TRANSMISSION, PROPERTIES AND HOSTRANGE OF SNAKE GOURD MOSAIC VIRUS

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DECLARATION

I hereby declare that this thesis entitled "Transmission, properties and host-range of snake gourd 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.

1-2-89

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CERTIFICATE

Certified that this thesis entitled "Transmission, properties and host-range of snake gourd mosaic virus" is a record of research work done independently by Sri. D. Raghunadhan 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

Snake gourd (*Trichosanthes anguina* L.) is one of the major vegetable crops of Kerala and is being extensively cultivated throughout the other parts of India as well. A number of diseases affecting this crop have been reported from Kerala and other states of the country. Among them the virus diseases are known to cause serious damage to the crop wherever it is cultivated. Snake gourd plants with mosaic symptoms were reported from different parts of India by many scientists (Shankar *et al.*, 1969, 1972; Verma *et al.*, 1970; Jaganathan and Ramakrishnan, 1971; Nagarajan and Ramakrishnan, 1971a and Dubey *et al.*, 1974). Occurrence of snake gourd mosaic in Kerala was first reported by Pillai (1971).

Snake gourd mosaic is a very common and destructive disease of snake gourd seeen in many parts of India. This disease is found to cause severe damage to the crop cultivated in all parts of Kerala also. The characters of snake gourd mosaic reported from different parts of India are not very much similar and hence the identity of snake gourd mosaic virus isolates reported from different parts of India is still not very clear. Only very little attempt has been made so far to identify the snake gourd mosaic virus occurring in Kerala. Therefore in the present

investigation an attempt has been made to identify the virus and to study other aspects of the disease.

The following details have been worked out during the course of the investigation.

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

REVIEW OF LITERATURE

REVIEW OF LITERATURE

I. Symptomatology

Snake gourd plants with virus like symptoms have been observed in India since 1967. Shankar et al. (1969) reported a mosaic disease of snake gourd which was characterised by a mosaic pattern of irregular dark green and yellow chlorotic patches on the lamina. The affected plants were stunted, produced fewer flowers and showed leaf crinkling. The causal virus was identified as cucumber mosaic virus. Verma et al. (1970) while investigating the virus diseases of snake gourd in Uttar Pradesh, reported that the leaves of the infected snake gourd were variously malformed, reduced in size and showed dark green mosaic mottling symptoms. The virus was identified as Cucumis virus 2B. Nagarajan and Ramakrishnan (1971a) studied cucurbit viruses in Madras State and reported the occurrence of watermelon mosaic virus on snake gourd. The plants were stunted and leaves were affected by prominent mosaic mottling with considerable reduction in leaf size. In advanced stages the leaves were crowded together to give a bushy appearance. When young plants were infected considerable malformations were seen in leaves. The symptoms appeared within 7-9 days.

Pillai (1971) was the first to report a mosaic disease of snake gourd in Kerala. The symptoms consisted

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

Cucumber mosaic virus is wide spread and reported from many places. Pejcinovski (1978) observed that cucumber mosaic virus (CMV) caused mosaic, dwarfing and wilt symptoms on cucumber, melon and pumpkin in Macedonia. Makhouk and Lesemann (1980) reported a severe mosaic of cucumber in Lebanon with mottling, blistering and malformation caused by watermelon mosaic virus 1 (WMV-1). Weber et al. (1982) reported a disease of glass house cucumber (Cucumis sativus L.) characterised by light green yellowish indistinct spots with brown necrotic centres. The disease causing agent was

identified as cucumber leaf spot virus. Sharma et al. (1984) observed a mosaic disease of muskmelon in Punjab and identified the disease causing organism as a distinct strain of cucumber mosaic virus and designated as cucumber mosaic virus, muskmelon strain (CMV-mst).

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

Hariharasubramanian and Badami (1964) while investigating pumpkin mosaic virus disease observed that the disease was characterised by severe blistering, distortion and stunting of leaves. Jagenathan and Ramakrishnan (1971)

found that a virus isolate from pumpkin produced mottling and malformation of leaves. They also reported that plants infected early in the season remained dwarf and flowered sparingly. A few leaves exhibited dark green vein banding along the midrib and lateral veins of affected plants. Shankar et al. (1972) observed that the symptoms of pumpkin mosaic disease appeared first as mosaic mottling of the leaves, followed by chlorosis of vein and veinlets leaving interveinal area green. The leaf lamina was very much reduced and distorted, the veins and veinlets often extended beyond the margin giving the leaves a filiform shape and severely affected vines were extremely dwarfed. Ghosh and Mukhopadhyay (1979) isolated nine different strains of viruses from pumpkin (Cucurbita moschata) from West Bengal and among them the isolate A_7 produced characteristic mottling with mild green blisters and green vein banding in the leaves of infected plants.

II. Transmission of the virus

1. Sap transmission

Doolittle (1920) had shown that the transmission of cucumber mosaic virus in the field was effected by mechanical means during the training and thinning of plants and plucking of fruits. He also demonstrated the sap transmission of the virus. Pillai (1971) reported that a strain

of cucumber mosaic virus infecting snake gourd (Trichosanthes anguina L.) was transmitted mechanically to healthy plants. Nagarajan and Ramakrishnan (1971a) reported that watermelon mosaic virus (WMV) affecting snake gourd could be transmitted to healthy plants by sap inoculation. Dubey et al. (1974) showed that snake gourd mosaic caused by Cucumis virus 1 could be transmitted by sap inoculation. Mechanical inoculation of Cucumis virus 1 affecting snake gourd was reported by Joseph and Menon (1978).

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

Basillious et al. (1969) could successfully get mechanical transmission of watermelon mosaic virus to

cucumber, pumpkin, watermelon, pea and Lupinus termis. Quiot et al. (1971) reported that watermelon mosaic virus 1 found on cucumber, melon, watermelon and courgette was readily transmissible by mechanical inoculation. Nagarajan and Ramakrishnan (1975) successfully transmitted a strain of melon mosaic virus on Cucurbita lundelliana by sap inoculation. Arteaga et al. (1976) reported mechanical transmission of watermelon mosaic virus 2, and that of watermelon mosaic virus 1 was reported by Makkouk and Lesemann (1980).

Mechanical transmission of pumpkin mosaic virus was reported by Hariharasubramanian and Badami (1964). A mosaic disease of pumpkin commonly occurring in Delhi was found to be sap transmissible (Shankar et al., 1972). Ghosh and Mukhopadhyay (1979) isolated nine virus strains from pumpkin and reported that all the isolates were sap transmissible. Roy and Mukhopadhyay (1980) found that the spread of pumpkin mosaic virus was possible by mechanical contact between above ground portions.

Foster (1972) observed that buffers added to non purified cucumber mosaic virus preparations affected the number of local lesions produced on Chenopodium amaranticolor and greater infectivity was obtained with sodium/potassium phosphate buffer at pH 6 than at pH 8, and at pH 7 the response was intermediate. Sharma et al. (1984) described

that a new strain of cucumber mosaic virus causing mosaic disease of muskmelon was most infective (85%) in 0.01 M, pH 7 phosphate buffer and least in water (45%).

2. Seed transmission

Many of the plant viruses were found to be transmitted through seeds of the diseased plants. Some of the viruses infecting cucurbits were also reported to be transmitted through seeds. Nagarajan and Ramakrishnan (1971a) found that a mosaic disease of snake gourd caused by watermelon mosaic virus was transmitted to some extent by seed. Dubey et al. (1974) studied the seed transmission of snake gourd mosaic caused by Cucumis virus 1 and found that the disease could not be transmitted through seeds collected from diseased snake gourd.

Hani and Pelet (1970) stated that cucumber mosaic virus (CMV) is transmitted through seeds of chickweed (Stellaria media) upto 30%. Transmission of CMV through chickweed seed was reported by Tomlinson and Carter (1970). Kaiser and Danesh (1971) described that cucumber mosaic virus isolated from Cicer arietinum could not be transmitted through seeds. Sharma and Chohan (1973) studied the seed transmission of Cucumis virus 1 and Cucumis virus 3 through seeds of cucurbits and they reported that Cucumis virus 1 was seed borne in vegetable marrow, ash gourd and pumpkin,

and Cucumis virus 3 was found to be seed borne in bottle gourd. Goel and Varma (1973) isolated a new strain of CMV designated as Luffa strain from ridge gourd and found that it was not transmitted through seed. Pejcinovski (1978) described that cucumber mosaic virus could be transmitted by surface contamination of seed. Sharma et al. (1984) found a strain of cucumber mosaic virus causing mosaic disease of muskmelon in Punjab which was transmitted by seed.

Nagarajan and Ramakrishnan (1975) carried out investigations on the transmission of melon mosaic virus and found that it was transmitted to some extent through seeds. Hein (1977) working on watermelon mosaic virus 1 on Zucchini (Cucurbita pepo L. Var. giromontima Alef.) found that it was not seed transmissible. Similar report was also made by Wu and Su (1977). Ahmed (1981) described that watermelon mosaic virus 1 infecting cucurbits could not be transmitted through seeds. Almeida and Borges (1983) investigated on watermelon mosaic virus infecting pumpkin in Portugal and reported the seed transmission of the virus.

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

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

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

3. Graft transmission

Basillious et al. (1969) reported that watermelon mosaic virus could be transmitted to other cucurbits by grafting.

4. Insect transmission

Insect transmission of snake gourd mosaic virus was reported by many workers. Verma et al. (1970) studied on a severe mosaic of snake gourd and found that it was transmitted by Myzus persicae and Aphis gossypii. Nagarajan and Ramakrishnan (1971a) reported watermelon mosaic virus on snake gourd in Madras State and found it to be transmitted by Myzus persicae and Aphis gossypii, out of 8 species of aphids tested including Aphis craccivora. Pillai (1971) attempted to transmit the mosaic disease of snake gourd (CMV)

by Aphis craccivora and Myzus persicae but was not successful. Dubey et al. (1974) identified a snake gourd mosaic virus and designated as Cucumis virus 1 which was found to be transmitted by Aphis gossypii and Myzus persicae and not by Aphis craccivora and 3 other aphid species. Joseph and Menon (1978) investigated on snake gourd mosaic virus and reported that the virus could be transmitted by Aphis gossypii and Aphis craccivora.

Goel and Varma (1973) reported that a new strain of cucumber mosaic virus designated as Luffa strain could be transmitted by Myzus persicae, A. gossypii and Brevicoryne brassicae to ridge gourd (Luffa acutangula). Pejoinovski (1978) observed that cucumber mosaic virus on cucumber, pumpkin and melon was transmitted by Myzus persicae and Aphis fabae.

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

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

Lindberg et al. (1956) studied the transmission of squash mosaic virus and melon mosaic virus and reported that they were transmitted by Myzus persicae and Aphis gossypii. Squash mosaic virus in Japan was found to be transmitted by two species of aphids, viz., Aphis gossypii and Myzus persicae (Komuro, 1957). Allam (1965) found that squash mosaic virus could not be transmitted by Aphis craccivora.

Hariharasubramanian and Badami (1954) reported that pumpkin mosaic virus was transmitted by Aphis laburni and many other Aphis spp. Forghani et al. (1966) observed that viruses infecting Cucurbita pepo were transmitted by four insect vectors viz., Aphis fabae, Dysaulacorthum pseudosolani,

Macrosiphon solanifolii (Macrosiphlm euphorbiae) and Myzus persicae. Nagarajan and Ramakrishnan (1971b) found that the virus commonly occurring on pumpkin could be non-persistently transmitted by Myzus persicae, Aphis gossypii and Aphis nerii. Shankar et al. (1972) reported that a mosaic virus of pumpkin commonly occurring in Delhi was found to be transmitted by Myzus persicae and Sitobion rosaeformis. Roy and Mukhopadhyay (1980) found that pumpkin mosaic virus was transmitted by Aphis gossypii in a non-persistent manner. Singh (1981a, 1982) reported that pumpkin mosaic virus was transmitted by Aphis gossypii as well as Aphis craccivora.

III. Physical properties

Verma et al. (1970) while studying the physical properties of Cucumis virus 2B causing mosaic disease in snake gourd and bottle gourd, observed that the viruses had a thermal inactivation point (TIP) of 97.5°C and dilution end point (DEP) of 10^{-6}- 10^{-7}. The longevity in vitro (LIV) at 30°C was 9-10 days. Nagarajan and Ramakrishnan (1971a) isolated watermelon mosaic virus from snake gourd. It had a TIP of 52-54°C, DEP of 1:200-1:500 and LIV at 32°C was 4-6 days and at 5°C was 4-8 days. Pillai (1971) found that CMV causing mosaic diseases of snake gourd had a TIP of 60°C DEP of 1:10000 and LIV was upto 72 h at room

temperature. Dubey et al. (1974) isolated Cucumis virus 1 (CMV) from mosaic infected snake gourd. Its TIP was between 65-70°C, DEP was between 1:1000-1:5000 and LIV was 16-18 h at 34.6°-39°C and 8 days at 8°C. Joseph and Menon (1978) reported that Cucumis virus 1 (CMV) infecting snake gourd had TIP between 70-75°C, DEP 1:5000-1:10000 and LIV 72-96 h at room temperature and 144-168 h at 10°C.

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

Watermelon mosaic virus affecting almost all cucurbits was reported by many workers. Lindberg et al. (1956) studied

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

Hariharasubramanian and Badami (1964) observed that pumpkin mosaic virus had a TIP of 55°C and DEP 1:5000. The LIV at room temperature was 72 h but the virus could be maintained for more than six months in leaves kept at -20°C. Shankar et al. (1972) reported that pumpkin mosaic virus was inactivated when subjected to 56°C for 10 min. The DEP of the virus was found between 1:100 and 1:500. At room temperature (32-35°C) the virus was infective for 8 h only but the longevity of the virus was increased to 26 h at 8°C. March and Nome (1973) found that the virus infecting Cucurbita moschata had a TIP between 65°C and 70°C, DEP 4 x 10^{-5}, LIV 6 weeks. Ghosh and Mukhopadhyay (1979) studied the physical properties of nine isolates of pumpkin mosaic virus under laboratory conditions and observed that all the isolates had a TIP between 40-55°C, DEP between 10^{-1}-$10^{-3.5}$ and LIV at room temperature (24-32°C) between 6-168 h.

IV. Vector-virus relationships

The vector-virus relationship of a virus occurring on snake gourd was worked out by Nagarajan and Ramakrishnan (1971a). They observed that the virus was transmitted by Myzus persicae and Aphis gossypii in a non-persistent manner. Dubey et al. (1974) observed that Cucumis virus 1 causing mosaic disease in snake gourd was transmitted by Aphis gossypii

and Myzus persicae in a non-persistent manner. Joseph and Menon (1978) studied the vector-virus relationship of a virus isolated from snake gourd, transmitted by Aphis gossypii and Aphis craccivora. They found that A. craccivora acquired the virus within 5 min acquisition feeding and transmitted it within 5 min inoculation feeding on healthy plants. A minimum of 5 aphids were required for transmission and pre-acquisition starvation increased transmission efficiency, whereas post-acquisition starvation for 30 min reduced the same. The vector could not retain the virus for long periods, the relationship being non-persistent.

Kaiser and Danesh (1971) found that a single aphid (Aphis craccivora) was able to transmit cucumber mosaic virus but greater transmissions were obtained only by using larger number of aphids. Singh (1972) studied the relationship of watermelon mosaic virus strains with its vector Myzus persicae and found that the virus was transmitted in a typical non-persistent manner. The vector was most efficient after 4 h pre-acquisition fasting and 2 min acquisition feeding. Infectivity was lost after 2 h post-acquisition fasting. The nymphal forms were slightly more efficient in transmission than alate and apterous adults. Raychaudhuri and Varma (1977) showed that a strain of watermelon mosaic virus was transmitted by the vector Myzus persicae to vegetable marrow in a typical stylet-borne

manner. Pre-acquisition starving of vectors was not essential but increased the transmission rate. Although a single aphid could transmit the virus, more than 5 aphids per plant were required for 100 per cent transmission. A feeding period of only 30 s was needed for virus acquisition but when it was extended for one or two min there was maximum transmission. Almeida and Borges (1983) found that watermelon mosaic virus could be transmitted to watermelon, pumpkin, squash and cucumber by Myzus persicae in a non-persistent manner. They obtained 70 per cent transmission with one aphid and 100 per cent with seven or more aphids.

