue was extracted with five times the volume of 80 per cent ethanol and centrifuged. The supernatant was saved and evaporated to dryness. The residue was dissolved in 5 ml distilled water. An aliquot of 0.3 ml was pipetted out and made up to three ml with distilled water. Folin - Ciocalteau reagent (0.5 ml) was added and two ml of 20 per cent sodium carbonate solution was added to each tube after three minutes. This was mixed thoroughly and kept in boiling water for one minute. This was cooled and absorbance was measured at 650 nm against reagent blank. Standard curve was prepared using different concentrations of catechol and expressed in catechol equivalents as microgram per gram leaf tissue on fresh weight basis.

3.2.2.5 Estimation of defense related enzymes

3.2.2.5.1 Estimation of peroxidase (PO)

Peroxidase activity was determined according to the procedure described by Srivastava (1987).

Leaf sample of 200 mg was homogenised in one ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenizaiton was done at 4^{0}C using a mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for

15 minutes at 4^0C. The supernatant was used as the enzyme extract for the assay of PO activity.

The reaction mixture consisting of 1 ml 0.05 M pyrogallol, and 50 µl enzyme extract was taken in both reference and sample cuvettes, mixed and kept in a spectrophotometer and the reading was adjusted to zero at 420 nm. The enzyme reaction was started by adding 1 ml one per cent hydrogen peroxide (H_2O_2) into sample cuvettes and change in absorbance was measured at 30 seconds interval.

3.2.2.5.2 Estimation of polyphenol oxidase (PPO)

Polyphenol oxidase activity was determined as per procedure given by Mayer *et al.* (1965). The enzyme extract was prepared as per the procedure given for the estimation of peroxidase.

The reaction mixture contained 1.0 ml of 0.1 M sodium phosphate buffer pH 6.5 and 50 µl of enzyme extract. The cuvettes were placed in a spectrophotometer and absorbance was set to zero. The reaction was started after adding one ml of 0.01 M catechol. The changes in absorbance was recorded at 495 nm and PPO activity was expressed as changes in the absorbance of the reaction mixture per minute per gram on fresh weight basis.

3.2.2.5.3 Estimation of phenylalanine ammonialyase (PAL)

PAL activity was analysed based on the procedure described by Dickerson *et al.* (1984). The enzyme extract was prepared by homogenising one gram leaf

sample in five ml of 0.1 M sodium borate buffer (pH 8.8) containing a pinch of PVP, using chilled mortar and pestle. The homogenate was centrifuged at 10000 rpm for 20 minutes, at 4^0C. The supernatant was used for the assay of PAL activity. The reaction mixture contained three ml of 0.1 M sodium borate buffer pH 8.8, 0.2 ml enzyme extract and 0.1 ml of 12 mM L-phenyl alanine prepared in the same buffer. The blank contained three ml of 0.1 M sodium borate buffer pH 8.8 and 0.2 ml enzyme extract. The reaction mixture and blank was incubated at 40^0C for 30 minutes and reaction was stopped by adding 0.2 ml of 3N hydrochloric acid (HCl). The absorbance was read at 290 nm in a spectrophotometer.

PAL activity was expressed as microgram of cinnamic acid produced per minute per gram on fresh weight basis.

3.2.3 Induction of systemic resistance

Chemicals like salicylic acid, barium chloride ($BaCl_2$), manganese chloride ($MnCl_2$) and neem oil are known to induce resistance against viral infection in plants. This study was undertaken to evaluate these for the management of CABMV.

3.2.3.1 Bioassay in local lesion host

The efficacy of the chemicals and neem oil were evaluated in the local lesion host of the virus, *C. amaranticolor*. Both pre and post-inoculation treatments were done. In pre-inoculation treatment, the materials were sprayed prior to mechanical inoculation of virus. In post-inoculation treatment, the

materials were sprayed after inoculating the virus. Chemicals were prepared at a concentration of 50, 100 and 150 mg l^{-1} and neem oil emulsion at 10 per cent concentration. The treated plants were kept in glasshouse. Local lesions were recorded for evaluating the efficacy of chemicals and neem oil. From this, pre cent inhibition was calculated based on the formula.

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = number of lesions on control leaves

T = total number of lesions on treated leaves.

Control leaves were maintained without chemical and oil treatment.

3.2.3.2 Bioassay in cowpea plants

Induction of resistance was also done on cowpea plants (*V. unguiculata*) variety Sharika. A pot culture experiment was laid out in CRD with three replications. The chemicals and concentrations were same as that of previous experiment. Both pre and post-inoculation treatment of chemicals were done. A control was also maintained. The application was done at the primary leaf stage. Spraying of chemicals were done at ten days intervals and the effect of chemicals on the expression of symptom was recorded.

3.2.4 Statistical analysis

The data were analysed statistically for interpretation of results.

RESULTS

4. RESULTS

4.1 Symptomatology

Mechanically inoculated seedlings of cowpea cv. Sharika expressed symptoms of viral infection seven days after inoculation. Initial symptoms appeared in the form of vein clearing on the newly emerged trifoliate leaves. Typical light and dark green mottling was produced in all subsequent leaves. As the infection progressed, mosaic pattern with light green, dark green and yellow areas developed. Characteristic vein banding was also noticed. Severely infected leaves became blistered, distorted and reduced in size. Generally, the plants were stunted with shortened internodes, reduced flowering and fruiting (Plate 1, 2).

Symptoms in naturally infected plants were characterised by severe mosaic, dark green vein banding, blistering, distortion and cupping of leaves. The leaves were reduced in size. Growth of the plants were stunted.

4.2 Transmission

4.2.1 Mechanical transmission

The virus was transmitted successfully through mechanical inoculation. this was done both on cowpea and *C. amaranticolor*, the local lesion host of the virus (Plate 3). Infectivity of the virus in different buffers was rated based on the development of local lesions. Phosphate buffer (0.01 M, pH 7.0) gave maximum number of lesions (31.8) compared to other buffers tested (Table 1 Fig.1).

Plate 1. Systemic symptom on cowpea plants

Plate 2. Initial symptom on young trifoliate leaves

0 : Healthy
1 : Vein clearing


0
1

Table 1 Effect of different buffers on infectivity of CABMV on *C. amaranticolor*

Sl. No.	Buffer	Molar concentration	pH	Mean number of lesions
1.	Citrate	0.1	6.2	6.8
2.	Citrate phosphate	0.1	7.0	3.0
3.	Phosphate	0.01	7.0	31.8
4.	Borate	0.1	7.6	13.6
5.	Tris	0.1	7.2	5.2

Fig. 1 Effect of different buffers on infectivity of CABMV on *C. amaranticolor*

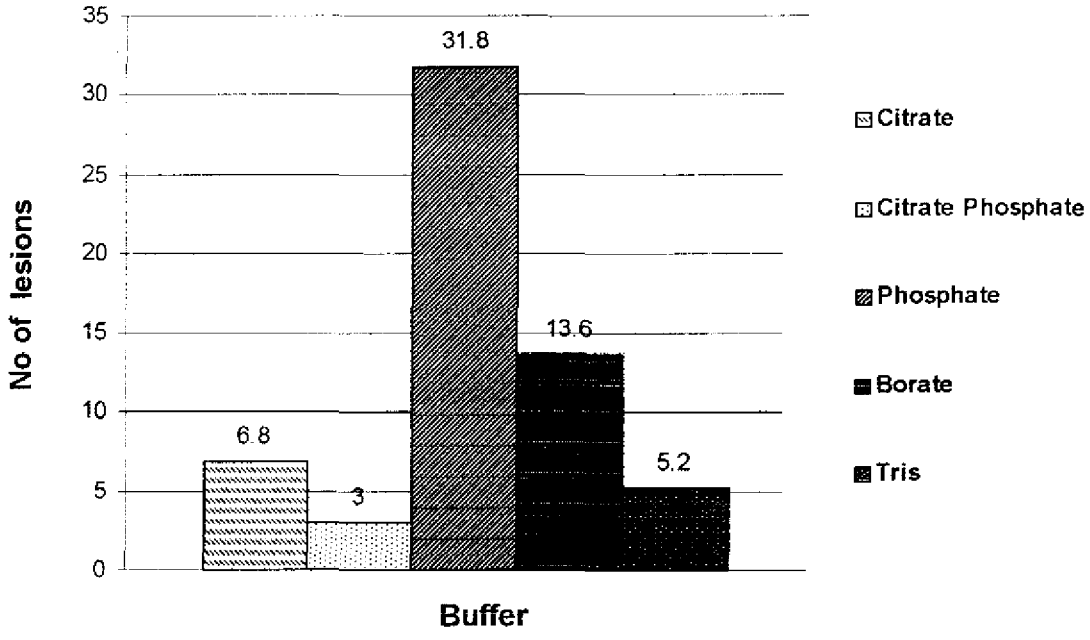


Plate 3. Local lesions on the leaf of *C. amaranticolor*


HEALTHY
DISEASED

Table 2 Insect transmission of CABMV

Sl. No.	Vector	Number of plants inoculated	Number of plants infected	Per cent transmission
1.	*A. craccivora*	10	5	50
2.	*A. gossypii*	10	2	20
3.	*M. persicae*	10	3	30

Extraction of sap for inoculation on cowpea seedlings was done using 0.01 M phosphate buffer (pH 7.0). Systemic infection developed in the inoculated plants seven days after inoculation.

4.2.2 Insect transmission

Insect transmission studies were carried out using three aphis species viz., *A. caraccivora*, *A. gossypii* and *M. persicae* to find out the comparative efficacy of transmission. The susceptible variety Sharika was used for the investigation. The results indicated that the virus was readily transmitted by the three aphid species tested. The observations showed that *A. craccivora* gave the highest per cent transmission (50) followed by *M. persicae* (30) and *A. gossypii* (20) (Table 2).

4.2.3 Seed transmission

Out of the 135 seeds collected from infected cowpea plants 118 germinated and 13 seedlings expressed mosaic symptoms on the primary leaves. The per cent seed transmission of cv. Sharika was found to be 11.

4.3 Physical properties

4.3.1 Dilution end point (DEP)

Serial dilutions of the infected sap was made, viz., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The different dilutions were used for inoculation on five fully opened leaves of *C. amaranticolor*. Maximum lesions were obtained for the undiluted sap (27.0) and minimum for 10^{-3} dilution. The data indicated that the dilution end point of the virus was between 10^{-3} and 10^{-4} (Table 3, Fig. 2).

Table 2 Insect transmission of CABMV

Sl. No.	Vector	Number of plants inoculated	Number of plants infected	Per cent transmission
1.	*A. craccivora*	10	5	50
2.	*A. gossypii*	10	2	20
3.	*M. persicae*	10	3	30

4.3.2 Thermal inactivation point (TIP)

The inoculum was subjected to different temperatures, viz., 35, 40, 45, 50, 55, 60, 65 and 70^0 C. The untreated and treated samples were inoculated on five fully opened leaves of *C. amaranticolor*. The results indicated that the virus was inactivated at a temperature range between 60 - 65^0 C (Table 4, Fig. 3, Plate 4 and 5).

4.3.3 Longevity *in vitro* (LIV)

The inoculum was kept at room temperature (28 ± 4^0 C) and also in a refrigerator (8^0 C). Inoculation was done on the leaves of *C. amaranticolor* at periods of 0, 2, 4, 6, 8, 12, 24, 48 and 72 h. The results indicated that the virus was infective for 4 h at room temperature and 6 h under refrigerated condition (Table 5, 6, Fig. 4).

4.4 Virus - vector relationship

This study was conducted to standardise the nature of transmission of the virus by its aphid vector, *A. craccivora*. Acquisition access period, inoculation access period, effect of starvation on the transmission of the virus and number of aphids required for effective transmission were studied (Plate 6).

4.4.1 Acquisition access period

Studies on acquisition access period indicated that the virus was acquired by the vector within a short period of five minutes. The results showed that the

Table 3 Dilution end point (DEP) of CABMV on *C. amaranticolor*

Sl. No.	Dilutions	Mean number of lesions
1.	0	27.0
2.	10^{-1}	8.6
3.	10^{-2}	8.4
4.	10^{-3}	5.4
5.	10^{-4}	0

Table 4 Thermal inactivation point (TIP) of CABMV on *C. amaranticolor*

Sl. No.	Temperature (^{0}C)	Mean number of lesions
1.	Control	63.8
2.	35	47.8
3.	40	32.4
4.	45	18.2
5.	50	8.8
6.	55	8.2
7.	60	0.2

