EFFECT OF SELECTED MEDICINAL PLANT EXTRACTS ON THE INCIDENCE OF PUMPKIN MOSAIC

By

VIMI LOUIS

THESIS

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DECLARATION

I hereby declare that the thesis entitled "Effect of selected medicinal plant extracts on the incidence of pumpkin 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

Vellanikkara
18 11 94

VIMI LOUIS

CERTIFICATE

Certified that the thesis entitled "Effect of selected medicinal plant extracts on the incidence of pumpkin mosaic " is a record of research work done independently by Ms Vimi Louis under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship

Vellayani
18 11 04

DR S BALAKRISHNAN
Chairman
Advisory Committee
Professor
Department of Plant Pathology
College of Agriculture
Vellayani

CERTIFICATE

We the undersigned members of the Advisory Committee of **Ms Vimi Louis** a candidate for the degree of **Master of Science in Agriculture** agree that this thesis entitled **'Effect of selected medicinal plant extracts on the incidence of pumpkin mosaic"** may be submitted by Ms V mi Louis in partial fulfilment of the requirements for the degree

Chairman

Dr S Balakrishnan
Professor
Dept of Plant Pathology
College of Agriculture
Vellayani

Members

1 Dr James Mathew
Professor & Head
Dept of Plant Pathology
College of Horticulture

2 Dr Sally K Mathew
Associate Professor
Dept of Plant Pathology
College of Horticulture

3 Dr A Augustin,
Assistant Professor
AICRP on M & AP
College of Horticulture

EXTERNAL EXAMINER

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VIMI LOUIS

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Introduction

1 INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch ex Poir) is an important vegetable crop cultivated in Kerala for its mature and immature fruits Its young leaves flowers and fruits are rich in carotene the precursor of vitamin A The crop is specially noted for its low cost of production and long keeping quality of fruits

A number of diseases affecting this crop have been reported from Kerala and other states of India Viral diseases are known to cause serious loss to this crop wherever it is cultivated and out of which pumpkin mosaic is the most serious one

For years, eradication of collateral hosts of the viruses altering planting dates and chemical protection against the insect vectors have been the principal means of control Cultivation of resistant varieties is the most efficient method for achieving maximum productivity by avoiding diseases None of the available pumpkin cultivars or varieties so far available or reported in the country possesses resistance to mosaic

There are many reports on the inhibitory effects of medicinal plant extracts on plant viruses Some of

these plant products are known to induce resistance in host plants against viruses Many plant extracts have been reported to possess insecticidal or insect repellent properties and thereby prevent the spread of vector borne viral diseases Plant products have the advantage over chemical protectents in that they are degraded in short period without leaving harmful residues (Verma, 1980)

No studies have been conducted so far on the inhibitory effects of medicinal plant extracts on the incidence of pumpkin mosaic In the present investigation, thirty species of plants were screened for their antiviral properties against pumpkin mosaic virus and promising ones were tested, at different concentrations, at different times of application, for their effect on vector transmission etc The present study was aimed at evolving an integrated management programme for pumpkin mosaic virus disease

Review of Literature

2 REVIEW OF LITERATURE

Pumpkin mosaic was first reported from India by Hariharasubramanian and Badami (1964) They observed that the disease was characterized by severe blistering distortion and stunting of leaves Jaganathan and Ramakrishnan (1971) found that a virus isolate from pumpkin produced mottling and malformation of leaves They also reported that plants infected early in the season remained dwarf and flowered sparingly A few leaves exhibited dark green vein banding along the midrib and lateral veins of affected plants Shankar *et al* (1972) reported that the first symptom of pumpkin mosaic virus (PMV) infection is mosaic mottling and other symptoms include late unfolding of young leaves and complete chlorosis followed by green vein banding The older leaves had dark green blisters with the rest of lamina being chlorotic Flowering was delayed and flower and fruit size were reduced They observed that the virus was transmitted by *Aphis gossypii* and through sap The virus resembled cucumis virus causing bottle gourd mosaic and cucumis virus 1 causing mosaic of vegetable marrow Umamaheswaran (1985) observed severe mottling and disfiguration of leaves of pumpkin (*Cucurbita*

moschata Duch) infected with pumpkin mosaic virus Dark green vein banding, irregular chlorotic spots and mottling with mild green blisters were the other symptoms Stunting of plants, sparse flowering and fruit setting were also noticed

2 1 Transmission

2 1 1 Mechanical transmission

Mechanical transmission of pumpkin mosaic virus was first reported by Hariharasubramanian and Badami (1964) Jaganathan and Ramakrishnan (1971) found that two virus isolates from musk melon (*Cucumis melo* L) and pumpkin (*C moschata* Duch) could be mechanically transmitted to healthy plants of the same species A mosaic virus of pumpkin (*C moschata* Duch) commonly occurring in Delhi was also found to be sap transmissible (Shankar *et al* , 1972) They prepared the inoculum for mechanical inoculation in 0 066 M phosphate buffer of pH 7 6 from mosaic affected leaves

Umamaheswaran (1985) reported that symptoms of pumpkin mosaic appeared 12-14 days after mechanical inoculation and the percentage of transmission varied with the extraction medium used

2 1 2 Insect transmission

Pumpkin mosaic virus was reported to be transmitted by a number of insect vectors Hariharasubramanian and Badami (1964) who reported pumpkin mosaic for the first time from India found that the causal virus was transmitted by *Aphis laburni* and many other *Aphis* spp Nagarajan and Ramakrishnan (1971a) found that the viruses commonly occurring in pumpkin could be non-persistently transmitted by *Myzus persicae, A gossypii* and *A nerii* Roy and Mukhopadhyay (1980) found that PMV was transmitted by *A gossypii* in a non-persistent manner Singh (1981, 1982) reported that pumpkin mosaic virus was transmitted by *Aphis gossypii* as well as by *A craccivora*

Umamaheswaran (1985) studied insect transmission of pumpkin mosaic virus using three vectors, viz , *Aphis gossypii, Aphis craccivora* and *Bemisia tabaci* Highest percentage of transmission was obtained with *Aphis gossypii* and *Bemisia tabaci* was found to be a non vector of PMV

The vector-virus relationship of a virus occurring on pumpkin was studied by Nagarajan and Ramakrishnan (1971b) They observed that virus was non-persistently transmitted by *M persicae, A gossypii* and *A nerii*

Umamaheswaran (1985) found that a short acquisition feeding period of 30 s was sufficient for the aphids (*A gossypii*) to become viruliferous, and optimum acquisition feeding period was 15 min Similarly minimum inoculation feeding period was one min whereas optimum was 15 min

2 2 Antiviral properties of plant extracts

Many plant extracts are known to possess inhibitory action against viruses causing plant diseases Leaf extract of *Phytolacca* sp was found inhibitory to TMV infection by Allard (1918) Since then many plant extracts have been reported to inhibit tobacco mosaic virus Kuntz and Walker (1947) 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 juice of cabbage infected with cabbage mosaic virus almost completely inhibited the infectivity of those juices Kassanis and Kleczkowski (1948) isolated a TMV inhibitor from the sap of *Phytolacca esculenta* It caused an immediate reduction in infectivity and the non-infective mixture regained infectivity when diluted Allen and Kahn (1957) reported the presence of inhibitors in the rice sap against tobacco mosaic virus

According to them the inhibitor extracted from leaves and roots was highly active at dilution up to 1 200 and that extracted from rice polish was active at dilution up to 1 6000 Inhibition of tobacco mosaic virus and tobacco necrosis virus by strawberry and raspberry leaf extracts was reported by Cadman (1959) Inhibitors from many succulent plants were found to retain antiviral property against TMV for 10 min at 80 C (Simons *et al* , 1962)

Thakur and Sastry (1971) reported the antiviral effects of extracts of *Boerhaavia diffusa, Bougainvillea spectabilis* and *Mirabilis jalapa* even at 1 10^4 dilution against petunia distortion strain of TMV Extracts were also effective when sprayed on hosts 24 h before or within 5 min after inoculation Latex of *Calotropis procera* reduced infectivity of TMV when sprayed on *Nicotiana glutinosa* before or after inoculation It was found to be stable against dilution and storage (Khurana and Singh 1972) Lal and Verma (1974) reported that latex of *Jatropha* sp significantly reduced infectivity of TMV on different hosts Verma and Mukerjee (1975) pointed out that brinjal leaf extract induced local and systemic resistance in *Nicotiana glutinosa* against TMV and in *N tabaccum* NP 31 against tobacco ring spot virus when applied 24 h before inoculation Dahlia leaf extract induced local and systemic resistance in *N glutinosa* against TMV

The local resistance persisted in the treated leaves up to eight days with 91 per cent inhibition of lesion production Inhibitory principle of the extract could move rapidly from treated to untreated portion of leaves (Srivastava *et al* , 1976) Okuyama *et al* (1978) found out a TMV inhibitor from *Yucca recurvifolia* Verma *et al* (1979) reported two virus inhibitors from *Euphorbia hirta* and *Boerhaavia diffusa* Both the plant extracts were found to be inhibitory to four viruses such as tobacco mosaic virus, sunnhemp rosette virus, gomphrena mosaic virus and tobacco ring spot virus in their hypersensitive hosts The inhibitory property of root extract of *B diffusa* was highly significant against elongated viruses (TMV and SRV) than against spherical viruses (GMV and TRSV)

Moreno (1980) reported the inhibitory effect of *Chenopodium quinoa, C amaranticolor, Opuntia* sp , potato, spinach *Solanum nigrum, Datura stramonium, Pelargonium geranium, Pennisetum claudestinum* and *Cuscuta* sp against TMV Germinating seeds of blackgram possessed virus in hibitory property against TMV in tobacco (Murthy and Nagarajan 1980) Virus inhibitors from *Datura metel* against tobacco mosaic virus were reported by Singh and Verma (1981) The inhibitory effect of prophylactic spraying of *Basella alba* leaf extract on the infection of tobacco by TMV was reported by Murthy *et al* (1981) and

Usharı *et al* (1982) Roychoudhury and Basu (1983) reported that crude extracts from *Solanum khasıanum* and *S nıgrum* were ınhıbıtory to tobacco mosaıc vırus and sunnhemp rosette vırus ın theır local lesıon hosts, *Nıcotıana glutınosa* and *Cyamopsıs tetragonoloba*, respectıvely Joharı *et al* (1983) poınted out vırus ınhıbıtory propertıes of leaf extracts of *Helıanthus annuus* agaınst TMV ın detached leaves of *Nıcotıana tabacum* var Samsun NN Induced resıstance was reported to be the maın mode of actıon of the ınhıbıtor Sprayıng wıth aqueous leaf extracts of *Bougaınvıllea spectabılıs* protected tomato, lemon and *Crotalarıa juncea* plants agaınst ınfectıon of TMV tomato yellow mottle mosaıc vırus, physalıs shoestrıng mosaıc vırus and cucumber green mottle mosaıc vırus (Verma and Dwıvedı, 1983) Habıb *et al* (1984) reported that latex of *Euphorbıa pulcherrıma* ınhıbited TMV Partıally clarıfıed aqueous extracts of *Pseuderanthemum bıcolor* leaves sprayed 1-6 days before ınoculatıon showed ınhıbıtory propertıes agaınst TMV ın tomato and tobacco, sunnhemp rosette vırus ın *Crotalarıa juncea* and cucumber green mottle mosaıc vırus ın *Cucumıs melo* var *momordıca* When only the lower leaves were treated, both lower and upper leaves developed resıstance whıch persısted for more than one week (Verma and Khan 1984) Rao *et al* (1984) found the antıvıral actıvity of coralloıd root

extract of *Cycas revoluta* against tobacco mosaic virus potato virus X potato virus Y and tomato ring spot virus when applied on tomato and *Chenopodium amaranticolor* test plants 24 h before virus inoculation, or when mixed with virus inocula before inoculation Pre-inoculation sprays of *Boerhaavia diffusa* extract was found to be effective against TMV in tobacco, cucumber mosaic virus and TMV in tomato cucumber green mottle mosaic virus in melon sunnhemp rosette virus in *Crotalaria juncea* and *Gomphrena globosa* (Awasthi *et al* , 1984) Pandey and Bhargava (1984) reported the effectiveness of *Ampelopteris prolifera* leaf extract against TMV and CMV Maximum inhibition was noticed when applied 24 h prior to virus inoculation in both local lesion and systemic hosts Roychoudhury (1984) found out a TMV inhibitor from *Solanum torvum*

Verma *et al* (1985) reported that leaf extracts of *Clerodendron aculeatum* induced local and systemic resistance in several host plants against TMV infection Verma and Srivastava (1985) reported a potent systemic inhibitor of tobacco mosaic virus and sunnhemp rosette virus from leaves of *Aerva sanguinolenta* Plant extract of *Celosia cristata* contains virus inhibitor against viruses belonging to tobamovirus group Inhibitory property of the plant extract was local and a high degree of inhibition was retained in the treated plants up to six days (Verma and

Baranwal 1985) Saigopal *et al* (1986) reported the antiviral activity of root and leaf extract of *Phyllanthus fraternus* against tobacco mosaic, peanut green mosaic and tobacco ring spot viruses TMV inhibition by extracts of *Artocarpus choplasha, Pentapanax leschenaultti* and *Syzygium arnottianum* was reported by Joshi *et al* (1986) Murthy and Nagarajan (1986) found out the inhibition of TMV in nursery and field grown tobacco by the twig extracts of *Pithecolobium dulce* leaf extract of *Peltophorum ferrugenium*, tannic acid and milk Alexander *et al* (1987) reported that leaf extract of *Alternanthera brasiliana, A ficoidea, Bougainvillea spectabilis, Chenopodium amaranticolor, C ambrosiodes, Iresine herbsti, Mirabilis jalapa* and *Phytolacca thirsiflora* are very effective when applied to *Phaseolus vulgaris* plants before mechanical inoculation with TMV, inhibiting infection by more than 70 per cent and even 100 per cent in some cases Doraisamy and Ramakrishnan (1988) screened 423 species of plants for antiviral principles against tobacco mosaic virus Out of these, 11 plant species, viz , *Peltophorum ferrugenium, Crassula indica, Eugenia jambosa, Turnera ulmifolia* var *elegans, Achras sapota, Bougainvillea spectabilis, Mirabilis jalapa, Pisonia alba, Beta vulgaris, Chenopodium murale* and *C ambrosiodes* gave 100 per cent inhibition

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 protected the crop from TMV infection up to 60 days Next best were leaf extracts of bougainvillea and clerodendron Shukla *et al* (1989) reported the antiviral effects of essential oils of *Foeniculum vulgare* and *Pimpinella anisum* against tobacco mosaic tobamovirus, tobacco ring spot nepovirus, potato X potyvirus in the hypersensitive host *Chenopodium amaranticolor* Both oils at 3000 ppm completely inhibited the formation of local lesions in the host Peshney and Moghe (1989) screened 46 plant species for their antiviral properties against TMV from chilli Among them, leaf extracts of *Polianthus tuberosa, Withania somnifera, Capsicum annuum* cv Perennial and *Abrus precatorius* gave 90-100 per cent inhibition Inhibitors from these plants were systemic in nature and capsicum plants were symptomless for 30 days following a single pre-inoculation treatment with crude leaf extract