Nagarajan and Ramakrishnan (1971b) studied on the vector-virus relationship of melon mosaic virus occurring on pumpkin. They observed that the virus was non-persistently transmitted by Myzus persicae, Aphis gossypii and Aphis nerii, and found that the optimum number of aphids required per plant was 20. Virus transmission by fasted vectors was greater than that of non-fasted ones. The length of the pre-acquisition fasting period, acquisition threshold and inoculation threshold was inversely related to efficiency. They also proved that a salivary inactivator was responsible for the reduction in transmission when vector number increased beyond optimum. Jaganathan and Ramakrishnan (1971) reported vector-virus relationship of two virus isolates from naturally infected melon and

pumpkin. They found that maximum transmission was obtained when the aphids (Aphis gossypii and Myzus persicae) were starved for 60 min. Starvation of aphids after acquisition resulted in reduced transmission of both muskmelon and pumpkin isolates and the viruliferous nature of the vector was lost 60 min after starvation. Minimum acquisition feeding of muskmelon isolate was 5 s while that of pumpkin isolate was 10 s. Minimum inoculation threshold was 5 s for both the isolates. Relationship of pumpkin mosaic virus with its aphid vector Aphis gossypii was reported by Singh (1981a). He found that pre-acquisition fasting of minimum 10 min and an optimum of 90 min was essential for transmission of the virus to pumpkin. Acquisition and transmission occurred in 20 s and 10 s, respectively. Although a single aphid could transmit the virus, transmission was maximum with 10 aphids per plant. Post-acquisition fasting of more than 2 h resulted in loss of infectivity. Singh (1982) conducted studies on the transmission of pumpkin mosaic virus by Aphis craccivora and showed that pre-acquisition fasting of vector was essential for virus transmission. Aphids acquired the virus within 20 s and inoculated it within 30 s. He found that a single aphid could transmit the virus, but maximum infection was obtained with 15 aphids. Aphids were infective only for 2 h and the transmission was therefore in a non-persistent manner.

V. Host range and local lesion hosts

Shanker et al. (1969) reported a mosaic disease of snake gourd (CMV) having an extensive host range which included Nicotiana glutinosa, Chenopodium amaranticolor and Cucurbita pepo. Verma et al. (1970) recorded a severe mosaic disease caused by Cucumis virus 2B on snake gourd and the host range was restricted to members of cucurbitaceae. But it produced local lesions on Chenopodium amaranticolor. Pillai (1971) identified a mosaic disease of snake gourd in Kerala and out of 51 species of plants in 7 families tested, 15 species in 6 families were found to be hosts of the virus. The causal virus was identified as a strain of cucumber mosaic virus (CMV). Nagarajan and Ramakrishnan (1971a) reported the occurrence of watermelon mosaic virus on snake gourd, the host range being restricted to cucurbitaceae. Dubey et al. (1974) identified a mosaic disease of snake gourd in Delhi caused by Cucumis virus 1. The virus was found to have its host range in cucurbitaceae, solanaceae, chenopodiaceae and compositae and produced systemic mosaic symptoms on Cucumis sativus L., C. anguria L., C. melo L. Var. Utilissima, Cucurbita pepo L., Lagenaria siceraria Standl. vars. Round and Long, Luffa acutangula Roxb., Citrullus vulgaris Schrad., C. vulgaris var. fistulosus Sks, Nicotiana tabacum L. var. White Burley, N. tabacum var. Xanthi, N. rustica L., N. glutinosa L., Capsicum annuum L.,

Solanum melongena L., Lycopersicon esculentum Mill., Datura stramonium L., Petunia hybrida var. violet, Zinnia elegans Jacq. and Spinacia oleraceae L. Luffa cylindrica Roem and N. tabacum L. var. Harrison Special carried the virus symptomlessly. The virus produced distinct necrotic local lesions on Chenopodium amaranticolor Coste and Reyn.

Allen and Fernald (1971) observed that wild cucumber mosaic virus could infect Marah oreganus. Foster (1972) reported that CMV produced local lesions on Chenopodium amaranticolor. Ehara and Misawa (1975) reported that cucumber mosaic virus produced local lesions on cowpea. Joshi and Dubey (1976) investigated on weed reservoirs of cucumber mosaic virus in Gorakhpur and reported that Amaranthus viridis, Nicotiana plumbaginifolia, Physalis minima, Salvia plebeia and Solanum nigrum were infected with CMV. Ignash (1977) reported that cucumber mosaic virus strain 1 isolated from cucumber and tulip produced local lesions on Chenopodium quinoa. Rao and Raychaudhuri (1977) reported that cucumber mosaic virus isolated from Vinca rosea produced local lesions on Chenopodium murale. Suteri et al. (1980) studied on the occurrence of CMV in Kumaon and reported that it affects the ornamental plants Amaranthus spinosus, Datura stramonium, Nicotiana sp, Solanum nigrum and Tagetes erecta. Sarjeet Singh (1981)

reported that a mutant of CMV produced severe systemic mosaic with puckering and leaf distortion symptoms in Datura stramonium. Sharma et al. (1984) observed that a new strain of cucumber mosaic virus (CMV-muskmelon strain) infected tobacco cultivars, white burley, Nicotiana glutinosa, N. rustica, Capsicum annuum and various cucurbits.

Toba (1962) found that Momordica balsamina was the commonest wild reservoir of watermelon mosaic virus in Hawaii. Zabla and Ramallo (1969) reported that watermelon mosaic virus infects Cucurbita sp, Cucumis sp and Chenopodium amaranticolor. Bhargava and Tewari (1970) reported that Trichosanthes dioica was the natural host of watermelon mosaic virus. Auger et al. (1974) observed that watermelon mosaic virus 2 was widely distributed in the cucurbit growing area in Central Chile infecting squash, (Cucurbita maxima) and Zucchini squash (Cucurbita pepo) and induced local lesions on Chenopodium amaranticolor and local lesions and systemic necrotic flecking on Lavatera trimestris. Bhargava et al. (1975) reported that watermelon mosaic virus could be perpetuated through commonly cultivated Trichosanthes dioica and Lagenaria vulgaris and the wild perennials Momordica dioica and Coccinia grandis. Nagarajan and Ramakrishnan (1975) concluded that WMV could infect only the members of cucurbitaceae viz., Cucurbita moschata,

C. maxima, C. pepo, Cucumis melo, Luffa acutangula, Trichosanthes anguina and Lagenaria vulgaris. They could not find any local lesion host for the virus. Tewari (1976) found that Zinnia elegans was a symptomless carrier of watermelon mosaic virus. Halliwell et al. (1979) observed that the weed Melothria peridula acted as a host of WMV-1. Makkouk and Lesemann (1980) reported that WMV-1 could induce local lesions without systemic spread in Chenopodium amaranticolor and C. quinoa and systemic infection in cucumber, squash, pumpkin and watermelon. Chang and Lee (1980) observed that watermelon mosaic virus could infect Sesamum indicum L. Ahmed (1981) reported that watermelon mosaic virus had a narrow host range confined to the family cucurbitaceae.

Lastra et al. (1975) reported that squash mosaic virus produced local lesions on Cucumis metuliferus. Lockhart et al. (1982) reported that squash mosaic virus could cause systemic infection in Chenopodium quinoa.

Shankar et al. (1972) inoculated pumpkin mosaic virus (PMV) on 76 plant species of 9 families. They found that its host range was restricted to the family cucurbitaceae and produced systemic mosaic symptoms on Cucurbita pepo, Cucumis melo, C. melo var. Utilissima, Lagenaria siceraria var. Round and Long, Luffa acutangula, Citrullus

vulgaris, Momordica charantia, Benincasa hispida and Trichosanthes anguina. Cucumis sativus was proved to be a symptomless carrier. Singh (1981b) reported that the host range of a virus causing mosaic disease of pumpkin was confined to the family cucurbitaceae.

VI. Serological properties of the virus

1. Purification of virus

Different methods of purification of viruses infecting cucurbits have been reported. Dubey et al. (1974) purified snake gourd mosaic virus by using butanol centrifugation method. Infected leaves were homogenised in 0.05 M phosphate buffer (pH 7.6) containing 0.1 per cent thioglycollic acid and subjected to differential centrifugation after adding 8.5 per cent n-butanol. The final pellet was suspended in 0.05 M phosphate buffer. Three other methods of purification viz., chloroform centrifugation using phosphate buffer with ascorbic acid (Gibbs et al., 1963) chloroform butanol centrifugation using phosphate buffer with ascorbic acid and DIECA (Brunt, 1966) and chloroform butanol centrifugation (Steere, 1956) were also attempted for their relative efficiency. The chloroform butanol centrifugation method gave the highest virus end point.

Lot et al. (1972) purified cucumber mosaic virus by polyethylene glycol (PEG) precipitation followed by two

centrifugation and resuspension of pellets in solutions of low sodium citrate concentration containing two per cent triton X-100. Shohara and Osaki (1974) reported that purified cucumber mosaic virus was obtained by repeated precipitation with 8 per cent PEG and 0.2 M sodium chloride (NaCl) followed by density gradient centrifugation. Omar et al. (1980) compared various methods of purification of cucumber mosaic virus and found that the best clarification was obtained with low speed centrifugation. Precipitation with ammonium sulphate gave the highest virus concentration followed by adsorption on PEG.

Wetter (1960) reported that some of the elongated plant viruses could be partially purified by using ether or carbon tetrachloride for preliminary sap clarification and they proved to be serologically active. Hebert (1963) and Van Kammen (1967) purified cowpea mosaic virus by PEG-NaCl method. The leaf extract was clarified by centrifugation at 1000 g and then PEG 6000 and NaCl were added. Van Kammen (1967) reported that PEG-NaCl method gave high yield of purified virus compared to butanol chloroform method of purification. Filigarova (1962) purified Arabis mosaic virus from infected leaves of Petunia hybrida, which were homogenised with phosphate buffer and the virus was precipitated with PEG and purified by density gradient centrifugation.

2. Serological tests

Dubey et al. (1974) found that the antiserum for snake gourd mosaic virus reacted positively and gave a precipitate of somatic (granular) type characteristic of spherical viruses with the diseased plant sap and purified virus preparation but not with healthy plant sap. The virus in the clarified plant sap gave reaction at a dilution of 1:128 whereas in the case of purified preparation it reacted upto 1:4096 dilution. No reaction was obtained in the diseased plant sap or purified virus preparation with normal serum. The titre of the serum was found to be 1:2048 with the purified virus preparation. In agar gel diffusion slides, the antiserum produced a single straight precipitation band when tested with diseased plant sap or purified virus preparation. Dubey and Nariani (1975) investigated the serological relations of 10 cucurbit virus isolates collected from Delhi and found that the viruses of snake gourd mosaic, cucumber mosaic, melon mosaic and bitter gourd mosaic formed a group of Cucumis virus 1, while bottle gourd and watermelon mosaic virus formed a group of Cucumis virus 2, pumpkin mosaic and vegetable marrow mosaic viruses comprised the unstable Cucumis virus 3 while a virus from tori (Luffa cylindrica) appeared to be distinct from these 3 groups.

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

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

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

VII. Varietal screening

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

Provvidenti et al. (1978) tested 14 wild cucurbita species against the common, often destructive viruses affecting squash in New York, and they found that two species

were highly resistant to WMV-1 and 2. They concluded that Cucurbita ecuadorensis and Cucurbita foetidissima appeared to be the most promising species resistant to CMV, WMV-1, WMV-2 and other viruses. Greber (1978) reported that watermelon mosaic virus 1 and 2 in Queensland could infect all commercially available watermelon, vegetable marrow and pumpkin cultivars. Halliwell et al. (1979) while investigating watermelon mosaic virus disease of squash, watermelon and pumpkin reported that WMV-1 was endemic to many vegetable growing areas in Texas and it severely limited the production of these crops. Pitrat and Dumas de Vaulx (1979) during their search for sources of resistance to cucumber mosaic virus and watermelon mosaic virus among cucurbita species found that Cucurbita lundelliana, C. martinezii, C. okeechobeensis and C. ecuadorensis were resistant to CMV and WMV. Sowell and Demski (1981) described that inoculation of muskmelon with WMV-2 produced significantly fewer infected seedlings in PI 403994, than in Hales Bert Jumbo. Sharma and Sharma (1982) tested 31 genotypes in field against natural infection of Cucumis virus 1 (Cucumber mosaic virus) and found that 12 were moderately resistant but none was immune. The lines 11-2-6-2, 10-1-2-4 and 17-1-2-1 which showed a low disease index and mild symptoms were found promising. Davis and Shifriss (1983) reported that under natural conditions

severity of virus diseases was constantly lower in the summer squash silvered-leaved breeding line NJ 260 than in the green leaved cultivar, early prolific straight neck (EPS). About six weeks after planting, 99 per cent of EPS and 28 per cent of NJ 260 plants showed symptoms of cucumber mosaic virus and clover yellow vein virus. By the end of the season all plants of both lines developed symptoms although EPS plants were much more severely affected.

VIII. Estimation of loss

Pillai (1971) investigated a mosaic disease of snake gourd and reported that the disease affected plants were stunted and produced fewer flowers and fruits. Dubey et al. (1974) studied on snake gourd mosaic virus and observed that the diseased plants sent out only a few weak runners and plants affected in an early stage blossom sparingly and set few fruits.

Hills et al. (1961) studied the effect of CMV on cantaloupe and recorded that inoculation of melon plants at the 6th leaf stage caused 40 per cent reduction in yield. Nelson (1962) while working with cantaloupe reported that when runners of 2-4 feet length were inoculated with CMV there was 75 per cent reduction in fresh plant weight and with WMV there was 50 per cent reduction. When plants were inoculated near maturity eventhough symptoms developed there

was no significant reduction in plant size or yield. Powell and Schlegel (1970) reported that cantaloupe plants infected with squash mosaic virus (SMV) significantly reduced fruit weight, size, seed number, seed weight and percentage germination, but no correlation was found between these reduction and the variable infection percentages. Singh and Mandahar (1971) reported that infection of Luffa aegyptica and Cucurbita moschata by CMV reduced leaf productivity. Thomas (1971) conducted field trial to study the economic importance of WMV-2 on cucurbits in New Zealand and reported that early infection reduced yield in Buttercup squash (63%), Golden Hebbard squash (53%) and pumpkin (49%) but not in cucumber. No yield reduction was recorded with late infection in any of the 4 cucurbits tested. Moskovets and Fegla (1972) found that cucurbits inoculated with WMV in early stages of growth had shorter runners and internodes, fewer side runners and lesser green weight. Demski and Chalkley (1974) while studying the effect of watermelon mosaic virus on watermelon observed that infected plants of 3 watermelon varieties had shorter runners and smaller leaves which reduced the fresh weight by over 55 per cent. It was also found that the fruit number and size were reduced due to infection. Alvarez and Campbell (1976) analysed the yield factors of cantaloupe infected by squash mosaic virus and recorded significent reduction in the number of fruits per

plant but had no influence on size, weight or edible quality of the fruits. However, retardation in fruit maturity was observed.

Singh and Dey (1976) assessed the loss due to bottle gourd mosaic virus in the yield of Lagenaria siceraria and found 64 per cent yield reduction. Bhargava (1977) reported that in field experiments early infection of vegetable marrow (Cucurbita pepo) plants with WMV caused greater loss in yield than late infection. It was also found that different strains of WMV varied in the extent of yield reduction caused by them. Karchi et al. (1978) found that infection of CMV in an early stage reduced the yield of susceptible cantaloupe (Cucumis melo) cvs. Noy yizreel by 73 per cent and resistant X-140 by 31 per cent. Yield reduction in Noy Yizreel was due to fewer fruits and lower fruit weight whereas in X-140 fruit weight was not affected although the number of fruits per plant was reduced. Jayasree (1984) found that yellow vein mosaic disease of pumpkin produced significant reduction in number of leaves, size of the leaves, internode length, number of branches, total length of vines and number of flowers.

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Symptomatology

Seeds of snake gourd (*Trichosanthes anguina* L.) obtained from the Instructional Farm, Vellayani and Department of Olericulture, College of Horticulture, Vellanikkara, were used for the study. They were sown in pots containing potting mixture of sand, red soil and cowdung in the ratio of 1:1:2. The culture of the virus causing snake gourd mosaic was collected from the field and the same was maintained by repeated transfers on young snake gourd plants in insect proof glass house by sap inoculation. The plants were inoculated in all cases, except otherwise stated, at 3-4 leaf stage. Symptomatology was studied by observing the development of symptoms in naturally infected as well as artificially inoculated snake gourd plants.

II. Transmission of the virus

1. Sap transmission

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

The standard sap was prepared by crushing the infected leaf of known weight into a fine pulp by adding one ml of sterile distilled water for every gram of diseased tissue. The pulp was filtered through fine muslin cloth and the filtrate was used for inoculation. When phosphate buffer (0.01 M, pH 7.0) and tris buffer (0.01 M, pH 7.0) were used as extraction media, the sap was extracted after adding one ml of the buffer in each case to every gram of infected leaf tissue.

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

2. Seed transmission

Seeds collected from mechanically inoculated plants showing clear symptoms of the disease were sown in pots and

kept in the insect proof glass house. One hundred and eighty seeds were sown and the plants were kept under observation for 45 days after germination.

3. Graft transmission

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

4. Insect transmission

Insect transmission studies were carried out by using Aphis craccivora Koch., Aphis gossypii Glov., Aphis malvae Koch., Pentalonia nigronervosa Coq. and Bemisia tabaci Genn. as vectors.

1) Inoculation using Aphis Spp.

