INHIBITORY EFFECTS OF CERTAIN PLANT EXTRACTS ON THE INCIDENCE OF COWPEA MOSAIC

BY

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DECLARATION

I hereby declare that this thesis entitled "Inhibitory effects of certain plant extracts on the incidence of cowpea mosaic" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "Inhibitory effects of certain plant extracts on the incidence of cowpea mosaic" is a record of research work done independently by Miss. S. MALLIKA DEVI 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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INTRODUCTION

INTRODUCTION

Pulses form an important part of Indian dietary. These supply vegetable proteins as essential adjuncts to predominantly starchy diets. Being leguminous crops possessing root nodules, they fix free nitrogen from the atmosphere and thereby enhance soil fertility. Among the pulse crops, cowpea is the most common one. But its production is very much reduced due to various diseases, out of which cowpea mosaic is a serious one causing heavy losses in yield.

Cowpea mosaic is known to be transmitted through sap, seeds and by vectors. A number of insecticides have been recommended for the control of the vectors. The insecticides are highly poisonous and are liable to cause environmental pollution. Plant materials have been reported to possess insecticidal properties. Some of these plant products are also known to inhibit certain plant viruses by inducing local or systemic resistance in plants.

Natural plant products have advantage over chemical protectants, in that they are degraded in a short period without leaving harmful residues (Verma, 1980).

In the present investigation, thirty non-host plants were screened for antiviral properties against cowpea mosaic virus and the promising ones were tested for insecticidal properties, if any against the vector, with a view to check the incidence and spread of the disease. The results obtained are presented in this dissertation.

REVIEW OF LITERATURE

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Mc Lean (1941) first reported cowpea mosaic from Arkansas, and the disease was characterised by dwarfed, slender growth of the affected plants with a tendency for excessive branching.

Vasudeva (1942) reported for the first time in India a mosaic disease of Vigna catjang from New Delhi. Capoor et al. (1947) reported the same disease from Poona. The disease was characterised by malformation, dwarfing and appearance of dark green areas alternating with light green areas on the leaves. Dale (1949) observed the occurrence of a mosaic disease of Vigna unquiculata from Trinidad in which the symptoms consisted of the appearance of dark and light green rings on the leaves, development of irregular yellowish and dark green mottling with blistering of the lamina. Sometimes, under green house conditions a reddish brown necrosis of the veins could be noticed.

Capoor and Varma (1956) reported a mosaic disease of Vigna cylindrica from Poona. Later, Chenulu et al. (1968) also reported the disease from Delhi. The symptoms consisted of typical mosaic mottling, yellowing, reduction and distortion of leaf lamina.

Govindaswamy et al. (1970) reported the symptoms of cowpea mosaic from Tamil Nadu. The symptoms consisted of vein clearing, development of mottling accompanied by slight rolling along the margins. In cases of severe infection, the plants became stunted and the terminal bud exhibited rugosity and wrinkling. There was shortening of internodes with necrosis of the main stem in a few instances. Finally the plant succumbed to the disease.

Sharma and Varma (1975) reported the symptoms of cowpea mosaic as mosaic mottling, crinkling, reduction in leaf size and vein banding.

Mali and Kulthe (1980) reported a seed borne poty virus causing mosaic of cowpea in India and described the symptoms as mild on primary leaves followed by irregular mosaic, puckering, slight distortion and arching of trifoliate leaves and slight stunting of the plants.

Ramachandran and Summanwar (1982) reported another symptoms of cowpea mosaic on cowpea cv. 'Prima', in which the younger leaves showed mild mottling, while older leaves had chlorotic lesions. There was no vein banding, stunting, puckering or twisting of leaves.

Transmission

First report about sap transmission of cowpea mosaic was published by Mc Lean (1941) from Arkansas. He also reported that the use of carborundum powder as an abrasive helped in the development of a high percentage of infection. Subsequently, many reports have been made on the sap transmission of cowpea mosaic virus from different parts of the world (Vasudeva, 1942; Harjono, 1959; Toler, 1964; Twardowicz-Jakuszowa and Anna, 1969; Govindaswamy et al., 1970; Sharma and Varma, 1975; Lima and Nelson, 1977; Ramachandran and Summanwar, 1982; Mazyad et al., 1984).

Cowpea mosaic virus has been 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.

Abeygunawardena and Perera (1964) reported that Aphis craccivora was the principal vector of cowpea mosaic virus in Ceylon and transmitted the virus in a non-persistent manner. Similar results were also obtained

by Klesser (1960) and Bock and Conti (1974).

Haque and Chenulu (1975) reported that the transmission of cowpea mosaic virus by A. craccivora was affected by the degree of susceptibility of the source plant. According to them younger plants were better virus sources for aphids.

Cowpea mosaic virus was found to produce local lesion on Chenopodium amaranticolor and C. album (Govindaswamy et al., 1970; Khatri and Singh, 1974), soybean, sunnhemp, C. amaranticolor and Cassia tora (Lima and Nelson, 1977). Mali and Kulthe (1980) studied a seed borne poty virus causing mosaic of cowpea in India and reported necrotic local lesions on Gomphrena globosa, Dolichos biflorus and Phaseolus vulgaris var. biela, chlorotic and necrotic local lesions on C. amaranticolor and C. quinoa and ring local lesions on C. murale.

Antiviral properties of plant extracts :-

Screening of extract from higher plants has shown that many of the plants contain highly potent inhibitors, giving local protection and also capable of inducing systemic resistance in many crops against several viruses (Verma, 1982).

Kuntz and Walker (1947) first reported that the extracts of leaves of spinach, garden beet, sugar beet and chard when mixed in equal parts with the juice of tobacco infected with tobacco mosaic virus and with the juice of cabbage infected with cabbage mosaic virus completely or almost completely inhibited the infectivity of those juices. They also reported that when spinach extract was mixed in equal parts with plant containing tobacco ring spot virus, potato necrotic ring spot virus and cucumber mosaic virus, the infectivity of those juices was also completely or nearly completely inhibited.

Kassanis and Kleczkowski (1948) reported a virus inhibitor from Phytolacca esculenta. They identified that the inhibitor was a glycoprotein. Sill and Webster (1951) conducted experiments with the sap from cucumber plants and found the presence of inhibitor in all cucumber varieties tested against Cucumber Virus 1. Among other cucurbits tested, watermelon was the only one having a comparable inhibitor.

Mc Keen (1956) reported the inhibition of cucumber mosaic virus and tobacco ring spot virus infections on cowpea and Chenopodium hybridum by extracts from Capsicum frutescens. Bawden (1954) reported that the virus inhibitors were present not only in the extracts

from leaves but also from other parts of the plants.

Benda (1956) while investigating the effect of Newzealand spinach juice on the infection of tobacco ring spot virus, found that it had an inhibitory effect on the particular virus on cowpea. It was due to the presence of a stable protein in the juice.

Allen and Kahn (1957) reported the presence of inhibitors in the rice juice against tobacco mosaic virus. According to them the inhibitor extracted from rice leaves and from roots was highly active in dilution upto 1:200 and that extracted from rice polish was active at dilution upto 1:6000.

Thresh (1956) and Cadman (1959) reported that the inhibition of tobacco necrosis virus and tobacco mosaic virus by strawberry and raspberry leaf extracts was due to the presence of phenolic tannins in the sap. Jones et al. (1959) used rice polish as source of TMV inhibitor on bean. The chemical nature of the plant virus inhibitor from rice polish was found to be a protein. Blaszczak et al. (1959) reported that the infectivity of PVX was wholly suppressed by juices from Pelargonium hortorum, Chenopodium album, C. amaranticolor and two varieties of Capsicum frutescens. Inhibition was very distinct with

juice of Datura stramonium, D. metel, Solanum integrifolium, S. tuberosum, Spinacia oleracea, Phaseolus vulgaris, Trifolium pratense and Vicia faba.

Semel (1960) reported an inhibitor of TMV from Begonia tuberhybrida and identified it as oxalic acid.

Ragetli and Weintraub (1962) reported a virus inhibitor from Dianthus caryophyllus, which showed characteristics of protein. Zaitlin and Siegel (1963) reported a virus inhibitor from tobacco leaf tissues which was a protein capable of inhibiting the infection of TMV on several hosts, viz. Nicotiana tabacum var. xanthi, Nicotiana glutinosa and Chenopodium amaranticolor.

Simons et al. (1963) assayed juices from leaves of seventy five plant species for the presence of inhibitors of TMV for transmission to Nicotiana glutinosa, and PVY to pepper or both. Juices from thirty species gave 95-100 per cent reduction in TMV lesion production.

El-Kandelgy and Wilcoxon (1966) found the inhibitory effect of red clover flower extract on the incidence of red clover vein mosaic virus (RCVMV) on Gomphrena globosa. They concluded that the extract contained no protein, but contained lipids and nine per cent glucose, five per cent galactose and three per cent xylose. These

sugars each inhibited infection of G. globosa when mixed with RCVMV. Ruppel (1967) reported the inhibition of cucumber mosaic virus by the extract of sugarbeet. Yoshi and Sako (1967) found out the inhibitory effect of Chenopodium sap on turnip mosaic virus infection.

Saksena and Mink (1969) observed that an inhibitor present in Chenopodium quinoa tissue extract prevented local lesion development by apple chlorotic leaf spot virus on Phaseolus vulgaris. Moreover, the inhibitor decreased local lesion production on C. quinoa itself. Wyatt and Shepherd (1969) reported a virus inhibitor from Phytolacca americana and indicated that the active material was not a glycoprotein. Tamura (1969) found inhibition of turnip mosaic virus by the juice extracted from Japanese black pine (Pinus thunbergii). Singh (1969) conducted experiment with crude sap of Chenopodium album, C. amaranticolor, Dahlia rosea and Spinacia oleracea against water melon mosaic virus and reported cent per cent inhibitory effect on the virus. The effect was reduced to 50-80 per cent by 1/1000 dilution of crude sap.

Smookler (1971) observed that leaf extracts of 29 species of the chenopodiales were shown to inhibit tobacco necrosis virus infection on Phaseolus vulgaris.

Verma and Raychaudhuri (1971) showed the inhibitory effect of tannins, catechol and caffeine on the infectivity of PVX *in vitro* but *in vivo* their effect was generally negligible.

According to Gupta and Raychaudhuri (1972) the leaf extracts of *Callistemon lanceolatus* and *Syzygium cumini* inhibited the local lesion production when mixed with potato virus Y infected sap.

Bark extract of *Ficus elastica* contained antiviral principle which prevented local lesion development on the leaves of *Chenopodium amaranticolor* by PVX (Singh and Singh, 1973). Fisher and Nienhaus (1973) found that crude leaf of *Capsicum annuum* inhibited the development of local lesions on *Nicotiana tabacum*, *N. glutinosa*, *Datura stramonium* and *Phaseolus vulgaris*, after TMV inoculation.

The latex of *Jatropha* species significantly reduced the infectivity of TMV on different hosts. The inhibitory principle was non-translocatable (Lal and Verma, 1974). Chandra *et al.* (1975) reported that some naturally occurring plant products such as flavanoids and coumarins inhibited sunnhemp mosaic virus on cluster bean. They found that coumarins such as tomentolide B from the nuts of *Caryophyllus tomentosum* and marmelosin from fruits of

Aegle marmelos effectively inhibited the number of local lesions on clusterbean by sunnhemp mosaic virus.

Verma and Mukerjee (1975) reported that brinjal leaf extract induced local and systemic resistance in Nicotiana glutinosa against TMV and in Nicotiana tabacum, var. N.P. 31 against tobacco ring spot virus, when applied 24 h before virus inoculation. Srivastava et al. (1976) observed that dahlia extract induced local resistance against TMV on N. gluttinosa. The plants became highly resistant (95-98 per cent) to TMV infection when inoculation was made after 4-6 h of treatment. The local resistance induced by dahlia extract persisted in the treated leaves upto 8 days showing 91 per cent inhibition of lesion production.

Tewari (1976) reported the effect of several bark extracts on the inactivation of three strains of watermelon mosaic virus. Grasso (1977) observed that a protein isolated from Phytolacca americana inhibited transmission of southern bean mosaic virus (SBMV) and cowpea mosaic virus to Phaseolus vulgaris and Chenopodium amaranticolor.

Gupta (1977) reported that plants belonging to families such as Aizoaceae, Amaranthaceae, Polygonaceae, Portulacaceae, and Rosaceae contained powerful antiviral agents in their leaf sap.

Grasso and Shepherd (1978) reported that 14 plant species taxonomically related to Phytolacca contained virus inhibitors.

Fukaya and Taniguchi (1979) found that an inhibitor from the leaves of Phytolacca americana which reduced the infection of TMV. Haji and Stevens (1979) suggested that the seed extracts from 15 legumes contained plant virus inhibitors. Verma and Awasthi (1979a) observed that the leaf extract of Euphorbia hirta inhibited the infection of tobacco mosaic, sunnhemp rosette, gomphrena mosaic and tobacco ring spot viruses on several hypersensitive hosts.

Roy et al. (1979) indicated that the juices of Ocimum sanctum, Dianthus caryophyllus, Capsicum annuum, Zingiber officinale and Nicotiana tabaccum possessed potent virus inhibitors against top necrosis virus of pea, effective at 1:1000 dilution of inhibitor-virus mixture.

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

Murthy and Nagarajan (1980) showed that the germinated seeds of pulses, groundnut and tobacco contained inhibitors of tobacco mosaic virus. Momin et al. (1980) reported that potent inhibitors were found to occur in seven varieties of red clover, three varieties of pea and one variety each of field bean, tomato and pepper against alfalfa mosaic virus.

Virus inhibitors from Datura metel against tobacco mosaic virus were reported by Singh and Varma (1981). The effect of prophylactic spraying of Basella alba leaf extract on the infection of tobacco by TMV, was reported by Murthy et al. (1981) and Ushari et al. (1982).

Erkan and Yorganci (1982) investigated the inhibition of potato virus X infectivity on Chenopodium amaranticolor, by extracts from chilli, grapevine and oats at the actively growing stage. Mukerjee et al. (1982) showed that the leaf extract of Datura metel inhibited infection by gomphrena mosaic virus and sunnhemp rosette virus in their hypersensitive hosts. Roychoudhury and Basu (1983) reported that crude extracts from Solanum khasianum and S. nigrum were inhibitory to tobacco mosaic virus and sunnhemp rosette virus in their two local lesion hosts Nicotiana glutinosa and Cyamopsis tetragonoloba respectively.

Bose et al. (1983) reported that leaf extract of Adenocalymma allicea contained an inhibitor of bean common mosaic virus infection on cowpea. The active principle was a yellow coloured oil with garlic like odour. It was more effective when used with virus or when sprayed before inoculation. Verma and Dwivedi (1983) reported that plant diseases caused by TMV, tomato yellow mottle mosaic virus, physalis shoestring mosaic virus and cucumber green mottle mosaic virus could be prevented by bougainvillea leaf extract.