Fig. 2 Dilution end point (DEP) of CABMV on *C. amaranticolor*

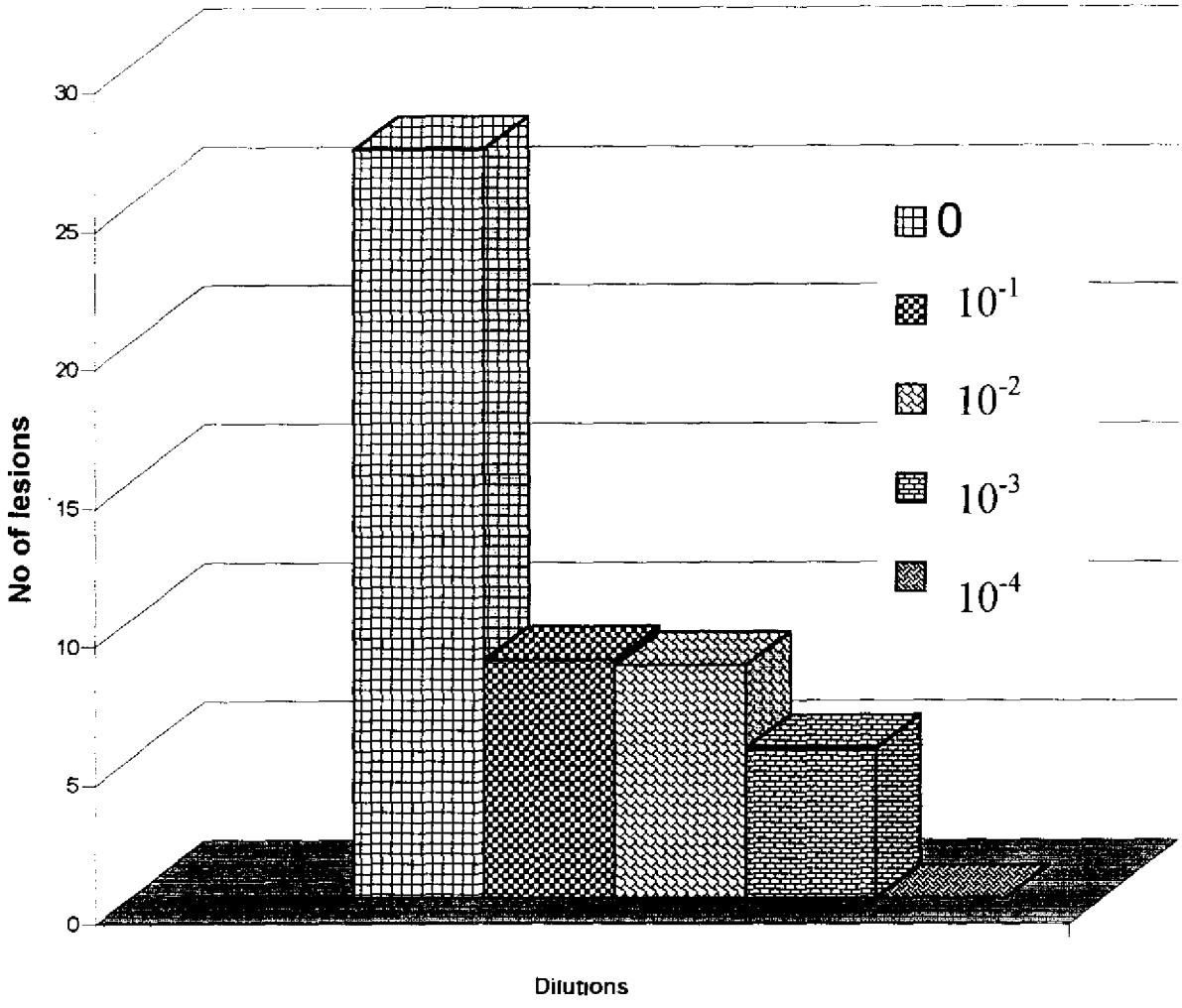


Fig. 3 Thermal inactivation point (TIP) of CABMV on *C. amaranticolor*

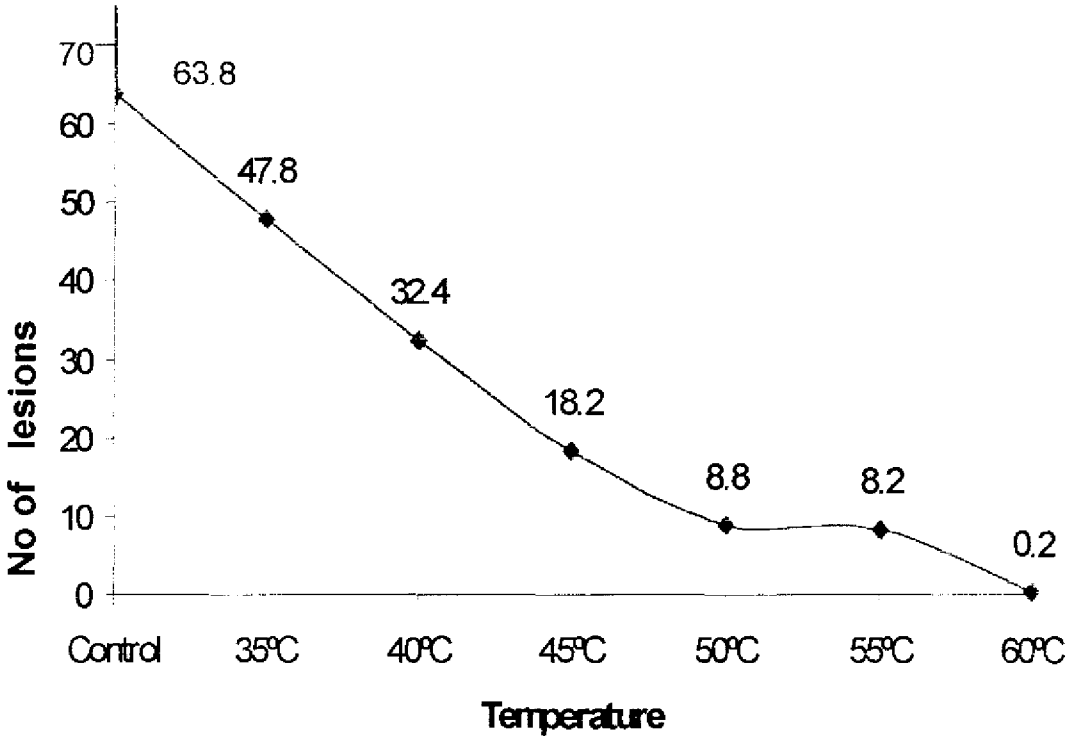
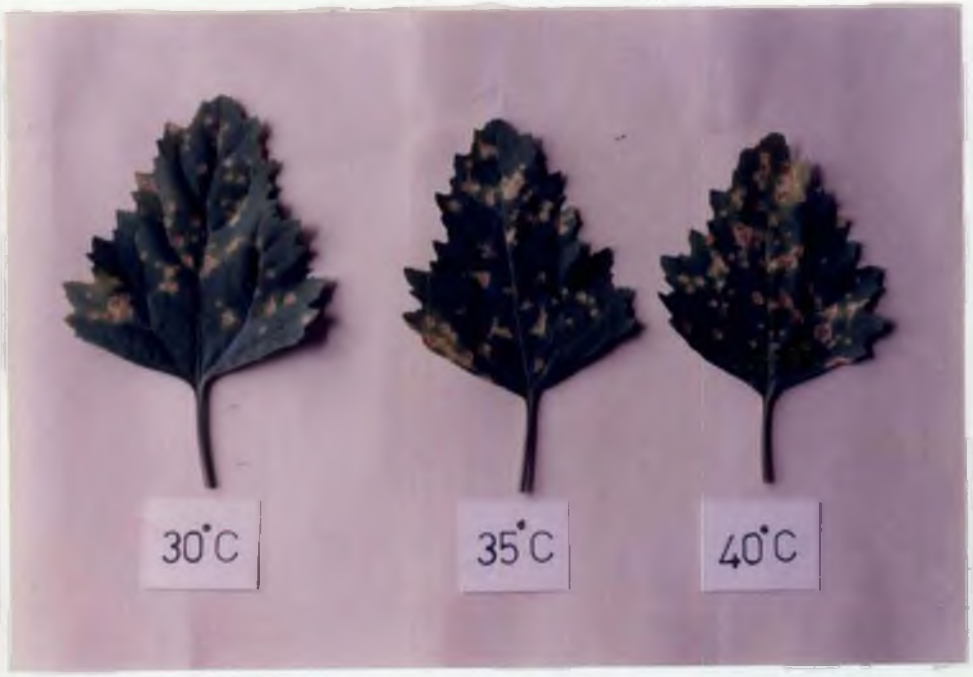


Plate 4. Local lesions on leaves of *C. amaranticolor* caused by infected sap subjected to different temperatures

Plate 5. Local lesions on leaves *C. amaranticolor* caused by infected sap subjected to different temperatures


30°C
35°C
40°C

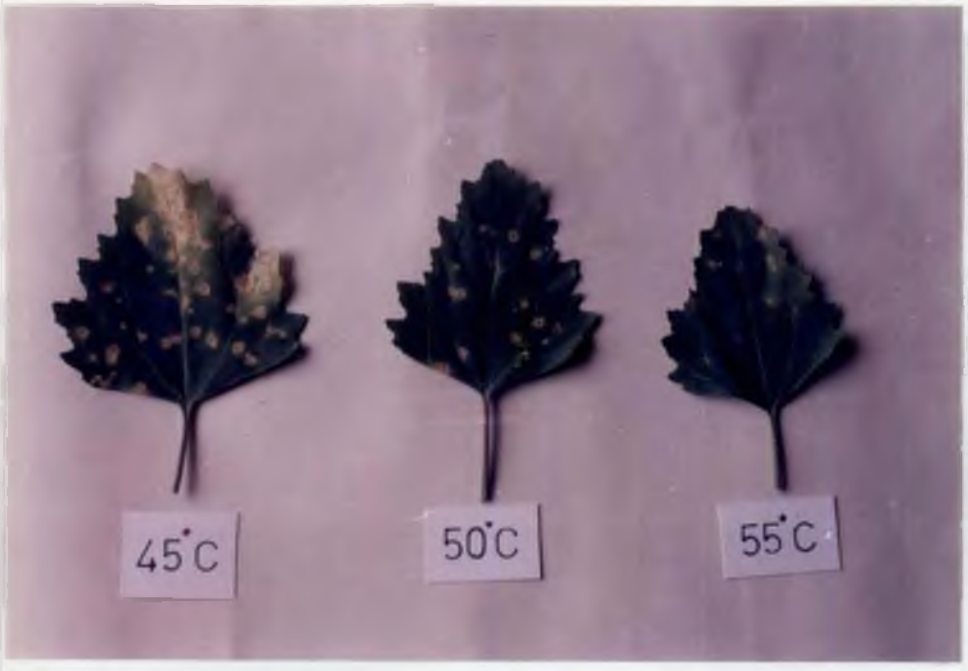

45°C
50°C
55°C

Table 5 Longevity *in vitro* (LIV) of CABMV at room temperature (28 ± 4^0 C)

Sl. No.	Aging in hours	Mean number of lesions
1.	0	14.00
2.	2	8.33
3.	4	4.00

Table 6 Longevity *in vitro* of CABMV under refrigerated condition (8^0 C)

Sl. No.	Aging in hours	Mean number of lesions
1.	0	14.00
2.	2	10.00
3.	4	5.67
4.	6	1.00

Fig. 4 Longevity *in vitro* (LIV) of CABMV at room temperature (28 ± 4^0 C) and under refrigerated condition (8^0 C)

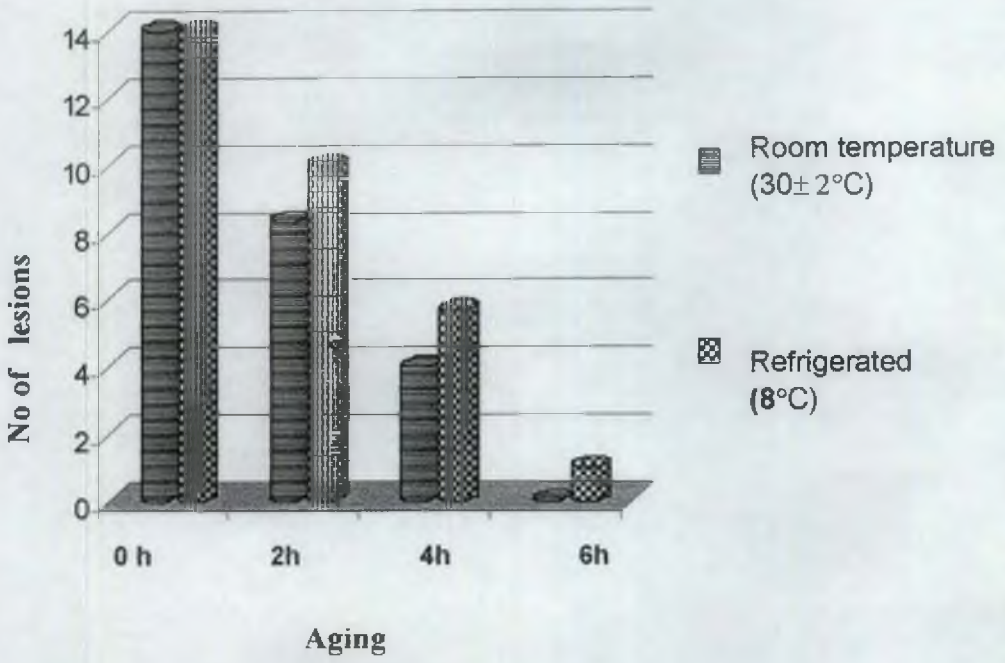


Plate 6. Colonies of *A. craccivora* on cowpea pods

aphid required an acquisition access period of 10 minutes for a maximum transmission of 70 per cent (Table 7, Fig. 5).

4.4.2 Inoculation access period

The results indicated that the viruliferous *A. craccivora* was able to transmit the virus even at short inoculation access period of one minute. Maximum transmission was obtained when the aphids were given an inoculation access period of ten minutes (Table 8, Fig. 6).

4.4.3 Effect of starvation before and after acquisition

4.4.3.1 Effect of pre-acquisition starvation

Pre-acquisition starvation of the vector enhanced the per cent transmission eventhough the vector could transmit the virus without starvation also. Maximum transmission of 80 per cent was obtained at one hour pre-acquisition starvation. The per cent transmission declined when starvation period was increased to two hours (Table 9 , Fig 7).

4.4.3.2 Effect of post-acquisition starvation

Post-acquisition starvation decreased the per cent transmission. Maximum transmission of 90 per cent was obtained when the aphids were released to healthy plants without starvation. Increase in starvation period decreased the rate of transmission (Table 10, Fig. 7).

Table 7 Effect of acquisition access period on the transmission of CABMV by *A. craccivora*

Acquisition access period (minutes)	Number of plants inoculated	Number of plants infected	Per cent transmission
1	10	0	0
5	10	6	60
10	10	7	70
15	10	6	60

Table 8 Effect of inoculation access period on transmission of CABMV by *A. craccivora*

Inoculation access period (minutes)	Number of plants inoculated	Number of plants infected	Per cent transmission
1	10	2	20
5	10	4	40
10	10	6	60
15	10	6	60

Fig.5. Effect of acquisition access period on the transmission of CABMV by *A. craccivora*

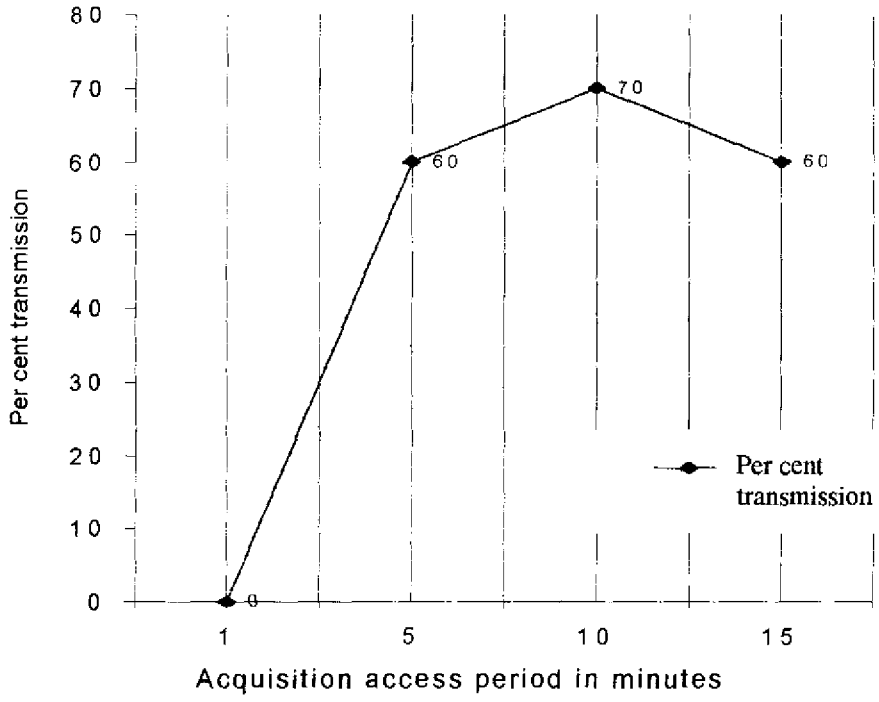


Fig.6. Effect of inoculation access period on transmission of CABMV by *A. craccivora*

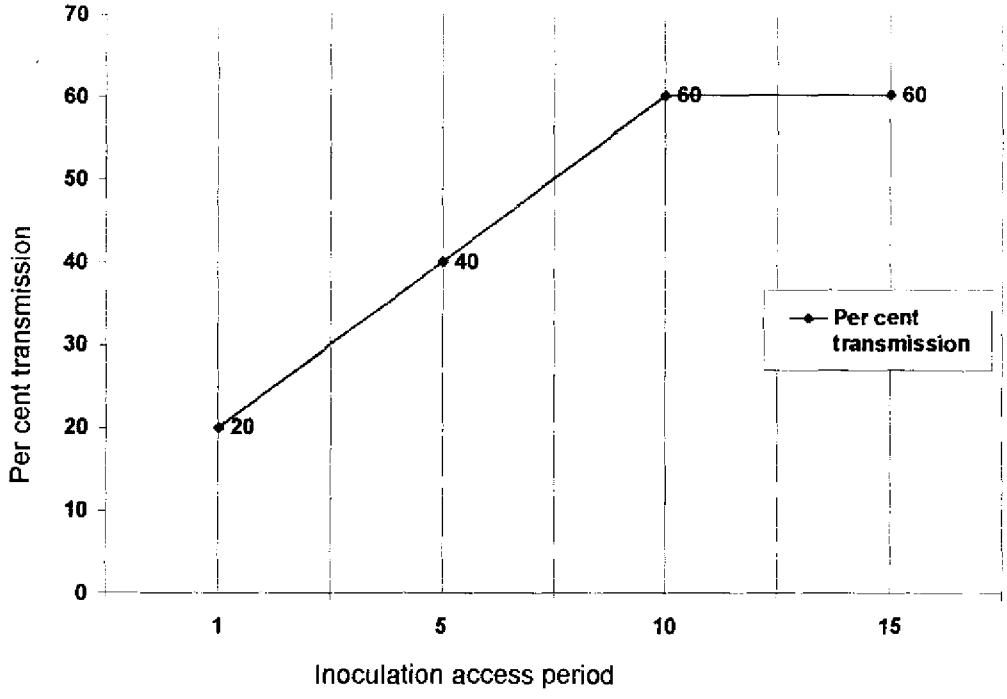


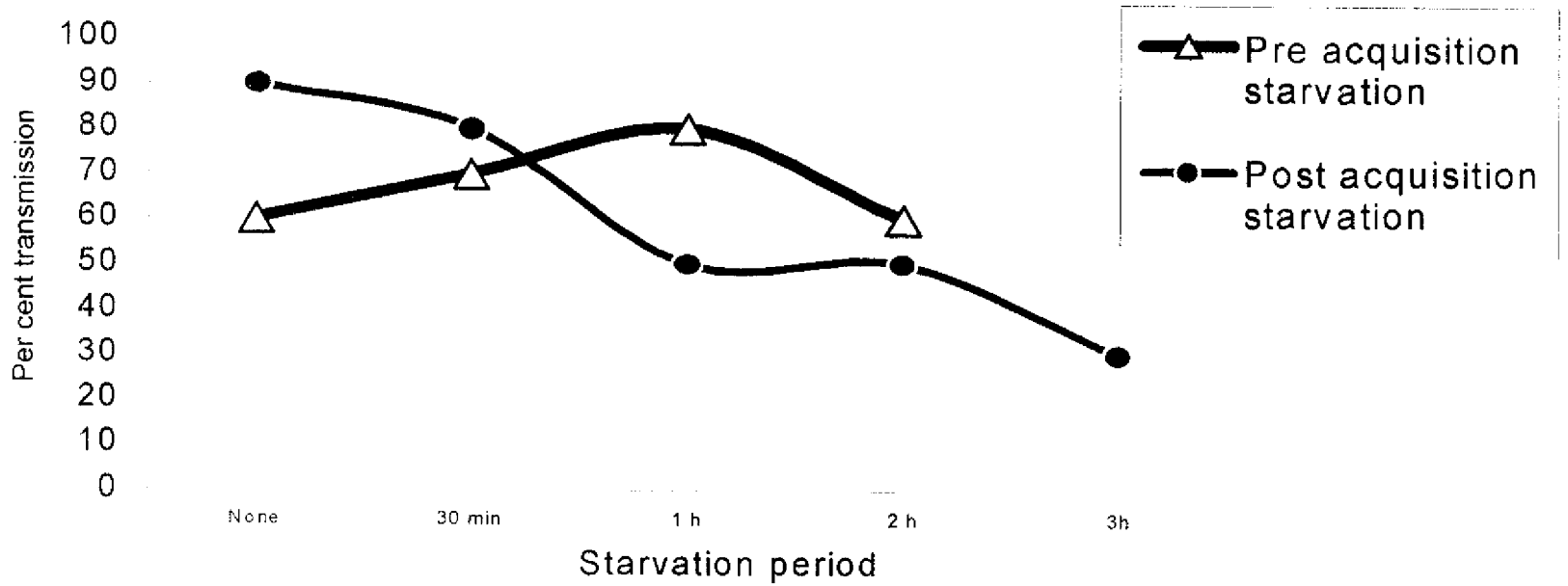
Table 9 Effect of pre-acquisition starvation period on the transmission of CABMV by *A. carccivora*

Pre-aquisition starvation period	Number of plants inoculated	Number of plants infected	Per cent transmission
Nil	10	6	60
30 min	10	7	70
1 h	10	8	80
2 h	10	6	60

Table 10 Effect of post-acquisition starvation period on the transmission of CABMV by *A. carccivora*

Post-aquisition starvation period	Number of plants inoculated	Number of plants infected	Per cent transmission
Nil	10	9	90
30 min	10	8	80
1 h	10	5	50
2 h	10	5	50
3 h	10	3	30

Fig. 7 Effect of pre and post-acquisition starvation period on the transmission of CABMV by *A. craccivora*



4.4.4 Minimum number of aphids required for transmission

The results indicated that though a single viruliferous aphid could transmit the virus to an extent of ten per cent, the maximum transmission of 50 per cent was obtained when a group of ten aphids were used for transmission (Table 11, Fig. 8).

4.5 Serodiagnosis and electron microscopy

4.5.1 Ouchterlony agar double diffusion test

For detecting the virus in sap extracts of infected cowpea leaves, Ouchterlony agar double diffusion test was carried out as per the procedure described by Noordam (1973). This test was performed in 0.8 per cent agarose gel taken in serological petri plates with properly arranged wells in it. The central well received the antiserum raised against the virus isolate under investigation. The surrounding wells 2, 4, 6 and 7 which received samples from diseased tissues gave thin precipitin lines between the central well. The well 3, which received extract from healthy tissue also gave a precipitin line. There was no band between the well, 5, which received the buffer control and central well (Plate 7).