Healthy colonies of Aphis craccivora were maintained

on cowpea (Vigna unguiculata), Aphis gossypii on brinjal (Solanum melongena), Aphis malvae on bhindi (Abelmoschus esculentus) and Pentalonia nigronervosa on banana (Musa paradisiaca) under insect proof conditions.

Healthy insects were collected and transferred to petriplates. They were starved for a period of one hour (Pre-acquisition fasting period) and then allowed to feed on detached young leaves of infected snake gourd plants so as to give them an acquisition feeding period of 30 min. A fixed number of infective aphids (20 nos.) were then transferred to young healthy plants, at the 3-4 leaf stage, for an inoculation feeding period of 24 h and after that they were killed by spraying 0.1% Quinalphos. As in the case of sap transmission an equal number of control plants were also maintained. The uninoculated controls were kept in separate cages. Only apterous form of vectors were used in the above mentioned trials. The inoculated plants were kept for observation under insect proof condition for 25 days.

ii) Inoculation using Bemisia tabaci

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

The top portion of young plants bearing 3-4 leaves was introduced into the transmission cage in such a way that the stem passed through the rectangular slit on the opening of the cage. Whiteflies were collected using an aspirator and were then released into the transmission cage. The transmission cage was covered by a black cloth except at the region of the wire netting which was kept facing light source while releasing the whiteflies. The cap of the transmission cage was immediately screwed on. The remaining portion of the rectangular slit of the cage was closed with modelling clay. The cage was kept in position by using two bamboo slivers and a rubber band. After the desired feeding period, the modelling clay was removed and the plant was disturbed by gently tapping it with a needle to disturb the whiteflies. This could induce the whiteflies to move to the side of the cage facing the light source. Pre-acquisition fasting and acquisition and inoculation feeding periods were given as mentioned under transmission with aphids.

Ten seedlings were used as test plants in each transmission experiment. A fixed number of whiteflies were released (20 nos) on each test plant for inoculation feeding. After inoculation feeding the insects were killed by spraying the plants with 0.1% Quinalphos. The inoculated plants were labelled and maintained in an insect proof

glass house. The experiments were done twice and observations were taken daily on the appearance of symptoms.

III. Physical properties

1. Dilution end point (DEP)

Infected snake gourd leaves of known weight were crushed into fine pulp by means of clean and sterile pestle and mortar, adding one ml of distilled water per gram of leaf material. The resulting pulp was strained through sterile cotton wool. The standard extract thus obtained was centrifuged at 3000 g for 30 min to remove the host material. The sap was diluted with sterilized distilled water in the ratio of 1:100, 1:500, 1:1000, 1:5000, 1:10000, 1:50000, 1:100000, 1:500000, 1:1000000. The different dilutions were used for inoculation on separate test plants starting from the highest dilution. Ten plants were inoculated with each of the dilutions and the experiment was repeated to confirm the results. The standard sap without dilution was used as control. The inoculated plants were labelled and kept under insect proof conditions and observed for the development of symptoms.

2. Thermal inactivation point (TIP)

The sap from the infected snake gourd plants was obtained as in the above experiment. Five ml each, of the

sap was pipetted into thin walled glass test tubes. Care was taken not to pour the sap on the sides of the tubes. The tube was then kept in thermostatically controlled water bath for 10 min at the required temperature in such a way that the level of the water in the water bath was 3 cm above the level of sap in the tube. The control was kept at room temperature (28-30°C). The sap was treated at different temperature ranges of 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 and 90°C and a thermometer was placed close to the tube in the bath to check the temperature. After 10 min in each case, the tube was removed and cooled immediately in running water. The treated sap was inoculated on young vigorously growing test plants of 3-4 leaf stage. The inoculation of plants was first done with sap treated at the highest temperature and then the other plants were inoculated with sap treated at low temperatures. Ten plants were inoculated in each set of treatment. Observation on the number of plants infected were recorded.

3. Longevity <u>in vitro</u> (LIV)

Infected leaves were ground with pestle and mortar and the sap was filtered through cotton wool. Five ml of the sap was pipetted into test tubes and closed with aluminium foil. The tubes were kept at room temperature (28-30°C) and also in a refrigerator (10°C). One tube 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 test plants. Ten plants were inoculated in each set of treatment and the experiment was repeated to confirm the results. The inoculated plants were kept under insect proof conditions and observed for the development of symptoms.

IV. Vector-virus relationships

The experiments to study the vector-virus relationships were conducted by using Aphis gossypii which was found to be the most efficient vector. Snake gourd plants showing typical symptoms of snake gourd mosaic virus were collected from the field and the culture of the virus was maintained in insect proof glass house by repeated transfers to healthy plants by mechanical inoculation. Virus free aphid colonies were maintained on brinjal plants in an insect rearing cage. In all the inoculation trials only fully grown apterous aphids were used. During feeding of the aphids the test plants were kept in insect proof cages. The aphids were killed at the end of the required feeding period by spraying the plants with 0.1% Quinalphos. In the case of short feeding periods of less than 5 min the individual aphids were watched through a magnifying lens and the time of feeding was determined with the help of a stop watch after aphids had settled down to feed.

1. Acquisition threshold

A large number of non viruliferous A. gossypii were collected and were given a pre-acquisition starvation of one hour. Groups of 10 aphids each were given acquisition feeding of 20 and 30 s, 1, 2, 5, 10, 15, 20, 30 and 45 min and 1 and 2 h on diseased snake gourd leaves before transferring them to healthy snake gourd plants. The aphids were then allowed to remain for 24 h on the test plants and were killed thereafter by spraying 0.1% Quinalphos.

2. Inoculation threshold

Non viruliferous aphids were given one hour pre-acquisition fasting and an acquisition feeding of 30 min. Then the viruliferous aphids in groups of 10 were transferred to individual healthy test plants. Each batch was given separate inoculation feeding periods, viz., 30 s, 1, 2, 5, 10, 15, 20, 30 and 45 min, 1, 2, 4, 8 and 24 h. The aphids were killed after the specific inoculation feeding period by spraying 0.1% Quinalphos.

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

In order to estimate the effect of pre-acquisition starvation on the efficiency of the vector to acquire the virus, the insects were starved for different periods,

viz., 30 min, 1, 2, 3, 4, 5, 6 and 12 h. Batches of 10 aphids from each of these categories were given an acquisition feeding period of 30 min and released on test plants to feed for 24 h. After the inoculation feeding period, the insects were killed by spraying 0.1% Quinalphos. The controls with equal number of aphids were maintained without pre-acquisition starvation. The plants were kept under observation in insect proof glass house.

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

A large number of aphids were starved for one hour and allowed an acquisition feeding period of 30 min. After that batches of 10 aphids were starved for different periods of 30 min, 1, 2, 3, 4, 5, 6 and 12 h. They were afterwards transferred to healthy test plants and allowed to feed for 24 h. After the inoculation feeding period, the insects were killed by spraying 0.1% Quinalphos, and the plants were kept under observation. The control was maintained with equal number of aphids without post-acquisition fasting.

5. Retention of infectivity by the vector

The experiments were conducted with viruliferous insects, which were transferred in succession to a series of healthy snake gourd plants after giving a definite inoculation feeding period on each plant. Groups of aphids were

starved for one hour and allowed an acquisition feeding period of 30 min to make them viruliferous. Groups of 10 aphids, were then transferred in succession to a series of five healthy plants transferring the insects after a definite interval. The different feeding intervals allowed in different series were 30 min, 1, 1½, 2, 2½ and 3 h. The aphids were killed from the fifth plant of the different series using 0.1% Quinalphos. The experiment was done twice.

6. Minimum number of aphids required for transmission

Single aphids as well as groups of 2, 3, 5, 10, 15, 20, 25 and 30 were released on each test plant, allowing a pre-acquisition starvation period of one hour, an acquisition feeding period of 30 min and an inoculation feeding period of 24 h to determine the minimum number of aphids required for transmission of the virus. After the inoculation feeding, the insects were killed by spraying 0.1% Quinalphos and the plants were kept under observation for the development of symptoms.

V. Host-range and local lesion hosts

To determine the host-range and local lesion hosts of snake gourd mosaic virus, healthy plants belonging to 70 species of 18 families were inoculated by sap inoculation.

Five plants were inoculated in each case. The plants which did not show symptoms after 4 weeks were indexed by back inoculation to Trichosanthes anguina L. to find whether they were symptomless carriers of the virus. Following plants were used for host-range and local lesion host studies.

1. Acanthaceae:

   a) Andrographis echioides (L.) Ness.

   b) Justicia prostrata Gramble.

2. Amaranthaceae:

   a) Amaranthus caudatus L.

   b) Amaranthus viridis L.

   c) Celosia sp.

   d) Gomphrena globosa L.

   e) Spinacia oleraceae L.

3. Apocynaceae:

   a) Cathranthus roseus G. Don.

   b) Vinca rosea L.

4. Araceae:

   a) Caladium sp.

   b) Colocasia esculenta L.

5. Asclepiadaceae:

   a) Calotropis gigantia R.Br.

   b) Hemidesmus indicus R.Br.

6. Balsaminaceae:

a) Impatiens balsamina L.

7. Capparidaceae:

a) Cleome viscosa L.

8. Chenopodiaceae:

a) Chenopodium amaranticolor Coste & Reyn.

b) Chenopodium murale L.

c) Chenopodium quinoa Willd.

9. Compositae:

a) Acanthospermum hispidum DC.

b) Ageratum conizoides L.

c) Emilia sonchifolia DC.

d) Eupatorium odoratum L.

e) Synedrella nodiflora Gaertin.

f) Tridax procumbens L.

g) Vernonia cinerea L.

h) Zinnia elegans Jacq.

10. Cucurbitaceae:

a) Benincasa hispida Cogn.

b) Citrullus vulgaris Schred. ex Eckl. & Zegh.

c) Cucurbita moschata Duch.

d) Cucurbita pepo L.

e) Cucumis sativus L.

f) Lagenaria siceraria (Mol) Standl.

g) Luffa acutangula Roxb.

h) Luffa cylindrica (L.) Roem.

i) Melothria perpusilla L.

j) Momordica charantia L.

11. Euphorbiaceae:

a) Acalypha indica L.

b) Croton sparsiflorus Morong.

c) Euphorbia geniculata Orteg.

d) Euphorbia hirta L.

e) Manihot esculenta Crantz.

f) Phyllanthus niruri L.

12. Malvaceae:

a) Abelmoschus esculentus (L.) Moench.

b) Abutilon indicum (L.) Sweet.

c) Sida acuta Burm F.

13. Labiatae:

a) Leucas aspera (Willd) Spreng.

14. Leguminosae:

i. Mimosaceae:

a) Mimosa pudica L.

14. Papilionaceae:

a) Arachis hypogaea L.

b) Cajanus cajan (L.) Millsp.

c) Calopogonium mucunoides Desv.

d) Canavalia ensiformis (L.) DC.

e) Clitoria ternatea L.

f) Crotalaria juncea L.

g) Dolichos biflorus Auct.

h) Vigna mungo (L.) Hepper.

i) Vigna radiata (L.) Wilczek.

j) Vigna unguiculata (L.) Walp.

15. Pedaliaceae:

a) Sesamum indicum L.

16. 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) Petunia hybrida Vilm.

h) Solanum melongena L.

17. Verbenaceae:

a) Clerodendron infortunatum L.

b) Lantana camara L.

c) Stachytarpheta indica L.

18. Zingiberaceae:

a) Zingiber officinale Rose.

VI. Serological properties of the virus

1. Purification of virus

The virus was purified following the method of Hebert (1963) and Van Kammen (1967). The inoculum was prepared by mincing the systemically infected, frozen leaves at the rate of 1 g/ml of 0.01 M phosphate buffer pH 7.0 in a clean sterile pestle and mortar. The homogenate was filtered through double layer muslin cloth and centrifuged at 10000 g for 15 min at 4°C using HIMAG refrigerated centrifuge model HCR20BA, to remove the host material. The clear supernatant was decanted and added polyethyleneglycol (PEG) to a final concentration of 4 per cent (w/v) and sodium chloride (NaCl) to give a concentration of 0.2 M. The mixture was stirred at room temperature to dissolve the PEG and NaCl and after one hour centrifuged at 10000 g for 15 min at 4°C. Both the fractions (supernatant and residue) were tested for infectivity separately and the residue was found to be highly

infective while the supernatant was not (residue was resuspended in phosphate buffer before test inoculation). The final virus preparation (residue obtained after the final centrifugation) was dissolved in 0.85 per cent saline and it was used as viral antigen for injecting rabbits.

2. Preparation of antiserum

Two healthy New Zealand white female rabbits weighing about 2 kg with conspicuous marginal ear vein were selected for immunization. The schedule of immunization consisted of five intramuscular injections at weekly intervals followed by one intravenous injection one week after the last intramuscular injection. In the case of intramuscular injection, the purified virus preparation suspended in 0.85 per cent saline was mixed with Freund's incomplete adjuvant (Difco), in the ratio 1:1 (V/V) and 4 ml of this emulsion was injected into the thigh muscle at a time. The final injection was given intravenously with 2 ml of 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 clot by keeping the tubes at room temperature for 2 h and after that the blood clot was loosened with the help of a sterilized glass rod and the samples were kept over night 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.

3. Serological tests

i) Microprecipitin test on slides

Thirty microlitres of antiserum and the same quantity of virus suspension were mixed on a microscopic slide. The mixture was incubated at 25°C under high humidity for 20-45 min and examined under microscope (Berckx et al., 1972). Isolates of snake gourd mosaic virus isolate I (isolated from diseased plants in the glass house) and isolate II (isolated from diseased plants in the field), cucumber mosaic virus, pumpkin mosaic virus, bitter gourd mosaic virus, cowpea mosaic virus and sword bean mosaic virus were tested against the antiserum of snake gourd mosaic virus. The above mentioned virus isolates were also tested with normal serum taken from healthy rabbits.

The virus suspension (snake gourd mosaic virus) was tested against three other antisera also viz., cucumber mosaic virus Arkansas isolate, cucumber mosaic virus-cowpea isolate (Arkansas) and cucumber mosaic virus ICRISAT isolate.

ii) Microprecipitin test in petridishes

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 using cotton wool and centrifuged at 5000 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% NaCl in 0.01 M Tris oxymethyl aminomethane buffer of pH 7.0) was added. The liquids were mixed by inverting the tube several times. This tube contained the sap diluted to $\frac{1}{2}$. Half of this dilution was transferred to next tube and an equal volume of saline buffer was added so asto make a dilution of $\frac{1}{4}$. This method was continued to make dilutions

of the series 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048 and 1/4096.

In the same way as with the sap from virus infected leaves, serial dilutions were made for the antiserum and healthy sap also. Healthy sap was used in the test as control.

A scheme was drawn on a paper with 10 mm squares and the sap and antiserum dilutions were marked as shown in the figure 1. A petridish 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 petridish at the point where the line labelled NaCl-buffer meet with the other lines. Using another pipette one drop each of the least concentrated sap (1/4096) was spotted at the intersections along the vertical line labelled 1/4096. 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 (1/4096) was taken in a fresh pipette and one drop was spotted to a saline drop and to the 13 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 scheme was followed for healthy sap also. The

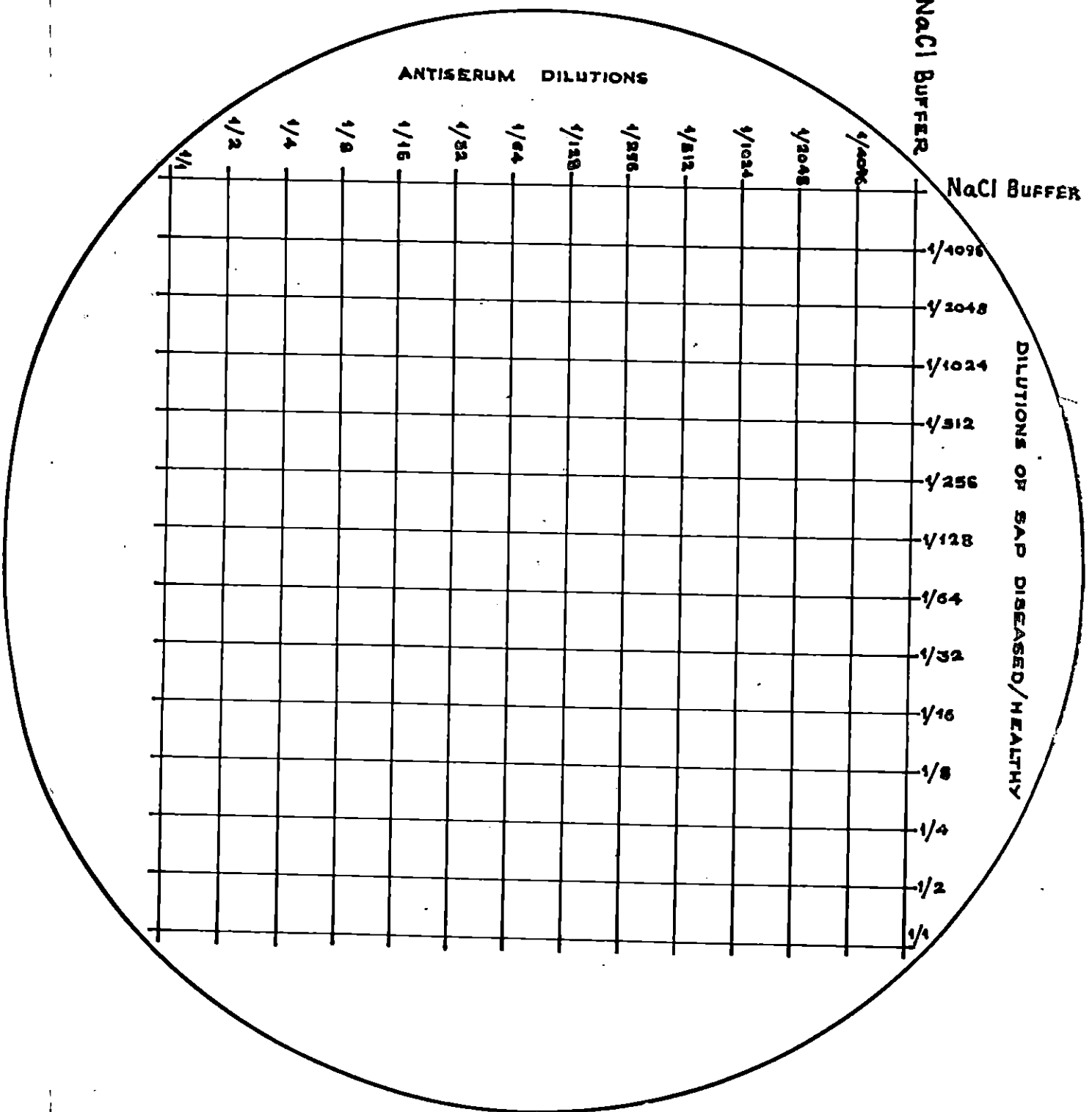

FIG. 1. MICROPRECIPITIN TEST IN PETRI DISHES

drops were covered with liquid paraffin to prevent evaporation. Liquid paraffin was added slowly through the sides of the petridish, so that the drops will not merge together. The petridishes were kept for 2 h at 28-30°C and examined after 2 h with a stereomicroscope with top light and black background. The intensity of the precipitate was evaluated based on a scale given below.

