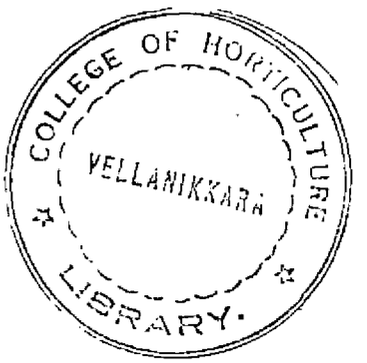
PROPERTIES, HOSTRANGE AND CONTROL OF COWPEA MOSAIC VIRUS



BY

SREELAKHA L.

THESIS

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1987

DECLARATION

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

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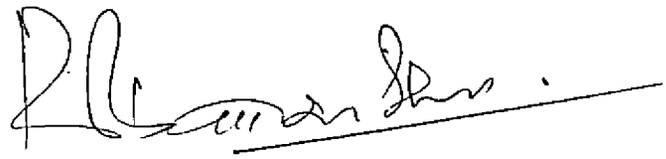
Vellayani,
4-4-1987.

CERTIFICATE

Certified that this thesis entitled "Properties, host-range and control of cowpea mosaic virus" is a record of research work done independently by Smt. L. SREELAKHA under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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Sreelakha

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INTRODUCTION

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is one of the major vegetable crops of Kerala and is being extensively cultivated throughout the other parts of India as well.

Cowpea plants are usually affected by different diseases. Among them the virus diseases are known to cause serious damage to the crop wherever it is cultivated. Characters of these viruses reported from different places in India and elsewhere are found to differ from each other in many aspects.

The occurrence of cowpea mosaic virus in India was first reported by Capoor and Varma(1956) on *Vigna cylindrica* from Poona and later Nariani and Kandaswami(1961) reported the virus on *V. sinensis* from Delhi. Afterwards, cowpea mosaic virus was reported from different parts of India by many scientists (Chenulu *et al.*, 1968; Govindaswamy *et al.*, 1970; Nene and Shankar, 1972; Sharma and Varma, 1975; Mali and Kulthe, 1980; Ramachandran and Summanwar, 1982).

Cowpea mosaic is a very common and destructive disease of cowpea, reported from different parts of India. The disease is found to cause serious damage to the crop cultivated in all parts of Kerala also. The identity of

the cowpea mosaic disease found in Kerala is not yet known and no studies have been conducted so far on this important disease occurring in Kerala. In the present investigations an attempt has been made to identify the virus and to study the other aspects of the disease.

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

1. Symptomatology
2. Transmission
3. Physical properties
4. Vector-virus relationship
5. Host-range of the virus
6. Varietal screening
7. Serology
8. Effect of virus on growth of the plant
9. Observation on the natural incidence of cowpea mosaic
10. Control of cowpea mosaic virus disease by leaf extract sprays.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cowpea (*Vigna unguiculata* (L.)Walp) is a very important pulse crop grown in Kerala and cowpea mosaic disease is a serious disease affecting this crop in all the areas where it is cultivated. The review of literature presented here pertains to the different types of cowpea mosaic diseases reported from different parts of India and elsewhere.

I. Symptomatology

Different types of cowpea mosaic symptoms have been reported from different places. Mc Lean(1941) described the symptoms of a cowpea mosaic as characterized by dwarfed slender growth and tendency for excessive branching. Snyder(1942) reported a seed-borne mosaic of asparagus bean having a pale and dark green foliar mosaic frequently accompanied by downward rolling of the leaves, mild rugosity or distortion, vein banding and stunting. Dale (1949) observed the occurrence of a mosaic disease of *Vigna unguiculata* from Trinidad in which symptoms were the appearance of dark and light green rings on the leaves, development of irregular yellowish and dark green mottling accompanied by blistering of the lamina. Sometimes under

glass house conditions a reddish brown necrosis of the veins had also been found to develop.

Capoor and Varma(1956) reported for the first time in India a mosaic disease of Vigna cylindrica from Poona and later Nariani and Kandaswami(1961) reported this disease on V. sinensis from Delhi. The disease was described as characterised by mosaic mottling of leaves accompanied by distortion and reduction of leaf size. The infected plants yield only few pods which were small, shrunken and containing only a few shrivelled seeds. Another cowpea mosaic disease was reported by Chenulu et al. (1968), also from Delhi. The symptoms consisted of typical mosaic mottling, yellowing, reduction and distortion of leaf lamina. The symptoms were seen as small chlorotic patches on the primary leaves of plants arising from diseased seeds. The affected leaves showed a tendency of marginal curling and cupping of the leaf. Nene and Shankar(1972) reported a cowpea mosaic virus infecting Vigna sinensis from Pantnagar. The disease was characterised by mosaic mottling, vein banding, puckering and distortion. Severe infection resulted in blistering and bleaching of the lamina. The pods became curved, twisted and reduced in size. The seeds in such pods were shrivelled and lesser in number. Sharma and Varma(1975) observed a

cowpea banding mosaic virus affecting cowpea (Vigna sinensis Savi.), which was characterized by mosaic mottling, crinkling and vein banding.

Klesser(1960) described three cowpea viruses in Bothalia and designated as cowpea mosaic virus A,B and C. Cowpea mosaic virus A showed stunting, small malformed leaves with vein banding or a mosaic with necrosis. Cowpea mosaic virus B showed dark green vein banding only. Cowpea mosaic virus C, which was a strain of cucumber mosaic virus showed a severe stunting and a vivid yellow mottling. Bock and Conti(1974) reported that the diseased cultivars show variable amounts of dark green vein banding or interveinal chlorosis, leaf distortion, blistering and stunting. They stated that the viruses that may be related to CAMV cause mosaic disease of adzuki bean (Phaseolus angularis) and aspargus bean (Vigna sesquipedalis).

II. Transmission

1. Sap transmission

Transmission of cowpea mosaic virus by mechanical methods was first reported by Mc Lean(1941) from Arkansas. He reported that the use of carborundum as an abrasive assisted the development of infection. Subsequently, many reports have been made from different parts of the

world on the sap transmission of cowpea mosaic viruses (Harjono, 1959; Toler, 1964; Adsuar, 1964; Debot and De Rojas, 1967; Twardowicz-Jakuszowa and Anna, 1969; Kvicala et al., 1970; Govindaswamy et al., 1970; Khatri and Singh, 1974; Diwakar and Mali, 1976; Sharma and Varma, 1976; Lima et al., 1977; Ramachandran and Summanwar,1982; Mazyad et al., 1984). Different types of inoculation media were used by different scientists for the mechanical transmission of cowpea mosaic virus. Phenol water extracts of diseased plants were used by Schlegel(1960), infected plant sap itself was used by Alconero and Santiago(1972). Sap extracted in 0.05 M phosphate buffer of pH 7 was used by Sharma and Varma(1976), sap extracted in cooled tris buffer was used by Mali and Kulthe(1980) and sap extracted in distilled water and diluted in the ratio 1:5 was used by Patel and Kuwaite(1982) and Patel(1982).

Abeygunawardena and Perera(1964) conducted studies on the virus diseases affecting cowpea in Ceylon and identified a new strain of cowpea mosaic virus which produced local lesions when sap inoculated on the varieties Victor K 798 and Brabham K 892. Guo et al.(1984) studied a C-1 isolate of cowpea aphid-borne mosaic virus obtained from asparagus bean and found that it was readily sap transmissible.

Rocha-Pena and Fulton(1984) while working on the propagation of an isolate of cowpea severe mosaic virus from Tabasco found that on mechanical inoculation six genotypes produced local lesions on inoculated primary leaves, followed by development of a severe mosaic on trifoliate leaves.

2. Seed transmission

Seed transmission of cowpea mosaic virus was first reported by Mc Lean(1941). He found that different varieties of cowpea showed different levels of seed transmission. In susceptible varieties like New Era, Whippoorwill and Briepea the levels of seed transmissions were 5, 4.5 and 6.8 per cent respectively, and in resistant varieties, Red Ripper, Black and Iron 0, 0 and 1 per cent respectively. Stevenson and Hagedorn (1970) reported that seed size has no effect on percentage of seed transmission. In the case of cowpea aphid-borne mosaic virus the seed transmission was found to be usually 0-3 per cent (Bock and Conti, 1974). But they have also recorded instances of 21.5 per cent seed transmission in cowpea cv. Kurodane 16. Phatak(1974) has reported seed transmission of 3-19 per cent in cowpea cv Pusa Phalguni for an Indian isolate of cowpea aphid-borne mosaic virus. Similar reports by Ladipo (1977) and Ata et al.(1982) confirmed the fact that the

transmission of cowpea mosaic virus through seed is influenced by the type of cultivar.

Different levels of seed transmission of cowpea mosaic virus were reported from different parts of the world. These were 37 per cent (Snyder,1942) from California, 5 per cent from Central Asia (Vlasol,1960), 26 per cent from Japan (Tzuchizaki et al., 1970), 0 to 73 per cent from Giza (Mazyad, 1971), 1.1 to 39.8 per cent from Iran (Kaiser and Mossahebi, 1975), 26 per cent from Morocco (Fischer and Lockhart, 1976), 17.5 per cent from Marathwada (Diwakar and Mali, 1976), 20.9 per cent from West Bengal (Ladipo, 1977), 41.6 per cent for a potyvirus causing mosaic of cowpea in India (Mali and Kulthe, 1980), 9 to 34 per cent for cowpea banding mosaic virus (Prakash and Joshi, 1980) and 14 per cent for cowpea aphid-borne mosaic virus from India (Mali and Kulthe, 1981). Reports have also been made on viruses causing mosaic of cowpea which were not transmissible through seeds (Harjono, 1959; Abeygunawardena and Perera, 1964; Kuhn, 1964).

Mazyad (1971) while studying the transmission of cowpea mosaic virus through seeds of cowpea plants reported that the time of storage of seeds has no effect on virus transmission. Haque and Chenulu(1972a)observed an inverse

relationship between the percentage of seed transmission and the age of cowpea plants at the time of inoculation. Guo et al.(1984) reported that cowpea mosaic virus can be transmitted through seeds of asparagus bean up to 3.1 per cent.

4. Insect transmission

Cowpea mosaic virus was reported to be transmitted by a number of vectors. Aphid transmission of cowpea mosaic virus was first reported by Mc Lean(1941) from Arkansas. The virus was found to be transmitted by Macrosiphum solanifolii, Aphis gossypii and Macrosiphum pisi to the extent of 60, 100 and 70 per cent, respectively. Anderson(1959) while studying the Vigna and Crotalaria viruses in Florida found that both beetle-borne and aphid-borne cowpea mosaic viruses existed separately. Abeyguna-wardena and Perera(1964) reported that A. craccivora is the principal vector of cowpea mosaic virus in Ceylon. They found that the transmission occurred in a non-persistent manner. Similar results were also obtained by Klesser(1960) and Bock and Conti(1974). Vidano and Conti(1965) reported that a mosaic virus of cowpea in Italy was found to be transmitted by Myzus persicae, A. fabae, A. medicaginis A. gossypii and Macrosiphum euphorbiae.

The cowpea mosaic virus was also found to be transmitted by Acyrthosiphon pisum (Kaiser and Mossahebi, 1975); Aphis craccivora (Khatri and Singh, 1974; Kaiser and Mossahebi, 1975; Sharma and Varma, 1976; Ramachandran and Summanvar, 1982; Guo et al., 1984; Mazyad et al., 1984); Aphis euronymi (Sharma and Varma, 1976), Aphis gossypii (Khatri and Singh, 1974; Sharma and Varma, 1976; Mali and Kulthe, 1980; Ramachandran and Summanvar, 1982), Aphis medicaginis (Harjono, 1959), Aphis sesbaniae (Kaiser and Mossahebi, 1975), Macrosiphum pisi (Snyder, 1942) and Myzus persicae (Khatri and Singh, 1974; Diwakar and Mali, 1976; Fischer and Lockhart, 1976; Sharma and Varma, 1976; Guo et al., 1984).

Eventhough in most cases the sap transmissible cowpea mosaic viruses were also transmitted by the aphids, A. craccivora, Macrosiphum pisi and Myzus persicae, there are reports about cowpea mosaic viruses which are not transmitted by these aphids (Toler, 1964; Shankar et al. 1973).

Beetle transmission of cowpea mosaic virus was reported by Walters and Barnett(1964) from Arkansas. Anjos and Lin (1984) studied the properties of cowpea mosaic virus sero-type I and found that it was transmitted

by the beetle Ceratoma arcuata. Similarly cowpea mosaic virus was reported to be transmitted by the beetles Andrector arcuatus and Andrector ruficornis (Debot and De Rojas, 1967), by Ceratoma ruficornis (Shepherd and Fulton, 1962; Kvicala et al., 1973), by C. ruficornis, Gynadrobrotica variabilis and C. artofasciata (Valverde et al. 1978).

III. Physical properties

McLean(1941) while studying the physical properties of cowpea mosaic virus observed that the virus had longevity in vitro (LIV) of 48 h, thermal inactivation point (TIP) between 72°C and 75°C and dilution end point (DEP) 1:1000. But Snyder (1942) studied a seed-borne mosaic of asparagus bean (Vigna sesquipedalis) and observed that the virus had TIP between 55 and 60°C, LIV - for 2 days at room temperature and DEP 1:1000. Similar physical properties were described for pea enation mosaic virus by Twardowicz - Jakuszowa and Anna(1969). They also reported that the virus could remain without inactivation in dried leaves for 7-9 days and in frozen leaves for 6-7 days. Capoor and Varma(1956) reported a mosaic disease of Vigna cylindrica from Poona and later Nariani and Kandaswami(1961) reported the same virus disease on V. sinensis from Delhi. The TIP of that virus was found to be

between 85-90°C, DEP 1:50000 and LIV as 19 days.

Harjono(1959) while studying the physical properties of a virus affecting cowpea (Vigna sinensis) reported that the inactivation of virus occurred after 10 min at 60°C, or at a dilution of 1:100000 or after 24 h at 25-30°C. Klesser(1960) while studying the virus diseases of cowpeas gave detailed descriptions of two viruses, none of which was identical with any of the previously recorded ones. One designated as cowpea mosaic virus A had a TIP between 62°C and 65°C, LIV 2-4 days and DEP 1:2000. Cowpea mosaic virus B which causes only vein banding symptom showed a TIP between 60 and 62°C, LIV 2-3 days and DEP 1:1000.

Yerks and Patino(1960) studied the physical properties of a severe bean mosaic affecting the bean crop in Mexico. The virus was able to withstand heating to 92°C, dilutions up to $1:4 \times 10^6$, ageing 7 months in dry tissue, 11 weeks in expressed sap and 10 weeks in frozen sap. Adsuar (1964) found that the virus infecting cowpea had a TIP of 60°C, DEP of 1:10000 and LIV of 48 h at 28-30°C. Abeygunawardena and Perera(1964) studied a new strain of cowpea mosaic virus affecting the cowpeas in Ceylon, the TIP of which was 55-60°C, DEP 1:3000 and LIV was more than 1-2 days.

Walters and Barnett(1964) while studying the properties of Arkansas cowpea mosaic virus reported that the virus was inactivated by dilution to 10^{-6}, heating to 75°C and storage for more than 5 days at 28°C. Chenulu et al. (1968) described a cowpea mosaic virus from Delhi the TIP of which was 55°C, DEP 1:500 to 1:1000 and LIV 6 h.

Kvicala et al.(1970) conducted studies on the physical properties of a cowpea mosaic virus isolated from Cuba and observed that the virus had a TIP between 65-70°C, DEP between $1:5 \times 10^5$ - $1:7 \times 10^5$ and LIV in crude sap 10-14 days. Govindaswamy et al.(1970) observed from Tamil Nadu a virus disease causing the mosaic mottling of leaves of cowpea and the causative virus was found to have a TIP of 50-55°C, DEP 1:1000 to 1:2000 and LIV 4-5 days. Nene and Shankar(1972) reported a cowpea mosaic virus infecting V. sinensis from Pantnagar, the TIP of which was 75°C, DEP 1:1000 to 1:5000 and LIV 56 h. Khatri and Singh (1974) reported the TIP of a cowpea mosaic virus as 70°C, DEP as 1:1000 and LIV as 96 h at room temperature and 120 h at 7-10°C. Diwakar and Mali(1976) studied the physical properties of a cowpea mosaic virus in Marathwada and reported the TIP of the virus as 65°C, DEP 1:500 and LIV 3 days at room temperature and 7-8 days at 4°C. Kaiser and Mossahebi (1975) while studying the properties of

cowpea aphid-borne mosaic virus from Iran reported the TIP as 55-60°C, DEP 10^{-4} to 10^{-5} and LIV 7 days at 20°C.

Sharma and Varma(1975) conducted investigations on three sap transmissible viruses on cowpea in India. The three viruses were cowpea chlorotic spot virus(CpCSV), cowpea banding mosaic virus (CpBMV) and cowpea necrosis virus (CpNV). CpBMV and CpNV were inactivated after 10 min at 50-55°C and CpCSV at 80-85°C. The DEP of CpBMV and CpNV were $1:10^3$ to $1:10^4$ and of CpCSV $1:10^6$ to $1:10^7$. The LIV of CpBMV, CpNV and CpCSV were 24 h, 2 days and 5 days at room temperature. Lima et al.(1979) studied a potyvirus on cowpea in Ceara, the TIP of which was 60°C, LIV 48 h and DEP 10^{-3}. Mali and Kulthe (1980) described the properties of another potyvirus from India and the TIP of that virus was 60-65°C, LIV 56 h and DEP 10^{-4}. Guo et al.(1984) studied the properties of cowpea aphid-borne mosaic virus obtained from asparagus bean and observed the TIP of the virus as 55-60°C, DEP 10^{-3} to 10^{-4} and LIV 1-2 days at room temperature.

IV. Vector-Virus relationship

The vector-virus relationship of a cowpea mosaic virus occurring on cowpea (Vigna sinensis) and transmitted by Aphis medicaginis was worked out by Harjono(1959).

The study revealed that the acquisition and inoculation thresholds were both 5 h and infectivity of the vector could be retained for 8 days.

