TRANSMISSION, PHYSICAL PROPERTIES AND HOST RANGE OF BRINJAL MOSAIC VIRUS

By

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THESIS

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1996

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I hereby declare that this thesis entitled Transmission physical properties and host range of brinjal mosaic virus is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship, fellowship or other similar title, of any other University or Society

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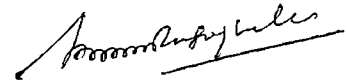
CERTIFICATE

Certified that this thesis entitled Transmission, physical properties and host range of brinjal mosaic virus is a record of research work done independently by Shri M SURENDRAN, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him



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INTRODUCTION

INTRODUCTION

Brinjal (Solanum melongena L) is one of the important vegetable crops grown in Kerala which is highly nutritious and forms an indispensable part of kitchen garden But the productivity of the crop is very low due to many factors of which one of them is the susceptibility of the crop to biotic stresses

Brinjal is affected by several fungal, bacterial viral and mycoplasmal diseases Viral diseases cause serious damage to the crop Many viruses causing mosaic disease of brinjal have been reported in India

Kulkarni (1924) first observed the incidence of brinjal mosaic disease in India So far more than 20 viruses have been reported to be the causal agents of mosaic disease of brinjal Singh and Singh (1975) observed that each 1 0 per cent disease incidence caused an yield loss of 1 5 per cent Sastry and Nayudu (1978) reported an yield loss of 72 3 per cent due to tobacco ring spot virus in brinjal Sriram (1994) observed that the causal agent of mosaic disease in brinjal was found to be a strain of cucumber mosaic virus The disease incidence was upto 30 per cent in the villages surrounding Coimbatore district

No study has been conducted about the brinjal mosaic disease seen in Kerala and no detailed information on this disease is available Hence an attempt was made to study the brinjal mosaic disease on the following areas

1 Symptomatology
2 Transmission of the virus
3 Vector virus relationships
4 Host range of the virus
5 Physical properties of the virus
6 Serological properties of the virus
7 Varietal screening
8 Natural incidence of brinjal mosaic disease
9 Effect of virus on growth of brinjal
10 Effect of certain plant extracts on the incidence of brinjal mosaic

REVIEW OF LITERATURE

REVIEW OF LITERATURE

1 Symptomatology

The symptoms of mosaic disease of brinjal include yellow lesions on leaves followed by irregular light and dark green mottling (Ferguson 1951) Verma and Rashmı Lal (1967) reported a mosaic disease of brinjal which was characterised by a light or dark green mosaic pattern A mosaic disease of brinjal in South Italy characterized by pronounced mottling and crinkling of the leaves and low yield was reported by Martelli in 1969

Usman and Mariappan (1977) observed that the symptoms of brinjal mosaic disease appeared as mild mosaic to severe necrosis resulting in brown streaks along the veins Martelli and Hamadi (1986) observed a mosaic disease of brinjal plants with crinkling of the leaves chlorotic mottling vein clearing, stunting and no fruit production in several locations of the Mitidja region (central coastal Algeria)

From South Beirute a brinjal mottled crinkle virus that induced mottling and malformation of leaves and stunted growth was isolated from brinjal (Makkouk et al 1981) Vyanjane and Mali (1981) reported a brinjal disease characterized by chlorotic vein banding oak leaf pattern, yellow blotches or mottle and leaf distortion In Hungary Salamon et al (1983) observed that alfalfa mosaic virus could cause mosaic yellows of brinjal

Iengo et al (1986) observed that stunting leaf narrowing vein chlorosis and severe crinkling of fruits were noted in brinjal plants Raj et al (1989) reported that brinjal mottled crinkle virus produced chlorotic rings line patterns and mosaic symptoms on brinjal leaves Al musa and Lockhart (1990) observed that brinjal mottled dwarf rhabdo virus caused severe vein yellowing with stunted growth of brinjal plants in Jordan Betti (1992) recently observed a tomato spotted wilt virus on brinjal in sicily The disease was characterized by deformation curling of top leaves stunting and produced malformed fruits

2 Transmission of the virus

2 1 Sap transmission

Gibbs and Harrison (1969) proved that brinjal mosaic virus could be transmitted by sap inoculation to several solanaceous and a few non solanaceous plant spp Martelli and Rana (1970) observed successful mechanical transmission of brinjal mosaic virus to tobacco Nicotiana glutinosa N rustica Petunia hybrida Chenopodium amaranticolor C quinoa and Gomphrena globosa and mechanically transmitted to several species of the families Amaranthaceae Chenopodiaceae Labiatae and Solanaceae

2 2 Graft transmission

Thomas (1938) reported that brinjal mosaic virus could be transmitted to brinjal and Datura fatuosa by grafting

Transmission of stolbur virus of pepper to brinjal by grafting was reported by Zirka in 1967 Martelli (1969) observed that brinjal mottled dwarf virus could be transmitted to brinjal by grafting Brinjal mild mosaic virus has been transmitted to brinjal by sap inoculation and grafting (Naqvi and Mahmood 1976)

2 3 Seed transmission

Seed transmission of brinjal mosaic virus to the extent of 41 8 per cent has been reported in India (Mayee 1974) After 120 days of storage of the seed there was a sharp decline in percentage of seed transmission (Mayee and Kharti 1975) Sriram (1994) also observed the seed borne nature of the brinjal mosaic virus

2 4 Insect transmission

Tobacco ringspot virus causing mosaic disease in brinjal was reported to be transmitted by Thrips tabaci L (Valleau 1951) Schuster (1963) reported that the mosaic disease of brinjal caused by a strain of tobacco ringspot virus was transmitted by tobacco flea beetle (Epitrix hirtipennis) Myzus persicae Sulz transmitted tobacco etch virus causing mosaic disease in brinjal (Verma and Rashmi Lal, 1967) A mosaic disease of brinjal caused by a strain of cucumber mosaic virus was reported by Seth et al (1967) The virus was transmitted by Aphis gossypii Aphis craccivora and M persicae The stolbur

virus on brinjal was transmitted by Hyalesthes obsoletus (Zirka 1967) Cucumber mosaic virus isolated from naturally infected Solanum melongena was transmitted by M persicae and Aphis fabae (Shawkat and Fegla 1979) The flea beetle Epitrix fuscula was a vector for the brinjal mosaic virus (Souza et al 1990)

3 Vector virus relationships

The vector virus relationships of a virus occuring on brinjal were studied by Seth et al (1967) They observed that the virus was transmitted by A gossypii A craccivora and M persicae in a non persistent manner Verma and Rashmi Lal (1967) studied the vector virus relationship of a virus isolated from brinjal which was transmitted only by M persicae and found that the vector transmitted the virus only after 5 10 minutes feeding on the infected plant and not when fed for 24 48 hours Zirka (1967) showed that a disease resembling stolbur in its external symptoms was transmitted to pepper (Capsicum) and brinjal by H obsoletus in a non persistent manner

Singh et al (1975) suggested that even a single viruliferous A craccivora and a minimum of 3 aphids of A gossypii were able to transmit Solanum torvum mosaic virus but maximum percentage of transmission was obtained with groups of 15 aphids per plant in both the species Preliminary fasting of both the aphid vectors increased the transmission efficiency An optimum of 90 min pre acquisition fasting was found to be

necessary to get the maximum transmission He concluded that A craccivora was more efficient in transmitting STMV than A gossypii Vyanjane and Mali (1981) showed that a strain of alfalfa mosaic virus was transmitted by the vector A gossypii to brinjal in a typical stylet borne manner

4 Host range of the virus

4 1 Crop plants as hosts

Ferguson (1951) reported that the red currant tomato (Lycopersicon pimpinellifolium) was found to produce the same symptoms as that on brinjal when inoculated with brinjal mosaic virus Verma and Rashmi Lal (1967) identified a mosaic symptom of vein clearing followed by yellow mosaic caused by brinjal mosaic virus in Nicotiana tabacum N rustica Lycopersicon esculantum and Solanum nigrum Naqvi and Mahmood (1976) reported that brinjal mild mosaic virus was found to infect Amaranthus hypochondriacus, Lycopersicon glandulosum, N tabacum and S nigrum Cucumis sativus, Vigna unguiculata and L esculentum were reported to be the hosts of brinjal mosaic virus (Kemp and Troup 1977) Rao and Shawkat (1979) observed that brinjal mosaic disease was sap transmissible to N tabacum L esculentum Luffa acutangula Momordica charantia and Vigna sinensis Igwegebe and Waterworth (1982) found that pepper vein mottle virus NMI strain isolated from brinjal was able to incite infection in S nigrum L esculentum Sesamum indicum and

Capsicum annuum L esculentum exhibited systemic symptoms when inoculated with brinjal mosaic virus (Souza et al 1990)

4 2 Indicator hosts

Ferguson (1951) found that brinjal mosaic virus produced necrotic circular lesions followed by chlorotic ring spots on N glutinosa L Usman and Mariappan (1977) reported that Datura ferox L was a indicator host for brinjal severe mosaic virus Verma and Rashmi Lal (1967) observed that brinjal mosaic virus produced local lesions on Chenopodium album L Kemp and Troup (1977) found that alfalfa mosaic virus from brinjal produced necrotic local lesions on Gomphrena globosa Citrullus lunatus and N tabacum Chlorotic yellow local lesions were developed in G globosa L on inoculation with brinjal mottled dwarf virus (Martelli and Hamadi 1986) Souza et al (1990) reported that tymovirus isolated from brinjal produced local lesions on C amaranticolor C quinoa, G globosa N benthamiana N glutinosa and Petunia hybrida Gupta et al (1992) observed that brinjal mosaic virus produced local lesions on Amaranthus tricolor C quinoa D stramonium and N tabacum cv white burley Sriram (1994) reported that brinjal mosaic virus could produce local necrotic lesions on C amaranticolor

4 3 Weed hosts

Datura metel and D stramonium produced vein clearing on inoculation with brinjal mosaic virus (Verma and Rashmi Lal

1967 Naqvi and Mahmood 1976) Debrot et al , (1977) observed that the weed Solanum seaforthianum acted as a host of brinjal mosaic virus in Venezuela D stramonium and Malva parviflora were reported to be the weed hosts of the brinjal strain of cucumber mosaic virus (Shawkat and Fegla 1979) Gupta et al (1992) reported that D stramonium and A spinosus were the hosts of brinjal necrosis mosaic disease caused by a tobacco mosaic virus strain Sriram (1994) conducted host range studies of brinjal mosaic virus on many weed plants and reported that Tridax procumbens Datura metel S nigrum and Coccinia indica were found to be the hosts of brinjal mosaic virus and produced systemic mosaic symptoms

5 Physical properties of the virus

Johnson and Grant (1932) studied the physical properties of Cucumis virus 1 infecting different host plants and reported that the virus had a thermal inactivation point (TIP) of 60-65°C dilution end point (DEP) of 1 10000 and longivity in vitro (LIV) at room temperature was 24 28 h Samson and Imle (1942) reported that tomato ringspot virus infecting different host plants including brinjal had a TIP of 56 58°C Dale (1954) found that brinjal mosaic virus infecting brinjal had a TIP of 78°C and LIV of 21 days at room temperature Kovachevski (1965) reported that Lucerne mosaic virus isolated from brinjal had a TIP of 63°C DEP of 1 5000 and LIV of 3 days in sap Verma and Rashmi Lal (1967) reported that brinjal mosaic virus infecting

Nicotiana tabacum had a TIP between 65 and 70°C and DEP of 1 1000 and LIV of 6 days at room temperature Martelli and Rana (1970) observed that brinjal mottled dwarf virus had a TIP between 51 57°C DEP between 10^3 and 10^4 and LIV 30 44 h at 4°C Shawkat and Fegla (1979) isolated cucumber mosaic virus from naturally infected brinjal and watermelon mosaic virus 2 from Cucurbita pepo and were found to be inactivated at 65°C Their DEP and LIV were 10^3 10^4 and 4 8 days respectively Vyanjane and Mali (1981) while studying the physical properties of alfalfa mosaic virus causing mosaic disease in brinjal observed that the virus had a TIP between 50 and 55°C DEP of 1 1000 and LIV was 24 h at room temperature Tobacco mosaic virus from brinjal had a TIP of 90°C DEP of 10^6 and LIV for a long period at room temperature (Gupta et al 1992)

6 Serological properties of the virus

6 1 Purification of virus

Debrot et al (1977) purified the brinjal mosaic virus from its weed host S seaforthianum Alfalfa mosaic virus causing natural infection on brinjal in Ontario was partially purified by Kemp and Troup (1977) employing the technique of Gibbs et al (1963) Brinjal mild mosaic virus was concentrated by Naqvi and Mahmood in 1976 using the procedure of Hebert (1963) Further purification was achieved by rate zonal density gradient centrifugation (Brakke 1960) A virus causing mosaic disease in brinjal which reacted serologically with bottlegourd

mosaic virus (cucumber vein yellowing virus) was purified by Rao (1978) Tobacco ringspot virus was purified from brinjal by Sastry and Nayudu (1978) 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 A mosaic virus from brinjal was purified by two cycles of differential centrifugation (10 000 g 10 min and 100 000g 2hr) by Khalil et al (1982) A1 strain of brinjal mosaic virus was purified using the method of Gooding and Hebert (1967) from chillies (Tobias et al 1982) The purification method of Lisa et al (1981) was used with some modifications to purify the virus causing Green mosaic disease in brinjal (Ladipo et al 1988) Brinjal mosaic virus isolates EMV AR (Arkansas strain) and EMV T (Tobacco strain of brinjal mosaic virus) which belong to tymovirus group were purified from N rustica and N tabacum respectively by Souza et al (1990) using the low pH method described by Scott (1976) for the desmodium yellow mottle virus Gupta et al (1992) purified the brinjal severe mosaic virus a strain of TMV by differential centrifugation followed by density gradient centrifugation

6 3 Serological tests

The virus which caused mosaic disease on brinjal at Lucknow differed serologically from other viruses that had been

reported earlier It was supposed to be a strain of Tobacco etch virus (Verma and Rashmi Lal 1967) Dubey and Nariani (1975) investigated the serological relations of 10 cucurbit virus isolates collected from Delhi and found that the viruses of snakegourd 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 vius 4 while a virus from tori (Luffa cylindrica) appeared to be distinct from these 3 groups EV 1 isolate of virus causing brinjal mosaic disease was serologically related to alfalfa mosaic virus EV 1 strain from brinjal and alfalfa mosaic virus isolate from alfalfa appeared to be serologically identical (Kemp and Troup 1977) S1 and S2 isolates of brinjal mosaic virus obtained from brinjal agreed in serological and physical properties and test plant reactions with cucumber mosaic virus (Mamula et al 1977)

Ghosh and Mukhopadhyay (1979) conducted agar gel diffusion method to identify the nine virus isolates of pumpkin PVMV NN2 isolate from brinjal was identified as a strain of pepper vein mottle virus based on serological tests (Igwegebe and Waterworth, 1982) Carla virus isolated from brinjal differed from other viruses like alfalfa mosaic virus cucumber mosaic virus brinjal mosaic virus tobacco etch virus tobacco mosaic virus and tobacco ring spot virus (Khalil et al 1982) Particle morphology cytopathogenic behaviour and serological

relationship clearly indicated that brinjal green mosaic virus belonged to a Poty virus group Other Poty viruses identified in brinjal were Potato virus Y (Sastry 1982) and Pepper vein mottle virus (Igwegbe and Waterworth 1982)

Brinjal mosaic disease observed in Algeria was shown to be caused by brinjal mottled dwarf virus based on serological studies (Martelli and Hamadi 1986) The causal agent of brinjal mosaic disease was a rhabdo virus which was very closely related or identical to Tunisian isolate of brinjal mottled dwarf virus which in turn was serologically indistinguishable from the original Italian culture (Martelli and Cherif 1987)

Isolates D and F from mosaic infected brinjal plants in Nigeria were serologically related to pepper vein mottle poty virus and Tobacco mosaic virus respectively (Marchie and Igwegbe 1987) The causal virus of brinjal mosaic in Bangalore differed from all the viruses reported earlier in serological properties (Pasannavar and Yarguntiah 1988) Eventhough there were differences in host range and symptomatology between the brinjal mosaic virus isolates from Arkansas and Abelia they were found to be serologically related (Souza et al 1990)

Brinjal necrotic mosaic virus (BNMV) isolate from Aligarh in India reacted serologically with antisera of type strain of TMV TMV U1 TMV A1 TMV WU1 and TMV D but differed from all these strains in symptom expression (Gupta et al 1992)