Chen *et al* (1991) found the effect of pokeweed antiviral proteins (PAP) against TMV cucumber mosaic cucumovirus, African cassava mosaic geminivirus and cauliflower mosaic caulimovirus Essential oil from *Blumea lacinata* inhibited tobacco mosaic tobamovirus in *Nicotiana*

glutinosa (Khanna et *al* , 1991) Baranwal and Verma (1992) reported that leaf extract of *Celosia cristata* showed antiviral activity against tobacco mosaic tobamovirus sunnhemp rosette virus and potato X potyvirus in several local lesion hosts

Many plant extracts contain inhibitors of other viruses like cucumber mosaic virus, potato virus X potato virus Y, rice tungro virus etc apart from tobacco mosaic virus

Mc Keen (1956) reported the inhibition of cucumber mosaic virus and tobacco ring spot virus infection on *Chenopodium hybridum* by extrtacts from *Capsicum frutescens* Foliar application of *Mirabilis jalapa* leaf extract caused marked suppression of disease symptoms improved growth and flowering and considerably reduced virus multiplication rate in the treated cucumber plants against cucumber mosaic virus cucumber green mottle mosaic virus and urd bean mosaic virus and tomato plants against tomato yellow mottle and tomato yellow mosaic virus (Verma and Kumar 1980)

Blaszezak et *al* (1959) reported the inhibitory property of *Capsicum frutescens, Chenopodium album, C amaranticolor, Solanum integrifolium,* potato, spinach broad bean *Phaseolus vulgaris* and clover against PVX

Extracts of *C frutescens* and *C amaranticolor* showed inhibitory property up to 1 500 and 1 100 dilution whereas pelargonium extract remained inhibitory up to a dilution of 1 2000 Bark extract of *Ficus elastica* contained antiviral principles which prevented local lesion development on *Chenopodium amaranticolor* by PVX (Singh and Singh, 1973) Essential oils from *Carum capticum* and *Cymbopogan citratus* reduced infectivity of PVX and PVY even up to a dilution of 4 1000 (Shukla *et al* , 1985) Rao and Shukla (1985a) observed that application of aqueous coralloid root extract of *Cycas revoluta* induced resistance against PVX infection in detached leaves of local lesion host, *Chenopodium amaranticolor*

According to Gupta and Raychaudhuri (1972) the leaf extracts of *Callistemon lanceolatus* and *Syzygium cumini* inhibited local lesion production when mixed with sap from potato virus Y infected plants Rao and Shukla (1985b) reported that aqueous extracts of dry coconut (copra) showed significant antiviral activity against PVY when applied 24 h before virus inoculation and tested on *Chenopodium amaranticolor* leaves No such inhibition was observed when extract was applied 24 h after virus inoculation Selvan and Narayanasamy (1987) pointed out that leaf extract of *Basella rubra* was most effective in inhibiting PVY infection in chilli followed by extracts from *B*

alba, Bougainvillea spectabilis and *Mirabilis jalapa*

Srinivasulu and Jeyarajan (1986) reported the inhibitory properties of leaf extracts of *Mirabilis jalapa*, coconut and sorghum against rice tungro virus (RTV) 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%) reduced RTV infection They also studied the effect of leaf extracts of *Vitex negundo, Mirabilis jalapa* and *Euphorbia jeniculata* and fruit extract of *Aegle marmelos* against RTV infection and found to inhibit the virus

Singh (1969) conducted experiments with crude sap of *Chenopodium album, C amaranticolor, Dahlia rosea* and *Spinacea oleracea* against watermelon mosaic virus and reported hundred per cent inhibitory effect on the virus The effect was reduced to 50-80 per cent by 1/1000 dilu tion of crude sap Tewari (1976) reported the effects of several bark extracts on the inactivation of three strains of watermelon mosaic virus

Tripati *et al* (1981) reported the antiviral activity of *Adathoda vasica* against bean mosaic virus Bose *et al* (1983) found that leaf extract of *Adenocalymma allicea* contained an inhibitor of common mosaic virus

infection on cowpea It was more effective when used with virus or when sprayed before inoculation

An antiviral principle against tomato spotted wilt virus was reported by Narayanasamy and Ramaiah (1983) from sorghum leaves Joi *et al* (1988) conducted studies on the inhibitory effects of the leaf extracts of 16 plant species on tomato spotted wilt virus and found that leaf extracts of chilli acacia, datura and chenopodium showed more than 80 per cent inhibition of the virus at 1 10 dilution Inhibition of tomato spotted wilt virus by plant extracts of *Crotalaria juncea, Morus alba, Delonix regia* and *Tecoma grandis* was reported by Velazhahan and Narayanasamy (1991) Khan and Zaim (1992) found that treatment of susceptible host plants with extracts from leaves of *Operulina turpethum* and bulbs of *Scilla indica* induced systemic resistance to subsequent challenge with tomato spotted wilt virus sunnhemp rosette virus tobacco mosaic tobamovirus and datura shoestring potyvirus

Leaf extract of *Prosopis chilensis* showed 100 per cent inhibition of tobacco necrosis virus (Sekar *et al* , 1991) Stevans and Reynolds (1992) reported the antiviral property of leaf extract of *Reynoutria japonica* against tobacco necrosis necrovirus in french bean

Sreelakha and Balakrishnan (1988) found that pre-inoculation sprayings with leaf extracts of *Bougainvillea* sp and *Eupatorium odoratum* were effective in controlling the incidence of cowpea mosaic disease By screening 30 non host plants against cowpea mosaic virus, Mallika Devi (1990) obtained maximum inhibitory effect of 80-90 per cent for the extracts of *Phyllanthus niruri, Clerodendron infortunatum* and *Vitex negundo*

Vasudeva and Nariani (1952) found out that leaf extract of *Datura stramonium, Capsicum annuum, Solanum nigrum* and *Lycopersicon esculentum* adversely affected the infectivity of bottlegourd mosaic virus The symptom expression of tobacco ring spot virus in cowpea was found to be inhibited by New Zealand spinach extract (Benda, 1956) Roy *et al* (1979) indicated that extracts of *Ocimum sanctum, Dianthus caryophyllus, Capsicum annuum, Zingiber officinale* and *Nicotiana tabaccum* possessed virus inhibitors against top necrosis virus of pea effective at 1 1000 dilution of inhibitor-virus mixture Mukerjee *et al* (1981) pointed out that *Datura metel* showed antiviral property against gomphrena mosaic and sunnhemp rosette viruses in their hypersensitive hosts

Singh *et al* (1985) reported that extracts from *Capsicum annuum* and *Datura stramonium* prevented infection

of arhar mosaic virus in arhar variety sharda when applied before inoculation Verma *et al* (1985) tested the inhibitory properties of leaf extracts of *Clerodendron fragrans* root extract of *Boerhaavia diffusa* for controlling natural viral infection of *Vigna radiata* and *Vigna mungo* and observed that four per cent foliar spray given at an interval of three to four days from seedling stage onwards reduced infection up to 60 per cent in the case of *C fragrans* but only delayed symptom expression when *B diffusa* root extract was used Yield of plants treated with *C fragrans* leaf extract was considerably enhanced Verma *et al* (1985) found out an inhibitor of yellow mosaic disease of beans from *Pseuderanthemum atropurpureum* Awasthi *et al* (1985) reported the control of virus diseases of vegetables like tomato, potato and french bean by an inhibitor isolated from *Boerhaavia diffusa* root extract when applied twice per week for one month starting from the seedling stage Plant extracts of *Syzygium cumini, Acacia arabica* and *Callistemon lanceolatus* have been found to inhibit turnip mosaic virus but the inhibition was not significant when the extracts were applied after inoculation (Pandey and Mohan 1986) An inhibitor of bean yellow mosaic virus infecting *Chenopodium quinoa* was reported from faba bean sap by Govier and White (1987)

Zaidi *et al* (1988) found that some medicinal plants have got inhibitory effects on spinach mosaic virus The inhibitory effects of plant extracts was directly correlated with increase in concentration Highest inhibition was achieved by applying leaf extract from *Ocimum sanctum* Vijayakumar and Narayanasamy (1988) observed that leaf extract of *Ocimum sanctum, Azadirachta indica, Cocos nucifera, Nerium indica, Calotropis gigantea, Eucalyptus globulus, Acacia arabica* and *Ficus bengalensis* were effective in reducing the percentage of infection by tomato leaf curl virus

Sewant and Ambekar (1990) tested the effects of leaf extracts of *Capsicum annuum, Acacia arabica, Datura metel, Azadirachta indica* and *Spinacea oleracea* against safflower mosaic virus on *Chenopodium amaranticolor* The leaf extract of *D. metel* at 1 1000 dilution produced maximum inhibition (38 7%) Two sprays before and after inoculation given at seven days interval after 10 days of sowing of safflower, showed maximum inhibition of the virus in *in vivo* experiments Alcohol extracts from callistemon, datura, agave and ginger showed good degree of suppression of bhindi yellow vein mosaic virus symptoms in bhindi (Choudhury *et al* 1992) Prevention of soybean mosaic virus infection in *Glycine max* by leaf extract of

Clerodendron aculeataum was reported by Verma *et al* (1992) Patel and Patel (1993) observed that *Clerodendron inerme, Parkinsonia eculeata* and *Ipomea carnea* completely inhibited chlorotic mottle mosaic virus infection in bidi tobacco

2 3 Effect of plant extracts on vectors of plant viruses

Apart from the inhibitory action of botanicals on viruses and their infection in plants by sap transmission, there are many reports on the inhibitory effects of botan icals on the transmission of viruses by vectors

Mariappan *et al* (1982) observed that many seed oils such as custard apple oil and neem oil possessed inhibitory action against rice tungo virus (RTV) Seed oils from *Azadirachta indica* and *Annona* sp at five per cent reduced RTV infection on seedlings of the cultivar TN-1 No insect survived on the sprayed plants after four days Saxena *et al* (1985) found that neem seed deriva- tives prevented the transmission of RTV by the green leaf hopper *Nephotettix virescens* Srinivasulu and Jeyarajan (1986) reported that pre-inoculation sprays of rice seedlings with leaf extracts of *Mirabilis jalapa,* coconut and sorghum reduced RTV transmission by the green leaf hopper, *N virescens* and increased the incubation period in the plants A pre inoculation spray with *Mirabilis*

jalapa leaf extract reduced transmission up to five days after spraying and maximum reduction in transmission was observed when the plants were inoculated one day after the treatment Ponnaiah *et al* (1988) observed that neem seed extract, neem oil cake extract and neem leaf extract also reduced the population of leaf hopper vector of RTV significantly Mariappan *et al* (1988) found that non-edible oils extracted from seeds of karanj (*Pongamia pinnata*) mahua (*Madhuca longifolia* var *latifolia*) and pinnai (*Calophyllum inophyllum*) were most effective than the seed oil of neem (*Azadirachta indica*) in reducing the survival of green leaf hopper *Nephotettix virescens* Insect mortality was 100 per cent four days after spraying compared to 60 per cent survival on control RTV infection was 17-35 per cent in treated plants compared to 51 per cent in control Gurubasavaraj (1988) reported that mahua oil and neem oil at the ratio of 1 4 was most effective than 1 1 and 2 1 proportions in decreasing the vector longivity and reduced RTV transmission

Dube and Nene (1974) reported that aphid transmission of cowpea mosaic virus was inhibited by oil sprays They found that castor oil (2 5%), 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* Mallika Devi (1990) got 100 per cent

inhibition of insect transmission of cowpea mosaic virus with extracts of *Azadirachta indica, Clerodendron infortunatum Phyllanthus niruri* and *Vitex negundo*

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* Srivastava *et al* (1986) found that crude margosa oil (0 5% water emulsion) inhibited the transmission rate of cucumber mosaic virus by single apterous aphid They also observed that oil did not affect the biological activity of the virus but it influenced the feeding behaviour of the aphids Roychoudhury and Jain (1989) found that neem oil (2 4%) and neem soap sprays caused complete mortality of the nymphs and alate forms of *Aphis rumicis* within 24 h Srimannarayana (1990) reported that derivatives from seed oils of indigenous trees like *Butea frondosa* and *Ailanthus excelsa* had promising insecticidal activity Subba Rao (1990) found that RD-9 Repelin consisting of neem, karanj and mahua oils had promising antifeedant repellant, antiviral and phyto tonic properties and also increased yields Nagasampagi *et al* (1990) reported the extraction of two Azadirachtin-rich fractions of neem seed oil of which fraction A2 exhibited insecticidal and acaricidal properties and fraction B2 exhibited antifeedant activity

Choudhury *et al* (1992) found that alcohol extracts from callistemon, datura agave and ginger showed good degree of suppression of symptoms of bhindi yellow vein mosaic virus by controlling the whitefly, *Bemisia tabaci* They got 20-80 per cent mortality of the whitefly, when they were confined for 30 min in a cage with plants treated with extracts Hunter and Ullman (1992) showed the effect of the neem product RD-Repelin on settling behaviour and transmission of zucchini yellow mosaic virus by the pea aphid *Acyrthosiphon pisum* RD-Repelin delayed symptom expression of zucchini yellow mosaic virus in 81 per cent plants treated with one per cent concentration although virus transmission was not prevented

2 4 Modes of action of plant extracts

Modes of action of inhibitors are mainly divided into two, viz , direct action on the virus and action via the host plant In the first process, the host is not involved in the suppression of disease symptoms and the effect of inhibitor is directly on the virus particles

Hirai (1949) observed that the inhibitory effect of chilli leaf extract was reversible after the dilution of the extract Franki (1964) found that a high molecular weight substance from the extract of cucumber leaves

caused aggregation of cucumber mosaic virus Coating of tobacco mosaic virus by a high molecular weight polysaccharide inhibitor present in the leaves of *Physarum polycephalum* and *Abutelon striatum* leaves was shown by electron micrograph studies by Mayhew and Ford (1971) and Moraes *et al* (1974) Several other naturally occurring compounds such as tannins, phenolics and saponins have been reported to interact with viruses forming a loose combination with viral RNA, causing aggregation of virus particles or denaturing of the nucleocapsid (Muftuoglu and Nienhaus, 1976)

Inhibitors which act via the host plant may induce local or systemic resistance to the host plant Plant extracts inducing local resistance act by preventing first stages of infection process such as adsorption or penetration of the virus into the host cell or by blocking or competing with the virus receptor sites on the leaf surface Kammen *et al* (1961) studied the mode of action of inhibitor from carnation (*Dianthus caryophyllus*) sap and the action of inhibitor was described as blocking virus receptors Verma and Awasthi (1979) reported that plant extracts affected the susceptibility of the host by altering its metabolism, so that the introduced virus particles are unable to multiply Verma and Baranwal (1985) and Baranwal and Verma (1992) observed that pre-inoculation

treatment with *Celosia cristata* leaf extract prevented lesion production by sunnhemp rosette virus tobacco mosaic tobamovirus and potato X potyvirus in several local lesion hosts The extract inhibited lesion formation only in treated areas and did not act on the virus directly but only via the host Semipermanent blocking of virus attachment sites was indicated as the mode of action Inhibition of early stages of infection of tobacco necrosis necrovirus in french beans by plant virus inhibitors from members of polygonaceae was reported by Stevans and Reynolds (1992)