\- = No reaction

T = Barely visible reaction

\+ = Slight reaction

\++ = Moderate reaction

\+++ = Heavy reaction

\++++ = Very heavy reaction

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

iii) Ouchterlony's agar double diffusion test

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

Sterilized petridishes were coated with a thin 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 dry. 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. Each well was 3 mm deep and 5 mm in diameter and the distance between adjacent wells was 10 mm. In the central well (well No. 1) of each plate 0.4 ml of antiserum was dispensed with a pasteur pipette and the antigens prepared from infected plants were dispensed in the surrounding wells as described below in five separate plates.

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

In the second plate, well 2 contained sap from infected snake gourd plants and the well 4 was filled with sap from healthy snake gourd plants, 3 with sword bean mosaic virus, 5 with cucumber mosaic virus and 6 with cowpea mosaic virus.

The wells 3 and 5 of the third plate contained healthy plant sap from snake gourd plants. Well 4 received sap from

bitter gourd mosaic affected plants, 6 received snake gourd mosaic virus obtained from plants grown in glass house and 2 received snake gourd mosaic virus obtained from plants grown in the field.

In the case of plate 4, wells 2 and 5 received snake gourd mosaic virus, 3 contained bitter gourd mosaic virus, 4 pumpkin mosaic virus and 6 healthy sap.

In plate 5, the experiment was done by using purified virus preparation diluted to ½ concentration. Wells 3 and 5 contained antigen for snake gourd mosaic virus, 4 contained cucumber mosaic virus and 2 pumpkin mosaic virus. Well 6 contained sap taken from healthy snake gourd plants diluted to a concentration of ½.

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

Before staining, the agar was soaked in two changes of 0.9 per cent phosphate buffered saline for 24 h and then

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

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

VII. Varietal screening

Seven varieties of snake gourd plants were tested for their resistance to snake gourd mosaic virus. The plants were grown in pots and kept in insect proof glass house. Ten plants of each variety were inoculated with the virus using standard sap as inoculum. The inoculum was prepared by grinding mosaic affected snake gourd leaves with sterilized pestle and mortar adding equal amount of distilled water (w/v). The sap was extracted from crushed pulp by squeezing through cotton wool and immediately inoculated on the leaves of test plants of 3-4 leaf stage. The experiment was conducted twice. Following were the varieties used for screening studies.

1. CO-1
2. TA-9
3. TA-10
4. TA-19
5. PKM-1
6. Extra long (Pocha)
7. Vellayani local

VIII. Estimation of loss

Experiments were conducted to estimate the effect of snake gourd mosaic virus infection at different stages of growth in commonly cultivated varieties under field condition. The experiment was laid out in Randomised Block Design in Instructional Farm attached to the College of Agriculture, Vellayani during 1985 (summer season). The lay out plan of the experiment is given in Fig. 2.

1. Varieties and seed materials

Two commonly cultivated varieties, viz., Vellayani local (V_1) and TA-19 (V_2) were used for conducting the experiment. The variety TA-19 was obtained from the Department of Olericulture, College of Horticulture, Vellanikkara, Trichur and the variety Vellayani local was obtained from the Instructional Farm, Vellayani. The following treatments were fixed to estimate the effects of infection at different growth stages of the plant.

Treatment-1 (V_1T_1) - Inoculation of Vellayani local variety on 15th day after planting.

Treatment-2 (V_1T_2) - Inoculation of Vellayani local variety on 45th day after planting.

Treatment-3 (V_1) - Vellayani local variety maintained without inoculation (control).

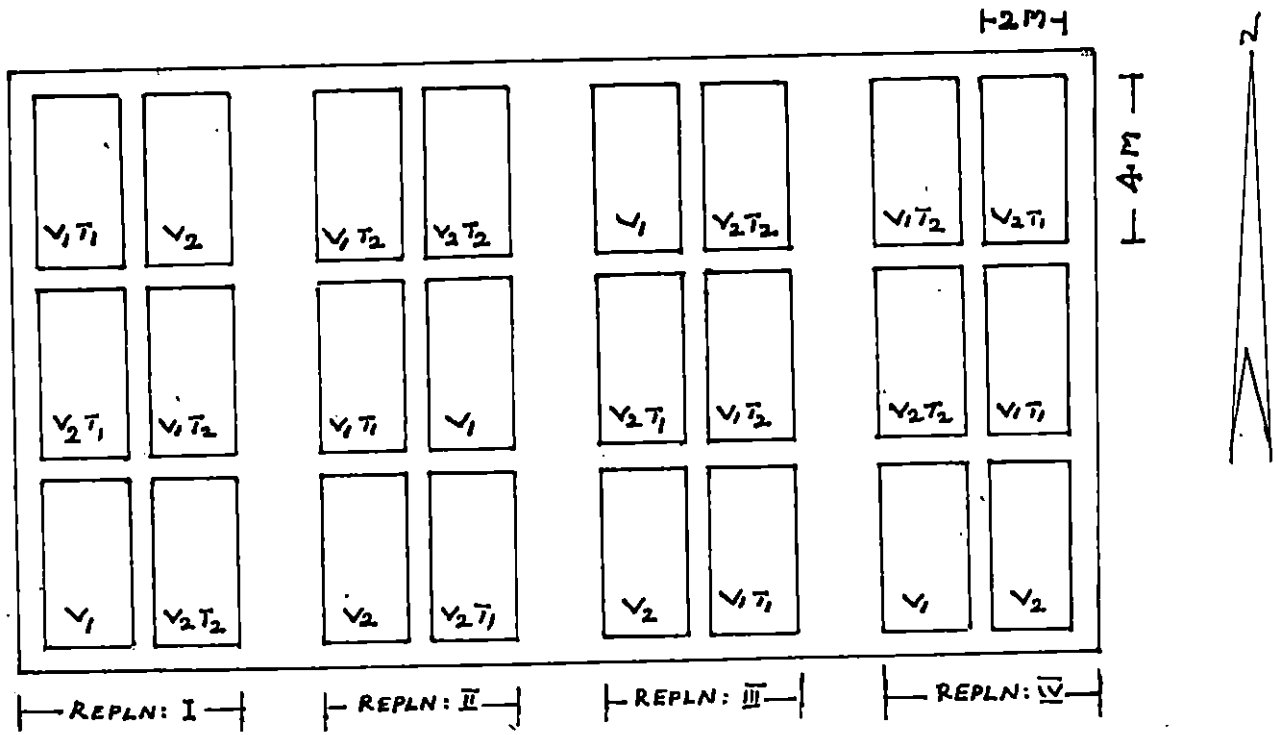

FIG. 2 LAY OUT OF THE FIELD EXPERIMENT TO ESTIMATE THE EFFECT OF INFECTION ON SNAKE GOURD BY SNAKE GOURD MOSAIC VIRUS AT DIFFERENT GROWTH STAGES

Treatment-4 (V_2T_1) - Inoculation of TA-19 on 15th day after planting.

Treatment-5 (V_2T_2) - Inoculation of TA-19 on 45th day after planting.

Treatment-6 (V_2) - TA-19 maintained without inoculation (control).

2. Field culture

The experimental field was dug twice, stubbles removed, clods broken and laid out into four blocks each with 6 plots. The plot size was 4 m x 2 m. The individual plots were thoroughly dug and levelled. Two pits were prepared in each plot at a spacing of two metre. 4-5 seeds were sown in each pit, but only two vigorously growing plants per pit were retained and used for the experiment.

Manures and fertilizers were applied according to the Package of Practice Recommendations of the Kerala Agricultural University (Anon, 1983). Separate pandals were constructed for each plot for training the plants. The plants were irrigated daily. All the plants were periodically sprayed with 0.1% Quinalphos to keep the plants free from insect infestation.

The crop was sown on 16-3-1985 and observations were recorded on the following aspects at an interval of 15 days

after virus inoculation, till the plants were 90 days old when harvesting completed.

a) Number of leaves formed
b) Leaf area
c) Internodal length
d) Thickness of vine
e) Total length of vine
f) Number of branches developed
g) Number of flowers formed (male and female)
h) Number of fruits formed
i) Length of the fruits
j) Girth of the fruits
k) Mean weight of fruit
l) Yield of fruits

3. Statistical analysis

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

During the 30^{th} and 45^{th} days of observation the treatments V_1T_2 and V_2T_2 were identical with V_1 and V_2 respectively. Hence the number of distinct treatments on these periods were only four. Necessary modifications were

made in the analysis to effect this change. The procedure adopted for the analysis is briefly described below.

The total sum of square and replication sum of square were calculated as per the procedure for Randomised Block Design (RBD). The treatment sum of square was calculated as in the case of Completely Randomised Design with unequal replications. The error sum of square (ESS) was obtained as follows.

$ESS = E_1SS + ASS + BSS$ where

ASS = Untreated within block sum of square of variety V_1

BSS = Untreated within block sum of square of variety V_2

E_1SS = Error sum of square of RBD

Corresponding changes in the degrees of freedom were also made in the case of data collected on 30th and 45th days.

The data on the number of leaves, number of branches, number of fruits and number of flowers formed were analysed after applying the square root transformation (Snedecor and Cochran, 1967).

RESULTS

RESULTS

I. Symptomatology

The leaves of naturally infected plants showed mosaic pattern of irregular dark green and yellow chlorotic patches on the lamina (Fig. 3). The symptoms appeared as small chlorotic areas which later coalesced together to form larger patches. In advanced stages of infection dark green raised blisters of varying size and shape developed on the lamina. In certain cases the leaves showed varying amount of dark green vein banding and interveinal chlorosis. 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.

On mechanical inoculation to snake gourd plants of 3-4 leaf stage, the symptoms appeared within 5-8 days. The symptoms first appeared as small greenish yellow areas, which are very thin compared to the healthy areas of the leaf. Typical mosaic mottling with dark green and light green patches was produced in all the subsequent leaves. In the older leaves, chlorotic areas were seen with less pronounced mottling. In some cases, the leaves had prominent vein banding and occasionally with dark green blisters. The growth of the infected plants was retarded and internodes

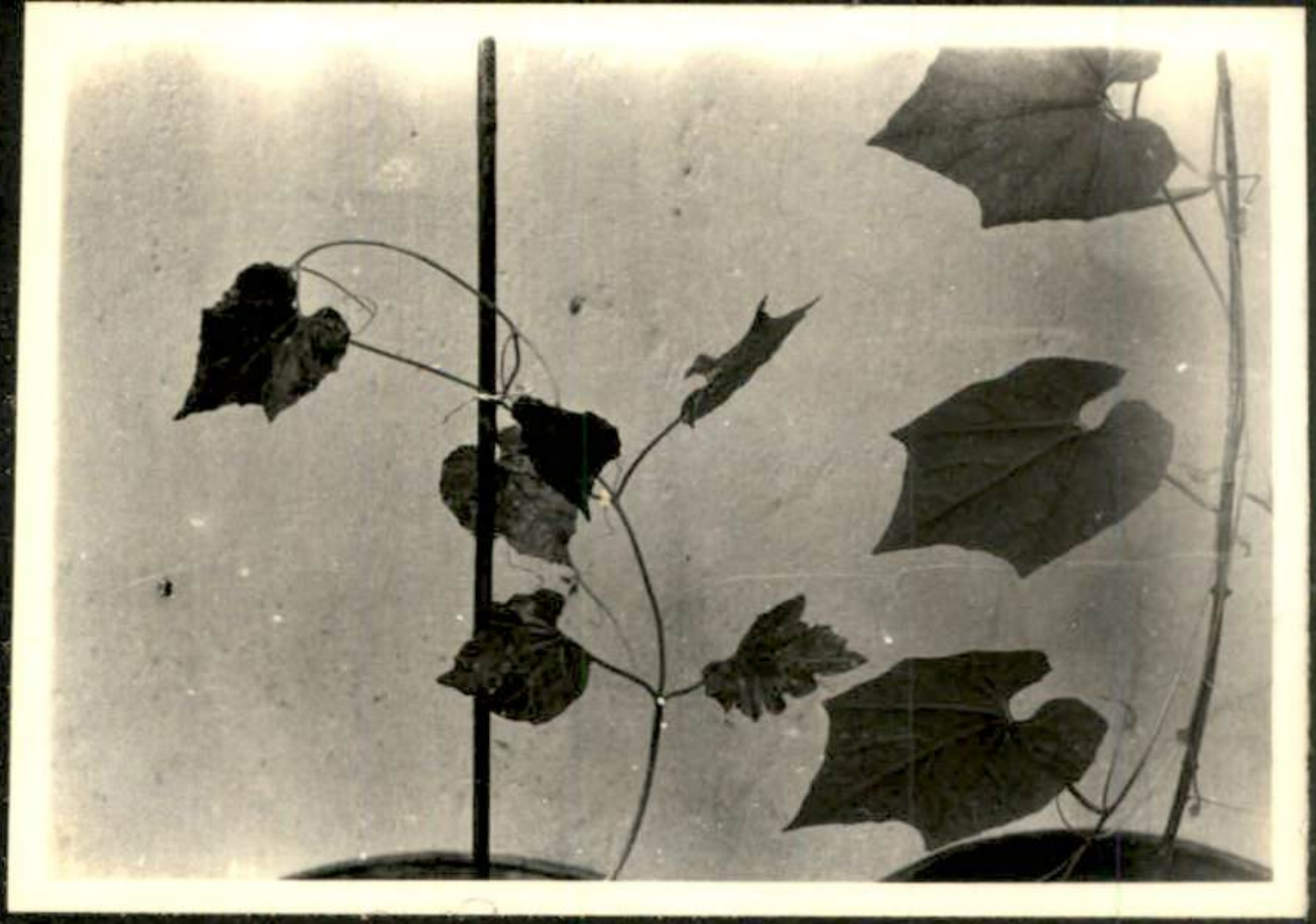

Fig. 4. Diseased and healthy snake gourd plants

shortened (Fig. 4). As in the case of naturally infected plants, the inoculated plants also produced only a few flowers and fruits.

II. Transmission of the virus

1. Sap transmission

The virus was found to be transmitted successfully by sap inoculation using standard sap, sap extracted in phosphate buffer (0.01 M, pH 7.0) and tris buffer (0.01 M, pH 7.0). The symptoms appeared 5-8 days after inoculation. Use of phosphate buffer and tris buffer did not show any significant increase in the infectivity of the virus (Table 1).

Table 1. Sap transmission of snake gourd mosaic virus

Sl. No.	Inoculum	Number of plants infected		Per cent infection
		Number of plants inoculated		
		Experiment I	Experiment II	
1.	Standard sap	9/10	10/10	95
2.	Sap extracted in phosphate buffer	10/10	9/10	95
3.	Sap extracted in tris buffer	9/10	10/10	95

2. Seed transmission

Out of the 180 seeds sown 134 seeds have germinated. None of the plants showed symptoms of snake gourd mosaic virus disease during the period of observation.