7 Varietal screening

Holmes (1932) while investigating the susceptibility of Black beauty brinjal to tobacco mosaic virus showed that 100 per cent of the plants were infected Ho and Li (1936) tested some varieties of brinjal in the field against natural infection of brinjal mosaic virus and found that the indigenous Pak and Hung varieties appeared to be resistant Florida High Bush brinjal plants were liable to severe infection by a typical mosaic causing stunting discoloration distortion of the foliage and reduction in size of the fruits Several varieties of brinjal viz Pusa kranti Pusa purple long R 34 S 5 H4 S 1 (A-bI) T2 S-4, Annamalai, Hybrid (Vijaya) local S-1, S-8, S 16 and Pusa purple round were susceptible to brinjal mosaic virus (Mayee and Khatri, 1975) Gupta et al , (1992) also reported that brinjal cultivars Banaras Giant white Black beauty BR 12 Pusa purple cluster and Pusa purple long were susceptible to a TMV strain causing brinjal necrotic mosaic disease

8 Observations on the natural incidence of brinjal mosaic virus disease

Singh and Singh (1975) reported that each 1 0 per cent incidence of brinjal mosaic disease resulted in yield loss of 1 5 per cent Sriram (1994) observed that the brinjal mosaic virus disease incidence was upto 30 per cent in the villages surrounding Coimbatore district In Anna district it was 90 per cent

9 Estimation of loss

Kulkarni (1924) reported that brinjal plants subjected to brinjal mosaic virus infection had malformation crowding of the leaves sterility and other abnormal conditions Holmes (1932) described the incidence of tobacco mosaic virus on young brinjal plants which produced systemic necrosis symptom Ho and Li (1936) investigated a mosaic disease of brinjal and reported that the disease affected plants were stunted with reduction in the size of the fruits Thomas (1938) conducted studies on brinjal mosaic virus and observed the reduction in number of nodes absence of flowers and sterility Dale (1954) conducted field trial to study the economic importance of brinjal mosaic virus on brinjal in Trinidad and reported a reduction in yield by 25 per cent Seth et al (1967) found that the brinjal mosaic virus infected plants exhibited mosaic symptoms with pronounced leaf yellowing stunting and reduced development of the flowers and fruits Sharma (1969) reported the incidence of brinjal mosaic in Pune with severe loss of this crop Morita et al (1974) while studying the effect of broad bean wilt virus on brinjal observed that the infected plants had twisted leaves and stunted growth Sastry and Nayudu (1978) recorded a yield loss of 55 2 per cent to 72 3 per cent due to infection by Tobacco ring spot virus on brinjal

10 Effect of certain plant extracts on the incidence of brinjal mosaic

Kassanis and Kleczkowski (1948) reported a virus inhibitor from Phytolacca esculenta They identified that the inhibitor was a glycoprotein Bawden (1954) established that the inhibitors present in the germinating seeds of pulses and groundnut like that of leaf and twig extracts of a large number of other plants inhibited TMV via host and not directly inactivated the virus Mckeen (1956) reported the inhibition of cucumber mosaic virus and tobacco ringspot virus infections on cowpea and Chenopodium hybridum by extracts from Capsicum frutescens Semel (1960) reported an inhibitor of TMV from Begonia tuberhybrida and identified it as oxalic acid Verma and Awasthi (1979a) observed that the leaf extract of Euphorbia hirta inhibited the infection of tobacco mosaic, sunnhemp rosette, gomphrena mosaic and tobacco ringspot viruses on several hypersensitive hosts

Verma and Awasthi (1979b) reported that the roots of Boerhaavia diffusa contained an antiviral agent active against several viruses including tobacco mosaic virus in Nicotiana glutinosa and the active principle was identified as a glycoprotein

Virus inhibitors from Datura metel against tobacco mosaic virus were reported by Singh and Varma (1981) Verma

(1982) reported that screening of extract from higher plants has shown that many of the plants contained highly potent inhibitors giving local protection and also capable of inducing systemic resistance in many crops against several viruses Mariappan et al (1982) reported that many seed oils such as custard apple oil and neem oil possessed inhibitory action against rice tungro virus Seed oils from Azadirachta indica and Annona sp at five per cent concentration reduced rice tungro virus infection on seedlings of the cultivar TN 1 No insect survived on the sprayed plants after four days Verma and Dwivedi (1983) reported that plant diseases caused by TMV tomato yellow mottle mosaic virus physalis shoe string mosaic virus and cucumber green mottle mosaic virus could be prevented by bougainvillea leaf extract Nagarajan and Murthy (1988) indicated that three sprayings of green leaf extract of Basella alba at 1 1000 dilution at ten days interval on tobacco commencing from 30 days after planting protected the crop from TMV infection upto 60 days

Doraisamy and Ramakrishnan (1988) screened 423 species of plants for antiviral principles against tobacco mosaic virus Of these 11 plant species viz Peltophorum ferrugenium Crassula indica Eugena jambosa Turnera ulmifolia var elegans Bougainvillea spectabilis Mirabilis jalapa Pisonia alba Beta vulgaris Chenopodium murale and C ambrosoides gave 100 per cent inhibition

Reddy et al (1988) reported that leaf extracts of Bougainvillea spectabilis and Asplenium nidus gave 100 per cent and 97 47 per cent inhibition respectively of the local lesions on Chenopodium amaranticolor by cucumber mosaic virus Incorporation of B spectabilis/A nidus extract with the virus inoculum gave more inhibition (100% / 97 47%) than either pre-inoculation (97 00% / 89 18%) or post inoculation (75 36% / 72 61%) treatments Raychaudhury and Jain (1989) reported that neem oil (2-4%) and neem soap sprays on Aphis rumicis caused complete mortality of the nymphs and alate forms within 24 h Mallika devi (1990) observed that leaf extracts of A indica and V negundo inhibited cowpea mosaic virus

Extracts from leaves, roots, green stems, bark seeds green fruits and rhizomes of seven flowering plants (Aconitum heterophyllum Azadirachta indica Capsicum annuum Chenopodium amaranticolor D metel, Glycyrrhiza glabra and Rawvolfia serpentina) markedly reduced the number of local lesions and systemic infection caused by brinjal necrotic mosaic virus on brinjal The extent of antiviral activity of the active substance was independent of the species concentration and methods of application (Gupta and Naqvi 1991)

Sriram (1994) found that leaf extracts of Azadirachta indica (10%) and A indica A Juss oil (5%) gave 25 79 per cent and 18 19 per cent reduction respectively in the incidence of brinjal mosaic over control after 10 days On 30th day, there was reduction over control by 28 08 per cent and 21 48 per cent respectively

MATERIALS AND METHODS

MATERIALS AND METHODS

1 Symptomatology

Seeds of brinjal (Solanum melongena L) variety SM-6, obtained from the Instructional Farm, College of Agriculture, Vellayani were used for the study They were planted in pots containing potting mixture of sand red soil and cowdung in the ratio of 1 1 2 Brinjal plants showing severe mosaic symptoms were collected and planted in the pots containing potting mixture and maintained in the glass house condition The virus inoculum was continuously maintained by repeated sap inoculations on young healthy brinjal plants under insect proof glass house conditions They were sprayed periodically with insecticides to guard against insect vectors Symptomatology was studied by the development of symptoms in naturally infected as well as on inoculated brinjal plants

2 Transmission of the virus

2 1 Sap transmission

Pure culture of brinjal mosaic virus maintained in the insect proof glass house was used for the studies Sap trans mission 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

The standard sap was prepared by adding one ml of sterile distilled water for every gram of infected leaves and crushed into pulp with the help of sterile pestle and mortar The pulp was squeezed through sterilized cotton wool and the sap used for inoculation Where as in the case of phosphate buffer (0 1 M, pH 7 0) and tris buffer (0 1 M, pH 7 0) used as extraction media the sap was obtained by adding one ml of the buffer in each case to every gram of diseased leaves

Carborundum powder was dusted uniformly on the leaves of test plants before inoculation Inoculation was done by gently rubbing the upper surface of the leaves with a small piece of sterilized absorbant cotton wool previously soaked in the inoculum The excess inoculum was washed immediately with distilled water Ten plants were inoculated for each experiment and equal number of healthy plants were maintained as control The plants were kept in an insect proof glass house under observation

2 2 Graft transmission

Small shoots showing mosaic symptom were used for preparing the scion The base of the scion was trimmed to a wedge shape and inserted into the cleft made on the stem of the healthy brinjal plants kept in the insect proof glass house

Thirty day old healthy plants were used as root stock 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 maintain humidity These plants were kept under observation for the development of systemic symptoms in the insect proof glass house

2 3 Seed transmission

Ripe brinjal fruits were collected from infected plants of SM 6 variety The seeds were separated dried under shade and sown in pots for testing the seed borne nature of the virus It was kept in the insect proof condition for observations

2 4 Insect transmission

For the insect transmission studies the following aphids and insects were used They were colonized on caged hosts noted against each

1	Myzus persicae Sulzer	Abelmoschus esculentus L
2	Aphis gossypii Glover	Solanum melongena L
3	Aphis craccivora koch	Vigna ungiculata (L)
4	Aphis malvae koch	Abelmoschus esculentus L
5	Henosepilachna vigintioctopunctata F	Solanum melongena L
6	Luperomorpha bombayensis Jac	Solanum melongena L
7	Bemisia tabaci Genn	Nicotiana tabacum L
8	Syndapteryx biguttula biguttula Inshida	Solanum melongena L

2 4 1 Inoculation using Aphid vectors

The apterous aphids were transferred to petri dishes and starved for a period of one hour (pre-acquisition fasting period) Then allowed the aphids to feed on young infected brinjal leaves for a period of 30 minutes (acquisition feeding period) After that ten aphids were transferred to each of the test plants and allowed to feed for 24 h Then they were killed by spraying 0 1 per cent monocrotophos Ten plants were inoculated in each experiment as in the case of sap transmission and an equal number of control plants were also maintained All the plants were maintained in an insect proof glass house and symptom development was recorded after 25 days along with percentage of infection

2 4 2 Inoculation using Bemisia tabaci

Whitefly colony (Bemisia tabaci) were collected and maintained on healthy tobacco plants (Nicotiana tabacum L) in an insect rearing cage and used for transmission trials Plastic transmission cages designed by Nene (1972) were used for transmission studies

The top portion of either the main stem or fresh branches showing typical symptoms was introduced into the transmission cage through the rectangular slit at the opening of the cage Whiteflies were collected with an aspirator and released into the transmission cage The transmission cage was

covered with a black cloth except at the region of the wire netting which was kept facing the light source while releasing the whiteflies The cap of the transmission cage was immediately screwed on and the remaining portion of the rectangular slit of the cage was kept closed by cotton wool The cages were kept in position with the help of two bamboo sticks and a rubber band After 24 h the cotton was removed and gently tapped with a glass rod to disturb the whiteflies This stimulated the whiteflies to move away from the leaves to the side of the cage facing the light source

Ten seedlings were used as test plants in each experiment Twenty viruliferous whiteflies were released on each test plant for inoculation feeding After the inoculation feeding the insects were killed by spraying the plants thoroughly with 0 1 per cent monocrotophos All the inoculated and uninoculated seedlings were labelled and kept for observation in the insect-proof house An equal number of healthy seedlings were maintained as control Spraying with 0 1 per cent monocrotophos was repeated every week The experiment was repeated twice

2 4 3 Inoculation using Henosepilachna vigintioctopunctata and Syndapteryx biguttula biguttula

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

The beetles were collected in vials and allowed to feed on young infected brinjal leaves for 30 min (Acquisition feeding period) Then they were transferred to test plants and allowed to feed for 24 h After that they were killed by spraying 0 1 per cent monocrotophos The inoculated and uninoculated plants were kept for observation under insect proof condition for 30 days

The leafhopper transmission was done as per the procedure described for the aphid transmission

3 Vector-virus relationships

Brinjal plants showing typical symptoms of brinjal 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 N tabacum in insect rearing cages In all the insect transmission trials only full 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 required feeding period by spraying the plants with 0 1 per cent nuvacron In the case of short feeding period of less than five minutes the individual aphids were watched through a magnifying lens and the time of feeding was determined with the help of a stop watch after the aphids had settled down to feed

Experiments on vector virus relationships were conducted by using M persicae which was found to be the most efficient vector

3 1 Acquisition threshold

Groups of non viruliferous aphids were collected and were given a pre-acquisition starvation of one hour Batches of ten aphids each were given acquisition feeding period of 20 and 30 s 1 5 10 15 20 25 30 and 45 min and 1 2 and 3 h on diseased plants before transferring them to healthy brinjal plants The aphids were then allowed to remain for 24 h on the test plants and were killed thereafter by spraying 0 1 per cent nuvacron

3 2 Inoculation threshold

Non-viruliferous aphids were given one hour pre acquisition starvation and an acquisition feeding of 25 minutes Then the viruliferous aphids were transferred in batches of ten to individual healthy test plants Each batch was given seperate inoculation feeding periods viz 20 and 30 s 1, 5 10 15 20 30 and 45 min 1 2 4 8 24 and 48 h The aphids were killed after specific inoculation feeding period by spraying 0 1 per cent nuvacron

3 3 Influence of fasting before acquisition and inoculation feedings

3 3 1 Pre-acquisition fasting

A large number of non viruliferous aphids were starved for different periods such as 15 and 30 min 1 2 3 4 5 6 12 and 24 h Then they were given an acquisition feeding for 25 minutes on diseased brinjal plants and subsequently they were confined in batches of ten to healthy test plants for inoculation feeding Effect of each pre-acquisition fasting period was tested on ten healthy test plants Control plants were also kept with an equal number of aphids but without any pre acquisition fasting After 1 h the aphids were killed by spraying with 0 1 per cent nuvacron The experiment was repeated to confirm the results

3 3 2 Post-acquisition fasting

A large number of aphids were starved for 1 h and given an acquisition feeding period of 25 minutes These viruliferous aphids were then starved in batches of ten for different periods such as 15 min 30 min 1 2 3 4 5 6 12 and 24 h Groups of ten aphids from each of these categories were transferred to each healthy test plant Effect of each post acquisition fasting period was tested on ten healthy test plants The aphids were killed after 1 h by spraying 0 1 per cent nuvacron The experiment was repeated to confirm the results The controls

were maintained with equal number of aphids with no post-acquisition fasting

3 4 Retention of infectivity by the vector

Groups of aphids were starved for one hour and allowed to feed on diseased brinjal plants for 25 min to make them viruliferous Groups of ten 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 15 and 30 min 1 1 5 2, 2 5 3 and 4 h The aphids were killed from the fifth plant of the different series by using 0 1 per cent nuvacron The experiment was done twice

3 5 Minimum number of aphids required for transmission

Single aphid as well as groups of 2, 3 5, 10 15, 20 25 30 and 40 were collected from a non viruliferous colony and were starved for one hour These aphids were made viruliferous by feeding them on diseased brinjal plants After an acquisition feeding period of 25 min, the aphids were transferred to healthy test plants and allowed to feed for one hour After that they were killed by spraying 0 1 per cent nuvacron

4 Host-range

To determine the host-range of brinjal mosaic virus, the plants belonging to 52 species of 17 families were inoculated

by sap inoculation Ten seedlings were inoculated in each case The plants which did not show visible symptoms of infection after four to six weeks were indexed by back inoculation to healthy brinjal plants to find out whether they were symptomless carriers of the virus Following plants were used for host range studies

1 Acanthaceae

(a) Andrographis echioides Nees

(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

Typhonium trilobatum Schott

5 Capparidaceae

Cleome viscosa L

6 Chenopodiaceae

(a) Chenopodium amaranticolor L

(b) Chenopodium quinoa Willd

7 Compositae

(a) Acanthospermum hispidum Dc

(b) Eupatorium odoratum L

(c) Tridax procumbens L

(d) Zinnia elegans Jacq

8 Cucurbitaceae

(a) Benincasa hispida Wild

(b) Citrullus vulgaris Schrad

(c) Cucurbita moschata Duch

(d) Cucumis sativus L

(e) Cucumis melo L

(f) Lagenaria siceraria Standl

(g) Luffa acutangula Roxb

(h) Momordica charantia L

(i) Trichosanthes anguina L

9 Euphorbiaceae

(a) Acalypa indica L

(b) Euphorbia geniculata Orteg

(c) Euphorbia hirta L

(d) Manihot esculenta Crantz

(e) Phyllanthus niruri L

10 Malvaceae

(a) Abelmoschus esculentus L

(b) Abutilon indicum Don

11 Labiatae

Leucas aspera Spr

12 Leguminosae

i Mimosaceae

Mimosa pudica L

ii Papilonaceae

(a) Arachis hypogaea L

(b) Cajanus cajan Adans

(c) Crotalaria juncea L

(d) Dolichos biflorus L

(e) Vigna mungo Hepper

(f) Vigna radiata Wilezek

13 Polygononaceae

Antigonon leptopus Hook and Arn

14 Pedaliaceae

Sesamum indicum L

15 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 nigrum

16 Verbenaceae

Clerodendron infortunatum L

17 Zingiberaceae

Zingiber officinale Rosc

5 Physical properties

5 1 Dilution end point (DEP)