Haque and Chenulu(1972b) studied the influence of aphid rearing plants and the developmental forms of aphid on the transmission of cowpea mosaic virus. There was little difference in transmission by Aphis craccivora reared on cowpea, broad bean and pea plants and all developmental forms were found to be equally efficient. Murugesan and Janaki(1972) studied the relationship of cowpea mosaic virus with its vector Myzus persicae Sulz. They found that the virus could be transmitted to healthy cowpea plants even by one viruliferous aphid. Maximum infection was obtained with 15 aphids and after one h acquisition feeding although the virus could be acquired in one sec. Preliminary fasting up to 2 h increased the efficiency of transmission only when followed by a short acquisition feeding of up to 5 min. Post acquisition fasting decreased the efficiency of the vector and the virus was retained only up to 4 h.

Sharma and Varma(1977) made studies on the vector-virus relationship of cowpea banding mosaic virus. Even a single viruliferous aphid (Aphis craccivora) was able to

transmit cowpea banding mosaic virus, but increase in number of aphids per plant increased the transmission. Optimum preacquisition fasting was found to be 3 h, eventhough the aphids could transmit the virus without pre-acquisition fasting. Aphids could acquire the virus in probes lasting for less than one min, but maximum transmission was obtained when given five min acquisition access time. The viruliferous aphids could transmit the virus within one min, but maximum transmission was recorded when given 30 min inoculation access time. Acquisition and inoculation thresholds were 20 and 25 seconds respectively, whereas transmission threshold was 50 seconds. Viruliferous aphids lost the virus in less than 2 min while feeding and in 2 h while fasting. Incubation period of virus in the host plant was reported to be 20-25 days (Govindaswamy et al., 1970), 4-6 weeks (Mali and Kulthe, 1980) and two weeks (Collins et al., 1984).

V. Host-range

Host-range of the virus causing mosaic of cowpea in different places differs significantly. Snyder(1942) while studying the virus disease of asparagus bean found that the virus could infect cowpea varieties, but other legumes were not infected. Harjono(1959) conducted studies

on a virus disease of cowpea (Vigna sinensis) and found Phaseolus lunatus and P. radiatus are susceptible to the virus. Subsequently, many reports have been made from different parts of the world on the host-range of cowpea mosaic virus. The cowpea mosaic virus was found to infect pigeon pea, Canavalia ensiformis, Desmodium distortum and D. gyroides in Puerto Rico (Adsuar, 1964); Canavalia ensiformis in Ceylon (Abeygunawardena and Perera, 1964); some plants of the family Leguminosae in Tamil Nadu (Govindaswamy et al., 1970); cowpea, bean (Phaseolus vulgaris) and Crotalaria juncea (Khatri and Singh, 1974); cowpea and dolichos bean in Marathwada (Diwakar and Mali, 1976); Centrosema brasilianum, Nicotiana benthamina and Phaseolus vulgaris in Ceara (Lima et al., 1982). Kvicala et al.(1970) studied the cowpea mosaic virus in Cuba and reported 33 plant species as hosts of the virus. It was also reported that on Phaseolus vulgaris the symptoms varied according to season. Kaiser and Mossahebi(1975) studied the cowpea aphid-borne mosaic virus in Iran and reported 15 host species belonging to six families. Systemic symptoms developed in Gomphrena globosa, Nicotiana glutinosa and samsun tobacco as well as in legume species. A seed-borne potyvirus causing mosaic of cowpea in India was

investigated by Mali and Kulthe(1980) and they found that the virus could infect plants belonging to the families Amaranthaceae, Chenopodiaceae and Leguminosae and systemic symptoms developed in different cowpea varieties and some other legumes. Mosaic symptom and mottling were observed in Glycine max, severe mosaic in Phaseolus lunatus, mosaic mottling in Phaseolus vulgaris and vein clearing and mottling in Vigna radiata.

Studies on the host reactions and transmission of two seed-borne cowpea viruses from Central Brazil were conducted by Lin et al.(1981). The viruses were blackeye cowpea mosaic (BICMV) virus and the cowpea isolate of cucumber mosaic virus. The BICMV infected three species of Amaranthaceae and three of Leguminosae on mechanical inoculation of 27 species belonging to 8 families. Of the 28 cowpea varieties and 11 bean cultivars inoculated, 19 and 10 respectively were found susceptible. The cowpea isolate of cucumber mosaic virus infected four members of the family Leguminosae, three of Solanaceae, two of Amaranthaceae and one blonging to Cucurbitaceae. Sanchez and Gonsalez(1981) found that the yellow strain of cowpea mosaic virus produced local lesions and apical necrosis in Phaseolus vulgaris and Stizolobium deeringianum and severe strain of cowpea mosaic virus

produced systemic mosaic on these two plants. Lin et al. (1984) carried out investigations on two serotypes of cowpea severe mosaic virus affecting the legumes in central Brazil. The serotype I of cowpea severe mosaic virus was detected in Calapagonium mucunoides, Centrosema pubescens and Vigna radiata var. radiata. Serotype II of cowpea severe mosaic virus occurred in Crotalaria juncea showing chlorotic mottling and leaf distortion. Both the serotypes I and II occurred in Crotalaria juncea and Vigna sesquipedalis. Ramachandran and Summanwar(1982) recorded a new cowpea mosaic virus from India which was detected in cultivar Prima, and was found restricted to cowpea varieties only. Guo et al.(1984) reported that the host-range of cowpea aphid-borne mosaic virus occurring in asparagus bean in Nanjing, included 12 species of Leguminosae and Chenopodiaceae.

Cowpea mosaic virus was reported to cause local lesions on certain hosts. The virus was found to produce local lesions on Chenopodium amaranticolor and Chenopodium album (Govindaswamy et al.,1970; Khatri and Singh, 1974); on soybean, sunhemp and Chenopodium amaranticolor (Diwakar and Mali,1976); on Chenopodium amaranticolor and on Cassia tora (Lima and Nelson, 1977). Mali and Kulthe(1980) studied a potyvirus causing mosaic of cowpea

in India and reported different types of local lesions on different hosts. They reported necrotic local lesions on Gomphrena globosa, Dolichos biflorus, Phaseolus vulgaris var. Prince and Vigna radiata var. Jalagaon-781. Chlorotic local lesions were reported on Glycine max var. Monetta, Phaseolus vulgaris var. Biela, Kockova and Perlicka. Chlorotic and Necrotic local lesions were reported in Chenopodium amaranticolor and Chenopodium quinoa and red local lesions in Chenopodium murale.

Certain common weeds have been reported as reservoirs of cowpea mosaic virus by some scientists. Two common weeds reported as reservoirs of cowpea mosaic virus are Euphorbia geniculata (Abeygunawardena and Perera, 1964) and Phaseolus lathyroides (Alconero and Santiago, 1972; Lima and Nelson, 1977).

VI. Varietal screening

Screening of cowpea varieties for resistance against different cowpea mosaic viruses has been done in different places.

Abeygunawardena and Perera(1964) conducted studies on the resistance of cowpea varieties to a virus disease affecting cowpea in Ceylon. The varieties Groit, Victor II,

Negron, Deip 8812, Deip 8862, Arlington and Birmingham were found highly resistant to the virus and the varieties Victor K 798 and Brabham K 892 developed local lesions. The variety Jackson Alabama showed a mild systemic mosaic and all the other local and introduced varieties tested were found highly susceptible. Govindaswamy et al.(1970) screened 112 varieties of cowpea for their resistance to cowpea mosaic virus and found 109 varieties as susceptible and three varieties as tolerant to virus infection. No variety was found to be immune to virus infection. Behncken and Maleevsky (1977) reported that all the 14 cultivars tested were found susceptible to cowpea aphid-borne mosaic virus in Queensland. Ladipo and Allen(1979) conducted glass house screening of different cowpea varieties for identification of resistance to Nigerian isolate of cowpea aphid-borne mosaic virus. In glass house screening, 52 lines were found immune, six found as tolerant and the rest either gave mixed reaction or were susceptible. None possessed hypersensitive resistance. Allen(1980) conducted varietal screening of 562 cowpea accessions for resistance to two isolates of cowpea mottle virus. Tolerance was the only type of resistance identified. More than 50 lines were identified as possessing resistance to both isolates. Of these five are resistant as

found by other workers also.

Mali et al.(1981) studied the resistance of 23 cowpea varieties to bean yellow mosaic, cowpea aphid-borne mosaic and tobacco ringspot virus and reported C-288 as the only variety immune to bean yellow mosaic virus and cowpea aphid-borne mosaic virus. Fulton and Allen (1982) reported that four cowpea accessions from the international cowpea disease nursery were found immune to three diverse isolates of cowpea severe mosaic virus from Arkansas, Costa Rica and Venezuela and another variety was found to possess resistance to six isolates of the virus. Patel et al.(1982) screened 249 cowpea cultivars by sap inoculation with veinbanding strain of cowpea mosaic virus in glass house and field conditions. Ten lines proved immune and eight found to be resistant. Of the rest, 12 lines proved moderately susceptible, 30 delayed susceptible, 176 susceptible to very susceptible and 13 showed heterogeneous reaction. Atiri and Thottappilly(1984) reported from Nigerea that mechanical inoculation is better than aphid transmission in screening studies. Collins et al.(1985) screened 16 cowpea cultivars for their resistance to black eye cowpea mosaic virus, cowpea chlorotic mottle virus, cowpea mosaic virus, cowpea severe mosaic virus, southern

bean mosaic virus (cowpea strain) and cucumber mosaic virus. Five cultivars showed promising levels of resistance to BICMV only. All the 16 cultivars were susceptible to the other five viruses.

VII. Serology

a. Purification

Different methods of purification of cowpea mosaic virus have been reported. Butanol-chloroform method, polyethylene glycol-Nacl method, a combination of these two methods, Butanol clarification of the virus and precipitation with PEG, using thioglycollic acid, Ammonium sulphate and Nacl are some of these methods. Steere(1956) purified cowpea mosaic virus using butanol-chloroform method. In this method the infected plant sap was extracted in 0.1 M phosphate buffer of pH 7. Van Kammen(1971) also purified cowpea mosaic virus employing butanol-chloroform method. But instead of using phosphate buffer he used 0.02 M potassium acetate buffer containing 0.002 M EDTA of pH 5.8 for leaf extract preparation.

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 yields of purified virus compared to butanol-chloroform method of purification.

Van Kammen and deJager(1978) used a method of purification of cowpea mosaic virus which was a combination of butanol-chloroform method of steere(1956) and PEG-Nacl method of Hebert(1963). Bock and Conti(1974) reported another method of purification of cowpea mosaic virus. They extracted the sap of infected leaves in 0.5 M sodium citrate buffer containing 1 per cent 2 mercapto ethanol of pH 8.1 and clarified by treatment with n-butanol and subjected to differential centrifugation. Lima and Nelson (1977) purified the cowpea mosaic virus by butanol clarification and precipitation with polyethelene glycol. Lima et al.(1979) reported that either n-butanol or a combination of chloroform and carbon tetrachloride can be used for the initial clarification of cowpea mosaic virus.

b. Serological tests

Several scientists worked out the relationship of viruses causing disease in cowpea and other legumes. Perez et al.(1971) reported that a virus causing mosaic in Puerto Rico was closely related to cowpea mosaic virus from Arkansas and Trinidad. He also found that the passive haemagglutination test is highly sensitive for the detection

of plant virus antigens. According to Bock and Conti(1974) cowpea aphid-borne mosaic virus belongs to potyvirus group, but no serological relationship exists between cowpea aphid-borne mosaic virus and other potyviruses, viz., potatoe virus Y, bean yellow mosaic virus, pea seed borne mosaic virus, clover yellow vein virus, soybean mosaic virus, sugarcane mosaic virus, tobacco severe etch virus and iris mosaic virus. Serological relationship of cowpea chlorotic mottle virus and bean yellow mosaic virus was reported by Fulton et al.(1975). Lima and Nelson(1977) found that purified sap extracts of cowpea mosaic virus infecting cowpea and Phaseolus lathyroides reacted with cowpea mosaic virus antiserum but not with antisera of bean pod mottle virus, broad bean mosaic virus, cowpea chlorotic mottle virus, southern bean mosaic virus or soybean mosaic virus in gel diffusion tests. It was also found that the cowpea mosaic virus isolates from cowpea and Phaseolus lathyroides were slightly different serologically as a spur was formed between the two when reacted against the antiserum specific to cowpea isolate.

Fulton and Scott(1979) put forth a serogrouping concept for legume comoviruses. Five serogroups have been recognised. Lima et al.(1979) found that the potyvirus

isolated from cowpea in Ceara was serologically related to but distinct from black eye cowpea mosaic and bean common mosaic virus. Immunodiffusion in agar gel containing sodium dodecyl sulphate was used for detection of cowpea viruses by Lima and Purcifull(1980) and Lin et al.(1981). Mali and Kulthe(1980) reported that the seed-borne potyvirus causing mosaic of cowpea in India is not related serologically to alfalfa mosaic virus, bean common mosaic virus, cucumber mosaic virus, tobacco mosaic virus and tobacco ringspot virus. Antiserum gave precipitin end point of 1:256 and the antiserum titre 1:1024. There existed a close immunological relationship between CM-11 cowpea virus antigen and broad bean isolate of bean yellow mosaic virus.

Nariani et al.(1980) reported that an aphid and seed-borne mosaic disease of cowpea showed a serological relationship with a strain of tobacco mosaic virus. Sanchez and Gonzales(1981) identified a close serological relationship between yellow and severe strains of cowpea mosaic virus. Taiwo and Gonsalves(1982) grouped the isolates of black eye cowpea mosaic virus and cowpea aphid-borne mosaic virus isolates into two serogroups. Mali(1983) reported that one of the isolates of seed-borne potyvirus causing mosaic of cowpea in India is serologically related

to CAMV of Bock and Conti(1974). Lin et al.(1984) reported that there are four distinct serotypes of cowpea severe mosaic virus isolates and that the four serotypes showed cross reactivity among them due to a common antigenic determinant. Rocha-Pena and Fulton(1984) reported a close serological relationship between cowpea mosaic virus isolate of Tabasco and isolates from Arkansas, Puerto Rico, El Salvador and Venezuela.

VIII. Effect of virus on growth of plant

Harrison and Gudauskas(1968) studied the effect of bean yellow mosaic virus, cowpea chlorotic mottle virus (CCMV) and cowpea mosaic virus (CpMV) individually and in mixed infections on growth and seed yield of the cowpea varieties 'Clay' and 'Early Ramshorn'. Only bean yellow mosaic virus caused significant reductions in growth and seed production of 'Early Ramshorn'. A mixed infection of CCMV and CpMV reduced seed yield whereas neither virus alone had any effect. None of the viruses alone or in combination affected growth or yield of 'Clay'. Khatri and Chenulu, (1970) reported that cowpea mosaic virus infection decreased the dry weight of leaves in resistant and susceptible varieties, moisture content in susceptible varieties and affected mineral metabolism. Gilmer et al. (1973) reported from Western Nigeria that early infections

of cowpea mosaic virus reduced the yields by 40-60 per cent and late infection caused reduction of only 5-10 per cent. Sharma and Varma(1976) observed a 41.8 per cent reduction in the yield of cowpea as a result of infection of cowpea chlorotic spot virus and cowpea banding mosaic virus. Fegla et al.(1981) studied the effect of cowpea aphid-borne mosaic virus and cucumber mosaic virus on growth and nodulation of cowpea. They observed that the shoot length, and fresh and dry weight of roots and shoots were affected by infection with the viruses individually or in combination. Nodulation was also reduced by combined infections. Vaverde et al.(1982) reported from Costa Rica that cowpea mosaic virus infection reduced the yield of cowpea by 84.8, 82.1 and 55.6 per cent, when infection occurred before, during and after flowering, respectively. Graham(1985) found that cowpea severe mosaic virus infection reduced the leaf area, shoot weight and nodule weight significantly in the case of early infections. It was also found that in diseased plants in the absence of fertilizer N_2 the pod yield of cowpea was also reduced significantly.

IX. Effect of leaf extract sprays on development of cowpea mosaic virus

The antiviral effect of leaf extracts have been reported by several scientists. The antiviral effects of

leaf extracts of Capsicum annuum and Datura stramonium were recorded by Raychaudhuri and Prasad(1965); and Sharma and Raychaudhuri(1968). There have been reports on the antiviral effect of leaf extracts of Mirabilis jalapa (Verma and Kumar,1980) and Boerhaavia diffusa (Verma Awasthi, 1980).

Verma and Dwivedi(1983) reported from Lucknow that the leaf extracts of Bougainvillea spectabilis protected Lycopersicon esculentum, Cucumis melo and Crotalaria juncea plants against the infection caused by tobacco mosaic virus, tomato yellow mottle virus, physalis shoestring mosaic virus and cucumber green mottle virus. They obtained complete protection against the diseases with six preinoculation sprays. They have extracted a virus inhibiting factor from the leaves of the host plants sprayed with Bougainvillea spectabilis leaf extract, and reported that the presence of virus inhibiting factor was the reason for the expression of antiviral effect by the treated plants.

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Symptomatology

Seeds of cowpea (*Vigna unguiculata*(L.)Walp.) variety C-152, obtained from the Department of Olericulture, College of Horticulture, Vellanikkara, Trichur, were sown in pots containing a potting mixture of sand, red soil and cowdung(1:1:2). Leaves of cowpea plants showing symptoms of cowpea mosaic virus disease were collected from the field. The culture of cowpea mosaic virus (CpMV) was maintained by repeated transfers on cowpea plants in insect proof glass house by sap inoculation. Symptomatology of cowpea mosaic virus disease was studied by observing the development of symptoms in naturally infected as well as artificially inoculated cowpea plants and *Chenopodium amaranticolor* Coste & Reyn.

II. Transmission

1. Sap transmission

The culture of the virus maintained in the insect proof glass house as mentioned above was used for the studies.

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. Young leaves of systemically infected plants showing typical symptoms were collected and triturated using a clean and sterile mortar and pestle. The resulting pulp was strained through sterilised cotton wool and used as the inoculum.