3. Graft transmission

Infected shoots were wedge grafted to 25 day old healthy plants, which were grown in insect proof glass house. The symptoms appeared 7-10 days after grafting was done. From the two trials conducted an average 90 per cent transmission was obtained from graft inoculation (Table 2).

Table 2. Graft transmission of snake gourd mosaic virus

Trials	Number of plants grafted	Number of plants infected	Per cent transmission
I	10	8	80
II	10	10	100

4. Insect transmission

Insect transmission studies of the virus were carried out using 5 vectors, viz., Aphis craccivora, Aphis gossypii, Aphis malvae, Pentalonia nigronervosa and Bemisia tabaci and the results are presented in Table 3. Aphis gossypii was found to transmit snake gourd mosaic virus very

efficiently followed by Aphis malvae. 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 7-8 days after inoculation.

The observations showed that the highest percentage of transmission (95%) was obtained with Aphis gossypii followed by A. malvae (85%) and A. craccivora (45%). Bemisia tabaci and Pentalonia nigronervosa were not able to transmit the virus.

Table 3. Insect transmission of snake gourd mosaic virus

Sl. No.	Vector	Number of plants infected / Number of plants inoculated		Per cent transmission
		Experiment I	Experiment II	
1.	Aphis craccivora	4/10	5/10	45
2.	Aphis gossypii	9/10	10/10	95
3.	Aphis malvae	9/10	8/10	85
4.	Bemisia tabaci	0/10	0/10	0
5.	Pentalonia nigronervosa	0/10	0/10	0

III. Physical properties

1. Dilution end point (DEP)

Serial dilutions of the infected sap was made viz.,

1:100, 1:500, 1:1000, 1:5000, 1:10000, 1:50000, 1:100000, 1:500000, 1:1000000. The different dilutions were used for inoculation on separate test plants starting from the highest dilution. Ten plants were inoculated with each of the dilutions and the experiment was repeated to confirm the result. The data indicated that the dilution end point of the virus was between 1:10000 and 1:50000 (Table 4).

Table 4. Dilution end point of snake gourd mosaic virus

Dilutions	Experiment I		Experiment II		Per cent transmission
	Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected	
0	10	9	10	10	95
1:100	10	10	10	10	100
1:500	10	10	10	10	100
1:1000	10	9	10	8	85
1:5000	10	7	10	6	65
1:10000	10	3	10	3	30
1:50000	10	0	10	0	0
1:100000	10	0	10	0	0
1:500000	10	0	10	0	0
1:1000000	10	0	10	0	0

2. Thermal inactivation point (TIP)

The infected sap was treated at different range of temperature, viz., 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 and 90°C. The treated and untreated (control at room temperature at 28-30°C) samples of the sap were inoculated on young vigorously growing test plants of 3-4 leaf stage. The results indicated that the virus was inactivated at temperature between 70 and 75°C (Table 5).

Table 5. Thermal inactivation point of snake gourd mosaic virus

Temperature	Experiment I		Experiment II		Per cent transmission
	Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected	
Control (28-30°C)	10	10	10	10	100
35°C	10	9	10	10	95
40°C	10	10	10	9	95
45°C	10	8	10	9	85
50°C	10	8	10	7	75
55°C	10	6	10	7	65
60°C	10	7	10	5	60
65°C	10	5	10	4	45
70°C	10	2	10	1	15
75°C	10	0	10	0	0
80°C	10	0	10	0	0
85°C	10	0	10	0	0
90°C	10	0	10	0	0

3. Longevity *in vitro* (LIV)

In order to find out the longevity *in vitro*, an experiment was conducted as described in materials and methods and the results are given in Tables 6 and 7. When the inoculum was stored at room temperature (28-30°C) for a period of 120 h, its infectivity was completely lost. About 35 per cent of the plants inoculated with sap kept for 96 h at room temperature developed symptoms. So the longevity *in vitro* of the virus stored at room temperature was between 96 and 120 h.

When the inoculum was stored in a refrigerator (10°C) the infectivity was retained upto 144 h, but the per cent of infected plants was considerably decreased. After 168 h of storage of the inoculum the infectivity was completely lost. So the longevity *in vitro* of the virus was between 144 and 168 h, when the sap was stored under refrigerated conditions.

IV. Vector-virus relationships

1. Acquisition threshold

This experiment was conducted to find out the minimum period required for the vector to acquire the virus and to become viruliferous. The results are presented in Table 8.

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

Ageing in hours	Experiment I		Experiment II		Per cent transmission
	Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected	
0	10	10	10	10	100
24	10	8	10	10	90
48	10	8	10	6	70
72	10	6	10	4	50
96	10	4	10	3	35
120	10	0	10	0	0
144	10	0	10	0	0
168	10	0	10	0	0
192	10	0	10	0	0

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

Ageing in hours	Experiment I		Experiment II		Per cent transmission
	Number of plants inoculated	Number of plants infected	Number of plants inoculated	Number of plants infected	
0	10	9	10	10	95
24	10	10	10	8	90
48	10	7	10	7	70
72	10	5	10	6	55
96	10	4	10	4	40
120	10	3	10	2	25
144	10	2	10	1	15
168	10	0	10	0	0
192	10	0	10	0	0

The results showed that a short acquisition feeding period of 30 s only is sufficient for the aphids to become viruliferous. The optimum acquisition feeding period which gave the maximum percentage of infection (90%) was found to be 20 min.

Table 8. Acquisition threshold of *Aphis gossypii* on the transmission of snake gourd mosaic virus

Acquisition feeding period	Number of plants infected		Number of plants infected out of 20	Per cent transmission
	Number of plants inoculated			
	Experiment I	Experiment II		
20 s	0/10	0/10	0	0
30 s	1/10	1/10	2	10
1 min	3/10	4/10	7	35
2 min	4/10	3/10	7	35
5 min	5/10	6/10	11	55
10 min	8/10	7/10	15	75
15 min	7/10	9/10	16	80
20 min	9/10	9/10	18	90
30 min	8/10	10/10	18	90
45 min	7/10	8/10	15	75
1 h	5/10	6/10	11	55
2 h	3/10	4/10	7	35

Pre-acquisition fasting - 1 h
Inoculation feeding - 24 h
Number of aphids per plant - 10

Table 9. Inoculation threshold of A. gossypii on the transmission of snake gourd mosaic virus

Inoculation feeding period	Number of plants infected		Number of plants infected out of 20	Per cent transmission
	Number of plants inoculated			
	Experiment I	Experiment II		
30 s	0/10	0/10	0	0
1 min	2/10	2/10	4	20
2 min	4/10	3/10	7	35
5 min	7/10	6/10	13	65
10 min	7/10	8/10	15	75
15 min	6/10	9/10	15	75
20 min	8/10	8/10	16	80
30 min	8/10	8/10	16	80
45 min	10/10	8/10	18	90
1 h	10/10	10/10	20	100
2 h	10/10	10/10	20	100
4 h	9/10	10/10	19	95
8 h	8/10	9/10	17	85
24 h	8/10	9/10	17	85

Pre-acquisition fasting - 1 h
Acquisition feeding - 30 min
Number of aphids per plant - 10

2. Inoculation threshold

In order to find out the minimum period required for the viruliferous aphid A. gossypii to transmit the virus successfully, an experiment was conducted as described in materials and methods and the results are presented in Table 9.

The data indicated that the viruliferous aphids were capable of transmitting the virus with one minute inoculation feeding on the test plant. Maximum infection of 100 per cent was obtained by feeding the vector for 1 h on test plants.

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

The data in Table 10 reveal that pre-acquisition fasting of aphids increased the efficiency of the vector to acquire and transmit the virus. Maximum efficiency was noted when insects were starved for a period of 1 h. Further increase of fasting period did not appreciably increase the per cent of infected plants and so also the efficiency of the vector to transmit the virus.

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

The results are presented in Table 11. It was observed

Table 9. Inoculation threshold of *A. gossypii* on the transmission of snake gourd mosaic virus

Inoculation feeding period	Number of plants infected		Number of plants infected out of 20	Per cent transmission
	Number of plants inoculated			
	Experiment I	Experiment II		
30 s	0/10	0/10	0	0
1 min	2/10	2/10	4	20
2 min	4/10	3/10	7	35
5 min	7/10	6/10	13	65
10 min	7/10	8/10	15	75
15 min	6/10	9/10	15	75
20 min	8/10	8/10	16	80
30 min	8/10	8/10	16	80
45 min	10/10	8/10	18	90
1 h	10/10	10/10	20	100
2 h	10/10	10/10	20	100
4 h	9/10	10/10	19	95
8 h	8/10	9/10	17	85
24 h	8/10	9/10	17	85

Pre-acquisition fasting - 1 h
Acquisition feeding - 30 min
Number of aphids per plant - 10

Table 10. Effect of pre-acquisition fasting of *A. gossypii* on the efficiency of transmission of snake gourd mosaic virus

Pre-acquisition fasting period	Number of plants infected / Number of plants inoculated		Number of plants infected out of 20	Per cent transmission
	Experiment I	Experiment II		
No fasting	5/10	6/10	11	25
30 min	7/10	7/10	14	70
1 h	9/10	10/10	19	95
2 h	8/10	9/10	17	85
3 h	5/10	5/10	10	50
4 h	5/10	4/10	9	45
5 h	3/10	4/10	7	35
6 h	0/10	0/10	0	0
12 h	0/10	0/10	0	0

Acquisition feeding - 30 min
Inoculation feeding - 24 h
Number of aphids per plant - 10

that post-acquisition fasting of the vector decreased the percentage of infection. Maximum infection of 90 per cent was obtained when the aphids were immediately transferred to test plants after acquisition feeding period and no infection was obtained when the aphids were given a post-acquisition fasting beyond 3 h.

Table 11. Effect of post-acquisition fasting of A. gossypii on the efficiency of transmission of snake gourd mosaic virus

Post-acquisition fasting	Number of plants infected		Number of plants infected out of 20	Per cent transmission
	Number of plants inoculated			
	Experiment I	Experiment II		
No fasting	9/10	9/10	18	20
30 min	7/10	6/10	13	65
1 h	4/10	4/10	8	40
2 h	3/10	5/10	8	40
3 h	2/10	2/10	4	20
4 h	0/10	0/10	0	0
5 h	0/10	0/10	0	0
6 h	0/10	0/10	0	0
12 h	0/10	0/10	0	0

Pre-acquisition fasting - 1 h
Acquisition feeding - 30 min
Inoculation feeding - 24 h
Number of aphids per plant - 10

5. Retension of infectivity by the vector

The results indicated that successful infection could be obtained upto the third plant of the first series in which aphids were transferred at intervals of 30 min and

upto 2nd plant when the interval was increased to 1 h. In all the other cases only the first plant of the series got infection, indicating that in most cases the viruliferous nature of the vector was lost after 1½ h (Table 12).

Table 12. Retension of infectivity by *A. gossypii*

Feeding period on each test plant		Infection produced in successive transfers				
		Serial number of plants tested				
		1	2	3	4	5
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	-	-	-	-	-

\+ Symptom produced

\- No symptom produced

6. 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 95 per cent was found to be 10 (Table 13).

Table 13. Minimum number of aphids (A. gossypii) required for the transmission of snake gourd mosaic virus

Number of aphids per plant	Number of plants infected / Number of plants inoculated		Number of plants infected out of 20	Per cent transmission
	Experiment I	Experiment II		
1	2/10	1/10	3	15
2	2/10	2/10	4	20
3	4/10	5/10	9	45
5	6/10	8/10	14	70
10	9/10	10/10	19	95
15	9/10	9/10	18	90
20	9/10	8/10	17	85
25	9/10	8/10	17	85
30	8/10	8/10	16	80

Pre-acquisition - 1 h
Acquisition feeding - 30 min
Inoculation feeding - 24 h

V. Host-range and local lesion hosts

Out of 70 plant species belonging to 18 families tested, 11 species of plants belonging to 3 families viz.,

compositae, cucurbitaceae and solanaceae produced systemic symptoms and 2 species of plants belonging to the family Chenopodiaceae produced local lesions.

1. Chenopodiaceae:

a) Chenopodium amaranticolor Coste & Reyn.

The inoculated plants produced local lesions within 2-3 days. The lesions appeared as chlorotic in the beginning, turned to necrotic with dark brown centre. The lesions were circular in shape and 1-2 mm in diamter (Fig. 5).

b) Chenopodium quinoa Willd.

The inoculated plants produced local lesions within 2-4 days. They appeared as circular chlorotic spots in the initial stage and soon enlarged and developed into necrotic with brown centre. The lesions were 2-3 mm in diameter (Fig. 6).

2. Compositae:

a) Ageratum conizoides L.

The inoculated plants produced systemic symptoms within 8-10 days after inoculation. Young leaves showed pale green areas and later developed dark green raised blisters. The size of leaves were reduced and plants were stunted (Fig. 7).

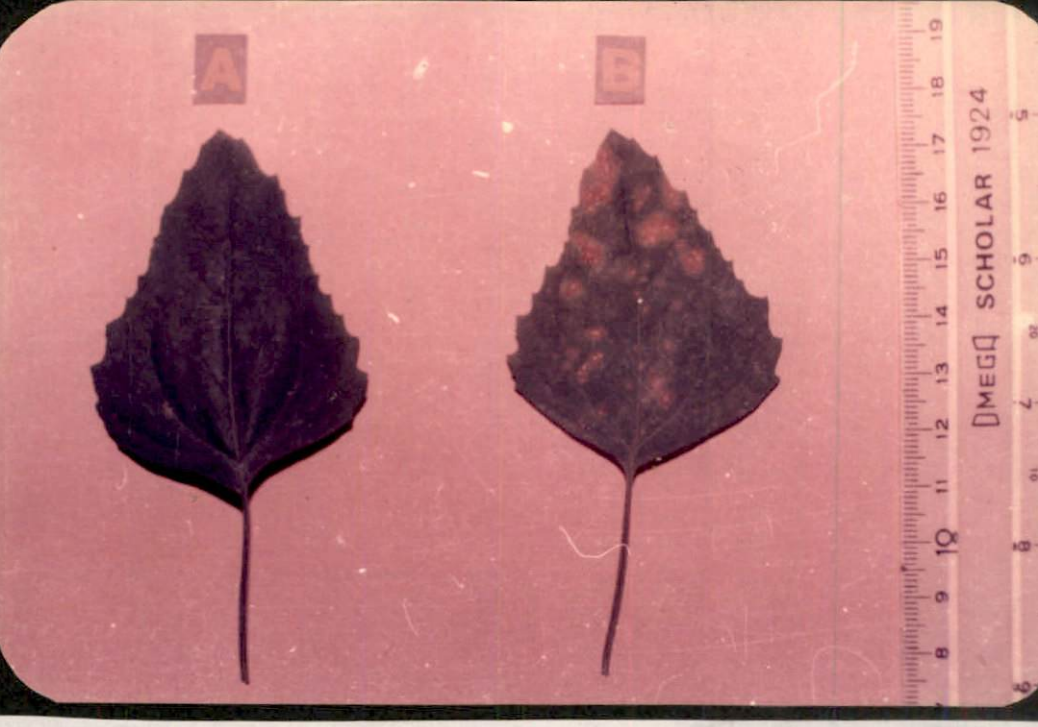

Fig. 5. Local lesions of snake gourd mosaic virus on Chenopodium amaranticolor

Fig. 6. Local lesions of snake gourd mosaic virus on Chenopodium quinoa

3. Cucurbitaceae

a) Cucurbita moschata Duch.

The inoculated plants produced symptoms within 5-7 days. Yellowish green chlorotic spots were developed on the lamina with less pronounced mottling. The chlorotic spots coalesced together to form larger patches. The size of leaves was reduced considerably, internodes were shortened and plants were stunted (Fig. 8).

b) Cucurbita pepo L.

The inoculated plants produced symptoms within 5-7 days. The leaves exhibited pale green spots which enlarged in size and developed into yellowish green patches. Reduction in the size of leaves, crinkling and distortion of lamina were also observed. The infected plants were stunted (Fig. 9).

c) Cucumis sativus L.

The symptoms were first noticed on the developing young leaves as small greenish yellow areas. Later characteristic yellow mottles appeared. The leaves were reduced in size, distorted, crinkled and in advanced stages there was downward curling of the edges of the lamina. The plants were stunted and internodes were shortened.

Fig. 7. Ageratum conizoides infected with snake gourd mosaic virus

Fig. 8. Cucurbita moschata infected with snake gourd mosaic virus

d) Lagenaria siceraria (Mol) & Standl.

The symptoms first appeared on the young developing leaves within 7-8 days after inoculation. Yellowish green chlorotic patches were formed on the lamina which later enlarged in size. Raised blisters were formed on the greenish darker portions. Reduction in size of leaves, crinkling and distortion of lamina were also observed (Fig. 10).

e) Luffa acutangula Roxb.