The standard sap was prepared by adding one ml of distilled water for every gram of infected brinjal leaves and crushed into pulp with the help of sterile pestle and mortar The pulp was squeezed through sterilised cotton wool The sap was diluted with sterilized distilled water in the ratio of 1 10 1 100 1 1000 1 10000 and 1 100000 The different dilutions were used for inoculation on seperate test plants starting from the highest dilution The local lesion host (Datura stramonium) was used as test plant Ten leaves were inoculated with each of the dilutions and the experiments repeated to confirm the results The inoculated leaves were labelled and kept under insect proof conditions and observed for the development of localised symptoms

5 2 Thermal inactivation point (TIP)

The sap from the infected brinjal plant was prepared as in the above experiment Five ml of the sap was pipetted into a thin walled glass test tube Care was taken not to smear the upper part of the test tube It was then placed in a waterbath provided with a thermostat The waterbath was filled with water until the level reached 3 cm above the level of the sap in the tubes The test tube was kept for ten minutes in the water bath maintained at 35°C The control was kept at room temperature

(28-30°C) In the same manner five ml lots of the sap were kept for ten minutes each at 40 45 50 55 60 65 70 and 80°C and a thermometer was placed close to the tube in the waterbath to check the temperature After ten minutes in each case the tube was removed and cooled immediately in running water The untreated and treated samples of the sap were used for inoculation on the test plants by smearing them on the leaves sprinkled with carborundum powder Inoculation was done on healthy leaves of two month old D stramonium Ten leaves were inoculated in each treatment and the experiment was repeated to confirm the results Observations on the number of local lesions produced on the leaves of D stramonium were recorded

5 3 Longevity in vitro (LIV)

The sap from the infected brinjal plant was prepared as in the above experiment Five ml of the sap were pipetted into test tubes and closed with aluminium foil The tubes were kept at room temperature (28 30°C) and also in refrigerator (10°C) One tube each containing the sap of each treatment was taken after specific periods viz 0 24 48 72 96 120 144 168 and 192 h and inoculated on the leaves of D stramonium Ten leaves were inoculated in each treatment and the experiment was repeated to confirm the results In all the experiments the inoculated plants were kept under insect proof conditions and observed for the development of symptoms

6 Serological properties of the virus

6 1 Purification of virus

The purification was done following the method of Hebert (1963) and Vankammen (1967) The inoculum was prepared by crushing the systemically infected frozen leaves at the rate of 1 g/ml of 0 01 M phosphate buffer pH 7 0 in a clean sterile pestle and mortar The pulp was squeezed through double layer muslin cloth and centrifuged at 10000 g for 15 min at 4°C using HIMAG refrigerated centrifuge model HCRZOBA, to remove the host material PEG and NaCl were added to the supernatant to get final concentrations of 4 per cent and 0 2 M respectively and centrifuged at 10000 g for 15 min at 4°C The precipitate was dissolved in 0 85 per cent saline solution and used as antigen for injecting rabbits The supernatant and precipitate were tested for their infectivity on healthy brinjal plants as well as on Datura stramonium

6 1 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 were injected into the thigh muscle at a time The final injection was given intravenously with 2 ml of antigen alone 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 15 ml tubes and were allowed to coagulate by keeping the tubes at room temperature for two hours The coagulated 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 5000 g for 30 min at 4°C to remove the remaining blood cells The supernatant antiserum was stored in small vials after adding a pinch of sodium azide and kept in freezer and used for other tests

6 2 Serological tests

6 2 1 Microprecipitin test on slides

Thirty microlitres of antiserum and the same quantity of virus suspension were mixed on a microscope slide The mixture was incubated at 25°C under high humidity for 20 45 minutes and examined under the microscope (Bercks et al 1972)

Antigens of brinjal mosaic virus isolate I (isolated from diseased plants in glass house) and isolate II (isolated from diseased plants in the field) cucumber mosaic virus snake gourd mosaic virus bitter gourd mosaic virus, pumpkin mosaic virus watermelon mosaic virus and chilli mosaic virus were tested against the antiserum of brinjal mosaic virus

The virus suspension (brinjal mosaic virus) was tested against the antiserum of bitter gourd mosaic virus available in the Department of Plant Pathology College of Agriculture Vellayani

6 2 2 Microprecipitin test in petri dishes

This test was mainly used to diagnose virus diseases to determine the titre of the antiserum with the virus to measure the end point of the virus the titre of antiserum with healthy sap and the end point of the healthy sap with antiserum The test was conducted as per the procedure described by Noordam (1973)

Leaves showing typical symptoms were ground using a clean sterile pestle and mortar and the sap was strained through cotton wool and centrifuged at 10000 g for 15 min to get clear supernatant It was transferred into a series of numbered corning glass test tubes with a capacity of 1 to 1 5 ml using a pasteur pipette The second tube was half filled with the sap

and an equal amount of saline buffer (0 85 per cent NaCl in 0 01M 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 1/8 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 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 NaCl buffer met with the other lines Using another pipette one drop each of the least concentrated 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 each 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 above mentioned

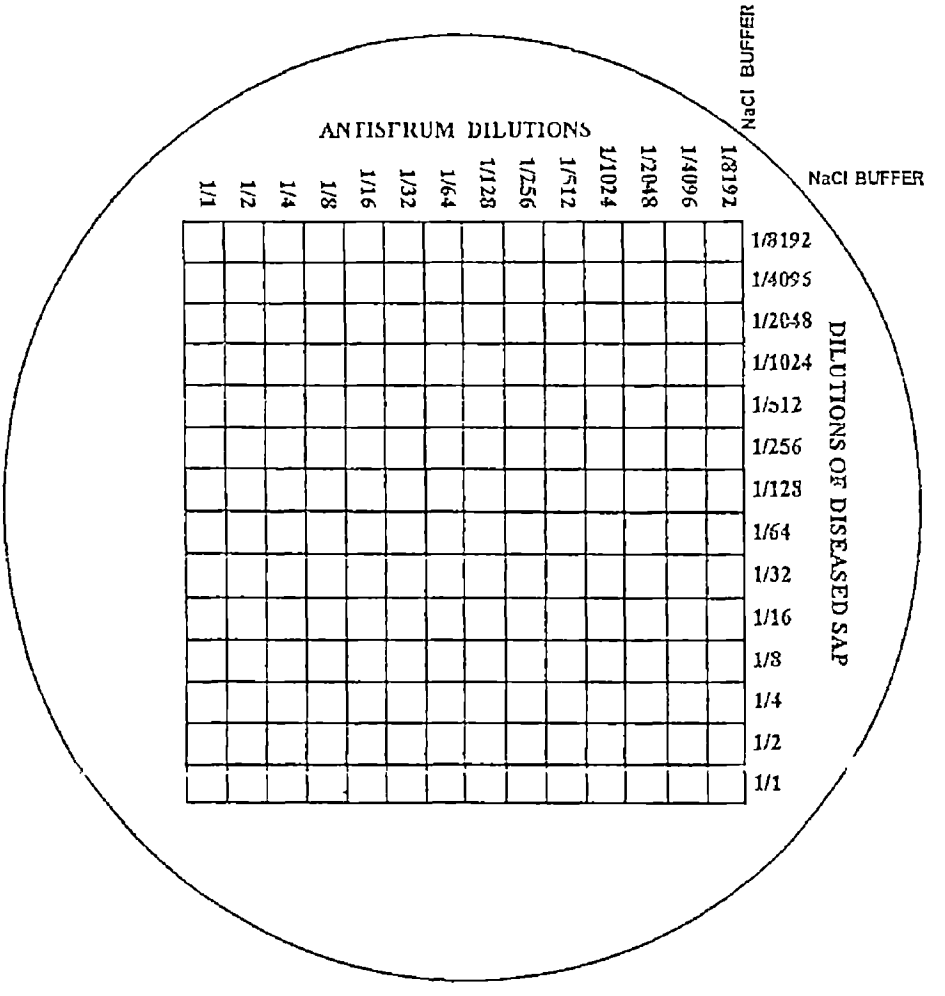

Fig 1 MICROPRECIPITIN TEST IN PETRI DISHES

scheme was followed for healthy sap also The drops were covered with liquid paraffin to prevent evaporation Liquid paraffin was added slowly through the sides 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 under a stereomicroscope with top light and black background The intensity of the precipitate formed was evaluated based on a scale given below

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

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

6 2 3 Outchterlony's agar double diffusion test

This test was done in serological petri dishes Antiserum and virus suspensions (0 4 ml each) were added to wells punched in agar

Sterilized petri dishes were coated with a layer of 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 soldify Above this layer 2 per cent melted agarose was again added to a thickness of 3 mm Thirty minutes 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 In the central well (Well No 1) of each plate 0 4 ml of antiserum was added with a pasteur pipette and the antigens in the surrounding wells as described below in three separate plates

a) In plate I wells 3 and 5 received distilled water 4 received phosphate buffer 6 received tris buffer and well 2 the clarified healthy plant sap

b) In the Plate II well 2 received sap from water melon mosaic virus 3 received brinjal mosaic virus 4 chilli mosaic virus 5 pumpkin mosaic virus and 6 cucumber mosaic virus

c) In plate III well 2 received bitter gourd mosaic virus 3 received cucumber mosaic virus 4 pumpkin mosaic virus 5 snake gourd mosaic virus and 6 brinjal mosaic virus

High percentage of relative humidity was maintained inside the petri dishes 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 detailed 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 57°C When the agar was completely dried, 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 discolouriser solution No 1 and 2 (Appendix - I) Each washing was of one hour duration The plates were then dried for one hour at 37°C and examined

7 Varietal screening

Four varieties and 11 indigenous collections of brinjal plants were tested for their resistance to brinjal mosaic virus Ten plants of each variety were inoculated with the virus using standard sap was inoculum The inoculum was prepared from leaves of systemically infected brinjal plants by grinding them with sterilized pestle and mortar adding distilled water The standard sap was sequeezed through cotton wool and immediately

inoculated on young test plants which were grown in insect proof glass house The experiment was done twice Following were the varieties and indigenous collections used for screening studies

1 SM 6
2 Palur 1
3 Varikai (TN local)
4 Anna malai
5 NIC 01190
6 NIC - 14101
7 NIC 14144
8 IC - 089822
9 IC 089847
10 IC - 089866
11 IC - 089911
12 IC - 089922
13 IC 111345 A
14 IC - 111449
15 IC - 111454

8 Observations on natural incidence of brinjal mosaic disease

Brinjal plants grown in and around Vellayani area were examined to find out the natural incidence of mosaic disease Observations were taken from 3 plots at 50 80 and 110 days after planting

9 Effect of virus on growth of the brinjal plants

Brinjal plants (SM 6 variety) grown in 2 acres of Instructional Farm of the Agricultural College Vellayani campus were examined to find out the yield loss due ot brinjal mosaic virus infection Manures and fertilizers were applied according to the package of practices recommendations of Kerala Agricultural University (1993) All the plants were periodically sprayed with 0 1 per cent Nuvacron and 0 2% Dithane M 45 to keep the plants free from pest and fungal diseases Ten healthy plants and equal number of diseased plants were randomly selected at 3 4 leaf stage for taking observations

The crop was planted on 29-3-94 and observation were recorded on the following aspects at an interval of one month

1) Plant height
2) Number of leaves formed
3) Number of branches developed
4) Internodal length
5) Leaf area
6) Thickness of branches
7) Number of flowers formed
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

The data were analysed statistically by applying the technique of analysis of variance for Completely Randomised Block Design and the significance was tested by F test Critical differences were calculated for comparing treatment means

10 Effect of certain plant extracts on the incidence of brinjal mosaic

10 1 Screening of non-host plant extracts for antiviral property against brinjal mosaic virus

(i) Virus inoculum was prepared (in phosphate buffer) as mentioned earlier

(ii) Preparation of leaf extracts

Plant extracts were prepared in distilled water by grinding the plant parts viz , leaves, roots bark using sterilized mortar and pestle by adding one ml of sterile distilled water for every gram of plant tissues and crushed into pulp and then squeezed through sterilized cotton wool The crude sap was centrifuged at 3000 g for 15 min and the supernatant was used for the study The following plants/products were used

	Botanical name	Common/local name	Family
1	Adathoda vasica Nees	Adathodai	Acanthaceae
2	Azadirachta indica A Juss	Veppu	Meliaceae
3	Azadirachta indica A Juss oil	Veppenna	Meliaceae
4	Azadirachta indica A Juss cake	Veppin pinnakku	Meliaceae
5	Boerhaavia diffusa L	Thazhuthama	Nyctaginaceae
6	Bougainvillea spectabilis Wild	Bougainvilla	Nyctaginaceae
7	Calatropis gigantea R Br	Erikku	Ascieipidaceae
8	Cassia fistula L	Kanikonnai	Leguminoceae
9	Chenopodium amaranticolor L	Chenopodium	Chenopodiaceae
10	Clerodendron infortunatum L	Peruvalam	Verbenaceae
11	Cocos nucifera L	Coconut	Palmae
12	Cyperus rotundus L	Muthanga	Cyperaceae
13	Eucalyptus grandis Schlecht	Eucalyptus	Myrtaceae
14	Eupatorium odoratum L	Communist pacha	Compositae
15	Moringa oleifera Lam	Moringa	Moringaceae
16	Ocimum sanctum L	Thulasi	Labiatae
17	Phyllanthus niruri L	Kizhanelli	Euphorbiaceae
18	Ricinus communis L	Castor	Euphorbiaceae
19	Thespesia populnea Correa	Poovarasu	Malvaceae
20	Vinca rosea L	Savakottathetti	Apocyanaceae
21	Vitex negundo L	Nochi	Verbenaceae

Datura stramonium (local lesion host of brinjal mosaic virus) was used as the test host plant for this experiment

The partially clarified extract (10 ml) of each of the above plants was mixed with equal quantity of virus inoculum, incubated at room temperature for 10 minutes Carborundum powder was dusted uniformly on the leaves of test plants before inoculation Inoculation was done by gently rubbing the upper surface of the leaves with a small piece of sterilized absorbant cotton wool previously soaked in the mixture of inoculum and plant extract Ten leaves were inoculated for each extract and same number of leaves inoculated with the virus inoculum alone maintained as control The inoculated plants were kept under observation in the insect proof glass house for the development of local lesions The efficacy of the plant extract having antiviral property against brinjal mosaic virus was estimated by applying the following formula

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

C Number of lesions produced in control

T = Number of lesions produced on treated leaves

10 2 Comparative efficacy of four concentrations of plant extracts/products on the symptom development by brinjal mosaic virus

Virus inoculum and extracts of 4 selected plants namely Azadirachta indica Clerodendron infortunatum Ocimum sanctum Vitex negundo and 2 products of Azadirachta indica namely

Acadirachta indica A Juss oil and Aradirachta indica A Juss cake were prepared as mentioned above The virus inoculum was mixed with equal quantity of four dilutions (10 20 30 and 40 per cent) of the extracts and incubated for 10 minutes at room temperature A small quantity of carborundum powder was dusted on the leaves of Datura stramonium before the application of the mixture of inoculum and plant extract Ten leaves were inoculated for each extract and equal number of leaves inoculated with virus inoculum alone maintained as control The inoculated plants were kept under observation in insect proof glass house for the development of local lesions

The extracts which caused 100 per cent inhibition of local lesions on Datura stramonium were further tested to confirm the results

10 3 Effect of time of application of plant extracts/products on the transmission of brinjal mosaic virus

Two promising plant extracts viz Azadirachta indica and Clerodendron infortunatum and two products viz A indica A Juss oil and A indica A Juss cake were sprayed on Datura stramonium at ten per cent dilution as given below

1 One day prior to the inoculation with the virus (pre inoculation application)

2 One day after inoculation with the virus (post inoculation application) and observations on the development of local lesions were recorded

10 4 Effect of plant extracts/product on the transmission of brinjal mosaic virus inoculated at different intervals