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. When tris buffer (0.1 M, pH 7.0), and phosphate buffer (0.05 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 used as the inoculum. Inoculation was done by gently rubbing on the upper surface of the leaves with inoculum. Plants were inoculated when they were in the two leaf stage. A small quantity of carborundum powder was sprinkled uniformly on the leaves before application of inoculum. Care was taken not to cause excess injury to the leaves during inoculation. Soon after the inoculations the leaves were washed with distilled water using a wash bottle.

Ten plants each were inoculated for every experiment and an equal number of uninoculated plants were kept as control. The experiments were done in cooled conditions also i.e. the inoculum was prepared from frozen leaves using previously chilled pestle and mortar. The experiments were done twice and the plants were kept under observation in insect proof conditions.

2. Seed transmission

Three hundred and eighteen seeds collected from cowpea mosaic virus (CpMV) infected cowpea plants were sown in pots in the insect proof glass house. The plants were kept under observation for three weeks after germination.

3. Graft transmission

Small shoots showing systemic symptoms were selected for preparing scion. The base of the scion was trimmed to a wedge shape and inserted into a cleft made on the stem of the stock grown in pots kept in the insect proof glass house. Thirty days old healthy plants were used as stock. Most of the leaves of the scion were removed and the base of the scion was inserted into the cleft of the stock. The graft was then tied properly with

a polythene bag to retain humidity.

4. Insect transmission

Insect transmission studies were carried out by using Aphis craccivora Koch, Aphis gossypii Glov. and Aphis malvae Koch, as vectors. The aphid species were identified at the department of Agricultural Entomology, College of Agriculture, Vellayani. Nonviruliferous aphids were collected from healthy host plants i.e., Aphis craccivora from healthy cowpea plants (Vigna unguiculata(L.) Walp), Aphis gossypii from snake gourd plants(Trichosanthes anguina L.) and Aphis malvae from bhindi plants (Abelmoschus esculentus (L)Moench).

Pre-acquisition fasting of one hour and an acquisition feeding of ten minutes were given. A fixed number of aphids were transferred to each of the test plants and allowed to feed for 24 h. After that they were killed by spraying 0.1 per cent methyl parathion. As in the case of mechanical inoculation an equal number of control plants were kept in separate cages. Only apterous form of aphids was used in these trials.

III. Physical properties

1. Dilution end point (DEP)

Infected cowpea leaves of known weight were crushed to fine pulp by means of clean and sterile mortar and pestle adding one ml of sterile distilled water per gram of leaf material. The resulting pulp was strained through cotton wool and thus the standard sap was obtained. Serial dilution of the standard sap (1:1) viz., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared as follows. Six test tubes were arranged in a row in a test tube rack. Nine ml of sterile distilled water was dispensed into each of the five test tubes starting from second test tube by using a sterilised pipette. The standard sap was poured into first test tube without adding sterile distilled water and kept as control. One ml of the standard sap was transferred to the second test tube with 9 ml distilled water to get a dilution of 10^{-1}. It was mixed thoroughly and one ml of the 10^{-1} dilution was transferred to the next test tube to prepare a dilution of 10^{-2}. The preparation of serial dilution was continued until a 10^{-5} dilution was made. All the transfers were made with sterilized pipettes. The different dilutions were used for inoculation on separate sets of test plants starting from the highest dilution.

Ten cowpea plants were inoculated with each of the dilutions. The inoculated plants were labelled and kept under insect proof conditions and observed for development of symptoms. The experiments were repeated for confirmation of results. The inoculation was also done on four leaves of the indicator host Chenopodium amaranticolor and the observations on the number of local lesions produced were recorded.

2. Thermal inactivation point (TIP)

The sap from the infected cowpea plants was prepared as in the above experiment. Five ml of the sap was pipetted into a thin walled glass test tube. Care was taken not to smear the upper part of the test tube. It was then placed in a waterbath with thermostat arrangement. The waterbath was filled with water until the level reached 3 cm above the level of the sap in the tubes. The test tube was kept for ten minutes in the waterbath maintained at 35°C. The control was kept at room temperature (28-30°C). In the same manner five ml lots of the sap were kept for ten minutes each at 35, 40, 45, 50, 55, 60, 70, 80 and 90°C and thermometer was placed close to the tube in the waterbath to check the temperature. After ten minutes in each case the tube was removed

and cooled immediately in running water. The untreated and treated samples of the sap were used for inoculation on the test plants by smearing them on leaves sprinkled with carborundum powder. Inoculation was done on healthy leaves of two months old Chenopodium amaranticolor. Five leaves were inoculated in each treatment, and the experiment was repeated to confirm the results. Observations on the number of local lesions produced on leaves of Chenopodium amaranticolor were recorded.

3. Longevity in vitro (LIV)

The sap from the infected cowpea plants was prepared as in the above experiment. 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 refrigerator (8°C). One tube each containing the sap of each treatment was taken after specific periods, viz., 0, 1, 2, 4, 6, 8, 12, 24, 48 and 72 h and inoculated on leaves of Chenopodium amaranticolor. Five leaves were inoculated in each treatment and the experiment was repeated to confirm the results. In all the experiments the inoculated plants were kept under insect proof conditions and observed for the development of symptoms.

Materials & Methods

IV. Vector-virus relationships

Cowpea plants showing typical symptoms of cowpea 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 suitable healthy host in insect rearing cages. Experiments on vector-virus relationships were conducted by using Aphis craccivora which was found to be the most efficient vector. In all the insect transmission trials only full grown apterous aphids were used. During feeding of the aphids the test plants were kept in insect proof cages. The aphids were killed at the end of required feeding period by spraying the plants with 0.1 per cent methyl parathion (metacid 50EC). In the case of short feeding periods of less than five minutes the individual aphids were watched through a mangifying lens and the time of feeding was determined with the help of a stop watch after the aphids had settled down to feed.

1. Minimum acquisition feeding period

A large number of nonviruliferous aphids were collected and were given a pre-acquisition starvation of one hour. Batches of ten aphids each were given acquisi-

tion feeding of 20 S, 30 S, 1, 2, 5, 10, 15 and 30 min and 1,2 4 and 24 h on diseased plants before transferring them to healthy cowpea plants. The aphids were then allowed to remain for 24 h on the test plants and were killed thereafter by spraying 0.1 per cent methyl parathion.

2. Minimum inoculation feeding period

Nonviruliferous aphids were given one hour pre-acquisition starvation and an acquisition feeding of 10 minutes. Then the viruliferous aphids were transferred in batches of ten to individual healthy test plants. Each batch was given separate inoculation feeding periods, viz., 15 S, 30 S, 1, 2, 5, 10, 15 and 30 min and 1 h. The aphids were killed after the specific inoculation feeding period by spraying 0.1 per cent methyl parathion.

3. Influence of fasting before acquisition and inoculation feedings

i. Pre-acquisition fasting

A large number of nonviruliferous aphids were starved for different periods such as 30 min, 1, 2, 4 and 24 h. Then they were allowed an acquisition feeding for ten minutes on diseased plants and subsequently they were

confined in batches of ten to healthy test plants for inoculation feeding. Effect of each pre-acquisition fasting period was tested on ten healthy test plants. Control plants were also kept with an equal number of aphids but without any pre-acquisition fasting. After 24 h the aphids were killed by spraying with 0.1 per cent methyl parathion. The experiment was repeated to confirm the results.

ii. Post-acquisition fasting

A large number of aphids were starved for 1 h and given an acquisition feeding period of 10 minutes. These viruliferous aphids were then starved in batches of ten for different periods such as 30 min 1,2, and 4 h. Groups of ten aphids from each of these categories were transferred to each healthy test plant. Effect of each post-acquisition fasting period was tested on ten healthy test plants. The controls were maintained with equal number of aphids with no post-acquisition fasting. The aphids were killed after 24 h by spraying 0.1 per cent methyl parathion. The experiment was repeated to confirm the results.

4.Retention of infectivity by the vector

The experiments were conducted with viruliferous insects which were transferred in succession to a series of healthy cowpea plants after a definite inoculation feeding period on each plant. A large number of aphids were starved for one hour and fed on diseased cowpea plants for 10 min to make them viruliferous. Groups of ten aphids were then transferred in succession to a series of five healthy plants transferring the insects after a definite interval. The different feeding intervals allowed in different series were 15 min, 30 min, 1 h and 2 h. The aphids were killed from the fifth plant of the different series by using 0.1 per cent methyl parathion. The experiment was done twice.

5. Minimum number of aphids required for transmission

Single aphids as well as groups of 3, 5, 10 and 15 aphids were collected from a nonviruliferous colony from the rearing cage and were starved for one hour. These aphids were made viruliferous by feeding them on diseased cowpea plants. After an acquisition feeding period of 10 min, the aphids were transferred to healthy test plants by using a camel hair brush without causing any injury to the insects and allowed to feed for 24 hours.

They were then killed by spraying the plants with 0.1 per cent methyl parathion.

6. Incubation period of virus in the host plant

Twenty healthy cowpea seedlings of two leaf stage were inoculated using Aphis craccivora and observations were taken on the date of appearance of symptoms.

V. Host-Range:

To determine the host-range of cowpea mosaic virus, plants belonging to 73 species of 17 families were inoculated by sap inoculation. Four to five seedlings were inoculated in each case. The plants which did not show symptoms after four weeks were indexed by back inoculation to Vigna unguiculata(L.)Walp. to find whether they were symptomless carriers of the virus. Following plants were used for host-range studies.

1. Acanthaceae:
   a) Andrographis echioides (L.)Ness
   b) Justicia prostrata Gramble

2. Amaranthaceae:
   a) Amaranthus caudatus L.
   b) Amaranthus viridis L.

c) Alternanthera sp.

d) Celosia sp.

e) Gomphrena globosa L.

f) Spinacia oleraceae L.

3. Apocynaceae:

a) Vinca rosea L.

4. Araceae:

a) Caladium sp.

b) Colocasia esculenta L.

5. Capparidaceae:

a) Cleome viscosa L.

6. Chenopodiaceae:

a) Chenopodium amaranticolor Coste & Reyn

b) Chenopodium quinoa Willd.

c) Chenopodium murale L.

7. Compositae:

a) Ageratum conizoides L.

b) Emilia sonchifolia DC

c) Eupatorium odoratum L.

d) Synedrella nodiflora Gaertin

e) Tridax procumbens L.

f) Vernonia cineria L.

g) Zinnia elegans Jacq.

h) Zinnia linnearis L.

8. Cucurbitaceae:

a) Cucurbita moschata Duch.

b) Cucurbita pepo L.

c) Cucumis sativus L.

d) Momordica charantia L.

e) Trichosanthes anguina L.

9. Euphorbiaceae:

a) Acalypha indica L.

b) Croton sparsiflorus Morong

c) Euphorbia hirta L.

d) Manihot esculenta Crantz.

e) Phyllanthus niruri L.

10. Graminae:

a) Echinochloa colona (L.)Link.

b) Oryza sativa L.

c) Panicum repens L.

11. Malvaceae:

a) Abelmoschus esculentus (L.)Moench.

b) Sida acuta Burm F.

12. Labiatae:

a) Leucas aspera (Willd)Spreng.

b) Ocimum sanctum L.

13. Leguminosae:

1. Caesalpiniaceae:

a) Casssia occidentalis L.

2. Mimosaceae:

a) Mimosa pudica L.

3. Papilionaceae:

a) Arachis hypogaea L.

b) Cajanus cajan (L.)Millsp.

c) Calapagonium mucunoides Desv.

d) Canavalia ensiformis (L.)DC.

e) Clitoria ternatea L.

f) Crotalaria juncea L.

g) Cyamopsis tetragonaloba (L.)Taub.

h) Dolichos biflorus Auct.

i) Glycine max (L.)Merr.

j) Phaseolus vulgaris L.

k) Pisum sativum L.

l) Psophocarpus tetragonalobus (L.)DC

m) Stylosanthes guianensis (Aubl) SW.

n) Stylosanthes guineensis Schum & Thonn.

o) Vigna mungo (L.) Hepper

p) Vigna radiata (L.) Wilczek

q) Vigna sesquipedalis (L.) Fruw

14. Pedaliaceae:

a) Sesamum indicum L.

15. Solanaceae:

a) Capsicum annuum L.

b) Datura stramonium L.

c) Datura metel L.

d) Lycopersicon esculentum Mill.

e) Nicotiana glutinosa L.

f) Nicotiana tabacum L.

g) Petunia hybrida Vilm

h) Solanum melongena L.

16. Verbenaceae:

a) Clerodendron infortunatum L.

b) Lantana camara L.

c) Stachytarpheta indica L.

17. Zingiberaceae:

a) Curcuma domestica Val

b) Zingiber officinale Rose.

VI. Varietal screening

Ten varieties of cowpea plants obtained from Regional Agricultural Research Station, Pattambi, were tested for their resistance to cowpea mosaic virus. Forty plants of each variety were inoculated with the virus using standard sap as inoculum. The inoculum was prepared from systemically infected cowpea leaves by triturating them using mortar and pestle adding 0.1 M tris buffer of pH 7.0. The standard sap was strained through cotton-wool and immediately inoculated on leaves of test plants. Healthy cowpea plants of two leaf stage, grown in insect proof glass house were used as test plants. Following were the varieties used for screening studies.

1. New Era
2. KBC-1
3. V-240
4. Kanakamony
5. C-152
6. V-59
7. V-87
8. C.G.104
9. V-37
10. Kozhinjil (local variety)

Observations on the incidence of the disease were recorded on the 14th and 28th days after inoculation.

VII. Serology

1. Purification of virus

Two methods of purification of virus were tried. (1) The inoculum was prepared by triturating the systemically infected leaves at the rate of one g/ml of 0.1 M phosphate buffer of pH 7.0 using a clean and sterile mortar and pestle. The homogenate was then strained through a double layer of muslin cloth and thioglycollic acid was added to the filtered sap, at the rate of two ml per 100 ml of the sap. Then the sap was centrifuged at 5000 rpm for five minutes at 4°C using HIMAG refrigerated centrifuge model HCR 20BA, to remove the host material. The precipitate was discarded and the clear supernatant was taken and activated charcoal was added to that at the rate of 0.05 g/ml of the sap. The sap and activated charcoal were mixed thoroughly and allowed to settle for 15 min. Then it was centrifuged at 6000 rpm for 10 min. The precipitate was discarded and the clear supernatant was taken. Six per cent polyethylene glycol(PEG) and 0.2 per cent Nacl were added to the supernatant and kept at 4°C for 60 to 90 min. It was then centrifuged at 2500 rpm

for 10 min. The precipitate was dissolved in 0.1 M phosphate buffer of pH 7.0. The supernatant and precipitate were tested for their infectivity on cowpea plants as well as Chenopodium amaranticolor.

(ii) In the second method, purification was done by using PEG and Nacl. The inoculum was preared by triturating the systemically infected, frozen leaves at the rate of 1 g/ml of 0.01 M phosphate buffer of pH 7.0 using a clean and sterile mortar and pestle. The homogenate was then strained through a double layer of muslin cloth. The filtered sap was centrifuged for 15 min at 10,000 g. PEG and Nacl were added to the supernatant to get final concentrations of 4 per cent and 0.2 M, respectively, and centrifuged at 10,000 g for 15 min. The precipitate was dissolved in 0.01 M phosphate buffer of pH 7.0. The supernatant and precipitate were tested for their infectivity on cowpea plants as well as on Chenopodium amaranticolor.

The supernatant obtained from second method of purification, found to have highest infectivity, was used for injecting rabbits.

2. Preparation of antiserum

Two Newzealand white female rabbits were used for the production of antiserum. The purified virus preparation was emulsified with Freund's incomplete adjuvant (Difco), in the ratio 1:1 v/v. This emulsion was injected intramuscularly four times at an interval of 7-10 days. Four ml (2 ml antigen + 2 ml adjuvant) was injected at a time at the rate of 2 ml portions into each thigh muscle. A fifth injection was given intravenously 7 days after the last intramuscular injection. Two ml of antigen alone was injected into marginal ear vein of each rabbit.

Two weeks after the last injection the rabbits were bled. They were fasted for 12 h prior to bleeding. The marginal ear vein, widened temporarily by rubbing the ear with xylol, was severed with a razor blade for bleeding the rabbits. The blood samples were aseptically collected in 15 ml tubes and were allowed to coagulate by keeping the tubes at room temperature for two hours. The coagulated blood clot was loosened with the help of a sterilized glass rod and the samples were kept overnight at $4^{o}C$. The clear serum was decanted and centrifuged at 5000 g for 30 minutes at $4^{o}C$. Purified serum was pipetted out using a sterile pipette and dispensed to 5 ml vials.

Sodium azide was added to the clarified serum as a preservative, so as to make a final concentration of 0.01 per cent. Vials were then sealed, labelled and kept in a freezer.

Two weeks after the first bleeding one more intravenous injection was given and the rabbits were again bled after one week.

3. Serological tests

(i) Microprecipitin test on slides

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

The cowpea mosaic virus antigen was tested against six other antisera also, viz., cowpea severe mosaic virus, cowpea mosaic virus (USA), cucumber mosaic virus(cowpea isolate),southern bean mosaic virus, cowpea chlorotic mottle virus and cowpea mosaic virus El Salvador.

(ii) Microprecipitin test in petridishes

This test was used 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. Procedure was carried out as described by Noordam(1973).

Cowpea leaves showing typical symptoms were triturated using a clean and sterile mortar and pestle and the sap was strained through cotton-wool and centrifuged at 5000 g for 15 min to get clear supernatant. Using a pasteur pipette one ml of that was transferred into the first of a series of numbered corning glass tubes with a capacity of 1 to 1.5 ml. The second tube was half filled with the sap and an equal amount of saline buffer (0.85 per cent Nacl in 0.01 M Tris oxymethyl aminomethane buffer of pH 7.0). The liquids were mixed by inverting the tube several times. This tube contained the sap diluted to 1/2. Half of this dilution was transferred to next tube and an equal volume

of saline buffer was added so as to make a dilution of 1/4. This method was continued to make dilution 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 dilution series were made for 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 figure. 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 13 drops of the least concentrated sap (1/4096) were spotted at the intersections along with vertical line labelled 1/4096. The next dilution of the 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 lowest 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 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 petri dishes were kept for 2 h at 28-30°C and examined after 30 min and 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
¦	= Barely visible precipitate
+	= Slight reaction
++	= Moderate reaction
+++	= Heavy reaction
++++	= Very heavy reaction

The petri dishes were kept overnight in a refrigerator and evaluated for the second time. From the above mentioned 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 petri dishes. Antiserum and virus suspensions (0.4 ml each) were added to wells punched in agar.

