# MANAGEMENT OF PUMPKIN MOSAIC USING SELECTED MEDICINAL PLANT EXTRACTS

By

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**THESIS**

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COLLEGE OF HORTICULTURE

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# DECLARATION

I hereby declare that this thesis entitled **"MANAGEMENT OF PUMPKIN MOSAIC USING SELECTED MEDICINAL PLANT EXTRACTS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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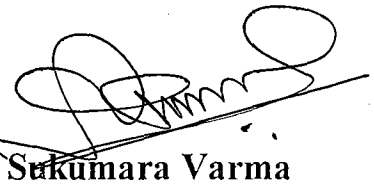
# CERTIFICATE

Certified that the thesis entitled **"MANAGEMENT OF PUMPKIN MOSAIC USING SELECTED MEDICINAL PLANT EXTRACTS"** is a record of research work done independently by **Smt. 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 to her.



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# *Introduction*

# 1. INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch ex Poir.) called as Khasiphal or Seetaphal, is one of the popular summer vegetables grown for its immature and mature fruits all over India. Because of its high carotene content and good keeping quality, it is considered as an important vegetable.

The crop known as 'Mathan' in Malayalam is an important crop of Kerala and is cultivated extensively in the summer rice fallows and in garden lands during rainy season. A number of diseases affecting this crop have been reported from Kerala and other states of India (Nair and Menon,1983; Rangaswami,1988). Viral diseases are known to cause serious loss to this crop wherever it is cultivated. Incidence of mosaic in pumpkin becomes very severe in Kerala especially during summer months and the farmers are reluctant to take up its cultivation during this otherwise most congenial season. The cultivation of pumpkin suffered a set back during last few years due to severe outbreak of mosaic diseases, particularly pumpkin mosaic and yellow vein mosaic (Balakrishnan, 1988). So the annual production of pumpkin is very meagre in Kerala and it was 33 thousand tonnes (personal communication, vegetable cell, Department of Agriculture, Kerala) during the year 2000-01 and is depending on other states for its major requirements.

Latha (1992) observed that yellow vein mosaic, cucumber mosaic, bottle gourd mosaic and pumpkin mosaic are the major mosaic diseases causing crop loss of pumpkin in Kerala. Of these, pumpkin mosaic caused by pumpkin mosaic virus (PMV) occurs in severe proportion wherever the crop is cultivated due to the high rate of insect transmission. The climatic conditions and high humidity existing in Kerala helps the build up of vector population and spread of the disease at a faster rate.

PMV, a definite member of family potyviridae and the genus potyvirus is ubiquitous and has become a major limiting factor preventing the realization of

high yield. It was first reported from pumpkin by Hariharasubramanian and Badami (1964). Since then, the disease has been observed wherever the pumpkin is grown (Shankar *et al.*, 1972; Singh, 1981).

Effective therapeutic agents have not yet been developed for the control of viral diseases of plants under field conditions even though many such diseases badly affect crop production. So cultivation of resistant varieties is the most accepted efficient method for achieving maximum productivity by avoiding the disease. None of the available pumpkin varieties possess resistance to mosaic.

Elimination of sources of viruses, use of virus free planting materials, manipulation of cultural practices and chemical control of insect vectors are some of the means for managing the virus disease. Indiscriminate use of chemical plant protectants to control vector population causes adverse impact on ecological balance of nature, human and animal health.

Many substances from biological sources such as plants, microorganisms and animals are reported to possess potential ability to control viral diseases of plants (Verma, 1985). Some of these products are known to induce resistance in host plants by stimulation of defense mechanisms existing in plants. These inducers are usually degraded in short time without leaving harmful residues. Many plant extracts have been reported to possess insecticidal or insect repellent properties and thereby prevent the spread of vector borne viral disease.

There are many reports on the inhibitory effect of medicinal plant extracts on plant viruses. Louis and Balakrishnan (1996) reported that five medicinal plants namely *Basella alba, Glycyrrhiza glabra, Phyllanthus fraternus, Plumbago rosea* and *Thespesia populnea* possess inhibitory property on PMV.

Serological methods of plant virus detection are efficient and cheap compared to molecular techniques. Standardisation of serodetection techniques will be useful to study the effect of antiviral agents.

Field level applications of antiviral plant products for the management of viral diseases of cucurbitaceous crops are lacking. So the present study was undertaken with the following objectives.

i) Selection of suitable medicinal plant having antiviral property to PMV

ii) Isolation of the inhibitory principle present in the medicinal plant

iii) Management of pumpkin mosaic using the plant extract

iv) Partial purification of PMV and serological studies.