The aqueous extract of leaves of the fern Ampetopteris prolifera showed maximum inhibition of TMV and CMV when applied on local lesion or systemic hosts 24 h prior to virus inoculation (Pandey and Bhargava, 1984).

Roychoudhury (1984) reported a TMV inhibitor from Solanum torvum. Awasthi et al. (1985) isolated a virus inhibitor of glycoprotein nature from the roots of Boerhaavia diffusa plants. Chowdhury and Saha (1985) reported the inhibition of urd bean leaf crinkle virus by extracts of ginger, garlic, onion, turmeric, in vitro and in vivo.

Rao et al. (1985) observed that Argemone mexicana, Azadirachta indica, Euphorbia milli, Jasminum sambac,

Lantana indica, Nerium indicum and Vinca rosea induced resistance to PVX in the hypersensitive host. Rao and Shukla (1985a) reported that the aqueous extracts of dry coconut (copra) showed significant antiviral activity against PVY when applied 24 h before virus inoculation or when mixed with virus inoculum and inoculated on Chenopodium amaranticolor leaves. No such inhibition was observed when extract was applied 24 h after virus inoculation. Rao and Shukla (1985b) observed that application of aqueous corolloid root extract of Cycas revoluta induced resistance against PVX infection in detached leaves of the local lesion host, Chenopodium amaranticolor.

Singh et al. (1985) conducted tests with leaf extracts of 50 plant species. Out of these, 42 showed inhibitory activity against the mild and severe strains of arhar mosaic virus. Pre-inoculation application of extracts from Capsicum annuum and Datura stramonium prevented the infection. Verma and Baranwal (1985) reported antiviral activity of the leaf extract of Celosia cristata against virus belonging to tobacco group in different hosts. The inhibitory activity was confined to only treated areas of the plants. Shukla et al. (1985) reported that volatile constituents of Carum copticum and Cymbopogon citratus reduced the infection of PVX and

PVY by 100 per cent upto a dilution of 1:250.

Verma et al. (1985a) reported that Clerodendron aculeatum leaf extract induced local and systemic resistance in several host plants against TMV infection. Verma et al. (1985b) reported that a potent systemic inhibitor was present in the leaves of Aerva sanguinolenta. Verma et al. (1985c) claimed that yellow mosaic disease of mung and urd beans under natural conditions was suppressed by aqueous partially clarified leaf extracts of Clerodendron fragrans and Aerva sanguinolenta and root extract of Boerhaavia diffusa. Verma et al. (1985d) reported that Pseuderanthemum atropurpureum also contained virus inhibitor against yellow mosaic disease.

Pandey and Mohan (1986) reported that leaf extracts of Callistemon lanceolatus, Acacia arabica and Syzigium cumini showed higher degree of inhibition of turnip mosaic virus. According to Prasad (1986), partially clarified aqueous leaf extract of Clerodendron aculeatum showed antiviral activity against tobacco mosaic virus in Samsun NN tobacco when applied to the leaf surface.

Extracts of Artocarpus chaplasa, Pentapanax leschenulttii and Sygium arnottianums showed higher activity against Ranikhet disease in animal system and

TMV infection in plants (Joshi et al., 1986). Srinivasulu and Jeyarajan (1986) found that pre-inoculation application with leaf extract of Mirabilis jalapa, coconut and sorghum reduced rice tungro virus transmission. Saigopal et al. (1986) reported that both leaf and root extracts of Phyllanthus niruri which is used in curing human jaundice were inhibitory to infection by tobacco mosaic, groundnut green mosaic and tobacco ring spot viruses on Chenopodium amaranticolor, Phaseolus vulgaris and Vigna sinensis respectively. Direct action of inhibitor was more effective than the pre-inoculation or post-inoculation application of the inhibitor.

Selvan and Narayanasamy (1987) reported that the leaf extract of Basella rubra was most effective in inhibiting PVY infection followed by extracts from B. alba, Bougainvillea spectabilis and Mirabilis jalapa. Sreelakha (1987) found that pre-inoculation sprayings with leaf extracts of Bougainvillea sp and Eupatorium odoratum were effective in controlling the incidence of cowpea mosaic disease.

Nagarajan and Murthy (1988) indicated that three sprays of green leaf extract of Basella alba at 1:1000 dilution at ten days interval commencing from 30 days after planting tobacco protected the crop from TMV

infection upto 60 days. Next best were green leaf extract of bougainvillea and clerodendron.

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

Zaidi et al. (1988) found that some of the medicinal plants have got inhibitory effects on spinach mosaic virus. The inhibition effect of plant extracts was directly correlated with increase in concentration. Highest inhibition was achieved by applying the leaf extract from Ocimum sanctum. Joi et al. (1988) conducted inhibition studies on tomato spotted wilt virus infection with leaf extracts of sixteen plant species. Among these, the leaf extracts of chilli, acacia, datura and chenopodium showed more than 80 per cent inhibition of the virus at 1:10 dilution.

Vijayakumar and Narayanasamy (1988) observed that the leaf extracts of Ocimum sanctum, Azadirachta indica, Cocos nucifera, Nerium indica, Calotropis gigantea,

Eucalyptus globurus, Acacia arabica and Ficus bengalensis were effective in reducing the percentage of infection by tomato leaf curl virus.

Aiyanathan and Narayanasamy (1988) studied the effect of neem oil on Rice tungro virus infection and observed that the pre-inoculation as well as post-inoculation spray of neem oil (5 per cent) reduced RTV infection. Aiyanathan and Narayanasamy (1988) studied the effect of antiviral principles on Rice tungro virus infection and reported that the leaf extracts of Vitex negundo, Mirabilis jalapa and Euphorbia jeniculata and the fruit extracts of Aegle marmelos reduced the RTV infection considerably. According to Eswaramurthy et al. (1988), sorghum leaf extract and coconut leaf extract possessed antiviral principles against groundnut bud blight.

Gurubasavaraj (1988) noted that the extract of Vitex negundo was inhibitory to rice tungro virus. Rao (1988) observed that the extract of Polyalthia longifolia possessed inhibitory properties against yellow dwarf disease of rice.

Mode of action of plant extracts against plant viruses

Bawden (1954) established that the inhibitors present in the germinating seeds of pulses and groundnut,

like that of leaf and twig extracts of a large number of other plants inhibited TMV via host and not directly inactivated the virus.

Kahn et al. (1960) described the inhibitory action of extracts obtained from rice on tobacco mosaic virus. They suggested that the inhibitory action was a host response rather than the inhibition of TMV.

Owens et al. (1973) isolated an inhibitor of virus transmission from poke weed. This inhibitor acted in vivo by blocking the messenger function of infective virus RNA. Verma et al. (1979a) found that extracts from various plant parts inhibited infection by tobacco mosaic, gomphrena mosaic, sunnhemp rosette and tobacco ringspot viruses in hypersensitive and systemic hosts by inducing systemic resistance.

Potato virus x inhibition by leaf extract of Cedrus deodara was reported by Singh and Singh (1979). Verma and Mukerjee (1979) observed that datura leaf extract reduced lesion number in the upper non-treated leaves of N. glutinosa and N. tabacum var. Np.31 when the two lower leaves of plants were rubbed with plant extract twenty four hours prior to virus inoculation. Verma et al. (1982) observed that the inhibition of virus with the plant

extracts was due to the inhibitory agent in the extracts, or protective substances formed as a result of extract treatment, and that the inhibitory response of extracts could be reversed by Actinomycin D.

Erkan and Yorganci (1982) also reported that the inhibitors from Capsicum annuum appeared to affect the host rather than the virus. Bose et al. (1983) showed that the leaf extract of Adenocalymma allicea exerted direct action on bean common mosaic virus. Besides, the pre-inoculation application of this extract also prevented the virus infection.

Verma and Prasad (1983) reported that the inhibitors of plant viruses present in plant extracts might inhibit the virus infectivity in vitro and in vivo or they might affect the virus replication also. In vitro, the inhibitors either formed a complex with the virus, or denatured the virus. The inhibitors which affected the virus infection in vivo mostly altered the essential process of infection.

Verma and Dwivedi (1983) reported that the extract from Bougainvillea spectabilis induced resistance against TMV, tomato yellow mottle mosaic virus and physalis shoestring mosaic virus and it was due to the formation of

some virus interfering substances in the plants.

Johari et al. (1983) observed that the leaf extract of sunflower inhibited TMV infection when it was applied 48 h before the application of virus. They also reported that tobacco mosaic virus was inhibited by leaf extract of sunflower by the induction of resistance. Rao et al. (1985) found that flower extracts of Argemone maxica, Azadirachta indica, Euphorbia milli, Jasminum sambac, Lantana indica, Nerium indicum and Vinca rosea induced resistance to PVX in Chenopodium amaranticolor. They also found that the highest resistance of 87.2 per cent was obtained in tomato with Azadirachta indica. The induced resistance was sensitive to Actinomycin D.

Rao and Shukla (1985a) reported that pre-inoculation application of copra extract was effective against potato virus Y in Chenopodium amaranticolor. But the post-inoculation application of copra extract with a time gap of 24 h had no effect against potato virus Y. Rao and Shukla (1985b) proved that corolloid root extract of Cycas revoluta induced production of antiviral factors against PVX in the host instead of directly interfering with the infection process. The induced resistance in the extract applied portion of the same leaf was also observed in untreated half indicating the diffusible nature of unknown factor.

Saigopal et al. (1986) reported that pre-inoculation and post-inoculation application of root and leaf extract of Phyllanthus niruri were less effective than mixing with the virus inoculation.

Arjunan et al. (1988) showed that spraying one per cent Iluppai Oil (Madhuca latifolia) 15 and 30 days after sowing had lowest yellow mosaic of 24.4 per cent.

Leaf extracts of Bougainvillea spectabilis and Asplenium nidus gave 100 per cent and 97.47 per cent inhibition respectively of the local lesions on Chenopodium amaranticolor by Cucumber mosaic virus. Incorporation of B. spectabilis/A. nidus extract with the virus inoculum gave more inhibition (100% / 97.47%) than either pre-inoculation (97.00% / 89.18%) or post-inoculation (75.36% / 72.61%) treatments (Reddy et al., 1988).

Effect of plant extracts on vectors

Dubey and Nene (1974) reported that aphid transmission of cowpea mosaic virus was inhibited by oil sprays. They found that castor oil 2.5 per cent, light paraffin (3, 3.5 and 4%) and non-emulsifiable oils (2.5 and 3.0%) completely prevented transmission of the virus by Aphis craccivora.

According to Khatri et al. (1977) the transmission of cowpea mosaic virus by Aphis craccivora was completely inhibited when glass house grown cowpea plants were sprayed with one per cent aqueous suspension of mineral oils.

Mariappan et al. (1982) reported that many seed oils such as custard apple oil and neem oil possessed inhibitory action against rice tungro virus. Seed oils from Azadirachta indica and Annona sp. at five per cent reduced rice tungro virus infection on seedlings of the cultivar TN-1. No insect survived on the sprayed plants after four days.

Bose et al. (1983) reported that an inhibitor present in the leaf extract of Adenocalymma allicea prevented the acquisition of bean common mosaic virus by Aphis gossypii. Saxena et al. (1985) found that neem seed derivatives prevented the transmission of rice tungro virus by the green leaf hopper. Makkouk and Menassa (1986) observed that application of 15 per cent Sunoco 7E/V oil reduced zuchini yellow virus spread in cucurbits by aphids in Labanon and also inhibited virus acquisition. Srivastava et al. (1986) found that crude margosa oil (0.5% water emulsion) inhibited the transmission rate of

CMV by single apterous aphid. They also observed that oil did not affect the biological activity of the virus, but it influence on the feeding behaviour of the aphids.

Ponnaiah et al. (1988) reported that neem seed extract, neem cake extract, neem leaf extract also reduced the population of leaf hopper vector of rice tungro virus significantly. Narasimhan et al. (1988) also showed that ten per cent leaf extract from nochi (Vitex negundo) sprayed rice plants showed minimum infection (18.8%) of RTV as compared to the untreated control (70%).

Pillayarsamy et al. (1988) tested extracts of seventeen plant species against cowpea aphid borne mosaic virus. Out of these, Mirabilis jalapa gave 90 per cent inhibition of the virus. Bougainvillea spectabilis gave 85 per cent inhibition. Leucena leucoephala, Tribulus terrestris, Achyranthus aspera, Alternathera echinata, Phyllanthus niruri, Pisonia alba, Prosopis sp. Datura metel and Azadirachta indica gave 70-80 per cent inhibition.

Subbaraja and Arumugan (1988) observed that banana plants treated with pseudostem injection of Cocos nucifea and Areca catachu extracts reduced the severity of the banana bunchy top symptoms. Shinde et al. (1988) tried leaf extracts of six plant species to find out the

possibilities of inhibition of tomato mosaic virus and found that leaf extracts from Datura metal and Capsicum annuum produced maximum inhibition of the virus infection.

Roychoudhury and Jain (1989) reported that neem oil (2.4%) and neem soap sprays on Aphis rumicis caused complete mortality of the nymphs and alate forms within 24 h.

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Symptomatology

Symptomatology was studied by observing the development of symptoms in artificially inoculated cowpea seedlings. Seeds of cowpea (Vigna unguiculata (L) Walp.) variety C-152 obtained from National Seed Corporation, Regional Office, Trivandrum were sown in 25 cm diameter earthen pots containing potting mixture of sand, red soil and cowdung in the ratio of 1:1:2. The culture of cowpea mosaic virus (CpMV) obtained from naturally infected cowpea plant in the Instructional Farm, Vellayani was maintained in an insect proof glass house by repeated transfers through sap inoculation on cowpea seedlings at two leaf stage.

II. Transmission of the virus

1. Sap transmission

Sap transmission trials were conducted by using the standard sap, sap extracted in phosphate buffer and tris buffer. Six hundred mesh carborundum powder was used as abrasive for sap transmission.

The standard sap was prepared by crushing the infected leaf of known weight into fine pulp by adding

one ml of sterile distilled water for every gram of diseased tissue. The extraction of sap was made by using a chilled mortar and pestle. For this the pestle and mortar was placed in an enamel tray containing water and kept in a freezer till the water was frozen. The buffers were also kept in the freezer before using. Phosphate buffer (0.1 M, pH 7) and tris buffer (0.1 M, pH 7) were used as extraction media and the sap was extracted after adding one ml of the buffer in each case to every gram of infected leaf tissue. The extract was filtered through fine musline cloth and the filtrate was centrifuged at 3000 g for 15 min and the supernatant was used as inoculum.