The inoculated plants produced symptoms within 8-9 days and was characterised by the development of slightly irregular and small yellow spots in the dark green background of the leaf. The chlorotic patches coalesced together to form larger patches. Reduction in the size of leaves and stunted growth of the plants were noticed (Fig. 11).

f) Melothria perpusilla L.

The symptoms developed within 7-9 days on young developing leaves after inoculation. The leaves produced pale green spots which enlarged in size and developed into yellowish green patches. The leaves were reduced in size, distorted and in advanced stages there was upward curling of the edges of the lamina. The plants were stunted and internodes were shortened (Fig. 12).

Fig. 10. Lagenaria siceraria infected with snake gourd mosaic virus

Fig. 12. *Melothria perpusilla* infected with snake gourd mosaic virus

g) Momordica charantia L.

The symptoms appeared within 5-7 days as yellowish green chlorotic patches which later coalesced to ether to form larger patches of infected tissue. The yellowing gradually spread over the entire leaf in the interveinal area and produced vein banding. Reduction in size of leaves, crinkling and distortion of lamina were also observed. The infected plants were stunted (Fig. 13).

4. Solanaceae:

a) Datura stramonium L.

The symptoms appeared 7-9 days after inoculation as irregular yellow patches on the leaf lamina which later on enlarged in size. The newly emerged leaves were highly reduced in size and were curled. The plants showed severe stunting and the internodes were shortened (Fig. 14).

b) Nicotiana glutinosa L.

The inoculated plants showed mottling within 5-6 days. A large number of dark green raised blisters were produced on the lamina. The newly emerged leaves were reduced in size and plants showed severe stunting. The internodes were shortened (Fig. 15).

Fig. 13. Momordica charantia infected with snake gourd mosaic virus

Fig. 14. Datura stramonium infected with snake gourd mosaic virus

Fig. 16. Nicotiana tabacum infected with snake gourd mosaic virus

c) Nicotiana tabacum L.

The inoculated plants showed vein clearing symptoms mingled with large number of dark green raised blisters on the lamina. In advanced stages the yellowing spread to other parts of the leaves (Fig. 16).

VI. Serological properties of the virus

1. Microprecipitin test on slides

Thirty microlitres of antiserum prepared as described under materials and methods was mixed with equal volume of antigen from different virus infected crop plants. The antigens of snake gourd mosaic virus Isolate I, Isolate II, cucumber mosaic virus and pumpkin mosaic virus produced dense precipitate with the antiserum specific to snake gourd mosaic virus. Antigens of bitter gourd mosaic virus, cowpea mosaic virus and sword bean mosaic virus did not produce any precipitate (Table 14).

When the snake gourd mosaic virus antigen was tested against the antisera of cucumber mosaic virus Arkansas isolate, cucumber mosaic virus-cowpea isolate (Arkansas) and cucumber mosaic virus ICRISAT isolate no precipitate was formed.

Table 14. Microprecipitin test on slides

Sl. No.	Antigen	Reaction with anti-serum	Reaction with normal serum
1.	Snake gourd mosaic virus (Isolate I)	+	-
2.	Snake gourd mosaic virus (Isolate II)	+	-
3.	Cucumber mosaic virus	+	-
4.	Pumpkin mosaic virus	+	-
5.	Bitter gourd mosaic virus	-	-
6.	Cowpea mosaic virus	-	-
7.	Sword bean mosaic virus	-	-

\+ Positive precipitin reaction
\- No precipitate formed

2. Microprecipitin test in petridishes

Series of dilution mixtures of virus and antiserum were spotted in petridishes at regular intervals. The precipitate was observed after 4 h under a stereomicroscope with top light and black background. The intensity of the precipitate was graded. It was observed that the antiserum titre was between 1:2048 and 1:4096 and the virus end point was between 1:1024 and 1:2048 (Table 15). The above experiment with healthy snake gourd plant sap did not produce any precipitate.

Table 15. Microprecipitin test of snake gourd mosaic virus and its antiserum

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

The curved line encloses the area of precipitates visible under microscope

++++ Very heavy reaction ++ Moderate reaction 1 Barely visible reaction

+++ Heavy reaction + Slight reaction - No reaction

3. Ouchterlony's agar double diffusion test

This test was performed in agarose taken in petri-dishes. The precipitates formed due to antiserum-antigen interaction were stained using amidoblack and the types of precipitate formed were recorded.

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

In the second plate there were dark thick line between the wells 1 and 2, and 1 and 5 (Fig. 17). Here the well 2 received snake gourd mosaic virus and the well 4 was filled with sap from healthy snake gourd plant, well 3 with sword bean mosaic virus, well 5 with cucumber mosaic virus and well 6 with cowpea mosaic virus.

In the third plate, well 1 contained antiserum of snake gourd mosaic virus and wells 3 and 5 were filled with sap from healthy snake gourd plant. Well 4 received sap from bitter gourd mosaic virus affected plants and wells 2 and 6 received snake gourd mosaic virus obtained from plants grown on the field and glass house respectively. Precipitin band was formed in between wells 1 and 2, and 1 and 6. The

Fig. 17. Well 1 received antiserum of snake gourd mosaic virus, well 2 received snake gourd mosaic virus, 3 sword bean mosaic virus, 4 sap from healthy snake gourd plant, 5 cucumber mosaic virus and 6 cowpea mosaic virus.

Fig. 18. Well 1 contained antiserum of snake gourd mosaic virus, wells 3 and 5 filled with sap from healthy snake gourd plant, well 4 received bitter gourd mosaic virus and, wells 2 and 6 received snake gourd mosaic virus obtained from plants grown on the field and glass house respectively.

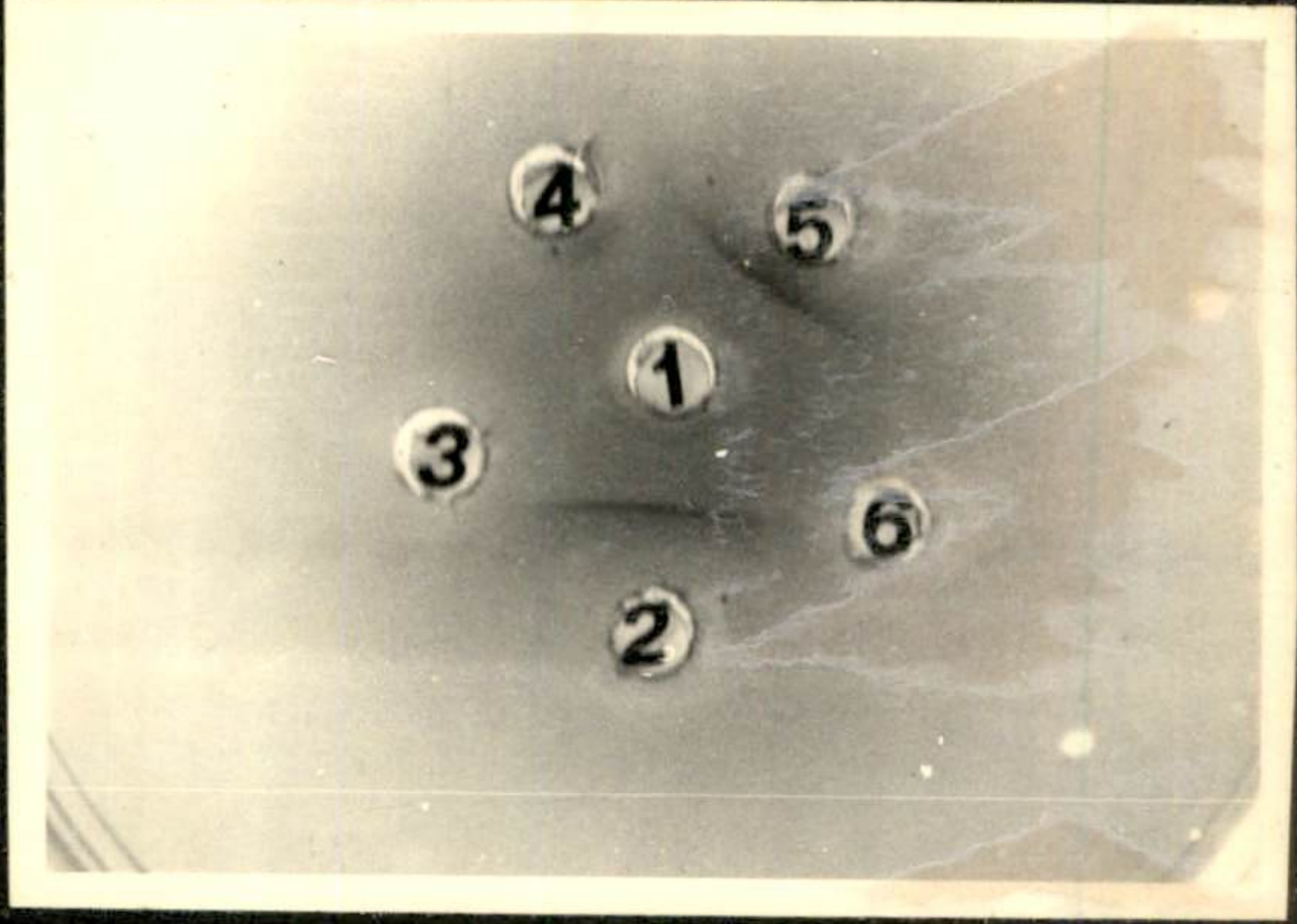

Fig. 17. Serologic reactions of snake gourd mosaic virus

fusion o the two precipitin line indicates that the virus isolate' from field and 'hat obtained from inoc late plants in the glass house are one and the same ig. 19).

In the case of plate 4, well 1 contained antiserum of snake gourd mosaic virus, wells 2 and 5 received snake gourd mosaic virus, 3 contained bi ter ourd mosaic virus, 4 pumpkin mosaic virus and 6 healthy sap of snake gourd plant (Fig. 19). The precipitin lines formed between 1 and 4 and 1 and 5 fused together showing that the are related, ie. pumpkin mosaic virus is serologically related to snake gourd mosaic virus. he precipitin line formed between well 1 and 2 is due to the antigen and antiserum reaction of snake gourd mosaic virus.

Fig. 20 shows the type of precipitin line in plate 5. This experiment was done by using purified virus preparations diluted to 1 concentration. ells 3 and 5 contained snake ourd mosaic virus antigen, 4 contained cucumber mosaic virus antigen and 2 pumpkin mosaic virus anti en. ell 6 contained sap from healthy snake gourd plants diluted to a concentration of . hin dark blue distinct bands were formed between wells 1 and 2, 1 and 3, 1 and 4 and 1 and 5. All the bands formed are fused together showing that cucumber mosaic virus and pumpkin mosaic virus are serologically related to snake gourd mosaic virus.

i . 1 . ell 1 contained antiserum of sna e ourd mosaic virus, wells ? and received sna e ourd osa c virus, ³ contained bitter our mosaic virus, 4 pumpkin mosaic virus and healthy sa of snake gourd plant.

i-. 2 . he experiment as one by u in urified virus preparation diluted to concentration. ell 1 contained antiserum of snake ourd mosaic virus diluted to , ells an contained sna e ourd mosaic virus antigen diluted to , contains cucu ber mosaic virus and ? umpkin mosaic virus, both dilute to . ell 6 contains ea from health snake our plant dilute to .

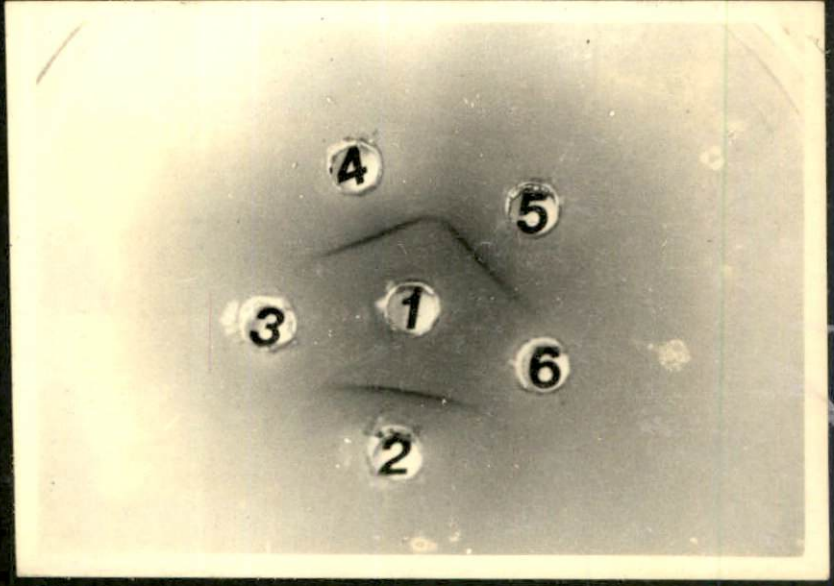

Fig. 19. Serologic reactions of snake gourd mosaic virus

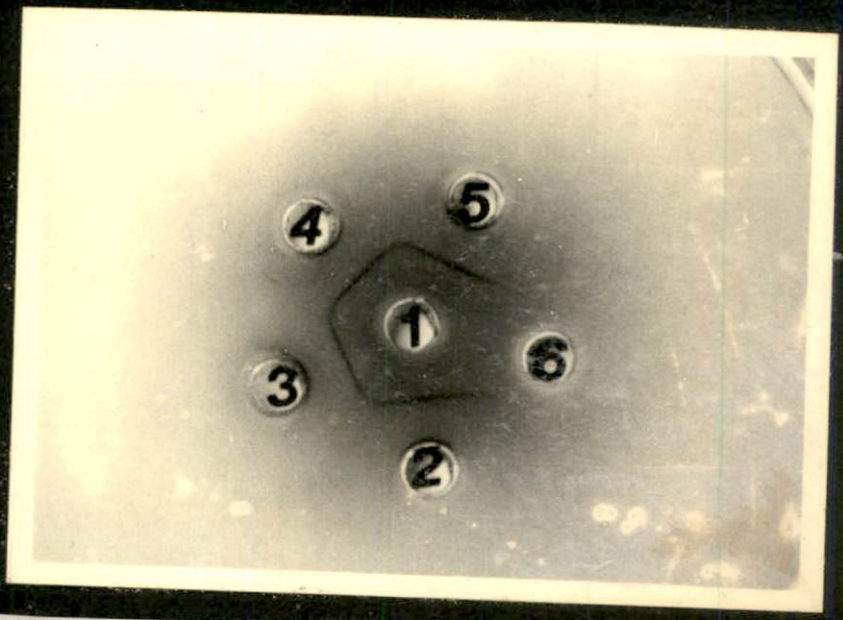

Fig. 20. Serologic reactions of snake gourd mosaic virus

VII. Varietal screening

Seven varieties were inoculated mechanically using snake gourd mosaic virus. Observations indicated that none of the varieties was found resistant to snake gourd mosaic virus. Appearance of symptoms on the newly emerged leaves varied from 5-8 days depending on the variety. Some varieties were more susceptible and produced severe symptoms compared to other varieties. Extra-long (Pocha) produced symptoms 5 days after inoculation and was very severe. It got 100 per cent infection. Co-1 was least susceptible which produced symptoms 8 days after inoculation and showed 65 per cent infection. The susceptibility of other varieties were between 80-95 per cent (Table 16).

Table 16. Incidence of snake gourd mosaic disease in different varieties of snake gourd

Varieties	Number of plants infected / Number of plants inoculated		Per cent infection	Time taken for appearance of symptom (days)
	Experiment I	Experiment II		
1. Co-1	7/10	6/10	65	8
2. TA-9	8/10	8/10	80	7
3. TA-10	9/10	7/10	80	7
4. TA-19	8/10	9/10	85	6
5. PKM-1	9/10	10/10	95	6
6. Extra-long (Pocha)	10/10	10/10	100	5
7. Vellayani local	9/10	10/10	95	6

VIII. Estimation of loss

1. Effect of snake gourd mosaic virus infection on number of leaves.

The observations of the experiment to find out the effect of virus infection of 15 and 45 day old plants on the number of leaves formed are given in Table 17. The data showed that the virus inoculation of 15 day old plants reduced the number of leaves significantly as compared to control and other treatment. Treatment V_1T_1 showed reduction in number of leaves from 30th day onwards whereas treatment V_2T_1 showed reduction on number of leaves from 45th day onwards. It was observed that the number of leaves were not reduced significantly over control when 45 day old plants were inoculated.

2. Effect of snake gourd mosaic virus infection on leaf area

The effect of virus infection on snake gourd plants at different growth stages on leaf area was recorded and presented in Table 18. The results showed that the leaf area was reduced significantly when 15 day old plants were inoculated. There was significant reduction on the leaf area when 45 day old plants were also inoculated.