Sterilized petri dishes were coated with a layer of 2 per cent agarose(prepared in 0.01 M tris buffer containing 0.85 per cent Nacl and sodium azide to get a final concentration of 0.02 per cent) to a thickness of 1 mm and allowed to soldify. Above this layer 2 per cent melted agarose was again added to a thickness of 3 mm. Thirty minutes after pouring of agarose, with the help of a sterilized gel cutter, six wells (one well in the centre and the other five wells around it) were made in each plate. 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 undiluted antiserum was dispensed with a pasteur pipette. In the surrounding wells antigens prepared from infected plants were dispensed as described below in five separate plates.

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

b) In the plate II, well 2 received sap from healthy cowpea plants, 3 received cowpea mosaic virus, 4

cucumer mosaic virus, 5 snake gourd mosaic virus and 6 bitter gourd mosaic virus.

c) In Plate III, well 2 received sword bean mosaic virus, 3 received sap from healthy cowpea plants, 4 bitter gourd mosaic virus, 5 cluster bean mosaic virus and 6 snake gourd mosaic virus.

d) In plate IV, well 2 received CpMV isolate I, 4 pumpkin mosaic virus and 5 cucumber mosaic virus, wells 3 and 6 received CpMV isolate II and CpMV isolate III, respectively.

e) In plate V, the experiment was done by using purified virus preparation diluted to 1/4 concentration. Well 2 contained the antigen of CpMV isolate I diluted to 1/4. Well 4 contained CpMV isolate II and well 5 CpMV isolate III each diluted to 1/4 concentration. Well 3 contained healthy cowpea plant sap diluted to 1/8 and well 6 contained buffer.

The petri dishes were kept humid by placing a moistened filter paper on the inner side of the lids. The experiments were performed twice. The dishes were kept in stacks with ordinary paper in between them to prevent any scratches and incubated at room temperature and examined

periodically for the appearance of characteristic precipitin bands up to 14 days. After that precipitin bands were stained using amido black as detailed below.

Before staining, the agar was soaked in two changes of 0.9 per cent phosphate buffered saline for 24 h and then in distilled water for another 24 h. Water was drained out and the agar was covered with a Whatman No.1 filterpaper and dried at 37°C. When the agar was completely dry, the filterpaper was stripped off. The dried agar was then immersed in amidoblack stain (Appendix-1) for 15 minutes.

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

(iv) Reaction of cowpea mosaic virus antigen with different antisera.

The relationship of cowpea mosaic virus antigen with the antisera obtained from different places, was also studied using Ouchterlony's agar double diffusion tests. Here antigen of cowpea mosaic virus was taken in the central well. Different antisera were dispensed in the

surrounding wells in two plates as follows.

First plate:

Well 2. Antiserum of cowpea mosaic virus (PMV) isolate.
" 3. Antiserum of cowpea severe mosaic virus
" 4. Antiserum of southern bean mosaic virus
" 5. Antiserum of cucumber mosaic virus cowpea isolate
" 6. Antiserum of cowpea mosaic virus prepared in our laboratory.

Second plate:

Well 2. Antiserum of cowpea mosaic virus El Salvador.
" 3. Antiserum of cucumber mosaic virus
" 4. Pumpkin mosaic virus antiserum prepared in our laboratory.
" 5. Antiserum of cucumber mosaic virus 'So afr str'
" 6. Antiserum of cowpea chlorotic mottle virus.

VIII. Effect of virus infection on growth of cowpea plants

A pot culture experiment was laid out in completely randomised design to estimate the effect of virus infection on growth of cowpea plants. There were ten varieties and two treatments, viz., uninoculated and inoculated. The following ten varieties of cowpea were used in the study.

V_1 C-152

V_2 V 37

V_3 CG 104

V_4 Kanakamony

V_5 V 87

V_6 V 59

V_7 KBC-1

V_8 V-240

V_9 Kozhinjil (local variety)

V_{10} New era

The plants were raised in insect proof glass house, and were sap inoculated when they were at two leaf stage. The uninoculated plants were kept separately from the inoculated plants. Observations were taken on leaf area, height of the plants, number of pods produced and length of the pods. Observations on leaf area were taken from the most susceptible variety C-152. The average of the leaf area of top, middle and bottom leaves of each plant was calculated and that was taken as the average leaf area of the plant. Observations were taken from ten plants of each variety, averages were calculated and statistical analysis was conducted.

IX. Observations on natural incidence of cowpea mosaic virus.

Cowpea plants grown in the germplasm collection of the Department of Olericulture, College of Horticulture, Vellanikkara, Trichur, were examined to find out the natural incidence of mosaic diseases of cowpea. Cowpea plants were grown in a total area of 4.5 acres. Observations were taken on 45 day old plants of 5 plots for the incidence of cowpea mosaic, cowpea chlorotic mottle, southern bean mosaic and cowpea yellow mosaic based on the symptoms.

X. Control of cowpea mosaic disease by leaf extract sprays.

A pot culture experiment as described by Verma and Dwivedi(1983) was conducted to find out the effects of leaf extract sprays on development of cowpea mosaic disease. Leaf extracts of Bougainvillea sp. and Eupatorium odoratum selected from preliminary experiments were used for the study. Leaf extracts were prepared by grinding 200 g of fresh leaves in a grinder with 400 ml of 0.05 M phosphate buffer, pH 7. The pulp was squeezed through two folds of muslin cloth and the filtrate centrifuged at 5000 g for 15 min. The clear supernatant was diluted up to 1:5 with 0.05 M phosphate buffer of pH 7.0 and was sprayed on test

host plants with the help of a hand sprayer.

Cowpea plants of two leaf stage were selected and divided into 11 lots of 20 plants each. Six lots of cowpea plants were given two, four and six pre-inoculation sprayings with each of the two extracts. The sprayings were given at an interval of four h and the plants were inoculated 24 h after last spraying. The next four lots of cowpea plants were given four and six post-inoculation sprayings with each of the plant extracts. One lot was inoculated without any spraying. Observations on the appearance of symptoms were recorded.

RESULTS

RESULTS

I. Symptomatology

The leaves of the naturally infected plants showed varying amount of dark green vein banding and interveinal chlorosis. Slight distortion of leaves and stunting of the plants were also noticed. In certain cases the pale green leaf lamina exhibited a net work like pattern with the veins and veinlets appearing green in colour. In some cases the infected plants appeared chlorotic even when observed from a considerable distance. Diseased plants produced only a few pods which were small in size.

On mechanical inoculation to cowpea plants of two leaf stage, the symptoms appeared within 14 days. The young trifoliate leaves showed complete chlorosis and in some cases a mild vein clearing. Subsequent leaves showed mosaic mottling with dark green and light green patches. In most cases leaves showed prominent vein banding (Fig.1). In some cases the interveinal areas were yellowish. Plants infected at the early stages remained stunted and flowering and pod formation were very rare. The virus could produce local lesions on the leaves of Chenopodium amaranticolor. The lesions appeared

Fig.1. Symptoms of cowpea mosaic virus on cowpea leaves.

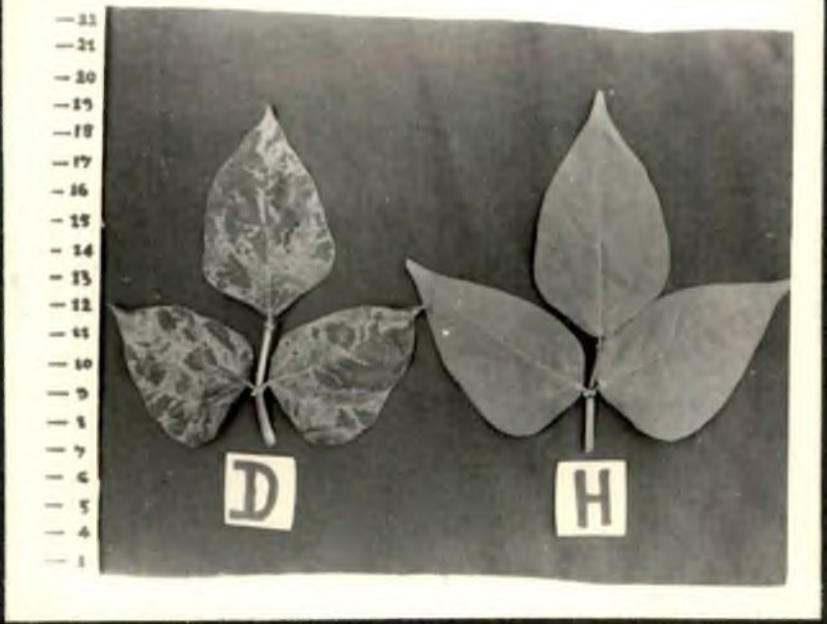

Fig.3. Diseased and healthy cowpea leaves

7-8 days after inoculation. The lesions first appeared as yellowish spots. Later they became necrotic spots (Fig.5).

II. Transmission

1. Sap transmission

The virus was found to be transmitted successfully through mechanical inoculation. The symptoms appeared 8-14 days after inoculation. The percentage of transmission varied with the extraction medium used. Standard sap gave 65 per cent transmission. Tris buffer used in cooled condition gave the maximum infection of 90 per cent and phosphate buffer at room temperature gave minimum infection of 55 per cent (Table 1).

2. Seed transmission

Out of the 318 seeds sown, 181 seeds have germinated and among them 10 seedlings showed symptoms of cowpea mosaic during the period of observation. Therefore there was 5.5 per cent seed transmission.

3. Graft transmission

Infected shoots were wedge grafted to 30 days old healthy cowpea plants grown in pots kept in insect proof

Table 1. Sap transmission of Cowpea Mosaic Virus

Sl. No.	Type of inoculum	Number of plants infected out of ten		Total number of plants infected	Per cent trans-mission
		Exp. No.I	Exp. No.II		
1.	Standard sap	7	6	13	65
2.	Sap extracted in tris buffer	8	7	15	75
3.	Sap extracted in phosphate buffer	6	5	11	55
4.	Standard sap(Cooled condition)	8	7	15	75
5.	Sap extracted in tris buffer (cooled condition)	9	9	18	90
6.	Sap extracted in phosphate buffer (Cooled condition)	8	6	14	70

Fig.5. Local lesions of cowpea mosaic virus on leaves of Chenopodium amaranticolor.

Table 2. Graft transmission of cowpea mosaic virus

Trials	No. of plants grafted	No. of plants infected	Per cent transmission
1	10	3	30
2	10	4	40
Total	20	7	35

Table 3. Insect transmission of cowpea mosaic virus

Sl. No.	Vector	Number of plants infected out of ten		Total number of plants infected	Per cent trans-mission
		Exp. No.I	Exp. No.II		
1.	Aphis craccivora	9	9	18	90
2.	Aphis gossypii	7	6	13	65
3.	Aphis malvae	5	4	9	45

glass house. There was no proper graft union established between stock and scion. But symptoms appeared in some plants 15-16 days after grafting, when new leaves were produced. From the two experiments conducted 35 per cent transmission was obtained by graft inoculation (Table 2).

4. Insect transmission

Experiments on insect transmission of the virus were carried out using three species of vectors, viz., Aphis craccivora Koch., Aphis gossypii Glov. and Aphis malvae Koch. All the three species of aphids were found to transmit the virus. The symptoms appeared 7-14 days after inoculation. The observations showed that 90 per cent transmission was obtained with Aphis craccivora, 65 per cent with Aphis gossypii and 45 per cent with Aphis malvae (Table 3).

III. Physical properties

1. Dilution end point (DEP)

Serial dilutions of the standard sap were made, viz., 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The different dilutions were used for inoculation on leaves of cowpea plants as well as C. amaranticolor starting from the highest dilution. Except 1:1 and 10^{-1} which gave 65 and 35 per cent trans-

Table 4. Dilution end point of cowpea mosaic virus on cowpea

Dilutions	Number of plants infected out of ten		Total number of plants infected	Per cent transmission
	Exp.No.I	Exp.No.II		
1:1	7	6	13	65
10^{-1}	4	3	7	35
10^{-2}	0	0	0	0
10^{-3}	0	0	0	0
10^{-4}	0	0	0	0
10^{-5}	0	0	0	0

Table 5. Dilution end point of cowpea mosaic virus on *Chenopodium amaranticolor*

Dilutions	Number of local lesions on leaves of *Chenopodium amaranticolor*				Total
	1	2	3	4	
1:1	5	3	3	4	15
10^{-1}	2	1	1	2	6
10^{-2}	0	0	0	0	0
10^{-3}	0	0	0	0	0
10^{-4}	0	0	0	0	0
10^{-5}	0	0	0	0	0

Table 6. Thermal inactivation point of cowpea mosaic virus

Temperature	Number of local lesions on five leaves of Chenopodium amaranticolor		Total
	Exp.No.I	Exp.No.II	
Room temp. (28-30°C)	13	15	28
35°C	9	11	20
40°C	3	6	9
45°C	3	3	6
50°C	1	1	2
55°C	0	0	0
60°C	0	0	0
70°C	0	0	0
80°C	0	0	0
90°C	0	0	0

missions respectively, all the other dilutions did not give any transmission, when the DEP was tested using susceptible cowpea plants (Table 4). When the DEP was tested with C. amaranticolor also positive transmissions, viz., 15 and 6 local lesions were observed in dilutions 1:1 and 10^{-1} only (Table 5).

2. Thermal inactivation point (TIP)

The thermal inactivation point of the virus was tested on leaves of C. amaranticolor. The inoculum was subjected to different temperatures, viz., room temperature (28-30°C), 35, 40, 45, 50, 55, 60, 70, 80 and 90°C. The results indicated that the virus was inactivated at a temperature between 50 and 55°C (Table 6).

3. Longevity in vitro

The inoculum was kept at room temperature (28-30°C) and also in refrigerator (8°C). It was then inoculated at specific intervals on leaves of Chenopodium amaranticolor. Inoculations were done after keeping the inoculum for 0,1, 2,4,6,8,12,24,48 and 72 h. The longevity in vitro was 8 h at room temperature and 24 h at 8°C (Table 7).

Table 7. Longevity *in vitro* of cowpea mosaic virus

Ageing in hours	No. of local lesions produced on five leaves	
	Room temperature (28-30°C)	8°C
0	60	62
1	52	53
2	23	64
4	13	16
6	1	4
8	1	2
12	0	1
24	0	1
48	0	0
72	0	0

IV. Vector-virus relationships

1. Minimum acquisition feeding period

The results showed that a short acquisition feeding period of 30 S is sufficient for the aphids to become viruliferous. The optimum acquisition feeding period which gave the maximum infection, viz., 70 per cent was found to be 10 minutes (Table 8).

2. Minimum inoculation feeding period

The viruliferous aphids were capable of transmitting the virus within 30 S inoculation feeding on the test plant. Maximum infection of 70 per cent was obtained by feeding the vector for a period of 15 minutes on test plants (Table 9).

3. Influence of fasting before and after acquisition feedings

i) Pre-acquisition fasting

The fasting of aphids before acquisition resulted in an increase in percentage of infection. Maximum infection of 75 per cent was obtained with two hour fasting. The percentage of transmission decreased with fasting for more than 2 h (Table 10).

ii) Post-acquisition fasting

It was observed that the percentage of infection was decreased due to post-acquisition fasting. Maximum

Table 8. Acquisition feeding period of *Aphis craccivora*

Acquisition feeding period	Number of plants infected out of ten		Total number of plants infected	Per cent trans-mission
	Exp.I	Exp.II		
20 s	0	0	0	0
30 s	1	1	2	10
1 min	4	2	6	30
2 min	6	4	10	50
5 min	6	5	11	55
10 min	8	6	14	70
15 min	7	5	12	60
30 min	5	4	9	45
1 h	4	3	7	35
2 h	3	2	5	25
4 h	0	1	1	5
24 h	0	0	0	0

Fig.6. Effect of acquisition feeding period on efficiency of transmission of cowpea mosaic virus by Aphis craccivora.