A small quantity of carborundum powder (600 mesh/ sq. inch) was dusted uniformly on the upper surface of the leaves of the test plants at two leaf stage before the application of inoculum. Care was taken not to injure the leaf tissue during inoculation. Inoculation was done by gently rubbing on the upper surface of leaves with a piece of sterilized absorbent cotton wool previously soaked in the inoculum. Soon after inoculation the inoculated 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 twice and the plants were kept under observation in the insect proof glass house. The local lesion host Chenopodium amaranticolor Coste and Reyn was inoculated by adopting the same procedure.

II. Aphid transmission

Aphid transmission studies were conducted by using the culture of vector Aphis craccivora Koch, which was maintained on cowpea plants under insect proof glass house conditions. Healthy insects were collected in petriplates by using a camel hair brush, the tip of which was moistened slightly. They were starved for a period of 2 h (pre-acquisition fasting period) and then allowed to feed on detached young leaves of mosaic affected cowpea plants for an acquisition feeding period of 30 min. A fixed number of infective aphids (10 Nos.) were then transferred to young healthy plants of two leaf stage kept in cages for an inoculation feeding period of 24 h and after that they were killed by spraying 0.05 per cent Quinalphos. As in the case of sap transmission studies an equal number of control plants were also maintained in separate cages. The experiments were done twice and the plants were kept under observation in insect proof glass house.

III. Preliminary screening of non-host plant extracts for antiviral property against cowpea mosaic virus

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

ii. Preparation of leaf extracts: Plant extracts were prepared in distilled water by grinding the plant parts viz., leaves, roots, bark or flower using sterilized mortar and pestle. For each gram of plant tissue one ml of distilled water was added, crushed into pulp and then squeezed through two layers of muslin cloth. The crude sap was centrifuged at 3000 g for 15 min and the supernatant was used for the study. The following plants were used.

Botanical name	Common/local name	Family
1. Adathoda vasica Nees.	Adathodai	Acanthaceae
2. Adenocalyma allicea Linn.	Ornamental garlic	Begnonaceae
3. Andrographis paniculata Nees.	Neela veppu	Acanthaceae
4. Azadirachta indica A.Juss.	Veppu	Meliaceae
5. Boerhaavia diffusa Linn.	Thazhuthama	Nyctaginaceae
6. Bougainvillea spectabilis Wild.	Bougainvilla	Nyctaginaceae

7.	Calotropis gigantea Linn.	Erikku	Ascleipidaceae
8.	Clerodendron infortunatum Linn.	Peruvalam	Verbenaceae
9.	Curcuma longa Linn.	Turmeric	Zingiberaceae
10.	Cyperus rotundus Linn.	Muthanga	Cyperaceae
11.	Eupatorium odoratum Linn.	Communist pacha	Compositae
12.	Ferula foetida Regel.	Assafoetida	Umbelliferae
13.	Hydrocotyle asiatica Linn.	Kodangal	Umbelliferae
14.	Ixora coccinea Linn.	Thetti	Rubiaceae
15.	Lawsonia alba Lam.	Mylanji	Lythraceae
16.	Mirabilis jalapa Linn.	Nalumani	Nyctaginaceae
17.	Moringa olifera Lam.	Moringa	Moringaceae
18.	Ocimum sanctum Linn.	Thulasi	Labiatae
19.	Ocimum gratissimum Linn.	Ramathulasi	Labiatae
20.	Phyllanthus niruri Linn.	Kizhanelli	Euphorbiaceae
21.	Polyalthia longifolia Benth & Hook.	Aranamaram	Annonaceae
22.	Ruta graveolens Linn.	Arutha	Rutaceae
23.	Salvinia molesta Linn.	African payal	Salviniaceae
24.	Saraca indica Linn.	Asokathetti	Leguminosae
25.	Solanum indicum Linn.	Puthirichunda	Solanaceae
26.	Thespesia populnea Soland ex.Correa.	Poovarasu	Malvaceae

27.	Vetiveria zizanoides Linn.	Ramacham	Graminaceae
28.	Vinca rosea Linn.	Savakottathetti	Apocyanaceae
29.	Vitex negundo Linn.	Nochi	Verbenaceae
30.	Vitis quadracularis Wall.	Changalam piranda	Vitaceae

Chenopodium amaranticolor the local lesion host of CpMV raised in earthen pots in an insect proof glass house, was used as the test host.

The partially clarified extract (10 ml) of each of the above plants was mixed with equal quantity of virus inoculum, incubated at room temperature for 15 min, and was then rubbed on the leaves of C. amaranticolor. A small quantity of carborundum powder was sprinkled uniformly on the leaves before the application of inoculum. After 15 min, the leaves were washed with distilled water using a wash bottle. The inoculated plants were kept under observation in insect proof glass house for the development of local lesions. Plants inoculated with the virus inoculum alone were kept as control. The efficacy of the plant extract having antiviral property against cowpea mosaic virus was estimated by applying the following formula.

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

C = Number of lesions produced in control

T = Number of lesions produced on treated plant

IV. Comparative efficacy of two concentrations of plant extracts on the symptom development by cowpea mosaic virus

Virus inoculum and extracts of 16 selected plants namely Adenocalyma allicea, Azadirachta indica, Boerhaavia diffusa, Bougainvillea spectabilis, Calotropis gigantea, Clerodendron infortunatum, Curcuma longa, Eupatorium odoratum, Ferula indica, Mirabilis jalapa, Moringa olifera, Phyllanthus niruri, Polyalthia longifolia, Solanium indicum, Thespesia populnea and Vitex negundo were prepared as mentioned above. The virus inoculum was mixed with equal quantity of two dilutions (5 and 10 per cent) of the plant extracts, incubated at room temperature for 15 min, and then rubbed on the upper surface of the leaves of Chenopodium amaranticolor, previously dusted with 600 mesh carborundum powder. The plants inoculated with virus inoculum alone were kept as control. The inoculated leaves were washed with distilled water using a wash bottle and kept under observation in insect proof glass house.

The extracts which caused 100 per cent inhibition of local lesions on C. amaranticolor were further tested on the primary leaves of cowpea seedlings to test their effect in the original host plant.

V. Effect of time of application of plant extracts on the transmission of cowpea mosaic virus

Extracts of ten plants namely, A. indica, B. diffusa, B. spectabilis, C. gigantea, C. infortunatum, C. longa, M. jalapa, P. niruri, S. indicum and V. negundo were sprayed on cowpea seedlings at ten per cent dilution as given below:

1. One day prior to the inoculation with the virus (pre-inoculation application). 2. One day after inoculation with the virus (post-inoculation application).

In one set of plants the virus was inoculated by sap transmission method and in other set the virus was inoculated by using viruliferous insect A. craccivora as described earlier. Observations were recorded two weeks after inoculation.

VI. Effect of plant extracts on the acquisition and transmission of cowpea mosaic virus by aphids

Plant extracts (ten per cent) were applied on mosaic affected cowpea plants maintained in the insect proof glass house. After 24 h, groups of 50-60 virus

free aphids, A. craccivora were allowed to feed on these plants. Before releasing on the infected plants the aphids were subjected to fasting for 2 h. After 30 min of acquisition feeding, the aphids were released on healthy cowpea seedlings at the rate of ten per seedlings. After 24 h, the plants were sprayed with 0.05 per cent Quinalphos to kill the insects. The inoculated seedlings were kept on insect proof glass house benches for observation.

VII. Effect of plant extracts on the transmission of cowpea mosaic virus inoculated at different intervals.

Extracts of the following five promising non-host plants selected on the basis of their ability to inhibit the transmission of cowpea mosaic virus on cowpea plants, when tested by sap as well as vector were used in this experiment.

1. A. indica
2. B. spectabilis
3. C. infortunatum
4. P. niruri
5. V. negundo

Cowpea seedlings grown in 25 cm diameter earthen pots, each containing two seedlings were arranged into

seven groups of five pots (Each group having ten plants). These pots were kept in the insect proof glass house. Ten per cent each of the plant extracts were sprayed on the six groups of test plants by means of an atomiser. The seventh group was kept as control. Soon after spraying with the plant extract (zero hour), the first set of plants and the control plants were inoculated with the virus by sap transmission method. The other five sets of plants were inoculated at intervals of 6 h, one day, two days, four days and six days respectively. The test plants were observed for the expression of disease symptoms at intervals of seven days, 14 days, and 21 days after inoculation.

A similar experiment was laid out, wherein virus inoculation was made by means of viruliferous aphid, A. craccivora. Observations were taken as mentioned above.

VIII. Effect of plant extracts on the survival of insect vector on cowpea.

Extracts of A. indica, B. spectabilis, C. infortunatum, P. niruri, V. negundo were sprayed on cowpea seedlings grown in 25 cm diameter earthen pots, each containing two seedlings. The pots were arranged in six groups, each group having five pots. These pots

were kept in the insect proof glass house. Plant extracts each at ten per cent concentrations were sprayed on five groups of the test plants by means of an atomiser. The sixth group served as control and was sprayed with the distilled water. Viruliferous aphids (A. craccivora) at the rate of two per plant were released on the seedlings of the first group after 3 h, second group after 6 h, third group after 24 h, fourth group after 48 h and fifth group after 72 h. The plants were placed in insect cages after releasing the insects. In each case the number of insects survived was noticed after 24 h.

IX. Systemic effect of plant extracts on the transmission of cowpea mosaic virus.

Extracts of A. indica, B. spectabilis, C. infortunatum, P. niruri and V. negundo at ten per cent concentration were carefully applied on the cotyledonous leaves of cowpea seedlings at the first true leaf stage, by means of cotton wool dipped in the extract. These test plants were inoculated with cowpea mosaic virus, on the first trifoliate leaf, by sap inoculation method, at intervals of 3 h, 6 h and 24 h after the application of plant extracts. In the group of plants which served as control, distilled water was applied on the cotyledonous leaves and the virus inoculum was applied on the trifoliate

leaf. The test plants were kept in insect proof glass house for further observation.

X. Effect of repeated application of plant extracts on the incidence of cowpea mosaic virus

The three plant extracts, C. infortunatum, P. niruri and V. negundo which exhibited maximum inhibitory effect against CpMV in experiment on the effect of time of application of plant extracts on the transmission of cowpea mosaic virus were used in this experiment.

Cowpea seedlings were raised in 80 earthen pots of 30 cm diameter. Three seedlings were maintained in each pot. Experiment was laid out in two groups, in Completely Randomised Design namely pre-inoculation application series and post-inoculation application series.

In the pre-inoculation series, the plant extracts were first sprayed on the seedlings and were inoculated with the cowpea mosaic virus after one day. The plants were sprayed with extracts four more times at weekly intervals.

In the post-inoculation series, the plants were inoculated with the CpMV at two leaf stage by sap inoculation method, one day prior to the first application of

plant extract. Four more sprayings were given at an interval of one week. Twelve plants were maintained for each series. Similar number of plants were kept as control. Observations on the disease development were taken 45 days after the first spraying.

A similar experiment was also conducted, wherein the plants were inoculated by means of viruliferous aphids. Observations were also taken as mentioned above.

XI. Effect of plant extracts on certain agronomic characters and the development of root nodules in virus inoculated and healthy cowpea plants.

The plants used in the experiment to study the repeated application of plant extract on the incidence of CpMV were observed for characters like:

1. Height of the plant
2. Weight of the plant (wet weight and dry weight)
3. Number and weight of the pods (wet weight and dry weight)
4. Number and wet weight of root nodules

RESULTS

RESULTS

I. Symptomatology

The infected plants exhibited mosaic mottling, slight distortion of leaves and stunted growth. Only few flowers and pods were produced by these plants (Fig. 1 and 2).

When cowpea seedlings were artificially inoculated with virus by sap transmission method at the cotyledonary leaf stage, the symptoms initially appeared on the first trifoliate leaves as mild vein clearing within 6-7 days after inoculation. Typical mosaic symptoms were developed on the leaf subsequently.

Similar symptoms were also produced on the leaves of seedlings inoculated with viruliferous insects, Aphis craccivora.

II. 1. Effect of buffer on sap transmission

Maximum percentage (85) of infected seedlings was obtained, when infective sap was extracted in tris buffer. This was followed by phosphate buffer. Sap extracted in distilled water gave minimum number of infected seedlings (Table 1).

Fig. 1. C. Cowpea mosaic virus infected plant.
A. Healthy cowpea plant.

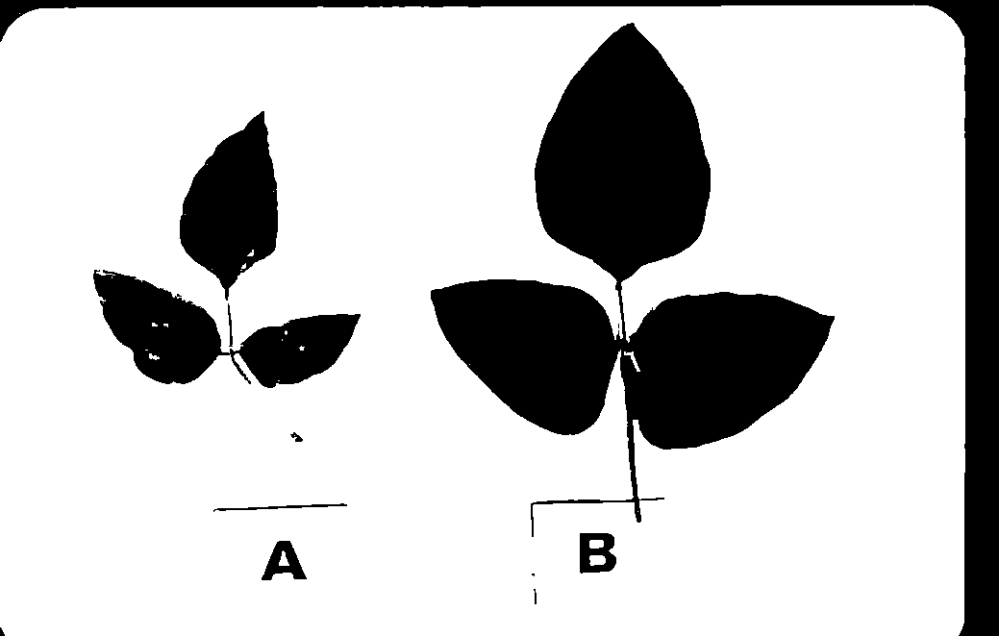


Table 1. Sap transmission of cowpea mosaic virus

Sl. No.	Inoculum	Number of plants infected / Number of plants inoculated		Per cent infection
		Experiment I	Experiment II	
1.	Sap in distilled water	$\frac{6}{10}$	$\frac{6}{10}$	60
2.	Sap in phosphate buffer	$\frac{6}{10}$	$\frac{7}{10}$	65
3.	Sap in tris buffer	$\frac{8}{10}$	$\frac{9}{10}$	85
4.	Control (uninoculated)	$\frac{0}{10}$	$\frac{0}{10}$	0

2. Aphid transmission

When cowpea seedlings were inoculated by means of viruliferous aphids (A. craccivora), at the rate of ten per seedling, 95 per cent of the seedlings were infected within 7-10 days whereas, none of the uninoculated plants produced symptoms of cowpea mosaic (Table 2).