# *Review of Literature*

# 2. REVIEW OF LITERATURE

Pumpkin (*Cucurbita moschata* Duch ex Poir.) is an important cucurbitaceous vegetable of tropics cultivated for its immature and mature fruit and is one of the most popular vegetables grown in Kerala. It is known for its high vitamin A content and suitability for prolonged storage. Tripathi and Joshi (1985) reported the occurrence of several mosaic viruses in pumpkin viz., watermelon mosaic virus, yellow vein mosaic virus, bottle gourd mosaic virus, cucumber mosaic virus, cucumber mottle mosaic virus, cucumber necrosis virus and squash mosaic virus. Latha (1992) observed that pumpkin mosaic, yellow vein mosaic, bottle gourd mosaic and cucumber mosaic are the major mosaic diseases of pumpkin causing crop loss in Kerala. Of these, pumpkin mosaic caused by pumpkin mosaic virus, is very serious in the field and its incidence was considerably more than that of yellow vein mosaic (Umamaheswaran, 1985). Considering the importance of crop and significance of this virus disease in the cultivation of pumpkin, investigations have been undertaken on this. A review of literature pertaining to PMV and its management using medicinal plant extracts is presented in this chapter.

## 2.1 SYMPTOMATOLOGY

Pumpkin mosaic disease 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) observed that the symptoms of pumpkin mosaic virus disease first appeared as mosaic mottling of the leaves. Sometimes leaves showed chlorosis of veins and veinlets leaving interveinal areas green. The leaf lamina was very often distorted and reduced. The veins and veinlets often extended beyond the margin

giving the leaves a filiform shape. Ragozzino and Stefanis (1977) studied the effect of watermelon mosaic virus-I (WMV-1) infection on vegetable marrow and reported that severe leaf narrowing and distortion and dome-shaped protuberances on the fruits were the symptoms. Ghosh and Mukhopadhyay (1979a) isolated nine different strains of viruses from pumpkin (*Cucurbita moschata*) from West Bengal, and among them, the isolate – A7 produced characteristic mottling with mild green blisters and green vein banding in the leaves of infected plants. Singh (1981) reported a mosaic disease of pumpkin characterized by severe mosaic mottling, deformation and blistering of leaves. The virus was identified as a strain of WMV-1. Almeida and Borges (1983) reported that WMV produced mosaic and severe distortion of leaves of pumpkin. Tripathi and Joshi (1985) explained symptoms of artificial inoculation of PMV as vein clearing followed by vein banding, on newly emerging leaves of pumpkin. At later stages of disease development, leaves showed mosaic symptoms, got distorted and reduced in size. The growth of plant was stunted. Umamaheswaran (1985) observed severe mottling and disfiguration of leaves of pumpkin infected with PMV. Very often the leaf lamina showed severe mottling with mild green blisters. Infected plants flowered very sparingly and produced less number of fruits. The fruits were reduced in size and often misshapen. Louis (1994) explained symptoms of natural infection and artificial inoculation of PMV. The leaves of naturally infected plants showed mottled green blisters which later coalesced resulting in disfiguration of leaves. The infected seedlings remained stunted. They flowered very sparingly and that also with less number of female flowers, and reduced fruit setting. The fruits were reduced in size and often misshapen. On mechanical inoculation of pumpkin seedlings at two leaf stage, symptoms were appeared 12-14 days after inoculation. Typical mosaic pattern with dark green and light green patches was produced in all the newly emerged leaves. The growth of plants subsequent to infection was much reduced and branches produced were very weak. Yuki *et al.* (2000) reported five viruses infecting cucurbits as cucumber mosaic virus (CMV), papaya ring spot virus – type W (PRSV-W), watermelon mosaic virus 2 (WMV-2), zucchini lethal chlorosis virus (ZLCV) and zucchini yellow mosaic virus (ZYMV). Of these, PRSV-W and ZYMV were the most frequently found viruses.