3. Effect of snake gourd mosaic virus infection on internodal length

The results on the internodal length are given in

Table 17. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on number of leaves

(Mean number of leaves)

Treatment	Time of observation (days)				
	30	45	60	75	90
V_1T_1	15.24 (3.90)	45.02 (6.71)	107.87 (10.39)	137.90 (11.74)	167.82 (12.95)
V_1T_2	••	••	128.08 (11.32)	170.22 (13.05)	210.60 (14.51)
V_1	17.40 (4.17)	57.71 (7.60)	129.66 (11.39)	173.08 (13.16)	206.78 (14.38)
V_2T_1	16.30 (4.04)	47.84 (6.92)	120.59 (10.98)	167.48 (12.94)	213.09 (14.60)
V_2T_2	••	••	137.67 (11.73)	197.41 (14.05)	260.07 (16.13)
V_2	17.43 (4.17)	61.16 (7.82)	142.97 (11.96)	205.67 (14.34)	268.22 (16.38)
$CD_{(0.05)}$	0.22	0.67	0.50	0.68	1.03

Figures in parenthesis are transformed values

Table 18. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on leaf area

(Mean leaf area in cm^2)

Treatments	Time of observation (days)				
	30	45	60	75	90
V_1T_1	113.81	166.67	213.91	193.56	161.84
V_1T_2	..	..	249.08	219.80	161.11
V_1	184.58	361.60	311.70	306.95	235.60
V_2T_1	81.99	186.10	207.34	201.19	119.35
V_2T_2	..	..	250.32	210.25	128.98
V_2	197.24	259.02	322.22	314.73	229.85
$CD_{(0.05)}$	27.37	62.56	29.34	24.18	27.42

Table 19. It was observed that the internodal length reduced significantly over control and other treatment when 15 day old plants were inoculated. Inoculation of 45 day old plants also reduced the internodal length significantly over control.

Table 19. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on internodal length

(Mean length in cm)

Treatment	Time of observation (days)				
	30	45	60	75	90
V_1T_1	9.98	15.31	15.41	14.84	14.15
V_1T_2	..	..	17.05	16.03	15.03
V_1	13.02	17.86	18.97	18.05	17.05
V_2T_1	8.97	14.78	15.44	14.55	13.61
V_2T_2	..	..	17.19	15.97	14.68
V_2	12.44	18.44	18.39	17.90	17.48
$CD_{(0.05)}$	2.51	0.95	1.18	0.67	0.85

4. Effect of snake gourd mosaic virus infection on thickness of vine

The results are given in Table 20. The results showed that inoculation of the plants at different growth

stages did not exert any significant effect on the thickness of vine as compared to control.

Table 20. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on thickness of vine

(Mean thickness in cm)

Treatment	Time of observation (days)				
	30	45	60	75	90
V_1T_1	1.79	2.02	1.91	1.81	1.70
V_1T_2	..	..	1.90	1.81	1.72
V_1	1.77	2.04	1.96	1.84	1.74
V_2T_1	1.77	2.03	1.90	1.81	1.73
V_2T_2	..	..	1.91	1.83	1.79
V_2	1.77	2.08	1.92	1.79	1.72
$CD_{(0.05)}$	NS	NS	NS	NS	NS

NS Not significant

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

The results are presented in Table 21. Inoculation of 15 day old plants reduced the length of vine significantly as compared to control. Treatment V_1T_1 showed significant reduction on the length of vine from 45th day onwards whereas treatment V_2T_1 produced reduction on length of vine

from 60th day onwards. Inoculation of 45 day old plants did not show any significant effect on length of vine as compared to control.

Table 21. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on total length of vine

(Mean length in metre)

Treatment	Time of observation (days)				
	30	45	60	75	90
V_1T_1	1.24	3.77	5.31	5.79	6.05
V_1T_2	..	..	6.00	6.55	6.82
V_1	1.63	4.48	6.65	7.22	7.56
V_2T_1	1.08	3.79	5.18	5.98	6.45
V_2T_2	..	..	6.73	7.11	7.35
V_2	1.47	4.28	7.24	7.74	7.94
$CD_{(0.05)}$	0.41	0.56	0.78	0.75	0.82

6. Effect of snake gourd mosaic virus infection on number of branches

The results on the effect of virus inoculation at different growth stages of the plants on the number of branches produced are given in Table 22. The results showed that inoculation of the plants with the virus at different stages of growth did not affect the production of branches significantly as compared with untreated plants.

Table 22. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on number of branches

(Mean number of branches)

Treatment	Time of observation (days)				
	30	45	60	75	90
V_1T_1	**	2.12 (1.46)	4.12 (2.03)	5.06 (2.25)	5.44 (2.33)
V_1T_2	**	**	4.66 (2.16)	5.85 (2.42)	6.16 (2.48)
V_1	**	2.37 (1.54)	4.49 (2.12)	5.49 (2.34)	5.86 (2.42)
V_2T_1	**	2.56 (1.60)	4.74 (2.18)	6.05 (2.46)	6.42 (2.53)
V_2T_2	**	**	5.41 (2.32)	6.25 (2.50)	6.92 (2.63)
V_2	**	2.96 (1.72)	5.50 (2.34)	6.87 (2.62)	7.48 (2.74)
$CD_{(0.05)}$	**	NS	NS	NS	NS

NS Not significant

Figures in parenthesis are transformed values

7. Effect of snake gourd mosaic virus infection on number of flowers

The results are presented in Table 23. It was observed that the number of flowers (male and female) produced was significantly reduced over control and other

treatment by those plants inoculated on the 15th day. Inoculation of 45 day old plants did not show any significant effect on the number of flowers produced over control.

Table 23. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on number of flowers produced

(Mean number of flowers)

Treatment	Male flowers	Female flowers
V_1T_1	1195.78 (34.88)	27.35 (5.23)
V_1T_2	1756.45 (41.91)	38.44 (6.20)
V_1	1826.71 (42.74)	41.86 (6.47)
V_2T_1	1196.47 (34.59)	32.83 (5.73)
V_2T_2	1583.24 (39.79)	41.09 (6.41)
V_2	1655.68 (40.69)	46.38 (6.81)
$CD_{(0.05)}$	2.92	0.55

Figures in parenthesis are transformed values

8. Effect of snake gourd mosaic virus infection on number of fruits

The results are presented in Table 24. When 15 day

old plants were inoculated the number of fruits produced were reduced significantly over control and other treatment. There was no significant reduction over control on the number of fruits produced on plants inoculated on 45th day. So early infection resulted in significant reduction on fruit set whereas late infection did not exert significant effect on fruit set.

Table 24. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on number of fruits produced

(Mean number of fruits)

Treatment	Number of fruits
V_1T_1	15.21 (3.90)
V_1T_2	22.94 (4.79)
V_1	26.73 (5.17)
V_2T_1	18.40 (4.29)
V_2T_2	24.90 (4.99)
V_2	28.73 (5.36)
$CD_{(0.05)}$	0.45

Figures in parenthesis are transformed values

9. Effect of snake gourd mosaic virus infection on length of fruits

The results are presented in Table 25. The results showed that inoculation of the plants with the virus at different stages of growth did not affect the length of fruits significantly as compared with untreated plants.

Table 25. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on the length of fruits produced

Treatment	Length of fruits
V_1T_1	71.71
V_1T_2	70.43
V_1	71.23
V_2T_1	59.24
V_2T_2	64.03
V_2	62.76
$CD_{(0.05)}$	NS

NS Not significant

10. Effect of snake gourd mosaic virus infection on girth of fruits

The results are presented in Table 26. It was observed that inoculation of the plants at different stages

of growth did not affect the girth of the fruits significantly over control.

Table 26. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on girth of fruits

(Mean girth in cm)

Treatment	Girth of fruits
V_1T_1	19.89
V_1T_2	19.43
V_1	19.06
V_2T_1	18.61
V_2T_2	20.18
V_2	19.5
$CD_{(0.05)}$	NS

NS Not significant

11. Effect of snake gourd mosaic virus infection on mean weight of fruit

The results presented in Table 27 showed that the mean weight of fruit was not significantly affected by the inoculation of plants with virus at different stages of growth. So the virus infection has no effect on the average fruit weight compared to untreated plants.

Table 27. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on weight of fruit

(Mean weight in kg)

Treatment	Mean weight of fruit
V_1T_1	0.74
V_1T_2	0.74
V_1	0.75
V_2T_1	0.64
V_2T_2	0.62
V_2	0.61
$CD_{(0.05)}$	NS

NS Not significant

12. Effect of snake gourd mosaic virus infection on fruit yield

It was observed from the data presented in Table 28 that when plants were inoculated on 15th day of growth, the quantity of fruits produced were significantly reduced over control and other treatment. There was no significant reduction on the yield of fruits when the plants were inoculated at 45th day of growth. Hence early infection resulted in significant reduction on yield of fruits whereas late infection did not exert significant reduction.

Table 28. Effect of infection on snake gourd by snake gourd mosaic virus at different growth stages on fruit yield

(Yield of fruits in kg)

Treatment	Yield of fruits
V_1T_1	9.02
V_1T_2	17.07
V_1	20.17
V_2T_1	11.09
V_2T_2	15.45
V_2	17.57
$CD_{(0.05)}$	3.25

DISCUSSION

DISCUSSION

The virus causing mosaic disease of snake gourd (Trichosanthes anguina L.) was investigated. The disease was found to be wide spread in Kerala causing severe damage to the crop. The main symptoms of the disease were mosaic pattern of irregular dark green and yellow chlorotic patches on the lamina similar to those described by Shankar et al. (1969). It also resembled the symptoms produced on snake gourd by a strain of cucumber mosaic virus (CMV) reported by Pillai (1971). Dubey et al. (1974) reported that snake gourd mosaic virus infection produced mosaic mottling accompanied by chlorosis, vein banding and blistering of leaf lamina. Diseased plants produced only a few weak runners and plants affected in an early stage blossom sparingly and set few fruits. Joseph and Menon (1978) also reported similar symptoms while studying the mosaic disease of snake gourd. A comparison of the symptoms of the snake gourd mosaic disease reported by the earlier workers with those of the snake gourd mosaic disease found in Kerala showed that the symptoms described from different places are more or less similar.

The virus was found to be transmissible through sap inoculation. It could be transmitted easily using distilled water as the extraction medium (standard sap). Sap transmission of snake gourd mosaic virus has been reported earlier

by many workers (Pillai, 1971; Dubey et al., 1974; Joseph and Menon, 1978). Use of phosphate buffer and tris buffer did not show any significant increase in the infectivity of the virus. This is not in agreement with the observations of the earlier workers (Foster, 1972; Sharma et al., 1984). They reported several fold increase in the infectivity of CMV with the use of phosphate buffer.

In the experiment to study the seed transmission of the virus, out of 180 seeds sown 134 seeds have germinated and none of these plants showed the symptoms of snake gourd mosaic disease. This shows that snake gourd mosaic virus is not transmissible through seed. Nagarajan and Ramakrishnan (1971a) found that mosaic disease of snake gourd caused by watermelon mosaic virus was transmitted through seed. Seed transmission of CMV through the seeds of their respective hosts was reported by many workers (Hani and Pelet, 1970; Tomlinson and Carter, 1970; Sharma et al., 1984). But Kaiser and Danesh (1971) found that CMV isolated from Cicer arietinum could not be transmitted through seed. Sharma and Chohan (1973) while investigating the seed transmission of cucurbit viruses reported that Cucumis virus 1 was seed borne in vegetable marrow, ash gourd, and pumpkin. But Dubey et al. (1974) found that mosaic disease of snake gourd caused by Cucumis virus 1 was not seed transmissible. It is therefore, difficult to come at a conclusion regarding

the seed transmission, as a general character of Cucumis virus 1. However in the present investigation, due to the failure of seed transmission, the virus causing mosaic disease of snake gourd resembles Cucumis virus 1 as reported by Dubey et al. (1974).

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

Studies on the insect transmission of snake gourd mosaic virus was conducted using A. gossypii, A. craccivora, A. malvae, Pentalonia nigronervosa and Bemisia tabaci. A. gossypii was found to transmit snake gourd mosaic virus in a very efficient manner giving 95 per cent followed by A. malvae giving 85 per cent and A. craccivora giving only 45 per cent transmission. The aphid Pentalonia nigronervosa

and the whitefly Bemisia tabaci failed to transmit the virus.

In the field conditions generally A. gossypii and A. malvae were infesting snake gourd plants and this must be responsible for the spread of the disease in field. Verma et al. (1970) reported that a severe mosaic of snake gourd was transmitted by the aphid A. gossypii. Transmission of Cucumis virus 1 causing snake gourd mosaic, by the vector A. gossypii was reported by Dubey et al. (1974) and by the vectors A. gossypii and A. craccivora was reported by Joseph and Menon (1978). Kaiser and Danesh (1971) also could obtain transmission of CMV infecting Cicer arietinum by A. craccivora in a stylet borne manner. However, failure to transmit viruses infecting snake gourd by A. craccivora has been reported by Verma et al. (1970), Pillai (1971), and Dubey et al. (1974). In the present investigation A. craccivora was not found infesting snake gourd plants but it could transmit snake gourd mosaic virus. Pentalonia nigronervosa and Bemisia tabaci were not reported to be vectors of snake gourd mosaic virus.

The physical properties of the virus, viz., dilution end point (DEP), thermal inactivation point (TIP) and longevity in vitro (LIV) were studied. It was found that the virus remained infective at a dilution of 1:10000. With regard to DEP the virus resembled a strain of CMV having a DEP of 1:10000 (Pillai, 1971).

The TIP of the virus was 70-75°C. Pillai (1971) reported the TIP of a strain of CMV as 60°C. However, Dubey et al. (1974) found it to be 65-70°C for Cucumis virus 1 and Joseph and Menon (1978) found it to be 70-75°C. The virus resembled Cucumis virus 1 (Dubey et al., 1974; Joseph and Menon, 1978) with regard to TIP.

The LIV of the virus was tested in two temperatures, viz., room temperature (28-30°C) and also under refrigeration (10°C). It was found that the virus was infective for 96 h at room temperature and 144 h under refrigeration. With regard to LIV the virus resembled Cucumis virus 1 (Joseph and Menon, 1978) having a LIV of 72-96 h and 144-168 h at room temperature and under refrigeration respectively.

The vector-virus relationship was studied using the most efficient vector, viz., A. gossypii. Acquisition threshold, inoculation threshold, effect of pre-acquisition and post-acquisition fasting of the vector on transmission of the virus, retention of infectivity by the vector and minimum number of aphids required for transmission were investigated.

Minimum acquisition feeding period required by A. gossypii for transmission of snake gourd mosaic virus was found to be 30 s. As the acquisition feeding period was increased the per cent transmission was also increased

and the maximum transmission of 90 per cent was obtained when an acquisition feeding period of 20 min was given. As the acquisition feeding period was further increased, the efficiency of transmission of the virus was reduced and it was only 35 per cent when the acquisition feeding period was 2 h (Table 8). The minimum inoculation threshold of snake gourd mosaic virus in the present studies was found to be 1 min (Table 9). The per cent transmission was found to increase with the increase in inoculation feeding period and reached the maximum of 100 per cent when 1 h inoculation feeding period was given. As the inoculation feeding period was further increased, the per cent transmission declined and reached 65 per cent with 8 h inoculation feeding. Non-persistent manner of transmission of snake gourd mosaic virus (Cucumis virus 1) by A. gossypii and A. craccivora was reported by Dubey et al. (1974), and Joseph and Menon (1978) respectively. Jaganathan and Ramakrishnan (1971) observed that the viruliferous aphid A. gossypii can pick up the melon mosaic virus with a short acquisition feeding period of 5 s and transmit it with a short inoculation feeding period of 5 s. According to the serological studies conducted by Dubey and Narieni (1975), melon mosaic virus, snake gourd mosaic virus, cucumber mosaic virus and bitter gourd mosaic virus are in the group of Cucumis virus 1. But in the present studies A. gossypii failed to acquire

and inoculate the virus at less than 30 s and 1 min respectively. This difference in the minimum acquisition feeding period and minimum inoculation feeding period of A. gossypii to transmit snake gourd mosaic virus may be due to environmental and other factors.

Investigations on the influence of pre-acquisition fasting showed that starvation of aphids before acquisition resulted in an increase in the per cent transmission. With the pre-acquisition fasting the increase in per cent transmission was found only till 1 h fasting and thereafter the per cent transmission declined. The efficiency of the vector to transmit the virus was completely lost when starved for 6 h. Therefore it is found that pre-acquisition fasting upto a period of 1 h increased the transmission efficiency of A. gossypii. The increase in efficiency of A. gossypii and other aphids in the transmission of many viruses including snake gourd mosaic virus due to pre-acquisition fasting has been reported by many workers (Nagarajan and Ramakrishnan, 1971a, 1971b; Jaganathan and Ramakrishnan, 1971; Singh, 1972, 1981a, 1982; Joseph and Menon, 1978). Jaganathan and Ramakrishnan (1971) observed that maximum transmission of melon mosaic virus was obtained when A. gossypii was given a pre-acquisition fasting of 1 h. The results of the present investigation also agree with this observation.

