INVESTIGATIONS ON MOSAIC DISEASE OF BITTER GOURD

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1994

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I hereby declare that this thesis entitled '**Investigations on mosaic disease of bitter gourd**' 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

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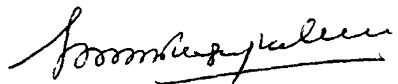

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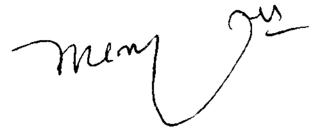
Dr. M. CHANDRASEKHARAN NAIR 

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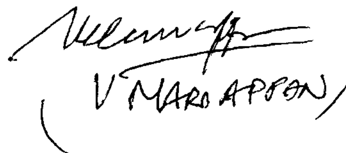
External Examiner 

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INTRODUCTION

INTRODUCTION

Bitter gourd (Momordica charantia L.) is one of the major vegetable crops of Kerala and is being extensively cultivated in many parts of India Bitter gourd fruit is a rich source of vitamins and minerals and is used as a dietery inclusion for persons suffering from some diseases A number of diseases affecting this crop have been reported from Kerala and other states of the Country (Singh, 1987) Among them the virus diseases are known to cause serious damage to the crop wherever it is cultivated Bitter gourd plants with mosaic symptoms were reported from different parts of India (Uppal, 1933, Nagarajan and Ramakrishnan, 1971a)

Mosaic disease of bitter gourd so far considered as a minor disease has gained importance in many parts of Kerala in the recent past No study has been made so far to identify the bitter gourd mosaic virus occurring in Kerala Therefore in the present investigation an attempt has been made to identify the virus and to study the following aspects pertaining to the disease, so as to evolve suitable viable management practice against the disease

1. Symptomatology
2. Transmission of the virus
3. Physical properties of the virus
4. Vector-virus relationship
5. Host-range and local lesion hosts
6. Serological properties of the virus
7. Varietal screening
8. Estimation of loss

REVIEW OF LITERATURE

REVIEW OF LITERATURE

I. Symptomatology

Bitter gourd with mosaic like symptoms has been observed in India since 1933 Uppal (1933) reported a mosaic disease of bitter gourd for the first time in India Nagarajan and Ramakrishnan (1971a) reported a mosaic disease of bitter gourd which was characterised by a mosaic pattern of irregular dark green and light green patches on the leaf lamina

Nagarajan and Ramakrishnan (1971 b) studied cucurbit viruses in Madras State and reported the occurrence of watermelon mosaic virus on snake gourd. The plants were stunted and leaves were affected by prominent mosaic mottling with considerable reduction in leaf size. In advanced stages the leaves were crowded together to give a bushy appearance When young plants were infected considerable malformation was seen in leaves The symptoms appeared within 7-9 days after inoculation

Pillai (1971) reported a mosaic disease of snake gourd in Kerala The symptoms consisted of a distinct mosaic

and crinkling and reduction in size of the leaves. Affected plants were stunted and produced fewer flowers and fruits. The causal virus was reported to be due to a strain of cucumber mosaic virus Dubey et al (1974) found a snake gourd mosaic disease in Delhi The chief symptoms were mosaic mottling accompanied by chlorosis, vein banding and blistering of leaf lamina Diseased plants produced only a few weak runners and plants affected in an early stage blossomed sparingly and set few fruits The causal virus was identified as Cucumis virus 1 Joseph and Menon (1978) while studying the mosaic disease of snake gourd in Kerala found that the symptoms were characterised by mosaic with dark green raised blisters on the leaf lamina, reduced leaf size, shortened and retarded growth

Cucumber mosaic virus disease was reported from many places Pejcinovski (1978) observed that cucumber mosaic virus (CMV) caused mosaic, dwarfing and wilt symptoms on cucumber, melon and pumpkin in Macedonia Makkouk and Leseman (1980) reported a severe mosaic of cucumber in Lebanon with mottling, blistering and malformation caused by watermelon mosaic virus 1 (WMV-1) Weber et al (1982) reported a disease of glass house cucumber (Cucumis sativus)

characterised by light green yellowish indistinot spots with brown necrotic centres The disease causing agent was identified as cucumber leaf spot virus Sharma et al (1984) observed a mosaic disease of muskmelon in Punjab and identified the disease causing organism as a distinct strain of cucumber mosaic virus and designated as cucumber mosaic virus muskmelon strain (CMV-mst)

Mosaic diseases were reported on watermelon plants from many places Bakker (1971) while conducting studies on East African plant virus diseases found that a strain of watermelon mosaic virus (WMV-K) caused dark green blisters on the leaves of courgette He also observed that it produced young leaves, similar to 'Shoe strings' and plants stunted and produced uneven fruits with yellow spots. Ahmed (1981) identified watermelon mosaic virus 1 causing mottling, leaf deformation and interveinal chlorosis in cucurbits Chen et al (1982) studied watermelon mosaic disease in China and observed the main symptoms as mosaic mottling, stunting and distortion and believed to be caused by watermelon mosaic virus Almeida and Borges (1983) reported that watermelon mosaic virus could produce mosaic and severe distortion of leaves on pumpkin

Hariharasubramanian and Badami (1964) while investigating pumpkin mosaic virus disease observed that the disease was characterised by severe blistering, distortion and stunting of leaves Jaganathan and Ramakrishnan (1971) found that a virus isolate from pumpkin produced mottling and malformation of leaves They also reported that plants infected early in the season remained dwarf and flowered sparingly A few leaves exhibited dark green vein banding along the midrib and lateral veins of affected plants Shankar et al (1972) observed that the symptoms of pumpkin mosaic disease appeared first as mosaic mottling of the leaves, followed by chlorosis of vein and veinlets leaving interveinal area green The leaf lamina was very much reduced and distorted, the veins and veinlets often extended beyond the margin giving the leaves a filiform shape Ghosh and Mukhopadhyay (1979a) isolated nine different strains of viruses from pumpkin from West Bengal and among them the isolate A7 produced characteristic mottling with mild green blisters and green vein banding in the leaves of infected plants

II. Transmission of the virus

1. Sap transmission

Doolittle (1920) had shown that the transmission of cucumber mosaic virus in the field was influenced by mechanical means during training and thinning of plants and plucking of fruits. He also demonstrated the sap transmission of the virus. Magee (1940) found that cucumber mosaic virus infecting banana could be readily transmitted mechanically from cucumber and squash to cucumber, squash and tobacco Further the virus was transmitted from infected tobacco to cucumber, squash and the seeded *Musa*.

Nagarajan and Ramakrishnan (1971a) reported that bitter gourd mosaic virus was not sap transmissible to bittergourd Pillai (1971) reported that a strain of cucumber mosaic virus infecting snake gourd (*Trichosanthes anguina*) was transmitted mechanically to healthy plants Nagarajan and Ramakrishnan (1971b) reported that watermelon mosaic virus affecting snake gourd could be transmitted to healthy plants by sap inoculation Dubey *et al* (1974) showed that snake gourd mosaic caused by *Cucumis* virus 1 could be transmitted

by sap inoculation Mechanical transmission of Cucumis virus 1 on snake gourd was reported by Joseph and Menon (1978)

Shukla and Singh (1971) reported that cucumber green mottle mosaic virus (cucumis virus 2D) could be transmitted to Lagenaria siceraria seedlings by pin prick inoculation on roots with infected sap and by submerging the roots in the sap for 24 h Goel and Varma (1973) observed that a new strain of cucumber mosaic virus designated as Luffa strain could be transmitted by mechanical inoculation. Pejcinovski (1978) proved that cucumber mosaic virus could be transmitted mechanically to cucumber, pumpkin and melon Raychaudhuri and Varma (1978) showed that muskmelon mosaic caused by cucumber green mottle mosaic virus could be transmitted by sap inoculation Weber et al (1982) described that cucumber leaf spot virus affecting glass house cucumber (Cucumis sativus) could be transmitted mechanically and by pruning implements

Basillious et al (1969) could successfully get mechanical transmission of squash mosaic virus to cucumber, pumpkin, watermelon, pea and Lupinus termis. Quiot et al (1971) reported that watermelon mosaic virus 1 found on

cucumber, melon, watermelon and courgette was readily transmissible by mechanical inoculation Nagarajan and Ramakrishnan (1975) successfully transmitted a strain of melon mosaic virus on Cucurbita lundelliana by sap inoculation Arteaga et al (1976) reported mechanical transmission of watermelon mosaic virus 2, and that of watermelon mosaic virus 1 was reported by Makkouk and Lesemann (1980)

Tripathi and Joshi (1985) found that the pumpkin plants infected with watermelon mosaic virus could be transmitted mechanically Jones et al. (1986) reported that the virus affecting watermelons and sweetmelons could be transmitted mechanically and the virus was identified as melon rugose mosaic virus

Mechanical transmission of pumpkin mosaic virus was reported by Hariharasubramanian and Badami (1964) Ghosh and Mukhopadhyay (1979a) isolated nine mosaic virus strains from pumpkin and reported that all the isolates were sap transmissible Roy and Mukhopadhyay (1980) found that the spread of pumpkin mosaic virus was possible by mechanical contact between above ground portions

Foster (1972) observed that buffers added to non purified cucumber mosaic virus preparations influenced the number of local lesions produced on _Chenopodium amaranticolor_ and greater infectivity was obtained with sodium/potassium phosphate buffer at pH 6 than at pH 8, and at pH 7 the response was intermediate. Shankar _et al_ (1972) while working with pumpkin mosaic virus found that the virus extracted in distilled water gave more percentage of infection when compared with Kirkpatrik and Lindner buffer, phosphate buffer, phosphate ascorbic acid buffer and sodium borate buffer Sharma _et al_. (1984) found that a new strain of cucumber mosaic virus causing mosaic disease of muskmelon was most infective (85%) in 0 01 M, pH 7 phosphate buffer and least in water (45%)

2. Seed transmission

Many of the plant viruses were found to be transmitted through seeds of the diseased plants. Some of the viruses infecting cucurbits were also reported to be transmitted through seeds Nagarajan and Ramakrishnan (1971a) reported that bitter gourd mosaic virus was not seed transmissible Nagarajan and Ramakrishnan (1971b) found that

a mosaic disease of snakegourd caused by watermelon mosaic virus was transmitted to some extent through its seed. Dubey et al (1974) studied the seed transmission of snake gourd mosaic caused by Cucumis virus 1 and found that the disease could not be transmitted through seeds collected from diseased snake gourd

Doolittle (1921) found that cucumber mosaic virus could be transmitted through seeds of wild cucumber (Macrampelis lobata) Hani and Pelet (1970) reported the seed transmission of cucumber mosaic virus (CMV) through seeds of chickweed (Stellaria media) upto 30%. Transmission of CMV through chickweed seed was also reported by Tomlinson and Carter (1970) Kaiser and Danesh (1971) found that cucumber mosaic virus isolated from Cicer arietinum could not be transmitted through Seeds. Sharma and Chohan (1973) studied the seed transmission of Cucumis virus 1 and Cucumis virus 3 through seeds of Cucurbits and they found that Cucumis virus 1 was seed borne in vegetable marrow, ash gourd and pumpkin and Cucumis virus 3 was found to be seed borne in bottle gourd Goel and Varma (1973) isolated a new strain of CMV designated as Luffa strain from ridge gourd and found that it was not transmitted through seed Pejcinovski

(1978) reported that cucumber mosaic virus could be transmitted by surface contamination of cucumber, pumpkin and melon seeds Sharma et al (1984) found a strain of cucumber mosaic virus causing mosaic disease of muskmelon in Punjab which was transmitted by seed

Nagarajan and Ramakrishnan (1975) carried out investigations on the transmission of melon mosaic virus and found that it was transmitted to some extent through seeds of Cucurbita lundelliana Hein (1977) working with watermelon mosaic virus 1 on zucchini vegetable marrow (Cucurbita pepo var giromontima) found that it was not seed transmissible Ahmed (1981) reported that watermelon mosaic virus 1 infecting cucurbits could not be transmitted through seeds Almeida and Borges (1983) investigated watermelon mosaic virus infecting pumpkin in Portugal and reported the seed, transmission of the virus

Powell and Schlegel (1970) while investigating the factors influencing seed transmission of squash mosaic virus in cantaloupe found that out of 50 seed samples from infected cantaloupe plants 12 per cent contained squash mosaic virus Thomas (1973) studied seed transmission of squash mosaic

virus in New Zealand in Honey-dew rock melon plants and found that out of 287 seedlings grown from seeds 8 were infected with squash mosaic virus

Shanker _et al_ (1972) found that the mosaic virus of pumpkin commonly occurring in Delhi was not transmitted through seed Capoor and Ahmed (1976) observed that a virus designated as pumpkin yellow vein mosaic virus infecting field pumpkin, vegetable marrow and squash could not be transmitted through seed

3.Graft transmission

Basillious _et al_. (1969) reported that water melon mosaic virus could be transmitted to other cucurbits by grafting Umamaheswaran (1985) observed the transmission of pumpkin mosaic virus through wedge grafting Raghunadhan (1989) also found that snake gourd mosaic virus could be transmitted by wedge grafting.

4. Insect transmission

Magee (1940) reported that infectious chlorosis or heart-rot of banana caused by _Cucumis_ virus 1 could be

transmitted by Aphis gossypii, Macrosiphum gei Varma et al (1970) studied a severe mosaic of snakegourd and found that it was transmitted by Myzus persicae and A gossypii Nagarajan and Ramakrishnan (1971a) reported that bitter gourd mosaic virus could be transmitted by A gossypii, A malvae, A nerii, M persicae and Brevicoryne brassicae According to Nagarajan and Ramakrishnan (1971b), watermelon mosaic virus from snakegourd could be transmitted by M persicae and A gossypii Pillai (1971) reported that the mosaic disease of snake gourd caused by a strain of CMV was not transmitted by A craccivora and M persicae

Dubey et al (1974) identified snake gourd mosaic virus and designated as Cucumis virus 1 and was found to be transmitted by A gossypii and M persicae and not by A craccivora and other aphid species Joseph and Menon (1978) investigated snake gourd mosaic virus and reported that the virus could be transmitted by A gossypii and A craccivora

Goel and Varma (1973) reported that a new strain of cucumber mosaic virus designated as Luffa strain could be transmitted by M persciae, A gosssypii and B brassicae to ridge gourd (Luffa acutangula) Pejcinovski (1978) observed

that cucumber mosaic virus from cucumber, pumpkin and melon was transmitted by M persicae and A fabae

Lastra (1968) reported that water melon mosaic virus 2, cucumber mosaic virus and squash mosaic virus were transmitted by the vector Acalymma thiemei thiemei Greber (1969) could transmit watermelon mosaic virus 2 to pumpkin and squash by a lady bird beetle (Henosepilachna vigintioctopunctata) in a persistent manner WMV could also be transmitted to cucumber, pumpkin, watermelon, pea and Lupinus termis by A gossypii and A punica (Basillious et al , 1969)

Transmission of WMV was found to be by M persicae and A gossypii (Nagarajan and Ramakrishnan, 1975, Tewari, 1976, Sako et al , 1976, Arteaga et al , 1976, Hein, 1977, Makkouk and Lesemann, 1980, Karl, 1981, Rizk et al , 1981, Almeida and Borges, 1983) Lipaphis erysimi (Sako et al , 1976, Tewari, 1976), A nerii (Nagarajan and Ramakrishnan, 1975, A craccivora (Sako et al , 1976) and a Dipteran Liriomyza sativae (Zitter and Tsai, 1977) Wyman (1979) reported that Acyrthosiphon kondoi could transmit WMV-1 and 2 to squash, watermelon, cantaloupe and vegetable marrow

Thomas (1980) reported the transmission of water melon mosaic virus to bitter gourd by *M persicae* in the Coale Islands

Linderberg *et al* (1956) studied the transmission of squash mosaic virus and melon mosaic virus and reported that they were transmitted by *M. persicae* and *A gossypii*. Squash mosaic virus in Japan was found to be transmitted by two species of aphids, viz , *A. gossypii* and *M. persicae* (Komuro, 1957) Bishnoi *et al* (1985) found that the summer squash mosaic could be transmitted by *A. gossypii*, *A malvoides* and *M persicae*

Hariharasubramanian and Badami (1964) reported that pumpkin mosaic virus was transmitted by *A laburni* and by many other *Aphis* spp Forghani *et al*. (1966) observed that viruses infecting *Cucurbita pepo* were transmitted by four insect vectors viz , *A fabae*, *Dyssulacorthum pseudosolani*, *Macrosiphon solanifolii*, and *M persicae*

Shankar *et al* (1972) found that pumpkin mosaic virus commonly occurring in Delhi could be transmitted by *M. persicae* and *Sitobion rosaeformis*. Roy and Mukhopadhyay (1930) studied that pumpkin mosaic virus was transmitted by

<u>A</u> <u>gossypii</u> in a non-persistent manner Singh (1981a, 1982) reported that pumpkin mosaic virus was transmitted by <u>A</u> <u>gossypii</u> as well as <u>A</u>. <u>craccivora</u>

III. Physical properties

Johnson and Grant (1932) reported that <u>Cucumis</u> virus 1 infecting different host plants had TIP of 60 - 65°C, DEP of 1 10000 and LIV at room temperature was 24-28 h Verma <u>et</u> <u>al</u> (1970) while studying the physical properties of <u>Cucumis</u> virus 2B causing mosaic disease in snake gourd and bottle gourd, observed that the viruses had a thermal inactivation point of 97 5°C and dilution end point of 10^{-6} - 10^{-7} The longivity <u>in</u> <u>vitro</u> at 30°C was 9-10 days. Nagarajan and Ramakrishnan (1971b) isolated watermelon mosaic virus from snakegourd It had a TIP of 52-54°C, DEP of 1·200 - 1·500 and LIV at 32°C was 4-6 days and at 5°C was 4-8 days Pillai (1971) found that CMV causing mosaic disease of snakegourd had a TIP of 60°C, DEP of 1:10000 and LIV was 72 h at room temperature. Dubey <u>et</u> <u>al</u>. (1974) isolated <u>Cucumis</u> virus 1 from mosaic infected snake gourd Its TIP was between 65-70°C , DEP was between 1 1000-1·5000 and LIV was 16-18 h at 34 6-39°C and 8 days at 8°C Joseph and Menon (1978)

reported that <u>Cucumis</u> virus 1 infecting snakegourd had TIP between 70-75°C, DEP 1 5000 - 1·10000 and LIV 72-96 h at room temperature and 144-168 h at 10°C.

Chen and Wei (1959) found that cucumber mosaic virus had a higher resistance to temperature (80-85°C) with DEP between 10^{-5} - $5x10^{-5}$ and LIV of 30 days Goel and Varma (1973) isolated a new strain of cucumber mosaic virus, designated as <u>Luffa</u> strain from ridge gourd and reported that the TIP was between 80-90°C, DEP between 10^{-4}-10^{-5} and LIV between 35-48 h at room temperature and 72-93 h under freezing conditions Pejcinovski (1978) found that CMV infecting cucumber, pumpkin and melon had a TIP between 55 and 65°C and DEP 1·50000 - 1 70000, LIV 6-14 days in sap and 12-25 days in dry leaf tissues at room temperature Shawkat and Fegla (1979) isolated cucumber mosaic virus from naturally infected egg plant and watermelon mosaic virus 2 from <u>Cucurbita pepo</u> and were found to be inactivated at 65°C Their DEP and LIV were 10^{-3} - 10^{-4} and 4 -8 days respectively

Linderberg <u>et al</u> (1956) studied the physical properties of watermelon and squash mosaic viruses and reported that their activity was lost in 10 min at 60°C,

during 28 days LIV and at 5 x 10^{-3} dilution But the later had a dilution end point of 10^{-4} - 10^{-5} . Basillious et al (1969) reported that squash mosaic virus isolated from squash had a TIP between 61 and 62°C and DEP of 1·1500 At room temperature, the LIV was 4 days Auger et al. (1974) conducted studies on WMV-2 infecting squash (Cucurbita maxima) and zucchini squash (C. pepo) and reported that the virus had a TIP between 55 and 60°C and DEP between 10^{-3} and 10^{-4} Nagarajan and Ramakrishnan (1975) studied a strain of melon mosaic virus on Cucurbita lundelliana and observed that the virus had a TIP of 52-54°C, DEP of 1:2500 - 1 5000 and LIV of 4 days at room temperature and 6 days at 5°C. Bhargava (1976) while investigating the effect of ageing on the activity of WMV under varying conditions found that the LIV at room temperature was 8 days In dried leaves stored at -1°C the virus was infective for at least 6 months although activity began to decrease after 5 months. Almeida and Borges (1983) reported that WMV infecting cucumber, watermelon, pumpkin and squash had a TIP of 50°C, DEP of 5 x 10^{-4} and LIV of 10 days Dikova et al (1983) isolated WMV from cucumber and reported that the virus had a TIP between 58-60°C, DEP 10^{-4} and LIV 6-7 days Bishnoi et al (1985) found that a strain of watermelon mosaic virus infecting summer

squash had a DEP between 10^{-3}-10^{-4}, TIP between 45-50°C and LIV at room temperature was between 24-38 h Tripathi and Joshi (1985) reported that a strain of watermelon mosaic virus infecting pumpkin in Utterpradesh had a DEP between 10^{-5}-10^{-6}, TIP between 60-65°C and LIV at 32-34°C was between 26-27 days and at 17-19°C was 42 days

Hariharasubramanian and Badami (1964) observed that pumpkin mosaic virus had a TIP of 55°C and DEP 1 5000. The LIV at room temperature was 72 h but the virus could be maintained for more than six months in leaves kept at -20°C. Shankar *et al* (1972) reported that pumpkin mosaic virus was inactivated when subjected to 56°C for 10 min. The DEP of the virus was between 1 100 and 1 500. At room temperature (32-35°C) the virus was infective for 8 h only but the longevity of the virus was increased to 26 h at 8°C. March and Nome (1973) found that the virus infecting *Cucurbita moschata* had a TIP between 65°C and 70°C, DEP 4 x 10^{-5} and LIV 6 weeks Ghosh and Mukhopadhyay (1979a) studied the physical properties of nine isolates of pumpkin mosaic virus under laboratory conditions and observed that all the isolates had a TIP between 40-55°C, DEP between 10^{-1} - $10^{-3\ 5}$ and LIV at room temperature (24-32°C) between 6-168 h.