## 2.2 TRANSMISSION

### 2.2.1 Sap Transmission

Mechanical transmission of PMV was first reported by Hariharasubramanian and Badami (1964). Jaganathan and Ramakrishnan (1971) found that two virus isolates from muskmelon (*Cucumis melo*) and pumpkin (*C. moschata*) could be mechanically transmitted to healthy plants of same species. A mosaic virus of pumpkin (*C. moschata*) commonly occurring in Delhi was found to be sap transmissible (Shankar *et al.*, 1972). Roy and Mukhopadhyay (1979) found that the spread of PMV was possible by mechanical contact. They reported 100 per cent transmission within 15 days of inoculation from young leaves, old leaves and male flowers of infected pumpkin plant, when extracted in 0.1 M citrate phosphate buffer, pH 8.0. Tripathi and Joshi (1985) reported a sap transmissible virus producing mosaic and leaf distortion symptoms on pumpkin. On mechanical inoculation using standard extract of diseased leaf in distilled water, symptoms appeared 7-10 days later as vein clearing followed by vein banding. Umamaheswaran (1985) reported that PMV found to be transmitted successfully when inoculated on fully opened cotyledons of ten days old pumpkin seedlings. The percentage of transmission varied with extraction medium used and standard sap gave maximum infection of 85 per cent. He also reported that initial symptoms appeared 14 days after inoculation in the form of mild vein clearing and typical mosaic mottling with dark green and light green patches. Maluf *et al.* (1986) conducted mechanical transmission from infected plants of *Cucurbita pepo* to screen different cucurbita species against WMV-1 at first true leaf stage. Symptoms were scored 14, 21 and 28 days after inoculation. Louis (1994) conducted mechanical transmission of PMV and reported that phosphate buffer 0.1M, pH 7.2 gave maximum transmission.

### 2.2.2 Insect Transmission

Pumpkin mosaic virus was reported to be transmitted by number of vectors. Hariharasubramanian and Badami (1964) who reported pumpkin mosaic for the first time in India found that it was transmitted by *Aphis laburni* and many other

*Aphis* spp. Nagarajan and Ramakrishnan (1971) found that the viruses commonly occurring on pumpkin could be non-persistently transmitted by *Myzus persicae, Aphis gossypii* and *A. nerii.*

Similar results were also reported by Jaganathan and Ramakrishnan (1971). Shankar *et al.* (1972) reported that a mosaic virus of pumpkin (*C. moschata*) commonly occurring in Delhi was found to be transmissible by *M. persicae* and *Sitobion rosaeformis.* Tewari (1976) studied the transmission of WMV by aphids and reported that *A. gossypii* Glov. was the most efficient vector and the virus was transmitted in a non-persistent manner. Bhargava (1977) also reported that *A. gossypii* is the field vector of WMV. Ghosh and Mukhopadhyay (1979a) isolated nine virus strains and reported that isolate A3 was transmissible by *Bemisia tabaci*, isolate A4 and A6 by *M. persicae*, isolate A5 and A7 by A. *gossypii.* Umamaheswaran (1985) carried out insect transmission of PMV using three vectors, viz., *A. gossypii, A. craccivora* and *B. tabaci.* Highest percentage of transmission (65 per cent) was obtained with *A. gossypii* when compared to that 25 per cent with *A. craccivora. Bemisia tabaci* was not able to transmit the virus. He also reported that preliminary fasting of the aphids resulted in an increase in per cent transmission. Yamamoto (1986) reported that WMV was transmitted to cucumber seedlings by *A. gossypii* at a high rate from many kinds of cucurbit crops infected with WMV. Louis (1994) obtained 62.5 per cent transmission of PMV by *A. gossypii* when 10 nos. viruliferous aphids were used per seedling.

### 2.2.3 Seed Transmission

Jaganathan and Ramakrishnan (1971) carried out investigations on the transmission of melon mosaic virus and found that it was transmitted to some extent through seeds. Shankar *et al.* (1972) found that mosaic virus of pumpkin was not transmitted through seeds. Sharma and Chohan (1973) studied seed transmission of cucumis virus 1 and cucumis virus 3 through seeds of cucurbits and they reported that cucumis virus 1 was seed borne in vegetable marrow, ash gourd and pumpkin and cucumis virus 3 was found to be seed borne in bottle gourd. Seed transmission of squash mosaic virus was reported by Thomas (1973)

and Kemp *et al.* (1974). Nagarajan and Ramakrishnan (1975) carried out investigations on transmission of melon mosaic virus through seeds and found that it was transmitted to some extent through seeds. Hein (1977) working on WMV-1 in *C. pepo* L. var. *giromontima* Alef. found that it was not seed transmissible. Similar report was also made by Wu and Su (1977). Singh (1981) reported that the primary infection of pumpkin mosaic is through infected seeds and secondary infection by aphid vectors. Mukhopadhyay (1985) reported that WMV can be transmitted through seeds but efficiency of transmission is low.