4.5.2 Enzyme linked immunosorbent assay of the virus

Polyclonal and monoclonal antibodies specific to CABMV and BICMV obtained from IITA, Ibadan, Nigeria were used for the studies. DAS and DAC ELISA were conducted to identify the virus causing mosaic disease on cowpea cultivated in Kerala. Crude sap from infected tissues was used as antigen source.

Table 11 Number of aphids required for transmisssion of CABMV

Number of aphids per plant	Number of plants inoculated	Number of plants infected	Per cent transmission
1	10	1	10
3	10	3	30
10	10	5	50

Fig. 8 Number of aphids required for transmisssion of CABMV

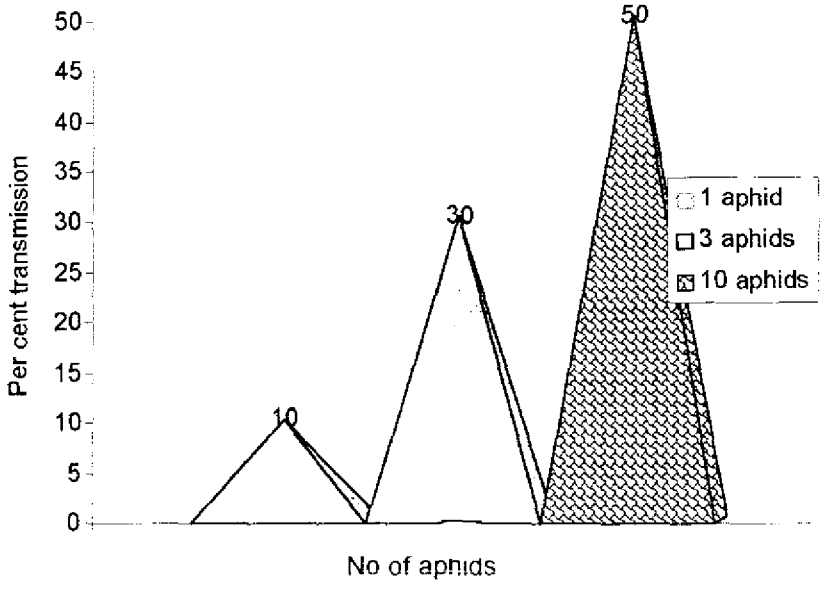
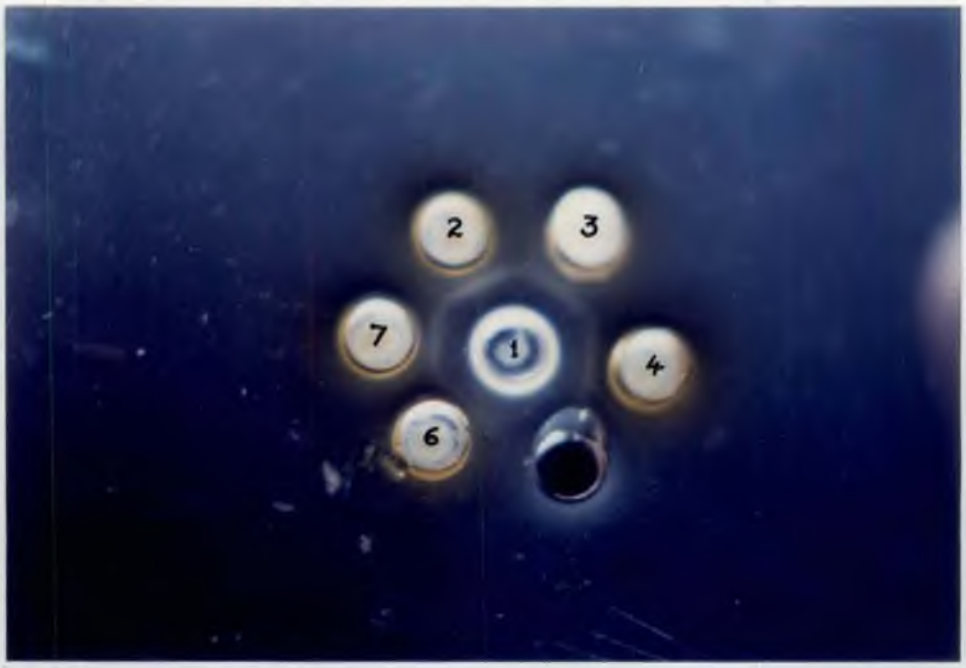


Plate 7. Reaction of virus antiserum with healthy and diseased plant sap

Well 1 : antiserum
Well 2 : diseased plant sap
Well 3 : healthy plant sap
Well 4 : diseased plant sap
Well 5 : buffer
Well 6 : diseased plant sap
Well 7 : diseased plant sap


2
3
7
1
4
6

4.5.2.1 DAS - ELISA

The result of this assay indicated that the monoclonal antibody 10G5 specific to BlCMV showed a high reactivity towards the virus isolate from Vellayani. The antigen prepared at different dilutions viz, 1 : 5,1 : 10 and 1 : 100 read an absorbance of 2.461, 3.183 and 1.819 respectively at 405 nm (Table 12, Fig. 9, Plate 8).

With the monoclonal antibody, 5H5, specific to CABMV the antigen prepared at different dilutions viz., 1 :5, 1 : 10 and 1 : 100 read an absorbance of 0.118, 0.134 and 0.164 respectively and was on par with the healthy control.

The absorbance measured for healthy sap on reaction with 10G5 and 5H5 was 0.134 and 0.106 respectively.

4.5.2.2 DAC - ELISA

Monoclonal antibodies specific to BlCMV (10G5 and 16G5) and monoclonal antibodies specific to CABMV (5H5, 7D9 and 6C10) were used as detecting antibodies in DAC-ELISA. The antibody 10G5, gave an absorbance of 0.229 at 405 nm while the healthy sap read an absorbance of 0.216. Negative reaction was given by antibodies specific to CAMBV (Plate 9).

4.5.2.3 Electron microscopy

The electron micrographs revealed the presence of flexuous, filamentous particles of 750 nm in length (Plate 10).

Table 12 Reaction of CABMV isolate in DAS - ELISA

Treatments		Absorbance at 405 nm
Dilution of antigen	Monoclonal antibody	
1 : 5	10G5 BlCMV	2.461
1 : 10	10G5 BlCMV	3.183
1 : 100	10G5 BlCMV	1.819
1 : 5	5H5 CABMV	0.118
1 : 10	5H5 CABMV	0.134
1 : 100	5H5 CABMV	0.164
Healthy	(1) 10 G5	0.134
	(2) 5H5	0.106

Fig. 9 Reaction of CABMV isolate in DAS - ELISA

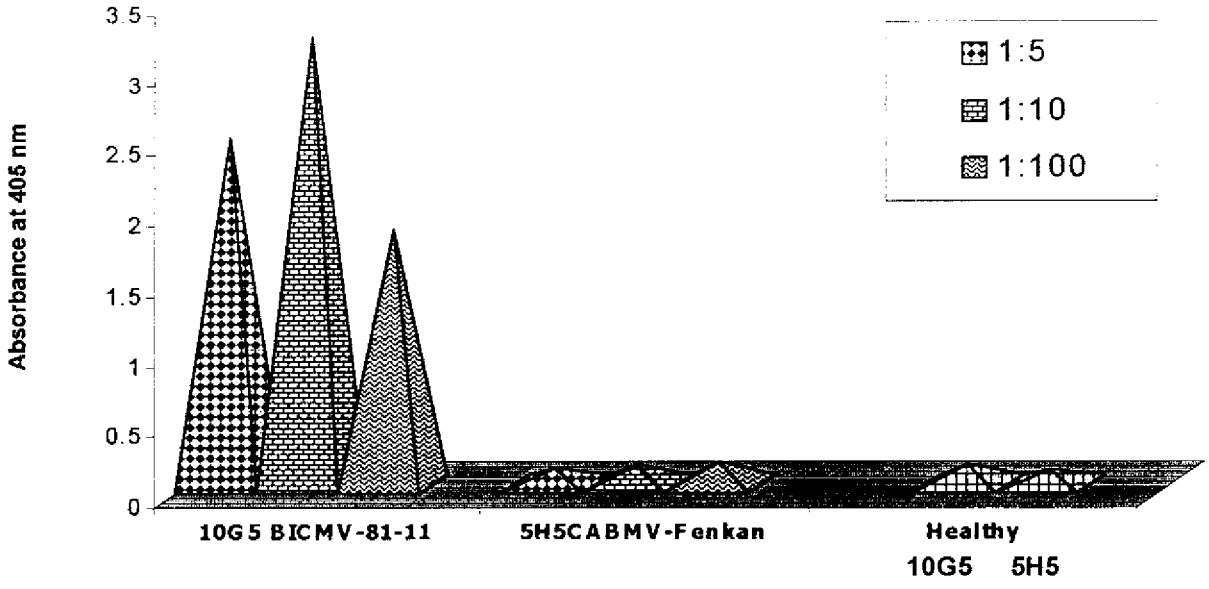
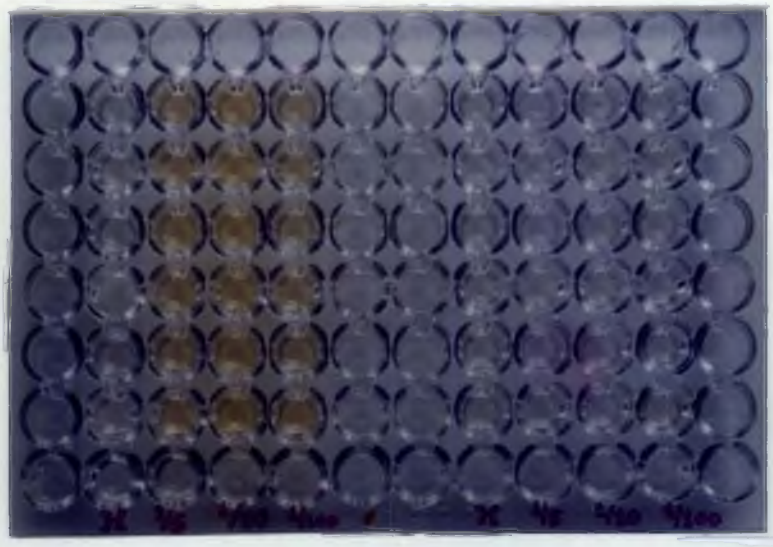


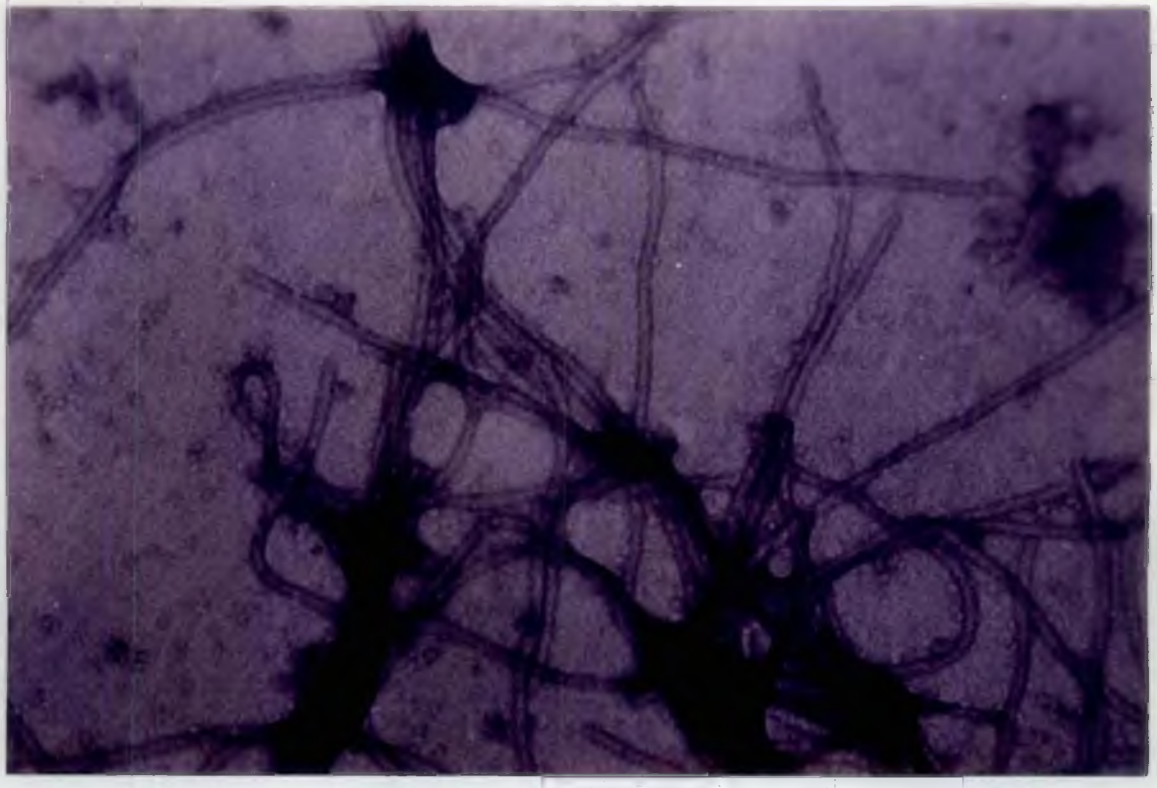
Plate 8. Reaction of virus in DAS-ELISA

Plate 9. Reaction of virus in DAC-ELISA




2

Plate 10. Electron micrograph of the virus



4.6 Disease resistance

4.6.1 Screening the sources of virus resistance

Sixty five varieties were screened for their reaction to CABMV infection under glasshouse condition. Assessment of the degree of resistance in cowpea cultivars was done based on 0 - 5 scale proposed by Bos (1982) (Plate 11).

The vulnerability index ranged between 0 - 86.67, the lowest for Co-6 and Co selection and highest for C-152. The varieties Co-6 and Co selection were classified under the group of no symptom expression with a score of zero. Thirty three varieties were classified as medium resistant, 24 as medium susceptible and six as susceptible (Table 13).

4.6.1.1 Incorporation of disease resistance

The F_1 of the crosses between Sharika and Co-6 (cross 1) and Co-Selection and Sharika (cross 2) were resistant to the virus (Plate 12, 13). The seggregating F_2 populations of both the crosses were grouped based on the length of pods and resistance to the virus. The cross between Sharika and Co-6, 53 progenies were long (more than 20 cm) poded and 61 were medium (10 - 20 cm) long poded. All were resistant to the virus (Table14). In the cross between Co-Selection and Sharika, 25 progenies were grouped as long poded and 21 as medium long and all were resistant to the virus (Table 15).

Joint seggregation for pod length and resistance to the virus was also observed for both the crosses. Fifty three progenies in cross 1 and 25 progenies in cross 2 were long poded with resistance to the virus (Table 16, 17 and Plate 14, 15).

Plate 11. Disease scoring

0 : no symptom
1 : vein clearing
2 : light and dark green mottling
3 : blistering
4 : distortion
5 : reduced leaf size


0
1
2
3
4
5

Table 13 Screening the sources of resistance to CABMV

Sl. No.	Genotypes	Vulnerability index	Range of score
1.	VS - 1	38.33	1-2
2.	VS - 3	40.00	2
3.	VS - 5	73.33	3-4
4.	VS - 6	60.00	3
5.	VS - 7	60.00	3
6.	VS - 8	53.33	2-3
7.	VS - 9	50.00	1-4
8.	VS - 9 (i)	30.00	1-2
9	VS - 11	40.00	2
10	VS - 12	43.64	1-3
11.	VS - 13	80.00	4
12.	VS - 14	45.00	2-3
13.	VS - 16	40.00	2
14.	VS - 17	43.33	1-3
15.	VS - 19	40.00	1-4
16.	VS - 20	42.00	1-2
17.	VS - 22	30.00	1-2
18.	VS - 23	40.00	1-3
19.	VS - 24	40.00	2
20.	VS - 25	30.00	3
21.	VS - 26	40.00	2
22.	VS - 27	30.00	3
23.	VS - 28	36.00	1-3
24.	VS- 28-1-2	36.67	1-2
25.	VS - 29	36.00	1-2

Sl. No.	Varieties	Vulnerability index	Range of score
26	VS - 30	40.00	2
27	VS - 36	28.33	1-3
28	VS - 48	44.00	1-3
29	VS - 53	40.00	2
30	VS - 81	44.60	2-3
31	VS - 88	26.67	1-2
32	VS - selection	60.00	3
33	Co - 3	45.00	2-3
34	Co 6	0.00	0
35	Co selection	0.00	0
36	IC 201085	40.00	2
37	IC 201095	40.00	2
38	IC 201097	40.00	2
39	IC 202184	40.00	2
40	IC 202865	40.00	2
41	IC 202918	40.00	2
42	IC 367703	40.00	2
43	KM 1	40.00	2
44	Ko-1	44.00	1-3
45	C - 152	86.67	2-3
46	CPD - 2	40.00	2
47	CAZC (B)	60.00	3
48	HC - 9588	40.00	2

Sl. No.	Varieties	Vulnerability index	Range of score
49	T1 - GC 8969-1	50.00	4
50	Malika	44.44	2-3
51	Sharika	46.67	1-3
52	Charrodi	40.00	2
53	Pusa komal	37.14	1-3
54	Kolingipayar	43.33	1-3
55	Covu selection	28.00	1-2
56	Cheeral local	40.00	2
57	Malappuram Local	40.00	2
58	Marayoor Local	40.00	2
59	Mitraniketan variety	56.00	2-3
60	Nedumangad Local -1	40.00	2
61	Nedumangad Local - 2	40.00	2
62	Nedumangad Local - 3	48.00	2-3
63	Thrissur Local	40.00	2
64	Vellayani selection Manikantan	40.00	2
65	Vettumpally Ela Local	65.00	3-4

Plate 12. F_1 of the cross between Sharika and Co-6

Plate 13. F_1 of the cross between Co-Selection and Sharika

Table 14 Seggregation of F_2 population of the cross Sharika x Co 6 for pod length and mosaic resistance

Generation	Number of plants						
	Long poded	Medium long	Short poded	Total	Resis-tant	Suscep-tible	Total
P_1	118	-	-	118	-	118	118
P_2	-	134	-	134	134	-	134
F_1	-	6	-	6	6	-	6
F_2	53	61		114	114		114