IV. Vector-virus relationships

The vector-virus relationship of a virus occurring on bitter gourd was studied by Nagarajan and Ramakrishnan (1971c) They observed that the virus was transmitted by _M persicae_ and _A gossypii_ in a non-persistent manner Dubey _et al_ (1974) observed that _Cucumis_ virus 1 causing mosaic disease in snake gourd was transmitted by _A gossypii_ and _M persicae_ in a non-persistent manner Joseph and Menon (1978) studied the vector-virus relationship of a virus isolated from snakegourd, transmitted by _A gossypii_ and _A craccivora_ and they found that _A craccivora_ acquired the virus within 5 min acquisition feeding and transmitted it within 5 min inoculation feeding on healthy plants A minimum of 5 aphids were required for the transmission and pre-acquisition starvation increased the transmission efficiency, where as post-acquisition starvation for 30 min reduced the same. The vector could not retain the virus for long period, the relationship being non-persistent

Kaiser and Danesh (1971) found that a single aphid (_A craccivora_) was able to transmit cucumber mosaic virus but greater transmissions were obtained only by using larger

number of aphids Singh (1972) studied the relationship of watermelon mosaic virus strains with its vector *M persicae* and found that the virus was transmitted in a typical non-persistent manner The vector was most efficient after 4 h pre-acquisition fasting and 2 min acquisition fasting. Infectivity was lost after 2 h post-acquisition fasting The nymphal forms were slightly more efficient in transmission than alate and apterous adults Raychaudhuri and Varma (1977) showed that a strain of watermelon mosaic virus was transmitted by the vector *M persicae* to vegetable marrow in a typical stylet-borne manner Pre-acquisition starving of vectors was not essential but increased the transmission rate Although a single aphid could transmit the virus, more than 5 aphids per plant were required for 100 per cent transmission A feeding period of only 30 s was needed for virus acquisition but when it was extended for one or two min there was maximum transmission Almeida and Borges (1983) found that watermelon virus could be transmitted to watermelon, pumpkin, squash and cucumber by *M persicae* in a non-persistent manner They obtained 70 per cent transmission with one aphid and 100 per cent with seven or more aphids

Nagarajan and Ramakrishnan (1971d) studied the vector-virus relationship of melon mosaic virus occurring on pumpkin They observed that the virus was non-persistently transmitted by M persicae, A gossypii and A nerii and found that the optimum number of aphids required per plant was 20 Virus transmission by fasted vectors was greater than that of non-fasted ones Jaganathan and Ramakrishnan (1971) working on vector-virus relationship of two virus isolates from naturally infected melon found that maximum transmission was obtained when the aphids (A gossypii and M persicae) were given 60 min pre-acquisition fasting. Sixty min post-acquisition fasting resulted the loss of viruliferous nature of the vector Minimum acquisition feeding of muskmelon isolate was 5 s while that of pumpkin isolate was 10 s Minimum inoculation threshold was 5 s for both the isolates. Relationship of pumpkin mosaic virus with its aphid vector A. gossypii was reported by Singh (1981a). He found that minimum pre-acquisition fasting of 10 min and an optimum of 90 min was essential for transmission of the virus to pumpkin. Acquisition and transmission occurred in 20 s and 10 s respectively Although a single aphid could transmit the virus, transmission was maximum with 10 aphids per plant. Post-acquisition fasting of more than 2 h resulted in loss of

infectivity Singh (1982) conducted studies on the transmission of pumpkin mosaic virus by A. craccivora and showed that pre-acquisition fasting of vector was essential for virus transmission Aphids acquired the virus within 20 s and inoculated it within 30 s. He found that a single aphid could transmit the virus, but maximum infection was obtained with 15 aphids Aphids were infective only for 2 h and the transmission was therefore in a non-persistent manner.

V. Host range and local lesion hosts

Magee (1940) reported that infectious chlorosis of banana caused by Cucumis virus 1 was found to infect cucumber, squash, tobacco and seeded Musa Nagarajan and Ramakrishnan (1971a) reported that a mosaic disease of bittergourd caused by bittergourd mosaic virus had a narrow host range confined to the family cucurbitaceae Shanker et al (1969) reported a mosaic disease of snake gourd caused by CMV having an extensive host range which included Nicotiana glutinosa, Chenopodium amaranticolor and Cucurbita pepo Verma et al (1970) recorded a severe mosaic disease caused by Cucumis virus 2B on snake gourd and the host range was restricted to members of cucurbitaceae But it produced

local lesions on *C amaranticolor* Pillai (1971) identified a mosaic disease of snake gourd in Kerala and out of 31 species of plants in 7 families tested, 15 species in 6 families were found to be hosts of the virus The causal virus was identified as a strain of cucumber mosaic virus Nagarajan and Ramakrishnan (1971b) reported that the host range of water melon mosaic virus was restricted to cucurbitaceae Dubey *et al* (1974) identified a mosaic disease of snake gourd in Delhi caused by *Cucumis* virus 1 The virus was found to have its host range in cucurbitaceae, solanaceae, chenopodiaceae and compositae and produced systemic mosaic symptoms on *Cucumis sativus*, *C anguria*, *C melo* var Utilissima, *Cucurbita pepo*, *Lagenaria siceraria*, *Luffa acutangula*, *Citrullus vulgaris*, *Nicotiana tabacum* var White Burley, *N tabacum* var Xanthi, *N rustica*, *N glutinosa*, *Capsicum annuum*, *Solanum melongena*, *Lycopersicon esculentum*, O*Datura stramonium*, *Petunia hybrida* var violet, *Zinnia elegans* and *Spinacia oleraceae*, *Luffa cylindrica* and *N tabacum* var Harrison special carried the virus symptomlessly The virus produced distinct necrotic local lesions on *C amaranticolor*

Allen and Fernald (1971) observed that wild cucumber mosaic virus could infect Marah oreganus Foster (1972) reported that CMV produced local lesions on C. amaranticolor Ehara and Misawa (1975) reported that cucumber mosaic virus produced local lesions on cowpea Joshi and Dubey (1976) conducted investigations on weed reservoirs of cucumber mosaic virus in Gorakhpur and reported that Amaranthus viridis, Nicotiana plumbaginifolia, Physalis minima, Salvia plebeia and Solanum nigrum were infected with CMV Ignash (1977) found that cucumber mosaic virus strain 1 isolated from cucumber and tulip produced local lesions on C quinoa Rao and Raychaudhuri (1977) reported that cucumber mosaic virus isolated from Vinca rosea produced local lesions on C murale Ghosh and Mukhopadhyay (1979 b) found that squirting cucumber mosaic virus infecting cucumber produced local necrotic spots followed by systemic infection on Datura stramonium and C amaranticolor. Sarjeet Singh (1981) found that a mutant of CMV produced severe systemic mosaic with puckering and leaf distortion symptoms in D stramonium Sharma et al (1984) observed that a new strain of cucumber mosaic virus (CMV-muskmelon strain) infected tobacco cultivars white burley, N glutinosa, N rustica, Capsicum annuum and various cucurbits

Toba (1962) found that *Momordica balsamina* was the common wild reservoir of watermelon mosaic virus in Hawaii Zabla and Ramallo (1969) reported that watermelon mosaic virus could infect *Cucurbita* sp, *Cucumis* sp and *C amaranticolour* Bhargava and Tewari (1970) reported that *Trichosanthes dioica* was the natural host of watermelon mosaic virus Adlerz (1972) reported that *M charantia* acted as a source of watermelon mosaic virus in Florida

Auger *et al* (1974) found that watermelon mosaic virus 2 was widely distributed in the cucurbit growing area in Central Chile infecting squash (*Cucurbita maxima*) and zucchini squash (*Cucurbita pepo*) and induced local lesions on *C amaranticolor*, both local lesions and systemic necrotic flecking on *Lavatera trimestris* Nagarajan and Ramakrishnan (1975) concluded that WMV could infect only the members of Cucurbitaceae viz , *Cucurbita moschata*, *C maxima*, *C pepo*, *Cucumis melo*, *Luffa acutangula*, *Trichosanthes anguina* and *Lagenaria vulgaris* They could not find any local lesion host for the virus Tewari (1976) found that *Zinnia elegans* was a symptomless carrier of watermelon mosaic virus. Halliwell *et al* (1979) observed that the weed *Melothria pendula* acted as a host of WMV-1 Makkouk and Lesemann (1980)

reported that WMV-1 could induce local lesions on C amaranticolor and C quinoa and systemic infection in cucumber, squash, pumpkin and watermelon.

Chang and Lee (1980) found that watermelon mosaic virus could infect Sesamum indicum L. Ahmed (1981) reported that watermelon mosaic virus had a narrow host range confined to the family cucurbitaceae Latera et al (1975) reported that squash mosaic virus produced local lesions on Cucumis metuliferus Lockhart et al (1982) found that squash mosaic virus could cause systemic infection on C. quinoa

Shanker et al (1972) inoculated pumpkin mosaic virus (PMV) on 76 plant speices of 9 families They observed that its host range was restricted to the family cucurbitaceae and produced systemic mosaic symptoms on C. pepo, C melo, L siceraria var Round and Long, L acutangula, C vulgaris, M charantia, Benincasa hispida and Trichosanthes anguina Cucumis sativus was proved to be a symptomless carrier Singh (1981b) reported that the host range of a virus causing mosaic disease of pumpkin was confined to the family cucurbitaceae

Vasudeva et al (1949) reported that M charantia could act as symtomless carrier of cucumber green mottle mosaic virus (Cucumis virus -2) Rahimian and Izadpanah (1977) found that cucumber green mottle mosaic virus infecting melon plants was confined to the family cucurbitaceae The virus produced systemic mosaic symptoms on cantaloupe, melon, cucumber and watermelon and chlorotic spots in Luffa acutangula and squash was found to be immune Horvarth (1985) reported M charantia as the new host of tobacco rattle virus and tomato ring spot virus

Nagaraju and Reddy (1983) studied the occurrence of a strain of cucumber mosaic virus in bell pepper for the first time in India Ullman et al. (1991) reported that the cucumber mosaic virus, zucchini yellow mosaic virus and an isolate of papaya ring spot virus infecting watermelon were found to infect three species of the family cucurbitaceae viz , M charantia, C dipsaceus and L siceraria.

VI. Serological properties of the virus

1. Purification of virus

Different methods of purification of viruses infecting cucurbits have been reported. Dubey et al. (1974)

purified snake gourd mosaic virus by using butanol centrifugation method Infected leaves were homogenised in 0 05 M phosphate buffer (pH 7.6) containing 0.1 per cent thioglycollic acid and subjected to differential centrifugation after adding 8.5 per cent n-butanol The final pellet was suspended in 0.05 M phosphate buffer Three other methods of purification viz , chloroform centrifugation using phosphate buffer with ascorbic acid (Gibbs et al 1963) chloroform butanol centrifugation using phosphate buffer with ascorbic acid and DIECA (Brunt, 1966) and chloroform butanol centrifugation (Steere, 1956) were also attempted for their relative efficiency The chloroform butanol centrifugation method gave the highest virus end point

Lot et al (1972) purified cucumber mosaic virus by polyethylene glycol (PEG) precipitation followed by two centrifugation and resuspension of pellets in solutions of low sodium citrate concentration containing two per cent triton X-100 Shohara and Osaki (1974) reported that purified cucumber mosaic virus was obtained by repeated precipitation with 8 per cent PEG and 0 2 M sodium chloride followed by density gradient centrifugation Omar et al (1980) compared various methods of purification of cucumber

mosaic virus and found that the best clarification was obtained with low speed centrifugation. Precipitation with ammonium sulphate gave the highest virus concentration followed by adsorption of PEG.

Wetter (1960) reported that some of the elongated plant viruses could be partially purified by using ether or carbon tetrachloride for preliminary sap clarification and they proved to be serologically active. Hebert (1963) and Van Kammen (1967) purified cowpea mosaic virus by PEG - NaCl method. The leaf extract was clarified by centrifugation at 10000 g and the PEG 6000 and NaCl were added Van Kammen (1967) reported that PEG - NaCl method gave high yield of purified virus compared to butanol chloroform method of purification Filigarova (1982) purified Arabis mosaic virus from infected leaves of Petunia hybrida which were homogenised with phosphate buffer and the virus was precipitated with PEG and purified by density gradient centrifugation

2. Serological tests

Dubey et al (1974) reported that the antiserum for snake gourd mosaic virus reacted positively and gave a

precipitate of somatic (granular) type characteristic of spherical viruses with the diseased plant sap and purified virus preparation but not with healthy plant sap The virus in the clarified plant sap gave reaction at a dilution of 1 128 whereas in the case of purified preparation it reacted upto 1·4096 dilution No reaction was obtained in the diseased plant sap or purified virus preparation with normal serum The titre of the serum was found to be 1 2048 with the purified virus preparation. In agar gel diffusion slides, the antiserum produced a single straight precipitation band when tested with diseased plant sap or purified virus preparation. Dubey and Nariani (1975) investigated the serological relations of 10 cucurbit virus isolates collected from Delhi and found that the viruses of snake gourd mosaic, cucumber mosaic, melon mosaic and bitter gourd mosaic formed a group of Cucumis virus 1, while bottle gourd and watermelon mosaic viruses formed a group of Cucumis virus 2, pumpkin mosaic and vegetable marrow mosaic viruses comprised the unstable Cucumis virus 4 while a virus from tori (Luffa cylindrica) appeared to be distinct from these 3 groups

Milne and Grogan (1969) while investigating the characterisation of watermelon mosaic virus strains by

serology found that WMV-1 and WMV-2 were related and an isolate of papaw ring spot virus was also serologically related to WMV Qureshi and Mayee (1980) while studying the characterisation of a virus inciting mosaic in _L. siceraria_ in Maharashtra found that the antiserum, produced specific to the virus had a titre of 1·32 in the tube precipitin tests It did not react with antisera of melon mosaic or cucumber mosaic virus Almeida and Borges (1983) reported that watermelon mosaic virus causing severe distorting mosaic on pumpkin, when serologically tested the antiserum had a titre of 1 16000

Shankar _et al_ (1972) reported that the antiserum of pumpkin mosaic virus produced flagellar type of precipitate typical of rod shaped virus in the tube precipitin tests when tested with diseased plant sap and purified virus preparations. They could not find any reaction between the antiserum and the clarified healthy plant sap or between normal serum and the plant sap or purified virus preparations The antiserum had a titre of 1:2048. They found that antiserum did not react with the other cucurbit viruses reported Ghosh and Mukhopadhyay (1979a) conducted agar gel diffusion method to identify the nine virus isolates of pumpkin

VII. Varietal screening

Shanmugasundaram _et_ _al_. (1969) while studying cucurbit viruses in Hawaii found that a cucumber breeding line Hawaii 64-A-15 was resistant to WMV-1, WMV-2, CMV and a mixture of CMV and WMV-1, but it was less resistant to the kauai strain of watermelon mosaic virus. Sowell and Demski (1969) reported that all the 59 watermelon cultivars tested were proved to be susceptible to WMV-2, but they found that some infected plants recovered Demski and Sowell (1970) while investigating the susceptibility of _Cucurbita_ _pepo_ and _Citrullus_ _lanatus_ to WMV-2 showed that 30 - 100 per cent of the plants of each introduction were susceptible to WMV-2. Moskovets and Fegla (1972) while studying the effect of watermelon mosaic virus on the growth of cucurbits, reported that none of the watermelon and pumpkin varieties tested was immune to the virus Fischer and Lockhart (1974) also reported that all varieties of watermelon,were susceptible to WMV-2 Provvidenti and Robinson (1974) could prove that two _Cucumis_ _metuliferus_ varieties viz., PI 20268 and PI 292140 were highly resistant to WMV-1 and squash mosaic virus

Provvidenti _et al_ (1978) tested 14 wild cucurbita species against the common, often destructive viruses affecting squash in New York and they found that two species were highly resistant to WMV-1 and 2 They concluded that _Cucurbita ecuadorensis_ and _Cucurbita foetidissima_ appeared to be the most promising species resistant to CMV, WMV-1, WMV-2 and other viruses Greber (1978) reported that watermelon mosaic virus 1 and 2 in Queensland could infect all commercially available watermelon, vegetable marrow and pumpkin cultivars Halliwell _et al_ (1979) while investigating mosaic disease of squash, watermelon and pumpkin, reported that WMV-1 was endemic to many vegetable growing areas in Texas and it severely limited the production of these crops Pitrat and Dumas de Vaulx (1979) during their search for sources of resistance to cucumber mosaic virus and watermelon mosaic virus among _Cucurbita_ species found that _C lundelliana_, _C martinegii_, _C okeechobeensis_ and _C ecuadorensis_ were resistant to CMV and WMV Sharma and Sharma (1982) tested 31 genotypes of summer squash in the field against natural infection of _Cucumis_ virus 1 and found that 12 were moderately resistant but none was immune. The lines 11-2-6-2, 10-1-2-4 and 17-1-2-1 which showed a low disease index and mild symptoms were found promising. Maluf

et al (1986) tested 29 cultivars of C pepo, C maxima, C moschata and a single specimen of C ecuadorensis against the watermelon mosaic virus (WMV) They found that C ecuadorensis, 4 cultivars of C moschata and 4 cultivars of C maxima were resistant

VIII Estimation of loss

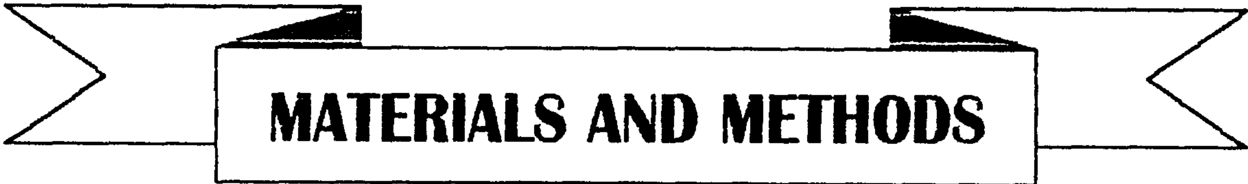
Pillai (1971) investigated a mosaic disease of snake gourd and reported that the disease affected plants were stunted and produced fewer flowers and fruits Dubey et al (1974) studied on snake gourd mosaic virus and observed that the diseased plants produced only a few weak runners and plants affected in an early stage blossomed sparingly and set few fruits Joseph and Menon (1981) reported that cucumber mosaic virus infection on snake gourd in the early stage resulted complete failure of fruit set where as in the late infected plants yielded fewer fruits Raghunadhan (1989) studied the snake gourd mosaic disease and found that the plants infected with virus at the early stage significantly reduced the number of leaves, leaf area, length of vines, number of flowers, number of fruits and yield

Hills *et al* (1961) studied the effect of CMV on cantaloupe and recorded that inoculation of melon plants at the sixth leaf stage caused 40 per cent reduction in yield. Nelson (1962) while working with cantaloupe reported that when runners of 2-4 feet length were inoculated with CMV there was 75 per cent reduction in fresh plant weight and with WMV there was 50 per cent reduction. When plants were inoculated near maturity there was no significant reduction in plant size or yield Powell and Schlegel (1970) reported that cantaloupe plants infected with squash mosaic virus (SMV) significantly reduced fruit weight, size, seed number, seed weight and germination percentage, but no correlation was found between these reductions and the variable infection percentages Singh and Mandahar (1971) reported that infection of *Luffa aegyptica* and *Cucurbita moschata* by CMV reduced leaf productivity Thomas (1971) conducted field trial to study the economic importance of WMV-2 on cucurbits in New zealand and reported that early infection reduced yield in Butter cup squash (63%), Golden Hebbard squash (53 1%) and pumpkin (49%) but not in cucumber No yield reduction was recorded with late infection in any of the 4 cucurbits tested Moskovets and Fegla (1972) found that cucurbits inoculated with WMV in early stages of growth had

shorter runners and internodes, fewer side runners and lesser green weight Demski and Chalkley (1974) while studying the effect of watermelon mosaic virus on watermelon observed that infected plants of 3 watermelon varieties had shorter runners and smaller leaves which reduced the fresh weight by over 55 per cent It was also found that the fruit number and size were reduced due to infection Alvarez and Campbell (1976) analysed the yield factors of cantaloupe infected by squash mosaic virus and recorded significant reduction in the number of fruits per plant but had no influence on size, weight or edible quality of the fruits However, retardation in fruit maturity was observed.

Singh and Dey (1976) assessed the loss due to bottle gourd mosaic virus infection in the yield of *L siceraria* and found 64 per cent yield reduction Bhargava (1977) reported that in field experiments early infection of vegetable marrow plants with WMV caused greater loss in yield than late infection It was also found that different strains of WMV varied in the extent of yield reduction caused by them Karchi *et al* (1978) found that infection of CMV in an early stage reduced the yield of susceptible cantaloupe (*Cucumis melo*) cvs Noy Yizre'el by 73 per cent and tolerant

X_v-140 by 31 per cent Yield reduction in Noy Yizre'el was due to fewer fruits and lower fruit weight where as in X-140 fruit weight was not affected although the number of fruits per plant was reduced Jayasree (1984) found that yellow vein mosaic disease of pumpkin produced significant reduction in number of leaves, size of the leaves, internode length, number of branches, total length of vines and number of flowers Singh (1986) assessed the loss due to watermelon mosaic virus in pumpkin and found that plants inoculated at 20, 30, 40, 50, 60 and 70 days after planting yielded 2, 2, 3, 4, 4 and 5 fruits per plant respectively. It was also found that plants inoculated at early stages of growth produced shorter runners and internodes. Singh (1989) studied the loss due to water melon mosaic virus in the yield of L siceraria and observed 100 per cent yield reduction during early stage of infection


MATERIALS AND METHODS

MATERIALS AND METHODS

I. Symptomatology

Seeds of bitter gourd (Momordica charantia L) of the variety priya obtained from the Instructional Farm, Vellayani were used for the study They were sown in pots containing potting mixture of sand, red soil and cowdung in the ratio of 1 1 2 The culture of the bitter gourd mosaic virus was collected from the field and the same was maintained by repeated transfers on young bitter gourd plants at two leaf stage, in insect proof glass house by sap inoculation Symptomatology was studied by observing the development of symptoms in naturally infected as well as artificially inoculated bitter gourd plants

II. Transmission of the virus

1. Sap transmission

Sap transmission studies were conducted using standard sap, sap extracted in phosphate buffer and tris buffer In all sap inoculation studies 600 mesh carborundum powder was used as abrasive (Costa, 1944)

The standard sap was prepared by crushing the infected leaf of known weight into a fine pulp by adding one ml of sterile distilled water for every gram of diseased leaves For crushing the leaves a sterile pestle and mortar was used The pulp was filtered through fine muslin cloth and the filterate was used for inoculation. When phosphate buffer (0 01 M, pH 7 0) and tris buffer (0.01 M, pH 7 0) were used as extraction media, the sap was extracted after adding one ml of the buffer in each case to every gram of infected leaf tissue

The expressed sap after initial clarification was inoculated by gently rubbing on the upper surface of the fully formed young leaves of the test plants with a swab of absorbant cotton moistened with the sap Carborundum powder was dusted uniformly on the leaves before the application of inoculum Care was taken not to injure the leaf tissue during inoculation Soon after the inoculation, the excess sap on the leaves was washed off using distilled water. Ten plants were inoculated for each experiment and an equal number of uninoculated plants were kept as control. The experiments were repeated twice and the plants were kept under observation in an insect proof glass house

2 Seed transmission

Seeds collected from mechanically inoculated plants showing clear symptoms of the disease were sown in pots and kept in an insect proof glass house One hundred and ten seeds were sown and the plants were kept under observation for 25 days after germination

3. Graft transmission

Small shoots showing systemic symptom were selected as scion The base of the scion was trimmed to a wedge, before inserting into the cleft made on the stem (root stock) of the healthy bitter gourd plant of 30 days old The cut on the stock was made through a node, since the stem was hollow at the centre Most of the leaves of the scion were removed and the base of the scion was inserted into the cleft of the stock The graft was then tied with a polythene strip and the grafted portion and the scion were covered with a polythene bag to retain humidity These plants were kept in the insect proof glass house under observation for the development of symptoms for 25 days

4. Insect transmission

Insect transmission studies were carried out by using *Aphis craccivora* Koch , *Aphis gossypii* Glov , *Aphis malvae* Koch , *Myzus persicae* Sclz , *Bemisia tabaci* Genn , *Sundapteryx biguttula biguttula* Inshida and *Henosepilachna vigintioctopunctata* F as vectors

(i) Inoculation using *Aphis* Spp.