Besides affording local protection against different viruses, a few of the plant extracts were also capable of inducing systemic resistance in plants It was Mc Keen (1956) who for the first time demonstrated that the application of pepper (*Capsicum frutescens* L) extract at some distance from the point of virus inoculation inhibited lesion formation on cowpea and *Chenopodium* Induction of systemic resistance by leaf extracts of higher plants was demonstrated for the first time by Verma and Mukerjee (1975) They reported that brinjal leaf extract induced local as well as systemic resistance in *Nicotiana glutinosa* against TMV and in *N tabaccum* against tobacco ring spot virus when applied 24 h before virus inoculation This induced resistance was reversed by

simultaneous application of Actinomycin-D Lal *et al* (1973) found that a time lapse between brinjal leaf extract application and virus challenge was essential for the development of resistance It was demonstrated that brinjal leaves contained two inhibitors The proteinaceous, low molecular weight inhibitor showed pronounced activity at remote site, while the other polysaccharide inhibitor having high molecular weight showed greater activity at site of application

Verma and Awasthi (1980) were able to demonstrate a strong and highly potent antiviral agent from roots of *Boerhaavia diffusa* inducing systemic resistance in several host virus combinations The induced resistance was sensitive to Actinomycin-D treatment indicating thereby the synthesis of virus inhibitory substances which are translocated in treated host plants They also isolated the induced virus inhibitory agent and suggested it to be proteinaceous Mukerjee *et al* (1982) reported that *Datura metel* leaf extract induces resistance in treated host plants against viral infection Antiviral activity of treated host plants was maximum 32 h after treatment Johari *et al* (1983) reported the induction of resistance against tobacco mosaic virus in detached leaves of *Nicotiana tabaccum* var *samsun* NN by leaf extract of *Helianthus annus* Verma and Dwivedi (1983, 1984) found that

development of resistance in plants treated with *Bougainvillea* leaf extract was associated with formation of some virus interfering substances Leaf extract from treated host plants could inhibit virus infectivity The inhibitory stimulus of *Aerva sanguinolenta* leaf extract moved from treated to untreated leaves within 1 h of application and the resistance induced was both of systemic and of long duration (Verma and Srivastava 1985) Verma *et al* (1985) found that induction of resistance by *Pseuderanthemum atropurpureum* and *Bougainvillea spectabilis* against several viruses was host-mediated The activity was lost by the simultaneous application of Actinomycin-D

Khan and Verma (1990) pointed out that aqueous leaf extracts of *Pseuderanthemum bicolor* when sprayed on *Cyamopsis tetragonoloba* provoked synthesis of a virus inhibitory agent (VIA) and purified VIA showed characteristics of proteins Treatment of susceptible host plants with extracts from leaves of *Operulina turpethum* and bulbs of *Scilla indica* induced systemic resistance to subsequent challenge with sunnhemp rosette virus, tobacco mosaic tobamovirus, datura shoestring potyvirus and tomato spotted wilt virus Resistance was significantly reversed by application of actinomycin-D at the same time of treatment with extracts (Khan and Zaim, 1992)

2 5 Mode of spread of pumpkin mosaic in the field

The concept of the gradient of disease incidence resulting from dispersal from a source was applied to plant viruses by Gregory and Read (1949) Spread of viruses within a plot is described in several ways showing the actual layout of infected and uninfected plants (Garret and Mc Lean, 1983) progress curves in which the number of infections or respective rate is plotted against time or distance (Thresh, 1974) and mathematical expression which incorporate and quantify parameters which are involved in the spread (Vanderplank, 1963 and 1982)

The spatial distribution of infected plants in a plot is indicative of the form of spread Incoming vectors are found to inoculate the plants in the field at random Secondary infections are found mostly around an infected source Therefore infections appear in clusters and a new infection is more likely to be found close to, rather than at a distance from an infection source (Raccah, 1986)

The distance from the source of virus is important and the nearer the source the more are the chances of a crop being infected early It is particularly true for nonpersistent aphid-borne viruses, which are usually

carried over short distances measurable in terms of meters (Basu and Giri, 1983) Dissemination of non-persistent viruses is along a spatial gradient and inversely and exponentially related to distance from the source of infection So the minimum effective isolation distance can be estimated from quantification of disease gradients (Thresh, 1976)

Materials and Methods

3 MATERIALS AND METHODS

3 1 Symptomatology

Development of symptoms of pumpkin mosaic virus infection was studied by observing naturally infected and artificially inoculated pumpkin seedlings Seeds of pumpkin (*Cucurbita moschata* var Ambili) obtained from Department of Olericulture College of Horticulture Vellanikkara were sown in polybags containing potting mixture consisting of sand soil and cowdung in 1 1 1 ratio The culture of pumpkin mosaic virus obtained from naturally infected pumpkin plants was maintained in an insect proof net house by repeated transfers through sap inoculation on pumpkin seedlings at two leaf stage

3 2 Transmission of the virus

3 2 1 Sap transmission

Sap transmission trials were conducted by using standard aqueous extract and sap extracted in different buffers of the phosphatic group viz phosphate buffer potassium phosphate buffer Sorenson's phosphate buffer and potassium phosphate sodium hydroxide buffer at 0 1 M and pH 7 2 Carborundum powder (600 mesh) was used as abrasive for sap inoculation

The standard aqueous extract was prepared by triturating young leaves of plants showing typical symptoms of pumpkin mosaic virus (PMV) infection into fine pulp by adding one ml of sterile distilled water for every gram of diseased tissue The extraction of sap was done by using sterilized chilled mortar and pestle When buffers (0 1 M pH 7 2) were used as the extraction medium sap was extracted after adding one ml of the buffer to every gram of infected leaf tissue

The sap extracted was filtered through fine muslin cloth and the filtrate was centrifuged at 3000 g for 20 min and supernatant was used as inoculum

A small quantity of carborundum powder was dusted uniformly on the upper surface of the leaves of the 10 day old pumpkin seedlings at two leaf stage before application of inoculum Inoculation was done by gently rubbing on the upper surface of leaves with a piece of absorbent cotton wool soaked in the inoculum Soon after inoculation the inoculated leaves were washed with distilled water using a wash bottle

3 2 2 Aphid transmission

Aphid transmission studies were conducted by using

the culture of vector *Aphis gossypii*, reared on healthy pumpkin (*Cucurbita moschata*) plants in insect proof cages

The aphids were collected by giving a gentle tap to the plants to disturb them from their feeding position The moving aphids were transferred to petridishes by using a camel hair brush They were starved for a period of one hour (pre-acquisition fasting) and then allowed to feed on young leaves of pumpkin plants infected with pumpkin mosaic virus for an acquisition access period of 30 min in insect proof cages A fixed number of the viruliferous aphids (ten numbers per plant) were then transferred to young healthy pumpkin plants of two leaf stage kept in insect proof cages for an inoculation access period of 24 h and after that they were killed by spraying quinalphos (0 05%) Twenty seedlings were inoculated like this and an equal number of seedlings were kept as control The experiment was repeated to confirm the results

3 3 Preliminary screening of medicinal plant extracts for antiviral property against pumpkin mosaic virus

Virus inoculum was prepared in phosphate buffer as mentioned under 3 2 1

Plant extracts were prepared in distilled water by triturating the plant part, viz leaves, roots and stem using sterilized mortar and pestle For each gram of plant

tissue one ml of distilled water was added triturated into a pulp and then filtered through muslin cloth The crude sap was centrifuged at 3000 g for 20 min and supernatant was used for study The following plants were used for the experiment

Botanical name	*Common name	Family	Part used
1	2	3	4
1 *Adathoda vasica* Nees	Vasaka (Adalodakam)	Acanthaceae	Leaf
2 *Adenocalymma allicea* Linn	Ornamental garlic (Veluthullichedi)	Begnonaceae	Leaf
3 *Aegle marmelos* Corr	Bael (Koovalam)	Rutaceae	Root
4 *Alstonia venenatus* R Br	(Analivegam)	Apocynaceae	Bark
5 *Anamirta cocculus* W et A	Fish berry (Polla)	Menispermaceae	Fruit
6 *Aristolochia indica* Linn	Birth wort (Karalakam)	Aristolochiaceae	Root
7 *Asperagus officinalis* Linn	Asperagus (Shathavari)	Liliaceae	Tuber
8 *Azadirachta indica* A Juss	Neem tree	Meliaceae	Leaf
9 *Basella alba* Linn	Spinach Indian (Vallikkeera)	Basellaceae	Leaf

Contd

1	2	3	4
10 *Boerhaavia diffusa* Linn	Horse Purslane (Thazhuthama	Nyctaginaceae	Root
11 *Calotropis procera* Linn	Akund (Vella erukku)	Asclepiadaceae	Leaf
12 *Chromalaena odorata* Linn	Eupatorium (Venapacha)	Compositae	Leaf
13 *Coscinium fenestratum* Colebr)	Calumbe, False (Maramanjal)	Menispermaceae	Dried stem
14 *Curcuma longa* Linn	Turmeric	Zingiberaceae	Rhizome
15 *Cymbopogon citratus* Stapf	Lemongrass	Gramineae	Leaf
16 *Ferula asafoetida* Regel	Asafoetida	Umbelliferae	Commercial product
17 *Glycyrrhiza glabra* Linn	Liquorice (Erattimadhuram)	Papilionaceae	Dried root
18 *Indigofera tinctoria* Linn	Indigo (Neela amari)	Papilionaceae	Leaf
19 *Mirabilis jalapa* Linn	Four O'clock plant (Nalumani)	Nyctaginaceae	Tuber
20 *Moringa oleifera* Lam	Drumstick (Muringa)	Moringaceae	Root bark
21 *Ocimum sanctum* Linn	Sacred Basil (Thulasi)	Lamiceae	Leaf

Contd

1	2	3	4
22	*Pandanus odoratissimus* Linn	Screw pine (Kaitha)	Pendanaceae	Young leaf
23	*Phyllanthus fraternus* Webst	Jarmala (Keezhanelli)	Euphorbiaceae	Whole plant
24	*Plumbago rosea* Linn	Leadwort, (Chethikoduveli)	Plumbaginaceae	Tuber
25	*Solanum xanthocarpum* Schrad & Wendll	Nightshade yellow (Kandakari Chunda)	Solanaceae	Fruit
26	*Strychnos nux-vomica* Linn	Nuxvomica (Kanjiram)	Loganiaceae	Root
27	*Thespesia populnea* Soland ex Correa	Bhindi tree (Poovarasu)	Malvaceae	Leaf
28	*Thuja orientalis* Linn	Arbor-vitae oriental	Cuppressaceae	Leaf
29	*Vitex negundo* Linn	Chaste tree-chinese (Karinochi)	Verbenaceae	Leaf
30	*Withania somnifera* Dunal	Ashwagandha (Amukkuram)	Solanaceae	Dried Root

* Names given in paranthesis are Malayalam names

Chenopodium amaranticolor was at first used as the test host but since consistent results could not be obtained in the control also pumpkin seedlings at the two

leaf stage raised in polybags were then used as the test host

The partially clarified extract of each of the above plants was mixed with equal quantity of virus inoculum, incubated at room temperature for 15 min and was then inoculated on the leaves of pumpkin seedlings Soon after inoculation the leaves were washed with distilled water using a wash bottle The inoculated plants were kept under observation in insect proof nethouse for the development of symptom Pumpkin seedlings inoculated with the inoculum alone were kept as control The antiviral property of plant extracts against pumpkin mosaic virus was estimated using the following formula

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

C = Number of plants infected in the control

T = Number of plants infected in the treatment

3.4 Comparative efficacy of selected medicinal plant extracts on pumpkin mosaic virus infection

Extracts of five selected medicinal plants viz *B alba, G glabra, P fraternus, P rosea* and *T populnea* were prepared as mentioned above From this, two dilutions of 5 per cent and 10 per cent were prepared and sprayed on

ten pumpkin seedlings at the two leaf stage Virus inoculum prepared as mentioned earlier was inoculated on the plants after 24 h of treatment with plant extracts An equal number of plants previously sprayed with distilled water and inoculated as above were kept as control The experiment was repeated to confirm the results

3 5 Effect of selected medicinal plant extracts on the partially purified PMV

The virus was purified following the method of Van Kammen (1967) The inoculum was prepared by triturating the infected young frozen leaves at the rate of one g/ml of 0 01 M phosphate buffer of pH 7 0 using a clean sterile mortar and pestle The homogenate was filtered through double layer muslin cloth and centrifuged at 10000 g for 15 min at 5 C to remove the host material The clear supernatant was decanted and added poly ethylene glycol (PEG) of M W 6000 to a final concentration of four per cent (w/v) and sodium chloride (NaCl) to give a concentration of 0 2 M The mixture was stirred at room temperature to dissolve the PEG and NaCl and after one hour centrifuged at 10,000 g for 15 min at 5 C Both the fractions (supernatant and residue) were tested for infectivity separately and the supernatant was found to be highly infective while the residue gave only very low percentage

of infection (residue was resuspended in phosphate buffer before inoculation) The final virus preparation was clear with a light greenish tinge

Medicinal plant extracts were prepared as mentioned under 3 3 The partially clarified extract of each of the selected medicinal plants was mixed with equal volume of partially purified virus preparation incubated at room temperature for 15 min and was then inoculated on the leaves of 10-day old pumpkin seedlings Soon after inoculation, the leaves were washed with distilled water using a wash bottle Pumpkin seedlings inoculated without the plant extracts were kept as control

3 6 Effect of pre and post inoculation application of selected medicinal plant extracts on sap transmission of PMV

Extracts of five plants namely *B alba, G glabra, P fraternus, P rosea, T populnea* were sprayed on pumpkin seedlings at ten per cent concentration as given below

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

(ii) One day after inoculation with the virus (post-inoculation application)

The inoculations were done as mentioned under

3 2 1 and the plants were kept under observation in insect proof net house

3 7 Effect of selected medicinal plant extracts on sap transmission of PMV inoculated at different time intervals

Pumpkin seedlings grown in polybags arranged into seven groups of twenty plants each were kept in insect proof net house Plant extracts at ten per cent concentration were sprayed on six groups of test plants using an atomiser The seventh group of plants was kept as control

Immediately after spraying plant extracts (zero hour), the first set of plants and the control plants were sap inoculated with the virus as mentioned under 3 2 1 The other five sets of plants were inoculated at intervals of 6 h, one day, 2 days, 4 days and 6 days respectively The test plants were observed for the expression of disease symptoms at intervals of two weeks, three weeks and four weeks after inoculation

3 8 Systemic effect of selected medicinal plant extracts on sap transmission of PMV

Extracts of *B alba, G glabra, P fraternus, P rosea* and *T populnea* at ten per cent concentration were carefully applied on the cotyledonous leaves of pumpkin seedlings by means of cotton wool dipped in the extracts