Table 15 Seggregation of F_2 population of the cross CO Selection x Sharika for pod length and mosaic resistance

Generation	Number of plants						
	Long poded	Medium long	Short poded	Total	Resis-tant	Suscep-tible	Total
P_1	-	74	-	74	74	-	74
P_2	118	-	-	118	-	118	118
F_1	-	2	-	2	2	-	2
F_2	25	21	-	46	46	-	46

Long poded - more than 20 cm
Medium long - 10 to 20 cm
Short poded - less than 10 cm

Table 16 Joint seggregation of F_2 population for pod length and mosaic resistance in the cross Sharika x Co 6

Generation	Number of plants								
	Long poded			Medium long poded			Short poded		
	Resistant	Susceptible	Total	Resistant	Susceptible	Total	Resistant	Susceptible	Total
P_1	-	118	118	-	-	-	-	-	-
P_2	-	-	-	134	-	134	-	-	-
F_1	-	-	-	6	-	6	-	-	-
F_2	53	-	53	61	-	61	-	-	-

Table 17 Joint seggregation of F_2 population for pod length and mosaic resistance in the cross CO Selection x Sharika

Generation	Number of plants								
	Long poded			Medium long poded			Short poded		
	Resistant	Susceptible	Total	Resistant	Susceptible	Total	Resistant	Susceptible	Total
P_1	-	-	-	74	-	74	-	-	-
P_2	-	118	118	-	-	-	-	-	-
F_1	-	-	-	2	-	2	-	-	-
F_2	25	-	25	21	-	21	-	-	-

Plate 14. Variation in pod length : Sharika and Co-6

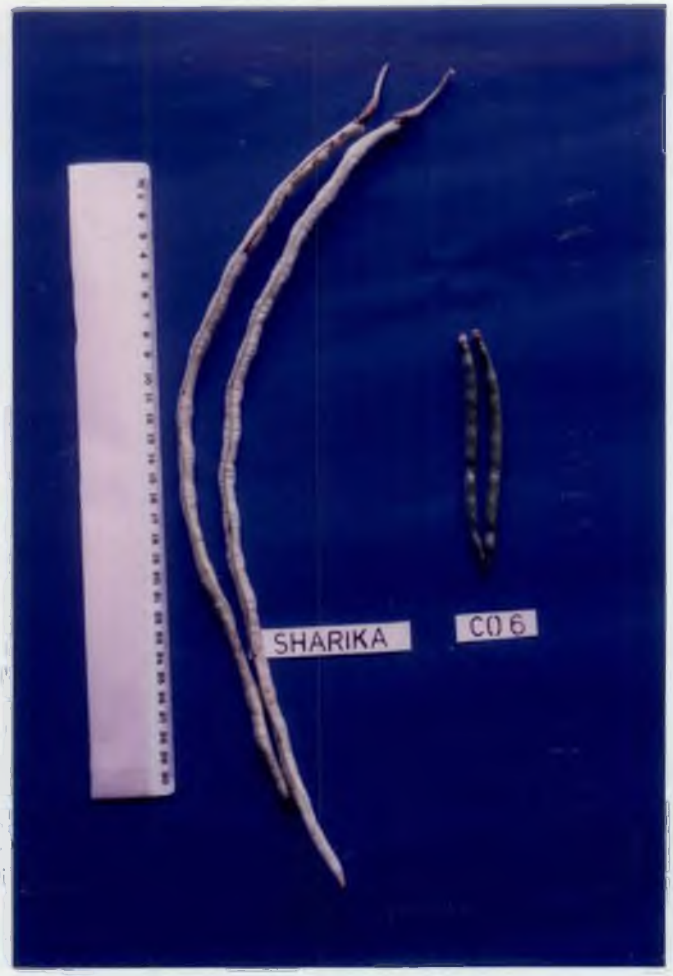

SHARIKA
CO 6

Plate 15. Variation in pod length : Co-Selection and Sharika


CO SELN
SHARIKA

4.7 Biochemical changes of host pathogen interaction

4.7.1 Estimation of total carbohydrate

The estimation was done according to the procedure given by Hedge and Hofreiter (1962). In the case of resistant variety, Co-6, carbohydrate value decreased from 51 mg g^{-1} at one day after inoculation to 11 mg g^{-1} at fifteen days after inoculation. The value increased from 11 mg g^{-1} at one day after inoculation to 25.67 mg g^{-1} at fifteen days after inoculation in the case of susceptible variety, Sharika (Table 18, Fig. 10).

4.7.2 Estimation of chlorophyll

The chlorophyll contents of resistant and susceptible cultivars were estimated as per the procedure given by Arnon (1949). The samples were estimated for chlorophyll a, chlorophyll b and total chlorophyll at different days after inoculation. Chlorophyll a for resistant variety showed an increase from 0.798 mg g^{-1} at one day after inoculation to 0.899 mg g^{-1} at fifteen days after inoculation. Chlorophyll b for the resistant variety decreased from 0.334 mg g^{-1} at one day to 0.283 mg g^{-1} at fifteen days after inoculation. The total chlorophyll also increased from 1.132 mg g^{-1} to 1.182 mg g^{-1}. Chlorophyll a for susceptible variety was reduced from 0.725 mg g^{-1} at one day to 0.163 mg g^{-1} at fifteen days after inoculation. Chlorophyll b was also reduced from 0.293 mg g^{-1} to 0.185 mg g^{-1} in susceptible variety. Total chlorophyll was reduced from 1.017 mg g^{-1} to 0.803 mg g^{-1} at one day to fifteen days after inoculation. The total chlorophyll of

Table 18 Changes in total carbohydrate content of cowpea leaves in response to CABMV inoculation

Days after inoculation	Changes in carbohydrate contents (mg g^{-1} fresh weight of tissue)	
	Resistant (Co 6)	Susceptible (Sharika)
1	51.00	11.00
5	17.00	17.00
10	14.67	16.67
15	11.00	25.67
	CD - Varieties	6.432
	Days interval	5.360

Fig. 10 Changes in total carbohydrate content of cowpea leaves in response to CABMV inoculation

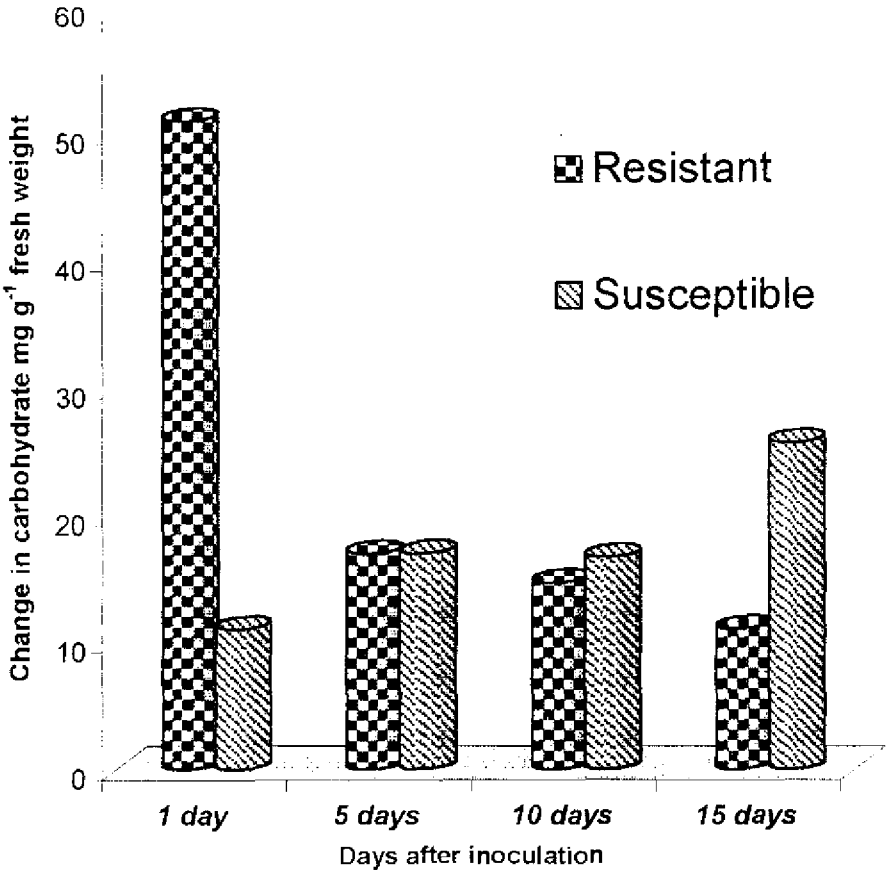


Table 19 Changes in chlorophyll contents of cowpea leaves in response to CABMV inoculation

Days after inoculation	Changes in chlorophyll contents (mg g^{-1} fresh weight)					
	Resistant variety (Co 6)			Susceptible variety (Sharika)		
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Chlorophyll a	Chlorophyll b	Total Chlorophyll
1	0.798	0.334	1.132	0.725	0.293	1.017
5	0.387	0.144	0.531	0.606	0.170	0.776
10	0.857	0.266	1.122	0.552	0.177	0.728
15	0.899	0.283	1.182	0.619	0.185	0.803
				CD (Varieties)		0.0188
				Days interval		0.01881

resistant variety showed a high value of 1.182 mg g^{-1} fresh tissue at fifteen days after inoculation in comparison to 0.803 mg g^{-1} for susceptible variety (Table 19).

4.7.3 Estimation of protein

Estimation of protein was carried out as per the procedure given by Bradford (1976). Increase in protein content was noted for the susceptible and resistant variety. The protein content of resistant variety increased from 78.0 on first day after inoculation to 128.67 at fifteen days after inoculation. The value for susceptible increased from 85.3 to 120.00 after fifteen days after inoculation. The protein content was higher for susceptible (116.67) than for resistant (99.7) ten days after inoculation (Table 20, Fig.11).

4.7.4 Estimation of phenol

Estimation of phenol was carried out as per the procedure given by Bray and Thorpe (1954). Results indicated that there was not much change in the phenolic compounds in resistant and susceptible varieties. Maximum value of 100 µg g^{-1} fresh leaf tissue was noted for resistant variety. In the case of susceptible variety, the initial value at one day after inoculation was 88 µg g^{-1} and it increased to 98 µg g^{-1} on five days after inoculation. The value decreased to 89 µg g^{-1} fresh weight at fifteen days after inoculation (Table 21, Fig. 12).

Table 20 Changes in total soluble protein content of cowpea leaves in response CABMV inoculation

Days after inoculation	Changes in soluble protein contents ($\mu g\ g^{-1}$ fresh weight of tissue)	
	Resistant (Co 6)	Susceptible (Sharika)
1	78.0	85.33
5	105.67	143.67
10	99.7	116.67
15	128.67	120.00
	CD - Varieties	15.10
	Days interval	11.67

Fig. 11 Changes in total soluble protein content of cowpea leaves in response CABMV inoculation

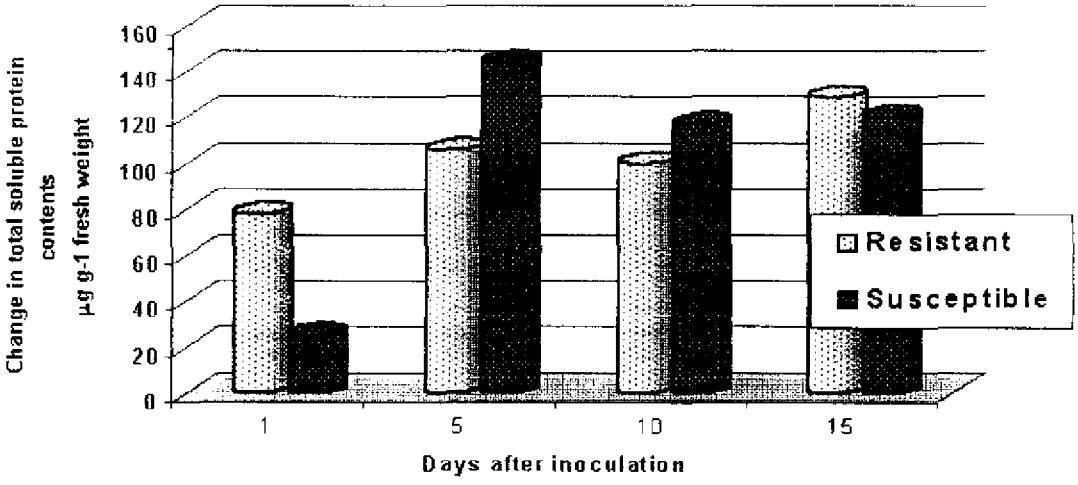
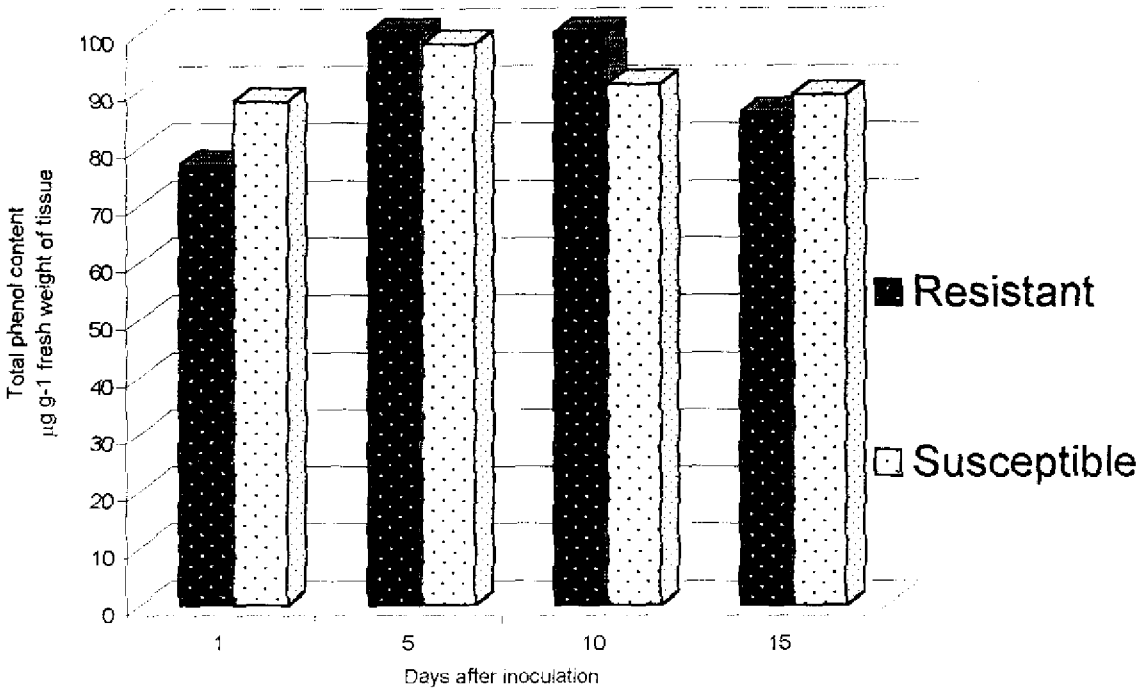


Table 21 Changes in total phenol content of cowpea leaves in response to CABMV inoculation

Days after inoculation	Changes in total phenol contents ($\mu g\ g^{-1}$ fresh weight of tissue)	
	Resistant (C0-6)	Susceptible (Sharika)
1	77	88
5	100	98
10	100	91
15	86	89
	CD - Varieties	0.801
	Days interval	0.8895

Fig. 12 Changes in total phenol content of cowpea leaves in response to CABMV inoculation



4.7.5 Defence related enzymes

4.7.5.1 Peroxidase

Estimation of peroxidase activity was carried out as per the procedure given by Srivastava (1987). There was reduced peroxidase activity (27.25 g^{-1} min^{-1}) in the resistant variety during the initial one day after inoculation. The peroxidase activity of susceptible variety was more during initial one day (44.9 g^{-1} min^{-1}) and reduced to 24.2 during five days after inoculation. For the resistant variety, maximum value of 59.80 was obtained at ten days after inoculation. Activity was more in resistant than in susceptible (44.8) on the final day of observation (Table 22, Fig. 13).

4.7.5.2 Polyphenol oxidase

Estimation of polyphenol oxidase activity was conducted as per the procedure given by Mayer *et al.* (1965). The activity showed a gradual increase from 1.3 g^{-1} min^{-1} in the resistant variety at one day after inoculation to 7.6 g^{-1} min^{-1} at ten days after inoculation. Similar increase from 2.1 at one day to 7.2 at ten days was observed in the case of Sharika also. The value reduced to 5.5 fifteen days after inoculation for the resistant and 2.5 for the susceptible (Table 23, Fig. 13).