Umamaheswaran (1985) tested seed transmission of PMV and reported that none of the plants showed symptom of mosaic during 45 days of observation.

## 2.3 HOST RANGE

Jaganathan and Ramakrishnan (1971) while studying the virus diseases of cucurbits found that melon mosaic virus was confined to the family cucurbitaceae and the virus could not be recovered from the other species by back inoculation to the test plants. Shankar *et al.* (1972) inoculated PMV on 76 plant species of nine families. They found that its host range was restricted to family cucurbitaceae. They also found that it produced systemic mosaic symptoms on *Cucurbita pepo, Cucumis melo, C. melo* var. *utilissima, Lagenaria siceraria* var. Round and long, *Luffa acutangula, Citrullus vulgaris, Momordica charantia, Benincasa hispida* and *Trichosanthes anguina. Cucumis sativus* was proved to be a symptomless carrier. Adlerz (1972) reported *M. charantia* as a source of WMV-I. Wu and Su (1977) reported that WMV could infect 16 plants coming under the family cucurbitaceae but wax gourd (*Benincasa cerifera*) was hypersensitive and calabash (*Cucurbita lagenaria*) was immune. Bhargava and Bhargava (1977) described that pumpkin yellow vein mosaic virus (PYVMV), PMV, cucumis virus-3 (cucumber green mottle mosaic virus), 3 strains of CMV and 7 of WMV had their host range basically restricted to cucurbitaceae, but they also infected cowpea, cv. Black Turtle. *Zinnia elegans* was a symptomless carrier of the WMV strain from *Trichosanthes dioica.* Makkouk and Lesemann (1980) reported that WMV-1 could induce local lesions without systemic spread in *Chenopodium amaranticolor* and

*C. quinoa* and systemic infection in cucumber, squash, pumpkin and watermelon. Tripathi and Joshi (1985) conducted host range studies of WMV covering 52 different plants belonging to different families and reported that the virus infect plant species in the family chenopodiaceae, compositae, cucurbitaceae and fabaceae. Umamaheswaran (1985) studied host range of PMV covering 56 species of 16 families and reported that host range was restricted to the members of family cucurbitaceae. Kirde and Lokhande (1996) reported that WMV showed systemic infection in ridge gourd (*Luffa cylindrica*), bottle gourd (*L. siceraria*), bitter gourd (*M. charantia*), pumpkin, cucumber, musk melon and water melon 7-20 days after inoculation. Soybean, cowpea and radish showed systemic symptom within 10-12 days. Back inoculation showed no transmission of disease.

Sastry (1982) reported host range of PVY isolate of brinjal on different crops and explained symptoms as severe mosaic mottle in brinjal, mosaic mottle in chilli, *Nicotiana glutinosa*, and *Datura metel*. Necrotic local lesions were reported in *Physalis floridana* and *C. amaranticolor*.

Yeh *et al.* (1984) studied host range of nine papaw ringspot virus (PRSV) isolates from Taiwan, Hawaii, Fla and Ecuador and reported that it included members of chenopodiaceae, cucurbitaceae and caricaceae. *Cucumis metuliferus*, *C. melo* and *C. sativus* cv. Surinam which possess genes resistant to WMV-I reacted identically to all isolates of WMV-1 and PRSV.

WMV has a very restricted host range, and it infects only the members of cucurbitaceae. Isolates which can infect plants belonging to fabaceae have also been reported (Anderson, 1954). Webb and Scott (1965) divided WMV into two groups, WMV-I and WMV-II. WMV-I cannot infect plants other than members of cucurbitaceae and WMV-II can infect plants belonging to cucurbitaceae as well fabaceae.

## 2.4 ELECTRON MICROSCOPIC STUDY

Shankar *et al.* (1972) studied particle morphology of PMV using partially purified virus preparation and partially clarified virus preparation. Electron micrographs of partially purified virus preparation showed aggregation of virus

particles, whereas partially clarified virus preparations gave uniform size particles with least aggregation. The modal length of the virus was determined to be 840 nm where as the average width was 15 nm. Makkouk and Lesemann (1980) isolated WMV-1 from cucumber leaves which showed systemic infection in pumpkin. Electron microscopy of negatively stained extracts from infected pumpkin revealed the presence of flexuous particles 750-800 nm long. As per the report of Mukhopadhyay (1985) the particle length of WMV-I is 700-800 nm and WMV-II is 700-760 nm. Meer *et al.* (1987) reported flexuous rod shaped virus, 706-770 nm long from cucurbit leaves showing chlorotic mottling, vein banding, dark green blisters and stunting. Singh (1987) on the basis of serology and electron microscopy identified the pathogen of pumpkin mosaic as a strain of WMV-I. He noticed flexuous rods of 725x15 nm in purified preparations from infected pumpkin leaves.