These test plants were inoculated with PMV on the first true leaf by sap inoculation as mentioned under 3 2 1, after application of plant extract Twenty pumpkin seedlings were kept for each treatment and control In the control distilled water was applied on the cotyledonous leaves and inoculation was done on the first true leaf The test plants were kept in insect proof net house for observation

3 9 Effect of application of selected medicinal plant extracts on the transmission of PMV by *Aphis gossypii*

3 9 1 Application of plant extracts before acquisition feeding

Nonviruliferous aphids were collected in petridishes using camel hair brush as mentioned under 3 2 2 They were starved for one hour and then allowed acquisition access period of 30 min on young leaves of mosaic affected pumpkin plants which were sprayed with extracts of selected medicinal plants viz *B alba, G glabra, P fraternus P rosea* and *T populnea* at ten per cent cocentration Acquisition access was given 3 h and 24 h after application of plant extracts The aphids were then transferred to healthy pumpkin seedlings in the two leaf stage at the rate of ten per plant

Ten plants were inoculated for each plant extract

along with a set of ten plants sprayed with distilled water, kept as control This was done for both 3 h and 24 h after application of plant extracts The plants were kept in insect proof cages and aphids were allowed to feed on the plants for 24 h and after that the aphids were killed by spraying quinalphos (0 05%) The experiment was done twice

3 9 2 Application of plant extracts before inoculation feeding

As mentioned under 3 9 1 nonviruliferous aphids were collected starved for one hour and given an acquisition access period of 30 min on pumpkin mosaic affected plant Viruliferous aphids were then transferred to healthy plants, at the rate of ten per plant which were previously sprayed with extracts of medicinal plants viz *B alba, G glabra, P fraternus, P rosea* and *T populnea* at ten per cent concentration Inoculation access was given 3 h and 24 h after application of plant extracts

Ten plants were inoculated for each plant extract along with ten plants sprayed with distilled water was kept as control The plants were kept in insect proof cages for 24 h and then sprayed quinalphos 0 05 per cent to kill the aphids The experiment was done twice

3 9 3 Application of plant extracts after inoculation feeding

As mentioned under 3 9 1 nonviruliferous aphids were collected, starved for one hour and given an acquisition access period of 30 min on pumpkin mosaic affected plant Viruliferous aphids were then transferred to healthy plants at the rate of ten per plant Three and 24 h after the inoculation access period, plants were sprayed with ten per cent of extracts of *B alba, G glabra, P fraternus, P rosea* and *T populnea* Ten plants were tested for each plant extract along with ten plants sprayed distilled water was kept as control The experiment was done twice

3 10 Effect of the selected medicinal plant extracts on PMV by sap and vector transmission

A comparison of the effectiveness of plant extracts against PMV by sap and vector transmission at pre and post inoculation applications was made The data from Table 6 and 12 were analysed for this purpose

3 11 Mode of spread of pumpkin mosaic in the field

Observations on the incidence of pumpkin mosaic were taken from the vegetable field of College of Horticulture Vellanikkara in which pumpkin plants were grown at a spacing of 4 5 x 2 m^2 during the period from November

1993 to January 1994 for studying the spread of the virus A sketch of the pumpkin plot was prepared and mosaic affected plants were marked in the sketch at monthly intervals starting from two weeks after planting The plants were denoted as A, B C for first, second and third incidence respectively on the occurrence of the disease as given by Gibbs (1983)

A convolution diagram was prepared by plotting the distribution of all the newly affected plants (B plants) around each of the affected plants found originally (A plants) This is the summary of the position of the B plants relative to all the A plants This was extended to further infected plants also (C plants) Convolution diagrams were prepared to know the mode of spread of disease from A to B plants and B to C plants

From the convolution diagrams, data were analysed by One Sample Runs Test (Siegel, 1956) using the following formula

$$Z = \frac{r - \left(\frac{2n_1n_2}{n_1 + n_2} + 1\right)}{\sqrt{\frac{2n_1n_2\,(2n_1n_2 - n_1 - n_2)}{(n_1 + n_2)^2\,(n_1 + n_2 - 1)}}}$$

where

Z = Standard normal variation

r - Number of runs

n_1 - Number of infected plants

n_2 - Number of uninfected plants

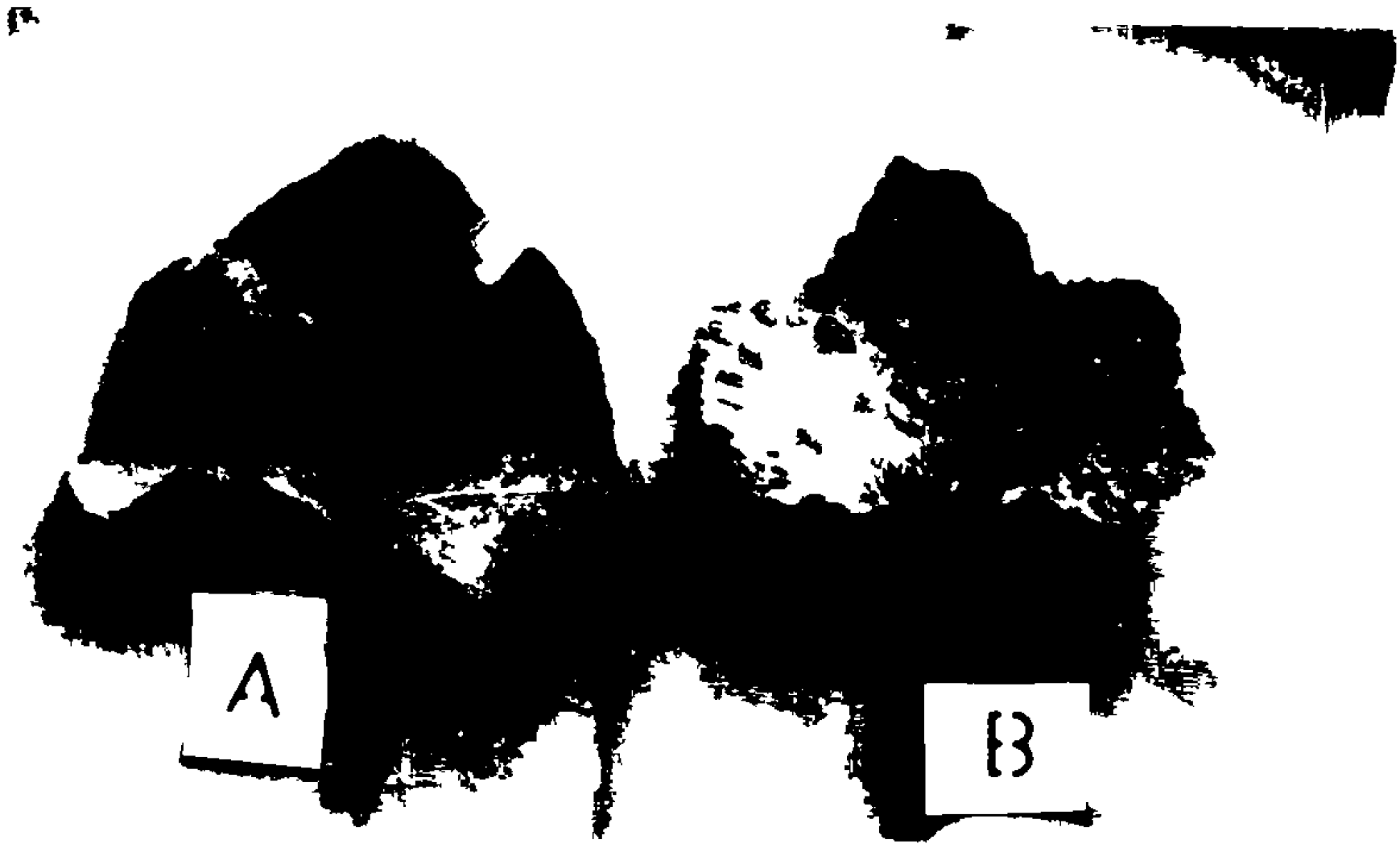
4 RESULTS

4 1 Symptomatology

Naturally ınfected as well as artıfıcıally ınocu-lated pumpkın seedlıngs were kept under observatıon for studyıng the development of symptoms The leaves of natu-rally ınfected plants showed mottled green blısters whıch later coalesced resultıng ın dısfıguratıon of leaves The ınfected seedlıngs remaıned stunted They flowered very sparıngly and that also wıth less number of female flowers and reduced fruıt settıng The fruıts were reduced ın sıze and often mısshapen

On mechanıcal ınoculatıon, symptoms were appeared 12-14 days after ınoculatıon Typıcal mosaıc pattern wıth dark green and lıght green patches was produced ın all the newly emerged leaves In older leaves, only chlorotıc areas were seen and mosaıc symptom was less pronounced The ınternodes were shortened, leaf lamına became thınner and narrower The growth of plants subsequent to ınfectıon was much reduced and the branches produced were very weak Flowerıng was delayed and sıze of flowers was much re-duced Many of the dıseased plants produced only male flowers The ınfected plants dıd not bear any fruıts (Plate 1)

Plate 1 A Healthy pumpkın leaf
B Pumpkın mosaıc affected leaf


A
B

4 2 Transmission of the virus

4 2 1 Sap transmission

Standard aqueous extract and sap extracted in different buffers were used for transmission trials The virus was found to be successfully transmitted by mechanical inoculation The percentage of transmission varied with the extraction medium used Among the four buffers tested at pH 7 2 and molarity 0 1 M, phosphate buffer was found to be most effective with 75 per cent transmission followed by potassium phosphate buffer Standard aqueous extract also gave a comparatively good percentage of infected seedlings (Table 1)

4 2 2 Aphid transmission

When pumpkin seedlings were inoculated by means of viruliferous aphids (*Aphis gossypii*) at the rate of ten per seedling 62 5per cent plants showed the symptom (Table 2)

4 3 Preliminary screening of medicinal plant extracts for antiviral property against PMV

Inorder to find out the antiviral property of medicinal plant extracts against PMV, experiment was conducted as given in 3 3 by preparing the extracts of 30 plants in distilled water

Table 1 Comparative efficacy of different buffers in the sap transmission of PMV

Sl No	Extraction medium	Per cent infection		Mean per cent infection
		Exp I	Expt II	
1	Phosphate buffer	70	80	75
2	Potassium phosphate buffer	60	80	70
3	Sorenson's phosphate buffer	40	40	40
4	Potassium phosphate sodium hydroxide buffer	40	30	35
5	Standard aqueous extract	60	70	65

Table 2 Transmission of PMV by *Aphis gossypii*

Tests	Per cent infection	Mean per cent infection
Experiment I	60	62 5
Experiment II	65	
Control	0	

It was found that extracts of eight species of medicinal plants, viz , *Anamirta cocculus Basella alba, Coscinium fenestratum, Glycyrrhiza glabra, Indigofera tinctoria, Phyllanthus fraternus, Plumbago rosea* and *Thespesia populnea* inhibited symptom expression of PMV by 80 per cent and above Extracts of nine species of plants showed inhibition of 50 per cent and above and that of twelve species of plants showed inhibition of less than 50 per cent *Calotropis procera* leaf extract did not have any antiviral properties against PMV (Table 3)

Based on the results of the preliminary screening the following five medicinal plants (Plates 2-6) which showed maximum inhibitory property against PMV were selected for detailed investigations on their antiviral properties against PMV

i) *Basella alba*

ii) *Glycyrrhiza glabra*

iii) *Phyllanthus fraternus*

iv) *Plumbago rosea*

v) *Thespesia populnea*

4 4 Comparative efficacy of selected medicinal plant extracts on PMV infection

Extracts of five selected medicinal plants were

Table 3 Effect of medicinal plant extracts against PMV infection

Sl No	Name of plant	Per cent inhibition over control		Mean per cent inhibition
		Exp I	Exp II	
1	*Adathoda vasica*	40 00	60 00	50 00
2	*Adenocalymma allicea*	0	16 67	8 34
3	*Aegle marmelos*	50 00	50 00	50 00
4	*Alstonia venenatus*	40 00	40 00	40 00
5	*Anamirta cocculus*	80 00	80 00	80 00
6	*Aristolochia indica*	50 00	40 00	45 00
7	*Asperagus officinalis*	12 50	33 30	22 90
8	*Azadirachta indica*	40 00	20 00	30 00
9	*Basella alba*	100 00	100 00	100 00
10	*Boerhaavia diffusa*	62 50	66 70	64 60
11	*Calotropis procera*	0	0	0
12	*Chromalena odorata*	62 50	60 00	61 25
13	*Coscinium fenestratum*	80 00	80 00	80 00
14	*Curcuma longa*	60 00	60 00	60 00
15	*Cymbopogan citratus*	37 50	50 00	43 75
16	*Ferula asafoetida*	80 00	60 00	70 00
17	*Glycyrrhiza glabra*	100 00	80 00	90 00
18	*Indigofera tinctoria*	80 00	80 00	80 00
19	*Mirabilis jalapa*	20 00	20 00	20 00
20	*Moringa oleifera*	40 00	20 00	30 00
21	*Ocimum sanctum*	62 50	60 00	61 25
22	*Pandanus odoratissimus*	0	16 67	8 33
23	*Phyllanthus fraternus*	100 00	80 00	90 00
24	*Plumbago rosea*	87 50	80 00	83 75
25	*Solanum xanthocarpum*	58 37	50 00	54 19
26	*Strychnos nux-vomica*	40 00	50 00	45 00
27	*Thespesia populnea*	80 00	100 00	90 00
28	*Thuja orientalis*	60 00	40 00	50 00
29	*Vitex negundo*	87 50	66 70	77 10
30	*Withania somnifera*	40 00	40 00	40 00