The following three promising plant extracts/product on the basis of their ability to inhibit the transmission of brinjal mosaic virus on brinjal plants was used in this experiment

1 Azadirachta indica
2 Clerodendron infortunatum
3 Azadirachta indica A Juss oil

The brinjal seedlings planted in earthern pots were arranged into seven groups of five pots (Each group having ten plants) Ten per cent of each of the above extracts/product were sprayed on the six groups of test plants by means of an atomiser The seventh group was kept as control Soon after spraying with the extract/product (zero hour), the first group of plants and the control plants were inoculated with the virus by sap transmission method The other five groups of plants were inoculated at intervals of 2 h, 4 h, 12 h, one day and two days respectively The test plants were observed for the expression of disease symptoms at 21 days after inoculation

RESULTS

RESULTS

1 Symptomatology

The initial symptoms of naturally infected plants appeared as mild mosaic pattern on the leaf lamina In advanced stages of infection light or dark green patches were produced along the leaf lamina The leaves were very much reduced in size and variously crinkled and deformed Diseased plants remained stunted and produced only a few flowers and fruits (Fig 2 3 and 4)

On sap inoculation of brinjal plants at 3 4 leaf stage the symptoms appeared within 16 20 days The symptoms first appeared as small light green areas followed by mosaic mottling Subsequent leaves showed mosaic mottling with dark green and light green patches The growth of the infected plants was retarded and internodes shortened As in the case of naturally infected plants the inoculated plants also produced only a few flowers and small fruits

2 Transmission of the virus

2 1 Sap transmission

The virus was found to be transmitted successfully by sap inoculation using standard sap, sap extracted in phosphate buffer (0 1M pH 7 0) and tris buffer (0 1 M pH 7 0) The symptoms appeared within 16 20 days after inoculation The percentage of transmission varied with the extraction medium used

(Table 1) Sap extracted in phosphate buffer gave maximum infection of 60 per cent followed by standard sap giving 50 per cent infection while tris buffer gave the minimum infection of 30 per cent

Table 1 Sap transmission of brinjal mosaic virus

Sl No	Inoculum	Number of plants infected out of 10 plants inoculated		Per cent transmission
		Experiment I	Experiment II	
1	Sap extracted in phosphate buffer	6	6	60
2	Standard sap	5	5	50
3	Sap extracted in tris buffer	3	3	30

2 2 Graft transmission

Mosaic affected brinjal shoots were wedge grafted to 30 day old healthy plants and the grafted plants were grown in insect proof glass house and observed for symptom development The symptoms appeared 18 20 days after grafting From the three trials conducted 90 per cent transmission was obtained (Table 2)

Table 2 Graft transmission of brinjal mosaic virus

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

Fig 2 Healthy and di eased brınjal leaves

2 3 Seed transmission

Seeds collected from infected plants were sown and observed for seed transmission in insect proof glass house The symptoms appeared 16-18 days after germination From the three trials conducted 40 per cent seed transmission was obtained (Table 3)

Table 3 Seed transmission of brinjal mosaic virus

Experiments	Number seeds sown	Number of seeds germinated	Number of plants infected	Per cent transmission
I	50	38	19	50
II	35	25	10	40
III	50	40	12	30

2 4 Insect transmission

Insect transmission studies of the virus were carried out using 8 vectors viz M persicae A gossypii A craccivora A malvae, H vigintioctopunctata, L bombayensis, B tabaci and S biguttula biguttula and the results are presented in Table 4 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 16-20 days after inoculation

The observations showed that the highest percentage of transmision (60%) was obtained with M persicae followed by

A gossypii (40%) A craccivora (25%) A malvae (20%) and H vigintioctopunctata (20%) L bombayensis B tabaci and S biguttula biguttula could not transmit the virus (Table 4)

Table 4 Insect transmission of brinjal mosaic virus

Sl No	Vector	Number of plants infected out of 10 plants inoculated Experiment I	Experiment II	Per cent trans mission
1	Myzus persicae	6	6	60
2	Aphis gossypii	4	4	40
3	Aphis craccivora	2	3	25
4	Aphis malvae	2	2	20
5	Henosepilachna vigintioctopunctata	2	2	20
6	Luperomorpha bombayensis	0	0	0
7	Bemisia tabaci	0	0	0
8	Syndapteryx biguttula biguttula	0	0	0

3 Vector virus relationships

3 1 Acquisition threshold

This experiment was conducted to find out the minimum period required for the vector Myzus persicae to acquire the virus and become viruliferous The results are presented in Table 5 The results showed that a short acquisition feeding period of 20 s only was sufficient for the aphids to become viruliferous The optimum acquisition feeding period which gave

the maximum percentage of infection (65%) was found to be 25 min As the acquisition feeding period further increased the efficiency of the vector to transmit the virus was reduced considerably and it was only 5 per cent when the acquisition feeding period was 3 h

Table 5 Acquisition threshold of Myzus persicae on the transmission of brinjal mosaic virus

Acquisition feeding period	Number of plants infected out of 10 plants inoculated		Total number of plants infected	Per cent transmission
	Experiment I	Experiment II		
10 s	0	0	0	0
20 s	0	1	1	5
30 s	1	2	3	15
1 min	3	2	5	25
2 min	4	2	6	30
5 min	4	4	8	40
10 min	5	4	9	45
15 min	5	6	11	55
20 min	6	6	12	60
25 min	7	6	13	65
30 min	6	6	12	60
45 min	6	5	11	55
1 h	5	5	10	50
2 h	3	2	5	25
3 h	1	0	1	5

Pre acquisition fasting	30 min
Inoculation feeding	24 h
Number of aphids per plant	10

3 2 Inoculation threshold

In order to find out the minimum period required for the viruliferous vector M persicae to transmit the virus successfully an experiment was conducted as described in the materials and methods and the results are presented in Table 6

The data indicated that the viruliferous aphids were capable of transmitting the virus with 30 s inoculation feeding on the test plant The maximum infection of 85 per cent was obtained by feeding the vector for 1 h on test plants

Table 6 Inoculation threshold of Myzus persicae on the transmission of brinjal mosaic virus

Inoculation feeding period	Number of plants infected out of 10 plants inoculated		Total Number of plants infected	Per cent transmission
	Experiment I	Experiment II		
20 s	0	0	0	0
30 s	0	1	1	5
1 min	2	2	4	20
5 min	3	4	7	35
10 min	4	4	8	40
15 min	6	5	11	55
20 min	6	7	13	65
30 min	6	9	15	75
45 min	8	8	16	80
1 h	9	8	17	85
2 h	8	8	16	80
4 h	8	7	15	75
8 h	7	7	14	70
24 h	7	6	13	65
48 h	5	6	11	55

Pre acquisition fasting 30 min
Acquisition feeding 25 min
Number of aphids per plant 10

3 3 1 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 The maximum efficiency was noted when insects were starved for a period of 1 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 7)

Table 7 Effect of pre acquisition fasting of Myzus persicae on the efficiency of transmission of brinjal mosaic virus

Pre-acquisition fasting period	Number of plants infected out of 10 plants inoculated		Total number of plants infected	Per cent transmission
	Experiment I	Experiment II		
No fasting	3	3	6	30
15 min	5	4	9	45
30 min	3	7	10	50
1 h	6	7	13	65
2 h	5	6	11	55
3 h	4	4	8	40
4 h	5	2	7	35
5 h	4	2	6	30
6 h	2	2	4	20
12 h	0	0	0	0
24 h	0	0	0	0

Acquisition feeding 25 min
Inoculation feeding 1 h
Number of aphids per plant 10

Fig 3. Brinjal plant infected with virus

Fig. 4. Healthy and diseased brinjal plants

3 3 2 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 50 per cent was obtained when the aphids were immediately transferred to test plants after acquisition feeding period and no infection was noted when the aphids were given a post acquisition fasting beyond 1 h (Table 8)

Table 8 Effect of post acquisition fasting of Myzus persicae on the efficiency of transmission of brinjal mosaic virus

Post acquisition fasting period	Number of plants infected out of 10 plants inoculated		Total number of plants infected	Per cent transmission
	Experiment I	Experiment II		
No fasting	3	7	10	50
15 min	4	4	8	40
30 min	3	2	5	25
1 h	1	2	3	15
2 h	0	0	0	0
3 h	0	0	0	0
4 h	0	0	0	0
5 h	0	0	0	0
6 h	0	0	0	0
12 h	0	0	0	0
24 h	0	0	0	0

Pre acquisition fasting	1 h
Acquisition feeding	25 min
Inoculation feeding	1 h
Number of aphids per plant	10

3 4 Retention of infectivity by the vector Myzus persicae

The results indicated that successful infection could be obtained upto the second plant of the first series in which aphids were transferred at an intervals of 15 min and 30 minutes In all 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 9)

Table 9 Retention of infectivity by Myzus persicae

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

a = replication 1 b replication 2

\+ Symptom produced - No symptom produced

3 5 Minimum number of aphids required for transmission

A single aphid was found to be capable of transmitting the virus to healthy test plants The optimum number of aphids required to produce maximum infection of 65 per cent was found to be 10 (Table 10)

Table 10 Minimum number of Myzus persicae required for the transmission of brinjal mosaic virus

Number of aphids per plant	Number of plants infected out of 10 plants inoculated		Total number of plants infected	Per cent trans mission
	Experiment I	Experiment II		
1	1	0	1	5
2	2	0	2	10
3	2	4	6	30
5	4	6	10	50
10	6	7	13	65
15	6	6	12	60
20	5	6	11	55
25	5	5	10	50
30	3	5	8	40
40	3	4	7	35

Pre acquisition fasting	1 h
Acquisition feeding	25 min
Inoculation feeding	1 h

4 Host range of the virus

Host range studies were conducted with 52 plant species belonging to 17 families The results showed that 11 species belonging to 3 families viz Chenopodiaceae Cucurbitaceae and Solanaceae produced symptoms of virus disease

1 Chenopodiaceae

Chenopodium quinoa Wild

Chlorotic local lesions appeared on the inoculated leaves 6 days after virus inoculation Later the lesions turned necrotic with brown centre The lesions were circular in shape and 1 2 mm in diameter (Fig 5)

2 Cucurbitaceae

a) Cucurbita moschata Duch

The symptoms first appeared on the young leaves within 15 18 days after inoculation as light green area followed by dark green patches In advanced stage, the leaf size was reduced internodes were shortened and plants stunted (Fig 6)

b) Cucumis sativus L

The symptoms noticed within 20 25 days after inoculation The younger leaves showed pronounced mosaic mottling and the older leaves showed only mild mottling The infected plants were stunted and internodes shortened (Fig 7)

c) Momordica charantia Standl

The symptoms first appeared 12 15 days after inocu lation as small light green areas followed by mosaic mottling Typical mosaic patches with dark green and light green blisters

Fig. 5. Local lesions of brinjal mosaic virus on Chenopodium quinoa

Fig. 6. Cucurbita moschata infected with brinjal mosaic virus

were produced in all the subsequent leaves In advanced stage the infected plants were retarded and internodes shortened

d) Trichosanthes anguina L

The initial symptom appeared in 18 20 days after inoculation as light greenish area on the leaf lamina Later it developed into typical mosaic mottling of dark green patches alternate with light green patches (Fig 8)

3 **Solanaceae**

a) Capsicum annuum L

The symptom was noticed about 2 weeks after inoculation The initial symptom appeared as mosaic mottling with dark green and yellow areas on the young leaf surface In the later stage of infection the leaves were reduced in size and were curled (Fig 9)

b) Datura stramonium L

The inoculated plants produced local lesions within 7 9 days after inoculation The lesions appeared as chlorotic in the beginning turned necrotic and 1 mm in diameter (Fig 10)

c) Nicotiana glutinosa L

The inoculated plants showed symptoms 8 10 days after inoculation The initial symptom appeared as light green patches on the leaf lamina In the advanced stage the newly emerging

leaves showed characteristic vein clearing yellowing and downward curling of leaves Leaf size was extremely reduced and plant growth was considerably checked (Fig 11)

d) Nicotiana tabacum L

The systemic symptoms were noticed 10 12 days after inoculation and were characterised by light yellow patches on the leaf lamina In the advanced stage the infected plants were stunted the internodes shortened and the leaves showed vein-banding The size of the leaves was very much reduced (Fig 12)

e) Physalis minima L

The initial symptom was noticed 15 16 days after inoculation and was characterised by mosaic patches on the leaf lamina In the later stage of infection curling and crinkling of the leaves were observed The infected plants produced only a few flowers (Fig 13)

f) Solanum nigrum L

The initial symptoms appeared as light green areas followed by mosaic mottling Later it turned into dark green alternate with light green patches In advanced stage curling and crinkling of leaves were observed and produced only a few flowers and fruits (Fig 14)

Fig. 7. Cucumis sativus infected with brinjal mosaic virus

Fig. 8. Trichosanthes anguina infected with brinjal mosaic virus

5 Physical properties of the virus

5 1 Dilution end point (DEP)

Serial dilutions of the standard sap (1 1) viz 1 0 1 100 1 1000 1 10000 1 100000 and 1 1000000 were prepared The different dilutions were used for inoculation on separate leaves of Datura stramonium starting from the highest dilution Ten leaves 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 1000 (Table 11)

Table 11 Dilution end point of brinjal mosaic virus

Dilutions	Experiment I		Experiment II		Per centrans mission
	Number of leaves inoculated	Number of leaves infected	Number of leaves inoculated	Number of leaves infected	
0	10	9	10	9	90
1 10	10	8	10	8	80
1 100	10	8	10	7	75
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

5 2 Thermal inactivation point (TIP)

The sap from infected brinjal plant was subjected different ranges of temperature viz 35 40 45 0 55 6 65 70 and 80°C The treated and untreated (Control at r

temperature at 28 30°C) samples of the sap were inoculated on young tender leaves of Datura stramonium The results indicated that the virus was infective at 60°C but not at 65°C (Table 12)

Table 12 Thermal inactivation point of brinjal mosaic virus

Temperature (°C)	Experiment I		Experiment II		Per cent transmission
	Number of leaves inoculated	Number of leaves infected	Number of leaves inoculated	Number of leaves infected	
Control (Room temperature 28 30)	10	9	10	9	90
35	10	8	10	8	80
40	10	8	10	7	70
45	10	6	10	7	60
50	10	6	10	6	60
55	10	5	10	5	0
60	10	4	10	4	40
65	10	0	10	0	0
70	10	0	10	0	0
80	10	0	10	0	0

5 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 13 and 14 When the inoculum was stored at room temperature (28 30°C) for a period of 1 0 h its infectivity was completely lost About 40 per cent of the

Fig. 9. Capsicum annuum infected with brinjal mosaic virus

Fig. 10. Local lesions of brinjal mosaic virus on Datura stramonium

leaves inoculated with the sap kept for 96 h at room temperatur developed local lesion symptoms and after 120 h of st rage tl infectivity of the inoculum was completely lost So tl longevity *in vitro* of the virus stored at room temperature wa between 96 and 120 h

Table 13 Longevity *in vitro* of bringal mosaic virus at room temperature (28 30°C)

Ageing in hours	Experiment I		Experiment II		Ier ce t trar missior
	Number of leaves inoculated	Number of leaves infected	Number of leaves inoculated	Number of leaves infected	
0	10	9	10	9	90
24	10	8	10	8	8
48	10	6	10	7	65
72	10	6	10	6	60
96	10	4	10	4	40
120	10	0	10	0	0
144	10	0	10	0	0
168	10	0	10	0	0
192	10	0	10	0	0

When the inoculum was stored in a refrigerator (1(°C) the infectivity was retained upto 144 h but the percentage of infected leaves was considerably decreased After 168 l of storage of the inoculum the infectivity was completely lo t So the longevity *in vitro* of the virus was between 144 and 168 h when the sap was stored under refrigerator conditions

Table 14 Longevity in vitro of brinjal mosaic virus at 10 C

Ageing in hours	Experiment I		Experiment II		Per cent transmission
	Number of leaves inoculated	Number of leaves infected	Number of leaves inoculated	Number of leaves infected	
0	10	9	10	9	90
24	10	8	10	8	80
48	10	7	10	7	70
72	10	5	10	6	55
96	10	5	10	5	50
120	10	4	10	4	40
144	10	3	10	3	30
168	10	0	10	0	0
192	10	0	10	0	0