## 2.5 SEROLOGY

### 2.5.1 Purification of PMV

Shankar *et al.* (1972) tried many methods of purification of PMV and standardized the method using 0.1 M citric acid phosphate buffer (pH 8.0) containing 10 m M Diethyl dithio carbamate (DIECA) as the extractant. The homogenate was passed through double layer muslin cloth and centrifuged at 5000 g for 20 min. The supernatant obtained was centrifuged at 78000 g for 90 min to sediment the virus. The pellet which was greenish in colour was suspended in 0.1 M citric acid phosphate buffer (pH 8.0) and was subjected to two more cycles of differential centrifugation. The final pellet was resuspended in 0.1 M citric acid phosphate buffer (pH 8.0) and centrifuged at 2000 g for 15 min.

Sako *et al.* (1980a) purified WMV from pumpkin using 0.3 M potassium phosphate buffer at pH 8.8 containing 0.01 M DIECA and 0.1 per cent mercaptoethanol and with 15 per cent carbon tetra chloride and 2 per cent triton x-100. Water melon mosaic virus was concentrated by two cycles of differential centrifugation using 20 per cent sucrose cushion and further purified by zonal sucrose density gradient centrifugation. The UV absorption spectrum of the

purified preparation was typical of other potyviruses with a maximum at 260 nm and minimum at 246 nm. The yield of purified WMV was 2-3.5 mg per 100 g of infected pumpkin leaves.

### 2.5.2 Antiserum Production

Shankar *et al.* (1972) injected purified virus preparation emulsified with Freunds incomplete adjuvant as five intramuscular injections at 3-4 days interval to the rabbit and was bled 10 days after final injection and collected antiserum. The antiserum obtained was specific and no reaction was obtained between the antiserum and clarified healthy plant sap or between normal serum and the diseased plant sap or purified virus preparation. The antiserum had a titre of 1:2048 when tested in serial dilutions with purified virus preparation (1:16 dilution). The maximum dilution of clarified diseased plant sap which reacted positively with antiserum was 1:64 and that of the purified virus preparation which gave positive precipitate was 1:2024.

Umamaheswaran (1985) used partially purified PMV for immunization of rabbits. The emulsion of the purified virus and Freund's incomplete adjuvant (1:1 ratio) was given as five intramuscular injections into the thigh muscles at the rate of 4 ml at a time and rabbits were bled three weeks after final injection and antiserum was collected. The antiserum had a titre of 1:512, when tested in serial dilutions and the virus end point was between 1:512 and 1:256. Khurana *et al.* (1987) reviewed the production of antiserum to potato virus Y.

### 2.5.3 Serodiagnosis

The serodiagnosis of plant viruses has been extensively reviewed by Noordam (1977), Hill (1984) and Van-Regenmortel (1982). Singh and Sharma (1989) reviewed the serodiagnosis of horticultural crops in India.

Umamaheswaran (1985) reported that PMV was related to viruses causing mosaic diseases of bitter gourd and snake gourd. It was also reported that PMV was not related to cucumber mosaic virus (CMV) ICRISAT isolate and "CMV So Afr. Str" and cowpea mosaic virus EL Salvador.

Milne and Grogan (1969) while studying the characterization of WMV strains by serology found that WMV-1 and WMV-2 were related and an isolate of PRSV was also serologically related to WMV. Purcifull and Hiebert (1979) while testing the antisera of purified preparations of Florida isolates of WMV type 1 (WMV-IFL) and WMV type 2 (WMV-2FL) with extracts from diseased leaves from a variety of host plants also found that PRSV was closely related serologically to WMV-IFL but non reactive with WMV-2FL, whereas soybean mosaic virus was unrelated to WMV-IFL but related to although distinct from WMV-2FL. Makkouk and Lesemann (1980) when used Derrick technique of immune electron microscopy of WMV isolated from cucumber leaves, strong specific trapping was observed when grids were coated with WMV-1 antiserum but no trapping with WMV-2 or bean yellow mosaic virus antisera. Using a decoration technique of immune electron microscopy, a strong effect was observed with WMV-1 antiserum and a weak effect with bean yellow mosaic virus and no effect with WMV-2 antisera. Yeh *et al.* (1984) tested isolates of PRSV and WMV-1 and found that they were serologically indistinguishable as determined by agar immuno diffusion tests with antisera to PRSV and WMV-1. They reported that the similarities in resistant susceptible host reaction and in serology, strongly indicates that PRSV and WMV-1 are very closely related.