Plate 2 *Basella alba*

Plate 3 Root bits of *Glycyrrhiza glabra*

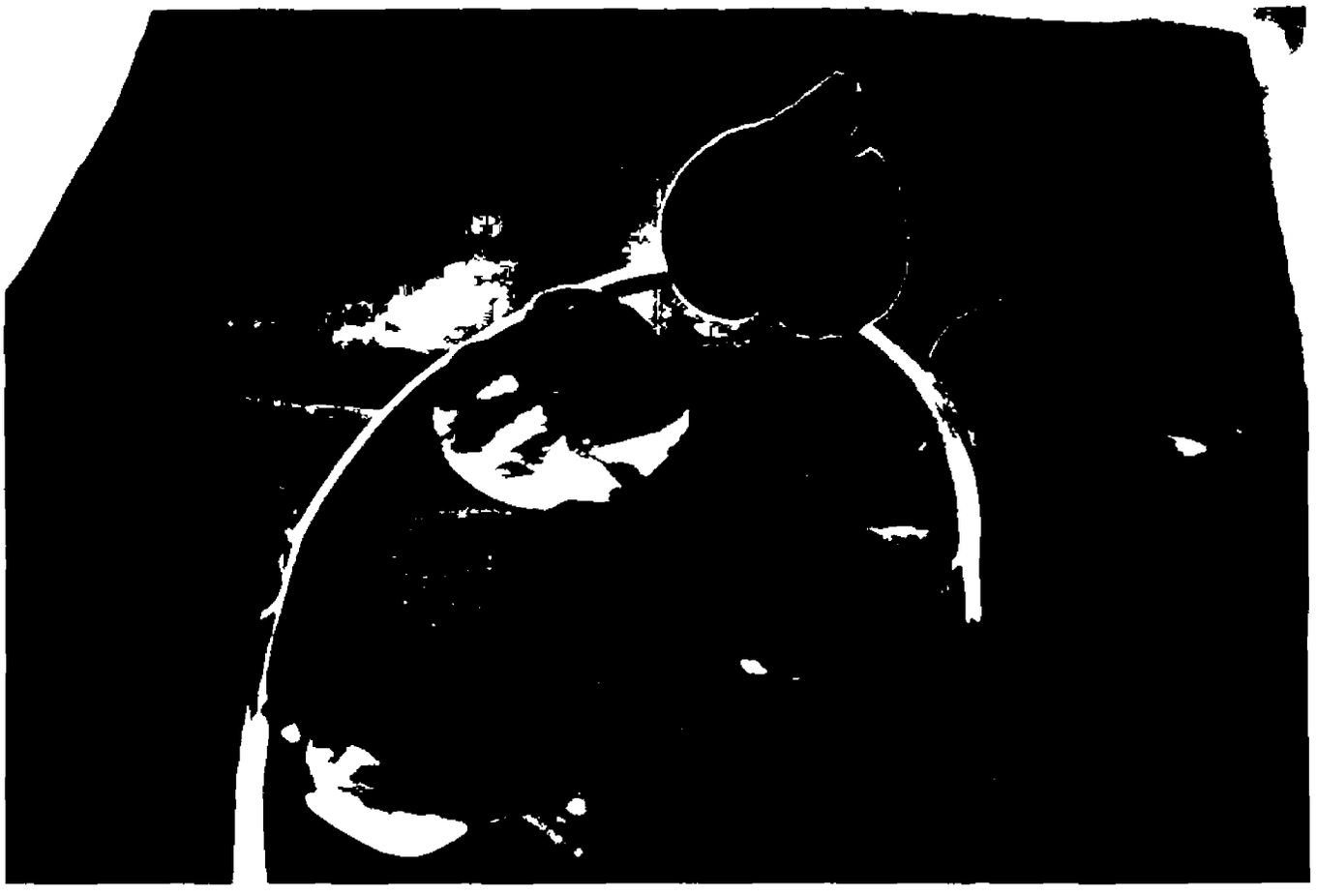


Plate 4 *Phyllanthus fraternus*

Plate 5 *Thespesia populnea*

Plate 6 *Plumbago rosea*

prepared as described under 3.3 and five per cent and ten per cent dilutions of these were sprayed on pumpkin seedlings The experiment revealed that the inhibitory property of plant extracts against PMV infection did not vary significantly with the concentration used but there was an increase in the inhibitory action with decrease in concentration observed for the extracts of two plants, viz , *B alba* and *P rosea P fraternus* and *T populnea* extracts were more effective at ten per cent concentration than at five per cent *G glabra* showed equal effectiveness at both concentrations (Table 4)

4 5 Effect of selected medicinal plant extracts on partially purified virus preparation

Extracts of all the five species of plants exhibited more than 83 per cent inhibition of infection of the virus Among these, *B alba, G glabra* and *T populnea* caused maximum inhibition of more than 94 per cent (Table 5)

4 6 Effect of pre and post inoculation application of selected medicinal plant extracts on sap transmission of PMV

Pre and post inoculation applications of selected medicinal plant extracts were done as mentioned under 3 6 Statistical analysis of the data revealed that pre-inoculation application of medicinal plant extracts was

Table 4 Comparative efficacy of selected medicinal plant extracts against PMV infection

Sl No	Name of plant	5 per cent		10 per cent		Change in per cent inhibition
		Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	
1	*Basella alba*	1	93 75	3	81 25	-12 50
2	*Glycyrrhiza glabra*	2	87 50	2	87 50	0
3	*Phyllanthus fraternus*	4	75 00	2	87 50	12 50
4	*Plumbago rosea*	2	87 50	4	75 00	-12 50
5	*Thepesia populnea*	4	75 00	3	81 25	6 25
	Control	16				
Friedman Test			NS		NS	

NS - Not significant

Table 5 Effect of selected medicinal plant extracts on partially purified PMV

Sl No	Name of plant	Number of plants infected out of 20	Per cent inhibition over control
1	*Basella alba*	1	94 44
2	*Glycyrrhiza glabra*	1	94 44
3	*Phyllanthus fraternus*	2	88 89
4	*Plumbago rosea*	3	83 33
5	*Thespesia populnea*	1	94 44
Control		18	
Friedman Test			NS

NS - Not significant

far better than post-inoculation application in inhibiting PMV (Table 6, Fig 1) Post-inoculation application reduced the efficacy of the inhibitory property of medicinal plant extracts and maximum reduction was in the case of *P fraternus* and *B alba* Among these two, *P fraternus* showed lower per cent inhibition of the virus by post-inoculation application Minimum per cent reduction in the inhibitory property was found in the case of *P rosea* and *T populnea*

4 7 Effect of selected medicinal plant extracts on sap transmission of PMV inoculated at different time intervals

When extracts of the five selected medicinal plants were tested against PMV inoculated at different time intervals after the application of plant extract, there was significant variation in the inhibitory effect between the different time of applications as well as between plant extracts

Details of the effects of the five selected medicinal plant extracts are as follows

4 7 1 Application of *B alba* leaf extract

The per cent inhibition decreased gradually as the time interval between application of plant extract and virus inoculation increased On statistical analysis, it

Table 6 Effect of pre and post inoculation application of selected medicinal plant extracts on sap transmission of PMV

Name of plant	Pre inoculation application		Post inoculation application		Peduction in per cent inhibition
	Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	
Basella alba	2	86 67	7	53 33	33 34
Glycyrrhiza glabra	3	80 00	6	60 00	20 00
Phyllanthus fraternus	4	73 33	9	40 00	33 33
Plumbago rosea	2	86 67	4	73 33	13 34
Thespesia populnea	3	80 00	5	66 67	13 33
Control	15				
Friedman Test		S		S	

Significant

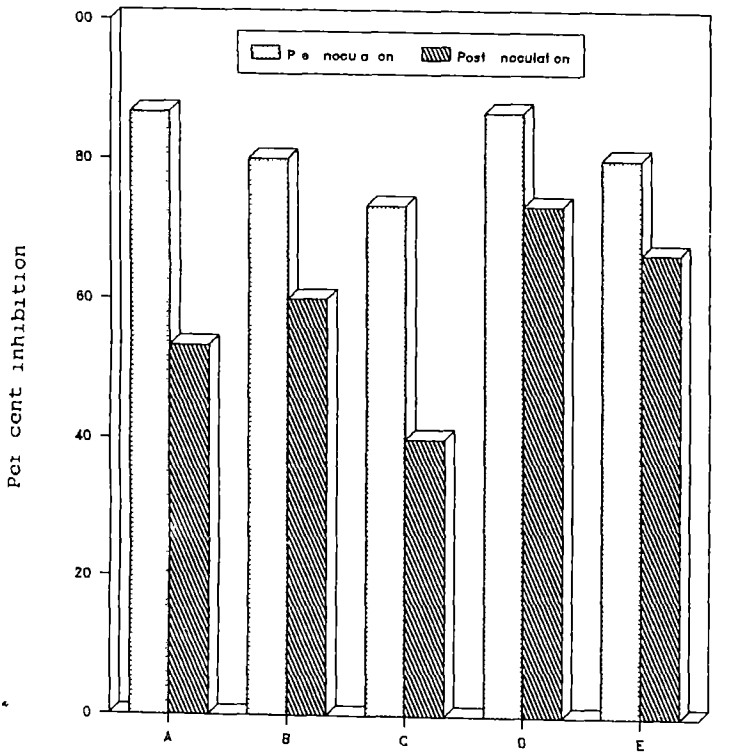


Fig 1 Effect of pre and post-inoculation applications of selected medicinal plant extracts on sap transmission of PMV

A *Basella alba* C *Phyllanthus fraternus* E *Thespesia populnea*

B *Glycyrrhiza glabra* D *Plumbago rosea*

was found that there is no significant difference in the inhibitory effect against PMV when virus inoculation was done 0, 6, 24 and 48 hours after application of plant extract But when the inoculation of the virus was done four and six days after the application of plant extracat the inhibitory effect was found to decrease significantly (Table 7a)

There was a gradual decrease in the inhibitory effect of *B alba* leaf extract along with increase in the number of days after inoculation as indicated by the increase in the number of plants showing symptom when the observations were taken two three and four weeks after inoculation of PMV

Hundred per cent inhibition was observed after two weeks when virus was inoculated immediately as well as six hours after the application of plant extract whereas only 75 per cent inhibition was observed when the plants were inoculated six days after the application of plant extract After four weeks of inoculation also similar trend of inhibition was observed

4 7 2 Application of *G glabra* root extract

In the case of application of the root extract of *G glabra* also, there was a gradual decrease in the in-

Table 7a Effect of pre inoculation application of *B alba* leaf extract against PMV at different time intervals

Sl No	Time of inoculation	Control (No of plants infected out of 20	Two weeks after inoculation		Three weeks after inoculation		Four weeks after inoculation		Mean	Friedman test
			Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control		
1	0 h	16	0	100 00	1	93 75	1	93 75	95 83	↑
2	6 h	16	0	100 00	1	93 75	1	93 75	95 83	NS
3	24 h	14	2	85 71	2	85 71	2	85 71	85 71	↓
4	48 h	15	2	86 67	2	86 67	3	80 00	84 45	↓
5	4 d	16	3	81 25	4	75 00	4	75 00	77 08	↑ S
6	6 d	16	4	75 00	7	56 25	7	56 25	62 50	↓
Friedman test				S		S		S		

S Significant
NS Not significant

hibitory effect on PMV infection as the time interval between the application of plant extract and virus inoculation increased There was no significant difference between the inhibitory effect when PMV was inoculated 0, 6 and 24 hours after the application of plant extract When the inoculation of the virus was done two, four and six days after the application of plant extract, the inhibitory effect has decreased significantly (Table 7b)

There was a gradual decrease in the inhibitory effect of *G glabra* extract also along with the increase in the number of days after inoculation as indicated by the increase in the number of plants showing the symptoms when the observations were taken two, three and four weeks after inoculation of PMV

There was 93 75 per cent inhibition when the observations were taken two weeks after in the case of inoculation of PMV immediately after the application of plant extract and there was only 71 43 per cent inhibition when the plants were inoculated six days after application of plant extract Similar trend of inhibition was observed four weeks after inoculation also

4 7 3 Application of *P fraternus* extract

As the time interval between the application of

Table 7b Effecet of pre inoculation application of *G glabra* oot extract against PMV at different time intervals

Sl No	Time of inoculation	Control (No of plants infected out of 20	Two weeks after inoculation		Three weeks after inoculation		Four weeks after inoculation		Mean	Friedman test
			Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control		
1	0 h	16	1	93 75	2	87 50	3	81 25	87 50	↑
2	6 h	16	2	87 50	2	87 50	3	81 25	85 42	NS
3	24 h	14	3	78 57	3	78 57	4	71 43	76 19	↓
4	48 h	15	3	80 00	4	73 33	4	73 33	75 56	↑
5	4 d	16	3	81 25	5	68 75	5	68 75	72 92	S
6	6 d	14	4	71 43	5	64 29	10	28 57	54 76	↓
Friedman test				S		S		S		

S Significant
NS Not significant

plant extract and inoculation of virus was increased up to six days, there was significant reduction in the per cent inhibition But it was found that when inoculation was done up to 24 hours after the application of plant extract, there was no significant reduction in the inhibition But the inhibitory effect obtained when PMV was inoculated two, four and six days after application of plant extract varied significantly Maximum inhibitory effect was observed when inoculation was done four days after application of plant extract (Table 7c) Although there was a decrease in the inhibitory effect of *P fraternus* extract up to two days after application, there was increase in the inhibition when the inoculation of PMV was done four days after application of the extract

In the case of *P fraternus* also the inhibitory effect has decreased along with the increase in the number of days after inoculation of PMV

Although there was only 87 5 per cent inhibition when the observations were taken two weeks after inoculation in the case of inoculation of PMV immediately after the application of plant extract, there was higher percentages of inhibition, viz 93 75 and 92 86 when the plants were inoculated four days and six days after application of plant extract A more or less similar trend was

Table 7c Effect of pre inoculation application of *P fraternus* extract against PMV at different time intervals

Sl No	Time of inoculation	Control (No of plants infected out of 20	Two weeks after inoculation		Three weeks after inoculation		Four weeks after inoculation		Mean	Friedman test
			Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control		
1	0 h	16	2	87 50	2	87 50	3	81 25	85 42	↑
2	6 h	16	2	87 50	3	81 25	4	75 00	81 25	NS
3	24 h	14	3	78 57	3	78 57	5	64 29	73 81	↓
4	48 h	15	5	66 67	5	66 67	6	60 00	64 45	↑
5	4 d	16	1	93 75	1	93 75	4	75 00	87 50	S
6	6 d	14	1	92 86	3	78 57	5	64 29	78 57	↓
Friedman test				S		S		S		

S Significant
NS Not significant

observed even four weeks after inoculation

4 7 4 Application of *P rosea* tuber extract

Significant variation in inhibition was noticed when the inoculation was done at different periods after the application of plant extract However when inoculation was done immediately after application of plant extract and 6 and 24 hours after application, there was no significant difference in the inhibitory effect When the inoculation was done four days after the application of plant extract, there was significant reduction in inhibition But there was an increase in the inhibitory effect when PMV was inoculated six days after application of *P rosea* tuber extract over that inoculated four days after the extract was applied (Table 7d) Maximum inhibition was observed when PMV was inoculated immediately after and six hours after application of plant extract

The inhibitory effect of *P rosea* tuber extract also decreased along with the increase in the number of days after inoculation of PMV

There was 87 50 per cent inhibition when the observation was taken two weeks after when the inoculation of PMV was done immediately as well as six hours after application of the extract The general trend was a

Table 7d Effect of pre inoculation application of *P rosea* tuber extract against PMV at different time intervals

Sl No	Time of inoculation	Control (No of plants infected out of 20	Two weeks after inoculation		Three weeks after inoculation		Four weeks after inoculation		Mean	Friedman test
			Number of plants infected out of 20	Per ent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control		
1	0 h	16	2	87 50	3	81 25	3	81 25	83 33	↑
2	6 h	16	2	87 50	3	81 25	3	81 25	83 33	NS
3	24 h	14	2	85 71	2	85 71	5	64 29	78 57	↓
4	48 h	15	2	86 67	3	80 00	4	73 33	80 00	↑
5	4 d	16	5	68 75	6	62 50	7	56 25	62 50	S
6	6 d	14	3	78 57	3	78 57	4	71 43	76 19	↓
Friedman test				S		S		S		

S Significant
NS Not significant

reduction in the inhibitory effect along with the increase in the time after the application of the extract This trend was observed four weeks after inoculation also