Treatments

T_1	20 s
T_2	30 s
T_3	1 min
T_4	2 min
T_5	5 min
T_6	10 min
T_7	15 min
T_8	30 min
T_9	1 h
T_{10}	2 h
T_{11}	4 h
T_{12}	24 h

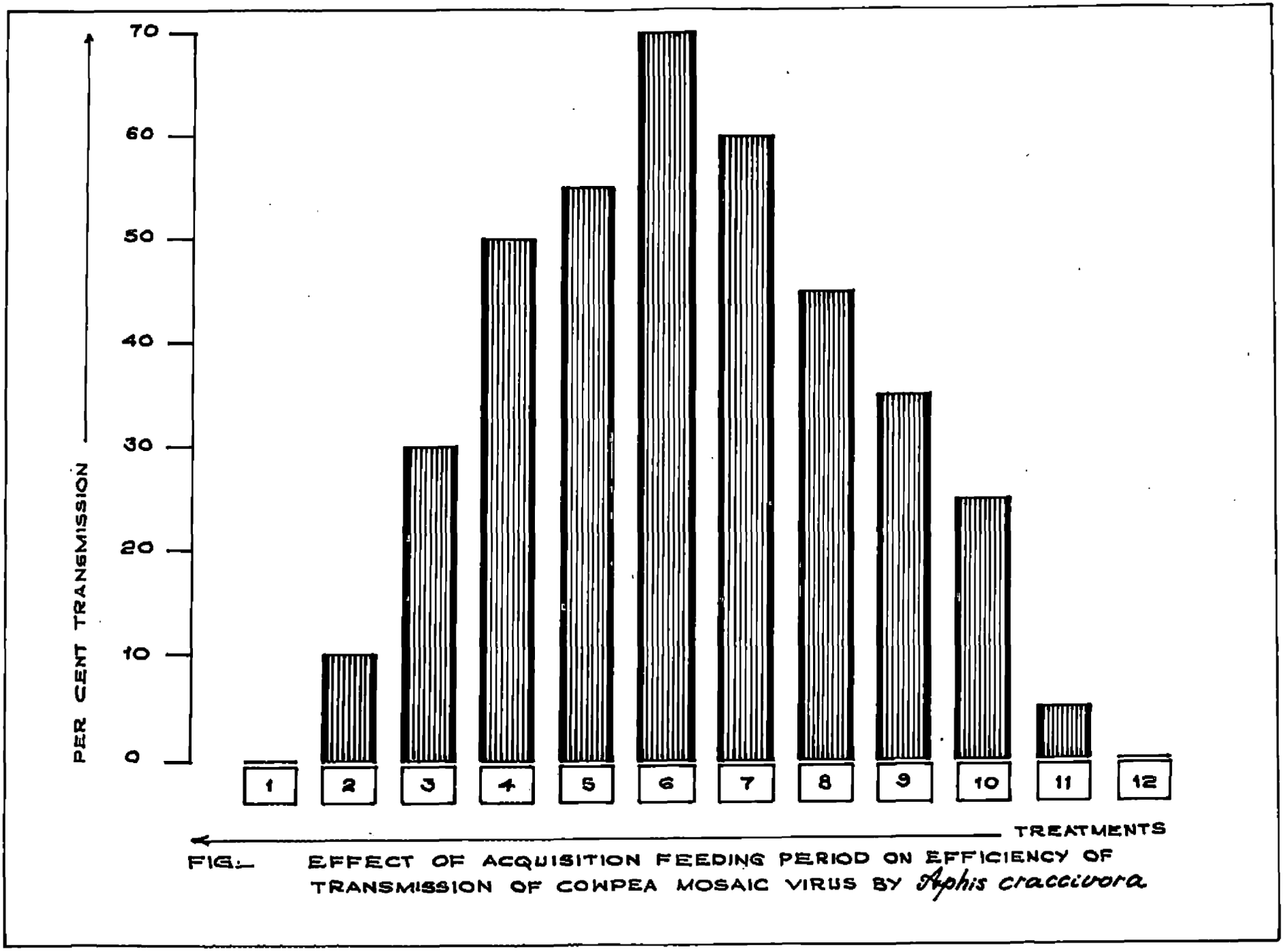

FIG. EFFECT OF ACQUISITION FEEDING PERIOD ON EFFICIENCY OF TRANSMISSION OF COWPEA MOSAIC VIRUS BY *Aphis craccivora*

Table 9. Inoculation feeding period of *Aphis craccivora*

Inoculation feeding period	Number of plants infected out of ten		Total number of plants infected	Per cent trans-mission
	Exp. I	Exp. II		
15 s	0	0	0	0
30 s	1	1	2	10
1 min	3	2	5	25
2 min	4	3	7	35
5 min	6	4	10	50
10 min	7	6	13	65
15 min	8	6	14	70
30 min	7	6	13	65
1 h	8	6	14	70

Table 10. Influence of pre-acquisition fasting on efficiency of transmission

Pre-acquisition fasting period	Number of plants infected out of ten		Total number of plants infected	Per cent transmission
	Exp. I	Exp. II		
0	3	2	5	25
30 min	3	2	5	25
1 h	7	7	14	70
2 h	8	7	15	75
4 h	2	1	3	15
24 h	0	0	0	0

Table 11. Influence of post-acquisition fasting on efficiency of transmission

Post-acquisition fasting period	Number of plants infected out of ten		Total number of plants infected	Per cent transmission
	Exp. I	Exp.II		
0	7	6	13	65
30 min	1	1	2	10
1 h	1	0	1	5
2 h	0	0	0	0
4 h	0	0	0	0

infection of 65 per cent was obtained when the aphids were immediately transferred after acquisition feeding and no infection obtained with a post-acquisition fasting of 2 hours and more (Table 11).

4. Retention of infectivity by the vector

Successful infection could be obtained up to the fourth plant of the first series in which the aphids were transferred at intervals of 15 minutes and up to the third plant when the interval was increased to 30 min. When the interval was increased to 1 h the infection was obtained up to the second plant of the series. With 2 h feeding only the first plant got infection (Table 12).

5. Minimum number of aphids required for transmission

A single viruliferous aphid was found to be capable of transmitting the virus to healthy test plants. The percentage of success obtained in this case was 15. The optimum number of aphids required to get maximum infection of 90 per cent was found to be ten (Table 13).

6. Incubation period of virus in the host plant

Twenty cowpea plants were inoculated using Aphis craccivora. Symptoms started appearing from the 7th day after inoculation and the maximum of 90 per cent infection was obtained on the 14th day after inoculation (Table 14).

Table 12. Retention of infectivity of Aphis craccivora

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

a. First series
b. Second series

Table 13. Minimum number of aphids required for transmission.

No. of aphids per plant	Number of plants infected out of ten		Total number of plants infected	Per cent trans-mission
	Exp. I	Exp. II		
1	2	1	3	15
3	2	2	4	20
5	7	6	13	65
10	9	9	18	90
15	8	7	15	75

Table 14. Incubation period of virus in the host plant

Days after inoculation	Number of plants infected out of 20	Per cent infection
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	1	5
8	1	5
9	3	15
10	7	35
11	11	55
12	11	55
13	16	80
14	18	90
15	18	90
16	18	90
17	18	90
18	18	90
19	18	90
20	18	90

Table 15. Screening of cowpea varieties for resistance to cowpea mosaic virus

Sl. No.	Variety	No.of plants inocula-ted.	No. of plants infected	Percentage of infection
1	New Era	40	38	95.00
2	KBC-1	40	35	87.50
3	Kanakamony	40	36	90.00
4	V-240	40	33	82.50
5	C-152	40	40	100.00
6	V-59	40	39	97.50
7	V-87	40	35	87.50
8	Kozhinjil (local variety)	55	44	80.00
9	CG.104	30	4	13.33
10	V-37	35	27	90.00

V. Host-Range

Host-range studies were conducted with 73 plant species belonging to 17 families. The results showed that the host range of the virus is restricted to the members of the family Leguminosae and Chenopodiaceae. The virus could produce systemic infection on different cowpea varities as well as asparagus bean (Vigna sesquipedalis) and local lesions on Chenopodium amaranticolor.

VI. Varietal Screening

Ten varieties of cowpea were inoculated with cowpea mosaic virus. Symptoms appeared 10-14 days after inoculation on the newly emerged leaves. Some varieties were more susceptible when compared to others. C-152 got 100 per cent infection and V-59 showed 97.5 per cent infection. The variety C.G.104 was found to be least susceptible, which showed only 13.33 per cent infection. Infection in other varieties was between 80 and 95 per cent (Table 15).

VII. Serology

1) Microprecipitin test on slides

Thirty microlitres of antiserum prepared as described under materials and methods, was mixed with equal

Table 16. Microprecipitin test on slides

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

+ positive reaction

- negative reaction

volume of antigens from different virus infected crop plants. It was observed that the antigens of cowpea mosaic virus isolate I, isolate II, isolate III, sword bean mosaic virus and cluster bean mosaic virus produced dense precipitate with the antiserum specific to cowpea mosaic virus. Antigens of cucumber mosaic virus, pumpkin mosaic virus, snake gourd mosaic virus and bitter gourd mosaic virus did not produce any precipitate.

The CpMV antigens were tested against the antisera of cowpea severe mosaic virus, cowpea mosaic virus(USA), cucumber mosaic virus (cowpea isolate), southern bean mosaic virus, cowpea chlorotic mottle virus and cowpea mosaic virus El Salvador. But no precipitate was obtained in these tests.

2) Microprecipitin test in petri dishes

A series of dilution mixtures of virus and antiserum were spotted in petri dish at regular intervals as described under materials and methods. The precipitate was observed after 30 minutes and after 2 h under a stereomicroscope with top light and black background. The intensity of the precipitate was graded. It was found that the antiserum titre was between 1:1024 and 1:2048 and the virus end point was between 1:512 and

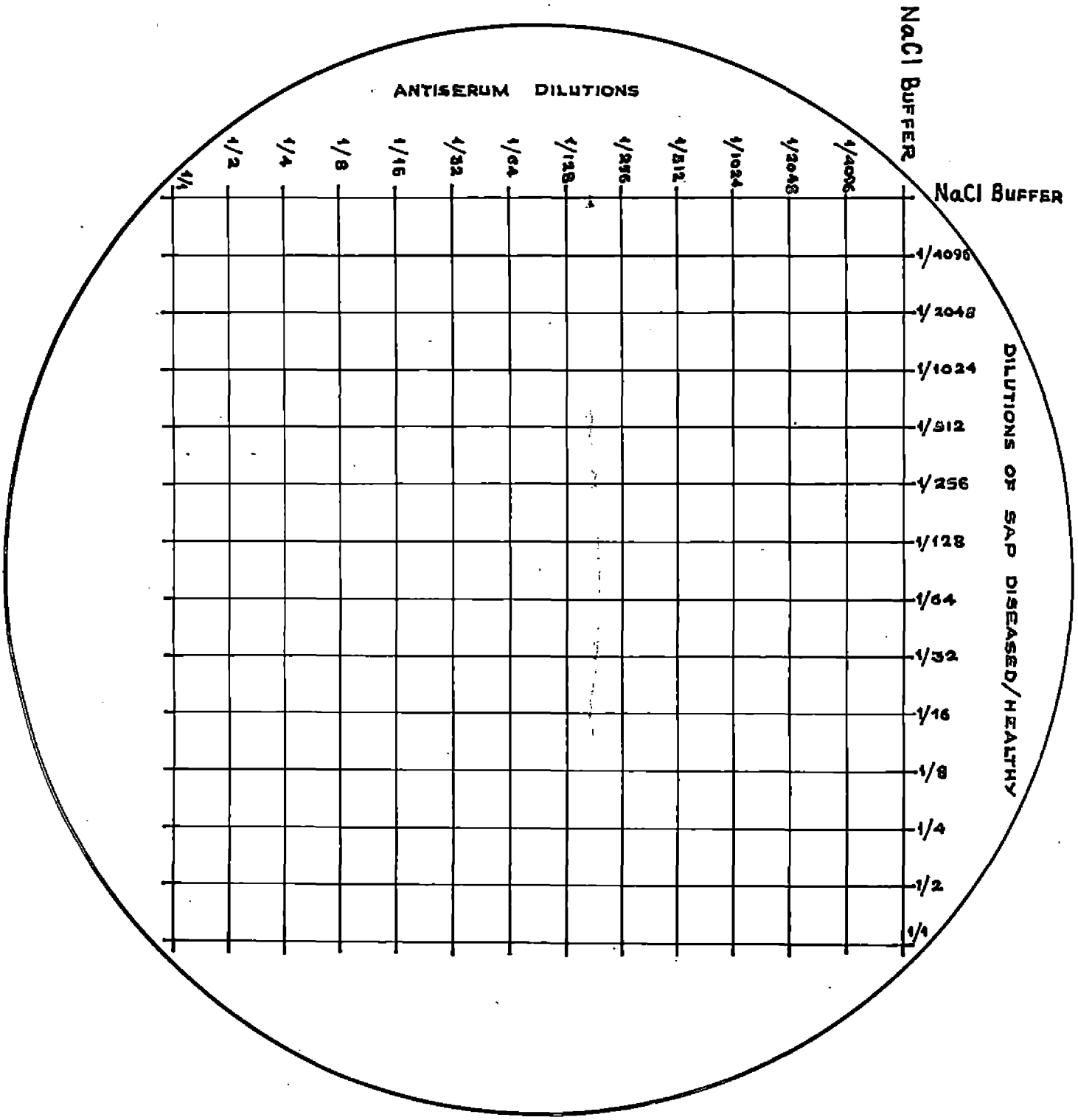

FIG.7 MICROPRECIPITIN TEST IN PETRI DISHES

Table 17. Microprecipitin test of cowpea mosaic virus and its antiserum

Anti serum dilutions	Dilutions of sap containing cowpea 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	++++	++++	++++	+++	+++	++	++	++	+	+	-	-	-
1/4	++++	++++	+++	+++	++	++	++	+	+	+	-	-	-
1/8	+++	+++	+++	++	++	++	++	++	++	+	-	-	-
1/16	++	++	+++	++	+	+	+	+	+	+	-	-	-
1/32	++	+	++	++	+	+	+	+	+	1	-	-	-
1/64	++	+	+	++	+	+	+	1	+	1	-	-	-
1/128	+	+	+	+	1	1	1	1	1	-	-	-	-
1/256	+	1	1	+	1	1	-	-	1	-	-	-	-
1/512	+	1	-	1	-	-	-	-	-	-	-	-	-
1/1024	+	-	-	-	-	-	-	-	-	-	-	-	-
1/2048	-	-	-	-	-	-	-	-	-	-	-	-	-
1/4096	-	-	-	-	-	-	-	-	-	-	-	-	-

The curved line encloses the area of precipitates visible under microscope.

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

Table 18. Microprecipitin test of healthy sap with cowpea mosaic virus antiserum

Antiserum dilutions	Dilutions of healthy sap						
	1/1	1/2	1/4	1/8	1/16	1/32	1/64
1/1	+++	+++	++	++	+	1	-
1/2	++	++	+	+	1	-	-
1/4	+	+	+	1	-	-	-
1/8	+	1	1	-	-	-	-
1/16	+	-	-	-	-	-	-
1/32	-	-	-	-	-	-	-
1/64	-	-	-	-	-	-	-
1/128	-	-	-	-	-	-	-
1/256	-	-	-	-	-	-	-
1/512	-	-	-	-	-	-	-
1/1024	-	-	-	-	-	-	-
1/2048	-	-	-	-	-	-	-
1/4096	-	-	-	-	-	-	-

1:1024 (Table 17). The titre of the antiserum with healthy sap was between 1:16 and 1:32 and the end point of the healthy sap with antiserum was between 1:32 and 1:64 (Table 18).

3) Ouchterlony's agar double-diffusion test

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

No precipitate was obtained in the first plate. Here wells 3 and 5 received distilled water, 4 and 6 buffer and 2 clarified healthy sap. In the second plate a precipitate was obtained between wells 1 and 3 only. Here well 3 received cowpea mosaic virus and well 2 clarified healthy sap. Wells 4,5 and 6 received, cucumber mosaic virus, snake gourd mosaic virus and bitter gourd mosaic virus respectively.

In the third plate precipitates were formed between wells 1 and 2 and 1 and 5. Well 2 contained sword bean mosaic virus and well 5 contained cluster bean mosaic virus. Wells 3, 4 and 6 contained clarified healthy sap, bitter gourd mosaic virus and snake gourd

Fig.8. Well 1 received antiserum of cowpea mosaic virus isolate I, well 2 received cowpea mosaic virus isolate I, 4 pumpkin mosaic virus and 5 cucumber mosaic virus, wells 3 and 6 received CpMV isolate II and CpMV isolate III respectively.

Fig.9. The experiment was done by using purified virus preparation diluted to ¼ concentration. In Well 1 antiserum of cowpea mosaic virus diluted to ¼ concentrati n was taken.

Well 2 contained the antigen of CpMV isolate I diluted to ¼. Well 4 contained CpMV isolate II and well 5 CpMV isolate III each diluted to ¼ concentration. Well 3 contained healthy cowpea plant sap diluted to 1/8 and well 6 contained buffer.

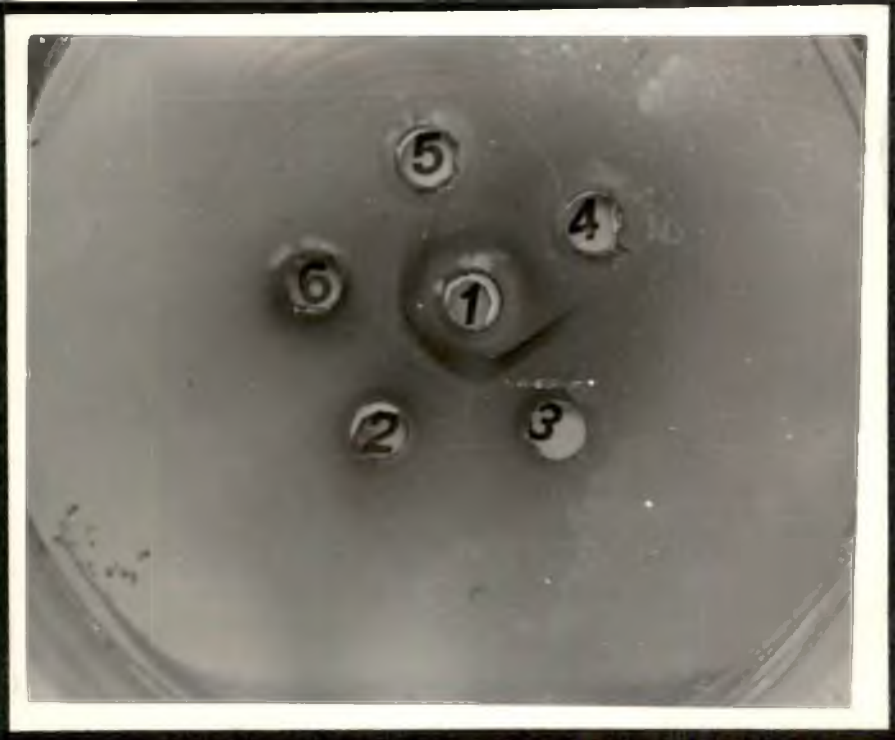

Fig 8. Serologic reactions of cowpea mosaic virus

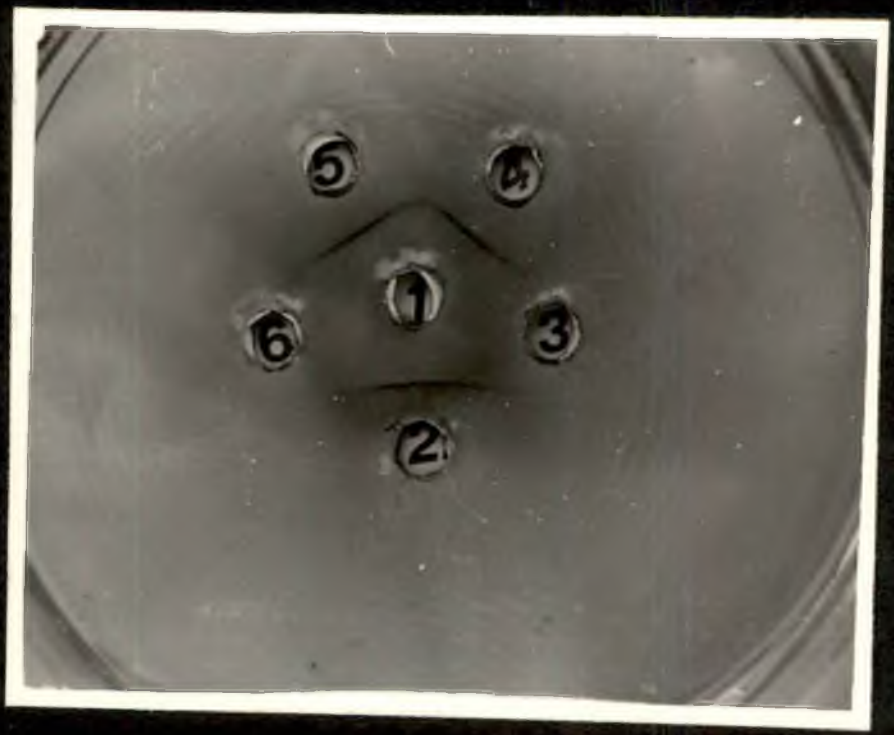

Fig.9. Serologic reactions of cowpea mosaic virus

mosaic virus respectively. In the fourth plate precipitates were formed between wells 1 and 2, 1 and 3 and 1 and 6. Here well 2 contained cowpea mosaic virus isolate I obtained from inoculated plants in the glass house. Wells 3 and 6 contained cowpea mosaic virus isolate II and III obtained from two different places. The fusion of the precipitin lines indicated that they were isolates of the same virus (Fig. 8). In the fifth plate precipitin lines were formed between plates 1 and 2, 1 and 4 and 1 and 5. There was a fusion of the precipitin lines formed between wells 1 and 4 and 1 and 5 (Fig. 9). Here the wells 2, 4 and 5 received 1/4 concentration of antigens of cowpea mosaic virus isolate I, cowpea mosaic virus isolate II and III respectively. In well 3 clarified healthy sap diluted to 1/8 concentration and in well 6 buffer were taken.