4.7.5.3 Phenylalanine ammonia-lyase

This was estimated as per the procedure developed by Dickerson *et al.* (1984). Maximum value (80.83 μg g^{-1}) was attained by resistant variety at ten

Table 22 Changes in peroxidase activity in cowpea plants in response to CABMV inoculation

Days after inoculation	Peroxidase activity (changes in absorbance min^{-1} g^{-1} fresh weight)	
	Resistant (C0-6)	Susceptible (Sharika)
1	27.25	44.90
5	23.80	24.20
10	59.80	41.00
15	47.20	44.80

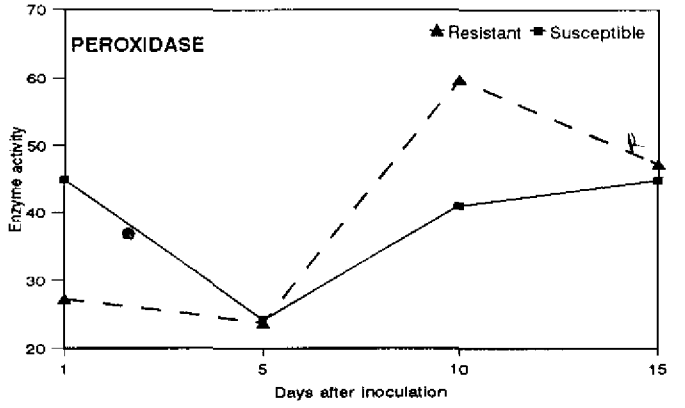
Table 23 Changes in polyphenol oxidase activity in cowpea plants in response to CABMV inoculation

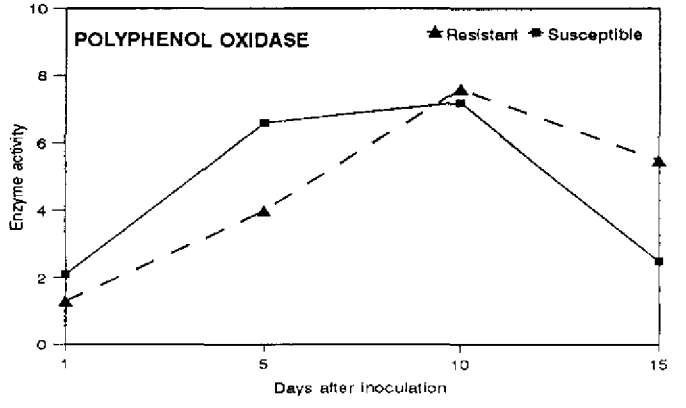
Days after inoculation	Activity of polyphenol oxidase (changes in absorbance min^{-1} g^{-1} fresh weight)	
	Resistant (C0-6)	Susceptible (Sharika)
1	1.3	2.1
5	4.0	6.6
10	7.6	7.2
15	5.5	2.5

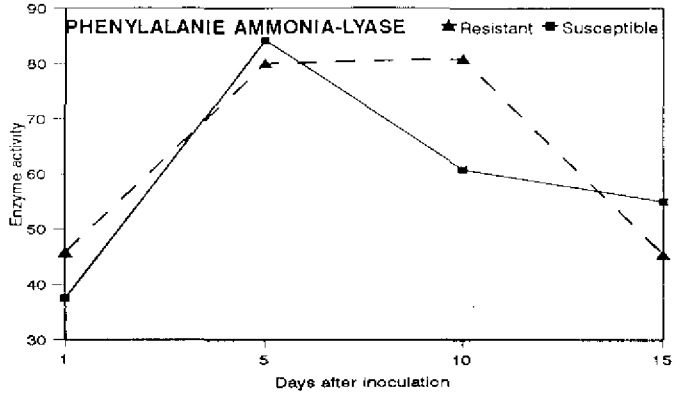
Table 24 Changes in phenylalanine ammonia-lyase (PAL) activity in cowpea plants in response to CABMV inoculation

Days after inoculation	Activity of PAL (μg g^{-1} min^{-1})	
	Resistant (C0-6)	Susceptible (Sharika)
1	45.83	37.50
5	80.00	84.17
10	80.83	60.83
15	45.42	55.00

Fig. 13 Changes in peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase activities in cowpea plants in response to CABMV inoculation







days after inoculation. Susceptible variety reached its maximum value of 84.17 μg g^{-1} at five days after inoculation (Table 24, Fig. 13). Resistant variety recorded a value of 45.42 μg g^{-1} at fifteen days while susceptible recorded a value of 55 μg g^{-1}.

4.8 Induction of systemic resistance

4.8.1 Bioassay on *C. amaranticolor*

4.8.1.1 Pre-inoculation treatment

Chemicals such as salicylic acid, manganese chloride and barium chloride at concentrations of 50 mg l^{-1}, 100 mg l^{-1} and 150 mg l^{-1} and neem oil emulsion at ten per cent concentration were used for pre-inoculation treatment. Statistical analysis of the data indicated that pre-treatment with neem oil (ten per cent) concentration was on par with $BaCl_2$ 150 mg l^{-1}, salicylic acid 100 mg l^{-1}, salicylic acid 150 mg l^{-1}, $MnCl_2$ 150 mg l^{-1}, $BaCl_2$ 50 mg l^{-1}, $MnCl_2$ 50 mg l^{-1} and $BaCl_2$ 100 mg l^{-1}. These recorded per cent inhibition of 67.2, 63.87, 60.19, 59.58, 57.52, 51.18 and 49.6 respectively against neem which recorded 68.92 per cent inhibition. Salicylic acid 100 and 150 mg l^{-1} were on par. Other treatments were on par among themselves (Table 25, Fig. 14).

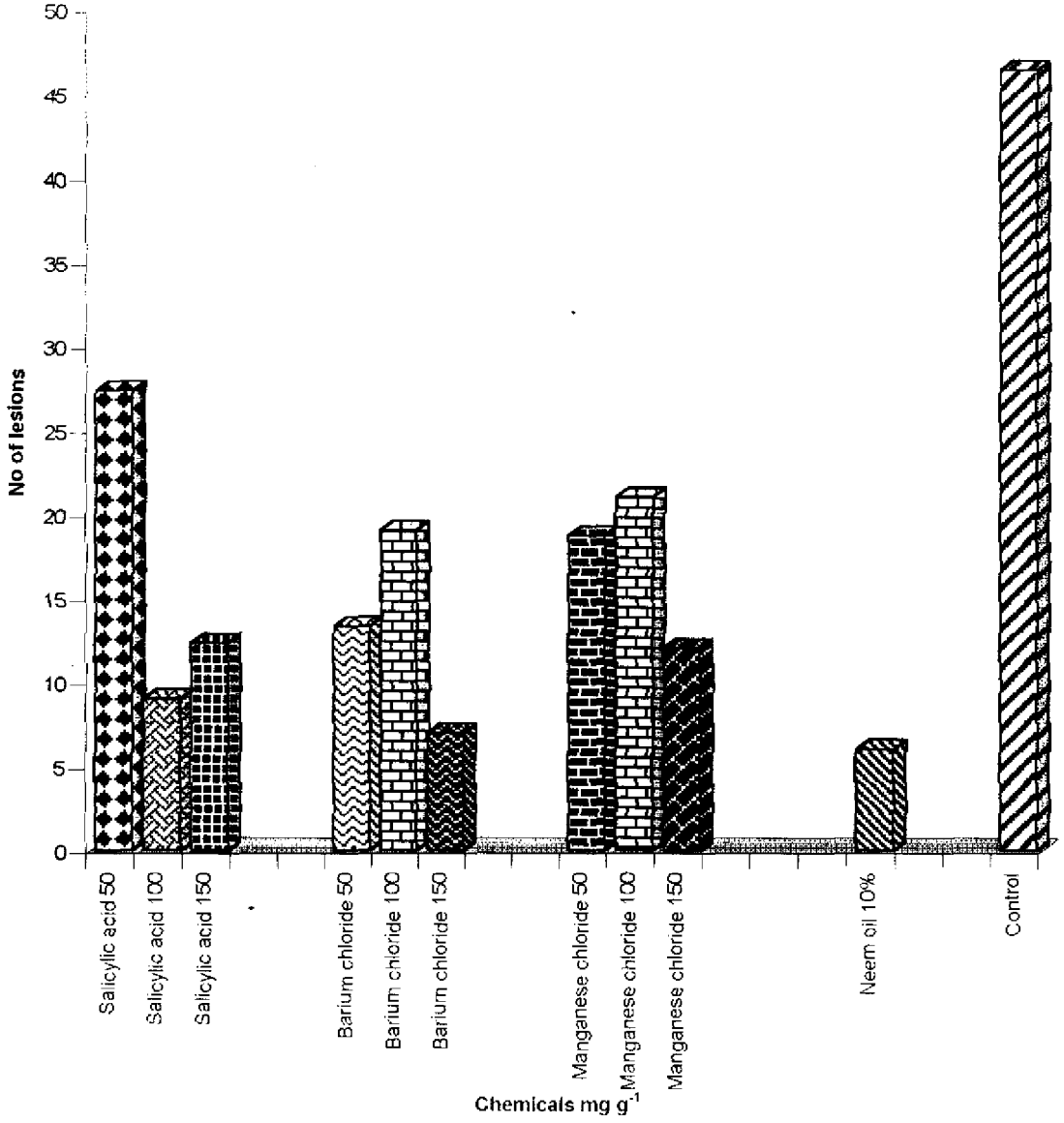
4.8.1.2 Post-inoculation treatment

Statistical analysis revealed that effect of $MnCl_2$ 150 mg l^{-1} was on par with the effects of salicylic acid 150 mg l^{-1}, salicylic acid 100 mg l^{-1}, $MnCl_2$ 50 mg l^{-1}, neem oil and $MnCl_2$ 100 mg l^{-1}. These recorded per cent inhibition of 60.19, 55.06, 49.72, 49.44 and 47.88 respectively. $MnCl_2$ 150 mg l^{-1} recorded 65.45 per

Table 25 Effect of pre-inoculation application of chemicals and neem oil on infection of *C. amaranticolor* by CABMV

Sl. No.	Chemical	Concentration	Mean number of lesions	Per cent inhibition
1.	Salicylicacid	50 mg l^{-1}	27.30	39.47
2.	Salicylicacid	100 mg l^{-1}	9.00	63.87
3.	Salicylicacid	150 mg l^{-1}	12.30	60.19
4.	Barium chloride	50 mg l^{-1}	13.30	57.52
5.	Barium chloride	100 mg l^{-1}	19.00	49.60
6.	Barium chloride	150 mg l^{-1}	7.00	67.52
7.	Manganese chloride	50 mg l^{-1}	18.67	51.17
8.	Manganese chloride	100 mg l^{-1}	21.00	47.68
9.	Manganese chloride	150 mg l^{-1}	12.00	59.58
10.	Neem oil	10 per cent	6.00	68.92
11.	Control	-	46.30	-
			CD -	19.39

Fig. 14 Effect of pre-inoculation application of chemicals and neem oil on infection of *C. amaranticolor* by CABMV



cent inhibition (Table 26, Fig.15). Comparison between different levels of chemicals indicated that they were on par among themselves.

4.8.2 Bioassay on cowpea plants

A pot culture experiment was laid out in completely randomised design to evaluate the effect of chemicals and neem oil on symptom expression in cowpea plants when they were applied before and after the inoculation of the virus. Three sprayings of the chemical and neem oil were given at ten days interval. The expression of symptoms were scored. Pre-inoculation application of neem oil ten per cent concentration was proved to be most effective. It recorded a score of 1.252 (Table 27).

Table 26 Effect of post-inoculation application of chemicals and neem oil on infection of *C. amaranticolor* by CABMV

Sl. No.	Chemical	Concentration	Mean number of lesions	Per cent inhibition
1.	Salicylic acid	50 mg l^{-1}	35.30	24.57
2.	Salicylic acid	100 "	15.30	55.06
3.	Salicylic acid	150 mg l^{-1}	11.60	60.19
4.	Barium chloride	50 mg l^{-1}	28.30	37.04
5.	Barium chloride	100 mg l^{-1}	30.30	37.77
6.	Barium chloride	150 mg l^{-1}	35.20	26.39
7.	Manganese chloride	50 mg l^{-1}	19.67	49.72
8.	Manganese chloride	100 mg l^{-1}	21.00	47.88
9.	Manganese chloride	150 mg l^{-1}	8.30	65.45
10.	Neem oil	10 per cent	19.60	49.44
11.	Control	-	46.30	-
				CD - 22.73

Fig. 15 Effect of post-inoculation application of chemicals and neem oil on infection of *C. amaranticolor* by CABMV

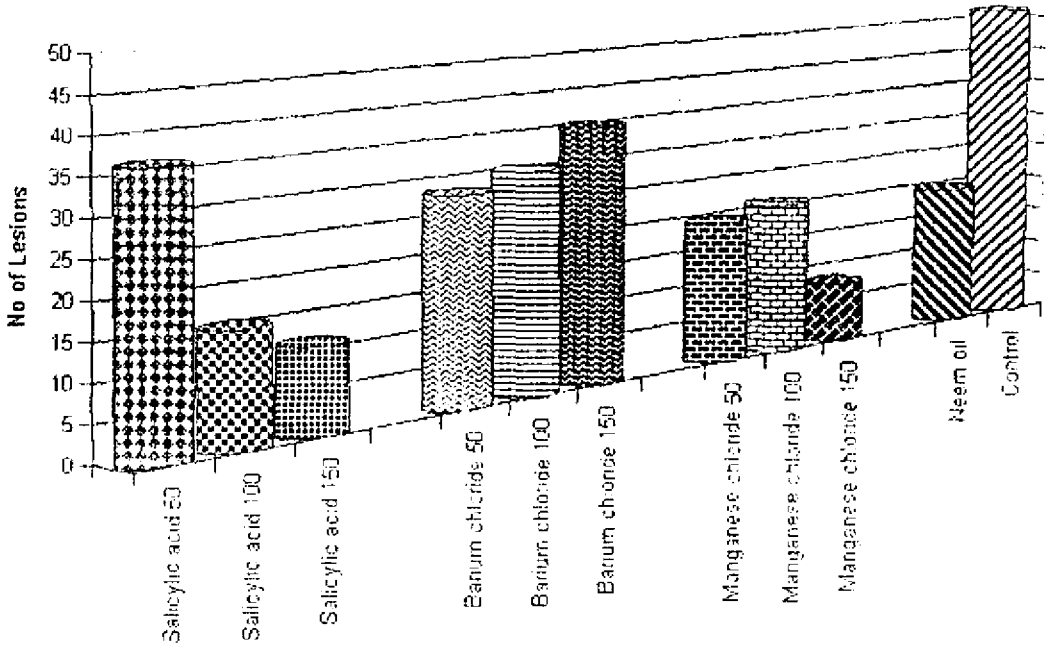


Table 27 Effect of pre and post-inoculation application of chemicals and neem oils on infection of cowpea by CABMV

Sl. No.	Treatment pre-application	Concentration	Mean disease score
1.	Salicylic acid	50 mg l^{-1}	1.516
2.	Salicylic acid	100 mg l^{-1}	1.644
3.	Salicylic acid	150 mg l^{-1}	1.697
4.	Manganese chloride	50 mg l^{-1}	1.618
5.	Manganese chloride	100 mg l^{-1}	1.622
6	Manganese chloride	150 mg l^{-1}	1.840
7.	Barium chloride	50 mg l^{-1}	1.675
8.	Manganese chloride	100 mg l^{-1}	1.600
9	Manganese chloride	150 mg l^{-1}	1.933
	Post application		
10.	Salicylic acid	50 mg l^{-1}	1.706
11.	Salicylic acid	100 mg l^{-1}	1.728
12.	Salicylic acid	150 mg l^{-1}	1.573
13.	Barium chloride	50 mg l^{-1}	1.777
14.	Barium chloride	100 mg l^{-1}	1.724
15.	Barium chloride	150 mg l^{-1}	1.799
16.	Manganese chloride	50 mg l^{-1}	1.732
17.	Manganese chloride	100 mg l^{-1}	1.706
18.	Manganese chloride	150 mg l^{-1}	1.675
19.	Pre-Neem oil	10 per cent	1.252
20.	Post-application Neem oil	10 per cent	1.531
21.	Control	-	1.799
			CD - 0.163

DISCUSSION

5. DISCUSSION

Cowpea is a major leguminous vegetable and grain crop cultivated in Kerala. Diseases and pests are a major constraint in increasing the production of the crop. Among the pathogens, viruses are a threat to the cultivars. Seed borne nature makes them very difficult to be controlled. This study was undertaken to identify and characterise the virus causing severe mosaic in cowpea and to study the resistance mechanism against the virus.

The characteristic symptoms caused by the virus under study included dark green vein banding, severe mosaic, blistering and distortion of leaves and stunting of the plant. Initial symptom appeared as vein clearing on the young trifoliates. These symptoms were similar to those caused by aphid-borne mosaic described earlier. Bock and Conti (1974) reported dark green vein banding, distortion, blistering and stunting of plants as a result of infection by CABMV. Mild mosaic on primary leaves have been reported by Mali and Kulthe (1980). Sudhakumari (1993) reported that primary symptom of CABMV appeared as mild vein clearing on primary leaves. Umamaheswaran (1996) have also reported vein clearing, mottling, chlorosis of leaflets and stunting as the major symptoms of CABMV infection. Thus, the comparison of symptoms produced by the virus under study and those earlier reported reveal that both the viruses are same or are related.

The virus could be transmitted more efficiently by mechanical means. Different buffers like 0.1 M citrate buffer (pH 6.2), 0.1 M citrate phosphate buffer (pH 7.0), 0.01 M phosphate buffer (pH 7.0), 0.1 M tris buffer (pH 7.2) and 0.1 M

borate buffer (pH 7.6) were used to test their effectiveness on transmitting the virus. 0.01 M phosphate buffer (pH 7.0) produced maximum number of local lesions (31.8) on *C. amaranticolor* (Table 1, Fig. 1). Sap for inoculating the cowpea plants was extracted using 0.01 M phosphate buffer (pH 7.0). Vein clearing was produced on the young trifoliate leaves seven days after inoculation. Many authors have reported the effective transfer of the virus using phosphate buffer (Kaiser and Mossahebi, 1975., Patel and Kuwaite, 1982., Sharma and Varma, 1975., Fischer and Lockhart, 1976). Phosphate buffer retained the infectivity of virus for long periods compared to other buffers used for inoculation.

The virus under study was transmitted through the seeds of cowpea cultivar Sharika at the rate of eleven per cent. Seed transmission of the virus has been reported by many authors. Mali *et al.* (1988) reported that the seed transmission ranged between 0 to 22.6 per cent for BlCMV and 0 to 18.5 per cent for CABMV. Mali *et al.* (1989) reported 3.1 to 20 per cent seed transmission of CABMV. This finding was in accordance with the earlier reports (Patil and Gupta, 1992., Thottappilly and Rossel, 1992., Bashir and Hampton, 1993, 1994).

Aphis craccivora is one of the major pest of cowpea cultivated in Kerala. Insect transmission was tried using *A. craccivora* and was found to transmit the virus at a rate of 50 per cent. The initial vein clearing symptom was observed on infected plants. Successful transmission of the CABMV by *A. craccivora* was reported by Bock and Conti (1974) and Ndiaye *et al.* (1993). Bashir and

Hampton (1994) obtained transmission in the range of 18 - 57 per cent with *A. craccivora*. Transmission as high as 88 per cent and 93.33 per cent for *A. craccivora* was reported by Nain *et al.* (1994) and Umamaheswaran (1996) respectively. They also reported that *A. craccivora* transmitted more efficiently when compared to other vectors tested.