6 Serological properties of the virus

6 2 1 Microprecipitin test on slides

Thirty micro litres of antiserum prepared as described under materials and methods were mixed with equal volume of antigens from different virus infected crop plant It was observed that the antigens of brinjal mosaic virus isolate I and isolate II cucumber mosaic virus snake gourd mosaic virus and pumpkin mosaic virus produced dense precipitate with the antiserum specific to brinjal mosaic virus Antigens of water melon mosaic virus chilli mosaic virus and bitter gourd mosaic virus did not produce any precipitate (Table 15)

Fig. 11. Nicotiana glutinosa infected with brinjal mosaic virus

Fig. 12. Nicotiana tabacum infected with brinjal mosaic virus

Table 15 Microprecipitin test of brinjal mosaic virus with the antiserum on slides

Sl No	Antigen used	Reaction with antiserum
1	Brinjal mosaic virus (Isolate I)	+
2	Brinjal mosaic virus (Isolate II)	+
3	Cucumber mosaic virus	+
4	Snake gourd mosaic virus	+
5	Bittergourd mosaic virus	
6	Pumpkin mosaic virus	+
7	Watermelon mosaic virus	
8	Chilli mosaic virus	

\+ Positive precipitin reaction
No precipitate formed

When the brinjal mosaic virus antigen was tested against the antiserum of bitter gourd mosaic virus no precipitate was formed indicating that the bitter gourd mosaic virus antiserum did not contain antibodies of brinjal mosaic virus

6 2 2 Microprecipitin test in petri dishes

A series of dilution of virus and antiserum were spotted in petri dish at regular intervals as described under materials and methods The precipitate was observed after 2 hr under a stereomicroscope with top light and black background The intensity of the precipitate was graded It was found that the antiserum titre was between 1 256 and 1 512 and the virus end point was between 1 128 and 1 256 (Table 16)

Table 16 M crop ec pitin test of brinjal mosaic v us w th its antiserum in petri dishes

Antiserum dilutions	Dilutions of sap containing b injal mosa c 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	1 819
1/1	++++	++++	+++	+++	+++	+++	+++	++						
1/2	++++	+++	+++	+++	+++	+++	+++	++						
1/4	+++	+++	+++	+++	+++	+++	++	+						
1/8	+++	+++	+++	+++	++	++	+	+						
1/16	++	+++	++	++	++	+	1	1						
1/32	++	++	+	+	+	1								
1/64	++	++	1	1	1									
1/128	+	1												
1/256	+													
1/512														
1/1024														
1/2048														
1/4096														
1/8192														

The cu ved l ne en loses the a ea of p ec p tates v s ble unde s c oscope

++ Very heavy reaction ++ Moderate reaction 1 Barely v s ble p ecip tate
++ Heavy reaction + Slight reaction No eaction

Fig. 13. Physalis minima infected with brinjal mosaic virus

Fig. 14. Solanum nigrum infected with brinjal mosaic virus

6 2 3 Ouchterlony s agar double diffusion test

This test was performed in agarose taken in petri dishes The precipitates formed due to antiserum a tige interaction were stained using amidoblack and recorded

No precipitate was obtained in the first plate Here wells 3 and 5 received distilled water 4 and 6 buffers and 2 clarified healthy sap

In the second plate thick precipitin bands were formed between the wells 1 and 3 1 and 5 and 1 and 6 (Fig 15) The central well 1 contained antiserum of brinjal mosaic virus The well 2 recieved watermelon mosaic virus and 4 recieved chilli mosaic virus Wells 3 5 and 6 received brinjal mosai virus pumpkin mosaic virus and cucumber mosaic virus respectively

In the third plate thin distinct bands were formed between the wells 1 and 3 1 and 4 1 and 5 and 1 a d 6 (Fig 16) The central well 1 contained antiserum of br al mosaic virus Well 2 received sap from infected bitter gourd plants and the well 3 was filled with cucumber mosaic vir 4 with pumpkin mosaic virus 5 with snake gourd mosaic viru a d 6 with brinjal mosaic virus All the bands formed wer fused together showing that cucumber mosaic virus pumpkin mosa c viru and snake gourd mosaic virus were serologically relate t brinjal mosaic virus No band was formed between the wells 1 a d

Fig 15 Well 1 contained antiserum of brinjal mosaic virus well 2 received water melon mosaic virus 3 with brinjal mosaic virus 4 with chilli mosaic virus 5 with pumpkin mosaic virus and 6 contained cucumber mosaic virus

Fig 16 Well 1 contained antiserum of brinjal mosaic virus well 2 received sap from infected bitter gourd plants well 3 was filled with cucumber mosaic virus 4 with pumpkin mosaic virus 5 with snake gourd mosaic virus and 6 with brinjal mosaic virus

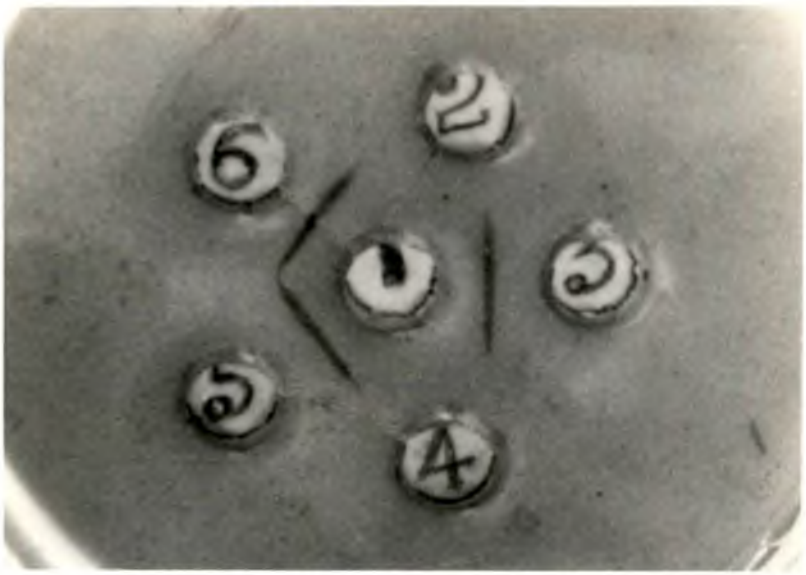

Fig. 15.

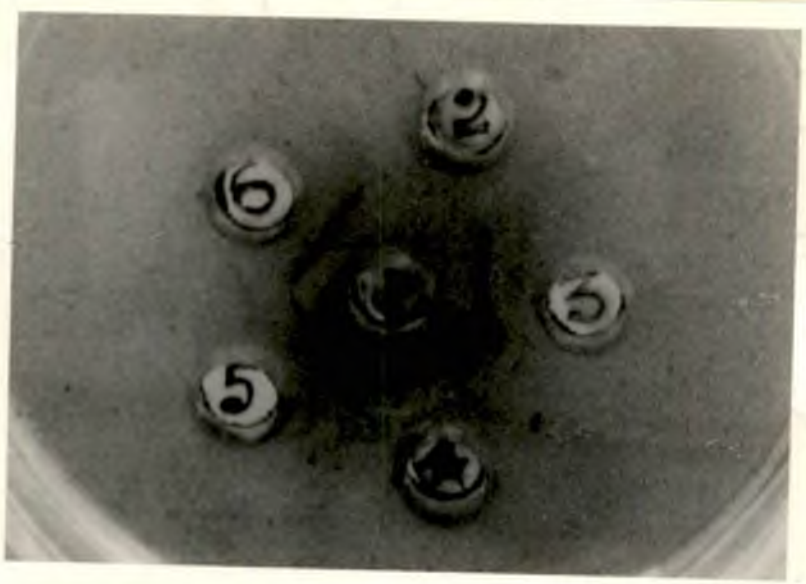

Fig. 16.

2 indicating that bitter gourd mosaic virus was not serologically related to brinjal mosaic virus

7 Varietal screening

Four varieties and eleven indigenous collections were inoculated mechanically using brinjal mosaic virus Twenty days after inoculation symptoms appeared on the newly emerged leaves Observations indicated that none of the varieties was found to be resistant to brinjal mosaic virus 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 varikai (TN local) and indigenous collection IC-089866 IC 089911 were highly susceptible with 90 per cent infection followed by NIC 01190 IC-089922 IC 111345 A IC-111449 IC 089847 and Palur 1 and the per cent infection varied from 75 80 The varieties Annamalai SM 6 and the collections IC 089822 and IC 111454 were susceptible with 55 60 per cent infection But the collections NIC 14101 and NIC 14144 got only 30 per cent infection when compared to others These two indigenous collections were somewhat tolerant to brinjal mosaic virus and it produced only mild symptoms (Table 17)

Tbale 17 Incidence of brinjal mosaic disease in different varieties and indigenous collections

Sl No	Varieties	Number of plants infected out of 10 plants inoculated		Per cent infection	Type of symptom	Time taken for symptom expression (days)
		Experiment I	Experiment II			
1	Annamalai	6	6	60	Severe	35
2	Palur-1	8	8	80	Severe	26
3	SM 6	6	6	60	Severe	28
4	Varikai	9	9	90	Severe	20
5	NIC 01190	8	8	80	Severe	26
6	NIC 14101	3	3	30	Mild	32
7	NIC-14144	3	3	30	Mild	40
8	IC 089822	5	6	55	Severe	35
9	IC 089847	7	8	75	Severe	42
10	IC 089866	9	9	90	Severe	22
11	IC 089911	9	9	90	Severe	23
12	IC 089922	8	8	80	Severe	27
13	IC 111345 A	8	8	80	Severe	26
14	IC 111449	8	8	80	Severe	40
15	IC 111454	5	6	55	Severe	33

8 **Observations on natural incidence of brinjal mosaic disease**

The natural incidence of brinjal mosaic disease due to brinjal mosaic virus are presented in Table 18 Out of 181 plants observed, 93 plants were found diseased at 110 days after planting The percentage of diseased plants at 50 days after planting was 16 02 whereas the percentage increased to 51 38 at 110 days after planting

Table 18 Observations on natural incidence of brinjal mosaic virus

Plot No	Total number of plants observed	Number of plants diseased			Healthy
		50 DAP	80 DAP	110 DAP	
1	72	12	21	38	34
2	48	9	16	26	22
3	61	8	15	29	32
Total	181	29	53	93	88
Percentage		16 02	29 28	51 38	48 61

DAP Days After Planting

9 Effect of virus on growth of the brinjal plants

9 1 Effect of infection of brinjal mosaic virus on the plant height

There was significant reduction in plant height due to the infection of brinjal mosaic virus The mean height of the infected plants was 44 26 cm as compared to the healthy plants (61 16 cm) Reduction in plant height due to infection was also observed at different days after planting The mean height of infected plants were 28 0 50 1 and 54 7 cm at 50 80 and 110 DAP respectively whereas the healthy plants had the height of 34 7 71 9 and 76 9 cm for the respective periods (Table 19)

Table 19 Effect of infection of brinjal mosaic virus on the plant height (in cm)

Treatment	Days after planting (D)			Mean
	50	80	110	
I_0 (Control)	34 7	71 9	76 9	61 16
I_1 (Infected)	28 0	50 1	54 7	44 26
Mean	31 35	61 00	65 80	

CD (I) = 3 69 CD (IxD) - 6 41 CD(D) 4 53

9 2 Effect of infection of brinjal mosaic virus on the number of leaves

Significant reduction in number of leaves was observed due to infection The mean number of leaves of the infected plants was 65 93 whereas it was 109 76 for the control healthy plants Significant differences between the means at different days interval were also observed with respect to control and infected plants The mean number of leaves for the infected plants were 50 2 66 3 and 81 3 at 50 80 110 days after planting respectively whereas the control plants produced as much as 76 2 112 4 and 140 7 leaves (Table 20)

Table 20 Effect of infection of brinjal mosaic virus on the number of leaves

Treatment	Days after planting (D)			Mean
	50	80	110	
I_0 (Control)	76 2	112 4	140 7	109 76
I_1 (Infected)	50 2	66 3	81 3	65 93
Mean	63 20	89 35	111 00	

CD (I) 9 15 CD (IxD) - 15 85 CD(D) 11 20

9 3 Effect of infection of brinjal mosaic virus on the number of branches

Viral infection had produced significant reduction in the number of branches per plant The mean number of branches produced by infected plants (8 36) was significantly lesser than that of the control plants (12 03) The interaction between control and diseased was also found to be insignificantly different The means of healthy and infected plants at different intervals are presented in Table 21

Table 21 Effect of infection of brinjal mosaic virus on the number of branches
(Mean number of branches)

Treatment	Days after planting (D)			Mean
	50	80	110	
I_0 (Control)	8 6	12 6	14 9	12 03
I_1 (Infected)	6 0	8 6	10 5	8 36
Mean	7 30	10 60	12 70	

CD (I) 1 37 CD (IxD) 2 38 CD(D) 1 68

9 4 Effect of infection of brinjal mosaic virus on the internodal length

It was observed that the infection was having a significant negative effect on the mean internodal length The mean internodal length of the infected and healthy plants were 3 6 and 6 86 cm respectively Significant difference in internodal length was noticed between infected and healthy plants with respect to days after planting and the results are presented in Table 22

Table 22 Effect of infection of brinjal mosaic virus on the internodal length
(Mean length in cm)

Treatment	Days after planting (D)			Mean
	50	80	110	
I_0 (Control)	6 52	6 84	7 23	6 86
I_1 (Infected)	4 17	3 57	3 08	3 60
Mean	5 34	5 20	5 15	

CD (I) 0 41 CD (IxD) 0 72 CD(D) - 0 51

9 5 **Effect of infection of brinjal mosaic virus on the leaf area**

The table on leaf area shows a reduction in leaf area due to infection The mean leaf area of the infected plants was 18 13 cm^2 where as it was 33 32 cm^2 for the control plants The virus infection x period interaction was found to be significant only at 110 days after planting There was no significant difference in the leaf area at 50 and 80 days after planting (Table 23)

Table 23 Effect of infection of brinjal mosaic virus on the leaf area

(Mean leaf area in cm^2)

Treatment	Days after planting (D)			Mean
	50	80	110	
I_0 (Control)	32 7	33 2	34 1	33 32
I_1 (Infected)	29 1	17 0	8 3	18 13
Mean	30 90	25 09	21 20	
CD (I) 12 85	CD (IxD) - 22 26		CD(D) - 15 74	

9 6 Effect of infection of brinjal mosaic virus on the thickness of branches

It was found that virus infection significantly reduced the thickness of branches The mean thickness of the treated plants was 2 46 cm whereas 3 23 cm for the control plants There was a considerable reduction in the thickness of branches in the infected plants at 80 days after planting as compared to healthy ones The mean thickness of branches for the treated plants were 1 68 2 21 and 3 49 cm while that of control plants were 1 77 3 62 and 4 30 cm at 50 80 and 110 DAP respectively (Table 24)

Table 24 Effect of infection of brinjal mosaic virus on the thickness of branches

(Mean thickness in cm)

Treatment	Days after planting (D)			Mean
	50	80	110	
I_0 (Control)	1 77	3 62	4 30	3 23
I_1 (Infected)	1 68	2 21	3 49	2 46
Mean	1 72	2 91	3 89	

CD (I) = 0 76 CD (IxD) = 1 32 CD(D) 0 93

9 7 Effect of infection of brinjal mosaic virus on the number of flowers

The number of flowers produced per plant was less in the case of infected plants (11 03) as compared to healthy ones (17 56) The infected plants had significantly lesser number of flowers in each of the period under study (50 80 and 110 DAP) The mean number of flowers for the infected plants were 6 2 14 3 and 12 6 at 50 80 110 days after planting whereas the control plants produced as much as 9 6 22 6 and 20 5 flowers (Table 25)

Table 25 Effect of infection of brinjal mosaic virus on the number of flowers

(Mean number of flowers)

Treatment	Days after planting (D)			Mean
	50	80	110	
I_0 (Control)	9 6	22 6	20 5	17 56
I_1 (Infected)	6 2	14 3	12 6	11 03
Mean	7 90	18 45	16 55	

CD (I) = 1 43 CD (IxD) 2 49 CD(D) 1 76

9 8 Effect of infection of brinjal mosaic virus on the fruit characters of brinjal