4) Reaction of cowpea mosaic virus antigen with different antisera

The antigen of cowpea mosaic virus (isolate I) was taken in the central well (well No.1) of two plates and antisera of viruses obtained from different places were kept in the surrounding wells as described under materials and methods.

A precipitin line was obtained in the first plate between wells 1 and 6. That precipitin line was formed due to the interaction of cowpea mosaic virus isolate I antigen and the antisera prepared specific to that virus. No precipitate was obtained between the cowpea mosaic virus antigen and the other antisera tested.

VIII. Effect of virus infection on growth of cowpea plants.

1) Effect of virus infection on plant height

There was significant reduction in plant height due to virus attack. A maximum of 22.48 per cent reduction in plant height was found in the variety V_9. In variety V_2 a reduction of 21.07 per cent was noticed. The variety V_6 showed least reduction in plant height, viz., 2.06 per cent and in variety V_3 the reduction was 4.13 per cent (Table 19).

2) Effect of virus infection on number of pods

There was a significant reduction in the number of pods in the diseased plants. A maximum reduction of 54.74 per cent was noticed in the variety V_2 and the least reduction of 6.62 per cent was noticed in the variety V_7 (Table 20).

Table 19. Effect of virus infection on plant height

Sl. No.	Variety	Height of plants (cm)		Per cent reduction
		Healthy (X)	Diseased (Y)	
1	V_1	124.95	105.65	15.45
2	V_2	161.4	127.4	21.07
3	V_3	145.4	139.4	4.13
4	V_4	168.3	142.1	15.57
5	V_5	180.3	166.8	7.49
6	V_6	162.3	158.95	2.06
7	V_7	152.95	136.25	10.92
8	V_8	162.5	148.1	8.86
9	V_9	102	79.15	22.48
10	V_{10}	176.2	167.55	4.91

Mean X = 153.63

Mean Y = 137.135

't' value = 5.509406

Table value of $t_{.05}$ = 2.262

Table 20. Effect of virus infection on number of pods produced by the plant

Sl. No.	Variety	Average number of pods produced		Per cent reduction
		Healthy (X)	Diseased (Y)	
1.	V_1	13.7	8.6	37.23
2.	V_2	13.7	6.2	54.74
3.	V_3	16.9	14.6	13.61
4.	V_4	18.7	11.1	40.64
5.	V_5	18.0	11.9	33.89
6.	V_6	16.5	14.3	13.33
7.	V_7	15.1	14.1	6.62
8.	V_8	13.8	12.4	10.14
9.	V_9	20.8	15.2	26.92
10.	V_{10}	14.3	10.7	25.17

Mean X = 16.15

Mean Y = 11.91

't' value = 5.430506

Table value of $t_{.05}$ = 2.262

Table 21. Effect of virus infection on pod length

Sl. No.	Variety	Pod length in Cm		Per cent reduction
		Healthy (X)	Diseased (Y)	
1	V_1	15.3	11.9	22.22
2	V_2	15.85	12.2	23.03
3	V_3	15.2	14.9	1.97
4	V_4	16.95	12.75	24.78
5	V_5	14.3	12.6	11.89
6	V_6	14.6	12.6	13.7
7	V_7	16.8	13.25	21.13
8	V_8	15.35	13.7	10.75
9	V_9	11.85	9.05	23.63
10	V_{10}	17.35	14.2	18.16

Mean X = 15.355

Mean Y = 12.715

't' value = 6.974796

Table value of $t_{.05}$ = 2.262

Table 22. Effect of virus infection on leaf area of cowpea variety C-152.

Sl. No.	Leaf area (sq. cm.)	
	Healthy (X)	Diseased (Y)
1.	58.34	40.2
2.	53.09	41.58
3.	45.5	38.33
4.	46.44	52.13
5.	43.11	43.19
6.	53.91	35.23
7.	48.82	36.27
8.	62.72	36.27
9.	38.66	51.6
10.	37.15	43.63
Mean	48.774	41.751

't' value = 1.785157

Table value of 't' = 2.262

Fig 10. Effect of cowpea mosaic virus infection on plant height.

Treatments

1) V_1 uninoculated
2) V_1 inoculated
3) V_2 uninoculated
4) V_2 inoculated
5) V_3 Uninoculated
6) V_3 inoculated
7) V_4 Uninoculated
8) V_4 inoculated
9) V_5 Uninoculated
10) V_5 inoculated
11) V_6 Uninoculated
12) V_6 inoculated
13) V_7 Uninoculated
14) V_7 inoculated
15) V_8 Uninoculated
16) V_8 inoculated
17) V_9 Unioculated
18) V_9 inoculated
19) V_{10} Uninoculated
20) V_{10} inoculated

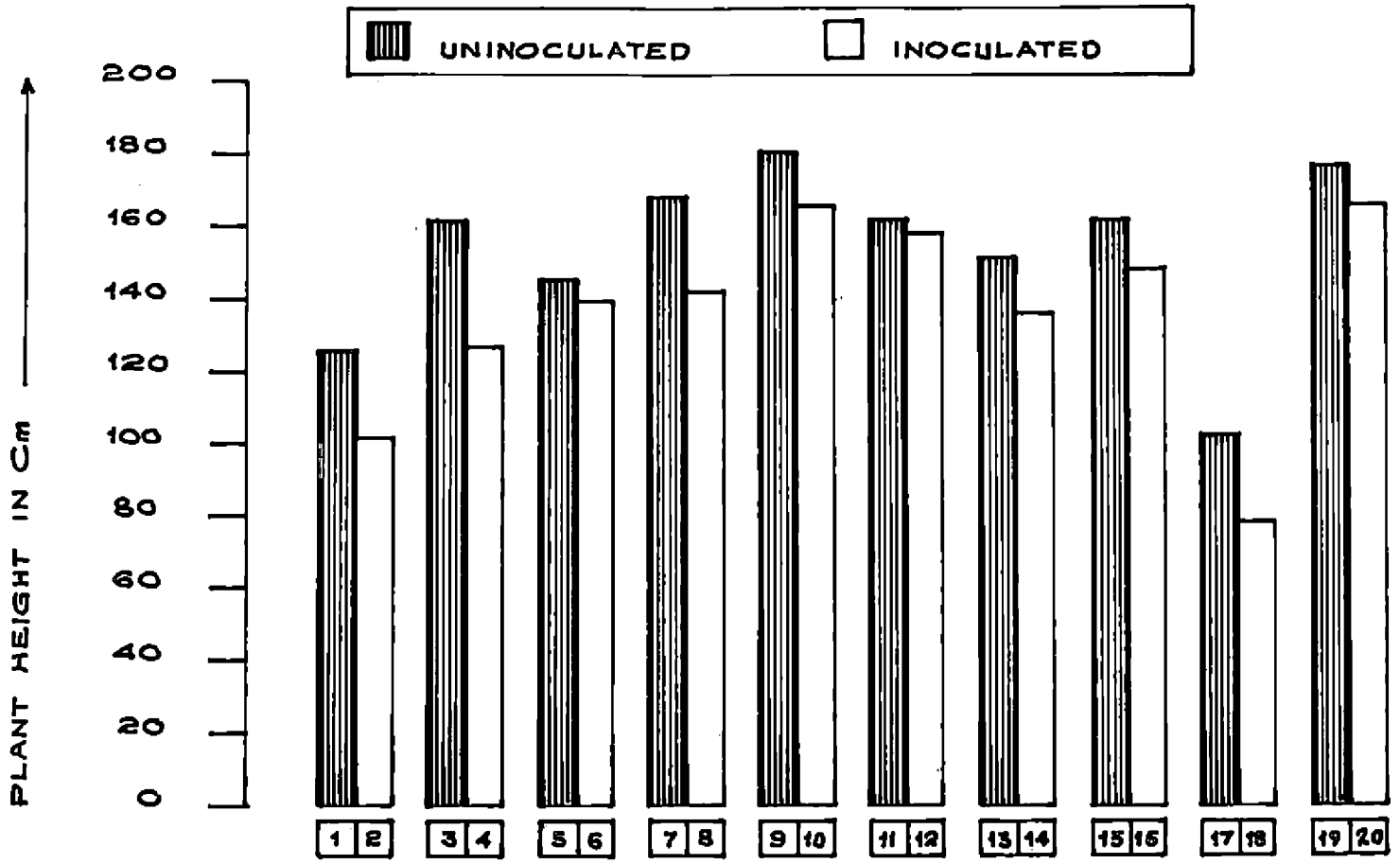

FIG:-10. EFFECT OF COWPEA MOSAIC VIRUS INFECTION ON PLANT HEIGHT

Fig. 11. Effect of cowpea mosaic virus infection on pod length.

Treatments

1) V_1 Uninoculated
2) V_1 inoculated
3) V_2 Uninoculated
4) V_2 inoculated
5) V_3 Uninoculated
6) V_3 inoculated
7) V_4 Uninoculated
8) V_4 inoculated
9) V_5 Uninoculated
10) V_5 inoculated
11) V_6 Uninoculated
12) V_6 inoculated
13) V_7 Uninoculated
14) V_7 inoculated
15) V_8 Uninoculated
16) V_8 inoculated
17) V_9 Uninoculated
18) V_9 inoculated
19) V_{10} Uninoculated
20) V_{10} inoculated

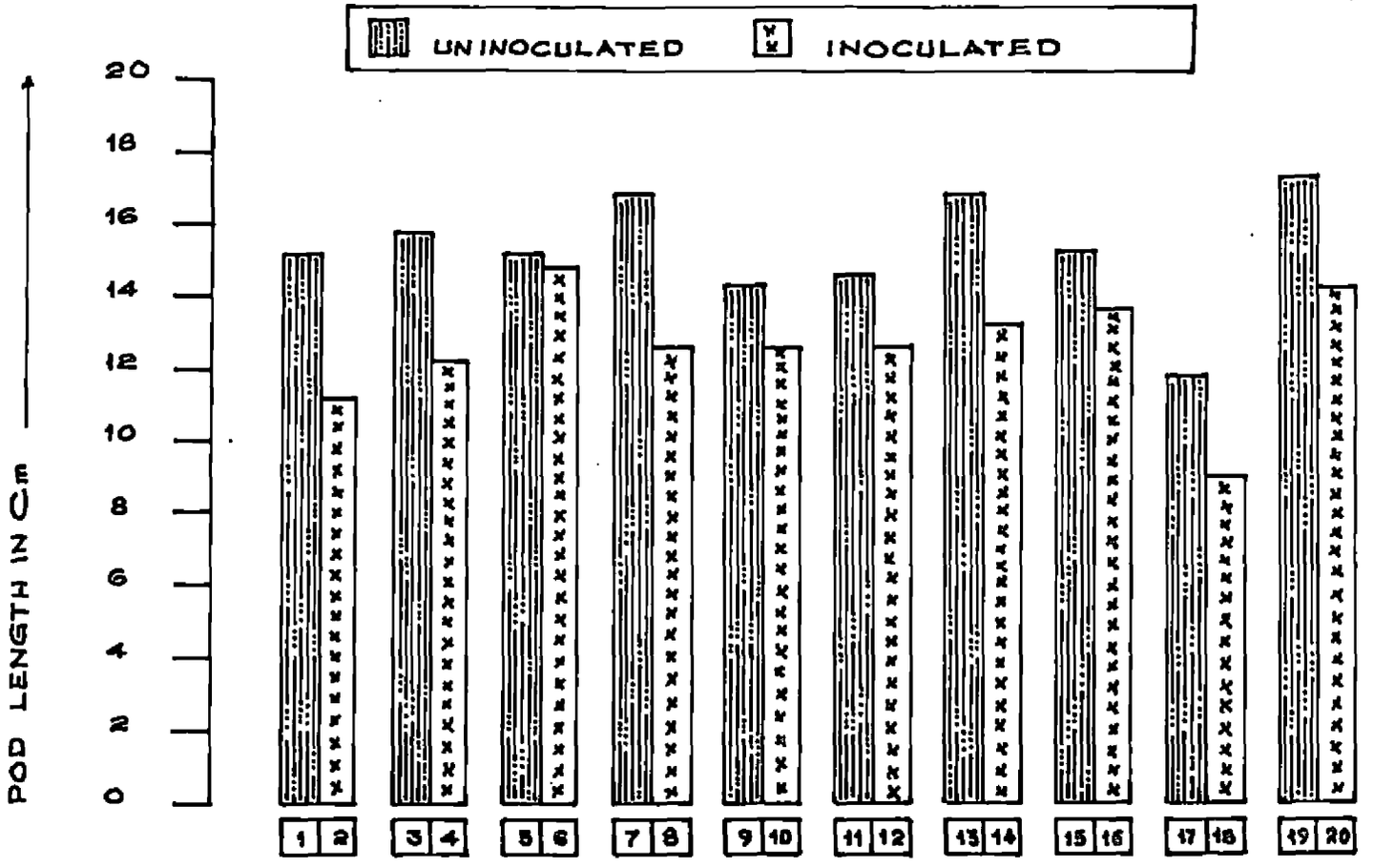

FIG-11 EFFECT OF COWPEA MOSAIC VIRUS INFECTION ON POD LENGTH

Fig.12. Effect of cowpea mosaic virus infection on number of pods.

Treatments

1) V_1 Uninoculated
2) V_1 inoculated
3) V_2 Uninoculated
4) V_2 inoculated
5) V_3 Uninoculated
6) V_3 inoculated
7) V_4 Uninoculated
8) V_4 inoculated
9) V_5 Uninoculated
10) V_5 inoculated
11) V_6 Uninoculated
12) V_6 inoculated
13) V_7 Uninoculated
14) V_7 inoculated
15) V_8 Uninoculated
16) V_8 inoculated
17) V_9 Uninoculated
18) V_9 inoculated
19) V_{10} Uninoculated
20) V_{10} inoculated

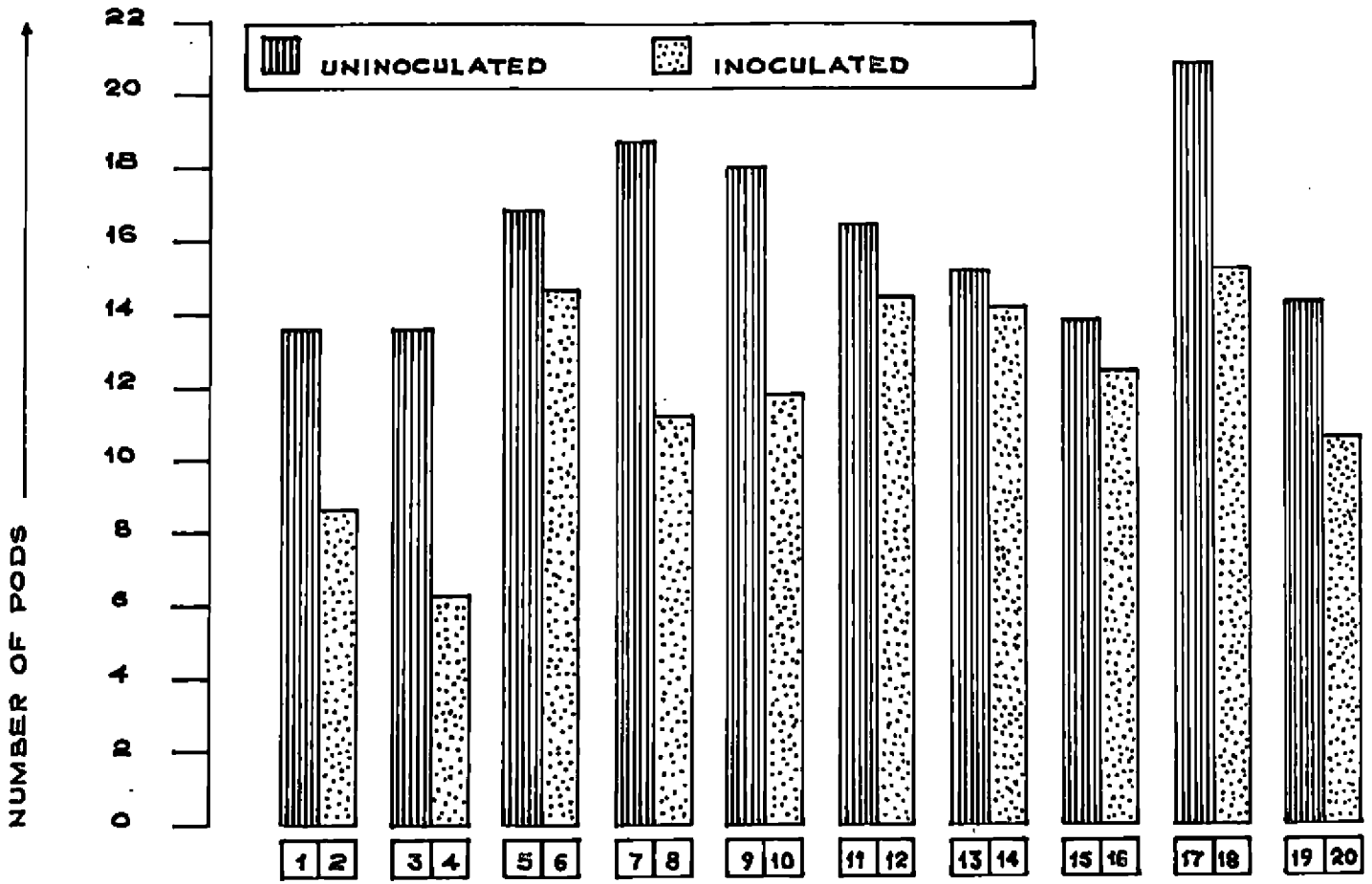

FIG-12. EFFECT OF COWPEA MOBAIC VIRUS INFECTION ON NUMBER OF PODS

3) Effect of virus infection on length of pods

There was a significant reduction in length of pods also, in the case of diseased plants. The highest reduction of 24.78 per cent was noticed in variety V_4; followed by V_9 and V_2, viz., 23.63 per cent and 23.03 per cent, respectively. The minimum reduction of 1.97 per cent has been observed in V_3 (Table 21).