3. Development of local lesions on Chenopodium amaranticolor

Local lesions were produced within 3-5 days on the inoculated leaves of C. amaranticolor. The lesions appeared as chlorotic, when the inoculated plants were maintained inside insect proof glass house. When the plants were placed under direct sunlight, the lesions became necrotic with brown centre and reddish margin, measuring 2-3 mm in diameter (Fig. 3).

III. Preliminary screening of non-host plants for antiviral property against cowpea mosaic virus

In order to find out the antiviral property of plant extracts, an experiment was conducted as described in materials and methods and the results are presented in Table 3.

Out of the 30 crude plant extracts tested, 16 plant extracts inhibited production of local lesions on C. amaranticolor, indicating that the extracts of these

Table 2. Transmission of cowpea mosaic virus by Aphis craccivora

Sl. No.	Number of plants inoculated	Number of plants infected	Per cent transmission	Mean
1.	10	9	90	95
2.	10	10	100	
3.	10 (control)	0	0	-

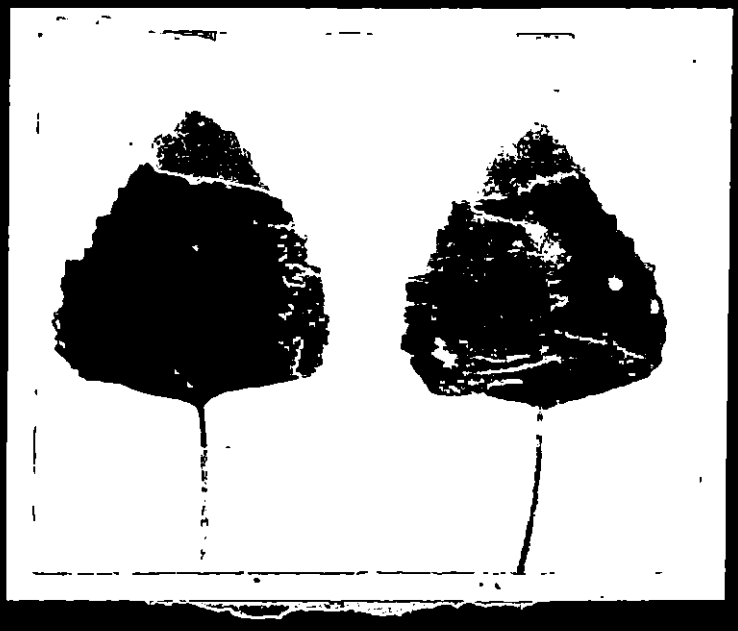


Fig. 3. Local lesions of cowpea mosaic virus on leaves of *Chenopodium amaranticolor*

Table 3. Preliminary screening of non-host plant extracts for antiviral property against cowpea mosaic virus

Local lesions developed on virus inoculated leaves of Chenopodium amaranticolor

Sl. No.	Extract of	Parts used	Average number of lesions		Per cent inhibition over control $\frac{C-T}{C} \times 100$
			Inoculum alone (Control = C)	Inoculum + plant extract (T)	
1.	Adathoda vasica	leaf	13	1	92.30
2.	Adenocalyma allicea	leaf	13	0	100.00
3.	Andrographis paniculata	leaf	10	1	90.00
4.	Azadirachta indica	leaf	13	1	92.30
		seed	12	0	100.00
5.	Boerhaavia diffusa	root	12	0	100.00
6.	Bougainvillea spectabilis	leaf	12	0	100.00
7.	Calotropis gigantea	leaf	8	0	100.00
8.	Clerodendron infortunatum	leaf	18	0	100.00
9.	Curcuma longa	rhizome	7	0	100.00
10.	Cyperus rotundus	root	7	1	85.70
11.	Eupatorium odoratum	leaf	13	0	100.00
12.	Ferrula indica	leaf	10	0	100.00
13.	Hydrocotyl asiatica	leaf	19	1	94.70
14.	Ixora indica	flower	8	4	50.00
15.	Losonia alba	leaf	15	3	80.00
16.	Mirabilis jalapa	leaf	18	0	100.00
17.	Moringa olifera	bark of the root	9	0	100.00
18.	Ocimum gratissimum	leaf	7	1	85.70
19.	Ocimum sanctum	leaf	16	1	93.70
20.	Phyllanthus niruri	leaf	11	0	100.00
21.	Polyalthia longifolia	leaf	9	0	100.00
22.	Ruta graveolens	leaf	22	1	95.45
23.	Saraca indicum	flower	10	1	90.00
24.	Salvinia molesta	leaf	9	1	88.80
25.	Solanum indica	root	13	0	100.00
26.	Thespesia populnea	leaf	7	0	100.00
27.	Vetiveria zizanoides	root	10	3	70.00
28.	Vinca rosea	leaf	18	1	94.70
29.	Vitis quadracularis	leaf	10	1	90.00
30.	Vitex negundo	leaf	15	0	100.00

plants possessed antiviral property against cowpea mosaic virus (CpMV). These included

1. Adenocalyma allicea
2. Azadirachta indica
3. Boerhaavia diffusa
4. Bougainvillea spectabilis
5. Calotropis gigantea
6. Clerodendron infortunatum
7. Curcuma longa
8. Eupatorium odoratum
9. Ferrula indica
10. Mirabilis jalapa
11. Moringa olifera
12. Phyllanthus niruri
13. Polyalthia longifolia
14. Solanum indicum
15. Thespesia populnea
16. Vitex negundo

IV. Comparative efficacy of two concentrations of plant extracts on symptom development by cowpea mosaic virus

The data revealed that the extracts of ten plants namely A. indica, B. diffusa, B. spectabilis, C. gigantea, C. infortunatum, C. longa, M. jalapa, P. niruri, S. indicum

and V. negundo caused 100 per cent inhibition of production of local lesions on C. amaranticolor at both dilutions tested (Table 4).

The extracts of the above ten plants when tested on the primary leaves of cowpea seedlings it was noticed that the inhibitory effect was less pronounced in the original host plant (Table 5). Ten per cent extract was more effective than five per cent, except in C. gigantea and C. longa where both dilutions had similar effect.

The extracts of B. diffusa and C. longa were comparatively less effective than those of the other plants tested on cowpea seedlings.

V. Effect of time of application of plant extracts on the transmission of cowpea mosaic virus

Results of the experiment conducted to study the effect of time of application of plant extracts on the transmission of cowpea mosaic virus by sap are presented in Table 6a. It was noticed that pre-inoculation application of plant extracts was more effective than post-inoculation application. Maximum inhibitory effect (90%) was noticed for the extract of P. niruri. This was closely followed by C. infortunatum and then by V. negundo.

Table 4. Comparative efficacy of two concentrations of plant extracts on symptom development by cowpea mosaic virus in Chenopodium amaranticolor

Sl. No.	Extracts of	Control (inoculum alone)	Number of local lesions			
			5 per cent plant extract		10 per cent plant extract	
			Treated (extract + inoculum) T	Per cent inhibition over control $\frac{C-T}{C} \times 100$	Treated (extract + inoculum) T	per cent inhibition over control $\frac{C-T}{C} \times 100$
1.	Adenocalyma allicea	23	1	96	1	96
2.	Azadirachta indica	13	0	100	0	100
3.	Boerhaavia diffusa	9	0	100	0	100
4.	Bougainvillea spectabilis	12	0	100	0	100
5.	Calotropis gigantea	13	0	100	0	100
6.	Clerodendron infortunatum	18	0	100	0	100
7.	Curcuma longa	10	0	100	0	100
8.	Eupatorium odoratum	13	2	85	1	92
9.	Ferrula indica	15	1	93	2	87
10.	Mirabilis jalapa	18	0	100	0	100
11.	Moringa olifera	9	1	89	1	89
12.	Phyllanthus niruri	11	0	100	0	100
13.	Polyalthia longifolia	25	3	88	1	96
14.	Solanum indicum	13	0	100	0	100
15.	Thespesia populnea	10	2	80	2	80
16.	Vitex negundo	15	0	100	0	100

Table 5. Comparative efficacy of two concentrations of plant extracts on symptom development by cowpea mosaic virus in cowpea

Sl. No.	Extracts of	Number of plants infected				
		5% plant extract			10% plant extract	
		Control (inoculum alone)	Treated (inoculum + plant extract)	Per cent inhibition over control	Treated (inoculum + plant extract)	Per cent inhibition over control
		C	T	$\frac{C-T}{C} \times 100$	T	$\frac{C-T}{C} \times 100$
1.	A. indica	16	16	62.50	5	68.70
2.	B. diffusa	18	16	11.10	12	33.30
3.	B. spectabilis	16	6	62.50	5	68.70
4.	C. gigantea	18	6	66.60	6	66.60
5.	C. infortunatum	18	6	66.60	4	78.00
6.	C. longa	16	8	50.00	8	50.00
7.	M. jalapa	18	9	50.00	6	66.60
8.	P. niruri	18	7	61.00	5	73.00
9.	S. indicum	18	10	44.40	6	66.60
10.	V. negundo	20	7	65.00	5	75.00

Table 6a. Effect of time of application of plant extracts on the sap transmission of cowpea mosaic virus

Sl. No.	Extracts of	Number of plants infected					
		Pre-inoculation application			Post-inoculation application		
		Control (inoculum alone)	Treated with plant extract	Per cent inhibition over control	Control (inoculum alone)	Treated with plant extract	Per cent inhibition over control
1.	A. indica	16	4	75.00	16	7	69.00
2.	B. diffusa	18	6	67.00	18	10	44.00
3.	B. spectabilis	16	4	75.00	16	6	63.00
4.	C. gigantea	18	6	67.00	18	8	50.00
5.	C. infortunatum	16	2	88.00	16	7	69.00
6.	C. longa	16	6	63.00	16	8	50.00
7.	M. jalapa	16	5	67.00	16	8	50.00
8.	P. niruri	20	2	90.00	20	4	80.00
9.	S. indicum	18	8	55.50	18	10	44.40
10.	V. negundo	20	4	80.00	20	6	70.00

When the effect of time of application of plant extracts on the transmission of cowpea mosaic virus by the vector A. craccivora was tested, cent per cent inhibition was noticed with A. indica, C. infortunatum, P. niruri and V. negundo in pre-inoculation application. Here also, the pre-inoculation application was found to be more effective than post-inoculation application, except with C. infortunatum wherein cent per cent inhibition was noticed at both the periods of application (Table 6b).

VI. Effect of plant extracts on the acquisition and transmission of cowpea mosaic virus by aphids

The results revealed that all the ten plant extracts caused reduction in the acquisition and per cent transmission of CpMV by A. craccivora (Table 7). The extract of P. niruri effected 100 per cent reduction over control. This was followed by C. gigantea and C. infortunatum (90%) and then by S. indicum and V. negundo (89%). B. diffusa showed only 66.6 per cent reduction over control.

VII. Effect of plant extracts on the transmission of cowpea mosaic virus inoculated at different intervals

When the effect of five plant extracts on the transmission of CpMV was tested by sap transmission method,

Table 6b. Effect of time of application of plant extracts on the insect transmission of cowpea mosaic virus

Sl. No.	Extracts of	Number of plants infected					
		Pre-inoculation application			Post-inoculation application		
		Control (inoculum alone)	Treated with plant extract	Per cent inhibition over control	Control (inoculum alone)	Treated with plant extracts	Per cent inhibition over control
1.	A. indica	10	0	100.00	10	1	90.00
2.	B. diffusa	10	2	80.00	9	2	78.00
3.	B. spectabilis	10	1	90.00	10	1	90.00
4.	C. gigantea	10	1	90.00	10	1	90.00
5.	C. infortunatum	20	0	100.00	20	0	100.00
6.	C. longa	18	4	78.00	18	6	67.00
7.	M. jalapa	13	2	84.60	13	2	84.60
8.	P. niruri	14	0	100.00	14	1	93.00
9.	S. indicum	18	4	78.00	18	4	78.00
10.	V. negundo	20	0	100.00	18	2	88.20

Table 7. Effect of plant extracts on the acquisition and transmission of cowpea mosaic virus by *Aphis craccivora*

Sl. No.	Extracts of	Treated with 10% plant extract		Control (untreated)		Per cent reduction over control
		Number of plants inoculated	infected	Number of plants inoculated	infected	
1.	*A. indica*	12	2	12	12	83.30
2.	*B. diffusa*	20	6	20	18	66.60
3.	*B. spectabilis*	20	2	20	18	89.00
4.	*C. gigantea*	10	1	10	10	90.00
5.	*C. infortunatum*	20	2	20	20	90.00
6.	*C. longa*	10	2	10	9	78.00
7.	*M. jalapa*	13	2	13	13	85.00
8.	*P. niruri*	20	0	20	20	100.00
9.	*S. indicum*	20	2	20	18	89.00
10.	*V. negundo*	20	2	20	18	89.00

90 per cent inhibition of disease development was noticed in plants inoculated upto two days after the application of the extracts of A. indica, P. niruri and V. negundo when observations were recorded seven days after inoculation. The inhibitory effect of the plant extracts was slightly reduced, when the inoculations were conducted at four days and six days after the application of plant extracts. Even at six days, the extract of P. niruri exhibited higher inhibitory effect (80%) than the other plant extracts. In the control, all the ten inoculated plants became infected within seven days after virus inoculation.

When the inhibitory effect of the above plant extracts was tested using A. craccivora, 75 to 78 per cent inhibition was noticed in plants inoculated upto two days after the application of extracts of C. infortunatum, P. niruri and V. negundo when observations were taken seven days after inoculation. In the case of C. infortunatum and P. niruri the inhibitory effect was not reduced even when inoculations were conducted six days after application of extracts. All the plants in control exhibited disease symptoms within seven days.

The inhibitory effect of plant extracts was found to be reduced when observations were taken 14 and 21 days

after the inoculation in both sap and vector transmission (Tables 8a to 8e, 9, 10a to 10e and 11).

VIII. Effect of plant extracts on the survival of insect vector on cowpea

When viruliferous aphids were released 3 h after the application of plant extracts on cowpea plants, minimum number of insects survived on plant sprayed with the extract of V. negundo. At other intervals of 6 h, 24 h, 48 h and 72 h also plants sprayed with V. negundo extract had lesser number of surviving aphids than those sprayed with other plant extracts. Extract of A. indica also caused considerable reduction in the number of insects survived. It was also noticed that in general, disease incidence was reduced as the number of surviving insects decreased. As the time interval of the release of insects after the application of plant extracts increased, the number of insects surviving on the plant was also found to be increased (Table 12 and Fig. 4).