The results are presented in Table 26

a) Mean number of fruits

The infected plants showed a reduction in yielding ability as it was observed from number of fruits per plant The mean number of the fruits of the infected plants (5 6) was significantly lesser than that of control plants (7 36)

b) Mean length of fruits

Significant difference was observed in the mean length of fruits due to infection The mean length of the fruits of the

infected plants (6 40 cm) was significantly lesser than that of control (9 34 cm)

c) Mean girth of fruits

The results showed that there was significant reduction in the girth of fruits of the infected plants than that of the control The mean girth of the fruits of the infected plants (12 97 cm) was significantly lesser than that of control (16 81 cm)

d) Mean weight of fruits

It was noticed that the infected plants produced light fruits while the normal healthy plant produced heavy fruits The mean fruit weight of the infected plants was 0 059 kg which was less than that of the control plants (0 086 kg)

e) Total yield of fruits

With regard to total yield in relation to infection of plants by mosaic virus it was observed that the fruit yield was reduced significantly The total fruit yield of the infected plants was 8 17 kg which was significantly less than that of the control plants (9 68 kg)

Table 26 Effect of infection of brinjal mosaic virus on fruit characters

Treatment	Mean number of fruit				Mean length of fruit (cm)				Mean girth of fruit (cm)				Mean weight of fruit (kg)				Total yield of fruit (kg)			Grand total (kg)
	Days after planting			Mean	Days after planting			Mean	Days after planting			Mean	Days after planting			Mean	Days after planting			
	50	80	110		50	80	110		50	80	110		50	80	110		50	80	110	
I_0 (Control)	3 50	7 50	11 1	7 36	10 51	9 48	8 05	9 34	15 13	16 61	18 71	16 81	1 01	0 85	0 73	0 086	3 50	6 30	8 06	17 86
I_1 (Infected)	2 50	7 30	7 00	5 60	6 98	6 47	5 76	6 40	10 62	13 54	14 74	12 97	0 88	0 52	0 39	0 059	2 17	3 44	2 57	8 18
Mean	3 00	7 40	9 05		8 74	7 94	6 91		12 87	15 07	16 73		0 094	0 068	0 056		5 67	9 73	10 63	
	CD (I)	1 42			CD (I)	0 32			CD (I)	0 60			CD (I)	0 0064			CD (I)	0 219		
	CD (D)	1 74			CD (D)	1 26			CD (D)	0 73			CD (D)	0 0070			CD (D)	0 269		
	CD (IxD)	2 46			CD (IxD)	0 56			CD (IxD)	1 04			CD (IxD)	0 0110			CD (IxD)	0 379		

10 Effect of certain plant extracts on the incidence of brinjal mosaic

10 1 Preliminary screening of non host plant extracts/products for antiviral property against brinjal mosaic virus

To find out the antiviral property of plant extracts a study was conducted as described in materials and methods and the results are presented in Table 27

Table 27 Preliminary screening of non host plant extracts/ products for antiviral property against brinjal mosaic virus

Sl No	Extract/product of	Parts used	Average number of local lesions		per cent inhibition over control
			Inoculum alone (Control-C)	Inoculum + plant extract	C T x100 C
1	Adathoda vasica	leaf	18	2	88 88
2	Azadirachta indica	leaf	15	0	100 00
3	Azadirachta indica	Oil	15	0	100 00
4	Azadirachta indica	Cake	14	0	100 00
5	Boerhaavia diffusor	root	12	1	91 66
6	Bougainvillea spectabilis	leaf	12	1	91 66
7	Calotropis gigantea	leaf	10	2	80 00
8	Cassia pistula	leaf	13	2	84 61
9	Chenopodium amaranticolor	leaf	10	1	90 00
10	Clerodendron infortunatum	leaf	17	0	100 00
11	Cocos nucifera	water	15	2	86 66
12	Cyperus rotundus	root	17	1	94 11
13	Eucalyptus grandis	leaf	14	3	78 57
14	Eupatorium odoratum	leaf	18	3	83 33
15	Moringa olifera	Bark	12	2	83 33
16	Ocimum sanctum	leaf	12	0	100 00
17	Phyllanthus niruri	leaf	13	2	84 61
18	Ricinus communis	leaf	13	1	92 30
19	Thespesia populnea	leaf	12	1	91 66
20	Vinca rosea	leaf	13	1	92 30
21	Vitex negundo	leaf	15	0	100 00

Nineteen plant extracts and 2 products of Azadirachta indica were tested Among them 4 plant extracts viz Azadirachta indica Clerodendron infortunatum Ocimum sanctum Vitex negundo and 2 products of A indica viz A indica A Juss oil A indica A Juss cake inhibited the production of local lesions on Datura stramonium indicating that the extracts of these plants and products possessed antiviral property against brinjal mosaic virus

10 2 Comparative efficacy of four concentrations of plant extracts/products on the symptom development by brinjal mosaic virus

The results showed that the plant extracts of A indica C infortunatum and two products of A indica namely A indica A Juss oil, A indica A Juss cake caused 100 per cent inhibition of production of local lesions on D stramonium at four dilutions (10 20 30 and 40 per cent) tested But the extracts of other two plants namely O sanctum and V negundo caused 100 per cent inhibition of production of local lesions on D stramonium at three dilutions (20 30 and 40 per cent) only

Thus the extracts of O sanctum and V negundo were comparatively less effective than those of the other plants tested (Table 28)

Table 28 Comparative efficacy of four concentrations of plant extracts/products on the symptom development in brinjal by mosaic virus (BMV)

Sl No	Extracts of	Control (inoculum alone) (C)	Number of local lesions							
			10% plant extract		20% plant extract		30% plant extract		40% plant extract	
			Treated (extract+ inoculum)	Per cent inhibition over control $\frac{C-T}{C} \times 100$	Treated (extract+ inoculum)	Per cent inhibition over control $\frac{C-T}{C} \times 100$	Treated (extract+ inoculum)	Per cent inhibition over control $\frac{C-T}{C} \times 100$	Treated (extract+ inoculum)	Per cent inhibition over control $\frac{C-T}{C} \times 100$
1	A indica	15	0	100 00	0	100	0	100	0	100
2	A indica A Juss oil	17	0	100 00	0	100	0	100	0	100
3	A indica A Juss cake	14	0	100 00	0	100	0	100	0	100
4	C infortunatus	18	0	100 00	0	100	0	100	0	100
5	O sanctum	11	2	81 81	0	100	0	100	0	100
6	V negundo	13	2	84 61	0	100	0	100	0	100

10 3 Effect of time of application of plant extracts/products on the transmission of brinjal mosaic virus

In order to find out the effect of time of application of plant extracts/products on the transmission of brinjal mosaic virus by sap an experiment was conducted as per the procedure mentioned under materials and methods and the results are presented in Table 29 It was observed that pre inoculation application of plant extracts was more effective than post inoculation application The maximum inhibitory effect (80%) was noticed for the extract of A indica This was closely followed by C infortunatum and then by A indica A Juss oil

Table 29 Effect of time of application of plant extracts/products on the sap transmission of brinjal mosaic virus in Datura stramonium

Sl No	Extracts of	Number of local lesions					
		Pre inoculation application			Post inoculation application		
		Control (inoculum alone)	Treated with plant extract	Per cent inhibition over control	Control (inoculum alone)	Treated with plant extract	Per cent inhibition over control
1	A indica	15	3	80 00	15	6	60 00
2	A indica A Juss oil	17	4	76 47	17	7	58 82
3	A indica A Juss cake	14	5	64 28	14	7	50 00
4	C infortunatum	18	4	77 77	18	6	66 66

10 4 Effect of plant extracts/product on the transmission of brinjal mosaic virus inoculated at different intervals

In the experiment conducted to study the effect of three selected plant extracts/product on the transmission of brinjal mosaic virus by sap transmission method 90 per cent inhibition of disease development was noticed in plants inoculated upto 4 h after the application of the extracts of A indica C infortunatum and A indica A Juss oil when observations were recorded 21 days after inoculation The inhibitory effect of the plant extracts was slightly reduced when the inoculations were conducted at 12 h one day and two days after the application of plant extracts In the control all the ten inoculated plants showed symptoms within 21 days after virus inoculation (Table 30)

Table 30 Effect of plant extracts/product on the sap transmission of brinjal mosaic virus inoculated at different intervals

Sl No	Time interval of virus inoculation after plant extract application	Number of plants inoculated	Extracts of A indica		C infortunatum		A indica A Juss oil	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1	0 h	10	1	90	1	90	1	90
2	2 h	10	1	90	1	90	1	90
3	4 h	10	1	90	1	90	1	90
4	12 h	10	2	80	2	80	2	80
5	1 day	10	2	80	3	70	3	70
6	2 days	10	3	70	4	60	3	70
	Control	10	10	0	10	0	10	0

DISCUSSION

DISCUSSION

The virus causing mosaic disease of brinjal (Solanum melongena L) was investigated The disease was noted in different parts of Kerala in severe form and as a result of this there was considerable reduction in the fruit yield The initial symptoms appeared as small light green areas followed by typical mosaic mottling Leaves emerged subsequent to infection showed the characteristic symptoms of mosaic mottling with dark green and light green patches The leaf size was very much reduced and deformed Diseased plants remained stunted and produced only a few flowers and fruits The symptoms of mosaic disease of brinjal observed in the present study are in close relationship with the findings of Ferguson (1951) Irregular light and dark green patches deformation crinkling of the leaves vein clearing and no fruit production have been observed due to brinjal mottled dwarf virus infection (Martelli and Hamadi 1986)

The virus was found to be transmissible through sap inoculation More percentage of virus transmission was obtained with inoculum prepared in phosphate buffer followed by distilled water Several fold increase in the infectivity of cucumber mosaic virus with the use of phosphate buffer has been reported (Foster 1972) But 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

The virus was transmitted to brinjal plants by wedge grafting Thomas (1938) found that brinjal mosaic virus could be transmitted to brinjal and Datura fatuosa by grafting Naqvi and Mahmood (1976) also observed the graft transmission of brinjal mild mosaic virus to brinjal

Seeds from fruits of mosaic infected brinjal plants were found to carry the virus The percentage of seed transmission was 40 in SM 6 variety The present finding is in agreement with that of Mayee (1974) and he reported the seed transmission of brinjal mosaic virus upto 41 8 per cent Sriram (1994) also observed the seed borne nature of brinjal mosaic virus and here the percentage of seed transmission was only 28 2

The insect transmission of brinjal mosaic virus was tested using M persicae A gossypii A craccivora A malvae H vigintioctopunctata L bombayensis B tabaci and S biguttula biguttula and it was found that M persicae could transmit brinjal mosaic virus in a very efficient manner giving 60 per cent transmission followed by A gossypii giving 40 per cent and A craccivora giving 25 per cent and A malvae H vigintioctopunctata giving only 20 per cent transmission The beetle L bombayensis the white fly B tabaci and the leaf hopper S biguttula biguttula failed to transmit the virus

During the survey M persicae A gossypii and A craccivora were seen on the brinjal plants and these insects must be the reason for the spread of the mosaic disease in the field Verma and Rashmi Lal (1967) found that the aphid M persicae could act as a vector of mosaic disease of brinjal Seth et al (1967) also observed that the cucumber mosaic virus infecting brinjal was transmitted by A gossypii A craccivora and M persicae Brinjal mosaic disease caused by CMV has been transmitted by M persicae and A fabae (Shawkat and Fegla 1979)

The vector-virus relationship was studied using the most efficient vector M persicae The minimum acquisition feeding period required by M persicae for the transmission of brinjal mosaic virus (BMV) was found to be 20 s If the acquisition feeding period was increased the percentage of virus transmission was also increased and the maximum transmission of 65 per cent was obtained when acquisition feeding period of 25 min was given If the acquisition feeding period was further increased the efficiency of transmission of the virus was reduced and it was only 5 per cent when the acquisition feeding period was 3 h (Table 5) The minimum inoculation threshold of brinjal mosaic virus required by M persicae was found to be 30s The per cent transmission was found to increase with increase in the inoculation feeding period and attained the maximum of 85 per cent when 1 h inoculation feeding period was given If the

inoculation feeding period was further increased the per cent transmission was decreased and reached 55 per cent with 48 h inoculation feeding period (Table 6) Verma and Rashmi Lal (1967) observed that the viruliferous aphid M persicae transmitted the brinjal mosaic virus only after 5 10 minutes feeding on the infected plant and not when fed for 24 48 hours Pumpkin mosaic virus could be transmitted by A gossypii with minimum acquisition feeding period and inoculation feeding period of 20 s and 10 s respectively (Singh 1981) In the present studies the influence of factors viz efficiency of the vector type of host climatic factors etc might be the reason for the differences in the minimum acquisition feeding period and inoculation feeding period required by M persicae to transmit brinjal mosaic virus

Investigations on the influence of pre acquisition fasting proved that fasting of M persicae before acquisition of the virus resulted an increase in the per cent of transmission of the virus The transmission percentage of the virus was found to increase with the increase in pre-acquisition fasting period and reached the maximum of 65 per cent when 1 h pre acquisition fasting was given and there after the same was decreased The efficiency of the vector to transmit the virus was completely lost when starved for 12 h (Table 7) 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 1971a Jaganathan and Ramakrishnan 1971 Singh 1972 1981 and 1982 Joseph and Menon 1978) Jaganathan and Ramakrishnan (1971) observed that the maximum transmission was obtained when A gossypii was starved for 60 min Singh et al (1975) found that an optimum of 90 min pre acquisition fasting was required by A gossypii and A craccivora for the maximum transmission of Solanum torvum mosaic virus The results of the present experiment also showed a similar trend

Post acquisition fasting of M persicae caused a steady decrease in the per cent transmission of brinjal mosaic virus The efficiency of the vector to transmit the virus was completely lost after 2 h post acquisition starvation The maximum infection of 50 per cent was obtained when no post acquisition fasting was given (Table 8) 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 effi ciency of the viruliferous vector A craccivora to transmit snake gourd mosaic virus was completely lost when starved for 4 h (Joseph and Menon 1978) Similar type of results were obtained in the present studies

Trials on the retention of infectivity by M persicae showed that the vector lost its infectivity after 1 h in all the 8 series of experiments carried out This proved the non persistent nature of the virus inside the vector for longer

period (Table 9) Seth et al (1967) observed that brinjal mosaic virus was transmitted by A gossypii A craccivora and M persicae in a non persistent manner A gossypii could retain the infectivity of pumpkin mosaic virus for 2 h (Singh 1981) Non persistent manner of transmission of snake gourd mosaic virus (Cucumis virus 1) by A gossypii and A craccivora had been observed (Dubey et al 1974) Since the vector (M persicae) lost its infectivity after 1 h of acquiring the virus the transmission of brinjal mosaic virus can be termed as non-persistent type as suggested by Nagarajan and Ramakrishnan (1971c)

The minimum number of aphids required for successful transmission of brinjal 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 Group of 10 aphids per plant could produce the maximum infection of 65 per cent and there after the per cent transmission was decreased (Table 10) Singh et al (1975) found that even a single viruliferous A craccivora and a minimum of 3 aphids of A gossypii were able to transmit Solanum torvum mosaic virus but the maximum percentage of transmission was obtained with groups of 15 aphids per plant in both the species Similar results were also obtained in the transmission of water melon mosaic virus to water melon pumpkin squash and cucumber by M persicae (Almeida and Borges 1983)

They got 70 per cent transmission with one aphid and 100 per cent with seven or more aphids

Host range studies of the virus with 52 plant species belonging to 17 families showed that the virus under study was confined to the members of the families chenopodiaceae cucurbitaceae and solanaceae It was found that brinjal mosaic virus could produce visible systemic symptoms on C moschata C sativus M charantia T anguina C annuum N glutinosa N tabacum P minima and S nigrum and local lesions on C quinoa and D stramonium Ferguson (1951) found that brinjal mosaic virus produced necrotic circular lesions followed by chlorotic ring spots on the leaves of N glutinosa Verma et al (1970) while working with Cucumis virus 2B causing mosaic disease of snake gourd observed that the virus could produce systemic symptoms only on the members of the family cucurbitaceae and local lesions on C amaranticolor Nagarajan and Ramakrishnan (1971b) observed that host range of water melon mosaic virus was found restricted to cucurbitaceae alone Shankar et al (1972) found that the host range of pumpkin mosaic virus was restricted to members of the family cucurbitaceae The host range of Cucumis virus I causing mosaic disease of snake gourd was restricted to members of the families cucurbitaceae compositae solanaceae and chenopodiaceae Among these distinct local lesions were produced on C quinoa and D stramonium belonging to chenopodiaceae and solanaceae and hosts belonging to other families produced systemic symptoms (Dubey et al 1974)