4) Effect of virus infection on leaf area

The effect of virus infection on the variety C-152 which is 100 per cent susceptible to the virus has been studied. It is found that the leaf area is not significantly reduced in inoculated plants (Table 22).

IX. Observations on natural incidence of cowpea mosaic virus.

Out of a total of 3807 plants 142 plants were found diseased. Among them 81 plants were infected with cowpea mosaic virus, 41 with cowpea yellow mosaic virus, 14 with cowpea chlorotic mottle and 6 with southern bean mosaic (Table 23).

Table 23. Observations on natural incidence of cowpea mosaic virus

Plot No.	Total Number of plants	Diseased	Cowpea mosaic	Southern bean mosaic	Cowpea chlorotic mottle	Cowpea yellow mosaic	Healthy
1	460	72	26	0	5	41	368
2	157	2	2	0	0	0	155
3	1015	46	41	0	5	0	969
4	1275	13	7	6	0	0	1262
5	900	9	5	0	4	0	891
Total	3807	142	81	6	14	41	3665
Percentage		3.73	2.13	0.157	0.367	1.08	86.27

Table 24. Effect of leaf extract sprays on development of cowpea mosaic

Sl. No.	Treatments	No. of plants inoculated.	No.of plants infected	Per cent control
1.	Two pre-inoculation sprayings of Bougainvillea leaf extract.	20	0	100
2.	Four "	20	0	100
3.	Six "	20	0	100
4.	Two pre-inoculation sprayings of Eupatorium leaf extract.	17	4	76.47
5.	Four "	19	0	100
6.	Six "	20	0	100
7.	Four post-inoculation sprayings of Bougainvillea leaf extract.	22	6	72.72
8.	Six "	22	3	86.36
9.	Four post-inoculation sprayings of Eupatorium leaf extract.	20	10	50
10.	Six "	20	10	50
11.	Control (without any spraying)	17	10	47.17

X. Effect of leaf extract sprays on development of cowpea mosaic.

A pot culture experiment was conducted to find out the effect of leaf extract sprays of Bougainvillea sp. and Eupatorium odoratum on the development of cowpea mosaic virus.

Cowpea plants in the treatments receiving 2, 4 and 6 pre-inoculation sprays of Bougainvillea leaf extract and 4 and 6 pre-inoculation sprays of Eupatorium leaf extract did not show any symptom of the disease. Two pre-inoculation sprayings of Eupatorium leaf extract gave 76.47 per cent control of the disease. With four and six post-inoculation sprays of Bougainvillea leaf extract 72.72 per cent and 86.36 per cent control of the disease, respectively, was obtained. When Eupatorium leaf extract sprays were given four and six times as post-inoculation sprays 50 per cent control was achieved. In the control plants there was no symptoms in 47.17 per cent of the plants (Table 24).

DISCUSSION

DISCUSSION

Cowpea mosaic virus disease is found throughout Kerala, causing severe damage to the crop. The main symptoms of the disease are vein banding, interveinal chlorosis, mosaic mottling and general stunting of the plants. A seed-borne mosaic of asparagus bean (Vigna sesquipedalis) having the above type of symptoms was reported by Snyder(1942). The symptoms of cowpea aphid-borne mosaic virus described by Bock and Conti(1974) also resemble to those of the cowpea mosaic virus disease found in Kerala. While discussing the host-range of cowpea aphid-borne mosaic virus they suggested that the viruses that may be related to cowpea aphid-borne mosaic virus (CAMV) could produce mosaic disease of adzuki bean (Phaseolus angularis) and asparagus bean (Vigna sesquipedalis).

The virus of the present studies produced local lesions on Chenopodium amaranticolor. Production of local lesions on Chenopodium amaranticolor by cowpea mosaic virus was reported by many other workers also (Harrison and Guduaskas, 1968; Govindaswamy et al., 1970; Khatri and Singh, 1974; Mali and Kulthe, 1980). The lesions first appeared as chlorotic spots which later turned necrotic.

This type of development of local lesions was reported by Bock and Conti(1974) also in the case of cowpea aphid-borne mosaic virus.

The virus was transmitted easily by mechanical inoculation. Mechanical transmission of cowpea mosaic virus using different preparations of inoculum has been reported earlier by many workers. Phenol-water extracts of diseased leaves in the case of several viruses (Schlegel, 1960), sap of diseased leaves in cowpea mosaic virus affecting Phaseolus lathyroides (Alconero and Santiago, 1972), 0.05M phosphate buffer in cowpea banding mosaic virus (Sharma and Varma, 1976), cooled tris buffer in seed-borne potyvirus causing mosaic of cowpea (Mali and Kulthe, 1980), and sap extracted in distilled water and diluted in the ratio 1:5 in cowpea aphid-borne mosaic virus (Patel and Kuwaite, 1982; Patel, 1982) were the preparations used earlier for mechanical inoculation. In the present studies distilled water, phosphate buffer and tris buffer under room temperature and in cooled condition were used as extraction media. Maximum percentage of transmission was obtained with cooled tris buffer followed by cooled distilled water. This is in confirmity with the results of Mali and Kulthe (1980).

In the experiment to study seed transmission, 318 seeds were sown, out of which 181 seeds have germinated. Among them, 10 seedlings showed symptoms of cowpea mosaic disease. Thus 5.5 per cent seed transmission was obtained. Seed-borne nature of cowpea mosaic viruses has been reported from different parts of the world. The extent of seed transmissions of cowpea mosaic virus in three susceptible varieties of cowpea, viz., Red Ripper, Black and Iron have been recorded as 5, 4.5 and 6.8 per cent, respectively, by McLean(1941). Kaiser and Mossahehi(1975) while conducting studies on cowpea aphid-borne mosaic virus disease in Iran observed 1.1 to 39.87 per cent seed transmission for the virus. In the case of an Indian isolate of cowpea aphid-borne mosaic virus, Phatak(1974) found 3 to 19 per cent transmission. The results of the present study are in agreement with this finding.

The virus could be transmitted through grafting, even-though there was no graft union in the horticultural sense. The extent of transmission obtained through grafting was as low as 35 per cent. This may be because of the hollow nature of the stem which made the graft union difficult. The reports on attempts of graft transmissions are scanty probably due to this reason. In the present study successful graft transmission was observed when grafting was done at the nodal region.

Studies on the insect transmission of cowpea mosaic virus was conducted using Aphis craccivora Koch., Aphis gossypii Glov. and Aphis malvae Koch. as vectors. Among these aphids, Aphis craccivora was found to transmit cowpea mosaic virus in an efficient manner giving up to 90 per cent transmission. Aphis gossypii and Aphis malvae gave 65 and 45 per cent transmissions respectively. Abeygunawardena and Perera(1964) observed Aphis craccivora as the principal vector of cowpea mosaic virus in Ceylon. Transmission of cowpea mosaic virus by Aphis gossypii and by Aphis craccivora has been reported from different parts of India by Capoor and Varma(1956), Nariani and Kandaswami(1961), Chenulu et al.(1968), Govindaswamy et al. (1970), Nene and Shankar(1972), Khatri and Singh(1974), Sharma and Varma(1976), Mali and Kulthe(1980) and Ramachandran and Summanwar(1982). A perusal of literature revealed that there is no earlier report on the transmission of cowpea mosaic virus by A. malvae. But in the present trials this was also included because this aphid was also found infesting the cowpea plants in Kerala along with A. craccivora and A. gossypii.

Anderson(1959) while studying the Vigna and Crotalaria viruses in Florida reported that there are separate aphid-borne and beetle-borne cowpea viruses. The results of the

present trials showed that the cowpea mosaic virus under study is also an aphid-borne mosaic virus. Cowpea mosaic virus was reported to be transmitted by the beetles Ceratoma ruficornis (Kvicala et al.(1970) and Ceratoma arcuata (Ajos and Lin, 1984). But they were not included in the present transmission trials, since infestation of these beetles on cowpea plants is not seen in Kerala.

The studies on physical properties, viz., dilution end point (DEP), thermal inactivation point(TIP) and longevity in vitro(LIV) revealed that the DEP of the virus was between 10^{-1} and 10^{-2}, TIP between 50 and 55°C and LIV 8 h at room temperature and 24 h at 8°C. Reports on the physical properties of cowpea mosaic viruses have been made from different parts of the world. The dilution end point of cowpea mosaic virus ranges between 10^{-3} to 10^{-4} as reported by Snyder(1942), Abeygunawardena and Perera(1964), Govindaswamy et al.(1970), Sharma and Varma(1976) and Guo et al.(1984). But Chenulu et al.(1968) reported a dilution end point of 1:500 - 1:1000 for a cowpea mosaic virus from Delhi. The differences between the DEP of the other Indian isolates of cowpea mosaic virus and the virus under study may probably be due to the difference in the host varieties and also to the difference in the environmental factors which might have affected the concentration of virus in the host.

The thermal inactivation point of cowpea mosaic virus as reported by several others is between 50 and 60°C (Snyder, 1942; Harjono, 1959; Adsuar, 1964; Abeygunawardena and Perera, 1964; Chenulu et al., 1968; Govindaswamy et al., 1970; Kaiser and Mossahebi,1975; Sharma and Varma, 1976; Guo et al., 1984). The TIP of the present virus is also in agreement with the above reports.

The LIV of cowpea mosaic virus at 25-30°C is reported to be ranging between 1-2 days (Snyder, 1942; Harjono,1959; Adsuar, 1964; Abeygunawardena and Perera, 1964; Govindaswamy et al., 1970; Sharma and Varma, 1976; Guo et al.,1984). But in the present studies the LIV of cowpea mosaic virus at room temperature was found to be 8 h and at 8°C it was found to be 24 h. The results of the present studies are not in agreement with those of the above workers but is very similar to the LIV reported by Chenulu et al.(1968), viz., 6 h of LIV at 25-30°C for a cowpea mosaic virus from Delhi.

Aphis craccivora was found to be the most efficient vector of the virus under study and hence the vector-virus relationship was worked out with that aphid only. Minimum and optimum acquisition feeding period, minimum inoculation feeding period, influence of fasting before and after

acquisition feeding on efficiency of transmission, retention of infectivity by the vector, minimum number of aphids required for transmission and the incubation period of virus in the host were investigated.

Minimum acquisition feeding period was found to be 30 s for Aphis craccivora for the transmission of cowpea mosaic virus. As the acquisition feeding period was increased there was an increase in per cent transmission also. The maximum transmission of 70 per cent was obtained when an acquisition feeding period of 10 min was given. Sharma and Varma (1977) reported an optimum acquisition feeding of 5 min in the case of transmission of cowpea banding mosaic virus by Aphis craccivora. In the present studies when acquisition feeding period was increased beyond 10 min there was a steady decline in the per cent transmission and it was only 5 per cent when acquisition feeding period was 4 h, and no transmission obtained after an acquisition feeding of 24 h. Similar observations have been made earlier by Murugesan and Janaki(1972) who were working on the vector-virus relationship of cowpea mosaic virus with the vector Myzus persicae. They found that one h acquisition feeding by M. persicae resulted in maximum percentage of transmission of the virus even-though the vector was able to acquire the virus withmone

second. There was a reduction in percentage of transmission when acquisition feeding period was increased beyond 1 h.

This phenomenon of decrease in the percentage of transmission with the increase in the acquisition feeding period was explained earlier by Watson and Roberts(1939). They postulated that an aphid feeding product formed during the feeding of the vector may reduce the efficiency of transmission. Another possibility suggested by them was that the tissues probed during short periods contain higher concentration of virus than those probed for a long period. They have explained that the formation of salivary sheath during prolonged feeding prevented the aphids from becoming infective. Yet another explanation given by them was that constant probing may cause the loss of infectivity of aphids.

The minimum inoculation feeding period required for Aphis craccivora to transmit cowpea mosaic virus was 30 s. The per cent transmission was found to increase with an increase in inoculation feeding period and maximum transmission was obtained with 15 min inoculation feeding period. Murugesan and Janaki (1972) reported 1 h as the optimum inoculation feeding period in the case of cowpea

mosaic virus by the vector Myzus persicae. But the vector was found to transmit the virus to healthy plants within one second. In the case of cowpea banding mosaic virus, Sharma and Varma (1977) reported that the minimum inoculation feeding period was 25 s, and the inoculation feeding period necessary to get maximum infection was 30 min. The inoculation feeding period of cowpea banding mosaic virus seems to be similar to that of cowpea mosaic virus of present studies.

Investigations on the incidence of starvation before acquisition and inoculation feedings showed that pre-acquisition fasting of the aphids resulted in an increase and post-acquisition fasting caused a steady decrease in the per cent transmissions. Even without pre-acquisition fasting the aphids were found to transmit the virus, but the percentage of transmission was very low. Up to 2 h of pre-acquisition fasting, increase in per cent transmission was obtained. This has been explained earlier by Murugesan and Janaki(1972) in the case of cowpea mosaic virus transmitted by Myzus persicae. The explanation given was based on inhibitor activity. The production of inhibitor by the vector is slow in fasted aphids. There was considerable reduction in per cent transmission when pre-acquisition fasting was increased beyond 2 h. This has also been reported by

Murugesan and Janaki(1972). The long pre-acquisition fasting might have affected the feeding behaviour of the insect and reduced the transmission efficiency. The post-acquisition fasting caused a considerable reduction in transmission efficiency. Maximum infection was obtained when no post-acquisition fasting was given. Murugesan and Janaki(1972) found that in the case of cowpea mosaic virus transmitted by Myzus persicae the percentage of infection obtained progressively decreased with increase in post-acquisition fasting. In the present trials the vector was found to lose the infectivity within 2 h of post-acquisition fasting. This finding is in agreement with that of Murugesan and Janaki(1972).

Experiments on retention of infectivity by A. craccivora revealed that the vector lost its infectivity within 1 to 2 h after acquisition, while feeding. Murugesan and Janaki(1972) reported that Myzus persicae lost cowpea mosaic virus within 4 h after acquisition and they have explained that the transmission of the virus by the vector was in a non-persistent manner. Since the infectivity was lost in the present studies within 2 h the transmission of cowpea mosaic virus by Aphis craccivora can also be termed as in a non-persistent manner as suggested by other workers (Abeygunawardena and Perera, 1964; Murugesan and Janaki,1972;

Bock and Conti, 1974; Sharma and Varma, 1977; Mali and Kulthe, 1980; Ramachandran and Summanwar, 1982; Guo et al. 1984).

Results of the experiments to find out the minimum number of aphids required to transmit cowpea mosaic virus and cause infection showed that a single viruliferous aphid was sufficient for successful transmission and infection. But there was an increase in the percentage of infection when the number of aphids was increased to 10. Similar results were obtained by earlier workers also who studied the vector virus relationship of non-persistent cowpea mosaic viruses. Haque and Chenulu (1972b)in the case of Aphis craccivora and Murugesan and Janaki(1972) in the case of Myzus persicae reported that even a single aphid could transmit the virus, but the percentage of transmission was maximum when the number of aphids was increased to 15.

The symptoms of cowpea mosaic virus disease appeared in the plants 14 days after inoculation and hence the incubation period of virus in the host plant is up to 14 days. This finding is in agreement with that of Collins et al. (1985) who also reported that the incubation period of cowpea mosaic virus in cowpea plants was up to 14 days.

In the host-range studies 73 plant species belonging to 17 families were tested and it was found that the virus produced systemic symptoms on different cowpea varieties as well as asparagus bean and local lesions on Chenopodium amaranticolor. Snyder (1942) while working with cowpea aphid-borne mosaic virus causing mosaic of asparagus bean observed that the virus could produce systemic symptoms on asparagus bean as well as on different varieties of cowpea. They did not find any other legumes infected with the virus. Cowpea mild mottle virus has been reported to infect groundnut, sugarbeet, redgram, soy bean and cocoa (VanKammen, 1971). Govindaswamy et al.(1970) found that the cowpea aphid-borne mosaic virus affecting cowpeas in Tamil Nadu produced systemic symptoms on three members of the family Leguminosae, viz., Canavalia ensiformis, Cyamopsis tetragonaloba and Phaseolus vulgaris and produced local lesions on Vicia faba, Chenopodium amaranticolor and Chenopodium album.