IX. Systemic effect of plant extracts on the transmission of cowpea mosaic virus

In the experiment conducted to study the systemic effect, if any, of plant extracts against CpMV, it was noticed that only ten per cent of the cowpea seedlings

Table 8a. Effect of plant extracts on the sap transmission of cowpea mosaic virus inoculated at different intervals. a. Azadirachta indica

Sl. No.	Time interval of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	1	90	5	50	7	30
2.	6 h	10	1	90	5	50	7	30
3.	1 day	10	1	90	3	70	6	40
4.	2 days	10	1	90	3	70	8	20
5.	4 days	10	2	80	4	60	8	20
6.	6 days	10	4	60	5	50	8	20
	Control	10	10	0	10	0	10	0

Table 8b. Effect of plant extracts on sap transmission of cowpea mosaic virus inoculated at different intervals. b. Bougainvillea spectabilis

Time interval of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
		7 days		14 days		21 days	
		Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
0 h	10	1	90	4	60	5	50
6 h	10	1	90	4	60	6	40
1 day	10	1	90	3	70	4	60
2 days	10	3	70	5	50	8	20
4 days	10	3	70	5	50	8	20
6 days	10	4	60	6	40	8	20
Control	10	10	0	10	0	10	0

Table 8c. Effect of plant extracts on sap transmission of cowpea mosaic virus inoculated at different intervals. c. Clerodendron infortunatum

Sl. No.	Time interval of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	1	90	4	60	5	50
2.	6 h	10	1	90	4	60	5	50
3.	1 day	10	1	90	4	60	7	30
4.	2 days	10	2	80	6	40	8	20
5.	4 days	10	2	80	6	40	8	20
6.	6 days	10	4	60	6	40	8	20
	Control	10	10	0	10	0	10	0

Table 8d. Effect of plant extracts on sap transmission of cowpea mosaic virus inoculated at different intervals. d. Phyllanthus niruri

Sl. No.	Time interval of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	1	90	2	80	3	70
2.	6 h	10	1	90	3	70	4	60
3.	1 day	10	1	90	3	70	4	60
4.	2 days	10	1	90	4	60	6	40
5.	4 days	10	2	80	5	50	7	30
6.	6 days	10	2	80	5	50	8	20
	Control	10	10	0	10	0	10	0

Table 8e. Effect of plant extracts on sap transmission of cowpea mosaic virus inoculated at different intervals. e. Vitex negundo

Sl. No.	Time interval of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	1	90	3	70	4	60
2.	6 h	10	1	90	2	80	3	70
3.	1 day	10	1	90	3	70	4	60
4.	2 days	10	1	90	4	60	5	50
5.	4 days	10	3	70	4	60	5	50
6.	6 days	10	3	70	5	50	7	30
	Control	10	10	0	10	0	10	0

Table 9. Effect of plant extracts on Sap transmission of cowpea mosaic virus inoculated at different intervals(pooled data)

Sl. No.	Time interval of virus inoculation after plant extract application.	Percentage of inhibition over control after														
		A. indica			B. spectabilis			C. infortunatum			P. niruri			V. negundo		
		7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
1.	0 h	90	50	30	90	60	50	90	60	50	90	80	70	90	70	60
2.	6 h	90	50	30	90	60	40	90	60	50	90	70	60	90	80	70
3.	1 day	90	70	40	90	70	60	90	60	30	90	70	60	90	70	60
4.	2 days	90	70	20	70	50	20	80	40	20	90	60	40	90	60	50
5.	4 days	80	60	20	70	50	20	80	40	20	80	50	30	70	60	50
6.	6 days	60	50	20	60	40	20	60	40	20	80	50	20	70	50	30
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 10a. Effect of plant extracts on the insect transmission of cowpea mosaic virus inoculated at different intervals. a. Azadirachta indica

Sl. No.	Time interval of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	1	90	3	70	5	50
2.	6 h	10	2	80	4	60	6	40
3.	1 day	10	2	80	4	60	6	40
4.	2 days	10	3	70	5	50	7	30
5.	4 days	10	4	60	5	50	8	20
6.	6 days	10	4	60	7	30	9	10
	Control	10	10	0	10	0	10	0

Table 10b. Effect of plant extracts on the insect transmission of cowpea mosaic virus inoculated at different intervals. b. Bougainvillea spectabilis

Sl. No.	Time interval of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	2	80	4	60	6	40
2.	6 h	10	2	80	4	60	6	40
3.	1 day	10	2	80	5	50	6	40
4.	2 days	10	3	70	5	50	7	30
5.	4 days	10	4	60	6	40	7	30
6.	6 days	10	4	60	7	30	9	10
	Control	10	10	0	10	0	10	0

Table 10c. Effect of plant extracts on the insect transmission of cowpea mosaic virus inoculated at different intervals. c. Clerodendron infortunatum

Sl. No.	Time interval of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	0	100.00	2	75.00	5	37.50
2.	6 h	10	1	87.50	2	75.00	5	37.50
3.	1 day	10	2	75.00	2	75.00	6	25.00
4.	2 days	10	2	75.00	3	62.50	4	50.00
5.	4 days	10	2	75.00	4	50.00	5	37.50
6.	6 days	10	2	75.00	5	37.50	7	12.50
	Control	10	8	20.00	8	20.00	8	20.00

Table 10d. Effect of plant extracts on the insect transmission of cowpea mosaic virus inoculated at different intervals. d. Phyllanthus niruri

Sl. No.	Time intervals of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	0	100.00	2	77.80	4	66.60
2.	6 h	10	1	88.80	2	77.80	4	66.66
3.	1 day	10	1	88.80	2	77.80	4	66.66
4.	2 days	10	2	77.80	4	66.66	6	33.30
5.	4 days	10	2	77.80	4	66.66	6	33.30
6.	6 days	10	2	77.80	5	44.44	8	22.22
	Control	10	9	10.00	9	10.00	9	10.00

Table 10e. Effect of plant extracts on the insect transmission of cowpea mosaic virus inoculated at different intervals. e. Vitex negundo

Sl. No.	Time intervals of virus inoculation after plant extract application	Number of plants inoculated	Number of plants infected after					
			7 days		14 days		21 days	
			Infected	Per cent inhibition over control	Infected	Per cent inhibition over control	Infected	Per cent inhibition over control
1.	0 h	10	0	100.00	2	75.00	3	62.50
2.	6 h	10	1	87.50	3	62.50	4	50.00
3.	1 day	10	2	75.00	2	75.00	4	50.00
4.	2 days	10	2	75.00	2	75.00	4	50.00
5.	4 days	10	3	62.50	4	50.00	5	37.50
6.	6 days	10	4	50.00	5	37.50	6	25.00
	Control	10	8	20.00	8	20.00	8	20.00

Table II. Effect of plant extracts on insect transmission of cowpea mosaic virus inoculated at different intervals (pooled data)

Sl. No.	Time intervals of virus inoculation after plant extract application	Percentage of inhibition over control after														
		A. indica			B. spectabilis			C. infortunatum			P. niruri			V. negundo		
		7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
1.	0 h	90	70	50	80	60	40	100.0	75.0	37.5	100.0	77.8	66.6	100.0	75.0	62.5
2.	6 h	80	60	40	80	60	40	87.5	75.0	37.5	88.8	77.8	66.6	87.5	62.5	50.0
3.	1 day	80	60	40	40	80	50	75.0	75.0	25.0	88.8	77.8	66.6	75.0	75.0	50.0
4.	2 days	70	50	30	70	50	30	75.0	62.5	50.0	77.7	66.6	33.3	75.0	75.0	50.0
5.	4 days	60	50	20	60	40	30	75.0	50.0	37.5	77.7	66.6	33.3	62.5	50.0	37.5
6.	6 days	60	30	10	60	30	10	75.0	37.5	12.5	77.7	44.4	22.2	50.0	37.5	25.0
	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 12. Effect of plant extracts on the survival of insect vector on cowpea

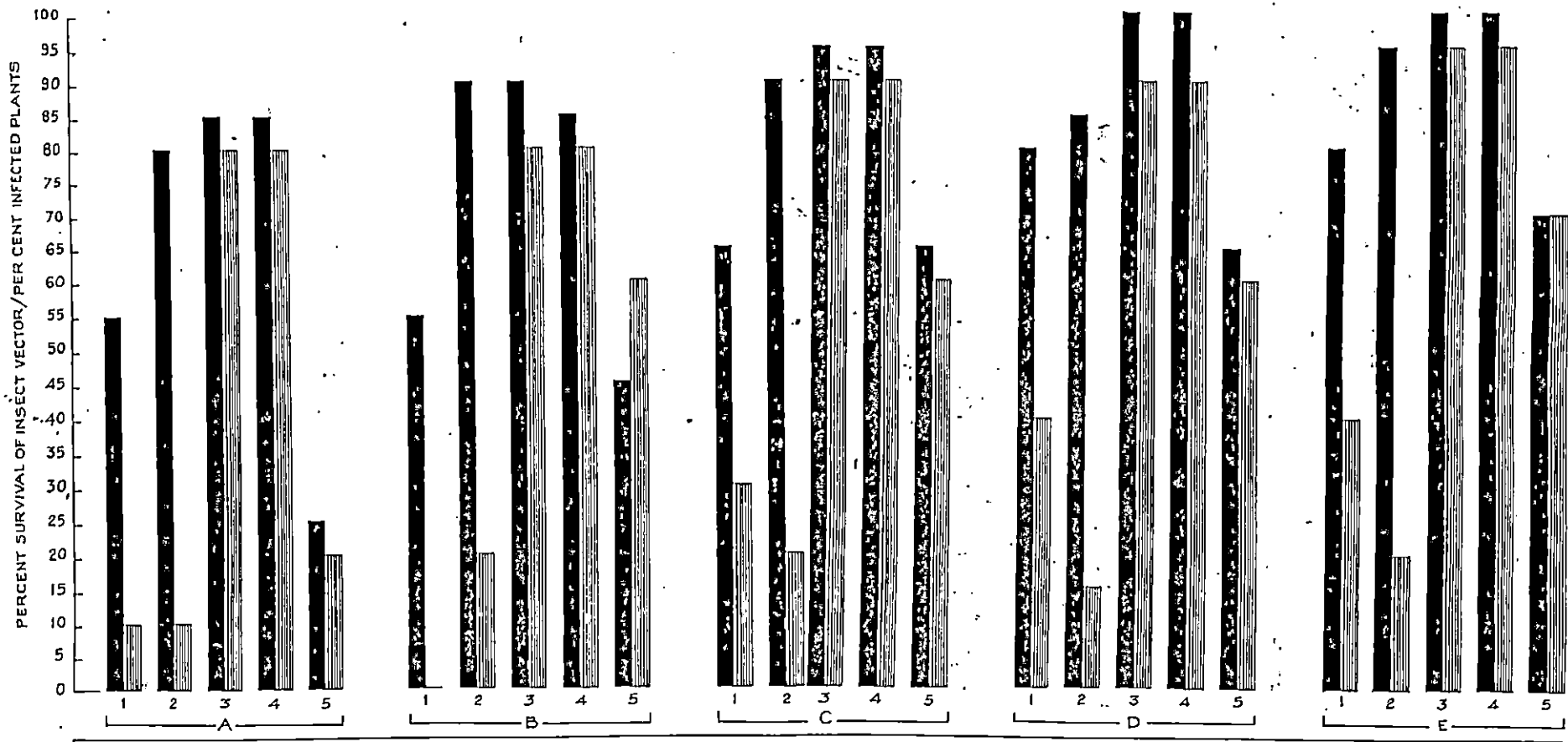
Extract of	Number of insects released	Interval of release of insects after application of extrat														
		3 hours			6 hours			24 hours			48 hours			72 hours		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
A. indica	20	11	55	10	11	55	0	13	65	30	16	80	40	16	80	40
B. spectabilis	20	16	80	10	18	90	20	18	90	20	18	90	20	19	95	20
C. infortunatum	20	17	85	80	18	90	80	19	95	90	20	100	90	20	100	90
P. niruri	20	17	85	80	17	85	80	19	95	90	20	100	90	20	100	90
V. negundo	20	5	25	20	9	45	60	13	65	60	13	65	60	14	70	70
Control	20	20	0	100	20	0	100	20	0	100	20	0	100	20	0	100

A - Number of insects survived

B - Percentage of survival

C - Percentage of infected plants

FIG. 4. EFFECT OF PLANT EXTRACTS ON THE SURVIVAL OF INSECT VECTOR ON COWPEA.



PLANT EXTRACTS

1. *Azadirachta indica*
2. *Bougainvillea spectabilis*
3. *Clerodendron infortunatum*
4. *Phyllanthus niruri*
5. *Vitex negundo*

TIME INTERVAL

A. 3 HOURS
B. 6 HOURS
C. 24 HOURS
D. 48 HOURS
E. 72 HOURS

PERCENT SURVIVAL OF INSECT VECTOR

PERCENT INFECTED PLANTS

inoculated with the virus 3 h after application of V. negundo extract exhibited mosaic symptoms when observations were taken seven days after inoculation.

In the case of seedlings treated with extracts of A. indica and B. spectabilis, 30 per cent became infected, while cent per cent of control plants as well as those treated with the extract of P. niruri exhibited mosaic symptoms within seven days.

In seedlings inoculated 6 h and 12 h after the application of plant extracts also minimum number (17%) of infected seedlings was obtained in the case of V. negundo and followed by B. spectabilis (20%).

In the case of C. infortunatum extract treated seedlings inoculated with CpMV 3 h after application of extract, 80 per cent plants were infected and this was reduced to 50 and 33.3 per cent at 6 h and 24 h respectively (Table 13).

X. Effect of repeated application of plant extracts on the incidence of cowpea mosaic virus

The data on the effect of repeated application of three plant extracts namely C. infortunatum, P. niruri and V. negundo on the transmission of CpMV are presented in Table 14. The results revealed that pre-inoculation

Table 13. Systemic effect of plant extracts on the transmission of cowpea mosaic virus

Sl. No.	Extracts of	A			B			C		
		No. of plants		Per cent infected plants	No. of plants		Per cent infected plants	No. of plants		Per cent infected plants
		Inoculated	Infected		Inoculated	Infected		Inoculated	Infected	
1.	A. indica	10	3	30	10	4	40	10	4	40
2.	B. spectabilis	10	3	30	10	2	20	10	2	20
3.	C. infortunatum	10	8	80	16	8	50	15	5	33.3
4.	P. niruri	15	15	100	10	6	60	12	10	83
5.	V. negundo	10	1	10	12	2	17	12	2	17
	Control	10	10	100	10	10	100	10	10	100

A - inoculated 3 h after the application of plant extract

B - " 6 h " " "

C - " 12 h " " "

Table 14. Effect of repeated application of plant extracts on the incidence of cowpea mosaic virus

Sl. No.	Treatments	Mean percentage of inhibition			
		Aphid transmission		Sap transmission	
		Pre-inoculation	Post-inoculation	Pre-inoculation	Post-inoculation
1.	*V. negundo*	100.00 (90)	66.66 (58.66)	100.00 (90)	66.66 (58.66)
2.	*C. infortunatum*	83.33 (72.35)	58.33 (49.35)	91.66 (81.17)	66.66 (58.66)
3.	*P. niruri*	74.99 (63.53)	58.33 (49.35)	91.66 (81.17)	58.33 (49.34)
4.	Control	0	0	0	8.33
	CD (P = 0.05)	29.62	29.62	20.30	20.30

Figures in parenthesis are the transformed values

application (followed by four subsequent applications) of the plant extracts exhibited higher inhibitory effect on disease development than post-inoculation applications, as evidenced by the number of infected plants obtained in both sap and aphid transmission trials. Of the three plant extracts tested, V. negundo had the maximum inhibitory effect (100%) on symptom development in cowpea plants.