N tabacum and M charantia were reported to be the hosts of brinjal mosaic virus (Verma and Rashmi Lal 1967 Naqvi and Mahmood 1976 Rao 1978) C sativus and C annuum were also reported to be the hosts of brinjal mosaic virus (Kemp and Troup, 1977 Igwegebe and Waterworth 1982) S nigrum was found to be the host of brinjal mosaic virus and produced systemic mosaic symptoms (Verma and Rashmi Lal 1967 Naqvi and Mahmood 1976 Igwegebe and Waterworth 1982 Sriram 1994) C quinoa was reported to produce local lesions when artificially inoculated with brinjal mosaic virus (Souza et al 1990 Gupta et al 1992) D stramonium was reported to be the weed host of the brinjal strain of cucumber mosaic virus (Naqvi and Mahmood 1976 Shawkat and Fegla 1979 Gupta et al 1992) A comparison of the host range and local lesion hosts of different viruses infecting brinjal plants revealed that the virus under study shows a close relationship 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)

The dilution end point (DEP) of the virus was between 1 1000 and 1 10000 and thermal inactivation point (TIP) was between 60 and 65°C and longevity in vitro (LIV) was between 96 and 120 h at room temperature (28 30°C) and 144 and 168 h under refrigeration (10°C) Tomato ring spot virus infecting different host plants including brinjal was found to have a TIP of 56 58°C (Samson and Imle 1942) Brinjal mosaic virus infecting brinjal

was found to have a TIP of 78°C and LIV of 21 days at room temperature (Dale 1954) According to Kovachevski (1965) lucerne mosaic virus isolated from brinjal had a TIP of 63°C DEP of 1 5000 and LIV of 3 days in sap Verma and Rashmi Lal (1967) found that the brinjal mosaic virus infecting N tabacum had a DEP of 1 1000 and TIP of 65°C and LIV of 6 days at room temperature Brinjal mottled dwarf virus had a DEP between 10^3 and 10^4 TIP between 51 and 57°C and LIV 30 and 44 h at 4°C (Martelli and Rana 1970) Shawkat and Fegla (1979) isolated cucumber mosaic virus from naturally infected brinjal and the DEP was 1 1000 TIP was 65°C and LIV was 4-8 days Vyanjane and Mali (1981) found that alfalfa mosaic virus infecting brinjal had a DEP of 1 1000 TIP of 50 55°C and LIV of 24 h at room temperature Tobacco mosaic virus isolated from brinjal was found to have a DEP of 10^6 TIP of 90°C and LIV for a long period at room temperature (Gupta et al 1992) A comparison of the physical properties of different viruses infecting brinjal plants revealed that the virus under study showed a close similarity with cucumber mosaic virus as described by Shawkat and Fegla (1979)

Serological studies were conducted with a view to identify the virus The results of the microprecipitin test on slides showed that antigens of brinjal mosaic virus isolate I (obtained from diseased plants in glass house) brinjal mosaic virus isolate II (obtained from diseased plants in the field) cucumber mosaic virus snake gourd mosaic virus and pumpkin

mosaic virus produced dense precipitate with the antiserum specific to brinjal mosaic virus and no serological relationship was obtained with bittergourd mosaic virus water melon mosaic virus and chilli mosaic virus

Microprecipitin test in petri dishes was useful to find out the antiserum titre and the virus end point of brinjal mosaic virus It was found that the antiserum titre was between 1 256 and 1 512 and the virus end point was between 1 128 and 1 256 In the present studies brinjal mosaic virus cucumber mosaic virus pumpkin mosaic virus and snake gourd mosaic virus reacted positively with the antiserum of brinjal mosaic virus This indicates that brinjal 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 In the first plate 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 antigens of water melon mosaic virus brinjal mosaic virus chilli mosaic virus pumpkin mosaic virus and cucumber mosaic virus The precipitin bands formed between the wells containing antiserum and brinjal mosaic virus antigen pumpkin mosaic virus antigen and cucumber mosaic virus antigen indicated the presence of antibodies specific to brinjal mosaic

virus, pumpkin mosaic virus and cucumber mosaic virus in the antiserum (Fig 15) The third plate contained antiserum for brinjal mosaic virus in the central well and the surrounding wells contained bitter gourd mosaic virus cucumber mosaic virus pumpkin mosaic virus, snake gourd mosaic virus and brinjal mosaic virus The appearance of preciptin bands between the wells containing antiserum and cucumber mosaic virus pumpkin mosaic virus snake gourd mosaic virus and brinjal mosaic virus and the fusion of all four bands indicated that all the four viruses were serologically very much related (Fig 16) It is 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 wells containing bitter gourd mosaic virus antigen water melon mosaic virus antigen and chilli mosaic virus antigen indicated that the antiserum did not contain antibodies against bitter gourd mosaic virus water melon mosaic virus and chilli mosaic virus and thus these viruses are not serologically related to brinjal mosaic virus

In the varietal trial carried out with four varieties and eleven indigenous collections of brinjal it was found that all the varieites and collections were susceptible to the virus in varying degrees The symptoms appeared within 20 42 days after inoculation Eventhough all the varieties and collections were susceptible to the virus there was some variation in the

percentage of infection in the inoculated plants Varikai IC-089866 and IC 089911 were the most susceptible variety and indigenous collections which gave 90 per cent infection The indigenous collections NIC-01190 IC 089922 IC 11345 A IC 111449 IC 089847 and the variety Palur-1 gave 75 80 per cent infection In the present studies the collection NIC 14101 and NIC-14144 were less susceptible with an infection of only 30 per cent (Table 17) Mayee and Khatri (1975) screened 8 varieties and 11 collections of brinjal to evaluate the resistance against brinjal mosaic virus but none of them were seen resistant to the virus

The observations on natural incidence of brinjal mosaic virus carried out in and around Vellayani area indicated that the percentage of brinjal mosaic virus infection was more at the later stage of the crop than compared to the initial stage At 110 days after planting there was 51 38 per cent virus infected plants This might be due to the increase in the inoculum and vector population in the field Sriram (1994) observed that the natural incidence of brinjal mosaic was upto 30 per cent in the villages surrounding Coimbatore district and in Anna district it was 90 per cent

The impact of brinjal mosaic virus on growth of brinjal plants using the variety SM 6 was studied and the observations on height, number of leaves number of branches, internodal length leaf area thickness of branches number of flowers and fruits

length, girth, weight and total yield of the fruits were taken at 50 80 and 110 days after planting There was significant reduction in the number of leaves leaf area and internodal length of brinjal plants due to virus infection The number of flowers and fruits produced in the virus infected plants were also significantly lesser than the control (healthy) plants Thomas (1938) while investigating mosaic disease of brinjal observed a considerable reduction in the number of nodes absence of flowers and sterility Brinjal mosaic virus infected plants exhibited pronounced leaf yellowing stunting and reduced development of the flowers and fruits (Seth et al 1967) In the present study in general there was considerable reduction in all the fruit characters of virus infected plants, viz length of fruits, girth of fruits weight of fruits and yield of fruits when compared to that of healthy plants The mean fruit yield of the healthy plants was 1 80 kg whereas in the case of infected plants it was only 0 78 kg, showing a reduction of 1 02 kg Brinjal mosaic virus affected plants were found to be stunted and size of the fruits was reduced (Ho and Li 1936) An yield reduction of 25 per cent due to brinjal mosaic virus infection on brinjal has been observed (Dale 1954) Each 1 0 per cent disease incidence of brinjal mosaic virus caused an yield loss of 1 5 per cent (Singh and Singh 1975)

The inhibitory effect of certain plant extracts/ products on the incidence of brinjal mosaic virus was studied

during the present investigation Extracts of A indica C infortunatum O sanctum and V negundo and 2 products of A indica viz , A indica A Juss oil and A indica A Juss cake were found to possess antiviral property against brinjal mosaic virus based on the ability to inhibit the production of local lesions on the leaves of D stramonium The leaf juice of O sanctum possessed potent virus inhibitors against top necrosis virus of pea and it was effective at 1 1000 dilution (Roy et al 1979) Seed oil from A indica inhibited rice tungro virus (Mariappan et al 1982) Leaf extracts of C fragrans C aculeatum and C inerme inhibited plant viruses like yellow mosaic of mung and urd beans sunnhemp rosette virus and tobacco mosaic virus respectively (Verma and Prasad 1987 and Nagarajan and Murthy 1988) Extracts of V negundo has been reported to have antiviral property against rice tungro virus (Gurubasavaraj 1988) Cowpea mosaic virus has been inhibited by extracts of A indica and V negundo (Mallika devi 1990) Brinjal mosaic virus has been inhibited by A indica and A indica A Juss oil in the field trial (Sriram 1994)

When four concentrations viz 10 20 30 and 40 per cent of the above 4 plant extracts and 2 products of A indica were further tested for inhibition of local lesion on the leaves of D stramonium it was noticed that all the concentrations of A indica A indica A Juss oil A indica A Juss cake and C infortunatum could cause cent per cent inhibition The extracts

of O sanctum and V negundo could cause cent per cent inhibition at all the concentrations tested except 10 per cent Saigopal et al (1986) observed that the percentage inhibition of TMV, tobacco ringspot virus and groundnut green mosaic virus was decreased as the dilution of extract of Phyllanthus niruri increased No inhibitory effect of the extract was noticed at 1 50 dilution Eighty per cent inhibition of tomato spotted wilt virus with 1 10 dilution of the leaf extracts of chilli acacia datura and chenopodium plants has been observed (Joi et al , 1988) Sriram (1994) found that leaf extracts of A indica (10%) and A indica A Juss oil (5%) gave 25 79 per cent and 18 19 per cent reduction in the incidence of brinjal mosaic disease respectively, over control

In the experiment conducted to study the comparative efficacy of pre inoculation and post inoculation application of plant extracts in inhibiting the brinjal mosaic virus infection in Datura plants It was observed that the pre inoculation application of plant extracts was more effective than post inoculation application The extract of A indica caused the maximum inhibition of 80 per cent closely followed by C infortunatum and A indica A Juss oil (Table 29) These results are in conformity with those reported by Verma and Mukerjee (1975) Rao and Shukla (1985) Aiyanathan and Narayana swamy (1988) and Mallika devi (1990)

When the extracts of two non host plants viz A indica and C infortunatum and one plant product A indica A Juss oil were tested against sap transmission of brinjal mosaic virus on brinjal plants at different time intervals 90 per cent of inhibition of disease development was noticed in plants applied with the above extracts upto 4 h after the application (Table 30) Mallika devi (1990) observed 90 per cent inhibition of cowpea mosaic disease development in cowpea plants treated with extracts of A indica P niruri and V negundo upto two days after the application Selvaraj (1990) found that antiviral principles from seed extracts of Tribulus terrestris and leaf extract of vitex negundo var purpurascense reduced rice tungro virus infection upto 45 per cent and 41 per cent respectively over control and also increased the incubation period and the extract of P niruri exhibited higher inhibitory effect than those of the other plants even upto six days after the application of extract

SUMMARY

SUMMARY

Mosaic disease of brinjal (Solanum melongera L) prevalent in Kerala was investigated

The initial symptoms appeared as small light green areas followed by mosaic mottling in 16 20 days after sap inoculation Typical mosaic mottling with dark green and light green patches was produced in all the leaves emerged to infection The leaves were very much reduced in size and deformed Diseased plants remained stunted and produced only a few flowers and fruits

Transmission studies showed that the virus could be transmitted through mechanical means grafting seeds of diseased brinjal plants and by vectors Sap extracted in phosphate buffer gave the maximum infection of 60 per cent followed by distilled water giving 50 per cent infection while tris buffer gave the minimum infection of 30 per cent There was 90 per cent graft transmission The virus was transmissible through seeds of diseased brinjal plants Seed transmission upto 40 per cent was recorded The virus was transmitted by the vectors Myzus persicae Aphis gossypii Aphis craccivora Aphis malvae and Henosepilachna vigintioctopunctata Among them M persicae was found to be the most efficient vector The percentages of transmission obtained by M persicae A gossypii A craccivora

A malvae and H vigintioctopunctata were 60 40 25 20 and 20 respectively

Studies on vector virus relationships showed that the minimum feeding period required for the vector (M persicae) to acquire the virus was 20 s and that the virus could be transmitted with an inoculation feeding period of 30 s But the percentage of transmission was the maximum when an acquisition feeding period of 25 min and inoculation feeding period of 1 h were given

Influence of fasting of the vector before acquisition and inoculation feedings proved that pre acquisition fasting for a period of 1 h resulted the maximum transmission whereas post acquisition fasting decreased the per cent infection The efficiency of the vector to transmit the virus was completely lost at 2 h post acquisition starvation The retention of infectivity by the vector was found to be 1 h Even a single viruliferous vector was able to transmit the virus to healthy test plants, but the maximum percentage of infection was obtained with 10 aphids

The virus was found to have its host range in the members of the families chenopodiaceae cucurbitaceae and solanaceae It produced systemic symptoms on Cucurbita moschata Duch Cucumis sativus L Momordica charantia L Trichosanthes anguina L Capsicum annuum L Nicotiana glutinosa L Nicotiana

tabacum L Physalis minima L and Solanum nigrum L Distinct necrotic local lesions were produced by the virus on Chenopodium quinoa Willd and Datura stramonium L Among these the plants Cucurbita moschata Trichosanthes anguina Nicotiana glutinosa and Physalis minima produced systemic symptoms were the new hosts of brinjal mosaic virus

Studies on the physical properties of the virus revealed that the dilution end point of the virus was between 1 1000 and 1 10000 and thermal inactivation point between 60 and 65°C Longevity in vitro of the virus was between 96 and 120 h at room temperature (28-30°C) and between 144 and 168 h at 10°C

Studies on serological properties indicated that brinjal mosaic virus is related to cucumber mosaic virus pumpkin mosaic virus and snake gourd mosaic virus The virus showed no serological relationship with other cucurbit viruses viz , bitter gourd mosaic virus and water melon mosaic virus and chilli mosaic virus The antiserum titre and virus end point in the present study were found to be between 1 256 and 1 512 and 1 128 and 1 256 respectively

Based on the results on symptomatology transmission physical properties host range and serological properties the brinjal mosaic virus under study was identified as Cucumis virus 1

The varietal screening carried out with four varieteis and eleven indigenous collections of brinjal revealed that all the varieties and collections were susceptible to brinjal mosaic virus NIC-14101 and NIC-14144 were least susceptible with 30 per cent infection whereas varikai, IC 089866 IC-089911, NIC 01190 IC 089922 IC-111345 A IC 111449 IC-089847 and Palur 1 were found to be highly susceptible

Brinjal mosaic virus disease was more serious in and around Vellayani area A high percentage of infected plants (51 38 per cent) proved that the brinjal mosaic virus was the most serious disease in this region

Studies on the effect of virus infection on growth of brinjal plants showed that there was significant reduction in the height number of leaves leaf area internodal length number of flowers, number of fruits and other fruit characters viz , length of the fruits, girth of the fruits weight of the fruits and total yield of the fruits

The inhibitory effect of certain plant extracts on the incidence of brinjal mosaic virus was studied during the present investigation and found that out of the nineteen crude plant extracts and two products of Azadirachta indica tested extracts of four plants viz A indica, Clerodendron infortunatum Ocimum sanctum Vitex negundo and two products of A indica viz A

indica A Juss oil and A indica A Juss cake inhibited the production of local lesions on the leaves of D stramonium indicating that these extracts possessed antiviral property against brinjal mosaic virus

When the extracts/products of the above plants were further tested at four concentrations (10 20 30 and 40 per cent) it was observed that the extracts of A indica A indica A Juss oil A indica A Juss cake C infortunatum caused 100 per cent inhibiton of the production of local lesions on the leaves of D stramonium even at 10 per cent concentration

Pre inoculation application of plant extracts was more effective than post inoculation application in checking the disease incidence The maximum inhibitory effect was obtained with extract of A indica closely followed by C infortunatum and A indica A Juss oil

The maximum inhibition of diease development was noticed in plants inoculated with brinjal mosaic virus upto 4 h after the application of ten per cent extracts of A indica C infortunatum and A indica A Juss oil The inhibitory effect of plant extracts was found to be reduced when virus inoculations were made at 12 h one day and two days after the application of plant extracts

REFERENCES

REFERENCES

Aiyanathan K E A and Narayanasamy P (1988) Effect of neem on RTV infection with different levels of resistance In Proceedings of National Seminar on Management of crop diseases with Plant Products/Biological agents Jan 11-12 pp 40

Almeida M M and Borges M (1983) Watermelon mosaic virus in Portugal Characteristics Serology Ultra structural aspects of the host Revista de Biologia 12 327 328

*Al-musa, A M and Lockhart, B (1990) Occurrence of eggplant mottled dwarf virus in Jordan J Phytopath 128 283 287