Mali and Kulthe(1980) studied a seed-borne potyvirus causing mosaic of cowpea in India and reported 42 host species belonging to Leguminosae, Amaranthceae and Chenopodiaceae. Lin et al.(1981) conducted trials on the host-range of black eye cowpea mosaic virus and the

cowpea isolate of cucumber mosaic virus. The black eye cowpea mosaic virus as per their observations infected three species of Amaranthaceae and three of Leguminosae as well. The cowpea isolate of cucumber mosaic virus infected four species of Leguminosae, three species of Solanaceae, two species of Amaranthaceae and one belonging to Cucurbitaceae. Sanchez and Gonsalez(1981) found that the yellow strain of cowpea mosaic virus produced local lesions and apical necrosis in Phaseolus vulgaris and Stizolobium deeringianum and severe strain of cowpea mosaic virus produced systemic mosaic on the two plants mentioned above.

A comparison of host-range of different viruses infecting cowpea revealed that the present virus shows a close similarity in its host-range to the cowpea aphid-borne mosaic virus described by Snyder(1942). It differs in host-range from black eye cowpea mosaic virus, cowpea isolate of cucumber mosaic virus, yellow and severe strains of cowpea mosaic virus and cowpea mild mottle virus.

In the varietal trial carried out with ten varieties of cowpea, it was found that all the varieties were susceptible to the virus to varying degrees. In all the varieties the symptoms appeared within 8-14 days after inoculation. Eventhough all the varieties were susceptible

to the virus there was some variation in the percentage of infection in the inoculated plants. The most susceptible varieties were C-152, V-59 and New Era which showed 100 per cent, 97.5 per cent and 95 per cent infections respectively. The variety C.G.104 showed some resistance with an infection of only 13.33 per cent. In the present study no variety was found immune to the infection of cowpea mosaic virus. Govindaswamy et al.(1970) screened 112 varieties of cowpea for their resistance to cowpea mosaic virus and found 109 varieties as suceptible and three varieties tolerant to virus infection. They also could not find any variety immune to virus infection. Mali et al.(1981) reported that C-288 is the only variety found immune to cowpea aphid-borne mosaic virus, out of the 23 cowpea varieties tested. In the present studies this variety was not included due to non-availability of seeds. Patel et al.(1982) screened 249 cowpea cultivars, by sap inoculation with vein banding strain of cowpea mosaic virus and found only ten lines showing immunity to virus infection. In the present studies, only the variety C.G.104 showed some extent of resistance.

Serological studies were conducted with a view to identify the virus. The results of the microprecipitin test showed that antigens of cowpea mosaic virus isolate I

(obtained from inoculated plants in the glass house), cowpea mosaic virus isolate II and isolate III (obtained from two locations), cluster bean mosaic virus and sword bean mosaic virus gave dense precipitates with antiserum specific to cowpea mosaic virus. This indicates the serological relationship of cowpea mosaic virus to cluster bean mosaic virus, sword bean mosaic virus and the cowpea mosaic virus obtained from different locations in Trivandrum. No serological relationship was obtained between cowpea mosaic virus and pumpkin mosaic virus, bittergourd mosaic virus, cucumber mosaic virus or snakegourd mosaic virus. The cowpea mosaic virus antigen did not show serological relationship with any of the antisera of the other viruses, viz., cowpea severe mosaic virus, cowpea mosaic virus El Salvador, cucumber mosaic virus (cowpea isolate), cowpea mosaic virus (USA), cowpea chlorotic mottle virus and southern bean mosaic virus.

Microprecipitin test in petri dishes was conducted to find out the antiserum titre, virus end point, titre of the antiserum with healthy sap and end point of healthy sap with antiserum. Different antiserum titres and virus end points have been reported from different places for cowpea mosaic virus. A virus end point of 1:128, for a seed borne virus on cowpea was reported from California (Shepherd and

Fulton, 1962). From India, an antiserum titre of 1:512 was reported for a cowpea mosaic virus by Chenulu et al. (1968). But Mali and Kulthe(1980) while working with a seed borne potyvirus causing mosaic of cowpea found an antiserum titre of 1:1024. In the present studies the titre of the antiserum was found to lie between 1:1024 and 1:2048 and virus end point between 1:512 and 1:1024 . The titre of the antiserum with healthy sap was between 1:16 and 1:32 and the end point of healthy sap with antiserum was between 1:32 and 1:64. The antiserum titre depends on the concentration of virus in the leaf tissue, method of purification adopted and whether the virus is weekly or strongly immunogenic. The cowpea mosaic virus reported by Mali and Kulthe(1980) also had the same antiserum titre as that of the present virus, but the virus end point of that virus was 1:64.

The present virus differs from the above virus in symptomatology, physical properties, host-range and serological relationships with other viruses. But Mali(1983) reported that one isolate of the seed-borne potyvirus causing mosaic of cowpea in Marathwada, described by Mali and Kulthe(1980), was found serologically related to cowpea aphid borne mosaic virus(CAMV). The present virus resembles the CAMV in its symptomatology,

modes of transmission, properties and host-range. The serological relationship of the present virus with CAMV has not been tried, since the antiserum to CAMV could not be obtained. But it is proved that the virus under study is serologically not related to most of the other important cowpea viruses like cowpea severe mosaic virus, cowpea mosaic virus (USA), cowpea chlorotic mottle virus, southern bean mosaic virus, cucumber mosaic virus (cowpea isolate) and cowpea mosaic virus El Salvador and also that the other properties of the present virus are similar to those reported for cowpea aphid-borne mosaic virus. Hence the results of the present investigations indicate that the virus under study may probably be an isolate of the cowpea aphid-borne mosaic virus.

The results of the Ouchterlony's agar double diffusion test have confirmed the findings of the micro-precipitin test on slides. No precipitate was obtained in the first plate in which the wells 3 and 5 contained distilled water, 4 and 6 buffer and 2 clarified healthy sap. In the second plate a precipitin line was formed between wells 1 and 3 only. Well 3 received cowpea mosaic virus and the precipitin line was formed due to the interaction of cowpea mosaic virus antigen with its

antiserum. In the third plate, precipitin lines were formed between wells 1 and 2 and 1 and 5. In well 2, sword bean mosaic virus and in well 5 cluster bean mosaic virus were taken. The formation of precipitin line indicated that these two viruses are serologically related to cowpea mosaic virus under study. The absence of precipitin line between wells 1 and 4 and 1 and 6 showed that cowpea mosaic virus is not serologically related to bitter gourd mosaic virus and snake gourd mosaic virus, respectively. In the fourth plate, precipitin lines were formed between wells 1 and 2, 1 and 3 and 1 and 6. Here well 2 contained cowpea mosaic virus obtained from inoculated plants in the glass house, well 3 and 6 contained two cowpea mosaic viruses obtained from two different locations. The fusion of the ends of the precipitin lines indicate that they are serologically related (Noordam,1973). Absence of precipitin lines between central well and wells 4 and 5 indicate that pumpkin mosaic virus and cucumber mosaic virus are serologically unrelated to cowpea mosaic virus. The fifth plate showed serological reactions between antiserum of cowpea mosaic virus and the diluted antigens of cowpea mosaic virus obtained from two different locations.

Ouchterlony agar double diffusion test was also conducted to find out the relationship of cowpea mosaic virus antigen with antisera of cowpea viruses obtained from different places. The results of this test also confirmed the results of microprecipitin test on slides, ie., there is no serological relationship between cowpea mosaic virus under study and the other viruses, viz., cowpea mosaic virus El Salvalor, cowpea mosaic virus(USA), cucumber mosaic virus (cowpea isolate), cowpea chlorotic mottle virus, southern bean mosaic virus, cowpea severe mosaic virus, cucumber mosaic virus and pumpkin mosaic virus. Bock and Conti(1974) reported that although cowpea aphid-borne mosaic virus belongs to potyvirus group, no serological relationship was observed between cowpea aphid-borne mosaic virus, and other potyviruses, viz., potatoe virus Y, bean yellow mosaic virus (BYMV), pea seed-borne mosaic virus, clover yellow vein mosaic virus, soy bean mosaic virus, sugarcane mosaic virus, tobacco severe etch virus and iris mosaic virus. Mali et al. (1985) reported a sword bean distortion mosaic virus from Marathwada which was unrelated to CAMV, but serologically identical with bean yellow mosaic virus (BYMV). The CAMV of Bock and Conti(1974) was serologically unrelated to BYMV. In the present host-range studies cluster bean and sword bean were not found to be the

hosts of cowpea mosaic virus. But serological relationship was observed for the virus with the sword bean mosaic virus and cluster bean mosaic virus. Detailed studies have to be conducted for the identification of sword bean mosaic and cluster bean mosaic diseases found in Kerala and their relationship to cowpea mosaic virus.

In an experiment to find out the effect of cowpea mosaic virus on growth of cowpea plants, ten varieties and two treatments, viz., uninoculated and inoculated were there. Observations on leaf area, height of the plant, number of pods produced and length of pod were taken. In general there was significant reduction in height of the plants, number of pods produced and length of pods of all the varieties tested. Several scientists reported earlier, that cowpea mosaic virus infection caused significant reduction in growth and yield of cowpeas (Khatri and Chenulu, 1970; Gilmer et al., 1975; Fegla et al., 1981; Vaverde et al., 1982; Graham, 1985). The variety V_1(C-152) was found to be the most susceptible variety in the varietal trial followed by V_6(V-59). The least susceptible variety was found to be V_3(CG.104). The maximum reduction of 22.48 per cent plant height was observed in the variety V_9 (V-59) followed by 21.07 per cent

reduction in the variety V_2 (V-37). V_1(C-152) showed 15.45 per cent reduction in plant height. In the case of number of pods, the maximum reduction of 54.74 per cent has been observed in the variety V_2(V-37) and least reduction in V_7 (KBC-1). Maximum reduction in the length of pods has been observed in the variety V_4 (Kanakamony), followed by V_9 (Kozhinjil) and V_2 (V-37).

The most susceptible variety C-152 also showed comparatively high percentage of reduction in plant height (15.45 per cent), number of pods (37.23 per cent) and length of pods (22.22 per cent). The least susceptible variety, CG.104 showed the least reduction in plant height (4.13 per cent) and pod length (1.97 per cent). The reduction in the number of pods also was not severe as in majority of other varieties. Therefore, it can be seen that the least susceptible variety in terms of percentage of infection is also the one which is least affected by the adverse effects of virus infection.

The observations on natural incidence of cowpea mosaic virus conducted at College of Horticulture, Vellanikkara, Trichur indicated that cowpea mosaic virus disease was more serious compared to the other virus diseases affecting cowpea in that area. A high percentage

of infected plants (57 per cent) showed cowpea mosaic virus infection. This shows that cowpea mosaic disease is the most serious virus disease affecting the cowpeas in this region.

A trial was conducted to find out the effect of leaf extract sprays on development of cowpea mosaic virus. The antiviral effect of leaf extracts of Bougainvillea and some other plants have been reported earlier by several scientists (Raychaudhuri and Prasad, 1965; Sharma and Raychaudhuri, 1968; Verma and Kumar, 1980; Verma and Awasthi, 1980; Verma and Dwivedi, 1983). In the present studies complete protection against the infection of cowpea mosaic virus was achieved with two pre-inoculation sprayings with Bougainvillea leaf extract and four pre-inoculation sprayings with Eupatorium leaf extract. Verma and Dwivedi (1983) extracted a virus interfering substance from the sap of host plants sprayed with Bougainvillea leaf extract. No virus interfering substance was obtained from the control plants. Thus they have concluded that the reason for the antiviral effect of leaf extracts was the presence of virus interfering substances in the treated host plants.

Based on the results of the present studies detailed investigations have to be conducted to find out whether similar type of virus interfering substances are present in the Eupatorium leaf extract also. If satisfactory control of the disease can be achieved by the application of such cheap and easily available plant extracts it will be a very much promising method of disease control since it does not involve any hazards of atmospheric pollution caused by the application of pesticides.

SUMMARY

SUMMARY

Mosaic disease of cowpea (Vigna unguiculata(L.)Walp) prevalent in Vellayani and nearby localities, was investigated.

The symptoms appeared within 14 days after mechanical inoculation as chlorosis of the emerging leaflets and in some cases as a mild vein clearing. Subsequent leaves showed mosaic mottling with dark green and light green patches. In most cases leaves showed prominent vein banding and interveinal chlorosis. Plants infected at the early stages remained stunted and flowering and pod formation were very rare.

Transmission studies showed that the virus could be transmitted through mechanical means, aphids, grafting and seeds of diseased cowpea plants. The virus was transmitted by the aphids, Aphis craccivora Koch., Aphis gossypii Glov. and Aphis malvae Koch. Among the three species of aphids, Aphis craccivora was found to be the most efficient vector. The percentages of transmission obtained by A. craccivora, A. gossypii and A. malvae were 90, 65 and 45 respectively. There was 35 per cent graft transmission. The virus was found to be seed transmissible to the extent of 5.5 per cent.

Studies on the physical properties of the virus revealed that the thermal inactivation point of the virus was between 50 and 55°C and dilution end point between 10^{-1} and 10^{-2}. Longevity *in vitro* of the virus was 8 h at room temperature and 24 h at 8°C.

Studies on vector-virus relationships showed that the minimum acquisition feeding period required for the vector to acquire the virus was 30 s, and that the virus could be transmitted with an inoculation feeding period of 30 s. But the percentage of transmission was maximum when an acquisition feeding of 10 min and inoculation feeding of 15 min were given.

Influence of fasting of the vector before acquisition and inoculation feedings proved that pre-acquisition fasting for a period of 2 h produced the maximum transmission, whereas post-acquisition fasting decreased the per cent infection. The retention of infectivity by the vector was found to be 1-2 h. Even a single viruliferous vector was able to transmit the virus to healthy test plants, but maximum percentage of infection was obtained with 10 aphids. The incubation period of virus in the host plant was found to be 14 days.

Host-range studies showed that the virus could produce, systemic symptoms on different cowpea varieties as well as on asparagus bean (Vigna sesquipedalis) and local lesions on Chenopodium amaranticolor.

Screening trial with ten different varieties of cowpea showed that by and large, all the ten varieties were susceptible to the virus infection. But C-152 with 100 per cent infection and V-59 with 97.5 per cent infection were found to be the highly susceptible varieties. C.G.104 showed some resistance with 13.33 per cent infection.

In serological studies the cluster bean mosaic virus and sword bean mosaic virus were found to be related to cowpea mosaic virus. The antiserum titre and end point of virus in the present study were found to be between 1:1024 and 1:2048 and 1:512 and 1:1024, respectively. The virus showed no serological relationship with other cowpea viruses, viz., cowpea chlorotic mottle virus, cowpea severe mosaic virus, cucumber mosaic virus (cowpea isolate), cowpea mosaic virus (USA), cowpea mosaic virus El Salvador and southern bean mosaic virus.

The results of the studies on symptomatology, transmissions, physical properties and host-range indicate

that the virus may probably be an isolate of the cowpea aphid-borne mosaic virus.

Studies on the effect of virus infection on growth of cowpea plants showed that there was significant reduction in height of plants, number of pods produced and length of pods.

Observations on natural incidence of cowpea mosaic virus disease showed that it is the major disease among the different virus diseases affecting cowpea. Among the infected plants 57 per cent showed cowpea mosaic virus disease.

The results of the experiments to find out the effect of leaf extract sprays on cowpea mosaic virus infection indicated that the disease could be effectively controlled by pre-inoculation sprayings with leaf extracts of Bougainvillea sp. and Eupatorium odoratum.

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* Originals not seen.

APPENDIX

Appendix-1

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

PROPERTIES, HOSTRANGE AND CONTROL OF COWPEA MOSAIC VIRUS

BY

SREELAKHA L.

ABSTRACT OF THE THESIS

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ABSTRACT

Studies were conducted on the cowpea mosaic virus disease commonly occurring in cowpea plants (Vigna unguiculata (L.) Walp) in Kerala.

The major sysmptoms caused by cowpea mosaic virus infection in cowpea plants were vein banding, interveinal chlorosis, mosaic mottling and general stunting of the plants.

Transmission studies showed that the virus could be transmitted through mechanical means, grafting, through seeds and by means of aphid vectors. The virus was found to be transmitted by the aphids, Aphis craccivora, Aphis gossypii and Aphis malvae. Among the three species of aphids, Aphis craccivora was found to be the most efficient vector. The percentages of transmission obtained by A. craccivora, A. gossypii and A. malvae were 90, 65 and 45 respectively.

Studies on the physical properties of the virus revealed that the virus had a thermal inactivation point between 50 and 55°C, dilution end point between 10^{-1} and 10^{-2}, longevity in vitro of 8 h at room temperature and 24 h at 8°C.

The minimum, acquisition feeding and inoculation feeding period were found to be 30 s each. But the percentage of transmission was maximum when an acquisition feeding of 10 min and inoculation feeding of 15 min were given.

Pre-acquisition fasting up to a period of 2 h increased the percentage of transmission, whereas the post-acquisition fasting decreased the efficiency of transmission. The vector was found to retain the virus for 1-2 h. Maximum percentage of transmission was obtained with 10 aphids and the symptoms appeared within 14 days after inoculation.

Host-range studies revealed that the virus is restricted to the family Leguminosae and Chenopodiaceae.

Varietal screening trial with ten different varieties of cowpea showed that all the ten varieties were susceptible to virus infection. C-152 was found to have the highest susceptibility of 100 per cent infection. C.G.104 showed some resistance with 13.33 per cent infection.

In serological studies the cluster bean mosaic virus and sword bean mosaic virus were found to be related to cowpea mosaic virus. The antiserum titre and end point of virus in the present study were found to be between

1:1024 and 1:2048 and 1:512 and 1:1024 respectively.

Studies on the effect of virus infection on growth of cowpea plants showed that there was significant reduction in height of the plant, number of pods produced and length of pods.

Observations on natural incidence of cowpea mosaic virus indicated that cowpea mosaic virus is the major disease among the different virus diseases affecting cowpea. Among the infected plants 57 per cent showed cowpea mosaic virus.

The results of the experiments to find out the effect of leaf extract sprays on cowpea mosaic virus infection indicated that the disease could be effectively controlled by pre-inoculation sprayings with leaf extracts of Bougainvillea sp. and Eupatorium odoratum.