Healthy colonies of *A craccivora* were maintained on cowpea, *A gossypii* on Brinjal, *A malvae* and *M persicae* on bhindi under insect proof glass conditions

Healthy insects were collected and transferred to petri plates They were starved for a period of one hour (pre-acquisition fasting period) and then allowed to feed on young infected bitter gourd leaves so as to give them an acquisition feeding period of 30 min A fixed number of infective aphids (10 nos) were then transferred to young healthy plants, at two leaf stage, for an inoculation feeding period of 24 h and after that they were killed by spraying with 0 1 per cent Dimethoate As in the case of sap

transmission an equal number of control plants were also maintained The inoculated and uninoculated plants were kept for observation under insect proof condition for 25 days

(ii) Inoculation using Bemisia tabaci

Whiteflies (B tabaci) were reared on healthy tobacco plants (Nicotiana tabacum L) in an insect rearing cage and they were used for transmission trials Plastic transmission cages designed by Nene (1972) were used for transmission studies

The top portion of young plants bearing 3-4 leaves was introduced into the transmission cage in such a way that the stem passed through the rectangular slit on the opening of the cage Whiteflies were collected using an aspirator and were then released into the transmission cage The transmission cage was covered by a black cloth except at the region of the wire netting which was kept facing light source while releasing the whiteflies The cap of the transmission cage was immediately screwed on The remaining portion of the rectangular slit of the cage was closed with modelling clay The cage was kept in position by using bamboo silvers

and a rubber band After the desired feeding period, the modelling clay was removed and the plant was disturbed by gently tapping it with a needle to disturb the whiteflies. This could induce the whiteflies to move to the side of the cage facing the light source. Pre-acquisition fasting and acquisition and inoculation feeding periods were given as mentioned under transmission with aphids

Ten seedlings were used as test plants in each transmission experiment A fixed number of whiteflies were released (20 nos) on each test plant for inoculation feeding After inoculation feeding the insects were killed by spraying the plants with 0.1 % Dimethoate The inoculated plants were labelled and maintained in an insect proof glass house Equal number of the control plants were also maintained Experiments were done twice and observations on the appearance of symptoms were taken daily.

(iii) Inoculation using the beetle H. vigintioctopunctata and leaf hopper S. biguttula biguttula

The beetles and leaf hoppers were reared on healthy bitter gourd plants in an insect rearing cage and they were used for transmission trials

The beetles collected in vials were allowed to feed on young infected bitter gourd leaves for 24 hours After acquisition feeding period, they were released to healthy bitter gourd plants and allowed to feed for 24 h Then the beetles were killed by spraying with 0 1 % carbaryl The inoculated plants were kept under insect proof condition for 25 days

The leaf hopper transmission was done as per the procedure described under aphid transmission

III. Physical properties

1. Dilution end point (DEP)

Infected bitter gourd leaves of known weight were crushed into fine pulp by means of clean and sterile pestle and mortar, adding one ml of distilled water per gram of leaf tissue The resulting pulp was strained through sterile cotton wool The sap was diluted with sterilized distilled water in the ratio of 1 10, 1 100, 1 1000, 1 10000, 1 100000, 1 1000000 The different dilutions were used for inoculation Ten plants were inoculated with each of the

dilutions and the experiment was repeated to confirm the results The standard sap without dilution was used as control The inoculated plants were labelled and kept under insect proof conditions and observed for the development of symptoms

2. **Thermal inactivation point (TIP)**

The sap from the infected bitter gourd plants was obtained as in the previous experiment Five ml each of the sap was pipetted into thin walled glass test tubes Care was taken not to pour the sap on the sides of the tubes. The tubes were then kept in thermostatically controlled water bath for 10 min at the required temperature in such a way that the level of the water in the water bath was 3 cm above the level of sap in the tube. The control was kept at room temperature (28 - 30°C) The sap was treated at different temperature ranges of 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 and 90°C and a thermometer was placed close to the tube in the water bath to check the temperature. After 10 min in each case, the tube was removed and cooled immediately in running water The treated sap was inoculated on young vigorously growing test plants of two leaf stage. Ten plants

were inoculated in each set of treatment and kept in an insect proof glass house and observation on the number of plants infected were recorded

3. Longevity *in vitro* (LIV)

Infected leaves were ground with pestle and mortar and the sap was filtered through cotton wool. Five ml of the sap was pipetted into test tubes and closed with aluminium foil The tubes were kept at room temperature (28- 30°C) and also in a refrigerator (10°C) One tube containing the sap of each treatment was taken after specific periods, viz., 0, 2, 4, 8, 24, 48, 72 and 96 h and inoculated on the test plants Ten plants were inoculated in each set of treatment and the experiment was repeated to confirm the results. The inoculated plants were kept under insect proof conditions and observed for the development of symptoms.

IV Vector - virus relationships

The experiments to study the vector - virus relationships were conducted by using one of the efficient vectors, ie , *A malvae* Bitter gourd plants showing

typical symptoms of bitter gourd mosaic virus were collected from the field and the culture of the virus was maintained in insect proof glass house by repeated transfers to healthy plants by mechanical inoculation. Virus free aphid colonies were maintained on Solanum torvum plants in an insect rearing cage In all the inoculation trials only fully grown apterous aphids were used During feeding of the aphids, the test plants were kept in insect proof cages The aphids were killed at the end of the required feeding period by spraying the plants with 0 1 % Dimethoate In the case of short feeding periods of less than 5 min the individual aphids were watched through a magnifying lens and the time of feeding was determined with the help of stop watch after the aphids had settled down to feed

1. Acquisition threshold

A large number of non viruliferous aphids (A malvae) were collected and were given a pre-acquisition starvation for one hour Batches of 10 aphids each were given acquisition feeding of 20 and 30 s, 1, 2, 5, 10, 15, 30, and 45 min and 1 and 2 h on diseased leaves before transferring them to healthy bitter gourd plants The aphids

were then allowed to remain for 24 hours on the test plants and after that they were killed by spraying 0.1 % Dimethoate.

2. Inoculation threshold

Non viruliferous aphids were given one hour pre-acquisition fasting and an acquisition feeding period of 30 min Then the viruliferous aphids in groups of 10 were transferred to individual healthy test plants. Each batch was given separate inoculation feeding periods, viz., 30 s, 1, 2, 5, 10, 15, 20, 30 and 40 min, 1, 2, 4, 8 and 24 h The aphids were killed after specific inoculation feeding period by spraying 0 1 % Dimethoate.

3 Effect of pre-acquisition fasting of the vector on the transmission

In order to estimate the effect of pre-acquisition starvation on the efficiency of the vector to acquire the virus, the insects were starved for different periods, viz , 30 min, 1, 2, 3, 4, 5, 6 and 12 h Batches of 10 aphids from each of these categories were given an acquisition feeding period of 30 min and released on test plants to feed for 24

h After the inoculation feeding period, the insects were killed by spraying 0.1 % Dimethoate. The controls with equal number of aphids were maintained without pre-aquisition starvation. The plants were kept under observation in insect proof glass house.

4. Effect of post-acquisition fasting of the vector on the transmission

A large number of aphids were starved for one hour and allowed an acquisition feeding period of 30 min. After that batches of 10 aphids were starved for different periods of 30 min, 1, 2, 3, 4, 5, 6 and 12 h after that they were transferred to healthy test plants and allowed to feed for 24 h After the inoculation feeding period, the insects were killed by spraying 0 1 % Dimethoate and the plants were kept under observation The control was maintained with equal number of aphids without post-acquisition fasting

5. Retention of infectivity by the vector

The experiments were conducted with viruliferous insects, which were transferred in succession to a series of

healthy bitter gourd plants after giving a definite inoculation feeding period on each plant Groups of aphids were starved for one hour and allowed an acquisition feeding period of 30 min to make them viruliferous Groups of 10 aphids were then transferred in succession to a series of five healthy plants transferring the insects after a definite interval The different feeding intervals allowed in different series were 30 min, 1, 1 5, 2, 2 5 and 3 h The aphids were killed from the fifth plant of the different series using 0 1 % Dimethoate The experiments were done twice

6. Minimum number of aphids required for transmission

Single aphid as well as groups of 2, 3, 5, 10, 15, 20, 25 and 30 were released on each test plant after allowing a pre-acquisition starvation period of one hour, an acquisition feeding period of 30 min and an inoculation feeding period of 24 h to determine the minimum number of aphids required for the transmission of the virus After the inoculation feeding, the insects were killed by spraying 0 1 % Dimethoate and the plants were kept under observation for the development of symptoms

V. Hostrange and local lesion hosts

To determine the host-range and local lesion hosts of bitter gourd mosaic virus, healthy plants belonging to 68 species of 20 families were inoculated by sap inoculation The plants which did not show symptoms after 8 weeks were indexed by back inoculation to bitter gourd plants to find out whether they were symptomless carriers of the virus Following plants were used for host-range and local lesion host studies

1 Acanthaceae

(a) Andrographis echioides L

(b) Justicia prostrata Schlecht

2 Amaranthaceae

(a) Amaranthus caudatus L

(b) Amaranthus viridis L

(c) Gomphrena globosa L

3 Apocynaceae

Vinca rosea L

4 Araceae

(a) Caladium sp

(b) Colocasia esculenta L

(c) Typhonium trilobatum (L) Schott

5 Asclepiadaceae

(a) Calotropis gigantea RBr

(b) Hemidesmus indicus RBr

6 Balsaminaceae

Impatiens balsamina L

7 Capparidaceae

Cleome viscosa L

8 Chenopodiaceae

Chenopodium amaranticolor coste & Reyn

9 Compositae

(a) Acanthospermum hispidum Dc

(b) Ageratum conyzoides L

(c) Emilia sonchifolia (L) Dc

(d) Eupatorium odoratum L.

(e) Synedrella nodiflora (L.) Gaertin

(f) Tridax procumbens L

(g) Vernonia cineria (L) Less

(h) Zinnia elegans Jacq

10 Cucurbitaceae

(a) Benincasa hispida Thanb and Cogn

(b) Citrullus vulgaris Schrad

(c) Cucumis metuliferus E Mey

(d) Cucurbita moschata Duch

(e) Cucumis sativus L

(f) Cucumis melo L

(g) Lagenaria siceraria Standl

(h) Luffa acutangula Roxb

(i) Trichosanthes anguina L

11 Euphorbiaceae

(a) Acalypha indica L

(b) Croton sparsiflorus Morong

(c) Euphorbia geniculata Orteg

(d) Euphorbia hirta L

(e) <u>Manihot esculenta</u> Crantz

(f) <u>Phyllanthus niruri</u> L

12 Malvaceae

(a) <u>Abelmoschus esculentus</u> L

(b) <u>Abutilon indicum</u> (L) Sweet

(c) <u>Sida acuta</u> Burm f

13 Labiatae

<u>Leucas aspera</u> (Willd) Link

14 Musaceae

<u>Musa</u> sp (L) cv Palayankodan

15 Leguminosae

i Mimosaceae

<u>Mimosa pudica</u> L

ii Papilionaceae

(a) <u>Arachis hypogaea</u> L

(b) <u>Cajanus cajan</u> (L) Millsp

(c) *Calopogonium mucunoides* Desv

(d) *Canavalia ensiformis* (L) Dc

(e) *Clitoria ternatea* L

(f) *Crotalaria juncea* L

(g) *Dolichos biflorus* Auct

(h) *Vigna mungo* (L) Hepper

(i) *Vigna radiata* (L) Wilczek

(j) *Vigna unguiculata* (L) Walp

16 Polygononaceae

Antigonon leptopus Hook & Arn

17 Pedaliaceae

Sesamum indicum L

18 Solanaceae

(a) *Capsicum annuum* L

(b) *Datura stramonium* L

(c) *Datura metel* L

(d) *Lycopersicon esculentum* Mill

(e) *Nicotiana glutinosa* L

(f) *Nicotiana tabacum* L.

(g) *Physalis minima* L.

(h) *Physalis minima* var *indica* C B. Clarke

(i) *Solanum melongena* L.

19 Verbenaceae

(a) *Clerodendron infortunatum* Gaertn

(b) *Lantana camera* L.

(c) *Stachytarpheta indica* (L.) vahl

20 Zingiberaceae.

Zingiber officinale Rose.

VI. Serological properties of the virus

1. Purification of virus

The virus was purified following the method of Hebert (1963) and Van Kammen (1967) The inoculum was prepared by mincing the systemically infected, frozen leaves at the rate of 1 g/ml of 0 01M phosphate buffer pH 7.0 in a clean sterile pestle and mortar The homogenate was filtered

through double layer muslin cloth and centrifuged at 10000 g for 15 min at 4°C using HIMAG refrigerated centrifuge model HCR20BA, to remove the host material The clear supernatant was decanted and added polyethylene glycol (PEG) to a final concentration of 4 per cent (W/V) and sodium chloride (NaCl) to give a concentration of 0.2M The mixture was stirred at room temperature to dissolve the PEG and NaCl and after one hour, centrifuged at 10000 g for 15 min at 4°C. Both the fractions (supernatant and residue) were tested for infectivity separately The residue was resuspended in phosphate buffer before test inoculation The final virus preparation (residue obtained after the final centrifugation) was dissolved in 0 85 per cent saline and it was used as viral antigen for injecting rabbits

2. Preparation of antiserum

Two healthy New Zealand white female rabbits weighing about 2 kg with conspicuous marginal ear vein were selected for immunization The schedule of immunization consisted of five intramuscular injections at weekly intervals followed by one intravenous injection one week after the last intramuscular injection. In the case of

intramuscular injection, the purified virus preparation suspended in 0 85 per cent saline was mixed with Freund's incomplete adjuvant (Difco), in the ratio (1 1) (v/v) and 4 ml of this emulsion was injected into the thigh muscle at a time The final injection was given intravenously with 2 ml of virus preparation suspended in 0 85 per cent saline into the marginal left ear vein of each rabbit one week after the last intramuscular injection

Fifteen days after the last intravenous injection the rabbits were bled They were fasted for 12 h prior to bleeding The lateral vein of the right ear was incised with a razor blade and it was widened temporarily by rubbing the ear with xylol The blood samples were aseptically collected in 50 ml beaker and were allowed to clot by keeping the beaker at room temperature for 2 h and after that the blood clot was loosened with the help of a sterilized glass rod and the samples were kept overnight at 4°C The clear serum was decanted and centrifuged at 10000 g for 30 min at 4°C to remove the remaining blood cells This supernatant antiserum was stored in small vials after adding a pinch of sodium azide and kept in freezer and used for other tests

3. Serological tests

i) Microprecipitin test on slides

Thirty microlitres of antiserum and same quantity of virus suspension were mixed on a microscopic slide The mixture was incubated at 25°C under high humidity for 20-45 min and examined under microscope (Bercks et al , 1972) Antigens of bitter gourd mosaic virus isolate I (isolated from diseased plants in glass house) and isolate II (isolated from diseased plants in the field) cucumber mosaic virus, pumkin mosaic virus, cowpea mosaic virus and snake gourd mosaic virus were tested against the antiserum of bitter gourd mosaic virus

The virus suspension (bitter gourd mosaic virus) was tested against four other antisera of cucumber mosaic virus, cucumber green mottle mosaic virus, squash mosaic virus and tobacco mosaic virus type strain which were received from Danish Government Institute of Seed Pathology for developing countries (Denmark).

ii) Microprecipitin test in petri dishes

This test was conducted to determine the titre of the antiserum with the virus and to measure the end point of the virus with the antiserum, as per the procedure described by Noordam (1973)

Leaves showing typical mosaic symptoms were ground using a clean sterile pestle and mortar with distilled water (pH 7) at the rate of 1 ml per gram of leaf tissue, and the sap was strained using cotton wool and centrifuged at 10000 g for 10 min to get clear supernatant It was transferred in to a corning glass test tube of 1 to 1 5 ml capacity using a pasteur pipette The second tube was half filled with the sap and an equal amount of saline buffer (0.85 % NaCl in 0 01 M Tris oxymethyl aminomethane buffer of pH 7 0) was added. The liquids were mixed by inverting the tube several times This tube contained the sap diluted to 1/2. Half of this dilution was transferred to next tube and an equal volume of saline buffer was added so as to make a dilution of 1/4 This method was continued to make dilutions of the series 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096 and 1/8192 In the same way as with the sap from virus infected leaves, serial dilutions

were made for the antiserum also.

A scheme was drawn on a paper with 10 mm squares and the sap and antiserum dilutions were marked as shown in the figure 1 A petri dish of 19 cm diameter was kept on the top of the scheme, keeping the dish at 8°C Using a pasteur pipette drops of saline buffer were placed in the petri dish at the point where the line labelled NaCl - buffer met with other lines Using another pipette one drop each of the least concentrate sap (1/8192) was spotted at the intersections along the vertical line labelled 1/8192. The next dilution of sap was spotted with another pipette along that particular line which indicated that dilution This was continued until the scheme for sap was completed The least concentration of the antiserum (1/8192) was taken in a fresh pipette and one drop was spotted to a saline drop and to the 14 different dilutions of the sap at the point of intersection of two lines This process was continued until the scheme for the antiserum was completed The drops were covered with liquid paraffin to prevent evaporation. Liquid paraffin was added slowly through the side of the petri dish, so that the drops will not merge together. The petri dishes were kept for 2 h at 28 - 30°C and then examined with a

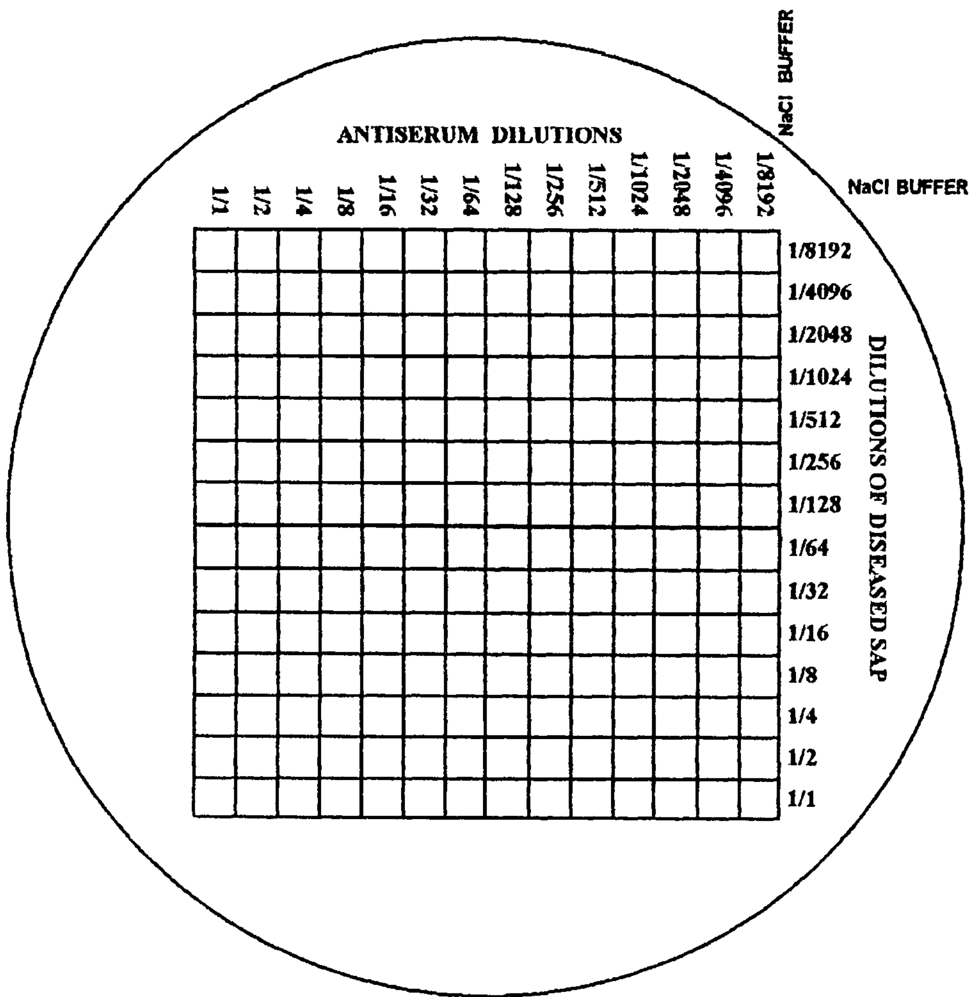


Fig. 1. MICROPRECIPITIN TEST IN PETRI DISHES

stereomicroscope with top light and black background The intensity of the precipitate was evaluated based on a scale as given below

- − = No reaction
- ± = Barely visible reaction
- + = Slight reaction
- ++ = Moderate reaction
- +++ = Heavy reaction
- ++++ = Very heavy reaction

These dishes were kept over night in a refrigerator and evaluated for the second time. From the above test the titre of the antiserum with diseased sap, virus end point with antiserum were determined.