4 7 5 Application of leaf extract of *T populnea*

Inhibitory property of the *T populnea* extract also varied significantly when inoculation was done at different periods after the application However it was found that variation was not significant when inoculation was made immediately after, six, and 24 hours of inoculation The maximum inhibition was observed, when inoculation was made 48 hours after application of the extract (Table 7e)

The inhibitory effect of *T populnea* leaf extract also decreased along with the increase in number of days after inoculation of PMV

Although there was only 87 5 per cent inhibition when the observation was taken two weeks after inoculation in the case of inoculation of PMV immediately as well as six hours after the application of plant extract there was higher percentages of inhibition (93 33 and 93 75) when the plants were inoculated two days and four days after the application of plant extract Similar trend was

Table 7e Effect of pre inoculation application of *T populnea* leaf extract against PMV at different time intervals

Sl No	Time of inoculation	Control (No of plants infected out of 20	Two weeks after inoculation		Three weeks after inoculation		Four weeks after inoculation		Mean	Friedman test
			Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control		
1	0 h	16	2	87 50	2	87 50	3	81 25	85 42	↑
2	6 h	16	2	87 50	3	81 25	3	81 25	83 33	NS
3	24 h	14	2	85 71	4	71 43	4	71 43	76 19	↓
4	48 h	15	1	93 33	2	86 67	2	86 67	88 89	↑
5	4 d	16	1	93 75	3	81 25	3	81 25	85 42	S
6	6 d	14	5	64 29	6	57 14	8	42 86	54 76	↓
Friedman test				S		S		S		

S Significant
NS Not significant

observed four weeks after inoculation also

4 7 6 Comparative efficacy of the five selected medicinal plants on sap transmission of PMV

By comparing all the five medicinal plants together (Table 8, Fig 2), it was found that *B alba* showed maximum inhibition of the virus, when the time interval between application of the extract and inoculation of the virus was zero, six and 24 hours *T populnea* was found to be most effective in inhibiting the virus when the interval of application of the extract and inoculation was 48 hours whereas *P fraternes* showed maximum inhibition of the virus at four and six days interval

4 8 Systemic effect of medicinal plant extracts against PMV

The experiment was conducted as mentioned under 3 8, by applying the medicinal plant extracts on the cotyledonous leaves and inoculating PMV on the first true leaf The data showed that all the five selected medicinal plants have considerable systemic effect in reducing PMV infection (Table 9, Fig 3) Maximum systemic effect was shown by *B alba* and minimum by *P fraternus* The inhibitory effect against PMV infection was highest when observation was taken two weeks after inoculation and it was found to be decreasing on third and fourth week after

Table 8 Comparative efficacy of pre-inoculation application of medicinal plant extract against PMV by sap transmission

Sl No	Name of plant	Mean per cent inhibition at different time of inoculation											
		0 h		6 h		24 h		48 h		4 d		6 d	
1	*Basella alba*	95	83	95	83	85	71	84	45	77	08	62	50
2	*Glycyrrhiza glabra*	87	50	85	42	76	19	75	56	72	92	54	76
3	*Phyllanthus fraternus*	85	42	81	25	73	81	64	45	87	50	78	57
4	*Plumbago rosea*	83	33	83	33	78	57	80	00	62	50	76	19
5	*Thespesia populnea*	85	42	83	33	76	19	88	89	85	42	54	76
	Friedman Test	---------- S ----------											

S Significant

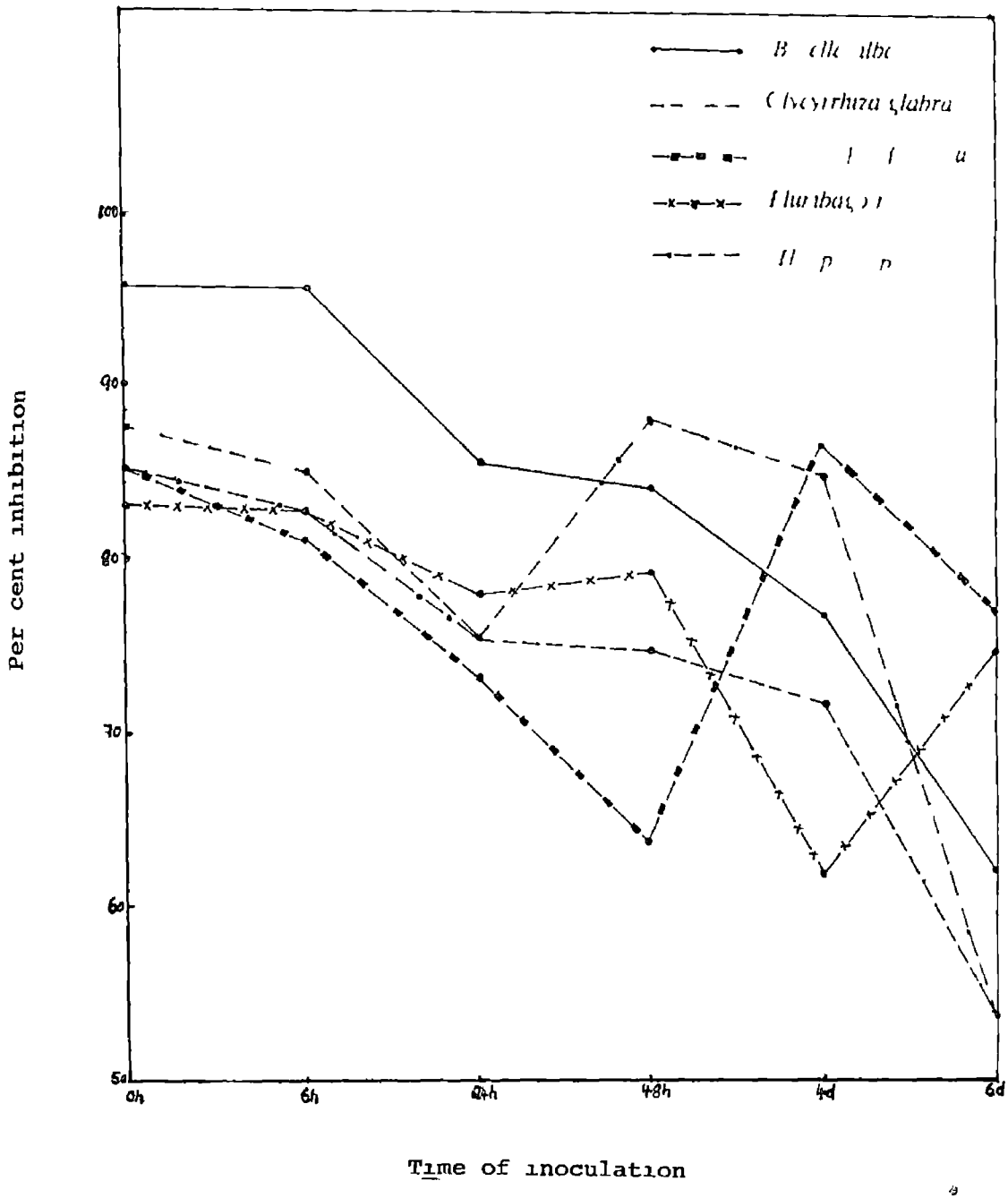


Fig 2 Comparative efficacy of five medicinal plant extracts on sap transmission of PMV

Table 9 Systemic effect of selected medicinal plant extracts against PMV infection

Sl No	Name of plant	Two weeks after inoculation		Three weeks after inoculation		Four weedks after inoculation		Mean
		Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	
1	*Basella alba*	1	92 86	2	85 71	2	85 71	88 09
2	*Glycyrrhiza glabra*	2	85 71	3	78 57	4	71 43	78 57
3	*Phyllanthus fraternus*	6	57 14	8	42 86	10	28 57	42 86
4	*Plumbago rosea*	2	85 71	4	71 43	6	57 14	71 43
5	*Thespesia populnea*	3	78 57	6	57 14	6	57 14	64 28
Control		14						
Friedman test			S		S		S	

S Significant

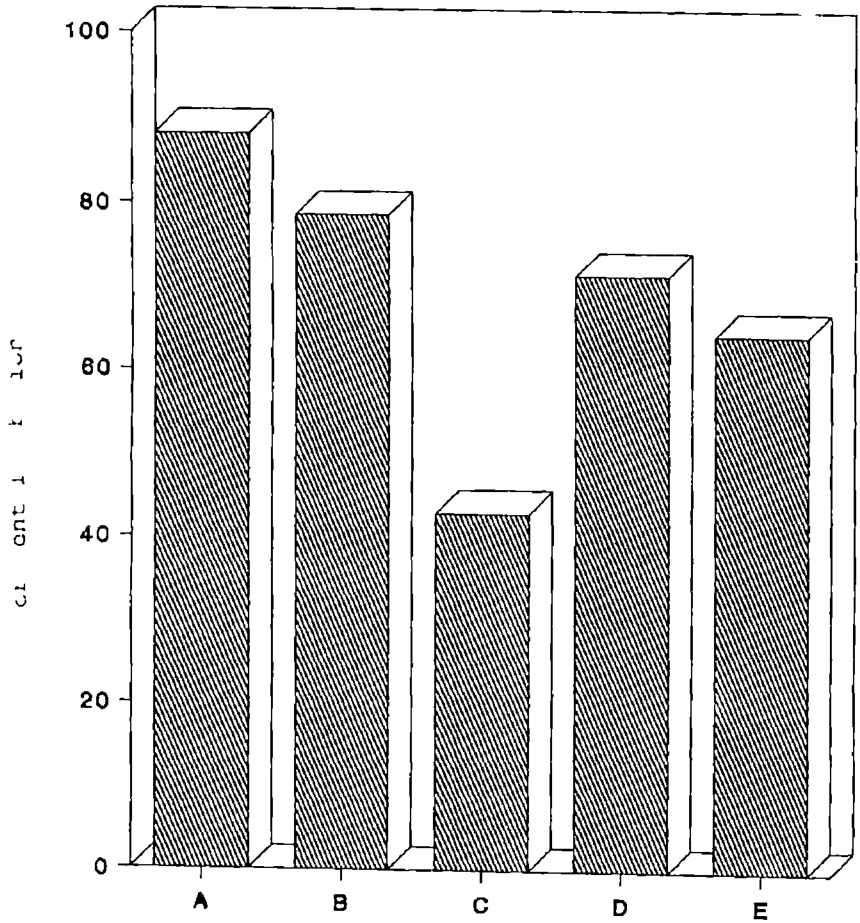


Fig 3 Systemic effect of selected medicinal plant extracts against PMV

A *Bi l i* C *Hyll athu frat i* E *The pe i p pul i*

B *Ch vrrl i g l d r i* D *Ilun bago ro ea*

inoculation Even four weeks after inoculation the extract of *B alba* and *G glabra* caused 85 7 and 71 4 per cent inhibition of PMV infection

4 9 Effect of application of selected medicinal plant extracts on the transmission of PMV by *Aphis gossypii*

4 9 1 Application of plant extracts before acquisition feeding

The experiment was conducted as mentioned under 3 9 1 The results revealed that all the five medicinal plants showed significant inhibitory effect on the acquisition of the virus by the vector *A gossypii* (Table 10) When the plant extracts were applied three hours before acquisition feeding of the vector maximum inhibitory effect of 80 per cent was shown by the extracts of three plants namely *B alba P rosea* and *P fraternus* The inhibitory effect was much reduced when plant extracts were applied 24 hours before acquisition feeding and the maximum inhibition was observed was only 60 per cent for *P fraternus* Reduction in inhibitory effect with the increase in the interval of application of plant extract and acquisition feeding was least in the case of *P fraternus*

Table 10 Effect of selected medicinal plant extracts against PMV infection when applied before acquisition feeding of *A gossypii*

Sl No	Name of plant	3 h after application of plant extract		24 h after application of plant extract		Reduction in per cent inhibition
		Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	
1	*Basella alba*	2	80	6	40	40
2	*Glycyrrhiza glabra*	6	40	10	0	40
3	*Phyllanthus fraternus*	2	80	4	60	20
4	*Plumbago rosea*	2	80	6	40	40
5	*Thespesia populnea*	4	60	8	20	40
	Control	10				
	Friedman Test		S		S	
	Significant					

4 9 2 Application of plant extracts before inoculation feeding

When PMV inoculation was done by feeding the viruliferous aphids on pumpkin seedlings sprayed with the plant extracts, in general, there was inhibitory effect on infection (Table 11) When the plant extracts were applied three hours before inoculation feeding of the vector maximum inhibitory effect of 83 33 per cent was shown by extracts of two plants viz *P rosea* and *T populnea* and minimum inhibition of 50 per cent was in *G glabra* The experiment when conducted by applying the plant extracts 24 h before inoculation feeding of the vector, maximum inhibitory effect of 83 33 per cent was shown by *P rosea* and minimum of 33 33 per cent by *P fraternus*

4 9 3 Application of plant extracts after inoculation feeding by *A gossypii*

The experiment was conducted as mentioned under 3 9 3 The results showed that the medicinal plant extracts varied significantly in their inhibitory effect against PMV infection when applied after inoculation feeding of the viruliferous vector (Table 12) When the plant extracts were applied three hours after inoculation feeding, *B alba, P rosea* and *T populnea* showed maximum inhibition of 80 per cent and *P fraternus* showed minimum inhibition of 40 per cent It was also found that as the

Table 11 Effect of selected medicinal plant extracts against PMV infection when applied before inoculation feeding of *A gossypii*

Sl No	Name of plant	Plant extracts applied 3 h before inoculation feeding		Plant extract applied 24 h before inoculation feeding		Reduction in per cent inhibition
		Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	
1	*Basella alba*	4	66 67	6	50 00	16 67
2	*Glycyrrhiza glabra*	6	50 00	6	50 00	0
3	*Phyllanthus fraternus*	4	66 67	8	33 33	33 34
4	*Plumbago rosea*	2	83 33	2	83 33	0
5	*Thespesia populnea*	2	83 33	4	66 67	16 66
	Control	12				
	Friedman Test		NS		NS	

NS Not significant

Table 12 Effect of selected medicinal plant extracts against PMV infection when applied after inoculation feeding of *A gossypii*

Name of plant	Plant extracts applied 3 h after inoculation feeding		Plant extract applied 24 h after inoculation feeding		Reduction in per cent inhibition
	Number of plants infected out of 20	Per cent inhibition over control	Number of plants infected out of 20	Per cent inhibition over control	
Basella alba	2	80	4	60	20
Glycyrrhiza glabra	4	60	6	40	20
Phyllanthus fraternus	6	40	8	20	20
Plumbago rosea	2	80	6	40	40
Thespesia populnea	2	80	4	60	20
Control	10				
Friedman Test		S		S	

Significant

time of application was delayed after inoculation the inhibitory effect of the plant extract was lesser and the least inhibitory effect was shown by *P fraternus* Reduction in the inhibitory effect with the increase in time after inoculation was maximum in the case of *P rosea*

4 9 4 Comparative efficacy of five selected medicinal plants in transmission of PMV by *A gossypii*