Thermal inactivation point (TIP) dilution end point (DEP) and longevity *in vitro* (LIV) are distinct to a particular virus. The virus studied recorded a TIP in the range of 60 - 65^0 C, DEP in the range of 10^{-3} - 10^{-4} and LIV for 4 h under room temperature (28 $\pm$ 4^0 C) and 6 h under refrigerated condition (8^0 C) (Table 3, 4, 5, 6 and Fig. 2,3,4). The TIP and DEP are in line with those elucidated by Mali and Kulthe (1980) and Boswell and Gibbs (1983) with slight variation in the LIV. Mali and Kulthe recorded TIP in the range of 60-65^{0}C, DEP at 10^{-4} and LIV for 64 h. Boswell and Gibbs (1983) reported TIP, DEP and LIV for BlCMV as 65^{0}C, 10^{-4} and 2 days respectively. The same authors recorded a TIP at 60^{0}C, DEP at 10^{-3} and LIV for 72 days in the case of CABMV. Since the finding of the present investigation is in accordance of the report of Boswell and Gibbs (1983) the virus strain under study could be BlCMV.

Studies on virus vector relationship was undertaken using the most efficient vector *A. craccivora*. The time for acquisition, inoculation feeding, effect of starvation on pre and post-acquisition and minimum number of aphids required for transmission were studied.

The study has shown that the virus was acquired by *A. craccivora* in a short period of five minutes (Table 7, Fig. 5) but maximum transmission was

obtained at ten minutes. Acquisition with in a second has been reported by Govindasamy *et al.*(1970). CABMV was acquired during brief probes by the vector as reported by Mali and Kulthe (1980) for *A. gossypii*, Atiri *et al.* (1984) for *A craccivora*, Nain *et al.* (1994) for nymphs of *A craccivora* and Umamaheswaran (1996) for adult apterous *A craccivora*. The present and former studies support the generalisation that virus was acquired in short acquisition feeding periods.

A. craccivora was able to transmit the virus when given an inoculation access of even one minute. Maximum transmission was observed at ten minutes time and the rate remained constant for fifteen minutes also (Table 8, Fig. 6). Mali and Kulthe reported a feeding time of four hours for the effective transmission by *A. gossypii* while Atiri *et al.*(1984) reported brief inoculation access periods for transmitting CABMV. A 24 h inoculation access was reported for successful transmission of CABMV by viruliferous aphid nymphs (Nain *et al.*, 1994). The results of the present experiment also confirmed the non-persistent mode of transmission by the vector associated with the crop.

Pre-acquisition starvation of the vector for 1 h increased the efficiency in transmitting the virus (Table 9, Fig. 7). This may be attributed to the more number of short probes by the vector in to the diseased source. This confirms the findings of Govindasamy *et al.* (1970), Kaiser and Mossahebi (1975) and Umamaheswaran (1996).

Post-acquisition starvation for even 30 minutes decreased the ability of the vector to transmit the virus. The transmission was maximum when no starvation was provided (Table 10, Fig. 7). This shows that the virus is transmitted in a non-

persistent manner or it is stylet borne. This is in line with the work conducted by Umamaheswaran (1996). The difference in the efficiency of transmission when different periods of starvation before acquisition and inoculation access period to transmit CABMV virus may be due to the nature of aphid probing and environmental factors.

Even a single aphid vector was capable of transmitting the virus even though a maximum transmission was obtained when a group of ten aphids were released (Table 11, Fig. 8). Transmission of the virus by a single *A. craccivora* and *A. gossypii* was reported by Kaiser and Mossahebi, (1975). Umamaheswaran (1996) reported that a single aphid could transmit the virus to healthy cowpea plants. Similar observation was made in the case of transmission of CABMV by Sharma and Varma (1977). Several workers have also demonstrated a correlation of the abundance of aphid vectors and virus spread (Thresh, 1974; Murphy *et al.* 1987; Nain *et al.* 1984).

The detection for the presence and assay of pathogen population are basic requirements for the elimination of the pathogen to minimise the disease incidence and its subsequent spread. In the present investigation an attempt has been made to develop a simple and reliable method of diagnosis of the virus in plants. Polyclonal antisera was raised against the partially purified CABMV isolate from Vellayani. Ouchterlony agar double diffusion test was performed and found that there was clear precipitin band between the wells which received infected sap and antiserum. Bands were also noticed between wells loaded with healthy sap and virus antiserum, indicating the presence of host proteins that interfered with the

reaction, which could have been eliminated by cross reacting with healthy host protein. Serodiagnosis using immunodiffusion has been conducted by Fischer and Lockhart (1976) to prove that an isolate of CABMV was not related to bean common mosaic virus. Lima *et al.* (1979) conducted immunodiffusion test to prove the relationship of BlCMV with other potyviruses and was found to be serologically related but distinct from other potyviruses. Lima and Purcifull (1980) used this method to detect the presence of CABMV in hypocotyls of infected cowpea seedlings. Kline and Anderson (1997 a) used immunodiffusion to detect CABMV in infected cowpea cultivated in United States.

ELISA is one of the quickest techniques to detect and characterise a virus. With a view to identify the virus causing aphid-borne mosaic disease in Kerala, ELISA test was conducted using monoclonal and polyclonal antibodies obtained from IITA, Ibadan. DAC and DAS-ELISA were conducted to identify the virus. The results indicated that there was high reactivity of the virus isolate to monoclonal antibody (10G5) raised specific to BlCMV (Table 12, Fig. 9). The virus isolate reacted feeble to the monoclonal antibody (5H5), produced specific to CABMV which was on par with the healthy control. In the present investigation, the virus was confirmed as BlCMV. Taiwo and Gonsalves (1982) conducted ELISA, immunoelectronmicroscopy and immunodiffusion tests to serologically group BlCMV and CABMV. They found that antisera to isolates of CABMV group did not detectably react to isolates of the BlCMV group.

Dijkstra *et al* (1987) compared two potyvirus isolates, one from germplasm of yard long bean and another one from soybean plants with two

isolates of BlCMV from the USA and a Moroccan isolate of BlCMV.

Huguenot *et al.* (1992) characterised potyviruses into two distinct serogroups, one containing BlCMV and other containing CABMV. Using biotin labelled monoclonal antibodies, BlCMV was subdivided into two serotypes and CABMV isolate was subdivided into five serotypes. Because, both CABMV and BlCMV induced very similar mosaic in cowpea, different ELISA procedures using mixed monoclonal antibodies were evaluated and a single protocol was developed for the reliable diagnosis of both viruses.

Electron micrographs of the virus isolate indicated that the virus particles were flexuous, flamentous particles of about 750 nm. Similar observations have been indicated for BlCMV and CABMV by Bock (1973), Kaiser and Mossahebi, 1975., Lima and Purcifull, 1980., Taiwo *et al.* 1982 and Bashir and Hampton, 1995. The result clearly indicated that the virus isolate belongs to BlCMV potyvirus.

Studies on physical properties, serodiagnosis and electron microscopy confirmed that the virus isolate causing severe mosaic of cowpea in Kerala is BlCMV. Eventhough the symptoms expressed by both viruses are similar, they are serogically grouped as distinct. The specific reaction to the monoclonal antibodies revealed that the virus belonged to the serotype A as it reacted with the coating antibody H3 and the detecting antibody 10G5, as reported by Huguenot *et al.* (1992).

Diseases caused by viruses are often the most destructive and difficult to control. Management of viral disease has always been a problem to cultivators and research workers. An universal strategy for their proper management is the development of improved varieties by imparting resistance to disease. Resistance to

viral disease of cowpea can be derived from natural sources. For achieving this, suitable sources of resistance need to be identified.

Sixty five genotypes were screened for resistance to the aphid-borne mosaic virus of cowpea. Two lines Co-6 and Co-Selection were grouped under no symptom expression category. Thirty three genotypes were classified as medium resistant, 24 as medium susceptible and six as susceptible (Table 13).

Co-6 and Co-Selection were used for crossing the locally popular variety Sharika which was susceptible to the disease. All the F_1 in both crosses were resistant and were selfed to developed F_2. The F_2 obtained was evaluated for long poded nature and resistance to the disease. Fifty three progenies from the cross Sharika and Co-6 and 25 from the cross Co-Selection and Sharika were long poded and resistant. Collins *et al.* (1985) identified cultivars like Brown Crowder, Magnolia Blackeye, Mississippi Silver, Magnolia Purple and Worthmore as tolerant to BlCMV. Varieties V-317 and V-276 was reported to be resistant to CABMV by Sudhakumari (1993). Improved cowpea varieties with multiple resistance to viruses have been developed at IITA. Cowpea varieties IT82D-889, IT838-818, IT83D-442 and IT85F-867-5 were reported as resistant to CPMV, CABMV, CGMV, CMV and SBMV (Singh *et al.*, 1987., Thottappilly *et al.*, 1988. and Singh *et al.*, 1997). Bashir and Hampton (1996) tested 51 lines of cowpea against seven isolates of BlCMV and identified five genotypes, IT 8082049, Big Boy, Corona, Serido and Tennessee Cream immune to all seven isolates.

Further selection of a genotype with resistance to virus and vegetable qualities can be done from the F_2 population.

The carbohydrate content of resistant variety decreased from 51 mg g^{-1} fresh weight of tissues at one day after virus inoculation to 11 mg g^{-1} at fifteen days after inoculation, while in the susceptible variety, the value increased from 11 mg g^{-1} on one day after inoculation to 25.67 mg g^{-1} on fifteen days after inoculation. Resistant variety recorded a value of 14.67 mg g^{-1} on tenth day while the value was 16.67 for susceptible (Table 18, Fig. 10). Thus a general increase in value was noted for susceptible and decrease in value was noted for resistant. Decrease in carbohydrate concentration due to viral infection in susceptible cultivars of cowpea has been reported by Ramiah (1978), Singh and Singh (1984) and Mayoral *et al.* (1989). Decreased photosynthesis and increased respiration occurs in virus infected tissues and lead to altered concentration of carbohydrates (Bhavani *et al.*, 1998). Narayanaswamy and Ramakrishnan (1966) suggested that reduction in the level of carbohydrate might be due to the breakdown of carbohydrates which have to provide the substrate for accelerated respiration in virus infected plants.

Viral infections are reported to cause reduction in chlorophyll in susceptible plants. Singh and Suhag (1982) reported reduction in chlorophyll content of mung and urd bean infected with viruses. Kaur *et al.* (1991) reported reduction in chlorophyll content of yellow mosaic infected soybean cultivars. The present investigation also indicated a reduction in chlorophyll content of susceptible variety, Sharika compared to the resistant variety, Co-6. Generally an increase was observed in the case of total chlorophyll content of resistant variety. The value increased from 1.132 at one day after to 1.182 at

fifteen days after inoculation in the resistant variety. Total chlorophyll in the leaves of Sharika reduced from 1.017 to 0.803 during fifteen days observation after inoculation (Table 19). The present study also agree with the findings of the earlier workers.

An increase in protein content was noted for the susceptible and resistant variety. In the case of resistant, the value increased from 78.0 at one day after inoculation to 128.67 after fifteen days of inoculation. In the case of susceptible variety the increase in value was from 85.3 in the first day to 120.00 in the last day of observation. Between the varieties, the value was more for susceptible (111.67) compared to resistant (99.7) at ten days after inoculation (Table 20, Fig. 11). Higher protein content due to virus infection has been reported by Singh *et al.* (1978)., Singh and Singh (1984)., Singh and Singh (1987)., Yadav, (1988)., Yadav and Sharma (1988)., Mayoral *et al.* (1989)., Patil and Sayyad, (1991). The reason for higher protein content in infected susceptible plants might be due virus related protein specifically found in the particulate fractions of the protoplasts as reported by Rottier *et al.* (1980).

There was not much change in phenol content between resistant and susceptible varieties. The phenol content increased from 77 to 100 $\mu g\ g^{-1}$ one day after inoculation to ten days after inoculation for the resistant variety. The value then decreased to 86 $\mu g\ g^{-1}$, 15 days after inoculation. In the case of susceptible variety, the value increased from 88 to 98 $\mu g\ g^{-1}$ during the first five days and then decreased to 89 $\mu g\ g^{-1}$ after fifteen days of inoculation. The rate of increase

of total phenol was more in resistant, compared to susceptible till ten days after inoculation and thereafter there was a decline in both the varieties (Table 21, Fig. 12). Padma et al. (1976) found higher phenol content in infected seeds of cowpea. Ramiah (1978) observed that the total phenol content increased in CABMV inoculated leaves of susceptible cultivars of cowpea. Enhanced level of phenol content has been observed in hypersensitive cowpea leaves infected with tobacco ring spot virus. There was much difference between varieties with respect to phenol was reported by Rathi *et al.* (1986). Sohal and Bajaj (1993) observed an increase in total phenols in both resistant and susceptible varieties of mungbean infected with yellow mosaic virus.

Investigation on changes in the activity of peroxidase, polyphenol oxidase, phenylalamine ammonia-lyase clearly indicated that there was significant increase in the activities of these enzymes in inoculated plants. Peroxidase (PO) activity increased in resistant variety and reached its maximum from 27.25 on first day after inoculation to 59.8 at ten days after inoculation. The value decreased to 47.20 at fifteen days after inoculation. The susceptible variety showed an activity of 44.9 at one day after inoculation and maintained a constant level of 44.8 at fifteen days after inoculation (Table 22, Fig. 13).

Polyphenol oxidase activity increased in the resistant variety and reached a maximum of 7.6 at ten days after inoculation. The susceptible variety also showed an increasing trend and reached a maximum of 7.02 at ten days after inoculation (Table 23, Fig. 13). There after there was a sudden decrease in the activity of PPO in resistant and susceptible varieties. The activity dropped to 5.5

$\mu g\ g^{-1}$ in resistant and 2.5 $\mu g\ g^{-1}$ in susceptible at fifteen days after inoculation.

PAL activity was increased to 80.83 at ten days after inoculation in resistant variety. The susceptible variety showed increased PAL activity (84.17) at five days after inoculation. The PAL activity was higher for susceptible (55.00) compared to resistant (45.42) at fifteen days after inoculation (Table 24, Fig. 13).

Khatri and Chenulu (1970) found higher peroxidase activity in inoculated susceptible cowpea varieties infected with cowpea mosaic virus. Similar trend was observed in cucumber plants infected with cucumber mosaic virus (Serova *et al.* 1972). Several workers have studied the activities of peroxidases and polyphenol oxidases and outlined quantitative changes in activity before, during, and after virus induced necrosis (Loebenstein, 1972., van Loon, 1982., Wagih and Coutts, 1982). Higher activities of peroxidase and polyphenol oxidase activities were noted in hypersensitive tobacco to virus infection (van Kammen and Brouwer, 1964). Verma and Prasad (1987) established that induced resistance was attributed to the enhanced activity of peroxidase and polyphenol oxidase enzymes.

Rathi *et al.* (1986) assayed peroxidase, polyphenol oxidase, and isozyme of peroxidase in pigeon pea cultivars resistant and susceptible to sterility mosaic disease. There was less difference between the varieties with respect to peroxidase Polyphenol oxidase activity increased in susceptible cultivar following infection. Sohal and Bajaj (1993) reported polyphenol oxidase activity in resistant variety of mungbean infected with yellow mosaic virus. The activity decreased in both resistant and susceptible varieties.

The oxidative enzymes at their peak activity oxidise phenolics to quinones, which in turn inactivate the virus (Hampton and Fulton, 1961., Minck and Saksena, 1971). The elevated activity of polyphenol oxidase and peroxidase lead to an accumulation of phenol-oxidised products and quinone which inactivated the virus (Verma and Prasad, 1987). The present investigation is also in concorrence with the findings of earlier reports. Inoculation of susceptible and resistant cowpea plants resulted in significant increase in enzymatic activities with increase in plant age. Hence this altered enzymatic metabolism in inoculated plants may be due to the defence reaction.

Various chemicals like salicylic acid, barium chloride, manganese chloride, and neem oil were tested for their effect on aphid-borne mosaic virus of cowpea. Pre and post-inoculation treatments were done on the local lesion host of the virus, *C. amaranticolor*. In pre-treatment studies neem oil (ten per cent) concentration produced 68.92 per cent inhibition and was on par with other treatments (Table 25, Fig. 14). In post-inoculation treatments, $MnCl_2$ 150 mg l^{-1} gave a maximum inhibition per cent of 65.44 (Table 26, Fig. 15).

Verma and Awasthi (1978) found that thio semicarbazone derivatives inhibited gomphrena mosaic virus in cowpea plants. They also found that the antiviral activity varied from host to host and was dependent on time of application.

Prakash and Joshi (1979) reported maximum inhibition of cowpea banding mosaic virus in cowpea by six sprays of gallic acid. White *et al.* (1986) reported that the chemicals salicylic acid, manganese chloride and barium chloride induced

resistance in Xanthi-nc tobacco, leaves due to TMV infection. Van Huijsduijnen *et al.* (1986) found that salicylic acid inhibited the systemic multiplication of alfalfa mosaic virus (AMV) in Samsun NN tobacco. Conti *et al.* (1988) reported that very low concentration of acetyl salicylic acid was effective in inducing resistance in *Datura* against TMV.

Neem seed oil and neem leaf extract have been reported to inhibit lesion production by mechanically transmitted virus when mixed with inoculum or when applied to test plants (Verma, 1974,., Choudhuri and Saha, 1985., Zaidi *et al.*, 1988). Pre-treatment with neem oil reduced local lesion production by tobacco mosaic virus on *N. glutinosa*, *N. tabacum*, var. Samsun N. N, *C. amaranticolor* and *Datura stramonium*. The test plants were sprayed before virus inoculation (Roychoudhury and Jain, 1993).

Bioassay of chemicals and neem oil was conducted as a pot culture experiment on cowpea plants in completely randomised design. Both pre and post inoculation treatment of chemicals were done. Statistical analysis revealed that pre-inoculation application of neem oil (ten per cent) concentration was highly effective in reducing the symptoms of aphid-borne mosaic in cowpea (Table 27).

Similar effects of neem oil in reducing symptoms of viral infection has been reported by many authors. Neem seed oil and custard apple seed soil reduced the transmission of rice fungro virus (Mariappan and Saxena, 1983). Aiyanathan and Narayanasamy (1988) reported that pre-inoculation of neem oil reduced the infection of rice tungro virus in rice varieties. Reghunath and Gokulapalan also reported the efficiency of neem oil in reducing symptoms of viral disease in cowpea.

SUMMARY

6. SUMMARY

Studies were conducted on the aphid-borne mosaic disease of cowpea in Kerala.