Statistical analysis of the data revealed that pre-inoculation application of plant extracts was significantly superior to post-inoculation application.

XI. Effect of plant extracts on certain agronomic characters and the development of root nodules in virus inoculated and healthy cowpea plants

1. Height of the plant

The height of cowpea plants treated with plant extracts was found to be more than that of the control plants in both sap and aphid transmission trials as well as in pre and post-inoculation application of extracts. The difference between pre and post-inoculation applications, was however not significant (Table 15).

2. Weight of the plant

The weight (wet and dry) of plants treated with plant extracts was in general more than that of the

Table 15. Effect of plant extracts on certain Agronomic characters and the development of root nodules in virus inoculated and healthy cowpea plants

1. Height of plants

Treatments	Mean value of height in centimetre	
	Aphid transmission	Sap transmission
1. C. infortunatum	72.40	75.50
2. P. niruri	72.08	68.38
3. V. negundo	75.58	79.19
4. Control	55.52	47.40
CD	14.97	11.47
5. Pre-inoculation application	69.89	69.10
6. Post-inoculation application	67.91	66.13
CD (P = 0.05)	10.58	8.10

control plants, except in the case of wet weight of P. niruri extract treated plants (Table 16). The difference between the pre and post-inoculation application was not significant.

3. Number and weight of the pods

Number and weight of the pods were found to be significantly increased as a result of application of plant extracts in aphid transmission trials, both in pre and post-inoculation application. The difference between pre and post-inoculation was however not significant in regard to the weight of the pods. In sap transmission trials, the difference in the number and weight of pods, between treated and control plants was also not significant (Table 17 and 18).

4. Number and weight of root nodules

The number and weight of root nodules of cowpea plants treated with plant extracts were found to be more than those of the control plants, in sap as well as in aphid transmission trials (Table 19).

However it was noticed that the difference between the pre and post-inoculation application was significant only in sap transmission trial, wherein the number and wet weight of nodules was higher in pre-inoculation application.

Table 16. Effect of plant extracts on certain Agronomic characters and the development of root nodules in virus inoculated and healthy cowpea plants

2. Weight of the plant

Sl. No.	Treatments	Mean value of the weight of the plant in gram			
		Aphid transmission		Sap transmission	
		wet weight	dry weight	wet weight	dry weight
1.	C. infortunatum	61.67	11.25	53.33	9.97
2.	P. niruri	54.79	10.91	61.25	10.43
3.	V. negundo	63.96	12.44	53.54	9.61
4.	Control	32.79	6.85	36.88	6.62
	CD	12.34	2.57	15.25	2.42
5.	Pre-inoculation application	54.27	10.82	48.02	8.38
6.	Post-inoculation application	52.33	9.91	54.48	9.93
	CD (P = 0.05)	8.73	1.82	10.78	1.71

Table 17. Effect of plant extracts on certain Agronomic characters and the development of root nodules in virus inoculated and healthy cowpea plants

3. Number of pods

Sl. No.	Treatments	Mean value of pod number	
		Aphid transmission	Sap transmission
1.	C. infortunatum	2.75	2.63
2.	P. niruri	2.58	2.79
3.	V. negundo	2.83	2.40
4.	Control	0.79	1.70
	CD	0.99	1.38
5.	Pre-inoculation	2.21	1.73
6.	Post-inoculation	2.27	3.00
	CD (P = 0.05)	6.70	0.35

Table 18. Effect of plant extracts on certain Agronomic characters and the development of root nodules in virus inoculated and healthy cowpea plants

4. Weight of pods

Sl. No.	Treatments	Mean value of weight of pods in gram			
		Aphid transmission		Sap transmission	
		wet weight	dry weight	wet weight	dry weight
1.	C. infortunatum	3.06	1.03	4.69	1.90
2.	P. niruri	3.27	1.29	5.73	2.53
3.	V. negundo	4.02	1.35	3.94	1.70
4.	Control	0.81	0.16	2.52	1.05
	CD	1.45	0.58	2.84	1.33
5.	Pre-inoculation	3.07	0.88	3.40	1.38
6.	Post-inoculation	2.51	1.04	5.04	2.22
	CD (P = 0.05)	1.03	0.41	2.01	0.94

Table 19. Effect of plant extracts on certain Agronomic characters and the development of root nodules in virus inoculated and healthy cowpea plants

5. Number and weight of nodules

Sl. No.	Treatments	Mean values of number and weight of nodules in gram			
		Aphid transmission		Sap transmission	
		Number of nodules	Wet weight of nodules	Number of nodules	Wet weight of nodules
1.	C. infortunatum	14.76	64.20	20.17	109.74
2.	P. niruri	12.08	67.17	18.96	118.80
3.	V. negundo	12.75	66.58	22.92	72.80
4.	Control	7.92	33.36	7.92	37.47
	CD	3.63	20.30	6.33	36.83
5.	Pre-inoculation	11.13	53.74	22.90	101.32
6.	Post-inoculation	12.60	61.92	12.08	68.09
	CD (P = 0.05)	2.57	14.35	4.48	23.92

DISCUSSION

DISCUSSION

The inhibitory effect of certain plant extracts on the incidence of cowpea mosaic virus, was studied during the present investigation. Based on symptomatology, transmission through sap as well as by means of the vector, Aphis craccivora and development of local lesions on the leaves of Chenopodium amaranticolor, the virus was identified as cowpea mosaic virus (CpMV) reported from Kerala by Sreelakha (1987).

Insect transmission trials were conducted only by using A. craccivora, since this vector has been reported to be more efficient than two other species tested viz., A. gossypii and A. malvae (Sreelakha, 1987). In the present studies, more than ninety per cent transmission could be obtained with A. craccivora.

Extracts of 16 plants (out of 30 tested) which were found to possess antiviral property against CpMV, based on the ability to inhibit the production of local lesions on the leaves of C. amaranticolor, were used for further experimentation. These included, A. allicea, A. indica, B. diffusa, B. spectabilis, C. gigantea, C. infortunatum, C. longa, E. odoratum, F. indica, M. jalapa, M. olifera, P. niruri, P. longifolia,

S. indicum, T. populnea and V. negundo.

The extract of Adenocalyma allicea has been reported to inhibit bean common mosaic virus (Bose et al., 1983) whereas those of Azadirachta indica and Mirabilis jalapa inhibited cowpea aphid borne virus (Pillayarswamy et al., 1988). Root and leaf extracts of Boerhaavia diffusa inhibited several viruses including TMV (Verma and Awasthi, 1979b; Nagarajan and Murthy, 1988).

Extract of Curcuma longa inhibited urd bean leaf crinkle virus (Chowdhury and Saha, 1985). Leaf extracts of Clerodendron fragrans, C. aculeatum and C. inerme inhibited plant viruses like yellow mosaic of mung and urd beans, sunnhemp rosette virus and tobacco mosaic virus respectively (Verma et al., 1985a & b; Verma and Prasad, 1987 and Nagarajan and Murthy, 1988). Extract of Phyllanthus niruri inhibited tobacco mosaic and tobacco ringspot viruses (Saigopal et al., 1986).

Cowpea plants which received pre-inoculation sprays of Bougainvillea and Eupatorium leaf extracts did not show any symptom of cowpea mosaic (Sreelakha, 1987). B. spectabilis exhibited 85 per cent inhibition of cowpea aphid borne mosaic virus (Pillayarswamy et al., 1988) and 100 per cent inhibition of cucumber mosaic virus (Reddy et al., 1988). Antiviral principle from Calotropis gigantea

reduced tomato leaf curl virus infection (Vijayakumar and Narayanaswamy, 1988). Extract of Solanum torvum inhibited the infection by tobacco mosaic and sunnhemp rosette viruses (Roychoudhury, 1984).

Extracts of V. negundo has been reported to have antiviral property against rice tungro virus (Gurubasavaraj, 1988). Rao (1988) observed that Polyalthia longifolia leaf extracts possessed inhibitory properties against rice yellow dwarf (MLO) disease.

The inhibitory effects of Ferrula indica, Moringa olifera and Thespesia populnea against plant viruses/MLOs have not been reported.

When five and ten per cent of the extracts of the sixteen plants were tested for inhibition of local lesion on the leaves of C. amaranticolor, it was noticed that those of A. allicea, A. indica, B. diffusa, B. spectabilis, C. gigantea, C. infortunatum, C. longa, E. odoratum, F. indica, M. jalapa, M. olifera, P. niruri, P. longifolia, S. indicum, T. populnea and V. negundo could cause cent per cent inhibition at both the concentrations tested.

When the efficacy of two concentrations of the above ten plant extracts was tested on the original host plant (cowpea) it was noticed that, ten per cent extract

was more effective than five per cent in inhibiting infection by the virus. The results further indicated that the inhibitory effect of plant extracts was less pronounced in the original host, than in the local lesion host. This is believed to be due to the differences in susceptibility/resistance of the test plants to infection by the virus. While studying the effect of different dilutions of leaf and root extracts of P. niruri against tobacco mosaic, peanut green mosaic and tobacco ring spot viruses, Saigopal et al. (1986) observed that the percentage of inhibition decreased as the dilution of extracts increased. No inhibitory effect was noticed for the extracts at 1:50 dilution. Joi et al. (1988) obtained eighty per cent inhibition of tomato spotted wilt virus with 1:10 dilution of the leaf extracts of chilli, acacia, datura and chenopodium plants.

In the experiment conducted to study the comparative efficacy of pre-inoculation and post-inoculation application of plant extracts to inhibit the virus infection in cowpea plants, it was noticed that pre-inoculation application was more effective than post-inoculation application, in both sap and insect transmission. These results are in confirmity with those reported by Verma and Mukerjee (1975), Rao and Shukla (1985) and Aiyanathan

and Narayanaswamy (1988). In pre-inoculation application tested, the extracts of C. infortunatum, P. niruri and V. negundo caused over 80 per cent inhibition of virus infection in sap transmission trials whereas, in insect transmission tests, the extract of the above three plants and A. indica also caused cent per cent inhibition of the virus infection. The inhibition of infection effected by the extract of A. indica observed in insect transmission trials is believed to be mainly due to its insecticidal property against the vector rather than the effect on the virus. Mariappan et al. (1988) reported insecticidal property of neem (A. indica) oil and purified preparation of neem seed extract against the green leaf hopper, Nephotettix virescens, the vector of rice tungro virus. Ponnaiah et al. (1988) reported significant reduction in the population of N. virescens by the application of neem leaf, seed and cake extracts.

In the case of C. infortunatum extract, cent per cent inhibition of infection was noticed in pre and post-inoculation application, in aphid transmission trials. No plausible reason could be attributed to this observation.

In studies on the acquisition and transmission of the virus by A. craccivora, from cowpea plants sprayed

with the plant extracts, 100 per cent inhibition of transmission was obtained with the extract of P. niruri, 90 per cent with that of C. gigantea and C. infortunatum and 89 per cent with that of S. indicum and V. negundo. The high percentage of inhibition of transmission obtained with the extracts of the above plants could either be due to prevention of acquisition of the virus by the vector (because of the film layer of extract formed on the source plant surface) or inactivation of the virus by some specific chemical substances present in the extracts. It is also possible that some of the plant extracts might have prevented the vector from acquiring the virus due to repellant/insecticidal properties. Bose et al. (1983) reported that an inhibitor present in the leaf extract of A. allicea prevented the acquisition of bean common mosaic virus. Makkok and Menassa (1980) reported that Sunoco oil caused reduction in the acquisition of Zuchini yellow mosaic virus by aphids. Narasimhan et al. (1988) noted that leaf extracts of V. negundo possessed insecticidal property against N. virescens, the vector of rice tungro virus. Mariappan et al. (1988) observed reduction in the survival of leaf hopper of rice (N. virescens) by spraying one per cent neem oil.

When the extracts of five non-host plants were tested against sap transmission of CpMV at different time intervals, ninety per cent of inhibition of disease development was noticed in plants inoculated with the extracts of A. indica, P. niruri and V. negundo upto two days after the application of extracts in observation taken 7 days after inoculation. Selvaraj (1990) observed that antiviral principles from seed extracts of Tribulus terrestris and leaf extract of Vitex negundo var. purpurascense reduced rice tungro virus infection upto 45 per cent and 41 per cent respectively over control and also increase the incubation period. The extract of P. niruri exhibited higher inhibitory effect than those of the other plants, even upto six days after the application of extract.

In transmission trials with A. craccivora, the extracts of C. infortunatum, P. niruri and V. negundo exhibited considerably high percentage of inhibition of symptom expression, when the test plants were inoculated upto two days after the application of extracts, in observations taken seven days after inoculation. The inhibitory effect of the extracts of C. infortunatum and P. niruri was not reduced even when inoculations were conducted 6 days after the application of extracts.

The inhibitory effect of plant extracts was found to be reduced in both sap and insect transmission, when observations were taken 14 and 21 days after inoculation. It is evident from the foregoing that, the plant extracts exerted inhibitory effect on cowpea mosaic virus and also caused delay in the expression of symptoms in the inoculated plants. Pandey and Mohan (1986) reported that the extract of Syzygium cumini inhibited the lesion production by turnip mosaic virus on the leaves of C. amaranticolor, when the virus was inoculated within four hours of application of the extract, while the extract of Acacia arabica decreased lesion production when inoculated within 18 hours of application. Extract of Callistemon lanceolatus had more prolonged inhibitory effect against turnip mosaic virus than the other two plant extracts.

Rao and Narayanasamy (1988) while studying the effect of plant extracts on the transmission of rice yellow dwarf (MLO) transmitted by green leaf hopper recorded considerably reduction in the percentage of infected plants as a result of application of leaf extracts of Mirabilis jalapa, Catheranthus roseus, Zea mays and Nerium sp. Further, they noted considerable delay in the symptom expression in plants inoculated

after treatment with the plant extracts. Reddy and Jeyarajan (1988) reported increase in the incubation period of yellow dwarf disease in rice plants sprayed with one per cent leaf extract of Polyalthia longifolia.