The effect of the different times of application of selected medicinal plant extracts namely before acquisition before inoculation and after inoculation feeding of the vector *A gossypii* did not vary significantly (Table 13) But among the means of per cent inhibition at the three times of application of plant extract it was found that *P rosea* is the best one When the efficacy of time of application of each plant extract was compared after 3 h interval, *P rosea* and *T populnea* showed maximum inhibition when applied before inoculation feeding whereas *P fraternus* and *G glabra* showed maximum inhibition before acquisition feeding and after inoculation feeding respectively *B alba* showed equal and maximum effectiveness when applied before acquisition feeding and after inoculation feeding After 24 h interval *G glabra, P rosea* and *T populnea* showed maximum inhibition when applied before inoculation feeding whereas that of *B alba* and *P fraternus* showed maximum inhibition

Table 13 Comparative efficacy of time of application of selected medicinal plant extracts against PMV infection by aphid transmission

Sl No	Name of plant	Per cent inhibition over control							
		3h				24h			
		A	B	C	D	A	B	C	D
1	*Basella alba*	80	66 67	80	75 56	40	50 00	60	50 00
2	*Glycyrrhiza glabra*	40	50 00	60	50 00	0	50 00	40	30 00
3	*Phyllanthus fraternus*	80	66 67	40	62 22	60	33 33	20	37 78
4	*Plumbago rosea*	80	83 33	80	81 11	40	83 33	40	54 44
5	*Thespesia populnea*	60	83 33	80	74 44	20	66 67	60	48 89
Friedman Test		- NS -				--- NS ---			

A Before acquisition feeding
B Before inoculation feeding
C after inoculation feeding
D - Mean per cent inhibition
NS - Not significant

when applied before inoculation feeding whereas that of *B alba* and *P fraternus* showed maximum inhibition when applied after inoculation feeding and before acquisition feeding respectively The inhibitory property of plant extracts was found to be decreasing when the time interval increased from 3 h to 24 h

4 10 Comparison of effectiveness of medicinal plant extracts against sap and vector transmission of PMV

Inhibitory properties of the medicinal plant extracts showed considerable variation when applied 24 h before sap as well as vector inoculation All the five medicinal plant extracts were in general considerably more inhibitory to PMV when inoculation was done mechanically than it was done by means of *A gossypii* But with *P rosea* the per cent inhibitions in sap and vector transmission were 86 67 and 83 33 respectively (Table 14a Fig 4)

When the plant extracts were applied as post-inoculation treatment i e after sap and vector transmission there was no significant variation in the inhibition of PMV obtained in respect of these two methods of transmissions The plant extracts were, in general more inhibitory to PMV when inoculation was done mechanically than it was done by means of *A gossypii* But with *B alba*

Table 14a Comparison of effectiveness of pre-inoculation application of medicinal plant extracts against PMV by sap and vector transmission

Sl No	Name of plant	Per cent inhibition over control		Reduction in per cent inhibition
		Sap transmission	Vector transmission	
1	*Basella alba*	86 67	50 00	36 67
2	*Glycyrrhiza glabra*	80 00	50 00	30 00
3	*Phyllanthus fraternus*	73 33	33 33	40 00
4	*Plumbago rosea*	86 67	83 33	3.34
5	*Thespesia populnea*	80 00	66 67	13 33
Friedman Test		S	S	-

S - Significant

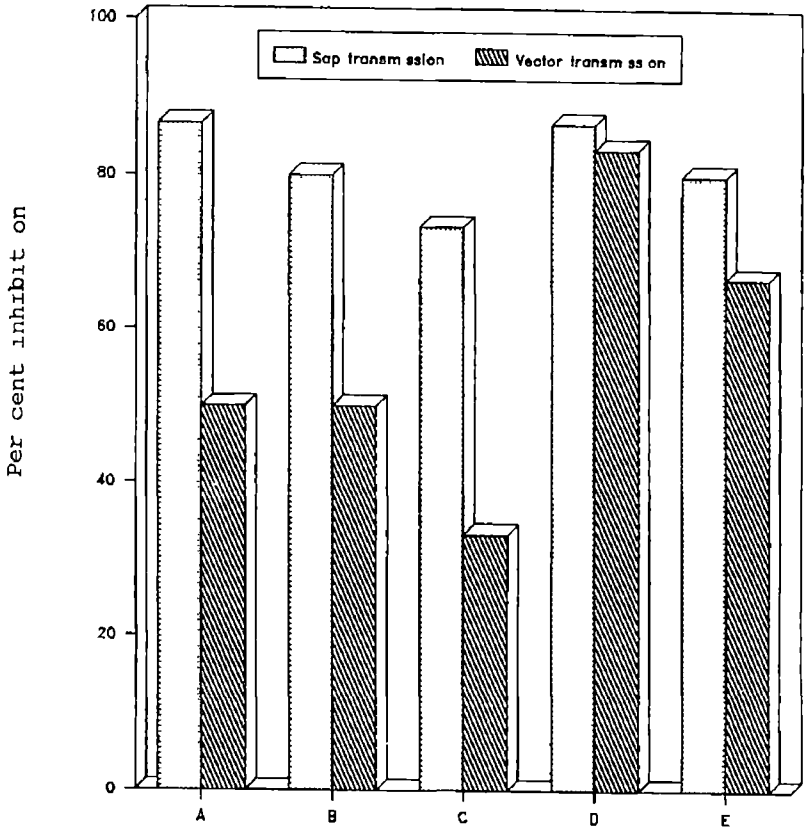


Fig 4 Pre-inoculation application of medicinal plant extracts against PMV by sap and vector transmission

A *Basella alba* C *Phyllanthus fraternus* E *Thespesia populnea*

B *Glycyrrhiza glabra* D *Plumbago rosea*

the per cent inhibition obtained were 53 33 and 60 00, respectively (Table 14b)

4 11 Mode of spread of pumpkin mosaic in the field

To study the mode of spread of pumpkin mosaic in the field, observations were taken as mentioned under 3 11 It was found that out of the total 97 plants observed number of A B and C plants were 30 14 and 23 respectively (Fig 5) Convolution diagrams were prepared from the data to understand the spread of the disease (Fig 6a and 6b)

When the data were analysed it was found that the spread of the disease was not at random It was more or less concentrated around the initially infected plants (A plants) To reassert the above point the spread of disease from B to C plants was also analysed in the same manner as described under 3 11

The values of Z obtained for A to B and B to C plants were 3 10 and 10 66 respecetively These values had a probability of occurrence of less than five per cent It could be concluded that the spread of disease was concentrated around the initially affected plants

Table 14b Comparison of effectiveness of post-inoculation application of medicinal plant extracts against PMV by sap and vector transmission

Sl No	Name of plant	Per cent inhibition over control		Change in per cent inhibition
		Sap transmission	Vector transmission	
1	*Basella alba*	53 33	60 00	-6 67
2	*Glycyrrhiza glabra*	60 00	40 00	20 00
3	*Phyllanthus fraternus*	40 00	20 00	20 00
4	*Plumbago rosea*	73 33	40 00	33 33
5	*Thespesia populnea*	66 67	60 00	6 67
Friedman Test		NS	NS	-

NS - Not significant

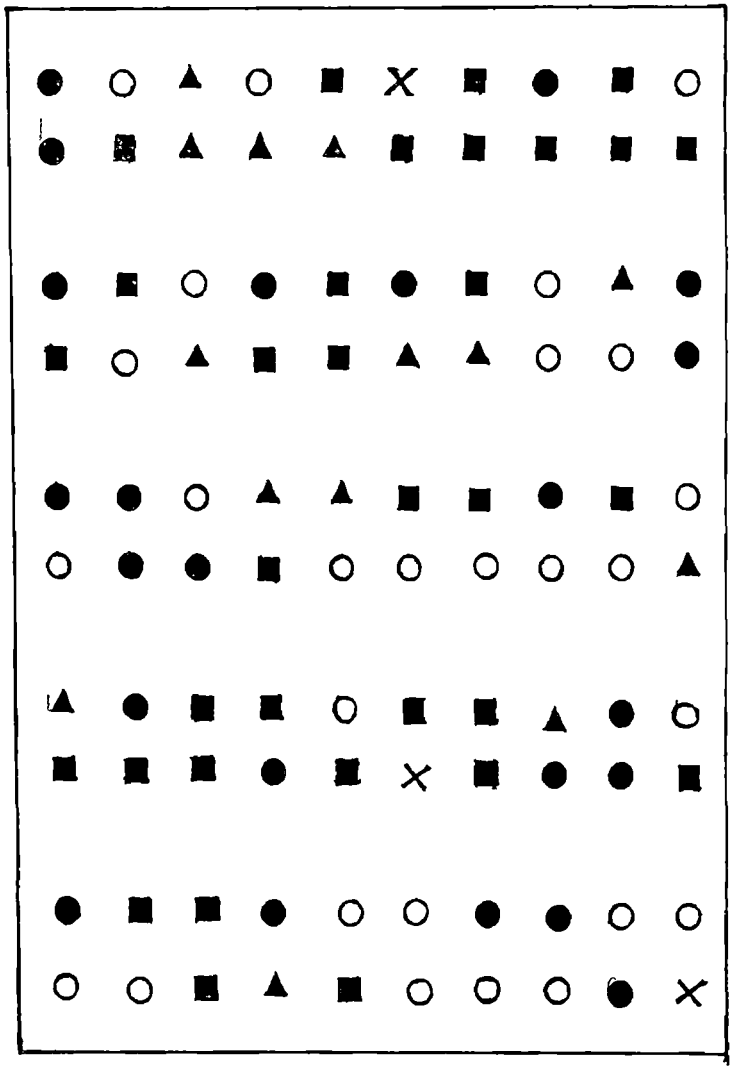


Fig 5 Map of pumpkin field effected by pumpkin mosaic disease

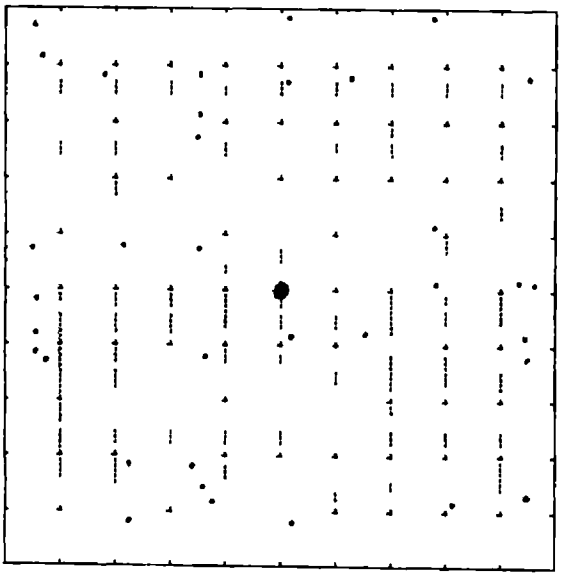
● A Plants

▲ B Plants

■ C Plants

○ Uneffected plants

✕ Missing plants



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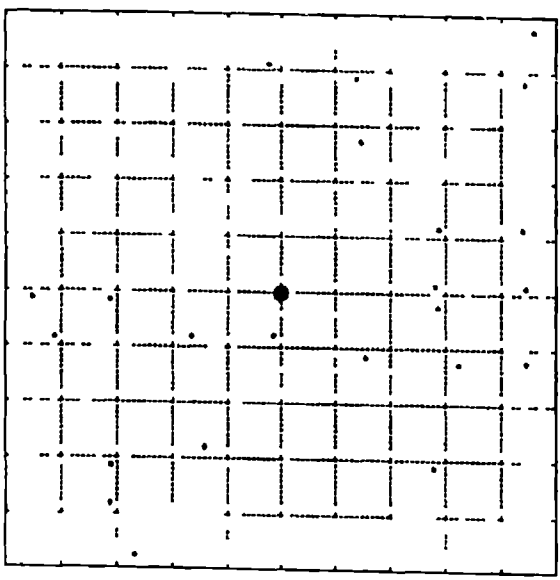
0 0 1 0 0 2 0 0 0 0

Fig 6a



1 0 1 2 1 1 1 1 0 1
2 0 0 1 1 1 2 1 0 2
0 1 0 1 1 0 1 0 0 1
0 0 1 0 1 0 1 1 0 1
1 1 0 2 0 0 1 1 0 1
2 1 0 2 1 0 1 1 0 0
0 0 0 1 0 0 1 0 1 1
0 0 0 1 0 0 0 0 0 0
2 2 0 2 2 0 2 2 0 1
2 0 2 2 2 0 2 2 0 1

Fig 6b

Fig 6 Convolution Diagrams of spread of pumpkin mosaic from A to B plants (Fig 6a) and from B to C plants (Fig 6b)

Discussion

5. DISCUSSION

Pumpkin (*Cucurbita moschata*) is one of the major vegetable crops cultivated in Kerala The productivity of this crop is very much reduced due to various diseases out of which pumpkin mosaic disease is the most serious one Umamaheswaran (1985) conducted studies on symptoms transmission physical properties serology and host range of the virus and concluded that pumpkin mosaic virus is a strain of cucumber mosaic virus He described the symptoms of the disease as the appearance of mottled green blisters which coalased and produced deformed leaves The infected plants become very much stunted producing only very few flowers with reduced fruit set The fruits are reduced in size and often misshapen When the plants were mechanically inoculated typical symptoms of pumpkin mosaic appeared within about 14 days after inoculation He also reported that among the different buffers used for the transmission 0 1 M Phosphate buffer of pH 7 2 was most effective The results of the present investigation are in agreement with the above findings *Aphis gossypii* was found to be the most efficient vector according to the studies conducted by Umamaheswaran (1985) Therefore in the present studies vector transmission trials were conducted only with *A gossypii*

In the preliminary screening trials to select medicinal plants with inhibitory effect against pumpkin mosaic virus (PMV) 30 species of plants were tested using ten-day-old pumpkin seedlings Among them eight species of plants showed 80 per cent and more inhibition of symptom expression of PMV infection Five species of plants namely, *Basella alba, Glycyrrhiza glabra, Phyllanthus fraternus, Plumbago rosea* and *Thespesia populnea* showing very high percentage of inhibition were selected for further studies The antiviral properties of of *B alba* leaf extract was first reported by Murthy *et al* (1981) Among the different extracts like basella bougainvillea and azadirachta extracts tested against TMV infection of tobacco, they found that basella leaf extract was significantly superior in inhibiting TMV and in enhancing yield of tobacco According to Selvan and Narayanasamy (1987) *B rubra* was most effective in inhibiting PVY infection of chilli followed by *B alba, Bougainvillea spectabilis* and *Mirabilis jalapa* Although the antiviral properties of *B alba* and *B rubra* against TMV and PVY have already beeen reported, inhibitory effect of *B alba* against PMV has not been reported so far In the present studies, the extract of *G glabra* was found to give 90 per cent inhibition of PMV over control *G glabra* is an ingredient in various ayurvedic preparation used in the treatment of

human diseases including viral diseases The inhibitory effect of *G glabra* extract obtained during the present studies is the first report on this aspect