Since the introduction of ELISA to plant virology by Clark and Adams (1977), it has been used widely for detection of plant viruses. Sako *et al.* (1980b) reported the use of ELISA for detection of WMV and CMV from cotyledons, leaves, hypocotyls and roots of cucurbitaceous plants. Application of ELISA to quantitative evaluation of virus resistance and screening of antiviral substances was reported by Shenk *et al.* (1988). Barker (1989) described the use of ELISA in plant virus diagnosis. Yilmaz and Sherwood (2000) compared the formats of three ELISA, Protein-A ELISA (PA-ELISA), antigen coated plate ELISA (ACP-ELISA) and indirect ELISA Kit for detection of viruses infecting cucurbits namely CMV, PRSV, WMV and ZYMV.

Clark (1981) explained different ways of interpretation of absorbance value of ELISA and to classify the samples as positive or negative. It may be the upper

negative limit or a value two or three times the mean absorbance value of the negative sample or mean of negative values plus two or three standard deviation.

## 2.6 BIOCHEMICAL CHANGES DUE TO HOST PATHOGEN INTERACTION

Ghosh and Mukhopadhyay (1979b) studied the biochemical alterations with respect to buffer soluble protein, reducing sugar and starch in *C. moschata* plants after inoculation with nine virus isolates, namely A1 and A2 (strains of bottle gourd mosaic virus), A3 (vein yellowing virus), A4 and A6 (strains of cucurbit latent virus), A5 (pumpkin cucurbit mosaic virus) and A7 (water melon mosaic virus), A8 (pumpkin enation mosaic virus) and A9 (pumpkin mild mosaic virus). Singh and Suhag (1982) reported reduction in protein content of mung and urd bean leaves infected with yellow mosaic virus, mung and urd mosaic virus-I and II and leaf crinkle virus. Sharma *et al.* (1987) studied metabolic changes in musk melon due to CMV infection. Protein levels were higher in infected plants while total N decreased at all stages of infection. Increased level of protein in CMV infected leaves and seeds of Amaranthus and Chenopodium was reported by Dhan-Prakash *et al.* (1995).

An increase in photosynthetic activity of TMV infected tobacco plants was reported (Owen, 1957; Zaitlin and Hesketh, 1965). Singh and Singh (1991) reported that infection of chillies by CMV reduced total chlorophyll, chlorophyll a and b. The reduction in chlorophyll was correlated with symptom development and disease severity. Wani *et al.* (1991) also recorded reduction in total chlorophyll in sorghum leaves infected with maize mosaic virus.

Rajan (1985) observed a negative correlation between resistance and total phenol content in tomato due to increase rate of oxidation of phenolics. Radhika (1999) reported an increase in total chlorophyll content of resistant, and a decrease in phenol content of resistant and susceptible cowpea varieties after 15 days of inoculation of cowpea aphid borne mosaic virus. Mali *et al.* (2000) reported that there was a significant decrease in the contents of total chlorophyll, starch, 0-dihydroxy phenols, dry matter and activities of catalase, peroxidase and nitrate

Extracts were effective when sprayed on hosts 24 h before or within five minutes after inoculation. Verma and Mukherjee (1975) pointed out that brinjal leaf extract induced local and systemic resistance in *Nicotiana glutinosa* against TMV and in *N. tabacum* NP 31 against tobacco ring spot virus when applied 24 h before inoculation. Murthy *et al.* (1981) studied the effect of spray of one per cent extract of *B. spectabilis, Basella alba* and *Azadirachta indica* in the inoculated and healthy tobacco seedlings and found that three sprayings of *B. alba* reduced both incidence and intensity of disease. The inhibitory effect of prophylactic spraying of *B. alba* leaf extract on the infection of TMV on tobacco was reported by Ushari *et al.* (1982). Johari *et al.* (1983) reported the effect of leaf extract of *Helianthus annuus* on TMV. The effect was negligible when it was mixed *in vitro* with clarified sap containing TMV and high percentage of inhibition was recorded when there was a gap of 48 h between treatment and challenge of the virus. The aqueous extract of the leaves of fern *Ampelopteris prolifera* showed high inhibition of TMV and CMV. Inhibition was maximum when applied on local lesion or systemic host 24 h prior to virus inoculation (Pandey and Bhargava, 1984).