(iii) Outchterlony's agar double diffusion test

This test was done in serological petri dishes Antiserum and virus suspensions (0.4 ml) were added to wells punched in agar

Sterilized petri dishes were layered using 2 per cent agarose (prepared in 0.01 M Tris buffer containing 0 85 per cent NaCl and Sodium azide to get a final concentration of 0 02 per cent) to a thickness of 1 mm and allowed to dry. Above this layer 2 per cent melted agarose was again added to a thickness of 3 mm. Thirty min after pouring of agarose, with the help of a sterilized gel cutter, six wells (one well in the centre and the other five wells around it) were made in each plate Each well was 3 mm deep and 5 mm in diameter and the distance between adjacent wells was 10 mm In the central well (well no 1) of each plate (except in plate no 4) 0 4 ml of antiserum was dispensed with a pasteur pipette and the antigens prepared from infected plants were dispensed in the surrounding wells as described below in four separate plates

In plate 1, well 2 contained the clarified healthy plant sap and well 3 and 5 received distilled water and 4 and 6 contained buffer

In the second plate, well 2 received pumpkin mosaic virus and 3 with healthy plant sap, 4 with cucumber mosaic virus, 5 with snake gourd mosaic virus and 6 contained

distilled water

In the third plate, well 2 contained sap from infected bitter gourd plants and the well 4 was filled with snake gourd mosaic virus, 3 with pumpkin mosaic virus, 5 with cucumber mosaic virus and 6 with cowpea mosaic virus In the case of plate 4, the well 1 contained sap from infected bitter gourd plants, 2 received cucumber mosaic virus antiserum, 3 with cucumber green mottle mosaic virus antiserum, 4 with squash mosaic virus antiserum, 5 with tobacco mosaic virus type strain antiserum and 6 received bitter gourd mosaic virus antiserum.

The petri dishes were kept humid by placing a moistened filter paper on the inner side of the lids. The experiments were performed twice. The dishes were kept in stacks with ordinary paper in between them to prevent any scratches and incubated at room temperature and examined periodically for the appearance of characteristic precipitin bands upto 14 days After that precipitin bands were stained using amidoblack as explained below

Before staining, the agar was soaked in two changes

of 0 9 per cent phosphate buffered saline for 24 h and then in distilled water for another 24 h. Water was drained out and the agar was covered with a whatman No. 1 filter paper and dried at 37°C. When the agar was completely dry, the filter paper was stripped off The dried agar was then immersed in amidoblack stain (Appendix - I) for 15 minutes

After staining it was washed two times each in decolouriser solutions No. 1 and 2 (Appendix - I) Each washing was 1 h duration. The plates were then dried for 1 h at 37°C and examined

VII. Varietal sreening

Varieties of bittergourd plants were tested for their resistance to bittergourd mosaic virus. The plants were grown in pots and kept in insect proof glass house Ten plants of each variety were inoculated with the virus using standard sap as inoculum The inoculum was prepared by grinding mosaic affected bitter gourd leaves with sterilized pestle and mortar after adding equal amount of distilled water (W/V) The sap was extracted from crushed pulp by squeezing through cotton wool and immediately inoculated on

the leaves of test plants of 2-3 leaf stage after dusting with carborundum The experiment was conducted twice Following 5 varieties and 20 indigenous collections were used for screening studies

1 Arka Harit
2 Co - 1
3 Priya
4 K Sona
5 Mc 84
6 12B green round IC 44410
7 36 green medium IC 44435
8 42B green medium IC 45338
9 50 green long IC 45346
10. 61 white medium IC 45358
11 78B white medium IC 85604
12 80B green medium IC 85605
13 87 green long IC 68234
14. 108 green long IC 68255
15 116 green medium IC 68263
16 139 green medium IC 68286
17 149 green long IC 68296
18. 159 green long IC 68306

19 175 green medium IC 68322

20 199A green long IC 85606

21 202 white medium IC 85608

22 2221A green medium IC 85616

23 259 white medium IC 85639

24 20 green long very good IC 44418

25 177 green medium IC 68324

VIII. Estimation of loss

Experiments were conducted to estimate the effect of bitter gourd mosaic virus infection on the growth of bitter gourd plant The experiment was laid out in a completely Randomised Block Design in cement pots in front of the Department of plant pathology college of Agriculture vellayani during 1993 The lay out plan of the experiment is given in Fig 2

1 Varieties and seed materials

One commonly cultivated variety viz , Priya (V_1) and another indigenous collection 177 green medium IC 68324 which was found least susceptible to bitter gourd mosaic

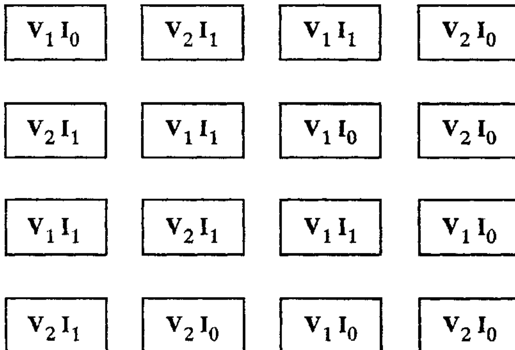


FIG. 2. LAY OUT OF THE POT EXPERIMENT TO ESTIMATE THE EFFECT OF INFECTION ON BITTERGOURD BY BITTER GOURD MOSAIC VIRUS

virus were used for conducting the experiment. The variety Priya was obtained from the Instructional Farm, College of Agriculture Vellayani and the collection 177 green medium IC 68324 was obtained from NBPGR Trichur. The following treatments were fixed to estimate the effects of virus infection on the growth of the plant.

Treatment 1 (V_1I_1) - Inoculation of Priya variety on tenth day after planting

Treatment 2 (V_1I_0) - Priya variety maintained with out inoculation (control)

Treatment 3 (V_2I_1) - Inoculation of 177 green medium IC 68324 on tenth day after planting

Treatment 4 (V_2I_0) - 177 green medium IC 68324 maintained without inoculation (control)

2. Pot culture

The cement pots having size 60 x 60 x 30 cm were filled with potting mixture of sand, red soil and cowdung in the ratio of 1 1·2 Four to five seeds were sown in each pot, but only two vigorously growing plants per pot were retained and used for the experiment.

Manures and fertilizers were applied according to the package of practices recommendations of the Kerala Agricultural University (1993) Separate standards were maintained for each pot for training the plants The plants were irrigated daily All the plants were periodically sprayed with 0 1 % Rogar and 0 2 % Dithane M-45 to keep the plants free from pest and fungal diseases

The crop was sown on 10-9-93 and observation were recorded on the following aspects at an interval of 1 month

1) Number of leaves formed

2) Leaf area

3) Internodal length

4) Thickness of vine

5) Total length of vine

6) Number of branches developed

7) Number of flowers formed (Male and female)

8) Fruit characters

a) Number of fruits formed

b) Length of the fruits

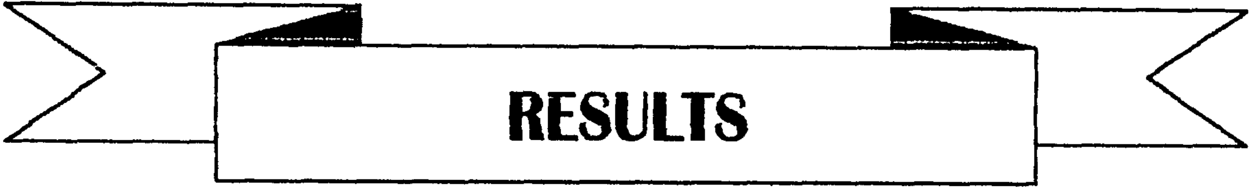
c) Girth of the fruits

d) Mean weight of fruit

e) Yield of fruits

3. Statistical analysis

The data were analysed statistically by applying the technique of analysis of variance for Completely Randomised Block Design in split plot fashion (Snedecor and Corhran 1967) and the significance was tested by F test Critical differences were calculated for comparing treatment means


RESULTS

RESULTS

I. Symptomatology

The initial symptoms of naturally infected plants appeared as clearing of vein and veinlets followed by mosaic mottling (Fig 3) In advanced stages of infection dark green raised blisters of varying size and shape developed on the lamina The leaves were very much reduced in size and showed filiform shape (Fig 4) Diseased plants remained stunted and produced only a few flowers and fruits

On mechanical inoculation of the infective sap to bitter gourd plants of two leaf stage, the symptoms appeared within 12-14 days The symptoms first appeared as small light green areas followed by mosaic mottling Typical mosaic patches with dark green and light green blisters were produced in all the subsequent leaves (Fig 5) In some cases the leaves had large area of light green patches, the growth of the infected plants was retarded and internodes shortened (Fig 6) As in the case of naturally infected plants, the inoculated plants also produced only a few flowers and small fruits

Fig 3 Healthy and diseased bitter gourd leaves

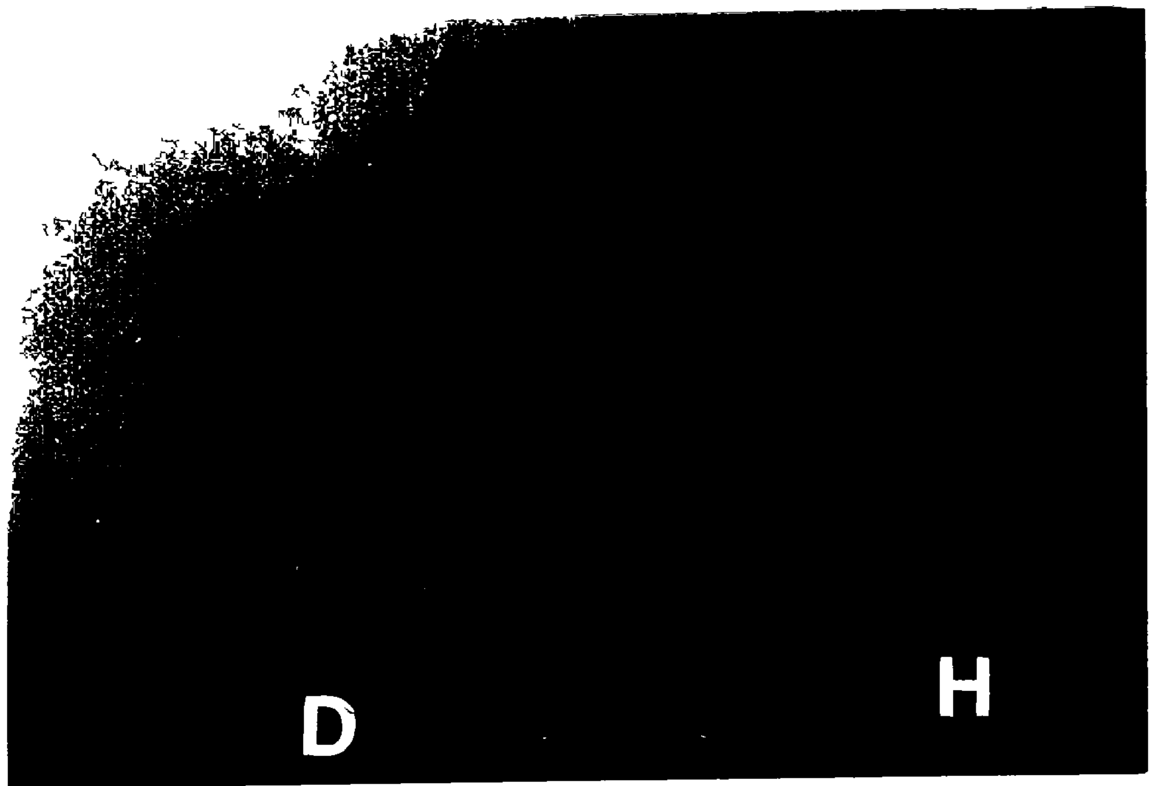


Fig 4 Bitter gourd leaves showing filiform shape

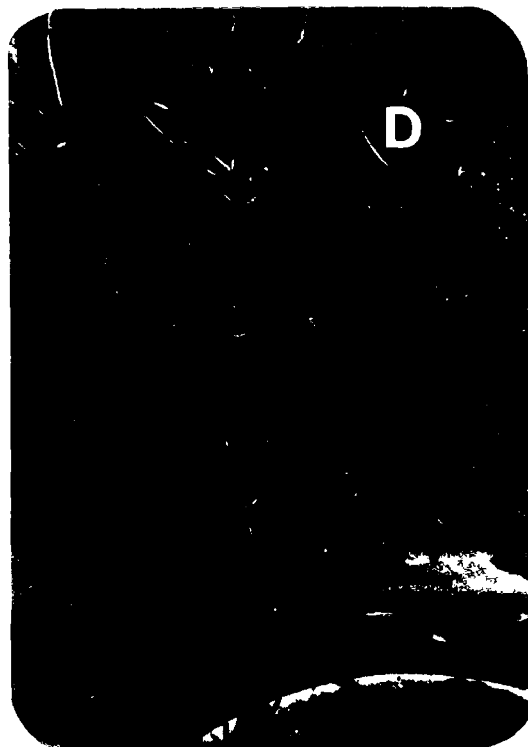


Fig 5 Bitter gourd plant infected with virus

Fig 6 Healthy and diseased bitter gourd plants

II Transmission of the virus

1. Sap transmission

The virus was found to be transmitted successfully by sap inoculation using standard sap, sap extracted in phosphate buffer (0 01M, pH 7 0) and tris buffer (0 01M, pH 7 0) The symptoms appeared within 12-14 days after inoculation The percentage of transmission varied with the extraction medium used (Table 1) Standard sap and sap extracted in phosphate buffer gave maximum infection of 90 per cent while tris buffer gave the minimum infection of 75 per cent

Table 1 Sap transmission of bitter gourd mosaic virus

Sl No	Inoculum	Number of plants infected / Number of plants inoculated: Experiment I	Number of plants infected / Number of plants inoculated: Experiment II	Per cent trans-mission
1	Standard sap	9/10	9/10	90
2	Sap extracted in phosphate buffer	9/10	9/10	90
3	Sap extracted in tris buffer	8/10	7/10	75

2. Seed transmission

Out of 110 seeds sown 107 seeds have germinated None of the plants showed symptoms of bitter gourd mosaic disease during the period of observation

3. Graft transmission

Infected shoots were wedge grafted to 30 days old healthy plants, which were grown in insect proof glass house The symptoms appeared 12-14 days after grafting From the two trials conducted 90 per cent transmission was obtained (Table 2)

Table 2 Graft transmission of bitter gourd mosaic virus

Experiment	Number of plants grafted	Number of plants infected	Per cent transmission
I	10	9	90
II	10	9	90

4. Insect transmission

Insect transmission studies of the virus were carried out using 7 vectors, viz , A craccivora, A

gossypii, A malvae, M. persicae, H vigintioctopunctata, S. biguttula biguttula and B. tabaci and the results are presented in Table 3. A. gossypii and A. malvae were found to transmit bitter gourd mosaic virus very efficiently followed by M persicae The insects were given a pre-acquisition fasting of 1 h, acquisition feeding period of 30 min and an inoculation feeding period of 24 h The symptoms appeared 8-12 days after inoculation.

The observations showed that the highest percentage of transmission (60%) was obtained with A gossypii and A. malvae, M persicae with (55%) and A. craccivora (30%) H. vigintioctopunctata, S biguttula biguttula and B. tabaci could not transmit the virus. (Table 3)

Table 3 Insect transmission of bitter gourd mosaic virus

Sl No	Vector	Number of plants infected / Number of plants inoculated		Per cent trans-mission
		Experiment I	Experiment II	
1.	Aphis craccivora	3/10	3/10	30
2.	Aphis gossypii	6/10	6/10	60
3	Aphis malvae	6/10	6/10	60
4	Myzus persicae	5/10	6/10	55
5	Henosepilachna vigintioctopunctata	0/10	0/10	0
6	Syndapteryx biguttula biguttula	0/10	0/10	0
7	Bemisia tabaci	0/10	0/10	0

III. Physical properties

1. Dilution end point (DEP)

Serial dilutions of the infected sap was made viz , 1 10, 1 100, 1:1000, 1·10000, 1:100000, 1:1000000 The different dilutions were used for inoculation on separate test plants starting from the highest dilution Ten plants were inoculated with each of the dilutions and the experiment was repeated to confirm the result The data indicated that the dilution end point of the virus was between 1·1000 and 1.10000 (Table 4)

Table 4 Dilution end point of bitter gourd mosaic virus

Dilutions	Experiment I		Experiment II		Per cent trans- mission
	Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected	
0	10	9	10	9	90
1 10	10	9	10	8	85
1 100	10	7	10	7	70
1.1000	10	6	10	6	60
1.10000	10	0	10	0	0
1 100000	10	0	10	0	0
1 1000000	10	0	10	0	0

2. **Thermal inactivation point (TIP)**

The infected sap was treated at different range of temperature, viz., 35, 40, 45, 50, 60, 70, 80 and 90°C. The treated and untreated (control at room temperature at 28-30°C) samples of the sap were inoculated on young vigorously growing test plants of two leaf stage The results indicated that the virus was inactivated at temperature between 50 and 60°C (Table 5)

Table 5 Thermal inactivation point of bitter gourd mosaic virus

Temperature (°C)	Experiment I		Experiment II		Per cent transmission
	Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected	
Control (28-30)	10	9	10	9	90
35	10	9	10	8	85
40	10	8	10	7	75
45	10	6	10	7	65
50	10	5	10	5	50
60	10	0	10	0	0
70	10	0	10	0	0
80	10	0	10	0	0
90	10	0	10	0	0

3. **Longevity *in vitro* (LIV)**

In order to find out the longevity *in vitro*, an experiment was conducted as described under materials and

methods and the results are given in Table 6 and 7 When the inoculum was stored at room temperature (28-30°C) for a period of 24 h, its infectivity was completely lost About 40 per cent of the plants inoculated with the sap kept for 12 h at room temperature developed symptoms and after 24 h of storage the infectivity of the inoculum was completely lost. So the longevity in vitro of the virus stored at room temperature was between 12 and 24 h.

When the inoculum was stored in a refrigerator (10°C) the infectivity was retained upto 48 h, but the percentage of infected plants was considerably decreased. After 72 h of storage of the inoculum the infectivity was completely lost So the longevity in vitro of the virus was between 48 and 72 h, when the sap was stored under refrigerated conditions

Table 6 Longevity in vitro of bitter gourd mosaic virus at room temperature (28-30°C)

Ageing in hours	Experiment I		Experiment II		Per cent trans-mission
	Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected	
0	10	9	10	9	90
2	10	8	10	7	75
4	10	7	10	7	70
6	10	6	10	7	65
8	10	6	10	6	60
12	10	4	10	4	40
24	10	0	10	0	0
48	10	0	10	0	0
72	10	0	10	0	0

Table 7. Longevity *in vitro* of bitter gourd mosaic virus at 10°C

Ageing in hours	Experiment I		Experiment II		Per cent trans-mission
	Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected	
0	10	9	10	9	90
2	10	9	10	9	90
4	10	8	10	8	80
6	10	8	10	7	75
8	10	7	10	7	70
12	10	7	10	6	65
24	10	6	10	5	55
48	10	5	10	4	45
72	10	0	10	0	0

IV. Vector - virus relationships

1. Acquisition threshold

This experiment was conducted to find out the minimum period required for the vector *Aphis malvae* to acquire the virus and to become viruliferous The results are presented in Table 8 The results showed that a short acquisition feeding period of 30 s only was sufficient for the aphids to become viruliferous. The optimum acquisition feeding period which gave the maximum percentage of infection (60%) was found to be 30 min As the acquisition feeding period further increased, the efficiency of the vector to

transmit the virus was reduced considerably and it was only 30 per cent when the acquisition feeding period was two hours

Table 8 Acquisition threshold of *Aphis malvae* on the transmission of bitter gourd mosaic virus

Acquisition feeding period	Number of plants infected / Number of plants inoculated Experiment I	Experiment II	Number of plants infected out of 20	Per cent trans-mission
20 s	0/10	0/10	0	0
30 s	1/10	1/10	2	10
1 min	3/10	2/10	5	25
2 min	3/10	3/10	6	30
5 min	4/10	3/10	7	35
10 min	5/10	4/10	9	45
15 min	5/10	5/10	10	50
20 min	5/10	6/10	11	55
30 min	6/10	6/10	12	60
45 min	6/10	5/10	11	55
1 h	5/10	4/10	9	45
2 h	3/10	3/10	6	30

Pre-acquisition fasting - 1 h

Inoculation feeding - 24 h

Number of aphids per plant - 10

2. Inoculation threshold

In order to find out the minimum period required for the viruliferous aphid *A malvae* to transmit the virus successfully, an experiment was conducted as described in

the materials and methods and the results are presented in (Table 9)

The data indicated that the viruliferous aphids were capable of transmitting the virus with one minute inoculation feeding on the test plant Maximum infection of 90 per cent was obtained by feeding the vector for 2 h on test plants

Table 9 Inoculation threshold of <u>Aphis malvae</u> on the transmission of bitter gourd mosaic virus

Inoculation feeding period	Number of plants infected / Number of plants inoculated		Number of plants infected out of 20	Per cent transmission
	Experiment I	Experiment II		
30 s	0	0	0	0
1 min	2/10	3/10	5	25
2 min	3/10	3/10	6	30
5 min	5/10	4/10	9	45
10 min	5/10	5/10	10	50
15 min	5/10	6/10	11	55
20 min	6/10	5/10	11	55
30 min	6/10	6/10	12	60
45 min	7/10	8/10	15	75
1 h	8/10	9/10	17	85
2 h	9/10	9/10	18	90
4 h	8/10	8/10	16	80
8 h	7/10	8/10	15	75
24 h	6/10	6/10	12	60

Pre-acquisition fasting - 1 h

Acquisition feeding - 30 min

Number of aphids per plant - 10

3. Effect of pre-acquisition fasting of the vector on the transmission

Pre-acquisition fasting of aphids increased the efficiency of the vector to acquire and transmit the virus. Maximum efficiency was noted when insects were starved for a period of 2 h Further increase of fasting period did not appreciably increase the percentage of infected plants and also the efficiency of the vector to transmit the virus (Table 10)

Table 10 Effect of pre-acquisition fasting of *Aphis malvae* on the efficiency of transmission of bitter gourd mosaic virus

Pre-acquisition fasting period	Number of plants infected / Number of plants inoculated		Number of plants infected out of 20	Per cent transmission
	Experiment I	Experiment II		
No fasting	4/10	3/10	7	35
30 min	7/10	4/10	11	55
1 h	6/10	6/10	12	60
2 h	8/10	7/10	15	75
3 h	6/10	5/10	11	55
4 h	4/10	3/10	7	35
5 h	2/10	2/10	4	20
6 h	0/10	0/10	0	0
12 h	0/10	0/10	0	0