The symptoms appeared as vein clearing on the young trifoliate leaves of cowpea. Typical symptoms appeared as light and dark green mottling, severe mosaic, dark green vein banding, blistering, distortion and reduction in leaf size. The plants became stunted with reduced flowering and pod production.

The virus could be transmitted mechanically through sap extracted in 0.01M phosphate buffer (pH 7.0). Insect transmission studies carried out with three aphids, viz., *A. craccivora*, *A. gossypii* and *M. persicae* indicated a high percentage of transmission by *A. craccivora*. The per cent transmission obtained for *A. craccivora*, *A. gossypi* and *M. persicae* were 50, 20 and 30 respectively. The virus was found to be transmitted through the seeds obtained from infected cowpea plants. Eleven per cent transmission was obtained for the cultivar Sharika.

The physical properties of the virus viz., dilution end point, thermal inactivation point and longevity *in vitro* were investigated and it was found that the virus was inactivated at dilutions between 10^{-3} and 10^{-4} and at temperatures between 60 and 65^{0} C. Longevity *in vitro* was recorded as four hours at 28 ± 4^{0} C and six hours at 8^{0} C.

Studies on virus-vector relationship showed that the virus could be efficiently transmitted with a short acquisition feeding period of ten minutes and an inoculation access of one minute. It was found that pre-acquisition starvation of one hour increased per cent transmission while post-acquisition starvation decreased per cent infection. A single aphid was capable of transmitting the virus to healthy cowpea plants.

The virus causing severe mosaic in cowpea was identified as blackeye cowpea mosaic virus by serological techniques. Electron micrographs indicated the presence of flexuous, filamentous virus particles of 750 nm in length.

Sixty five cowpea genotypes were screened for resistance to aphid-borne virus of cowpea. Two varieties, Co-6 and Co-Selection were grouped as no symptom producing, 33 as medium resistant, 24 as medium susceptible and six as susceptible. The varieties Co-6 and-Co Selection was crossed with the popular variety 'Sharika' to incorporate resistance against the virus. The F_2 progenies were evaluated for joint seggregation of pod length and resistance. Fifty three progenies of the cross Sharika and Co-6 and twenty five progenies of the cross Co-Selection and Sharika were long poded and resistant to virus.

The biochemical changes due to virus infection was studied in resistant (Co-6) and susceptible (Sharika) varieties. The carbohydrate content in leaves of resistant variety was lower than that of susceptible variety. Total chlorophyll in the leaves of resistant variety was higher than that of suceptible vareity. Increase in protein content was observed for both resistant and susceptible varieties. Not much change in phenol content was observed in both vareities. Activities of defence related

enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase was higher in resistant variety at ten days after inoculation compared to the susceptible.

Bioassay of chemicals and neem oil was conducted as a pot culture experiment to evaluate their efficacy in reducing the symptoms caused by the virus on *C. amaranticolor* and cowpea. Pre-inoculation treatment of chemicals and oil conducted on *C. amaranticolor* indicated that neem oil was better compared to other treatments. Post-inoculation treatment indicated that manganese chloride was better compared to other treatments.

Bioassay of chemicals and oil on cowpea plants was also conducted as a pot culture experiment. The results indicated that pre-inoculation application of ten per cent neem oil emulsion was effective in managing the viral disease cowpea.

REFERENCES

REFERENCES

Aboul-Ata, A. E., Allen, D. J., Thottappilly, G. and Rossel, H. W. 1982. Variation in rate of seed transmission of cowpea aphid-borne mosaic virus in cowpea. *Trop. Grain Leg. Bull.* **25** : 2-7

Aiyanathan, E. A. K. and Narayanasamy, P. 1988. Effect of neem oil on RTV infection in rice varieties with different levels of resistance. *Proc. Natl. Sem. Management of Crop Diseases with Plant Products Biological Agents*. TNAU, Madurai p. 40

Anderson, E. J., Kline, A. S., Morelock, T. E. and Mc New, R. W. 1996. Tolerance to blackeye cowpea mosaic potyvirus not correlated with decreased virus accumulation or protection from cowpea stunt disease. *Plant Dis.* **80** (8) : 847-852

Ando, Y., Ehara, Y. and Yamanaka, S. 1984. Release of antifungal phenolic compounds from cucumber mosaic virus-infected and non-infected cowpea protoplasts. *Phytopath. Z.* **110** : 354-359

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta Vulgaris*. *Plant Physiol.* **24** : 1-15

Atiri, G. I. 1984. Insect transmission characteristics of a Nigerian strain of cowpea aphid-borne mosaic virus. *Fitopatologia Brasileria* **9** : 495-503

Atiri, G. I., Ekpo, E. J. A. and Thottappilly, G. 1984. The effect of aphid resistance in cowpea on infestation and development of *Aphis craccivora* and transmission of cowpea aphid-borne mosaic virus. *Ann. appl. Biol.* **104** : 339-346

Atiri, G. I. and Thottappilly, G. 1984. Relative usefulness of mechanical and aphid inoculation as modes of screening cowpeas for resistance against cowpea aphid-borne mosaic virus. *Trop. Agric.* **61** : 289-292

Atiri, G. I., Enobakhare, D. A. and Thottappilly, G. 1986. The importance of colonizing and non-colonizing aphid vectors in the spread of cowpea aphid-borne mosaic virus. *Plant Prot.* **5** : 406-410

Bashir, M. and Hampton, R. O., 1994. Seed and aphid transmission of some isolates of blackeye cowpea and cowpea aphid-borne mosaic poly viruses. *Pakistan J. Phytopathol.* **6** (2) : 140-146

Bashir, M., Ahmed, Z., Zafar, R. and Malik, B. A. 1995. Sources of immunity in cowpea against blackeye cowpea mosaic potyvirus. *Pakistan J. Phytopathol.* **7** (2) : 94-97

Bashir, M. and Hampton, R. O. 1995. Purification and electron microscopy of some isolates of blackeye cowpea mosaic and cowpea aphid-borne mosaic potyvirus. *Pakistan J. Bot.* **27** (1) : 243-249

Bashir, M. and Hampton, R. O. 1996a. Sources of genetic resistance in cowpea (*Vigna unguiculata* (L.) Walp.) to cowpea aphid-borne mosaic potyvirus. *European J. Plant Pathol.* **102** (5) : 411-418

Bashir, M. and Hampton, R. O. 1996b. Identification of cowpea (*Vigna unguiculata*) cultivars and lines immune to variants of blackeye cowpea mosaic potyvirus. *Plant Pathol.* **45** (5) : 984-989

Batra, G. K. and Kuhn, C. W. 1975. Polyphenoloxidase and peroxidase activities associated with acquired resistance and its inhibition by 2 - thiouracil in virus infected soybean. *Physiol. Plant Pathol.* **5** : 239-248

Bauer, U., Kluge, S., Nuhn, P., Stenger, P. and Lettau, H. 1993. Enantio selective antiphytoviral activity of 1 - (α-carboxy alkyl) - 4, 5 - dimethyl imidazol-3-oxides in systemically infected host plants. *J. Phytopathol.* **137** : 309-316

Bhavani, U., Subbiah, K. V., Rao, A. S. and Sai Gopal, D. V. R. 1998. Studies on mosaic disease of sunflower : biochemical changes and growth parameters. *Indian Phytopath.* **51** (4) : 357-358

Bock, K. R. 1973. East African strains of cowpea aphid-borne mosaic virus. *Ann. of App. Biol.* **74** : 75-83

Bock, K. R. and Conti, M. 1974. Cowpea aphid-borne mosaic virus No. 134. In : *Description of Plant Viruses*. Commonwealth Mycological Institute, Association of Applied Biologists, Kew, Surrey, England p. 4

Bos, L. 1982. Crop losses caused by viruses. *Adv. Virus Research* **2** : 31-57

Boswell, K. F. and Gibbs, A. J. 1983. Viruses of Legumes - Descriptions and keys from VIDE. Canberra Publishing and Printing Co. p. 42-53

Bradford, M. M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilising the principle of protein - dye binding. *Anal. Biochem.* **72** : 248

Bray, G. G. and Thorpe, W. V. 1954. Analysis of phenolic compounds of interest in metabolism. *Methods Biochem. Anal.* **1** : 27-52

Burke, D. W., Ditshipi, P. and De Mooy, C. J. 1986. Virus diseases of cowpeas in dry land and irrigated plots in Botswana. *Plant Dis.* **70** (8) : 801

Caner, J., Fazio-G-de., Alexandre, M. A. V., Kudamatsu, M. and Vincente,M. 1985. Action of antiviral chemicals in the control of bean golden mosaic virus on *Phaseolus lunatus* L. *Arquivos - do - Instituto Biologico* **52** (1-4) : 39-43

Chang, C. A. and Kuo, Y. J. 1983. Cowpea aphid-borne mosaic virus and its effect on the yield and quality of asparagus bean. *J. Agrl. Res. China* **32** : 270-278

Chowdhury, A. K. and Saha, N. K. 1985 Inhibition of urd bean leaf crinkle virus by different plant extracts. *Indian Phytopath.* **38** : 566-568

Collins, M. H., Witcher, W., Barnett, O. W. and Ogle, W. L. 1985. Reactions of 16 cowpea cultivars to six viruses. *Plant Dis.* **69** : 18-20

Coutts, R. H. A. and Wagih, E. E. 1983. Induced Resistance to viral infection and soluble protein alterations in cucumber and cowpea plants. *Phytopath. Z.* **107** : 57-69

Dawson, W. O. 1984. Effects of animal antiviral chemicals on plant viruses. *Phytopathol.* **74** : 211-213

Dickerson, D. P., Pascholati, S. F., Hagerman, A. K., Butler, L. G. and Nicholsn, R. L. 1984. Phenylalanine ammonia - lyase hydroxycinnamate : CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiol. Plant Pathol.* **25** : 111-123

Dijkstra, J., Bos, L., Bouwneester, H. J., Hadiastono, T. and Lohuis, H. 1987. Identification of blackeye cowpea mosaic virus from germplasm of yardlong bean and from soybean and the relationships between blackeye cowpea mosaic virus and cowpea aphid-borne mosaic virus. *Neth. J. Plant Path.* **93** : 115-133

Fidan, U. and Yorganci, U. 1990. Investigation on the detection and seed transmission of the virus diseases occurring on pulse crops in Aegean Region. I. The identification of viruses infecting pulse crops in Aegean Region. *J. Turkish Phytopathol.* **18** : 93-105

Fischer, H. U. and Lockhart, B. E. 1976. A strain of cowpea aphid-borne mosaic virus isolated from cowpeas in Morocco. *Phytopath. Z.* **85** : 43-48

Gillaspie, A. G. Jr., Hopkins, M. S. and Pinnow, D. L. 1993. Relationship of cowpea seed-part infection and seed transmission of blackeye cowpea mosaic potyvirus in cowpea. *Plant Dis.* **77** : 875-877

Govindaswamy, C. V., Mariappan,V., Murugesan, S. S., Padmanabhan, C. Thangamani, G. and Janaki, I. P. 1970. Studies on a cowpea mosaic virus disease. *Madras agric. J.* **57** : 405-414

Gumodzoe, M. D. 1993. Major viruses of cowpea (*Vigna unguiculata* (L.) Walp.) in Togo. *Cahiers Agricultures* **2** (5) : 352-355

Gumedzoe, M. Y. D., Rossel, H. W., Thottappilly, G., Asselin, A. and Huguenot, C. 1998. Reaction of cowpea (*Vigna unguiculata* (L.) Walp.) to six isolates of blackeye cowpea mosaic virus (BlCMV) and cowpea aphid-borne mosaic virus (CAMV), two potyviruses infecting cowpea in Nigeria. *Int. J.Pest Management* **44** (1) : 11-16

Hampton, R. E. and Fulton, R. W. 1961. The relation of polyphenol oxidase to instability *in vitro* of prune dwarf and sour cherry necrotic ring spot viruses. *Virology* **13** : 44-52

Hayati, J. and Varma, J. P. 1985. Effect of some chemicals on Tomato leaf curl virus infection of tomato *Indian J. Virol.* **1(2)** : 152-156

Hedge, J. E. and Hofreiter, B. T. 1962. In : *Carbohydrate Chemistry 17* (Eds. Whistler, R. L. and Be Miller, J. N) Academic Press, New York

Huguelnot, C., Furneaux, M. T., Thottappilly, G., Rossel, H. W. and Hamilton, R. I. 1992. Evidence that cowpea aphid-borne mosaic and blackeye cowpea mosaic viruses are two different potyviruses. *J. of Gen. Virol.* **75** (3) : 335-340

Kaiser, W. J. and Mossahebi, G. H. 1975. Studies with cowpea aphid-borne mosaic virus and its effect on cowpea in Iran. *FAO Plant Prot. Bull.* **23** : 33-39

Kannan, N. R. and Doraiswamy, S. 1994. Screening cowpea entries for seed-borne infection of CAMV and to study the weed hosts of the virus. *Madras agric. J.* **81** (11) : 637-638

Kato, T., Yamaguchi, Y., Ohtani, T., Kabuto, Y., Uyehara, J., Ehara, Y., Oh, B. K. and Yoshikawa, M. 1993. Characterization of two phenolic compounds extracted from cowpea leaves infected with cucumber mosaic virus. *Ann. Phytopath. Soc.* Japan **59** : 209-213

Kaur, G., Gill, C. G., Rataul, H. S. and Raheja, R. K. 1991. Biochemical changes in soybean (*Glycine max* L.) cultivars infected with yellow mosaic virus. *Biochemie und Physiologie de pflanzen* **187** (5) : 357-371

Khatri, H. L. and Chenulu, V. V. 1969. Metabolism of resistant and susceptible cowpea varieties infected with cowpea mosaic virus. *Indian Phytopath.* **22** : 453-457

Khatri, H. L. and Chenulu, V. V. 1970. Metabolism of resistant and susceptible cowpea varieties infected with cowpea mosaic virus. II changes in peroxidase and catalase enzyme activity. *Indian Phytopath.* **23** : 553-557

Khurana, S. M. P., Singh, M. N. and Shiv Kumar, 1987. Purification of Potato virus Y and the production of antiserum. *Curr. Science* **56** (9) : 420-422

Kline, A. S. and Anderson, E. J. 1997a. First report of CABMV potyvirus from cowpeas grown commercially in the U.S. *Plant Dis.* **81** (8) : 959

Kline, A. S. and Anderson, E. J. 1997b. UARK-M2 : a line of cowpea cultivar Coronet with blackeye cowpea mosaic virus resistance. *Hort Science* **32** (5) : 945-946

Konate, G. and Neya, B. J. 1996. Rapid detection of cowpea aphid-borne mosaic virus in cowpea seeds. *Ann. appl. Biol.* **129** (2) : 261-266

Kovalenko, A. G., Grabina, T. D., Kolesnik, L. V., Didenko, L. T., Oleschenko, L. T., Olevinskaya, Z. M. and Telegeeva, T. A. 1993. Virus resistance induced with mannan sulphates in hypersensitive host plants. *J. Phytopathol.* **137** : 133-147

Ladipo, J. L. and Allen, D. J. 1979. Identifiction of resistance to cowpea aphid-borne mosaic virus. *Trop. Agric.* (Trinidad) **56** (4) : 358-359

Loebenstein, G. 1972. Localisation and induced resistance in virus infected plants. *Ann. Rev. Phytopath.* **10** : 177-206

Lima, J. A. A., Purcifull, D. E. and Hiebert, E. 1979. Purification, partial characterization and serology of blackeye cowpea mosaic virus. *Phytopathol.* **69** : 1252-1258

Lima, J. A. A. and Puricifull, D. E. 1980. Immunochemical and microscopical techniques for detecting blackeye cowpea mosaic and soybean mosaic viruses in hypocotyls of germinated seeds. *Phytopathol.***70** (2) : 142-147

Lima, J. A. A., Santos, C. D. G. and Silveira, L. F. C. 1986. Behaviour of cowpea genotypes with respect to two main viruses occurring in Ceara. *Fitopatologia Brasileira* **10** (1) : 141-161

Lovisolo, O. and Conti, M. 1966. Identification of an aphid-transmitted cowpea mosaic virus. *Neth. J. Plant Pathol.* **72** : 265-269

Mali, V. R. and Kulthe, K. S. 1980. Seed borne potyvirus causing mosaic of cowpea in India. *Plant Dis.* **64** : 925-928

Mali, V. R., Mundhe, G. E., Patil, N. S. and Kulthe, K. S. 1988. Detection and identification of blackeye cowpea mosaic and cowpea aphid-borne mosaic viruses in India. *Int. J. Trop. Plant Dis.* **6** (2) : 159-173

Mali, V. R., Mundhe, G. E and Shaikh, W. R. 1989. Sero-diagnosis of six cowpea seedborne viruses in India. *Indian J. Virol* **5** : 45-55

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

Mariappan, V., Saxena, R. C and Ling, K. C. 1982. Effect of custard apple oil and neem oil on the life span of rice tungro virus transmission by *Nephotettix virescens*. *Int. rice res. Newsl.* 7 : 13-14

Mayer, A. M., Harel, E. and Shaul, R. B. 1965. Assay of catechol oxidase, a critical comparison of methods. *Phytochemistry* **5** : 783-789

Mayoral, M. L., Mallorea, M. S. and Uzcategui, R. 1989. Comparative response of inoculated and nitrogen supplied cowpea (*Vigna unguiculata* (L.) Walp.) plants to infection by cowpea mosaic virus. *J. Expt. Bot.* **40** 159-165

Miller, J. C. and Scheuring, D. C. 1994. Texas Pink eye Purple Hull cowpea. *Hort. Science* **29** (8) : 926-927

Minck, G. I. and Saksena, K. N. 1971. Studies on the mechanism of oxidative inactivation of plant viruses by plant viruses by O-quinones. *Virology* **45** : 755-763

Murphy, J. F., Barnett, O. W. and Witcher, W. 1987. Characterization of a blackeye cowpea mosaic virus strain from South Carolina. *Plant Dis.* **71** : 243-248

Nain, P. S., Rishi, N., Bishnoi, S. S. 1994. Growth stages of cowpea (*Vigna unguiculata*) vis-a-vis seed transmission of viruses. *Indian J. Virol* **10** (1) : 37-43

Nain, P. S., Rishi, N. and Bishnoi, S. S. 1994. Profile of viral diseases of cowpea (*Vigna unguiculata*) in Northern India. *Indian J. Virol.* **10** : 128-136