Habib *et al.* (1984) reported that latex of *Euphorbia pulcherrima* inhibited TMV. Pre-inoculation sprays of *B. diffusa* root extract was found to be effective against CMV and TMV in tomato, cucumber green mottle mosaic virus (CGMMV) in melon, sunnhemp rosette virus in *Crotalaria juncea* and *Gomphrena globosa* (Awasthi *et al.*, 1984). 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. Baranwal and Verma (1992) reported that aqueous leaf extract of *Celosia cristata* showed antiviral activity against tobacco mosaic tobamovirus, sunnhemp rosette virus, potato X potyvirus in several local lesion hosts. Many plant extracts contain inhibitors of other viruses like potato virus Y (PVY), water melon mosaic virus (WMV), potato virus X (PVX) etc.

According to Gupta and Raychaudhuri (1971b) leaf extract of *Callistemon lanceolatus* and *Syzygium cumini* inhibited local lesion production when mixed with sap from PVY infected plants. Rao and Shukla (1985) reported that aqueous

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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 *B. rubra* was the most effective in inhibiting PVY infection in chilli followed by extracts from *B. alba, B. spectabilis* and *M. jalapa.*

Singh (1969) reported 100 per cent inhibition of WMV by the crude sap of *C. album, C. amaranticolor, Dahlia rosea* and *Spinacea oleracea.* The effect was reduced to 50-80 per cent by 1/1000 dilution of crude sap. Tewari (1976) reported the effects of several bark extracts on the inactivation of three strains of WMV. Louis and Balakrishnan (1995) reported that extracts of eight species of plants viz., *Anamirta cocculus, Basella alba, Coscinium fenestratum, Glycyrrhiza glabra, Indigofera tinctoria, Phyllanthus fraternus, Plumbago rosea* and *Thespesia populnea* showed 80 per cent or more inhibition of PMV, when the extracts were mixed with virus inoculum and applied on pumpkin seedlings. When compared pre and post inoculation application of *B. alba, G. glabra, P. fraternus, P. rosea and T. populnea,* pre-inoculation application was found to be more effective than post inoculation application (Louis and Balakrishnan, 1996).

Molina *et al.* (1986) injected extracts of *Phytolacca americana* into sorghum plants 24 h before, at the moment of and after inoculation with soybean mosaic potyvirus and results were evaluated by symptom and double antibody ELISA. Crude and seed extracts were most effective (75-100% inhibition) when applied before or at the time of inoculation and cell and callus extracts after inoculation. No significant difference in virus concentrations was found between the control and treated plants. By screening 30 plant species, Devi (1990) reported that 16 plant species including *P. fraternus* and *T. populnea* showed inhibition of cowpea mosaic virus.

Tewari *et al.* (2001) tested 13 species of ferns for their inhibitory effects against CGMMV on *Cucurbita pepo* cv. caserta. The extracts of *Adiantum caudatum, Dryoteris filix-mas, Polypodium parasiticum, P. obligatum* and *P. nitro-*

*carpum* showed highest inhibitory action. More than 90 per cent infectivity of the virus was inhibited *in vitro*.

### 2.7a Biophysical Characteristics of Virus Inhibitors from Plants

Virus inhibitory properties of plants depends on factors like plant part used, solvent of extraction, concentration of extract etc. Change in virus inhibitory activity may also occur due to heating of the extract.

Allen and Kahn (1957) demonstrated that TMV inhibitor was present in leaf, flower, polish, roots, culm and kernels of rice. The dilution end point of inhibitor was 1:3000 (fresh weight) for leaf and root extracts and 1:13000 (dry weight) for rice polish. Thermal inactivation studies suggested the presence of two or more inhibitors one labile above 60°C and one stable at 100°C. Singh and Varma (1981) tested the extracts of roots, stem, leaves, flower parts and seed of *Datura metel* on *C. amaranticolor* for inhibition of TMV, extracts from all parts except roots of the plant prevented TMV lesions. Gupta and Naqvi (1991) used different parts (leaves, roots, green stem, bark, seeds, green fruits and rhizomes) of seven flowering plants namely *Aconitum heterophyllum, Azadirachta indica, Capsicum annuum, Chenopodium amaranticolor, Datura metel, Glycyrrhiza glabra* and *Rauvolfia serpentina* against brinjal necrotic mosaic virus on auberigines. They decreased number of local lesions and systemic infection markedly.