The experiment to study the effect of plant extracts on the vector, A. craccivora revealed that the number of insects which survived on cowpea plants after spraying with the extract of V. negundo was minimum and closely followed by that of A. indica. Consequently, the number of infected plants was also reduced. It was further noticed that as the time interval of releasing insects after application of plant extracts increased, the number of surviving insects was also increased, thereby indicating that the insecticidal/repellent action of plant extracts on the insect vector was reduced rapidly within a period of 72 h. This is believed to be due to the fact that only ten per cent of the crude plant extract was used for treating the plants.

When the plant extracts were tested for systemic effect if any, on the transmission of CpMV only very few plants treated with the extract of V. negundo exhibited mosaic symptoms and this was followed by that of B. spectabilis. This indicated the possibility of some systemic effect for the extracts of the above plants.

Verma et al. (1985) reported systemic protection against sunnhemp rosette virus on Cyamopsis tetragonoloba and TMV on Nicotiana glutinosa by the extracts of Pseuderanthemum atropurpureum and B. spectabilis. Srivastava et al. (1976) showed that inhibitory principle of the dahlia extract could move rapidly from treated to untreated portion of leaves of N. glutinosa and induce local resistance against TMV.

The effect of repeated four application of plant extracts revealed that pre-inoculation application (followed by four subsequent applications) of the plant extracts had higher inhibitory effect on disease development than post-inoculation applications. This was in agreement with the results of Pandey and Mohan (1975) and Aiyanathan and Narayanasamy (1988).

Cowpea plants treated with plant extracts, exhibited increase in height and weight, number and weight of pods and root nodules. Verma et al. (1985c) reported similar results with the leaf extracts of Clerodendron fragrans, Aerva sanguinolenta and root extracts of Boerhaavia diffusa in the case of yellow mosaic affected mung and urd beans.

Results of the present investigation indicated the possibility of utilising extracts of certain non-host

plants for providing prophylactic protection against mosaic disease of cowpea prevalent in certain parts of Kerala. Plant extracts have the added advantage of being cheap and easily available without causing toxic hazards and environmental pollution.

SUMMARY

SUMMARY

Symptoms of cowpea mosaic appeared on the leaves of artificially inoculated seedlings as vein clearing within 6-7 days after inoculation. The virus was found to be transmitted through sap as well as by the vector A. craccivora.

In sap inoculation, maximum percentage of infected seedlings was obtained when infective sap was extracted in "Tris" buffer. Local lesions were produced within 3-5 days on sap inoculated leaves of C. amaranticolor.

Out of the thirty crude plant extracts tested, extracts of sixteen plants viz. Adenocalyma allicea, Azadirachta indica, Boerhaavia diffusa, Bougainvillea spectabilis, Calotropis gigantea, Clerodendron infortunatum, Curcuma longa, Eupatorium odoratum, Ferrula indica, Mirabilis jalapa, Moringa olifera, Phyllanthus niruri, Polyalthia longifolia, Solanum indicum, Thespesia populnea and Vitex negundo inhibited the production of local lesions on the leaves of C. amaranticolor, indicating that these extracts possessed antiviral property against cowpea mosaic virus.

When the extracts of the above sixteen plants were further tested at five and ten per cent concentrations,

it was noticed that the extracts of A. indica, B. diffusa, B. spectabilis, C. gigantea, C. infortunatum, C. longa, M. jalapa, P. niruri, S. indicum and V. negundo caused 100 per cent inhibition of the production of local lesions on the leaves of C. amaranticolor even at 5 per cent concentration. However, when tested on the primary leaves of cowpea seedlings, the inhibitory effect of the plant extracts was found to be less pronounced.

Pre-inoculation application of plant extracts was more effective than post-inoculation application in checking the disease incidence in both sap and insect transmission trials. In sap transmission, maximum inhibitory effect was noticed for the extract of P. niruri, closely followed by C. infortunatum and V. negundo. In insect transmission, cent per cent inhibition was obtained with the extracts of A. indica, C. infortunatum, P. niruri and V. negundo.

Extracts of the above mentioned ten plants caused reduction in the acquisition and per cent transmission of cowpea mosaic virus (CpMV) by A. craccivora. Of these, the extract of P. niruri effected 100 per cent reduction over control.

Maximum inhibition of disease development was noticed in plants which were sap inoculated with CpMV

upto two days after the application of extracts of A. indica, P. niruri and V. negundo, when observations were taken seven days after inoculation. But, in transmission studies with the vector, the extracts of C. infortunatum, P. niruri and V. negundo exhibited better inhibitory effect than the other extracts. The inhibitory effect of plant extracts was found to be reduced when observations were taken at 14 and 21 days after inoculation, in both sap as well as aphid transmission studies.

Extracts of V. negundo and A. indica caused considerable reduction in the number of insects survived on cowpea. It was also noticed that, disease incidence was also reduced as the number of surviving insects decreased.

The study of the systemic effect of plant extracts, indicated that the extracts of C. infortunatum possessed some systemic effect against CpMV.

Repeated application of plant extracts revealed that V. negundo had the maximum inhibitory effect on symptom development in cowpea plants in both sap and vector transmission trials.

Height and weight of plants, number and weight of pods and root nodules of cowpea plants treated with

plant extracts were found to be more than those of the control plants.

REFERENCE

REFERENCES

Abeygunawardana, D.V.W. and Perera, S.M.D. (1964). Virus disease affecting cowpea in Ceylon. Trop. Agric. 120: 181-204.

Aiyanathan, K.E.A. and Narayanasamy, P. (1988). Effect of neem oil on RTV infection in rice varieties with different levels of resistance. In Proceedings of National Seminar on Management of crop diseases with plant products/Biological agents. Jan. 11-12.

Aiyanathan, K.E.A. and Narayanasamy, P. (1988). Effect of antiviral principles on RTV infection. Indian J. Virol., 4(1-2): 97-99.

Allen, T.C. (Jr.) and Kahn, R.P. (1957). Tobacco mosaic virus inhibition by rice extracts (Abstr.). Phytophpathology 47: 515.

Arjunan, G., Samiyappan, R., Nara Simhan, V., Mariappan, V. and Jeyarajan, R. (1988). Studies on the effect of symptom suppressors on yellow mosaic in blackgram. In Proceedings of National Seminar on Management of crop diseases with plant products/Biological agents. Jan. 11-12.

Awasthi, L.P., Pathak, S.P. and Gautam, N.C. (1985). Control of virus diseases of vegetable crops by a glycoprotein isolated from Boerhaavia diffusa. Indian J. Plant Pathol. 3: 59-63.

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

Benda, G.T.A. (1956). The effect of Newzealand Spinash juice on the infection of cowpeas by tobacco ring spot virus. Virology 2: 438-454.

Blaszczak, W., Ross, A. and Larson, R.H. (1959). The inhibitory activity of plant juices on the infectivity of potato virus. Phytopathology 49: 784-791.

Bock, K.R. and Conti, M. (1974). Cowpea aphid-borne mosaic virus. C.M.I./A.A.B. Descriptions of Plant Viruses. Set 8, No. 134 - 4 pp.

Bose, K., Kulshreshtha, K. and Joshi, R.D. (1983). Some aspects of the inhibition of bean common mosaic virus by ornamental plants. AGRIC. Sci. Dig. 3: 195-198.

Cadman, C.H. (1959). Some properties of an inhibitor of virus infection from leaves of raspberry. J. Gen. Microbiol. 20: 113-128.

Capoor, S.P. and Varma, P.M. (1956). Studies on a mosaic disease of Vigna cylindrica Skeels. Indian J. Agric. Sci. 26: 95-103.

Capoor, S.P., Varma, P.M. and Uppal, B.M. (1947). A mosaic disease of Vigna catjang Walp. Curr. Sci. 16: 151.

Chandra, S., Singh, B.P., Nigam, S.K. and Srivastava, K.M. (1975). Effect of some naturally occurring plant products on Sunnhemp mosaic virus (SSMV). Curr. Sci. 44: 511-512.

Chenulu, V.V., Sachidananda, J. and Mehta, S.C. (1968). Studies on the mosaic disease of cowpea from India. Phytopath. Z. 63: 381-387.

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

Dale, W.T. (1949). Observations on a virus disease of cowpea in Trinidad. Ann. Appl. Biol. 36: 327-333.

Dubey, G.S. and Nene, Y.L. (1974). Aphid transmission of cowpea mosaic virus as influenced by oil sprays. Indian Phytopath. 27: 325-330.

Doraisamy, S. and Ramkrishnan, K. (1988). Inhibition of tobacco mosaic virus. In: Proceedings of National Seminar on management of crop diseases with plant products/biological agents 11-12. pp. 54.

EL-Kandelgy, S.M. and Wilcozson, R.D. (1966). Effect of red clover flower extract and glucose on infection of Gomphrena globosa by red clover vein mosaic virus. Phytopathology 56: 832-837.

Erkan, S. and Yorganci, U. (1982). Investigations on the inhibition of potato virus x (PVX) infectivity by some plant extracts I. The effectiveness of extracts from various plant species on potato virus x infection and effects on certain factors on the inhibitive activity of plant extract. J. Turkish Phytopathol. 11: 61-75.

Eswarmurthy, S., Muthusamy, M., Mariappan, V., Jeyasekar, M. and Natarajan, S. (1988). Effect of Sorghum and Coconut leaf extracts containing antiviral principles on Groundnut bud blight. In: Proceedings of national Seminar on management of crop diseases with plant products/biological agents. 11-12 pp. 48.

Fisher, H. and Nienhaus, F. (1973). Virus inhibiting principles in Paprika plants (Capsicum annuum L.). Phytopath. Z. 78: 25-41.

Fukaya, N. and Taniguchi, T. (1979). Some properties of an inhibitor of plant virus infection occurring in the leaves of Phytolacca americana. Phytopathol. Z 94: 132-138.

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

Grasso, S. (1977). Investigations on the action mechanism of a virus inhibitor from Phytolacca americana. Rev. Pat. Veg. 13: 77-84.

Grasso, S. and Shepherd, R.J. (1978). Isolation and partial characterisation of virus inhibitors from plant species taxonomically related to Phytolacca. Phytopathology 68: 199-205.

Gupta, B.M. (1977). Inhibition of plant virus infections by antiviral agents. In Aphids as virus vectors. Harris, K.F. and Maramorosch, K. (Eds). Academic Press, New York, pp. 455-471.

Gupta, V.K. and Raychaudhuri, S.P. (1972). Inhibition of potato virus Y by the leaf extracts of Callistemon lanceolatus and Syzygium cumim. Indian Phytopathol. 25: 108-112.

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

*Haji, B. and Stevens, W.A. (1979). The effects of legume seed extracts on plant virus infection. Experientia 35: 1460-1462.

Haque, S.Q. and Chenulu, V.V. (1975). Epidemiology of cowpea mosaic virus with special reference to influence of host factors on the transmission efficiency of Aphis craccivora Koch. 357-364.

*Harjono. (1959). Virus disease in cowpea (Vigna sinensis). Indonesia Abstr. 1: 84-85.

Johari, A.K., Raizada, R.K., Srivastava, K.M. and Singh, B.P. (1983). Induction of resistance against tobacco mosaic virus in detached leaves of Nicotiana tabacum var. Samsun NN by leaf extract of Helianthus annus. Curr. Sci. 52: 1022-1023.

Jones, W.A., Jacobson, M. and Kahn, R.P. (1959). Chemical nature of a plant-virus inhibitor from rice. Nature 184: 1146.

Joi, M.B., Patil, B.H. and Ruikar, S.K. (1988). Inhibition studies on tomato spotted wilt virus in tomato by leaf extracts. In: Proceedings of National Seminar on Management of crop disease with plant products// biological agents. 11-12. pp. 51.

Joshi, M.N., Chandra, K. and Verma, H.N. (1986). Demonstration of antiviral activity in some indigenous plant extracts against Ranikhet disease virus and tobacco mosaic virus. Indian J. Virolol. 2: 201-206.

Kahn, R.P., Allen, T.C. and Zeymeyu, W.J. (1960). Characteristics of plant virus inhibitors in rice, Oryza sativa. Phytopathology 50: 847-851.

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

Khatri, H.L. and Singh, L. (1974). Studies on a mosaic disease of Cowpea. J. Res. 11: 289-294.

Khatri, H.L., Singh, L. and Sekhon, I.S. (1977). Inhibitory effect of mineral oil spray on aphid transmission of cowpea mosaic virus. Indian Phytopathol. 29: 75-77.

*Klesser, P.T. (1960). Virus diseases of Cowpea. Bothalia, 7: 233-251.

Kuntz, J.E. and Walker, J.C. (1947). Virus inhibition by extracts of Spinash. Phytopathology 37: 561-579.

Lal, R. and Verma, G.S. (1974). Effect of plant latex on virus infectivity. Zentral bl Bakeriol. Parasiten k De Infektion Skr Hyg 1 ABT orig c 2 (1974) 129: 271-277.

Lima, J.A.A. and Nelson, M.R. (1977). Etiology and Epidemiology of mosaic of cowpea in Ceara, Brazil. Plant Dis. Rep. 61: 864-867.

Makkouk, K.M. and Menassa, R.E. (1986). Inhibitory on aphid - spread Zucchini yellow mosaic virus with oil sprays. J. Plant Dis. Prot. 93: 104-107.

Mali, V.R. and Kulthe, K.S. (1980). A seed borne poty virus causing mosaic of cowpea in India. Plant Dis., 64: 925-928.

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

Mariappan, V., Gurubasavaraj, T.M., Narasimhan, V., Sreemannarayana, G. and Muthusamy, M. (1988). Effect of neem and custard apple oil preparations on Nephotettix virescens Dist. In: Proceedings of National Seminar on Management of crop diseases with plant products/biological agents. 11-12 pp.2.

Mazyad, K.H.M., El-Hammady, M., El-Amretty, A.A. and El-Din, A.S.G. (1984). Studies on cowpea aphid-borne mosaic virus in Egypt. Agric. Res. Rev., 59: 167-178.

Mc Keen, C.D. (1956). The inhibitory activity of extract of Capsicum frutescens on plant virus infections. Can. J. Bot. 34: 891-903.

Mc Lean, D.M. (1941). Studies on mosaic of Cowpea (Vigna sinensis). Phytopathology 31: 420-430.

Momin, A., Robb, S.M. and Caldwell (1980). Natural inhibitors of alfalfa mosaic virus (AMV) infection. Indian Phytopathol. 33: 26-29.

Mukerjee, K., Awasthi, L.P. and Verma, H.N. (1982). Further studies on the antiviral resistance induced in host plants treated with the leaf extract of Datura metel L. Indian J. Bot. 5: 161-165.