Narayanasamy, P. and Ramakrishnan, K. 1966. Studies on sterility mosaic disease of pigonpea. II. Carbohydrate metabolism of infected plants. *Proc. Natl. Sem. Management of Diseases of Oilseed Crops*. TNAU, Madurai p. 13-15

Ndiaye, M., Bashir, M., Keller, K. E. and Hampton, R. O. 1993. Cowpea viruses in Senegal, West Africa : Identification, distribution, seed transmission and sources of genetic resistance. *Plant Dis.* **77** (10 : 999-1003

Ndiaye, M., Thiaw, S. and Hall, A. E. 1995. Registration of 'Mouride' cowpea. *Crop Science* **35** (4) : 1215-1216

Noordam, D. 1973. *Identification of Plant Viruses - Methods and Experiments*. Oxford and IBH Publishing Co. p. 103-134

Padma, R., Singh, S., Verma, S. and Upretty, D. C. 1976. Chemical composition of healthy and diseased seeds collected from mosaic affected cowpea plants. *Z. pflanizen. pflanzenschutz* **83** : 459-461

Patel, P. N. and Kuwaite, C. 1982. Prevalence of cowpea aphid-borne mosaic virus and two strains of cowpea mosaic virus in Tanzania. *Indian Phytopath.* ***35*** : 467-472

Patil, P. L. and Sayyad, A. N. 1991. A study on cowpea mosaic virus Rhizobium interaction in cowpea. *J. Maharashtra Agric. Univ.* **16** : 332-334

Ponte, J. J., Alves, M. E. and Da Ponte, J. J. 1994. Reaction of cowpea cultivar 'Pampo' (*Vigna unguiculata*) to three viruses. *Fitopatologia brasileira* **19** (1) : 92-94

Prakash, J. and Joshi, R. D. 1979. Inhibition of cowpea banding mosaic virus in cowpea (*Vigna sinensis* (L.) Savi ex. Hassk.) by some phenolic acids. *Indian J. Microbiol* **19** : 61-63

Quindere, M. A. W. and Barreto, P. D. 1988. Evaluation of cultivar and lines of cowpea, *Vigna unguiculata* (L.) Walp. and their reaction to disease. *Agropecuaria do Ceara* **11** : 15

Rachie, K. O. 1985. Introduction : *Cowpea Research, Production and Utilization* (Eds. S. R. Singh and K. O. Rachie) John Wiley and Sons

Ramiah, M. 1978. Studies on mosaic disease of cowpea in relation to disease resistance. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore p. 105

Rathi, Y. P. S., Bhatt, A. and Singh, U. S. 1986. Biochemical changes in pigeon pea (*Cajanus cajan* (L.) Millsp.) leaves in relation to resistance against sterility mosaic disease. *J. Biosci.* **10** (4) : 467-474

Rao, G. P., Shukla, K. and Gupta, V. N. P. 1988. Induction of resistance by 12 - tungstozincic (II) acid in hypersensitive and non hypersensitive hosts against legume viruses. *Indian J. Virol* **4** : 1-2, 64-68

Rao, G. P., Singh, R. K., Pandey, A. K. and Singh, R. 1985. Induction of local and systemic resistance of potato virus x by flower extracts. *Indian J. Virol.* **1** (2) : 174-182

Reghunath, P. and Gokulapalan, C. 1996. In : *Keedanashinikalillatha Pachakari Krishi*. Farm Information Bureau, Trivandrum p. 32

Roberts, J. M. F., Thottappilly, G. and Hodgson, C. J. 1993. The ability of *Aphis craccivora*, *A. gossypii* and *A. citricola* to transmit single and mixed viruses of cowpeas. *J. Phytopathol.* **138** : 164-170

Rossel, H. W. and Thottappilly, G. 1985. Virus diseases of important food crops in tropical Africa - IITA Publications series, IITA, Ibadan

Rottier, P. J. M., Rezelman, G. and Kammen, A. B. 1980. Protein synthesis in cowpea mosaic virus infected protoplasts : detection of virus - related proteins. *J. Gen. Virol.* **51** : 359-371

Roychoudhary, R. and Jain, R. K. 1993. Neem for the control of aphid and whitefly vectors and virus diseases of plants. In : *Neem and Environment* Vol. 2 (Eds. Singh, R. P., Chari, M. S., Raheja, A. K. and Kraus, W.) Oxford and IBH Publishing Co. p. 763-775

Sastry, K. S. and Nayudu, M. V. 1988. Studies on biochemical changes in cowpea (*Vigna unguiculata* (L.) Walp.) infected with tobacco ring spot virus. *Indian J. Virol.* **4** : 138-139

Serova, Z. Y., Ges, D. K. and Reutsk, L. M. 1972. Changes in peroxidase and catalase activity in virus infected tomato and cucumber. *Bia Navule.* **5** : 48-55

Schuster, G. 1986. Neccessities, problems and results of the development and application of antiphytoviral substances against tobacco viruses. *Acta phytopathologica et Entomologica Hungarica* **21** (1-2) : 15-22

Schuster, G. and Holy, A. 1988. Inhibitory effects of 9 - (2-3- dihydroxypropyl) adenine and 3 (adenin-9-yl)-2-hydroxy propanoic acid 2-methyl propylester on potato virus x replication. *Antiviral Res.* **9** : 329-334

Sharma, S. R. and Varma, A. 1975. Three sap transmissible viruses from cowpea in India. *Indian Phytopath.* **28** : 192-198

Sharma, S. R. and Varma, A. 1977. Factors affecting transmission of cowpea banding virus by aphids *J. Entomol. Res.* **1** : 29-35

Singh, A. K. and Singh, A. K. 1984. Effect of southern bean mosaic virus infection on the leaf protein concentration in cowpea cultivars. *Curr. Sci.* **53** : 390

Singh, A. K. and Singh, A. K. 1987. Chemical composition of cowpea seeds as influenced by southern bean mosaic virus and cowpea mosaic virus. *Phyton.* **26** : 165-170

Singh, A. K., Singh, M. and Singh, A. K. 1988. Antiviral activity and physical properties of the extracts of *Azadiracta indica* L. *Indian J. Virol.* **4** : 76-81

Singh, B. B., Chambliss, O. L. and Sharma, B. 1997. In : *Advances in Cowpea Research* (Eds. Singh, B. B., Mohan Raj, D. R., Dashiell, K. E. and Jackai, L. E. N.) IITA and JIRCAS p. 375

Singh, B. B., Thottappilly, G. and Rossel, H. W. 1987. Breeding for multiple virus resistance in cowpea. *Agronomy Abstracts* 79

Singh, H. C., Singh, B. R. and Ganguli, R. 1978. Studies on the nitrogen metabolism of cowpea leaves infected with southern bean mosaic virus. *Acta Botanica Indica* **6** : 213-214

Singh, J. P. and Suhag, L. S. 1982. Pigment, nucleic acid and protein concentration in the virus infected mung bean and urd bean leaves. *Indian J. Mycol. Pl. Pathol.* **12** (1) : 61-63

Singh, R. and Singh, H. C. 1981. Changes in nitrogenous constituents of cowpea fruit due to cowpea mosaic virus infection. *Natl. Acad. Sci. Lett.* **4** : 145-148

Sohal, B. S. and Bajaj, K. L. 1993. Effects of yellow mosaic virus on polyphenol metabolism in resistant and susceptible mung bean (*Vigna radiata* L. Wilczek) leaves. *Biochemie und Physiologie der Pflanzen* **188** (6) : 419-423

Sreelakha, L. 1987. Properties, host range and control of cowpea mosaic virus. M.Sc. (Ag.) thesis, Kerala Agricultural. University, Thrissur p. 121

Sreelakha, L. and Balakrishnan, S. 1988. Effect of plant leaf extract sprays on the incidence of cowpea mosaic disease. *Proc. Natl. Sem. Management* of crop diseases with Plant Products / Biological agents. TNAU, Madurai, p. 46

Srivastava, S. K. 1987. Peroxidase and polyphenol oxidase in *Brassica juncea* plants infected with *Macrophomina phaseolina* (Tassi) Goid and their implication in disease resistance. *Phytopath Z.* **120** : 249-254

Sudhakumari, J. S. 1993. Screening of cowpea (*Vigna unguiculata* (L.) Walp.) types for resistance to cowpea aphid-borne mosaic disease. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur p. 106

Taiwo, M. A. and Gonsalves, D. 1982. Serological grouping of isolates of blackeye cowpea mosaic and cowpea aphid-borne mosaic virus. *Pytopathol.* **72** : 583-589

Taiwo, M. A., Gonsalves, D., Provvidenti, R. and Thurston, H. D. 1982. Partial characterization and grouping of isolates of blackeye cowpea mosaic and cowpea aphid-borne mosaic viruses. *Phytopathol.* **72** : 590-596

Thresh, J.M. 1974. Temporal patterns of virus spread. *Ann. Rev. Phytopathol.* **12** : 111-128

Thottappilly, G. and Rossel, H. 1985. World wide occurrence and distribution of virus diseases In : *Cowpea Research, Production and Utilization* (Eds. S. R. Singh and K. O. Rachie, Eds.) John Wiley and Sons. p. 155-171

Thottappilly, G. and Rossel, H. W. 1992. Virus diseases of cowpea in tropical Africa. *Trop. Pest Management* **38** : 337-348

Tsuchizaki, T., Senboku, T., Iwaki, M., Pholaupora, S., Srithongchi, W., Oeema, N. and Ong, C. A. 1984. Blackeye cowpea mosaic virus from asparagus bean (*Vigna sesquipedalis*) in Thailand and Malaysia and their relationship to a Japanese isolate. *Ann. Phytopath. Soc. Japan.* **50** (4) : 461-468

Umamaheswaran, K. 1996. Management of cowpea aphid borne mosaic virus. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore

Van Huijsduijnen, R. A. M. H., Alblas, S. W., De Rijk, R.H. and Bol, A. F. 1986. Induction by salicylic acid of pathogenesis related proteins and resistance to alfalfa mosaic virus infection in various plant species. *J. Gen. Virol.* **67** : 2135-2143

van Kammen, A. and Brouwer, D. 1964. Increase of polyphenol oxidase activity by a local virus infection in uninoculated parts of leaves. *Virology* **22** : 9-14

van Loon, L. C. 1982. Active Defense Mechanisms in Plants Plenum press, New York p. 339

Verma, V. S. 1974. Chemical compounds from Azadiracta as inhibitors of potato virus x. *Acta Microbiol. Polonica* **6** : 9-13

Verma, H. N. and Prasad, V. 1987. Systemic induced antiviral resistance by plant extract alters physiology of susceptible test host. *Indian J. Plant Pathol.* **5** : 69-72

White, R. F., Antoniw, J. F., Carr, J. P. and Woods, R. D. 1983. The effects of aspirin and polyacrylic acid on the multiplication and spread of TMV in different cultivars of tobacco with and without N-gene. *Phytopath. Z.* **107** : 224-232

White, R. F., Duman,E., Shaw, P. and Antoniw, J. F. 1986. The chemical induction of PR (b) proteins and resistance to TMV infection in tobacco. *Antiviral Res.* **6** : 177-185

Wagih, E. E. and Coutts, R. H. A. 1982. Peroxidase, polyphenoloxidase and ribonuclease in tobacco necrosis virus infected or mannitol osmotically stressed cowpea and cucumber tissue. II. Qualitative alterations. *Phytopath. Z.* **104** : 124-137

Wani, S. P., Sivaramakrishnan, S., Naidu, R. A., Zambre, M. A., Lee, K. K. and Pande, S. 1991. Biochemical changes in sorghum (*Sorghum bicolor* L. Moench) plants infected wtih maize mosaic virus. *Indian J. Microbiol.* **31** (4) : 387-395

Yadav, A. K. 1988. Changes in biochemical status of cowpea infected with cowpea banding mosaic virus. *Indian J. Mycol. Plant Pathol.* **17** : 233-234

Yadav, A. K. and Sharma, S. 1988. Disturbance in phytochemical status in different plant parts of cowpea infected with cowpea mosaic virus. *Indian J. Mycol. Plant Pathol.* **17** : 182-183

Zaidi, Z. B., Gupta, V. P., Samad, A. and Naqui, Q. A. 1988. Inhibition of spinach mosaic virus by extracts of some medicinal plants. *Curr. Sci.* **57** : 151-153

Zhao, G. S., Baltensperger, D.D., Hiebert, E., Purcifull, D. E. and Edwardson, J. R. 1991. Purification, serology and *in vitro* translation of an Alyce-clover isolate of blackeye cowpea mosaic virus. *Plant Dis.* **75** : 254-257

APPENDICES

APPENDIX - 1

Buffers for sap extraction

0.1M citrate buffer (pH 6.2)

Stock solutions

A : 0.1 M solution of citric acid (19.21 g in 1000 ml)

B : 0.1 M solution of sodium citrate (29.41 g in 1000 ml)

7.2 ml of A is mixed with 42.8 ml of B and diluted to total of 100 ml

2. 0.1 M citrate - phosphate buffer (pH 7.0)

stock solution

A : 0.1 M solution of citric acid (19.21 g in 1000 ml)

B : 0.2 M solution of diabasic sodium phosphate

(53.65 g of Na_2HPO_4 $7H_2O$ in 1000 ml)

6.5 ml of A is mixed with 43.6 ml of B diluted to a total of 100 ml

3. 0.1 M Phosphate buffer (pH 7.2)

A : 0.2 M solution of monobasic sodium phosphate (27.8 g in 1000 ml)

B : 0.2 M solution of dibasic sodium phosphate

(53.65 g of Na_2HPO_4, $7H_2O$ or 71.7 g of Na_2HPO_4. $12H_2O$ in 1000 ml)

39.0 ml of A is mixed with 61.0 ml of B, diluted to a total of 200 ml.

4. 0.1 M Tris buffer (pH 7.2)

A : 0.2 M solution of Tris (24.2 g in 1000 ml)

B : 0.2 N HCl

50 ml of 4 is mixed with 44.2 ml B diluted to a total of 200 ml

5. 0.1 M Borate buffer (pH 7.6)

A : 0.2 M solution boric acid (12.4 g in 1000 ml)

B : 0.05 M solution of borax (19.05 g in 1000 ml., 0.2 M in terms of sodium borate)

50 ml of A is mixed with 2.0 ml of B and diluted to a total of 200 ml

APPENDIX - II

Buffers for ELISA

1. Phosphate buffered saline (PBS-T) pH 7.4

   8.0 g NaCl

   0.2 g KH_2PO_4

   1.1 g Na_2HPO_4

   0.2 g KCl

   0.2 g NaN_3

   In one litre water

   0.5 ml Tween 20 (0.05 per cent)

2. Coating buffer (pH 9.6)

   1.59 g $Na_2 CO_3$

   2.93 g Na HCO_3

   0.2 NaN_3

   in 1 litre water

3. Substrate buffer (pH 9.8)

   97 ml diethanolamine

   800 ml H_2O

   0.2 g NaN_3

   Add HCl to give pH 9.8

APPENDIX - III

Buffers for enzyme analysis

0.1 M sodium acetate (pH 4.7)

Stock solutions

A : 0.2 M solution of acetic acid (11.55 ml in 1000 ml)

B : 0.2 M solution of sodium acetate (16.4 g of $C_2H_3O_2$ Na Or 27.2 g of $C_2H_3O_2$ Na $3H_2O$ in 1000 ml).

22.7 ml of A is mixed with 27 ml of B, diluted to a total of 100 ml.

0.1 M Borate Buffer (pH 8.8)

A : 0.2 M solution boric acid (12.4 g in 1000 ml)

B : 0.05 M solution of borax (19.05 g in 1000 ml)

50 ml of A is mixed with 30 ml of B, diluted to a total of 200 ml.

DISEASE RESISTANCE IN THE MANAGEMENT OF COWPEA APHID-BORNE MOSAIC VIRUS

By

RADHIKA N.S.

ABSTRACT OF THE THESIS
SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR
THE DEGREE
MASTER OF SCIENCE IN AGRICULTURE
(PLANT PATHOLOGY)
FACULTY OF AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI
THIRUVANANTHAPURAM

1999

ABSTRACT

Investigations were undertaken on the virus causing severe mosaic on cowpea (*Vigna unguiculata* (L.) Walp) in Kerala. The characteristic symptoms appeared as vein clearing, light and dark green mottling, severe mosaic, dark green vein banding, blistering, distortion and reduction in leaf size. The virus was mechanically transmitted through sap extracted in 0.01 M phosphate buffer (pH 7.0). The virus was efficiently transmitted by the aphid vector, *Aphis craccivora*. Seed transmission of eleven per cent was recorded in the variety Sharika. Thermal inactivation point was recorded at a range of 60 - 65^0 C, dilution end point at a range of 10^{-3} - 10^{-4} and longevity *in vitro* for four hours at room temperature (28 ± 4^0 C) and six hours under refrigerated condition (8^0 C). *A. craccivora* could efficiently transmit the virus with an acquisition access of ten minutes and inoculation access of one minute. Pre-acquisition starvation increased the rate of transmission while post-acquisition starvation decreased the rate. A single aphid was capable of transmitting the virus. The virus causing severe mosaic was identified as blackeye cowpea mosaic virus by ELISA. The virus could also be detected by Ouchterlony immunodiffusion test. Electron microscopic studies revealed the presence of flexuous, filamentous particles of 750 nm in length. Two varieties Co-6 and Co-Selection were grouped as no symptom producing among 65 genotypes screened for resistance. Fifty three F_2 progenies of the cross Sharika

and Co-6 and twenty five F_2 progenies of the cross Co-Selection and Sharika were long poded and resistant. Biochemical changes indicated a lower carbohydrate content in resistant compared to susceptible. Chlorophyll content decreased in the susceptible variety due to virus infection. Increase in protein was observed in both resistant and susceptible. The phenol content did not show variation between the varieties. Peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase activities increased in the resistant variety. Bioassay of chemicals and neem oil on local lesion host (*C. amaranticolor*) indicated a per cent inhibition of 68.92 by neem oil in pre-inoculation application and 65.45 per cent inhibition by manganese chloride in post-inoculation application. On cowpea plants, pre-inoculation application of neem oil (ten per cent) concentration was found to be effective in reducing the symptoms due to viral infection.