Phyllanthus fraternus (*P niruri*) is also used in human medicine The antiviral property of the extract of this plant against plant virus was reported by Saigopal *et al* (1986) He found that leaf and root extracts of *P fraternus* were inhibitory to TMV, peanut green mosaic virus and tobacco ring spot virus Inhibitory effect of this plant against cowpea mosaic virus was observed by Mallika Devi (1990) but its inhibitory effect against PMV has not been reported earlier *Plumbago rosea* is also an important medicinal plant used in many ayurvedic preparations In the present studies root extract of this plant was found to have inhibitory effect against PMV This is the first report on the antiviral property of *P rosea* root extract against a plant virus

Thespesia populnea is a very common tree found in all parts of Kerala The leaf extract of this plant also was found inhibitory to PMV The antiviral property of *T populnea* against cowpea mosaic virus was observed by Mallika Devi (1990), but its antiviral property against PMV was not reported earlier

Most of the earlier reports on antiviral properties of plant extracts indicates that the inhibitory effect decreases with the increase in dilution of the extracts Hirai (1949) reported that the inhibitory effect of chilli leaf extract was reversible after dilution of extract Similar observations were made by Habib *et al* (1984) in the case of *Euphorbia pulcherrima* against TMV and Saigopal *et al* (1986) for *P fraternus* against TMV peanut green mosaic virus and tobacco ring spot virus Mallika Devi (1990) found that both five per cent and ten per cent extracts of *T populnea* were equally inhibitory against cowpea mosaic virus But in the present investigation, extract of *T populnea* was more effective at higher concentration Unlike that found in the earlier reports in the present study the lower concentrations of *B alba* and *P rosea* were more effective than the higher concentrations Although the exact reason for this type of effect cannot be elucidated from the results of the present investigations, it may probably be due to the activity of some other constituent in these plants which may be suppressing the antiviral property at higher concentrations At lower concentrations the suppressive effect might have been reduced and allowing the antiviral effect

to be expressed properly Detailed further work is necessary to reveal the mechanism involved in the above effects

When the inhibitory properties of selected medicinal plant extracts were tested against partially purified virus preparation, three plants namely *B alba*, *G glabra* and *T populnea* showed more than 94 per cent inhibition of infection and *P fraternus* and *P rosea* showed less than 90 per cent inhibition (Table 5) When the partially purified virus preparation is mixed with the plant extracts there are chances of closer interaction between virus particles and active ingredients of medicinal plant extracts The higher antiviral effects observed when the extract of *G glabra* and *T populnea* were used may be due to their direct action with the virus particles Although *B alba* leaf extract gave more than 94 per cent inhibition of infection when mixed with partially purified inoculum, there was a higher percentage of inhibition when this was mixed with crude preparation of inoculum Similarly extracts of *P fraternus* and *P rosea* also exhibited lesser inhibitory property when mixed with partially purified virus preparation than with crude virus preparation (Tables 3 and 5) Therefore, the inhibitory effects of these three plant extracts may not be the result of direct

action on the virus The modes of inhibitory action of these plant extracts may be mediated through the host plant (Tables 7a 7c and 7d) Detailed further investiga tions are required to arrive at definite conclusions on this aspect

When the selected five medicinal plant extracts were applied as pre-inoculation and post-inoculation sprays it was found that pre-inoculation application is better than post-inoculation application in all the cases Similar observations were made by many other workers also Verma and Mukerjee (1979) found that inhibition of TMV infection was highly significant when *Datura metel* leaf extract was applied 24 h before virus inoculation Rao and Shukla (1985b) reported that aqueous extracts of dry coconut showed significant antiviral activity against PVY when applied 24 h before virus inoculation and no such inhibition was observed when extract was applied 24 h after virus inoculation Extracts of *Sysygium cumini*, *Acacia arabica* and *Callistemon lanceolatus* decreased local lesion production by turnip mosaic virus on *C amaranti-color* by pre-inoculation treatments and no significant reduction was observed by post inoculation application (Pandey and Mohan 1986) The better antiviral effects obtained when the plant extracts were applied as pre-inoculation treatments may be because the inhibitory

activities in host tissues were stimulated sufficiently earlier to the entry of the virus in the plant cells

When PMV was inoculated at different intervals after the application of plant extract it was found that inhibitory property of the extract varied with time of application Among the five selected medicinal plants *B alba* extract showed maximum inhibition when inoculation was done immediately after, six and 24 hours after application *T populnea* was the most effective one when inoculated 48 h after application and *P fraternus* showed maximum inhibition when inoculated four and six days after application

When the change in the effectiveness of plant extract with the passage of time after application (before virus inoculation) was compared, it was found that, two plants namely *B alba* and *G glabra* showed a gradual reduction in inhibitory property This may be because the agents responsible for virus inhibitory action present in these plant extracts are in the active form at the time of application and as the time gap between the application of the extract and inoculation increased, the concentration of these agents decreased and hence the reduction in inhibitory effect

But in the case of extracts of *P fraternus* and *T populnea* a time gap was required between application and virus inoculation for the development of maximum inhibition *T populnea* and *P fraternus* showed maximum inhibition of infection when inoculated two and four days after application of plant extract Similar results were reported by other workers also

Johari *et al* (1983) found that the inhibitory activity of safflower leaf extract against TMV in *Nicotiana tabaccum* var Samsun was negligible when 15 min elapsed between application of extract and virus inoculation but it has increased with the increase in the time between the application and challenge so that with 48 h gap there was 85 per cent inhibition According to Verma and Srivastava (1985) a high degree of resistance (80-100% reduction) was observed when *Cyamopsis tetragonoloba* and *Datura stramonium* plants were mechanically inoculated with sunnhemp rosette virus or tobacco mosaic virus one to seven days after application of *Aerva sanguinolenta* extracts

Extract of *P rosea* showed maximum inhibition when inoculation was done immediately as well as six hours after application Eventhough there was a reduction in inhibitory effect after one and four days of application there was a slight increase also in two and six days after

application This indicated that in the case of *P rosea* extract there was no considerable change in the inhibitory effect as the time gap increased between its application and virus inoculation Therefore the inhibitory effect of *P rosea* extract on PMV may be a combination of the direct as well as indirect effect on the virus

When the systemic effect of selected medicinal plants was tested against PMV it was found that *B alba* showed maximum effect followed by *G glabra* The other three plant extracts also have shown systemic activity but to a lesser extent Verma and Srivastava (1985) found that the inhibitory stimulus of *Aerva sanguinolenta* leaf extract could move from treated to untreated leaves of *Cyamopsis tetragonoloba* and *Datura stramonium* plants within one hour after application and that the inhibitory effect was observed even after seven days Thus the resistance to virus infection induced by the extract is both systemic and of long duration Peshney and Moghe (1989) reported that leaf extract of *Polianthus tuberosa, Withania somnifera, Capsicum annuum* and *Abrus precatorius* showed 90-100 per cent inhibition of tobacco mosaic toba movirus of chilli and capsicum plants were symptomless for 30 days following a single pre-inoculation treatment with crude leaf extract Kubo *et al* (1990) found that the plant protein, designated as Mirabilis antiviral protein

(MAP) showed systemic resistance against plant viruses when applied to basal leaves 24 h before inoculation of upper ones The above reports also clearly show that the antiviral activity of many plant extracts have systemic effect

Many plant extracts are known to reduce the efficiency of the vectors in the transmission of plant viruses (Bose *et al* , 1983 Mallika Devi, 1990) In the present studies all the five selected medicinal plant extracts were capable of decreasing the percentage of transmission of PMV by *A gossypii* It was found that *P fraternus* and *P rosea* caused maximum inhibition in percentage of transmission of PMV when applied before acquisition and before inoculation feeding of the vector, respectively Bose *et al* (1983) reported that leaf extract of *Adenocalymma allicea* prevented the acquisition of bean common mosaic virus by the vector *Aphis gossypii* whereas in the present studies *A allicea* had no considerable inhibitory effect against PMV Mallika Devi (1990) reported that extract of *Phyllanthus niruri* caused 100 per cent inhibition in the acquisition and per cent transmission of cowpea mosaic virus by *Aphis craccivora*

Srinivasulu and Jeyarajan (1986) reported that pre-inoculation sprays of rice seedlings with leaf extract

of *Mirabilis jalapa* coconut and sorghum reduced RTV transmission by the green leaf hopper, *Nephotettix virescens* and increased the incubation period in the plants Hunter and Ullman (1992) reported that RD Repelin at concentrations of one four and ten per cent was highly repellent to *Acyrthosiphon indicum* and delayed symptom expression of zucchini yellow mosaic virus

Since the antiviral properties of the selected five medicinal plant extracts were tested in mechanical transmission as well as in vector transmission, a comparison of the inhibitory effects in both the types of inoculation was also made In the pre-inoculation application all the five medicinal plant extracts were more inhibitory to PMV when transmission was done by mechanical inoculation But in post-inoculation application although the plant extracts were in general, more inhibitory to PMV in the case of mechanical transmission the differences between the per cent inhibitions obtained in both the types of inoculation were statistically not significant

In the case of mechanical inoculation heavier doses of inoculum may enter the host plant cells when compared with the amount of inoculum in insect transmission Therefore the considerable antiviral effects observed in mechanical inoculation may be a more realistic

indication of the antiviral properties of the plant extracts The lesser per cent inhibition in insect transmission tests may probably be due to the faster systemic spread of the virus since the vector feeds from the conducting vessels

The distribution of virus-infected individual plants in a plant population reveals useful clues to the sources and mode of spread of plant viruses Usually the data on this aspect are not subjected to proper statistical analysis In the present investigations One Sample Runs Test by Siegel (1956) was used to analyse the data by the convolution method of analysis and it was found that the spread of the disease was concentrated around the initially affected plants Therefore roguing of the initially infected plants, i e , the A plants in the present experiment will be very much effective in substantially reducing the further incidence of pumpkin mosaic disease in the field Pumpkin mosaic virus is transmitted by aphids and their movement is very much restricted when compared with the movement of other vectors like whiteflies, leaf hoppers and plant hoppers If the spread of plant viruses transmitted by other types of vectors are also subjected to the convolution method of analysis a comparative idea about the pattern of spread of different viruses could be obtained

Summary

6. SUMMARY

Ten day old pumpkin seedlings were used as test plants to study the effect of medicinal plant extracts on the PMV infection Symptoms of pumpkin mosaic appeared within two weeks on inoculated pumpkin seedlings In sap inoculation maximum percentage of infected seedlings was obtained when the infective sap was extracted in 0 1 M phosphate buffer at pH 7 2

Out of thirty species of medicinal plants tested against PMV eight plants viz *A cocculus, B alba, C fenestratum, G glabra, I tinctoria, P fraternus, P rosea* and *T populnea* showed more than 80 per cent inhibition of infection Among these five medicinal plants namely *B alba, G glabra, P fraternus, P rosea* and *T populnea* which showed very high inhibition of infection were selected for further studies

The antiviral properties of *A cocculus, C fenes tratum, G glabra, I tinctoria* and *P rosea* observed against PMV was not reported earlier against any plant viruses Eventhough there are reports of the antiviral properties of the extracts of *B alba, P fraternus* and *T populnea* against different plant viruses, this is the

first report of antiviral properties of these extracts against PMV

When the inhibitory properties of selected medicinal plant extracts were tested at five and ten per cent concentrations, it was found that extracts of *B alba* and *P rosea* were more effective at five per cent and *P fraternus* and *T populnea* at ten per cent *G glabra* showed equal effectiveness at both concentrations

G glabra and *T populnea* extracts showed more inhibition of infection when tested on partially purified PMV than when used with crude virus preparation

Pre and post inoculation application of selected medicinal plant extracts revealed that pre-inoculation is better in the cases of all the five plants When pre and post inoculations were considered together, *P rosea* showed maximum inhibition followed by *T populnea*

When PMV was inoculated at different time intervals after the application of medicinal plant extracts it was found that *B alba* and *G glabra* showed a gradual reduction in inhibitory property But in the case of extracts of *P fraternus* and *T populnea* a time gap was required between application and virus inoculation for the development of maximum inhibition Extract of *P rosea*

showed maximum inhibition when inoculation was done immediately as well as six hours after application Eventhough there was a reduction in inhibitory effect one and four days after application there was a slight increase also in two and six days after application

B alba extract showed maximum inhibition when inoculation was done immediately after six and 24 hours after application At 48 hours after application *T populnea* showed maximum inhibition When inoculated four and six days after application *P fraternus* showed maximum inhibition

When the systemic effect of selected medicinal plant extracts were tested against PMV it was found that *B alba* showed maximum effect (88 09%) followed by *G glabra* (78 57%)

All the five selected medicinal plant extracts were capable of decreasing the percentage of transmission of PMV by *A gossypii* It was found that extracts of *P fraternus* and *P rosea* caused maximum inhibition in percentage of transmission of PMV when inoculated before acquisition and before inoculation feeding of the vector, respectively

When the effect of medicinal plant extracts on

mechanical and vector transmission was compared it was found that plant extracts showed more inhibition of infection when transmission was done mechanically

Mode of spread of pumpkin mosaic in the field was studied by the method given by Gibbs (1983) It was found that the spread of the disease was not random but was more or less concentrated around initially infected plants

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EFFECT OF SELECTED MEDICINAL PLANT EXTRACTS ON THE INCIDENCE OF PUMPKIN MOSAIC

By

VIMI LOUIS

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ABSTRACT

The inhibitory effect of certain medicinal plant extracts on the incidence of pumpkin mosaic was studied using ten day old pumpkin seedlings as the test host

Preliminary screening of 30 species of medicinal plant extracts against pumpkin mosaic virus (PMV) revealed that eight plants possessed 80 or more per cent inhibition of the infection Of these five plants namely, *Basella alba, Glycyrrhiza glabra, Phyllanthus fraternus, Plumbago rosea* and *Thespesia populnea* which showed very high virus inhibitory property were subjected to detailed studies

When the selected medicinal plant extracts were tested at five and ten per cent concentrations two plants, *B alba* and *P rosea* showed more inhibition at lower concentration *P fraternus* and *T populnea* were more effective at higher concentration *G glabra* showed equal effectiveness at both the concentrations

When the extracts of medicinal plants were mixed with partially purified virus preparation *B alba, G glabra* and *T populnea* showed more inhibition than when used crude virus preparation

All the five medicinal plant extracts showed more inhibition by pre-inoculation application than by post inoculation application

Pre-inoculation application of medicinal plant extracts at different time intervals revealed that inhibitory properties of *B alba* and *G glabra* decreased gradually, whereas, that of *P fraternus* and *T populnea* reached a maximum after a time gap Inhibitory property of *P rosea* did not show any gradual trend

Among the five selected medicinal plants *B alba* possessed a high degree of systemic effect (88 09%) followed by *G glabra* (78 57%)

The extracts of *P fraternus* and *P rosea* showed maximum reduction in percentage transmission of PMV by the vector (*Aphis gossypii*) when applied before acquisition and before inoculation feeding respectively

All the medicinal plant extracts showed more inhibition of infection in the case of mechanical transmission than in vector transmission

Spread of pumpkin mosaic in the field is not random and the data revealed that it is more or less concentrated around the initially infected plants