Murthy, N.S. and Nagarajan, K. (1980). Virus inhibitory effect of extracts from germinating seeds of flowering plants. Indian Phytopathol. 33: 615-617.

Murthy, N.S., Nagarajan, K. and Sastry, A.B. (1981). Effect of prophylactic sprays of leaf extracts on the infection of tobacco by tobacco mosaic virus. Indian J. Agric. Sci. 51: 792-795.

Nagarajan, K. and Murthy, N.S. (1988). Screening of plants for antiviral properties against Tobacco mosaic virus. In: Proceedings of National Seminar on Management of crop diseases with plant products/ biological agents. 11-12 pp. 52.

Narasimhan, V., Gurubasavaraj, T.M., Mariappan, V. and Alagianagalingam, M.N. (1988). In: Proceedings of National Seminar on management of crop diseases with plant products/biological agents. 11-12 pp. 41.

Owens, R.A., Bruening, G. and Shephard, R.J. (1973). A possible mechanism for the inhibition of plant viruses by a peptide from Phytolacca americana. Virology 56: 390-393.

Pandey, A.K. and Bhargava, K.S. (1984). Effect of Ampelopteris prolifera leaf extract on the activity of tobacco mosaic and cucumber mosaic viruses. Indian Phytopathol. 37: 271-277.

Pandey, B.P. and Mohan, J. (1986). Inhibition of Turnip mosaic virus by plant extracts. Indian Phytopathol. 39: 489-491.

Pillayarsamy, K., Rabindran, R., Narayanasamy, P., Jeyarajan, R. and Doraisamy, S. (1988). Control of cowpea aphid borne virus. In: Proceedings of National Seminar on Management of crop diseases with plant products/biological agents. 11-12 pp. 47.

Ponniah, S., Saroja, R. and Ariyavanamkatha Pillai, M. (1988). Effect of neem products and slow release of nitrogen on green leaf hopper, the vector of rice tungro virus. In: Proceedings of National Seminar on management of crop diseases with plant products/biological agents. 11-12 pp. 41.

Prasad, V. (1986). Alternations in enzyme activity during induced antiviral state by leaf extracts. J. Indian Bot. Soc. 65: 90-94.

Ragetli, H.W.J. and Weintraub, M. (1962). Purification and characteristics of virus inhibitor from Dianthus caryophyllus L.I. purification and activity. Virology. 18: 232-240.

Ramachandran, P. and Summanwar, A.S. (1982). A new record of cowpea mosaic virus in India. Indian Phytopathol. 35: 667-670.

Rao, N.G. (1988). Studies on mycoplasma disease of rice with special reference to resistance. Ph.D. thesis, Tamil Nadu Agril. University, Coimbatore.

Rao, N.G. and Narayanasamy, P. (1988). Effect of plant products and oils on rice yellow dwarf infection. In: Proceedings of National Seminar on the manage- of crop diseases with plant products/biological agents. 11-12 pp. 44.

Rao, G.P. and Shukla, K. (1985a). Antiviral activity of copra extract and physical properties of the inhibitor. Indian Phytopathol. 38: 623.

Rao, G.P. and Shukla, K. (1985b). Induction of resistance against PVX in detached leaves of Chenopodium amaranticolor by corolloid root extract of Cycas revoluta. Indian Phytopathol. 38: 624.

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

Reddy, V.A. and Jeyarajan (1988). Effect of leaf extracts of Polyalthia longifolia and neem on infection of rice plants by yellow dwarf pathogen. In: Proceedings of national seminar on management of crop diseases with plant products/biological agents. 11-12 p. 44.

Reddy, K.R., Rao, G.N. and Sastry, K.S. (1988). Inhibition of cucumber mosaic virus in Safflower by plant extracts. In: Proceedings of national seminar on management of crop diseases with plant products/ biological agents. 11-12 pp. 49.

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

Roychoudhury, R. (1984). Virus inhibitor from Solanum Torvum. Indian Phytopathol. 37: 665-668.

Roychoudhury, R. and Basu, P.K. (1983). Characterisation of plant virus inhibitor from two Solanum species. Indian J. Exp. Biol. 21: 212-215.

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

Ruppel, E.G. (1967). Inactivation and inhibition from two Solanum species. Indian J. Exp. Biol. 21: 212-215.

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

Saksena, K.N. and Mink, G.I. (1969). Properties of an inhibitor of apple chlorotic leaf spot virus from Chenopodium guinoa. Phytopathology 59: 61-63.

Saxena, R.C., Khan, Z.R. and Bajet, N.B. (1985). Neem seed derivatives for preventing rice tungro virus transmission by the green leaf hopper N. virescense. Philipp. Phytopathol. 21: 88-102.

Selvan, N.S. and Narayanaswamy, P. (1987). Inhibition of potato virus of infection on chilli by plant extract. Madras Agric. J. 74: 154-156.

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

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

Sharma, S.R. and Varma, A. (1975). Three sap transmissible viruses from Cowpea in India. Indian Phytopathol. 28: 192-198.

Shinde, P.B., Sawant, D.M. and Ruikar, R.K. (1988). In: Proceedings on national seminar on management of crop diseases with plant products/biological agents. 11-12: 40.

Shukla, H.S., Rao, G.P. and Tripathi, S.C. (1985). Reduction in potato virus x and potato virus y infectivity by volatile constituents. Analesde Edafologia'y Agrobiologia, 44: 1763-1766.

Sill and Webster H, Jr. (1951). Some characteristics of a virus inhibitor in Cucumber. Phytopathology, 41: 32.

Simons, J.N., Swindler, R. and Moss, L.M. (1963). Succulent type plants as sources of plant virus inhibitors. Phytopathology, 53: 677-683.

*Singh, R. (1969). The inhibitory activity of some plant juices on the infectivity of watermelon mosaic juice. Acta Virol. 13: 244-246.

Singh, R. and Singh, R. (1973). Properties of an inhibitor of potato virus x from bark of Ficus elastica. Indian Phytopathol., 26: 560-563.

Singh, R., Mall, T.P. and Singh, R.R. (1985). Inhibitory activity of leaf extracts on the infectivity of arhar (Pigeon pea) mosaic virus. Int. pigeon pea newsl. 4: 38-40.

Singh, A.K. and Singh, A.K. (1984). Distribution and inactivation of cowpea mosaic virus in seeds and flowers of cowpea cultivar. Indian Phytopathol., 37: 568.

Singh, V. and Singh, R.A. (1979). Potato virus x inhibition by leaf extract of Cedrus deodara. Indian J. Mycol. Plant Pathol. 7: 85-86.

Singh, I. and Varma, J.P. (1981). Virus inhibitor from Datura metel. Indian Phytopathol. 34: 452-457.

Smookler, M.M. (1971). Properties of inhibitors of plant virus infection occurring in the leaves of species in the chenopodiales. Ann. Appl. Biol. 69: 157-168.

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

Srinivasulu, B. and Jeyarajan, R. (1986). Effect of leaf extracts on the infection of rice tungro virus in Rice. Indian J. Virol. 2: 176-180.

Srivastava, K.M., Chandra, S., Singh, B.P. and Abidi, S.M.H. (1976). Induction of local and systemic resistance in Nicotiana glutinosa against tobacco mosaic virus by leaf extract of Dahlia. Indian J. Exp. Biol., 14: 377-378.

Srivastava, K.M., Rana, N.S., Dwadash Shreni, V.C. and Singh, B.P. (1986). Mechanism of inhibition of CMV by crude oil from Margosa. Azadirachta indica during inoculation with single apterous Aphis gossypii. Indian Phytopathol., 39: 20-25.

Subbaraja Raja, K.T. and Arumugam, R. (1988). Studies on the comparative effect of plant extracts on banana bunchytop control. In: Proceedings of national seminar on management of crop diseases with plant products/biological agents. 11-12. page 40.

Tamura, M. (1969). Inhibition of Turnip mosaic virus by the juice extracted from Japanese Black pine. (Pinus thunberga Pail). Ann. Phytopathol. Soc. Jpn. 35: 260-264.

Tewari, J.P. (1976). Inhibition of three strains of watermelon mosaic virus by bark extracts. Curr. Sci., 45: 696-697.

Thresh, J.M. (1956). Some effects of Tannic acid and of leaf extracts which contain tannins on the infectivity of tobacco mosaic and tobacco necrosis virus. Ann. appl. biol. 44: 608-618.

Toler, P.R.W. (1964). Identity of a mosaic virus of cowpea. Phytopathology 54: 886-913.

*Twardowicz-Jakuszowa and Anna (1969). Investigation on pea enatron mosaic virus. Zesz. Probl. Postep. Nauk. roln. 94: 169-194.

Ushari, V., Nagarajan, K. and Reddy, T.S.N. (1982). Isolation and characterisation of inhibitor to tobacco mosaic virus from Basella alba L. Tob. Res., 8: 39-43.

Vasudeva, R.S. (1942). A mosaic disease of Cowpea. Indian J. Agric. Sci. 12: 281.

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

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

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

Verma, H.N. and Baranwal, V.K. (1985). Studies on plant virus inhibitor occurring in leaves of Celosia cristata. Indian Phytopathol. 38: 626.

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

Verma, H.N. and Mukerjee, K. (1975). Brinjal leaf extract induced resistance to virus infection in plants. Indian J. Exp. Biol. 13: 416-417.

Verma, H.N. and Mukerjee, K. (1979). Induction of anti-viral resistance in host plants by datura leaf extract. Indian Phytopathol. 32: 95-97.

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

Verma, H.N. and Prasad, V. (1983). Inhibitors of viruses: systemic resistance inducers from higher plants. In: Recent Adv. Pl. Path. (Ed A. Husain, Singh, K., Singh, B.P. and Agnihotri, V.P.): 312-324. Print house (India), Lucknow.

Verma, V.S. and Raychoudhuri, S.P. (1971). Effect of tannins and other chemicals on the infectivity of potato virus x Phytopath. Mediterranea 10: 124-127.

Verma, H.N., Awasthi, L.P. and Mukerjee, K. (1979a). Prevention of virus infection and multiplication by extracts from medicinal plants. Phytopathol. Z., 96: 71-76.

Verma, H.N., Awasthi, L.P. and K. Mukerjee (1982). Characteristics and mode of action of natural inhibitors of virus infection. In. Advancing frontiers of Mycology and Plant Pathology. K.S. Bilgrami (ed). Today and Tomorrow's Printers and Publishers, New Delhi. pp. 255-264.

Verma, H.N., Chowdhury, B. and Prasad, V. (1985a). Clerodendron aculeatum leaf extract induced virus inhibitory agent (VIA) from treated and non treated leaves of healthy Nicotiana Spp. Indian Phytopathol. 38.

Verma, H.N., Khan, M.M.A.A. and Dwivedi, S.D. (1985d). Bio-physical properties of highly antiviral agents present in Pseuderanthemum atropurpureum and Bougainvillea spectabilis extracts. Indian Phytopathol. 3: 13-20.

Verma, H.N., Srivastava and Alka (1985b). A potent systemic inhibitor of plant virus infection from Aerva sanguinolenta Blume. Curr. Sci., 54: 526-528.

Verma, H.N., Rastogi, P., Prasad, V. and Srivastava, A. (1985c). Possible control of natural virus infection on Vigna radiatus and Vigna mungo by plant extracts. Indian J. Plant Pathol. 3: 21-24.

Vijayakumar, J.S. and Narayanasamy, P. (1988). Effect of leaf extracts, non edible oils and seed kernel extracts on tomato leaf Curl virus infection. In: Proceedings of national Seminar on management of crop diseases with plant products/biological agents. 11-12, 52.

Weintraub, M. and Willison, R.S. (1953). Studies on stone fruit viruses in Curcurbit hosts. III effect of Cucurbits extracts on infectivity. Phytopathology, 43: 328-332.

Wyatt, S.D. and Shepherd, R.J. (1969). Isolation and characterisation of a virus inhibitor from Phytolacca americana. Phytopathology, 59: 1787-1794.

Yoshi, H. and Sako, N. (1967). Inhibitory effect of chenopodium sap on virus infection. Hypersensitive reaction of plant cytoplasm against incompatible inhibitor chenopodium sap. Ann. Phytopathol. Soc. Jpn. 33: 244-252.

Zaidi, Z.B., Gupta, V.P., Samad, A. and Naqui, Q.A. (1988). Inhibition of spinash mosaic virus by extracts of some medicinal plants. Curr. Sci., 57.

Zaitlin, M. and Siegel, A. (1963). A virus inhibitor from tobacco. Phytopathology, 53: 224-227.

* Original not seen

INHIBITORY EFFECTS OF CERTAIN PLANT EXTRACTS ON THE INCIDENCE OF COWPEA MOSAIC

BY

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ABSTRACT

The inhibitory effect of certain plant extracts on the incidence of cowpea mosaic was studied.

Preliminary screening of non-host plants for anti-viral property against cowpea mosaic virus revealed that extracts of sixteen plants viz. Adenocalyma allicea, Azadirachta indica, Boerhaavia diffusa, Bougainvillea spectabilis, Calotropis gigantea, Clerodendron infortunatum, Curcuma longa, Eupatorium odoratum, Ferrula indica, Mirabilis jalapa, Moringa olifera, Phyllanthus niruri, Polyalthia longifolia, Solanum indicum, Thespesia populnea and Vitex negundo inhibited the production of local lesions on the leaves of Chenopodium amaranticolor indicating that these extracts possessed antiviral property.

The extracts of A. indica, B. diffusa, B. spectabilis, C. gigantea, C. infortunatum, C. longa, M. jalapa, P. niruri, S. indicum, and V. negundo caused 100 per cent inhibition of the production of local lesions on C. amaranticolor even at five per cent concentration. The inhibitory effect of the plant extracts was found to be less pronounced when tested on the primary leaves of cowpea seedlings.

Pre-inoculation application of plant extracts was found to be more effective than post-inoculation application in checking the incidence of cowpea mosaic. In insect transmission studies with Aphis craccivora cent per cent inhibition of disease incidence was obtained with the extracts of A. indicum, C. infortunatum, P. niruri and V. negundo.

Studies on the effect of plant extracts on the acquisition and transmission of cowpea mosaic virus by A. craccivora revealed that the extract of P. niruri caused 100 per cent reduction over control.

Maximum inhibition of disease development was obtained in plants inoculated with cowpea mosaic virus upto two days after the application of extracts of A. indica, P. niruri, V. negundo and C. infortunatum.

Extracts of V. negundo and A. indica caused considerable reduction in the survival of A. craccivora on cowpea.

The present studies indicated that, the extract of C. infortunatum had some systemic effect against cowpea mosaic virus.

Repeated application of plant extracts on cowpea plants revealed that V. negundo had the maximum inhibitory

effect on symptom development, in both sap and insect transmission trials.

Height and weight of plants, number and weight of pods and root nodules of cowpea plants treated with plant extracts were found to be more than that of the control.