Acquisition feeding - 30 min

Inoculation feeding - 24 h

Number of aphids per plant - 10

4. Effect of Post-acquisition fasting of the vector on the transmission

It was observed that post-acquisition fasting of the vector decreased the percentage of infection. Maximum infection of 60 per cent was obtained when the aphids were immediately transferred to test plants after acquisition feeding period and no infection was obtained when the aphids were given a post-acquisition fasting beyond 2 h (Table 11)

Table 11 Effect of post-acquisition fasting of *Aphis malvae* on the efficiency of transmission of bitter gourd mosaic virus

Post-acquisition fasting	Number of plants infected / Number of plants inoculated		Number of plants infected out of 20	Per cent transmission
	Experiment I	Experiment II		
No fasting	7/10	5/10	12	60
30 min	5/10	5/10	10	50
1 h	4/10	4/10	8	40
2 h	4/10	3/10	7	35
3 h	0/10	0/10	0	0
4 h	0/10	0/10	0	0
5 h	0/10	0/10	0	0
6 h	0/10	0/10	0	0
12 h	0/10	0/10	0	0

Pre-acquisition fasting - 1 h

Acquisition feeding - 30 min

Inoculation feeding - 24 h

Number of aphids per plant - 10

5. Retention of infectivity by the vector *Aphis malvae*

The results indicated that successful infection could be obtained up to the second plant of the first series in which aphids were transferred at an interval of 30 min and in all the other cases only the first plant of the series got infection, indicating that the viruliferous nature of the vector was lost after 1 h (Table 12)

Table 12 Retention of infectivity by *Aphis malvae*

Feeding period on each test plant		Infection in successive transfers				
		Serial number of plants tested				
		1	2	3	4	5
30 min	a	+	+	-	-	-
	b	+	+	-	-	-
1h	a	+	-	-	-	-
	b	+	-	-	-	-
1 h 30 min	a	+	-	-	-	-
	b	+	-	-	-	-
2 h	a	+	-	-	-	-
	b	+	-	-	-	-
2 h 30 min	a	+	-	-	-	-
	b	+	-	-	-	-
3 h	a	+	-	-	-	-
	b	+	-	-	-	-

a = replication 1 b = replication 2

+ = Symptom produced - = No symptom produced

6. Minimum number of aphids required for transmission

A single aphid was found to be capable of the virus transmitting the virus to healthy test plants The optimum number of aphids required to produce maximum infection of 60 per cent was found to be 10 (Table 13)

Table 13 Minimum number of *Aphis malvae* required for the transmission of bitter gourd mosaic virus

Number of aphids per plant	Number of plants infected / Number of plants inoculated		Number of plants infected out of 20	Per cent transmission
	Experiment I	Experiment II		
1	1/10	1/10	2	10
2	3/10	2/10	5	25
3	4/10	5/10	9	45
5	5/10	6/10	11	55
10	6/10	6/10	13	60
15	6/10	5/10	11	55
20	5/10	6/10	11	55
25	5/10	5/10	10	50
30	5/10	4/10	9	45

Pre-acquisition fasting - 1 h

Acquisition feeding - 30 min

Inoculation feeding - 24 h

V. Host range and local lesion hosts

Out of 68 plant species belonging to 20 families tested, 16 species belonging to six families viz , Araceae,

Chenopodiaceae, Cucurbitaceae, Musaceae, Polygonaceae and Solanaceae produced symptoms of virus disease, one plant species viz , Datura metel did not show any symptom but acted as symptomless carrier of bitter gourd mosaic virus.

1. Chenopodiaceae

Chenopodium amaranticolor Coste and Reyn

The inoculated plants produced local lesions within 5-7 days after inoculation The lesions appeared as chlorotic in the beginning, then turned to necrotic with brown centre The lesions were circular in shape with 1 - 2 mm in diameter (Fig 7)

2. Cucurbitaceae :

a) Cucurbita moschata Duch.

The inoculated plants produced symptoms in 22-25 days Initially light green patches were developed on the leaf lamina, later these patches coalesced together to form mosaic mottling In advanced stage of infection, the size of the leaves was reduced considerably, internodes were shortened and plants were stunted (Fig. 8)

b) Cucumis metuliferus E.Mey

The symptoms appeared 10-15 days after inoculation as irregular light yellow patches followed by mosaic

Fig 7 Local lesions of bitter gourd mosaic virus on Chenopodium amaranticolor

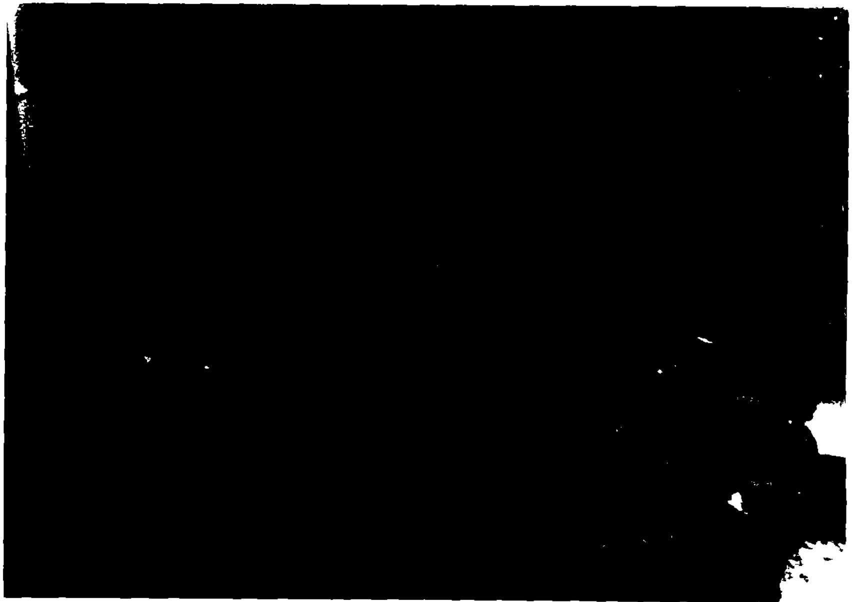


Fig 8 Cucurbita moschata infected with bitter gourd mosaic virus

mottling The newly formed leaves were reduced in size. The infected plants were stunted and internodes were shortened (Fig 9)

c) <u>Luffa acutangula</u> Roxb

The symptoms first appeared on the young leaves within 10-12 days after inoculation and was characterised by light green area followed by dark green patches. The infected plants produced top necrosis in the advanced stage (Fig. 10).

d) <u>Citrullus vulgaris</u> Schrad

The inoculated plants produced chlorotic spots after 7-8 days of inoculation. These spots coalesced together to form larger patches The size of the infected leaves was reduced considerably, internodes were shortened and plants were stunted

e) <u>Trichosanthes anguina</u> L.

The symptoms noticed within 21-23 days after inoculation The initial symptom appeared as light greenish area, on the leaf lamina Typical mosaic mottling with dark green and light green patches were produced in all the

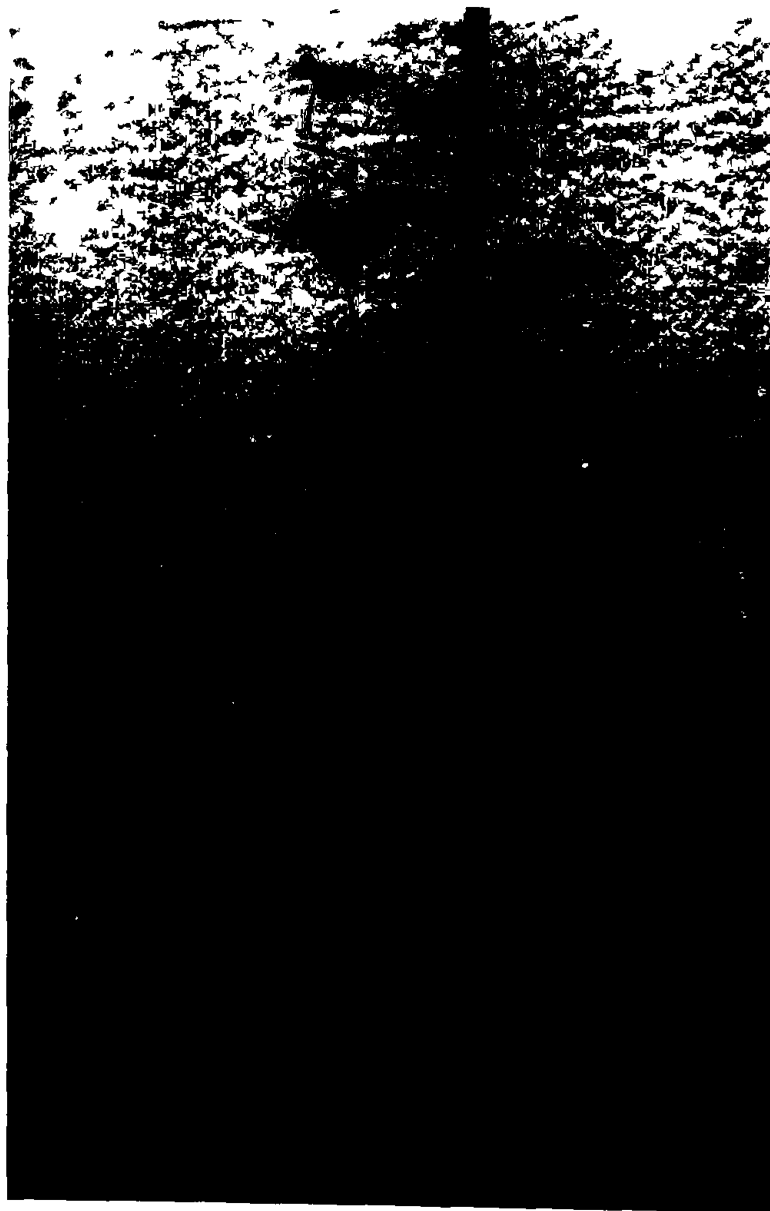


Fig 9 Cucumis metuliferus infected with bitter gourd mosaic virus

Fig 10 Luffa acutangula infected with bitter gourd mosaic virus

subsequent leaves The infected plants were stunted (Fig 11)

f) Cucumis melo L

The inoculated plants produced symptoms in 10-15 days after inoculation and was characterised by mosaic patches with light green and dark green area The infected plants were stunted and internodes were shortened

The following plants viz , Cucumis sativus L , Benincasa hispida Thanb and Cogn , Lagenaria siceraria Standl when inoculated with bitter gourd mosaic virus did not produce any symptom of mosaic and when they were again back inoculated to bitter gourd plants did not produce any symptom Thus the above plants were found to be immune to the virus

Musaceae.

Musa sp (L) cv Palayankodan

The healthy plants showed the symptoms one month after the inoculation of bitter gourd mosaic virus and was characterised by the chlorotic or yellowish - green bands on young leaves The infected plants were stunted in appearance (Fig 12)

Fig 11 *Trichosanthes anguina* infected with bitter gourd mosaic virus

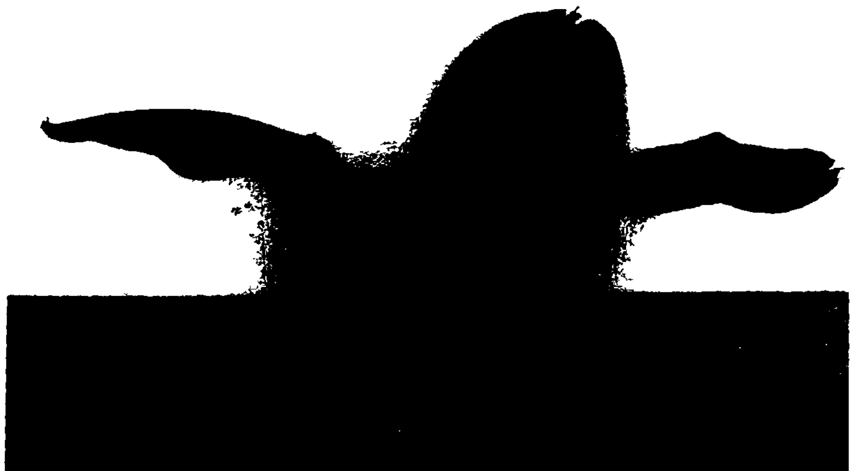


Fig 12 *Musa* sp L cv Palayankodan. infected with bitter gourd mosaic virus

Polygonaceae.

Antigonon leptopus Hook & Arn.

The symptom was noticed about 2 weeks after inoculation The initial symptom appeared as mosaic mottling with light green and dark green patches on the young leaves In advance stage of infection, the inoculated plants produced top necrosis and marginal necrosis of vine and leaves respectively (Fig 13)

Solanaceae

a) *Capsicum annuum* L

The initial symptom was noticed 7-8 days after inoculation and was characterised by mosaic patches on the leaf lamina In the later stage of infection the plants showed top necrosis symptom The leaves were reduced in size and were curled (Fig 14)

b) *Datura stramonium* L

The inoculated plants produced local lesions within 7 days after inoculation The lesions appeared as chlorotic spots initially then turned to necrotic lesions having 1 mm in diameter (Fig 15)

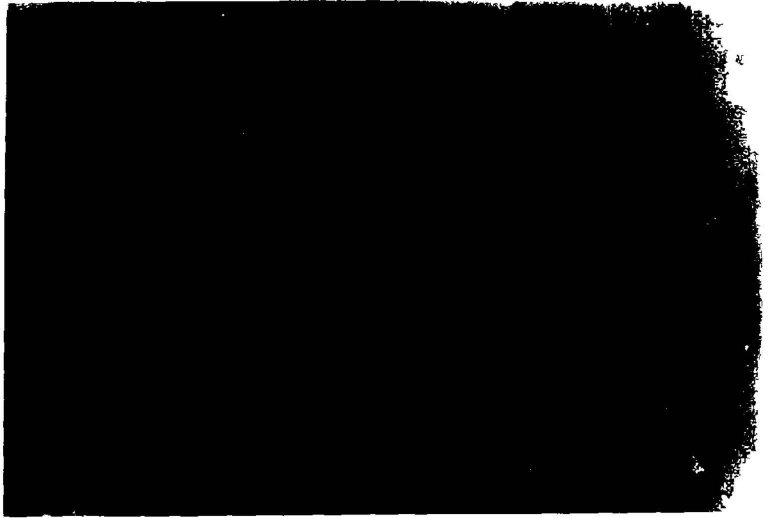


Fig 13 Antigonon leptopus infected with bitter gourd mosaic virus

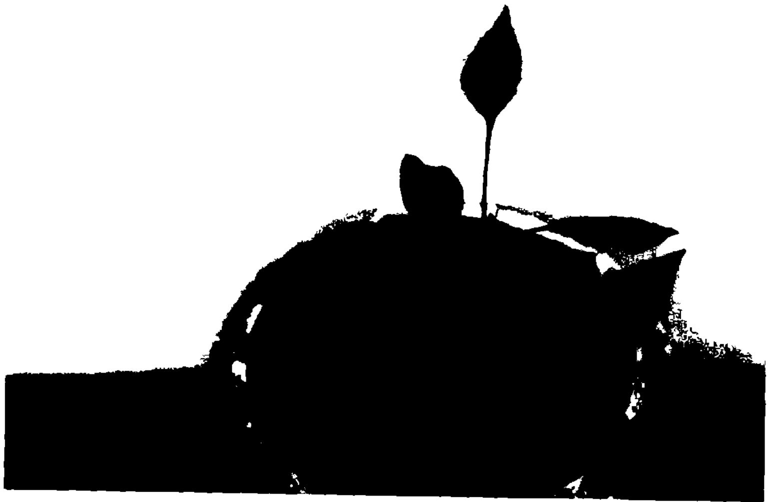


Fig 14 Capsicum annuum infected with bitter gourd mosaic virus

c) <u>Datura</u> <u>metel</u> L.

The inoculated plants did not show any visible symptom, but on back inoculation to bitter gourd plants produced mosaic mottling and thus acted as a symptomless carrier of bitter gourd mosaic virus

d) <u>Nicotiana</u> <u>tabacum</u> L.

The symptoms were noticed 8-10 days after inoculation and were characterised by light green patches on the leaf lamina In the advanced stage of infection the plants were stunted and the internodes were shortened. The size of the leaves was very much reduced (Fig 16)

e) <u>Nicotiana</u> <u>glutinosa</u> L.

The inoculated plants showed symptoms 7-8 days after inoculation The initial symptom appeared as light yellow patches on the leaf lamina. In the advanced stages thickening of vein and veinlets followed by curling of leaves were observed (Fig 17)

f) <u>Physalis</u> <u>minima</u> L

The symptoms appeared within 10-15 days after inoculation Light green patches were developed on the leaf lamina, followed by mosaic mottling with dark green area In

Fig 15 Local lesions of bitter gourd mosaic virus on *Datura stramonium*

Fig 16 *Nicotiana tabacum* infected with bitter gourd mosaic virus

advanced stage of infection curling and crinckling of the leaves were observed The infected plants produced only few flowers (Fig 18)

g) Physalis minima var. indica C.B. Clarke

The inoculated plants produced mild mottling symptom within 10-12 days after inoculation In advanced stage green and yellowish green patches were observed The leaves were curled and reduced in size (Fig 19)

Araceae

Typhonium trilobatum (L) Schott

The inoculated plants produced mild mosaic symptoms 15-20 days after inoculation The infected plants were stunted (Fig 20)

VI. Serological properties of the virus

1. Microprecipitin test on slides

Thirty microlitres of antiserum prepared as described under materials and methods was mixed with equal volume of antigen from different virus infected crop plants

Fig 17 Nicotiana glutinosa infected with bitter gourd mosaic virus

Fig 18 Physalis minima infected with bitter gourd mosaic virus

Fig 19 Physalis minima var indica infected with bitter gourd mosaic virus

Fig 20 Typonium trilobatum infected with bitter gourd mosaic virus

The antigens of bitter gourd mosaic virus isolate I, Isolate II, snake gourd mosaic virus, cucumber mosaic virus, pumpkin mosaic virus produced dense precipitate with the antiserum specific to bitter gourd mosaic virus Antigen of cowpea mosaic virus did not produce any precipitate

When the bitter gourd mosaic virus antigen was tested against the antisera of cucumber mosaic virus, cucumber green mottle mosaic virus, squash mosaic virus and tobacco mosaic virus type strain which were received from Danish Government Institute of Seed Pathology for developing countries Denmark no precipitate was formed, indicating that the antisera of the above four viruses did not contain antibodies of bitter gourd mosaic virus.

2. Microprecipitin test in petri dishes

Series of dilution of antigen and antiserum were spotted in petri dishes at regular intervals. The formation of precipitate was observed after 4 h under a stereomicroscope with top light and black background The intensity of the precipitate was graded It was observed that the antiserum titre was between 1 4096 and 1 8192 and the virus end point was between 1 1024 and 1 2048 (Table 15)

Table 14 Microprecipitin test of bitter gourd mosaic virus with its antiserum

Antiserum dilutions	Dilutions of sap containing bitter gourd mosaic virus													
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192
1/1	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++			
1/2	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	+			
1/4	++++	++++	++++	++++	++++	++++	++++	+++	+++	+++	+			
1/8	++++	++++	++++	++++	++++	+++	+++	+++	+++	+++				
1/16	++++	++++	+++	+++	+++	+++	+++	++	+++	++				
1/32	++++	+++	+++	+++	+++	+++	+++	++	++	++				
1/64	+++	+++	+++	+++	+++	+++	++	+	+	+				
1/128	+++	+++	+++	+++	++	++	+	+	|	|				
1/256	++	+++	++	++	++	+	|	|						
1/512	++	++	+	+	+	|								
1/1024	++	++	|	|	|									
1/2048	+	|												
1/4096	+													
1/8192														

The curved line encloses the area of precipitates visible under microscope

++++ very heavy reaction ++ moderate reaction | Barely visible reaction

+++ heavy reaction + slight reaction No reaction

3 **Ouchterlony's agar double diffusion test**

This test was performed in agarose taken in petri dishes The precipitates formed due to antiserum - antigen interaction were recorded

First plate did not show any band between the wells In this plate, well 2 contained clarified healthy plant sap and well 3 and 5 received distilled water and 4 and 6 contained buffer The central well 1 contained antiserum of bitter gourd mosaic virus

In the second plate, well 2 received pumpkin mosaic virus and 3 with healthy plant sap, 4 with cucumber mosaic virus, 5 with snake gourd mosaic virus and 6 contained distilled water The central well contained antiserum of bitter gourd mosaic virus Dark thick precipitin bands were formed between the wells 1 and 2, and 1 and 4, 1 and 5 (Fig 21)

In the third plate, well 2 received sap from infected bitter gourd plants and the well 4 was filled with snake gourd mosaic virus, 3 with pumpkin mosaic virus, 5 with cucumber mosaic virus and 6 with cowpea mosaic virus The central well 1 contained antiserum of bitter gourd mosaic virus Thin distinct bands were formed between wells 1 and

Fig 21 Well 1 contained antiserum of bitter gourd mosaic virus, well 2 received pumpkin mosaic virus, 3 with healthy plant sap, 4 with cucumber mosaic virus, 5 with snake gourd mosaic virus and 6 contained distilled water

Fig 22 Well 1 contained antiserum of bitter gourd mosaic virus, well 2 received sap from infected bitter gourd plants, well 4 was filled with snake gourd mosaic virus, 3 with pumpkin mosaic virus, 5 with cucumber mosaic virus and 6 with cowpea mosaic virus

Fig 21 Serological reactions of bitter gourd mosaic virus

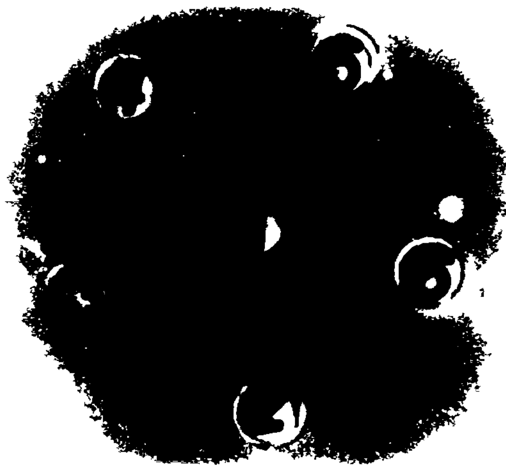


Fig 22 Serological reactions of bitter gourd mosaic virus

2, 1 and 3, 1 and 4 and 1 and 5 All the bands formed were fused together showing that snake gourd mosaic virus, pumpkin mosaic virus and cucumber mosaic virus are serologically related to bitter gourd mosaic virus No band was formed between the wells 1 and 6 indicating that cowpea mosaic virus is not serologically related to bitter gourd mosaic virus (Fig 22)