Bawden F C (1954) Inhibitors and plant viruses Adv Virus Res 2 31 57

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

Betti L (1992) Tomato spotted wilt virus on eggplant in Sicily Phytopathologia Mediterranea 31 119 120

Brakke M K (1960) Density gradient centrifugation and its application to plant viruses Adv Virus Res 7 193 224

Dale W T (1954) Sap transmissible mosaic diseases of solanaceous crops in Trinidad Ann appl Biol 41 240 247

Debrot E A Lastra R and de Ugcategul R C (1977) Solanum seaflorthianum a weed host of eggplant mosaic virus in Venezuela Plant Dis Rep 61 628 631

Doraisamy S and Ramakrishnan K (1988) Inhibition of tobacco mosaic virus In Proceedings of National Seminar on Management of crop diseases with Plant Products/Biological agents Jan 11 12 pp 54

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

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

Ferguson I A C (1951) Four virus diseases of solanaceous plants in Trinidad Plant Dis Rep 35 102

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

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

Gibbs A J Nixon H L and Woods R D (1963) Properties of purified preparations of lucerene mosaic virus Virology 19 441 449

Gibbs A J and Harrison B D (1969) Eggplant mosaic virus and its relationship to Andean Potato latent virus Ann appl Biol 64 225 231

Gooding G V and Hebert T T (1967) A simple technique for purification of tobacco mosaic virus in large quantities Phytopathology 57 1285

*Gupta V P and Naqvi Q A (1991) Inhibitory activities of plant extracts on virus infection Act Bot Indica 19 18 24

*Gupta V P Naqvi Q A and Garg I D (1992) A strain of tobacco mosaic virus causing necrotic mosaic disease of brinjal in India Int J Trop Plant Dis 10 51 59

Gurubasavaraj T M (1988) Effect of Botanicals for the control of rice tungro virus disease M Sc (Ag) Thesis Agricultural College and Research Institute Madurai

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

*Holmes F O (1932) Symptoms of tobacco mosaic disease Contrib Boyce Thompson Inst 3 323 357

*Ho W T H and Li L Y (1936) Preliminary notes on the virus diseases of some economic plants in Kwangtung Province Lingnan Sci 1 67 78

Iengo C Alioto D and Ragozzino A (1986) Mottled dwarf of aubergines in Campania Informatore Fitopatologico 36 37 41

Igwegbe E C K and Waterworth H E (1982) Properties and serology of a strain of pepper veinal necrotic virus isolated from eggplant (Solanum melongena L) in Nigeria Phytopath Z 103 9 12

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

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

Joi M B Patil B H and Ruikar S K (1988) Inhibition studies on tomato spotted wilt virus in tomato by leaf extracts In Proceedings of National Seminar on Management of crop diseases with Plant Products / Biological agents Jan 11 12 pp 51

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

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

Kassanis B and Kleczkowski A (1948) The isolation and some properties of a virus inhibiting protein from Phytolacca esculenta J Gen Microbiol 2 143 153

Kemp W J and Troup P A (1977) Alfalfa mosaic virus occurring naturally on eggplant in Ontaria Plant Dis Rep 61 393 396

Kerala Agricultural University (1993) Package of practices Recommendations 1993 Director of Extension Mannuthy 680 651 Kerala India pp 237

Khalil J K Nelson M R and Wheeler R E (1982) Host range purification serology and properties of a carlavirus from eggplant Phytopathology 72 1062 1068

*Kovachevski I (1965) Lucerne mosaic virus in Bulgaria Rastenier Nauki 2 89 108

*Kulkarni G S (1924) Mosaic and other diseases of crops in the Bombay presidency Poona Agric Coll Mag 16 6 12

Ladipo J L (1976) A green mosaic disease of eggplant (Solanum melongena) in Nigeria Plant Dis Rep 60 1068 1072

Ladipo J L Lesemann D E and Koenig R (1988) Further studies on a virus causing green mosaic disease of eggplant in Nigeria Phytopath Z 121 159 165

Lisa V Boccardo G Agostino G D Dellavake G and Aquilio M D (1981) Characterization of potyvirus that causes zucchini yellow mosaic Phytopathology 71 667 672

Makkouk K M Koening R and Leseman D E (1981) Characteri zation of a tombus virus isolated from eggplant Phytopathology 71 572 577

Mallika devi S (1990) Inhibitory effects of certain plant extracts on the incidence of cowpea mosaic M Sc (Ag) Thesis Kerala Agricultural University Thrissur

Mamula D Plese N and Juretic N (1977) Spontaneous occu rrence of CMV on Echinocystis lobata Leycesteria formosa and Solanum melongena in Yugoslavia Act Bot Croatica 36 29 37

Marchie D C and Igwegbe E C K (1987) Some properties of two viruses isolated from eggplant growing areas in Anombra state Nigeria Samari J Agri Res 5 51 57

Mariappan V Saxena R C and Ling K C (1982) Effect of custard apple oil and neem oil on the lifespan of the rice tungro virus transmission by Nephotettix virescens Int Rice Res Newsl 7 13 14

Martelli G P (1969) Bacilliform particles associated with mottled dwarf of eggplant (Solanum melongena L) J Gen Virol 5 319 320

Martelli G P and Cirulli M (1969) Mottled dwarf of eggplant (Solanum melongena L) a virus disease Annales de Phytopathologie 1 393 397

Martelli G P and Rana G L (1970) Mechanical transmission of the causal agent of eggplant mottled dwarf disease Phytopath Medit 9 187 191

Martelli G P and Hamadi A (1986) Occurrence of eggplant mottled dwarf virus in Algeria Plant Pathology 35 595 597

Martelli G P and Cherif C (1987) Eggplant mottled dwarf virus associated with vein yellowing of honeysuckle J Phytopathology 9 315 317

Mayee C D (1974) Systemic lesions of seed borne brinjal mosaic virus in some varieties of brinjal Curr Sci 43 691 693

Mayee C D and Khatri H L (1975) Seed transmission of brinjal mosaic virus in some varieties of brinjal Indian Phytopath 28 238 240

Mckeen C D (1956) The inhibitory activity of extract of Capsicum frutescens on plant virus infections Can J Bot 34 891-903

Morita K Arai K Doi Y and Hora K (1974) Necrotic leaf spot a new virus disease of eggplant caused by broad bean wilt virus Annals of the Phytopathological Society of Japan 40 288 294

Nagarajan K and Ramakrishnan K (1971a) 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 (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 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 Murthy N S (1988) Screening of plants for antiviral properties against Tobacco mosaic virus In Proceedings of National Seminar on Management of crop diseaes with Plant Products / Biological agents Jan 11 12 pp 52

Naqvi Q A and Mahmood K (1976) Studies on brinjal mild mosaic virus Indian Phytopath 29 138 142

Naqvi Q A and Mahmood K (1978) Purification and properties of brinjal mild mosaic virus Phytopath Z 93 86 89

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 plant 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

Pasannavar N B and Yarguntiah R C (1988) Studies on a mosaic disease of brinjal (Solanum melongena L) Plant Pathology Newsletter 6 49 51

Raj S K Aslam M Srivastava R M and Singh B (1989) Occurrence and identification of eggplant mottled crinkle mosaic virus in India Phytopathology 125 283 288

*Rao D R (1978) A new eggplant mosaic disease Z. pflkrankh. 85 313 316

Rao G P and Shukla K (1985) Antiviral activity of copra extract and physical properties of the inhibitor Indian Phytopath 38 623

Reddy V A and Jeyarajan (1988) Effect of leaf extracts of Polyalthia longifolia and neem on infection of rice plants by yellow dwarf pathogen In Proceedings of National Seminar on Management of crop diseases with Plant Products/Biological agents Jan 11 12 pp 44

Reddy K R Rao G N and Sastry K S (1988) Inhibition of cucumber mosaic virus in safflower by plant extracts In Proceedings of National Seminar on Management of crop diseases with Plant Products/Biological agents Jan 11 12 pp 49

Roy A N Sinha B P and Gupta K C (1979) The inhibitory effect of plant juices on the infectivity of top necrosis virus of pea Indian J Microbiol 19 198 201

Raychaudhury R and Jain R K (1989) Effect of neem and lemon grass products on some aphid vectors of plant viruses Indian Phytopathol 42 280

Saigopal D V R Prasad V S and Sreenivasulu P (1986) Antiviral activity in extracts of Phyllanthus fraterns Webst (P niruri) Curr Sci 55 264 265

*Salamon P Dezsery M and Bujdoso G (1983) Viruses and virus diseases of cultivated and wild Solanaceae in Hungary I Occurrence and aetiology of aubergine (Solanum melongena L) yellow mosaic Novenyvédelem 19 433 440

Samson R W and Imle E P (1942) A ring spot virus disease of Tomato Phytopathology 12 1037 1047

Sastry K S (1982) Studies on the identification of mosaic diseases of brinjal (Solanum melongena L) in Karnataka Curr Sci 51 568 569

Sastry K S and Nayudu M V (1978) Studies on Tobacco ring spot virus from brinjal (Solanum melongena L) with particular reference to purification and assessment of losses Curr Sci 22 846 848

Schuster M F (1963) Flea beetle transmission of tobacco ring spot virus in the Lower Rio Grande Valley Plant Dis Rep 47 510 511

*Scott H A (1976) Desmodium yellow mottle virus No 168 Descriptions of plant viruses Kew Surrey U K 5 pp

Selvaraj C (1990) Management of major rice diseases by plant extracts and chemicals Department of Plant Pathology Centre for plant protection studies Agricultural College and Research Institute TNAU CBE 641003

*Semel J (1960) Effect of the extract from Begonia tuberhybrida on the number of local lesions formed by tobacco mosaic and other viruses Bull Inst Agron Gembloax 28 433 444

Seth M L Raychaudhury S P and Nath R (1967) A new mosaic disease of brinjal (Solanum melongena L) Phytopath Z 69 385 389

Shankar G Nariani T K and Prakash N (1972) Studies on Pumpkin mosaic virus (PMV) with particular reference to purification electron microscopy and serology Indian J Microbiol 12 154 165

Sharma D C (1969) A comparative study of sap transmissible viruses isolated from mosaic infected brinjal (Solanum melongena L) Phytopath Z 65 341 357

Shawkat A L B and Fegla G I (1979) Identification of two viruses from eggplant and Cucurbita pepo in Iraq Plan Dis Rep 63 235 238

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

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

Singh S J (1982) Studies on the transmission of pumpkin mosaic virus by aphid (Aphis craccivora koch) Z Pflanzen Physiol 89 79 87

Singh S J Sastry S M and Sastry K S (1975) Studies on a mosaic disease of Solanum torvum L Indian Phytopath 28 209 211

Singh B R and Singh D R (1975) Brinjal mosaic incidence and effect on yield Indian J Plant Pathol **11** 21 29

Singh I and Varma J P (1981) Virus inhibitor from Datura metel Indian Phytopath **34** 452 457

Souza V B V DE Gergerich R C Kim K S and Langham M A C (1990) Properties and Cytopathology of a tymovirus isolated from eggplant Phytopathology **80** 1092 1098

Sriram S (1994) Studies on mosaic disease of brinjal (Solanum melongena L) M Sc thesis Tamil Nadu Agricultural University Coimbatore

Thomas K M (1938) Studies on brinjal mosaic virus with particular reference to graft transmission and yield loss Detailed administration Report of the Government Mycologist Madras pp 21

Tobias L Rast A Th B and Maat D Z (1982) Tobamoviruses of pepper eggplant and tobacco comparative host reactions and serological relationships Neth J Pl Path **88** 257 268

Usman K M and Mariappan V (1977) Datura ferox L A new indicator host for brinjal severe mosaic virus Sci and Cult **43** 496 497

Valleau W D (1951) Tobacco ringspot virus The cause of eggplant yellows Phytopathology **41** 209 212

Vankammen A (1967) Purification and properties of the Components of cowpea mosaic virus Virology **31** 633 642

Verma H N (1982) Inhibitors of plant viruses from higher plants In Current trends in plant pathology Ed by B P Singh and S P Raychaudhury Today and Tomorrows printers and publishers New Delhi 151 159

*Verma, H N and Awasthi L P (1979a) Prevention of virus infection and multiplication by leaf extract of Euphorbia hirta and the properties of virus inhibitor New Bot 6 49-59

Verma H N and Awasthi L P (1979b) Antiviral activity of Boerhaavia diffusa root extract and the physical properties of the virus inhibitor Can J Bot 57 926 932

Verma H N and Dwivedi S D (1983) Prevention of plant diseases in some economically important plants by Bougainvillea leaf extract Indian J Plant Pathol 1 97 100

Verma H N and Mukerjee K (1975) Brinjal leaf extract induced resistance to virus infection in plants Indian J Exptl Biol 13 415 417

Verma H N and Prasad V (1987) Inhibition of viruses Systemic resistance inducers from higher plants Recent Advances in Plant Patholoy Ed by A Hussain et al Print House (India) Lucknow pp 312 324

Verma G S and Rashmi Lal (1967) Occurrence of a mosaic disease on brinjal Indian Phytopath 20 243 247

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

Vyanjane N T and Mali V R (1981) Occurrence of alfalfa mosaic virus on brinjal in India Indian Phytopath **34** 164-168

Zirka, T I (1967) Transmission of stolbur virus of Pepper and Eggplant by grafting and by Hylasthes obsoletus Mykrobiol Zh **29** 334-338

* Original not seen

APPENDIX

Appendix I

Amidoblack stain for precipitin lines

Amidoblack 10 B	1 g
Sodium acetate acetic acid buffer 0 2 m 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

TRANSMISSION, PHYSICAL PROPERTIES AND HOST RANGE OF BRINJAL MOSAIC VIRUS

By

M SURENDRAN

ABSTRACT OF THE THESIS

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ABSTRACT

Studies were conducted on the brinjal mosaic virus disease commonly occurring in brinjal plants (Solanum melongena L) in Kerala

The symptoms appeared as typical mosaic mottling with dark green and light green patches The leaves were very much reduced in size and deformed 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 through seeds and by vectors The virus was found to be transmitted by the vectors Myzus persicae Aphis gossypii Aphis craccivora Aphis malvae and Henosepilachna vigintioctopunctata Among them M persicae was found to be the most efficient vector

The minimum acquisition feeding and inoculation feeding period were found to be 20 s and 30 s respectively But the percentage of transmission was the maximum when an acquisition feeding of 25 min and inoculation feeding of 1 h were given

Influence of starvation before acquisition and inoculation feeding proved that pre acquisition starvation for 1 h produced the maximum infection but post acquisition starvation decreased the per cent infection The vector was found to retain

the virus for 1 h A single aphid could transmit the virus to healthy test plants but the maximum percentage of transmission was obtained with 10 aphids

Host range studies showed that the virus was restricted to the members of the family chenopodiaceae cucurbitaceae and solanaceae

Investigations on the physical properties of the virus revealed that the virus had a dilution end point between 1 1000 and 1 10000, thermal inactivation point between 60-65°C, longevity in vitro between 96-120 h at room temperature (28 30°C) and between 144 168 h at 10°C

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

Varietal screening trial with four varieties and eleven indigenous collections of brinjal revealed that all the varieties were susceptible to brinjal mosaic virus infection The collections NIC-14101 and NIC 14144 were least susceptible with 30 per cent infection

The observations on natural incidence of brinjal mosaic virus disease in and around Vellayani area indicated that brinjal mosaic virus was the major disease of brinjal

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The observations on natural incidence of brinjal mosaic virus disease in and around Vellayani area indicated that brinjal mosaic virus was the major disease of brinjal

Studies on the effect of virus infection on growth of brinjal plants showed that there was significant reduction in height number of leaves leaf area internodal length number of flowers number of fruits and yield

The inhibitory effect of certain plant extracts on the incidence of brinjal mosaic was studied Preliminary screening of non host plants for antiviral property against brinjal mosaic virus revealed that extracts of four plants viz Azadirachta indica Clerodendron infortunatum Ocimum sanctum Vitex negundo and two products of A indica viz A indica A Juss oil, A indica A Juss cake inhibited the production of local lesions on the leaves of Datura stramonium indicating that these extracts possessed antiviral property

The extracts of A indica A indica A Juss oil A indica A Juss cake and C infortunatum caused 100 per cent inhibition of the production of local lesions on D stramonium even at ten per cent concentration

Pre inoculation application of plant extracts was found to be more effective than post inoculation application in checking the incidence of brinjal mosaic

The maximum inhibition of disease development was obtained in plants inoculated with brinjal mosaic virus upto 4 h after the application of ten per cent extracts of A indica C infortunatum and A indica A Juss oil