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

Experiments on retention of infectivity by A. gossypii revealed that the vector lost its infectivity after 1½ h in all the 6 series of experiments carried out. Non-persistent nature of retention of infectivity of mosaic viruses of cucurbits by the vector A. gossypii and other aphids were reported by many workers (Nagarajan and Ramakrishnan, 1971a, 1971b; Jaganathan and Ramakrishnan, 1971; Singh, 1972, 1981a, 1982; Ghosh and Mukhopadhyay, 1972). Jaganathan and Ramakrishnan (1971) reported that the aphid vector

retained the infectivity of melon mosaic virus for 1 h. The vector Myzus persicae could retain the infectivity of watermelon mosaic virus for 2 h (Singh, 1972). Singh (1981a) found that A. gossypii could retain the infectivity of pumpkin mosaic virus for 2 h. In the present investigation since the infectivity is lost after 1½ h, the transmission of snake gourd mosaic virus by A. gossypii can also be termed as non-persistent manner suggested by Jaganathan and Ramakrishnan (1971) and Singh (1981a).

The minimum number of aphids required for successful transmission of snake gourd mosaic virus was also worked out and it was found that a single viruliferous aphid could cause successful transmission of the virus (Table 13). Kaiser and Danesh (1971) found that a single aphid was able to transmit CMV but greater transmissions were obtained only by using comparatively larger number of aphids. Similar results were also obtained in the transmission of watermelon mosaic virus to vegetable marrow (Raychaudhuri and Varma, 1977), watermelon mosaic virus to watermelon, cucumber, pumpkin and squash by Myzus persicae (Almeida and Borges, 1983) and pumpkin mosaic virus by A. gossypii (Singh, 1981a).

Maximum infection of 95 per cent was obtained in the present study when 10 aphids were used. Nagarajan and Ramakrishnan (1971b) observed maximum infection when 20

aphids (Myzus persicae) were used in the case of pumpkin mosaic virus. Raychaudhuri and Varma (1977) obtained cent per cent transmission of watermelon mosaic virus with more than 5 aphids (A. gossypii). Singh (1981a) found maximum infection of pumpkin mosaic virus when 10 aphids (A. gossypii) were used.

Studies on host range and local lesion hosts conducted with 70 plant species belonging to 18 families showed that the virus under study was confined to the members of the families chenopodiaceae, compositae, cucurbitaceae and solanaceae. It produced systemic symptoms on Ageratum conizoides, Cucurbita moschata, Cucurbita pepo, Cucumis sativus, Lagenaria siceraria, Luffa acutangula, Melothria perousilla, Momordica charantia, Datura stramonium, Nicotiana glutinosa and Nicotiana tabacum. The virus produced distinct necrotic local lesions on Chenopodium amaranticolor and Chenopodium quinoa. Among these the weeds, Ageratum conizoides and Melothria perpusilla are first reports as hosts of snake gourd mosaic virus. Verma et al. (1970) while working with Cucumis virus 2B causing mosaic disease of snake gourd observed that the virus could produce systemic symptoms on members of the family cucurbitaceae, and local lesions on Chenopodium amaranticolor. Nagarajan and Ramakrishnan (1971a) reported the occurrence of watermelon

mosaic virus on snake gourd but its host range was found restricted to cucurbitaceae alone. Shankar et al. (1972) observed pumpkin mosaic virus on snake gourd, but its host range also was restricted to members of the family cucurbitaceae. Dubey et al. (1974) reported a mosaic disease of snake gourd from Delhi caused by Cucumis virus 1, the host range of which was restricted to members of the families cucurbitaceae, compositae, solanaceae and chenopodiaceae. Among these distinct local lesions were produced in Chenopodium amaranticolor belonging to chenopodiaceae and in all the hosts belonging to the other three families systemic symptoms were produced. Ignash (1977) reported that cucumber mosaic virus strain 1 produced local lesions on Chenopodium quinoa. A comparison of the host range and local lesion hosts of different viruses infecting snake gourd revealed that the virus under study shows a close similarity to Cucumis virus 1 described by Dubey et al. (1974). It differs in host range and local lesion hosts from Cucumis virus 2B, watermelon mosaic virus and pumpkin mosaic virus infecting snake gourd.

Serological studies were conducted with a view to identify the virus. The results of the microprecipitin test on slides showed that antigens of snake gourd mosaic virus isolate I (obtained from inoculated plants in the glass house), snake gourd mosaic virus isolate II (obtained

from diseased plants in the field), cucumber mosaic virus and pumpkin mosaic virus produced dense precipitate with the antiserum specific to snake gourd mosaic virus. This indicates the serological relationship of snake gourd mosaic virus to the viruses causing cucumber mosaic and pumpkin mosaic found in Kerala. No serological relationship was obtained between snake gourd mosaic virus and, bitter gourd mosaic virus, cowpea mosaic virus or sword bean mosaic virus. The snake gourd mosaic virus antigen did not show any serological relationship with any of the antisera of three other viruses, viz., cucumber mosaic virus Arkansas isolate, cucumber mosaic virus (cowpea isolate) Arkansas and cucumber mosaic virus ICRISAT isolate indicating that snake gourd mosaic virus found in Kerala is not serologically related to any of these viruses.

Microprecipitin test in petridishes were conducted to find out the antiserum titre, the virus end point, the titre of the antiserum with the healthy sap and the end point of healthy sap with the antiserum. In the present studies the titre of the antiserum was found to lie between 1:2048 and 1:4096 and virus end point between 1:1024 and 1:2048. Dubey et al. (1974) got an antiserum titre of 1:2048 and virus end point of 1:4096 with purified virus preparations of snake gourd mosaic occurring in Delhi caused

by Cucumis virus 1 and no positive reaction was obtained with healthy plant sap and antiserum. The virus end point of 1:4096 obtained by them indicates that the virus concentration was more in their preparation due to the use of purified virus preparation for the test. In the present investigation supernatent of diseased sap strained through cotton wool, subjected to centrifugation at 5000 g was used for the test and thus the virus concentration was low in the preparation. The results of the present investigation on antiserum titre and failure of healthy sap to react with antiserum are in agreement with the findings of Dubey et al. (1974).

According to the serological studies conducted by Dubey and Nariani (1975), snake gourd mosaic virus, cucumber mosaic virus, melon mosaic virus and bitter gourd mosaic are in the group of Cucumis virus 1. In the present studies, bitter gourd mosaic virus failed to react positively with snake gourd mosaic virus antiserum. This indicates that bitter gourd mosaic found in Kerala may not be caused by Cucumis virus 1. The serological relationship of the present virus with melon mosaic virus has not been tried, since the antigen to melon mosaic virus could not be obtained.

The results of the Ouchterlony's agar double diffusion test have confirmed the findings of the microprecipitin

test on slides. The appearance of dark blue precipitin bands between the wells containing antiserum and snake gourd mosaic virus antigen and cucumber mosaic virus antigen in the second plate clearly indicates the presence of antibodies specific to snake gourd mosaic virus and cucumber mosaic virus in the antiserum (Fig. 17). The absence of any band between the antiserum well and the wells containing sword bean mosaic virus and cowpea mosaic virus antigens indicated that the antiserum did not contain antibodies against any of these two viruses. In the third plate there was fusion of two precipitin lines formed between wells containing antiserum and snake gourd mosaic virus antigens obtained from infected plants in the glass house and field (Fig. 18). This shows that the snake gourd mosaic virus antigen obtained from glass house and field grown plants are of the same virus. It is a well established fact that fusion of precipitin lines shows the identical nature of antigens (Noordam, 1973).

The fourth plate contained antiserum for snake gourd mosaic virus in the central well and the surrounding wells contained snake gourd mosaic virus antigen, pumpkin mosaic virus antigen, bitter gourd mosaic virus antigen and healthy plant sap. The fusion of the precipitin lines formed between the antiserum well and the wells containing antigens

of snake gourd mosaic virus and pumpkin mosaic virus indicates that these two viruses are serologically very much related (Fig. 19).

The fifth plate shows the serological reactions between antiserum of snake gourd mosaic virus with the diluted antigens namely snake gourd mosaic virus, cucumber mosaic virus and pumpkin mosaic virus. The fusion of the dark blue precipitin lines formed between the antiserum well and all the antigen wells except the well containing healthy sap shows that the virus antigens are very much related (Fig. 20).

In the varietal screening trial carried out with seven varieties it was found that all the varieties were susceptible to snake gourd mosaic virus. Symptoms appeared within 5 to 8 days depending on the variety. Even though all the seven varieties were susceptible to the virus, there was some variation in the severity of symptoms produced, time taken for appearance of symptoms and percentage of infection in the inoculated plants. Extra-long (Pocha) was found highly susceptible which produced 100 per cent infection 5 days after inoculation followed by Vellayani local and PKM-1 which produced symptoms on 95 per cent of test plants 6 days after inoculation. CO-1 was found least susceptible with 65 per cent infection 8 days after inoculation. Sowell and Demski (1969) while working with watermelon mosaic

virus 2 found that none of the watermelon varieties tested was immune to the virus. Moskovets and Fegla (1972) also arrived at similar conclusion and reported that all the watermelon and pumpkin varieties tested were susceptible to watermelon mosaic virus. Graber (1978) from Queensland also found that all the commercially available watermelon, vegetable marrow and pumpkin cultivars were susceptible to watermelon mosaic virus 1 and 2.

Inoculation of snake gourd plants with virus at different stages of growth showed that the vegetative growth and yield were affected severely when inoculated at an early stage while the effect of virus inoculation was not significant at later stages of inoculation.

It was observed that the inoculation of 15 day old plants with virus significantly reduced the number of leaves of both the cultivars tested while the effect was not significant with 45 day old plants. Hence the reduction in number of leaves depended on the time of inoculation, ie. if the plants were inoculated at early stages of growth the number of leaves were reduced significantly. Variety V_1 showed reduction in number of leaves from 30th day onwards where as variety V_2 showed reduction in number of leaves from 45th day onwards. This variation may be due to varietal differences.

There was severe reduction of leaf area and internodal length due to virus infection at all stages of growth. But the virus infection had no effect on the number of branches produced and thickness of vine. The total length of the vine was reduced significantly when 15 day old plants were inoculated and resulted in stunting of the plants. This effect was not pronounced when infected at later stages of growth.

The number of flowers (male and female) produced was significantly reduced when 15 day old plants were inoculated with virus, whereas the inoculation of 45 day old plants did not show any significant reduction on flower production. Similarly there was significant reduction on the number of fruits produced when 15 day old plants were inoculated with virus. But the number of fruits produced was not affected with the inoculation of 45 day old plants. So early infection resulted in significant reduction on flower and fruit production whereas later infection did not exert significant effect on flower production and fruit set. It was also observed that the virus infection had no effect on the length, girth and weight of fruits produced.

It was observed that when plants were inoculated on 15th day of growth, the yield of fruit was significantly reduced over control and other treatments. There was no

significant reduction on fruit yield when the plants were inoculated at 45th day of growth. Hence early infection resulted in significant reduction on fruit yield while late infection did not exert any effect on fruit yield.

Pillai (1971) while investigating mosaic disease of snake gourd reported that the disease affected plants were stunted and produced fewer flowers and fruits. Similar result was also noticed by Dubey et al. (1974) in snake gourd plants affected with mosaic disease. Thomas (1971) from New Zealand reported that early infection of watermelon mosaic virus 2 reduced yield in Buttercup squash, Golden Hubbard squash and pumpkin but no yield reduction was observed with later infection. Demski and Chalkley (1974) while studying the effect of watermelon mosaic virus on watermelon observed that infected plants had shorter runners, smaller leaves and reduction in number of fruits produced. Alverez and Campbell (1976) while studying the effect of squash mosaic virus on cantaloupe recorded significant reduction in number of fruit per plant but had no influence on size, weight or edible quality of the fruits. Jayasree (1984) found that yellow vein mosaic disease of pumpkin produced significant reduction in number of leaves, size of the leaves, size of the leaves, internodal length, total length of vines, and number of flowers. The results of the present investigation also agree with these findings.

SUMMARY

SUMMARY

Mosaic disease of snake gourd (*Trichosanthes anguina* L.) prevalent in Kerala was investigated.

The symptoms appeared within 5-8 days after mechanical inoculation, as small chlorotic areas which later coalesced together to form larger patches. Typical mosaic mottling with dark green and light green patches was produced in all the subsequent leaves. In some cases, the leaves had prominent vein banding and occasionally with dark green blisters. 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 and by aphids. There was 90 per cent graft transmission. The virus was transmitted by the aphids *Aphis gossypii* Glov., *Aphis malvae* Koch., and *Aphis craccivora* Koch. Among the three species of aphids *A. gossypii* was found to be the most efficient vector. The percentage of transmission obtained by *A. gossypii*, *A. malvae* and *A. craccivora* were 95, 85 and 45 respectively. It was found that snake gourd mosaic virus is not transmitted through seeds, by the aphid *Pentalonia nigronervosa* Coq. or by the whitefly *Bemisia tabaci* Genn.

Studies on the physical properties of the virus revealed that the thermal inactivation point of the virus was between 70 and 75°C and dilution end point between 1:10000 and 1:50000. 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.

The results of the studies on vector-virus relationships showed that the minimum acquisition feeding period required for the vector (*A. gossypii*) to acquire the virus was 30 s, and that the virus could be transmitted with a minimum inoculation feeding period of 1 min. But the percentage of transmission was maximum when an acquisition feeding of 20 min and inoculation feeding of 1 h were given. It was found that pre-acquisition fasting of the vector for a period of 1 h resulted in maximum infection, whereas post-acquisition starvation decreased the per cent infection. The retension of infectivity by the vector was found to be 1½ h. A single aphid was found capable of transmitting the virus to healthy plants but 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, compositae, cucurbitaceae, and solanaceae. It produced systemic symptoms on *Ageratum conizoides* L., *Cucurbita moschata* Duch.,

Cucurbita pepo L., Cucumis sativus L., Lagenaria siceraria (Mol) & Standl., Luffa acutangula Roxb., Melothria perpusilla L., Momordica charantia L., Datura stramonium L., Nicotiana glutinosa L., and Nicotiana tabacum L. Distinct necrotic local lesions were produced by the virus on Chenopodium amaranticolor Coste & Reyn. and Chenopodium quinoa Willd. Among these the weeds Ageratum conizoides and Melothria perpusilla were first reports as hosts of snake gourd mosaic virus.

Studies on serological properties indicated that snake gourd mosaic virus is related to cucumber mosaic virus and pumpkin mosaic virus. The virus showed no serological relationship with other cucurbit viruses, viz., Bitter gourd mosaic virus, cucumber mosaic virus Arkansas isolate, cucumber mosaic virus-cowpea isolate (Arkansas) and cucumber mosaic virus ICRISAT isolate. The antiserum titre and virus end point in the present study were found to be between 1:2048 and 1:4096, and 1:1024 and 1:2048, respectively.

Based on the results on symptomatology, transmissions, physical properties, host range and serological properties, the mosaic virus of snake gourd under study was identified as Cucumis virus 1.

The varietal screening studies carried out with seven varieties of snake gourd revealed that all the varieties

were susceptible to snake gourd mosaic virus. But, Extra-long (pocha) with 100 per cent infection and PKM-1 and Vellayani local with 95 per cent infection were found to be very highly susceptible whereas CO-1 was least susceptible with 65 per cent infection.

The results of the field experiment to estimate the loss due to virus infection at different growth stages revealed that early infection reduced significantly the length of vines, number of leaves, number of flowers, number of fruits and yield, whereas late infection did not produce considerable effect on both the varieties tested. There was significant reduction on leaf area and internodal length on plants inoculated at all stages of growth.

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* Original not seen

APPENDICES

Appendix-I

Amidoblack stain for precipitin lines

Amidoblack 10B - 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

ABSTRACT

Snake gourd mosaic virus disease commonly occurring in Kerala was investigated.

The symptoms appeared as typical mosaic mottling with dark green and light green patches. The leaves were much reduced in size, variously crinkled 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 and by aphid vectors. The virus was found to be transmitted by the aphids, Aphis gossypii Glov., Aphis malvae Koch. and Aphis craccivora Koch. Among the three species of aphids, A. gossypii was found to be the most efficient vector.

Investigations on the physical properties of the virus revealed that the virus had a thermal inactivation point between 70-75°C, Dilution end point between 1:10000-1:50000, longevity in vitro between 96-120 h at room temperature (28-30°C), and between 144-168 h at 10°C.

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

acquisition feeding of 20 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 maximum infection but post-acquisition starvation decreased the per cent infection. The vector was found to retain the virus for 1½ h. A single aphid could transmit the virus to healthy test plants, but maximum percentage of transmission was obtained with 10 aphids.

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

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

Varietal screening trial with seven varieties of snake gourd revealed that all the varieties were susceptible to snake gourd mosaic virus infection.

Studies on estimation of loss revealed that early infection reduced significantly the length of vine, number of leaves, number of flowers, number of fruits and yield, whereas late infection did not produce significant effect, on both the varieties tested. There was significant reduction on leaf area and internodal length on plants inoculated at all stages of growth.