In the case of plate 4, the well 1 contained sap from infected bitter gourd plants, 2 received cucumber mosaic virus antiserum, 3 with tobacco mosaic virus type strain antiserum, 4 with squash mosaic virus antiserum, 5 with cucumber green mottle mosaic virus antiserum and 6 received bitter gourd mosaic virus antiserum, precipitin band was formed between wells 1 and 6 and no precipitin band was formed between the well 1 and 2, 1 and 3, 1 and 4, 1 and 5 indicating that the antisera obtained from Danish Government Institute of Seed Pathology for developing countries did not contain antibodies specific to bittergourd mosaic virus seen in Kerala

VII Varietal screening

Five varieties and twenty indigenous collections were inoculated mechanically using bitter gourd mosaic virus Observations showed that none of the varieties was found resistant to bitter gourd mosaic virus Appearance of initial

symptoms on the newly emerged leaves varied from 14 to 36 days depending on the variety and the collections Some of them were more susceptible and produced severe symptoms Eventhough all the varieties and indigenous collections were susceptible to the virus there was some variations in the percentage of infection in the inoculated plants Variety Priya produced symptoms 14 days after inoculation and was highly susceptible with 90 per cent infection, followed by 116 A green medium IC 68263, 149 green long IC 68296, K Sona, 42 B green medium IC 45339, 12 B green round IC 44410, 259 A white medium IC 85639, 36 green medium IC 44435, 139 green medium IC 68286 and the per cent infection varied from 80-85 But the variety Arka Harit and the collections 87 green long IC 68234, 61 white medium IC 45358 and 177 green medium IC 68324 were least susceptible with 40 per cent infection Among the above, least susceptible variety and collections Arka Harit, 61 white medium and 177 green medium produced only mild symptoms of bitter gourd mosaic virus disease (Table 15)

Table 15 Incidence of bitter gourd mosaic disease on different varieties and collections

Sl. No	Varieties	Number of plants infected / Number of plants inoculated Experiment I	Experiment II	per cent infection	Type of symptom	Time taken for symptom production (days)
1	Arka Harit	4/10	4/10	40	Mild	18
2.	Co-1	7/10	7/10	70	Severe	19
3	Priya	9/10	9/10	90	Severe	14
4	K Sona	8/10	8/10	80	Severe	15
5	MC 84	7/10	7/10	70	Severe	31
6	12B green round IC 44410	8/10	8/10	80	Severe	36
7	36 green medium IC 44435	8/10	8/10	80	Severe	23
8	42B green medium IC 45339	8/10	8/10	80	Severe	25
9	50 green long IC 45346	5/10	5/10	50	Severe	10
10	61 white medium IC 45358	4/10	4/10	40	Mild	21
11	78 B green medium IC 85604	6/10	6/10	60	Severe	35
12.	80 B green medium IC 85605	6/10	6/10	60	Severe	20
13.	87 green long IC 68234	4/10	4/10	40	Severe	34
14	108 green long IC 68255	6/10	6/10	60	Severe	23

Sl No	Varieties	Number of plants infected / Number of plants inoculated Experiment I	Experiment II	per cent infection	Type of symptom	Time taken for symptom production (days)
15	116 A green medium IC 68263	9/10	8/10	85	Severe	20
16	139 green medium IC 68286	8/10	8/10	80	Severe	24
17	149 green long IC 68296	8/10	9/10	85	Severe	24
18	159 green long IC 68306	6/10	6/10	60	Severe	24
19	175 green medium IC 68322	5/10	5/10	75	Severe	33
20	199 A green long IC 85606	8/10	7/10	75	Severe	30
21	202 white medium IC 85608	7/10	7/10	70	Severe	34
22	221 A green medium IC 85616	6/10	6/10	60	Severe	23
23	259 A white medium IC 85639	8/10	8/10	80	Severe	22
24	20 green long very good IC 44418	5/10	5/10	50	Severe	23
25.	177 green medium IC 68324	4/10	4/10	40	Mild	22

VIII. Estimation of loss

1. Effect of bitter gourd mosaic virus infection on the number of leaves

The inoculation of bitter gourd mosaic virus on the bitter gourd plants was found to have much significant reduction on the number of leaves. The mean number of leaves of the inoculated plants were 104 58 where as it was 134 38 for the uninoculated control plants

It was observed that the virus inoculation x period interaction was significant The inoculated plants had significantly lesser number of leaves in each of the period under study (40, 70 and 100 days after planting) The mean number of leaves for the inoculated plants were 53 38, 116 50 and 143 88 respectively, where as the control plants produced as much as 75 63, 145 63 and 181 88 leaves for the respective periods (Table 16)

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Table 16 Effect of infection on bitter gourd by bitter gourd mosaic virus on the number of leaves

(Mean number of leaves)

Variety	Days after planting (P) 40	70	100	I_0 (control)	I_1 (Inoculated)	Mean (v) CD (v) = 10 39
V1	70.75	144 38	175.50	150 42	110 00	130.21
V2	58 25	117 75	150 25	118 33	99 17	108 75

CD (VxP) = 6.19

	40	70	100	Mean (I)
I_0 (control)	75 63	145 63	181 88	134 38
I_1 (Inoculated)	53 38	116 50	143.88	104.58
Mean(P)	64 50	131 06	162.88	

CD (P) = 4.38 CD (IxP) = 6.20 CD (I) = 10.39

2. Effect of bitter gourd mosaic virus infection on the leaf area

The infection of bitter gourd plants with bitter gourd mosaic virus was found to have a significant negative effect on the leaf area of the plant The mean leaf area of the treated plants was 48 53 cm^2 as against a mean area of 86 80 cm^2 for the control plants

The treatments interacted significantly with both period and variety The variety V1 produced leaves with maximum leaf area in uninoculated control plants (91 20 cm^2) as compared to variety V2 (80.40 cm^2). While the variety V2 produced maximum leaf area for the infected plants (49.47 cm^2) as compared to V1 (46 9 cm^2). The difference in the leaf area between 40 and 100 days after planting in the control plants was found to be 39 65 cm^2 while that of treated plants it was only 2 53 cm^2 (Table 17)

Table 17 Effect of infection on bitter gourd by bitter gourd mosaic virus on the leaf area

(Mean leaf area in cm^2)

Variety	Days after planting (P)			I_0 (control)	I_1 (Inoculated)	Mean (v) CD (v) = 2 62
	40	70	100			
V1	56 45	74 60	76 13	91 20	46 98	69.09
V2	50 80	70 70	73 30	80.40	49.47	64.93
				Mean (I)		
I_0 (control)	62 35	96 05	102 00	86.80		
I_1 (Inoculated)	44 90	53 25	47 43	48 53		
Mean(P)	53 63	73 65	74 71			

CD (P) = 2 49 CD (IxP) = 3 52 CD (I) = 2 62

3. Effect of bitter gourd mosaic virus infection on the internodal length

The plants infected with bitter gourd mosaic virus was found to have a significantly reduced internodal length The mean internodal length of the infected plants was 7 80 cm which was less than that of the control plants by 3 12 cm

The virus inoculation x period interaction was found to be significant The inoculated plants had significantly lesser internodal length in each of the period under study (40, 70 and 100 days after planting)

The variety x virus inoculation effect was significant The uninoculated V1 had a higher internodal length of 11.30 cm But it was 10 53 cm for V2. Due to virus infection there was a reduction of 3 97 cm in the internodal length of V1 where as it was only 2 26 cm for V2 (Table 18)

Table 18 Effect of infection on bitter gourd by bitter gourd mosaic virus on the internodal length

(Mean length in cm)

Variety	Days after planting (P) 40	70	100	I_0 (control)	I_1 (Inoculated)	Mean (v)
V1	7 30	9 95	10 70	11 30	7 33	9 32
V2	8 05	9 30	10.85	10.53	8.27	9.40

CD (VxP) 0 17 CD (VxI) = 0.20

	40	70	100	Mean (I)
I_0 (control)	8 50	11.40	12 85	10.92
I_1 (Inoculated)	6.85	7 85	8 70	7.80
Mean(P)	7 68	9 63	10 78	

CD (P) = 0.12 CD (IxP) = 0.17 CD (I) = 0 14

4. Effect of bitter gourd mosaic virus infection on the thickness of vine

It was observed that the inoculation of plants with bitter gourd mosaic virus was found to have significant negative effect on the mean thickness of vine

The interaction of treatment x period was found to be significant for vine thickness There was a considerable

reduction in the thickness of vine in the inoculated plants at 40, 70 and 100 days after planting The mean thickness of vine for the treated plants were 1 20, 1.38 and 1 46 cm, while that of control plants were 1 44, 1 70 and 1 88 cm respectively (Table 19)

Table 19 Effect of infection on bitter gourd by bitter gourd mosaic virus on the thickness of vine

(Mean thickness in cm)

Variety	Days after planting (P) 40	70	100	I_0 (control)	I_1 (Inoculated)	Mean (v) CD(v) = 0 04
V1	1.48	1 67	1 81	1.88	1.42	1.65
V2	1 16	1 41	1 53	1.46	1.27	1 36
				Mean (I)		
I_0 (control)	1 44	1 70	1 88	1.67		
I_1 (Inoculated)	1 20	1 38	1 46	1.35		
Mean(P)	1 32	1 54	1 67			

CD (P) = 0 06 CD (IxP) = 0.086 CD (I) = 0 04

5. Effect of bitter gourd mosaic virus infection on the total length of vine

It was found that the inoculation of bitter gourd mosaic virus on the bitter gourd plants significantly reduced the total length of vine The mean total length of the treated plants was 2 78 M where as 3 23 M for control plants

The variety x treatment interaction was found to be significant The total vine length of the V1 was 3 63 M while V2 had only 2 82 M There was a reduction of 0 61 M in the total vine length of V1 due to the inoculation of virus, where as it was 0 28 M for V2

The treatment x period interaction was also found to be significant with regard to the total length of vine The infected plants had a mean vine length of 3 30 M as against 3 97 M of their control counter parts, at 100 days after planting (Table 20)

Table 20 Effect of ınfectıon on bitter gourd by bitter gourd mosaıc virus on the total length of vıne

(Mean length ın Metre)

Varıety	Days after plantıng (P) 40	70	100	I_0 (control)	I_1 (Inoculated)	Mean (v) CD (v) = 0.10
V1	2 43	3 63	3.93	3 62	3.02	3 32
V2	2 06	2 63	3.34	2.82	2 54	2 68
		CD (VxP) 0.18			CD (VxI) = 0.15	
				Mean (I)		
I_0 (control)	2 40	3 31	3.97	3.23		
I_1 (Inoculated)	2.09	2 95	3 30	2.78		
Mean(P)	2 24	3 13	3 63			

CD (P) = 0.13 CD (IxP) = 0.18 CD (I) = 0.10

6. Effect of bıtter gourd mosaıc vırus ınfectıon on the number of branches

The ınfectıon of plants wıth bıtter gourd mosaıc vırus sıgnıfıcantly reduced the productıon of branches. The inoculated plants produced only a mean number of 6 50 branches where as the control plants produced 9.76 branches.

The varıety x inoculation ınteractıon was found to be sıgnıfıcant The ınoculated plants of V1 and V2 produced a

mean number of 6 and 7 branches where as the control plants produced 10 44 and 9 08 branches respectively (Table 21)

Table 21 Effect of infection on bitter gourd by bitter gourd mosaic virus on the number of branches

(Mean number of branches)

Variety	Days after planting (P)			I_0 (control)	I_1 (Inoculated)	Mean (v)
	40	70	100			
V1	5 98	8 19	10.50	10.44	6.00	8 22
V2	5 88	8 25	10.00	9.08	7 00	8 04
					CD (VxI) = 0 96	
				Mean (I)		
I_0 (control)	7 10	9 69	12.50	9.76		
I_1 (Inoculated)	4.75	6 75	8 00	6.50		
Mean(P)	5 93	8 22	10 25			

CD (P) = 1 1 CD (I) = 0.68

7. Effect of bitter gourd mosaic virus infection on the number of flowers

Male flowers

As far as the number of male flowers produced is concerned, the varieties interacted significantly with the

process of inoculation. The inoculated plants of both varieties had produced significantly a lesser number of male flowers The inoculated plants of variety V1 produced as less as 107 male flowers (the control plants of variety V1 produced 585 male flowers), for V2 it was 105 flowers (the control plants of V2 produced 540 male flowers) the marginal effects of both variety and inoculation were significantly different from each other. The variety V1 produced much higher number of male flowers (531 50 flowers as against 487 50 of V2) The inoculation of virus affected the production of male flowers significantly (Table 22).

Female flowers

The inoculated plants of both varieties had produced a significantly lesser number of female flowers The inoculated plants of variety V1 produced only a mean number 59 50 female flowers where as the control plants produced 75 50 female flowers It was found that a reduction of 16 female flowers were noticed in the case of the inoculated plants The variety V2 inoculated with bitter gourd mosaic virus produced 41 45 female flowers but the control plants produced 48 50 female flowers In this case also a reduction of 7 05 flowers was observed (Table 22)

Table 22 Effect of infection on bitter gourd by bitter gourd mosaic virus on the production of flowers

(Mean number of flowers)

Variety	Male flowers			Female flowers		
	I_0 (control)	I_1 (inoculated)	Mean	I_0 (Control)	I_1 (Inoculated)	Mean
V1	585	478	531.50	78.50	59.50	67 50
V2	540	435	487.50	48.50	41.45	44.98
Mean	562.50	456 50		62.00	50 48	

CD (V) = 13 79 CD (V) = 1.7

CD (I) = 13 79 CD (I) = 1.7

CD (VxI)= 19.78 CD (VxI)= 2.40

8. Effect of bitter gourd mosaic virus infection on the fruit characters of bitter gourd

The results are presented in Table 23

a) Mean number of fruits

The inoculation of virus significantly affected the number of fruits The marginal mean number of the fruits of the inoculated plants (9 75) was significantly lesser than that of control plants (13 25)

b) Mean length of fruits

The marginal mean length of the fruits of the inoculated plants (13.00 cm) was significantly lesser than that of control (15.48 cm).

c) Mean girth of fruits

The results showed that there was significant reduction in the girth of fruits of the inoculated plants than the control The control plants of variety V1 produced much higher girth of fruits (17.52) than the variety V2 (10 58) It was found that there was a reduction of girth of fruits (5.32 cm) in the variety V1 and 1.34 cm in the variety V2 due to virus infection

d) Mean weight of fruits

The inoculated plants of both varieties showed a significant reduction in the mean weight of fruits than the uninoculated plants The control plants of V1 showed a mean fruit weight of 0 26 kg where as the virus inoculated plants of the same variety showed only 0.14 kg. The inoculated plants of V2 produced only a mean fruit weight of 0.12 kg where as control plants produced as much as 0 18 kg. A reduction in the mean fruit weight of 0.12 kg in the variety V1 and 0 06 kg in the variety V2 was observed.

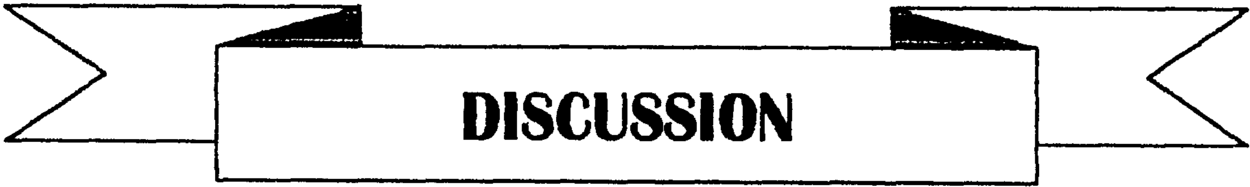
Total yield of fruits

The variety x treatment interaction was significant As far as the control plants are concerned the V1 yielded significantly higher weight of fruits (3 57 kg) as against (2 25 kg) of V2 The fruit yield was found to be reducing significantly by the inoculation of the plants with virus It was reduced by 2 25 kg in the case of V1 and 1 17 kg in the case of V2

Table 23. Effect of bitter gourd mosaic virus infection on fruit characters

Variety	Mean number of fruits			Mean length of fruit (cm)			Mean girth of fruit (cm)			Mean weight of fruit (kg)			Total yield of fruit (kg)		
	I_0 (Control)	I_1 (Inoculated)	Mean	I_0 (Control)	I_1 (Inoculated)	Mean	I_0 (Control)	I_1 (Inoculated)	Mean	I_0 (Control)	I_1 (Inoculated)	Mean	I_0 (Control)	I_1 (Inoculated)	Mean
V_1	14 00	10 50	12.25	17.50	13.90	15 70	17 52	12 20	14 86	0 26	0 14	0 20	3.57	1.32	2.44
V_2	12 50	9.00	12.50	13.45	12 10	12 78	10.58	9 24	9 91	0 18	0.12	0 15	2.25	1 08	1.67
Mean	13 25	9 75		15.48	13 00		14 10	10.72		0 22	0 13		2.91	1.20	

Mean number of fruits	Mean length of fruit	Mean girth of fruit	Mean weight of fruit	Total yield of fruit
CD(V) - 1 94	CD(V) 1.05	CD(V) 0.72	CD(V) 0.02	CD(V) 0 14
CD(I) 1 94	CD(I) 1.05	CD(I) 0 72	CD(I) 0 02	CD(I) 0 14
CD(VxI) = 2 74	CD(VxI) 1 49	CD(VxI) 1 02	CD(VxI) 0 03	CD(VxI) 0 19


DISCUSSION

DISCUSSION

The virus causing mosaic disease of bitter gourd (<u>Momordica</u> <u>charantia</u> L) was investigated The disease was found to be wide spread in Kerala causing severe damage to the crop The initial symptoms appeared as clearing of vein and veinlets followed by mosaic mottling Typical mosaic mottling with dark green and light green patches appeared in all the subsequent leaves The leaves were very much reduced in size and showed filiform shape Diseased plants were stunted and produced only a few flowers and fruits Few of the symptoms of mosaic disease of bitter gourd observed in the present study are in agreement with the findings of Nagarajan and Ramakrishnan (1971a) But the leaves were very much reduced in size and showed filiform shape Such type of symptom was also observed by Shanker <u>et</u> <u>al</u> (1972) while working with pumpkin mosaic virus

The virus was found to be transmissible through sap inoculation It could be transmitted easily, using distilled water as the extraction medium (standard sap) Use of

phosphate buffer as the extraction medium was equally effective as that of distilled water Several fold increase in the infectivity of cucumber mosaic virus with the use of phosphate buffer has been reported by Foster (1972) Shanker et al (1972) while working with pumpkin mosaic virus found that the virus extracted in distilled water gave more percentage of infection when compared with Kirkpatrik and Lindner buffer, phosphate buffer, phosphate ascorbic acid buffer and sodium borate buffer

In the experiment to study the seed transmission of the virus, out of 110 seeds sown 107 seeds have germinated and none of these plants showed the symptoms of bitter gourd mosaic disease This shows that the bitter gourd mosaic virus is not transmissible through seeds of bitter gourd This observation is in agreement with that of Nagarajan and Ramakrishnan (1971a) Shankar et al (1972) also found that pumpkin mosaic virus was not transmitted through seeds According to the serological studies conducted by Dubey and Nariani (1975) snake gourd mosaic virus, cucumber mosaic virus, melon mosaic virus and bitter gourd mosaic are in the group of Cucumis virus 1 Most of the work on viruses grouped under Cucumis virus 1 suggest that they are usually

seed borne But Dubey et al (1974) reported that snake gourd mosaic virus was not seed transmissible But some viruses like cucumber mosaic virus, cucumber green mottle mosaic virus, snake gourd mosaic virus infecting cucurbits are reported to be transmitted through seeds of their respective host plants (Doolittle, 1921; Nelson and Knuhtsen, 1973, Kemp et al , 1974) In the present studies, bitter gourd mosaic virus resembles Cucumis virus 1 as reported by Dubey et al (1974)

The virus could be transmitted through graft inoculation, eventhough there was no proper graft union in the horticultural sense Proper graft union was not obtained probably because of the hollow nature of the stem. Due to this difficulty the reports on attempts of graft transmission in the case of cucurbit viruses are scanty. The present studies revealed that wedge grafting can be done successfully in bitter gourd plants for virus transmission, if the grafting is done at the nodal region Such type of successful graft transmission has not been reported so far in the bitter gourd although Bacillious et al (1969) reported the graft transmission of water melon mosaic virus Umamaheswaran (1985) observed that pumpkin mosaic virus

could be transmitted through graft inoculation Similarly graft transmission of snake gourd mosaic virus was observed by Reghunadhan (1989)

Studies on the insect transmission of bitter gourd mosaic virus was conducted using A gossypii, A malvae, A craccivora, M persicae, H vigintioctopunctata, S biguttula biguttula and B tabaci A gossypii and A malvae were found to transmit bitter gourd mosaic virus in a very efficient manner giving 60 per cent followed by M persicae giving 55 per cent and A craccivora giving only 30 per cent transmission The beetle H vigintioctopunctata, the leaf hopper S biguttula biguttula and the white fly B tabaci failed to transmit the virus

In field conditions generally A gossypii and A. malvae were infesting bitter gourd plants and these insects must be responsible for the spread of the mosaic disease in the field Nagarajan and Ramakrishnan (1971a) found that bitter gourd mosaic virus could be transmitted to healthy bitter gourd plants by the aphids viz , A gossypii, A malvae, A nerii, M persicae and B brassicae Transmission of Cucumis virus 1 causing snake gourd mosaic by the vector

A gossypii was observed by Dubey *et al* (1974) Verma *et al* (1970) found that a severe mosaic of snake gourd was transmitted by *A gossypii* Kaiserh and Danesh (1971) observed that CMV infecting *Cicer arietinum* was transmitted by *A craccivora* in a stylet borne manner In the present investigation *A craccivora* was not found infesting bitter gourd plants but it could transmit bitter gourd mosaic virus *H vigintioctopunctata*, *S biguttula biguttula* and *B tabaci* were not reported to be the vectors of bitter gourd mosaic virus, but these were included in the transmission trials since the infestation of bitter gourd plants by the above insects is common in Kerala

The physical properties of the virus viz , dilution end point (DEP), thermal inactivation point (TIP) and longevity *in vitro* (LIV) were studied It was found that the DEP of the virus was between 1 1000 and 1 10000 and TIP was found between 50 and 60°C Shawkat and Fegla (1979) isolated cucumber mosaic virus from naturally infected egg plant and the DEP was 1 1000 and TIP was 65°C Pillai (1971) found that cucumber mosaic virus (*Cucumis* virus 1) infecting snake gourd had a DEP of 1 10000 and TIP of 60°C The bitter gourd mosaic virus found in Kerala is similar to cucumber mosaic

virus as reported by Shawkat and Fegla (1979) and Pillai (1971) with respect to of DEP and TIP

The LIV of the virus was tested in two temperatures viz , room temperature (28-30°C) and also under refrigeration (10°C) It was found that the LIV of the virus was between 12-24 h at room temperature and 48-72 h under refrigeration Johnson and Grant (1932) observed that the virulence of Cucumis virus 1 infecting different host plants was lost within 24-48 h at room temperature Dubey et al (1974) found that Cucumis virus 1 infecting snake gourd was infective up to 8 days at 8°C But Joseph and Menon (1978) found that the LIV of Cucumis virus 1 infecting snake gourd at 10°C was between 144-168 h

The vector-virus relationship was studied using one of the most efficient vectors, ie , Aphis malvae Acquisition threshold, inoculation threshold, effect of pre-acquisition and post-acquisition fasting of the vector and minimum number of aphids required for virus transmission were investigated

Minimum acquisition feeding period required by A malvae for the transmission of bitter gourd mosaic virus was

found to be 30 s As the acquisition feeding period was increased the per cent transmission was also increased and the maximum transmission of 60 per cent was obtained when acquisition feeding period of 30 min was given As the acquisition feeding period was further increased, the efficiency of transmission of the virus was reduced and it was only 30 per cent when the acquisition feeding period was 2 h (Table 8) In the present studies the minimum inoculation feeding period required by the vector to transmit bitter gourd mosaic virus found to be 1 min (Table 9) The per cent transmission was found to increase with the increase in inoculation feeding period and reached the maximum of 90 per cent when 2 h inoculation feeding period was given As the inoculation feeding period was further increased, the per cent transmission declined and reached 60 per cent with 24 h inoculation feeding period Jaganathan and Ramakrishnan (1971) observed that the viruliferous aphid *A gossypii* acquired the melon mosaic virus with a short acquisition feeding period of 5 s and transmitted with a short inoculation feeding period of 5 s Singh (1981 a) reported that *A gossypii* could transmit pumpkin mosaic virus with minimum acquisition feeding period and inoculation feeding period of 20 s and 10 s respectively In the present studies differences in the minimum acquisition

feeding period and inoculation feeding period of *A malvae* to transmit bitter gourd mosaic virus may be due to the factors viz , efficiency of the vector, type of host, climatic factors etc

Investigations on the influence of pre-acquisition fasting showed that starvation of *A malvae* before acquisition of virus resulted in an increase in the per cent of transmission The increase in per cent transmission was found only up to 2 h pre-acquisition fasting and there after the per cent transmission was decreased The efficiency of the vector to transmit the virus was completely lost when starved for 6 h (Table 11). The increase in the efficiency of *A gossypii* and other aphids in the transmission of many viruses due to pre-acquisition fasting has been reported by many workers (Nagarajan and Ramakrishnan, 1971c&d; Jaganathan and Ramakrishnan, 1971, Singh, 1972, 1981a, and 1982; Joseph and Menon, 1978) Jaganathan and Ramakrishnan (1971) observed that maximum transmission was obtained when *A gossypii* was starved for 60 min The results of the present study also showed a similar trend

Post-acquisition fasting of the vector caused a steady decrease in the per cent transmission of bitter gourd

mosaic virus The efficiency of the vector to transmit the virus was completely lost at 3 h post-acquisition starvation Maximum infection of 60 per cent (Table 11) was obtained when no post-acquisition fasting was given Jaganathan and Ramakrishnan (1971) found that when A gossypii and M persicae were starved post-acquisitionally for more than 5 min there was decrease in the transmission of melon mosaic virus but after a period of 1 h the viruliferous aphids ceased to transmit the virus. Singh (1972) also found that the infectivity of M persicae to transmit the melon mosaic virus was completely lost after 2 h post-acquisition fasting Similarly the efficiency of the vector A craccivora to transmit snake gourd mosaic virus was completely lost when starved for 4 h (Joseph and Menon, 1978) The results of the present study also showed a similar trend

Experiments on retention of infectivity by A. malvae revealed that the vector lost its infectivity after 1 h in all the 6 series of experiments carried out. Thus the virus could not persist inside the vector fo~ long period Non persistent nature of retention of infectivity of mosaic viruses of cucurbits by the vector A gossypii and other

aphids were reported by many workers (Nagarajan and Ramakrishnan, 1979c&d, Jaganathan and Ramakrishnan, 1971; Singh, 1972, 1981a, 1982). Jaganathan and Ramakrishnan (1971) reported that the *A gossypii* retained the infectivity of melon mosaic virus for 1 h But the vector *M. persicae* could retain the infectivity of water melon mosaic virus for 2 h (Singh, 1972) Singh (1981a) also found that *A. gossypii* could retain the infectivity of pumpkin mosaic virus for 2 h Since in the present studies the infectivity was lost after 1 h of acquiring the virus the transmission of bitter gourd mosaic virus by *A malvae* can be termed as non-persistent type as suggested by Nagarajan and Ramakrishnan (1971d) and Singh (1982) Non persistent manner of transmission of snake gourd mosaic virus (*Cucumis* virus 1) by *A gossypii* and *A craccivora* had been reported by (Dubey *et al* , 1974, Joseph and Menon, 1978)

The minimum number of aphids required for successful transmission of bitter gourd mosaic virus was also worked out and it was found that a single viruliferous aphid could cause successful transmission of the virus and the per cent transmission was more when the number of aphids per plant was increased (Table 13) Similar results were also

obtained in the transmission of a strain of water melon mosaic virus by the vector M persicae (Raychaudhuri and Verma, 1977), water melon mosaic virus to watermelon, cucumber, pumpkin and squash by M persicae (Almeida and Borges, 1983) and pumpkin mosaic virus to pumpkin by A gossypii (Singh, 1981a) Maximum infection of 60 per cent was obtained in the present study when 10 viruliferous aphids were used for the transmission of the virus

Studies on host range and local lesion hosts conducted with 68 plant species belonging to 20 families showed that the virus under study was confined to the members of the families Araceae, Cucurbitaceae, Musaceae, Ploygonaceae and Solanaceae It was found that bitter gourd mosaic virus could produce visible systemic symptoms on C melo, C moschata, C metuliferus, L acutangula, C vulgaris, T anguina, Musa sp cv palayankodan, A leptopus, C annuum, N tabacum, N glutinosa, P minima, P minima var indica and T trilobatum and local lesions on C amaranticolor and D stramonium But D metal acted as a symptomless carrier Three ' plant species coming under the family cucurbitaceae viz , C sativus, B hispida, L siceraria were found to be immune to bitter

gourd mosaic virus Nagarajan and Ramakrishnan (1971a) found that a mosaic disease of bitter gourd caused by bitter gourd mosaic virus in Tamilnadu had a narrow host range restricted to cucurbitaceae Verma et al. (1970) while working with Cucumis virus 2 B causing mosaic disease of snake gourd observed that the virus could produce systemic symptoms only on members of the family cucurbitaceae and local lession on C amaranticolor Nagarajan and Ramakrishnan (1971c) observed that host range of water melon mosaic virus was found restricted to cucurbitaceae alone. Similarly Shankar et al (1972) found that the host range of pumpkin mosaic virus was restricted to members of the family cucurbitaceae. But Dubey et al (1974) observed that the host range of the mosaic disease of snake gourd caused by Cucumis virus 1 was restricted to members of the families cucurbitaceae, compositae, solanaceae and chenopodiaceae Among these distinct local lesions were produced on C amaranticolor belonging to chenopodiaceae and in all the hosts belonging to the other three families systemic symptoms were produced. Ghosh and Mukhopadhyay (1979b) found that the cucumber mosaic virus infected cucumber produced local necrotic lesions followed by systematic infection in D stramonium and C amaranticolor Rahimian and Izadpanah (1977) observed

that cucumber green mottle mosaic virus infecting melon plants produced systemic mosaic symptoms in cantaloupe, melon, cucumber and water melon and chlorotic spots on *L acutangula* but squash was found to be immune A comparison of the host range and local lesion hosts of different viruses infecting cucurbits revealed that the virus under study shows a close similarity with *Cucumis* virus 1 as described by Dubey *et al* (1974) and it may not be similar to *Cucumis* virus 2B as reported by Verma *et al* (1970)

Serological studies were conducted with a view to identify the virus The results of the microprecipitin test on slides showed that antigens of bitter gourd mosaic virus isolate I (obtained from inoculated plants in the glass house), bitter gourd mosaic virus isolate II (obtained from diseased plants in the field), cucumber mosaic virus, pumpkin mosaic virus and snake gourd mosaic virus produced dense precipitate with the antiserum specific to bitter gourd mosaic virus This indicates the serological relationship of bitter gourd mosaic virus to the viruses causing cucumber mosaic, pumpkin mosaic and snake gourd mosaic found in Kerala The bitter gourd mosaic virus antigen did not show any serological relationship with any of the antisera of

cucumber mosaic virus, cucumber green mottle mosaic virus, squash mosaic virus and tobacco mosaic virus type strain which were received from Danish Government institute of Seed pathology for developing countries No serological relationship was obtained between bitter gourd mosaic virus and cowpea mosaic virus Microprecipitin test in petri dishes were conduced to find out the antiserum titre and the virus end point of bitter gourd mosaic virus It was found that the antiserum titre was between 1 4096 and 1 8192 and the virus end point was between 1 1024 and 1 2048 Dubey _et al_ (1974) obtained an antiserum titre of 1 2048 and virus and point of 1 4096 for the snake gourd mosaic virus occurring in Delhi The virus end point obtained by them was more due to the use of purified virus preparation for the test In the present investigation the infective sap was subjected to 10000 g for 10 min and the supernatant was taken as the purified antigen so the virus concentration was comparatively lesser According to serological studies conducted by Dubey and Nariani (1975) snake gourd mosaic virus, cucumber mosaic virus, melon mosaic virus and bitter gourd mosaic virus come under the group of _Cucumis_ virus 1 In the present studies, bitter gourd mosaic virus, snake gourd mosaic virus and cucumber mosaic virus reacted positively with the antiserum

of bitter gourd mosaic virus This indicates that bitter gourd mosaic virus disease found in Kerala may be caused by Cucumis virus 1

The results of the Ouchterlony's agar double diffusion test have confirmed the findings of the microprecipitin test on slides The first plate in the experiment, there was no precipitin line between the wells containing antiserum and healthy plant sap This indicated the absence of antibodies against healthy plant sap The second plate contained antiserum in the central well and the surrounding wells contained pumpkin mosaic virus, healthy plant sap, cucumber mosaic virus, snake gourd mosaic virus and distilled water The appearance of precipitin bands between the wells containing antiserum and pumpkin mosaic virus antigen, cucumber mosaic virus antigen and snake gourd mosaic virus antigen indicated the presence of antibodies specific to pumpkin mosaic virus, cucumber mosaic virus and snake gourd mosaic virus in the antiserum The third plate contained antiserum for bitter gourd mosaic virus in the central well and the surrounding wells contained bitter gourd mosaic virus, snake gourd mosaic virus, cucumber mosaic virus, pumpkin mosaic virus and cowpea mosaic virus

Percipitin bands were formed between the wells containing antiserum and bitter gourd mosaic virus, snake gourd mosaic virus, cucumber mosaic virus and pumpkin mosaic virus and the fusion of all four bands inducated that all the four viruses were serologically very much related (Fig 22) It is a well established fact that the fusion of precipitin lines shows the identical nature of antigens (Noordam, 1973) The absence of precipitin band between the antiserum well and the well containing cowpea mosaic virus antigen indicated that the antiserum did not contain antibodies against cowpea mosaic virus and thus this virus is not serologically related to bitter gourd mosaic virus In the forth plate central well contained bitter gourd mosaic virus antigen and the surrounding wells contained antisera of cucumber mosaic virus, cucumber green mottle mosaic virus, bitter gourd mosaic virus, squash mosaic virus and tobacco mosaic virus type strain and bitter gourd mosaic virus In this plate, a precipitin band formed between the wells containing bitter gourd mosaic virus antigen and antiserum of bitter gourd mosaic virus The absence of any precipitin bands between the wells containing bitter gourd mosaic virus antigen and other four antisera indicated that they are not related It is confirmed that bitter gourd mosaic virus seen in Kerala is

not serologically related to the viruses seen in Denmark namely, cucumber mosaic virus, cucumber green mottle mosaic virus, squash mosaic virus and tobacco mosaic virus type strain

In the varietal screening trial carried out with 5 varieties and 20 indigenous collections it was found that all the varieties and collections were susceptible to bitter gourd mosaic virus and in all the cases symptoms of bitter gourd mosaic virus disease appeared within 14-36 days after inoculation Even though all the varieties and collections were susceptible to the virus there was some variations in the percentage of infection in the inoculated plants The variety priya gave 90 per cent infection and the indigenous collections 116 A green medium IC68263, 149 green long IC68296, 42B green medium IC 45339, 12B green round IC 44410, 259 A white medium IC 85639, 36 green medium IC44435 and the variety K sona gave 80-85 per cent infection, whereas the variety Arka Harit and the collections 87 green long IC68234, 61 white medium IC45358 and 177 green medium IC68324 were least susceptible with 40 per cent infection Sowell and Demski (1969) while working with water melon mosaic virus 2 found that none of the water melon varieties tested were

immune to the virus Moskovets and Fegla (1972) also arrived at a similar conclusion and reported that all the water melon and pumpkin varieties tested were susceptible to water melon mosaic virus

In the present study among the least susceptible variety and collections Arka Harit, 61 white medium IC45358 and 177 green medium IC 68324 produced mild symptoms of bitter gourd mosaic virus disease, which is in agreement with the findings of Sharma and Sharma (1982) According to them some of the genotypes of summer squash produced mild symptoms of mosaic due to the infection with Cucumis virus 1

In the experiment to find out the effect of bitter gourd mosaic virus on the growth and yield of bitter gourd plants, two varieties (Priya and indigenous collection IC 68324) and two treatments namely inoculated and uninoculated (control) were included It was found that there was significant reduction in the number of leaves and leaf area in both the varieties inoculated with virus The mean number of leaves of the inoculated plants was significantly lesser than that of control plants at 40, 70 and 100 days after planting The mean leaf area of the inoculated plants was

48 38 cm^2 but the uninoculated plants had 86 80cm^2 Due to virus infection the variety V_1 showed a reduction of 3 97 cm in the mean internodal length but the variety V_2 had only a reduction of 2 26 cm This variation may be due to varietal differences Raghunadhan (1989) while studying with snake gourd mosaic virus also found that the plants infected at the early stage of growth, there was significant reduction in the number of leaves, leaf area and internodal length The results of the present study also agree with findings of Singh (1986) with respect to internodal length

It was observed that the bitter gourd plants inoculated with virus at the early stage, there was significant reduction in the vine thickness, number of branches formed and total length of vine in both varieties The variety V_1 showed a maximum reduction of 0 61 M in the mean length of vine, but the V_2 had a reduction of only 0 28 M This implies that the variety V_1 may be highly susceptible to virus than V_2 Jayasree (1984) found that yellow vein mosaic disease of pumpkin produced significant reduction in the number of leaves, size of leaves, internodal length and total length of vines. The results of the present investigation also agree with above the findings

The number of flowers (Male and Female) produced in the inoculated plants was significantly lesser than the control plants Pillai (1971) while investigating mosaic disease of snake gourd observed that the disease affected plants produced fewer flowers and fruits Dubey _et al_ (1974) also noticed similar type of findings In general there was considerable reduction in all the fruit characters viz , number of fruits, length of fruits, girth of fruits, weight of fruits and yield of fruits in both the varieties inoculated with virus when compared to the uninoculated plants The varieties V_1 and V_2 showed a reduction of 2 25 and 1.17 kg of yield respectively due to virus infection than the control plants Karchi _et al_ (1978) found that susceptible cantaloupe (_Cucumis melo_) cvs. Noy Yizre'el infected with CMV in an early stage, reduced the yield by 73 per cent and tolerent x_v-140 by 31 per cent The results of the present study also showed a similar trend

SUMMARY

SUMMARY

Mosaic disease of bitter gourd (*Momordica charantia* L) prevalent in Kerala was investigated

The initial symptom appeared as clearing of vein and veinlets followed by mosaic mottling 12 - 14 days after mechanical inoculation Typical mosaic patches with dark green and light green blisters were produced in all the subsequent leaves In some cases leaves had a large area of light green patches and the leaves were very much reduced in size and showed filiform shape Diseased plants remained stunted and produced only a few flowers and fruits

Transmission studies showed that the virus could be transmitted through mechanical means Sap extracted in distilled water and phosphate buffer gave maximum infection of 90 per cent while in tris buffer gave the minimum infection of 55 per cent When infected shoots were wedge grafted to 30 days old healthy plants produced the symptoms 12 - 14 days after grafting

Insect transmission studies of the virus were carried out using 7 vectors, viz , *Aphis craccivora*, *Aphis gossypii*, *Aphis malvae*, *Myzus persicae*, *Henosepilachna Vigintioctopunctata*, *Sundapteryx biguttula biguttula* and *Bemisia tabaci* Observations showed that the highest percentage of transmission was obtained with *A gossypii* and *A malvae* (60 %) *M persicae* (55%) and *A craccivora* (30%) The beetle *H vigintioctopunctata*, the leaf hopper *S biguttula biguttula* and the white fly *B tabaci* could not transmit the virus Studies on seed transmission revealed that the virus was not transmitted through seeds

The results on the physical properties of the virus showed that the dilution end point of the virus was between 1 1000 and 1 10000 and thermal inactivation point of the virus was between 50 and 60°c Longevity *in vitro* was between 12 and 24 h at room temperature (28 - 30°c) and between 48 and 72 h at 10°c

Studies on vector-virus relationships showed that the minimum acquistion feeding period required for the vector (*A malvae*) to acquire the virus was 30s and the virus could be transmitted with a minimum inoculation feeding period of 1

carrier of bitter gourd mosaic virus Three plant species coming under the family cucurbitaceae viz , Cucumis sativus, Benincasa hispida, Lagenaria siceraria were found to be immune to bitter gourd mosaic virus

Serological studies were conducted with a view to identify the virus The results of the microprecipitin test on slides showed that antigens of bitter gourd mosaic virus isolate I (obtained from plants in the green house), bitter gourd mosaic virus isolate II (obtained from diseased plants in the field), cucumber mosaic virus, pumpkin mosaic virus and snake gourd mosaic virus produced dense precipitate with the antiserum specific to bitter gourd mosaic virus This indicated the serological relationship of bitter gourd mosaic virus to the viruses causing cucumber mosaic, pumpkin mosaic and snake gourd mosaic found in Kerala No Serological relationship was obtained between bitter groud mosaic virus and cowpea mosaic virus The antiserum titre and virus end point in the present study were found to be between 1 4096 and 1 8192 and 1 1024 and 1 2048 respectively Serogical studies revealed that the antisera of cucumber mosaic virus, cucumber green mottle mosaic virus, squash mosaic virus and tobacco mosaic virus type strain received from Denmark did

not contain antibodies specific to bitter gourd mosaic virus which indicated that the above four viruses were not related to bitter gourd mosaic virus

Based on the results on transmission, physical properties, host range and serological properties, the bitter gourd mosaic virus under study was identified as <u>Cucumis</u> virus 1

The varietal screening carried out with five varieties and twenty indigenous collections of bitter gourd revealed that all the varieties and collections were susceptible to bitter gourd mosaic virus Among these the variety Priya was highly susceptible But the variety Arka Harit and the collections 87 green long IC 68234, 61 white medium IC 45358 and 177 green medium IC 68324 were least susceptible

The infection of bitter gourd plant at the early stage by bitter gourd mosaic virus resulted significant reduction in the number of leaves, leaf area, internodal length, thickness of vines, length of vines, number of branches, number of flowers, number of fruits and other fruit characters viz , length of fruits, girth of fruits, weight of fruits and total yield of fruits

REFERENCES

REFERENCES

Adlerz, W C (1972) Momordica charantia as a source of water melon mosaic virus -1 for cucurbit crops in Palm beach country, Florida Plant Dis 57 563-564

Ahmed, A H (1981) Occurrence of water melon mosaic virus in Sudan TPM 27 279-281

Allen, T C and Fernald, E K (1971) Recovery and partial characterisation of wild cucumber mosaic virus from Marah oreganus Plant Dis Rep 55 546-550

Almeida, M M and Borges, M (1983) Water melon mosaic virus in Portugal Characteristics, Serology, Ultra structural aspects of the host Revista de Biologia 12 327-338

Alvarez, M and Campbell, R N (1976) Influence of squash mosaic virus on yield and quality of cantaloupes Plant Dis Rep 60 636-639

Arteaga, M P L , Quiot, J B and Leroux, J P (1976) Detection of a strain of water melon mosaic virus II (WMV-II) in south eastern France Ann Phytopathol 8 347-353

Auger, J G , Escaffi, O and Nome, F S (1974) Occurrence of water melon mosaic virus 2 on cucurbits in Chile plant Dis Rep 58 599-602

Bakker, W (1971) Notes on East African plant virus diseases II Courgette leaf distortion incited by Water melon virus East Afr Agric For J 37 78-85

Basillious, H A , Soliman, A A and Nour-Eldin, F (1969) Studies of a squash mosaic virus disease in U A R I Properties of the causal virus Agric Res Rev Cairo 47 91-104

Bercks, R , Koenig, R and Querfurth, G (1972) Plant virus Serology In Principles and techniques in plant virology Kado, C I and Agrawal, H O (Eds), pp 446-490, Van Nostrand Reinhold Company, New York

Bhargava, B (1976) Effect of ageing on the activity of water melon mosaic virus under varying conditions Z Pflanzen Physiol 83 611-614

Bhargava, B (1977) Effect of water melon mosaic virus on the yield of Cucurbita pepo Acta Phytopathol Acad Sci Hung 12 165-168

Bhargava, K S and Tewari, J P (1970) Trichosanthes dioica, an additional natural host of water melon mosaic virus Plant Dis Rep 54 727-728

Bishnoi, S S , Jagadish, B and Narayan Rishi (1985) Studies on mosaic disease of summer squash (Cucurbita pepo L) in Haryana Indian J Virol 1 139-142

Brunt, A A (1966) Partial purification, morphology and Serology of Dahlia mosaic virus Virology 28 778-779

Capoor, S P and Ahmad, R V (1976) Yellow vein mosaic disease of field pumpkin and its relationship with the vector Bemisia tabaci Indian Phytopath 28 241-246

Chang, M V and Lee, C V (1980) A virus disease of sesame (Sesamum indicum L) caused by water melon mosaic virus (WMV) Korean J Plant Prot 19 193-198

Chen, Y X and Wei, C T (1959) A preliminary study on the identity of mosaic virus from cucurbits in the vicinity of Vanking Acta Phytopathol Sinica 5 1-6

Chen, Z Y , Zhun, B M and Yi, J H (1982) Studies on water melon mosaic disease Shanghai Agric Sci Tech 1 23

Costa, A S (1944) Quantitative studies with carborundum and its use in local lesion Phytopathology 34 228-300

Demski, J W and Chalkley, I H (1974) Influence of water melon mosaic virus on water melon Plant Dis Rep 58 195-198

Dikova, B , Kaitazova, P and Markov, M P (1983) Water melon mosaic virus on cucumber Gradinar Lozar 21 66-73

Demski, J W and Sowell, G (1970) Susceptibility of Cucurbita pepo and Citrullus lanatus introductions to water melon mosaic virus 2 Plant Dis Rep 54 880-881

Doolittle, S P (1920) The mosaic disease of cucurbits U S Dep Agric Bull 879

Doolittle, S P (1921) The relation of wild host plants to the over wintering of cucurbit mosaic Phytopathology (Abstr) 11 47

Dubey G S and Nariani, T K (1975) Serological reactions of ten cucurbit virus isolates collected from Delhi Indian Phytopath 28 118-119

Dubey, G S , Nariani, T K and Prakash, N (1974) Identification of snake gourd mosaic virus Indian Phytopath 27 470-474

Ehara, Y and Misawa, T (1975) Studies on the infection by cucumber mosaic virus VIII Effect of temperature on the virus spreading and the size of local lesions Ann Phytopath Soc Japan 41 15-23

Filigarova, M (1982) Purification of Arabis mosaic virus Sharnik UVTIZ - Ochana Rostlin 18 241-246

Fischer, H V and Lockhart, B E L (1974) Serious losses in cucurbits caused by water melon mosaic virus in Moroco Plant Dis Rep 58 143-146

Forghani, B , Sanger, H L and Crossmann, F (1966) Virus on pumpkin Nachr Bl dt Pfl Schutz Stultg 18· 113-116

Foster, R E (1972) Cucumber mosaic virus buffering affects Chenopodium amaranticolor response Plant Dis Rep 56 443-445

Ghosh, S K and Mukhopadhyay, S (1979a) Viruses of pumpkin (Cucurbita moschata) in West Bengal Phytopath Z 94 172-184

Ghosh, S K and Mukhopadhyay, S (1979b) Incidence of squirting cucumber mosaic virus of cucumber in West Bengal Curr Sci 48 788

Gibbs, A J , Nixon, H L and Woods, R D (1963) The relationship between barley stripe mosaic and Lychnis ring spot virus Virology 20 194-198

Goel, R K , Varma, J P (1973) Mosaic diseases of ridge gourd (Luffa acutangula Roxb) in Haryana Haryana Agric Univ J Res 3 135-144

Greber, R S (1969) Viruses infecting cucurbits in Queensland Queensl J Agric Anim Sci 26 145-171

Greber, R S (1978) Water melon mosaic virus 1 and 2 in Queensland cucurbit crops Aust J Agric Res 29 1235-1245

Halliwell, R S , Johnson, J. and Cotner, S. (1979) Water melon mosaic virus disease of squash, water melon and pumpkin _Misc Publ Texas Agric Exp Stn_ No 1435 5 pp

Hani, A and Pelet, F (1970) Cucumber mosaic virus in the Tessin _Rev Suisse Vitic Arboric_ 2· 136-139

Hariharasubramanian, V and Badami, R S (1964) A virus disease of cucurbits from India _Phytopath Z_ 51 274-279

Hebert, T T (1963) Precipitation of plant viruses by polyethylene glycol _Phytopathology_ 53 362

Hein, A (1977) Occurrence of water melon mosaic virus 1 on zucchini (_Cucurbita pepo_ L var giromontina Alef) in South Germany _Phytopath Z_ 221-228

Hills, O H , Coudriet, D L , Brubaker, R W and Keener, P D (1961) Effect of curly top and cucumber mosaic viruses on cantaloupes. _J Econ Ent_ 54 970-972

*Horvarth, J (1985) New artificial host virus relations between cucurbitaceous plants and virus IV _Acta phytopathologia Academiae Scientiarum Hungaricae_ 20 273-281

*Ignash, Y R (1977) Comparitive characteristics of some representatives of the cucumber mosaic virus group isolated in the Latvian SSR , _Tr Biol Pochven Inst Nauch Tsentr An SSSR_ 46 193-197

Jaganathan, T and Ramakrishnan, K (1971) Studies on virus diseases of cucurbits Madras Agric J 58 331-337

Jayasree, P K (1984) Yellow vein mosaic disease of pumpkin in Kerala M Sc (Ag) Thesis Kerala Agricultural University 74 pp

Johnson, J and Grant, T J (1932) The properties of plant viruses from different host species Phytopathology 22 741-757

Jones, P , Angood, S B and Carpender, J M (1986) Melon rugose mosaic virus, the cause of a disease of water melon and sweet melon Ann Appl Biol 108 303-307

Joseph, P J and Menon, M R (1978) Studies on the mosaic disease of snake gourd (Trichosanthes anguina L) Agric Res J Kerala 16 148-154

Joseph, P J and Menon, M R (1981) Effect of a strain of a cucumber mosaic virus on growth and yield of snake gourd Madras Agric J 67 65-66

Joshi, R D and Dubey, L N (1976) Some weed reservoirs of cucumber mosaic virus in Gorakhpur and adjacent areas Indian phytopath 28 568

Kaiser, W J and Danesh, D (1971) Biology of four viruses affecting Cicer arietinum in Iran Phytopathology 61 372-375

Karachi, Z , Cohen, S , Govers, A and Yoseph, R (1978) Response of susceptible and resistant cantaloupes (Cucumis melo) to cucumber mosaic virus under field conditions Phytoparasitica 6 9-13

*Karl, E (1981) Investigations on the infection of a test plant series with turnip mosaic virus or watermelon mosaic virus 1 by several aphid species Phytopathologie und pflanzenschutz 17 325-332

Kemp, W G , Wiebe J and Troup, P A (1974) Occurrence of squash mosaic virus in muskmelon seeds available in Ontario in 1973 Canadian plant Dis Survey 54 43-44

Kerala Agricultural University, (1993) Package of practices Recommendations 1993 Director of Extension, Mannuthy, 680651, Kerala, India, pp 237

Komuro Y (1957) Studies on a mosaic disease of squash in Japan Ann Phytopath Soc Japan 21 162-166

Latera, J R , Acosta J M and Uzcatigui, R D E (1975) Cucumis meuliferus as a local lesion host for squash mosaic virus Plant Dis Rep 4 205-212

Lastra R (1968) Occurrence of cucurbit viruses in Venezuela Plant Dis Rep 52 171-174

Linderberg, G D , Hall, D H and Walker, J C (1956) A study of melon and squash viruses Phytopathology 46 489-495

Lockhart, B E L , Ferji, Z and Hafidi, B (1982) Squash mosaic virus in Morocco Plant Dis 66 1191-1193

Lot, H , Marrou, J , Quiot, J B and Esvan, C (1972) Contribution to the study of cucumber mosaic virus II Rapid method of virus prufication Ann phytopath 4 25-38

Makkouk, K M and Lesemann, D E (1980) A severe mosaic of cucumber in Lebanon caused by water melon mosaic virus 1 Plant Dis 64 799-801

*Maluf, W F , Moura, W DEM , Silva, I S DA and Caskelo-Branco, M (1986) Screening of Cucurbita spp. accessions for resistance to watermelon mosaic virus-1 Revista Brasileira de Genetica 9 161-167

Magee, C J P (1940) Transmission of infectious chlorosis or heart-rot of the Banana and its relationship to cucumber mosaic J Aust Inst Agric Sci 6 44-47

March, G J and Nome, S F (1973) Squash (Cucurbita moschata Duch) mosaic virus in Argentina Argentina Revista de Investigaciones Agropecuaria 10 319-332

Miline, K S and Grogan, R C (1969) Characterization of watermelon mosaic virus strains by serology and other properties Phytopathology 50 809-818

*Moskovets, S U and Fegla, G I. (1972) Effect of water melon mosaic virus on the growth of cucurbits Mikrobiologichnyi Zhurnal 34 240-244

Nagarajan, K and Ramakrishnan, K (1971a) Studies on cucurbit viruses in Madras State I A new virus disease on bitter gourd (Momordica charantia L) Proc Indian Acad Sci B 73· 30-35

Nagarajan, K and Ramakrishnan, K (1971b) Studies on Cucurbit viruses in Madras State III Watermelon mosaic virus on snake gourd (Trichosanthes anguina L) J Indian Bot Soc 50 25-30

Nagarajan, K and Ramakrishnan, K (1971c) Studies on Cucurbit viruses in Madras State II vector - virus relationship of the bitter gourd mosaic virus Proc Indian Acad. Sci Sect B 73 84-95

Nagarajan, K and Ramakrishnan, K (1971d) Studies on Cucurbit viruses in Madras State IV Some aspects of the relationships of melon mosaic virus strain to its three aphid vectors Proc Indian Acad Sci Sect 74 194-207

Nagarajan, K and Ramakrishnan, K. (1975) Studies on Cucurbit viruses in Madras State V Strain of melon mosaic virus on Cucurbita lundelliana Bailg Madras Agric J 62 57-60.

Nagaraju and Reddy, H R (1983) Occurrence of cucumber mosaic virus in bell pepper Indian J Mycol Plant Pathol 12 217-219

Nelson, M R (1962) Effect of mosaic viruses on cantaloupes _Phytopathology_ (Abstr) 52 363

Nelson, M R and Knuhtsen, H K (1973) Squash mosaic virus variability epidemiological consequences of differences in seed transmission frequency between strains _Phytopathology_ 63 918-920

Nene, Y L (1972) A survey of viral diseases of pulse crops in Uttar Pradesh _Final Technical Report_, G B P U A T , Pantnagar, pp 191

Noordam, D (1973) _Identification of Pant viruses Methods and Experiments_ Oxford and IBH publishing Co pp 154

Omar, R A , EL-Khadem, M and Scdarous, S A (1980) Purification of cucumber mosaic virus (CMV) and preparation of diagnostic serum _Egyptian J Phytopathol_ 12 1-12

*Pejcinovski, F (1978) Cucumber mosaic virus on cucurbitaceae cultivated in Macedonia _Godisen Zemjodelskiot Fakultet na Univerzitetot vo skopje Zbornik na_ 28 97-116

Pillai, N G (1971) A mosaic disease of snake gourd (_Trichosanthes anguina_ L) _Sci_ & _Cult_ 37 46-47

Pitrat, M and Dumas de Vaulx, R (1979) Search for sources of resistance to powdery mildew and cucumber mosaic and watermelon mosaic viruses among _Cucurbita_ species _Planter_ 29 439-445

Powell, C C and Schlegel, D E (1970) Factors influencing seed transmission of squash mosaic virus in Cantaloupe Phytopathology 60 1466-1469

Provvidenti, R and Robinson, R W (1974) Resistance to squash mosaic virus and water melon mosaic virus 1 in Cucumis metuliferus Plant Dis Rep 58 735-738

Provvidenti, R , Robinson, R W and Munger, H M (1978) Resistance in feral species to six viruses infecting Cucurbita Plant Dis Rep 62 326-329

Quiot, J B , Kaan, F and Bermis, M (1971) Identification of a water melon mosaic virus I in the French West Indies Ann Phytopath 3 125-130

Qureshi, M A and Mayee, C D (1980) Characterisation of a virus inciting mosaic in Lagenaria siceraria in Maharashtra Indian phytopath 32 360-364

Raghunadhan, D (1989) Transmission, properties and host range of snake gourd mosaic virus MSc (Ag) thesis Kerala Agricultural University 121 pp

Rahimian, H and Izadpanah, K (1977) A new strain of cucumber green mottle mosaic virus from Iran. Iranian J Agric Res 5 25-34

Rao, D R and Roychaudhuri, S P (1977) Chenopodium murale a local lesion host for cucumber mosaic virus (CMV) isolates from Vinca rosea Curr Sci 46. 539

Raychaudhuri, M and Varma, A (1977) Vector-virus relationship of marrow mosaic virus with Myzus persicae Sulz Indian J Ent 37 247-250

Raychaudhuri, M and Varma, A (1978) Mosaic disease of muskmelon, caused by a minor variant of cucumber green mottle mosaic virus Phytopath Z 93 120-125

*Rizk, G N , Shawkat, A L B and Ahmad, K G (1981) Ecological studies on aphid vectors of water melon mosaic virus 2 on squash plants in Iraq Mespotamia J Agric 16 163-172

Roy, S and Mukhopadhyay, I (1980) Epidemiology of virus disease of pumpkin in West Bengal Indian phytopath 32 207-209

Sako, N , Okumura, T and Nonaka, F (1976) Aphid transmission of water melon mosaic irus. Agric Bull Saga Univ 42· 1-8

Sarjeet Singh (1981) Datura leaf distortion mosaic virus caused by a strain of Cucumber mosaic virus Indian phytopath 34· 92-94

Shankar, G , Nariani, T K and Prakash, N (1969) Detection and identification of cucurbit viruses by electron microscopy and serology Proc Int Symp Electron Microscopy in life Science Calcutta 27-28

Shankar, G , Narıanı, T K and Prakash, N (1972). Studies on Pumpkın mosaıc vırus (PMV) with particular reference to purıfıcatıon, electron mıcroscopy and serology Indian J Microbiol 12 154-165

Shanmugasundram, S Ishıı, M , Gılbert, J C and Nagaı, H (1969) Cucubıt vırus studıes ın Hawaıı Plant Dis Rep 53 70-74

Sharma, O P , Khatrı, H L , Bansal, R D and Komal, H S (1984) A new straın of cucumber mosaıc vırus causıng mosaıc dısease of muskmelon Phytopath Z 109 382-340.

Sharma, Y R and Chohan, J S (1973) Transmıssıon of Cucumıs vırus 1 and 3 through seeds of cucurbıts Indıan Phytopath 26 596-598

Shawkat, A L B and Fegla, G I. (1979) Identıfıcatıon of two vıruses from egg plant and Cucurbıta pepo ın Iraq Plant Dıs Rep. 63 235-238

Sharma, O P and Sharma, B R (1982) Note on fıeld evaluatıon of dıfferent genotypes of summer squash agınst vegetable marrow mosaıc vırus Indıan J Agrıc Scı 52 192-193

Shohara, K and Osakı, T (1974) Precıpıtatıon and purıfıcatıon of cucumber mosaıc vırus by polyethylene glycol (PEG) and reverse concentratıon by PEG gradıent centrıfugatıon Ann Phytopath Soc Japan 4 265-267

Shukla, T N and Singh, R A (1971) Transmission of Cucumis virus 2D by root inoculation Sci & Cult 37 295-296

Singh, J S and Mandahar, C L (1971) Primary productivity of three vegetable crops as influenced by virus infection Trop Ecol 12 223-227

Singh, R (1972) Relationship of watermelon mosaic virus strains with its vector Myzus persicae Sulz Phytopathol Mediterr 11 189-192

Singh, R A and Dey, S K (1976) Loss assessment due to bottle gourd mosaic Haryana J Hort Sci 5 57-59

*Singh, S J (1981a) Relationship of pumpkin mosaic virus with its aphid vector Aphis gossypii Glov J Turkish Phytopath 10 93-109

Singh, S J (1981b) Studies on a virus causing mosaic disease of pumpkin (Cucurbita maxima Duch) Phytopathol Mediterr. 20 184-188

Singh, S I (1982) Studies on the transmission of pumpkin mosaic virus by aphid (Aphis craccivora Koch) Z Pflanzen Physiol 89 79-87

Singh, S J (1986) Assessment of losses caused by a strain of watermelon mosaic virus in pumpkin (Cucurbita maxima Duch) Indian J Plant Pathol 4 165-167

Singh, R S (1987) Diseases of Vegetable crops Oxford and IBH publishing Co pp 362

Singh, S J (1989) Effect of watermelon mosaic virus on growth and yield of bottle gourd (Lagenaria siceraria) Indian J virol 5 73-78

Snedecor, G W and Cochran, W G. (1967) Statistical methods Oxford and IBH Publishing Co pp 593

Sowell, G and Demski, J W (1969) Susceptibility of water melon cultivars to water melon mosaic virus 2 Plant Dis Rep 53 208-209

Steere, R L (1956) Purification and properties of tobacco ring spot virus Phytopathology 46 60-69

Tewari, J P (1976) Zinnia elegans Jacq The latent host of water melon mosaic virus Curr Sci 45 37

Thomas, W (1971) Watermelon mosaic virus, a disease of cucurbits in New Zealand The incidence and economic importance of watermelon mosaic virus. New Zaland J Agric Res 14 235-247

Thomas, W (1973) Seed transmitted sqush mosaic virus New Zealand J Agric Res 16 235-297

Thomas, W (1980) Water melon mosaic virus the cause of serious disease of cucurbits in the coale Islands New Zealand J Exp Agric 8 309-312

Toba, H H (1962) Studies on the host range of water melon mosaic virus in Hawaii Plant Dis Rep 46 409-410

Tomlinson, J A and Carter, A L (1970) Studies on the seed transmission of cucumber mosaic virus in chick weed (Stellaria media), in relation to the etiology of the virus Ann Appl. Biol. 66 381-386

Tripathi, G and Joshi, R D (1985) Watermelon mosaic virus in Pumpkin (Cucurbita maxima). Indian Phytopath. 38 244-247

Ullman, D E , Cho, J J and German, T.L. (1991). Occurrence and distribution of cucurbit viruses in the Hawaiian Islands Plant Dis 75: 367-370

Umamaheswaran, K (1985) Transmission, Physical properties and host range of pumpkin mosaic virus M Sc (Ag) thesis Kerala Agricultural University 99 pp

Uppal, B N (1933) India· disease in the Bombay Presidency Internat Bull. of plant protect. 7: 103-104

Van Kammen, A (1967) Purification and properties of the Components of cowpea mosaic virus. Virology 31: 633-642

Vasudeva, R S , Raychudhuri, S P and Singh, J. (1949). A new strain of cucumis virus-2 Indian Phytopath. 2: 180-185

Verma, G S , Verma, H N , Rani, S , Verma, V.K. and Hajela, O P (1970) New record of virus diseases of some cucurbits Indian J Agric Sci. 40: 556-558.

Weber, I , Proll, E , Ostermann, W.D , Leiser, R M , Stamarius, A and kegler, H (1982) Characterisation of cucumber leaf spot virus, a virus unknown so far in glass house cucumber (Cucumis sativus L) Archiv fur Phytopathologie Und Pflanzenschutz 18 137-154

Wetter, C (1960) Partial purification of some elongated plant viruses Arch Mikrobiol 37 278-298

Wyman, J A (1979) Acyrthosiphon Kondoi a new vector of cucurbit viruses in Southern California Environ Entmol 8 549-551

Zabla, S and Ramallo, J C (1969) The mosaic of cucurbitaceae Rev Agron Noroeste Argent 6 197-208

Zitter, T A and Tsai, J H (1979) Transmission of three poty virus by the leaf miner, Liriomyza sativae (Diptera Agromyzidae) Plant Dis Rep 61 1025-1029

*   Original not seen

APPENDIX

Appendix - I

Amidoblack stain for precipitin lines

Amidoblack 10B	-	1 g
Sodium acetate acetic acid buffer 0 2M, pH 3 6	-	1000 ml

Decolorizer No.1

Methyl alcohol	-	45 parts
Glacial acetic acid	-	10 parts
Distilled water	-	50 parts

Decolorizer No. 2

Ethyl alcohol (Absolute)	-	40 parts
Glacial acetic acid	-	10 parts
Distilled water	-	50 parts

INVESTIGATIONS ON MOSAIC DISEASE OF BITTER GOURD

By

PURUSHOTHAMAN, S. M.

ABSTRACT OF THE THESIS
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ABSTRACT

Bitter gourd mosaic virus disease commonly occurring in Kerala was investigated The symptoms appeared as typical mosaic mottling with dark green and light green patches Diseased plants were severely stunted and produced only a few flowers and fruits

Transmission studies showed that the virus could be transmitted through mechanical means, grafting and by aphid vectors The virus was found to be transmitted by the aphids *Aphis gossypii* Glov , *Aphis malvae* Koch , *Myzus persicae* Sclz and *Aphis craccivora* Koch Among the four species of aphids, *A gossypii* and *A malvae* were found to be the most efficient vectors

Investigations on the physical properties of the virus revealed that the virus had a thermal inactivation point between 50 and 60°C, dilution end point between 1 1000 and 1 10000, longevity *in vitro* between 12 and 24 h at room temperature (28 - 30°C) and 48 and 72 h at 10°c

The minimum acquisition feeding and inoculation feeding period of the vector _A malvae_ were found to be 30s and 1 min respectively But the percentage of transmission was maximum when an acquisition feeding period of 30 min and inoculation feeding period of 2 h were given

Influence of starvation before acquisition and inoculation feeding period proved that pre-acquisition starvation for 2 h produced maximum infection but post-acquisition starvation decreased the per cent infection The vector could retain the virus for 1 h only A single aphid could transmit the virus to healthy test plants, but maximum percentage of transmission was obtained with 10 aphids

Host range studies showed that the virus was restricted to the members of the family Araceae, Chenopodiaceae, Cucurbitaceae, Musaceae, Polygonaceae and Solanaceae _Datura metel_ acted as a symptomless carrier of bitter gourd mosaic virus

Serological studies showed that bitter gourd mosaic virus is related to cucumber mosaic virus, pumpkin mosaic virus and snake gourd mosaic virus

Varietal screening showed that all the varieties and collections were susceptible to biter gourd mosaic virus infection, but the variety Arka Harit and the collections 87 green long IC 68234, 61 white medium IC 45358 and 177 green medium IC 68324 were least susceptible

Studies on estimation of loss revealed that early infection of bitter gourd plants by the virus significantly reduced the number of leaves, leaf area, internodal length, thickness of vines, length of vines, number of branches, number of flowers, number of fruits and other fruit characters viz , length of fruits, girth of fruits, weight of fruits and total yield of fruits