Varma (1973) reported that extract of cabbage leaves (*Brassica oleracea* var. wirsing) inhibited TMV infection when assayed on *N. tabacum* var. Xanthi-nc and *N. glutinosa*. The inhibitor was destroyed if the leaves were macerated in ethanol, methanol and petroleum ether but not in chloroform and water. An antiviral agent active against spherical and tubular viruses in hypersensitive and systemic hosts has been isolated from *B. diffusa*. The inability of solvents (ether, petroleum ether and chloroform) to extract any active material from the root extract indicated probable absence of lipid moiety (Verma *et al.*, 1979). Alcohol extracts from callistemon, datura, agave and ginger showed good degree of suppression of bhindi yellow vein mosaic virus symptom in bhindi (Choudhuri

*et al.*, 1992). Barakat and Ghanem (1996) reported that aqueous crude seed extracts of 18 species of legume seeds were inhibitory to tobacco necrosis virus on *Phaseolus vulgaris*. The inhibitory activity of majority of species decreased on dilution (1:1000) suggesting that they act as virus inhibitors and not inactivators.

Blaszezak *et al.*(1959) reported that infectivity of PVX was wholly suppressed by juices from *Pelargonium hortorum, C. album, C. amaranticolor* and two varieties of *Capsicum frutescens*. Juice of *P. hortorum* did not lose its inhibitory activity when heated 10 min at 100°C. The inhibition of the virus was complete when the leaves were inoculated 20 days after spray of *P. hortorum*. The inhibitory principles in *A. arabica* effective against PVY was thermostable and dialyzable. Its activity was sharply reduced on dilution but not by dessication for eight days (Gupta and Raychaudhuri, 1971a). Singh and Singh (1988) studied antiviral activity of leaf and bark extracts of *A. indica* against cowpea mosaic comovirus. The inhibitor was heat stable, non-dialysable through cellophane, remained active after 25 days of storage, was not destroyed by dessication for 50 days and showed appreciable loss of activity upon dilution. Manickam and Rajappan (1998) studied the effect of temperature on the effectiveness of antiviral principles from *Cocos nucifera, Sorghum vulgare, Prosopis chilensis, Euphorbia thymifolia* and *Croton sparsiflorus* in inhibiting infection of cowpea cv. C152 by tomato spotted wilt virus. All the antiviral principles (AVP) tested showed some loss in their inhibitory effect as the temperature increased and maximum loss was observed at 80°C indicating that all AVPs were thermolabile.

Simons *et al.* (1963) studied TMV inhibitory properties of succulent plants and reported that most of succulent plants with-stood 80°C for 10 min without apparent loss of activity. Activity was also demonstrable at dilutions of 1:250. Verma and Awasthi (1979) showed that the inhibitors in root extracts of *B. diffusa* was active upto a dilution of 1:500 and upto 15 days of storage at room temperature and was inactivated by heating at 95°C, for 10 min against TMV, sunnhemp rosette, gomphrena mosaic and tobacco ring spot virus in several hosts. According to Sawant and Ambekar (1990) safflower mosaic virus was inhibited by leaf extracts from *Capsicum annuum, Acacia arabica, Datura metel, Azadirachta*

*indica, Spinacea oleracea* at three dilutions viz., 1:10, 1:100 and 1:1000 and of these *D. metel* at 1:1000 dilution produced maximum inhibition of the virus. Tewari *et al.* (2001) reported that 90 per cent dilution of extracts of *Adiantum caudatum, Dryoteris filix-mas* and *Polypodium parasiticum* which showed more than 93 per cent inhibition of CGMMV on *Cucurbita pepo* cv. caserta resulted in 75,70 and 65 per cent inhibition of virus respectively.

**2.7b Biochemical Changes due to Application of Plant Extracts**

Prasad (1986) reported alterations in enzyme activities by aqueous extract of *C. aculeatum* against TMV in *Nicotiana tabacum* cv. Samsun NN. Verma *et al.* (1987) reported that *C. aculeatum* induced resistance against sunnhemp rosette virus in *Cyamopsis tetragonoloba* plants and studied enzymatic changes due to the application of the extract. It was observed that higher catalase and peroxidase activity at the treatment site, polyphenol oxidase activity at treatment and distant sites two hours after application. Verma and Prasad (1988) studied the effect of *B. diffusa* and *C. fragrans* application on protein and enzymes of *C. tetragonoloba.* Catalase, polyphenol oxidase, peroxidases and proteins showed acyclical pattern with two peak values and first peak corresponded with the highest point of antiviral activity. The products of the enzyme catalysed reactions said to have a role in antiviral activity on sunnhemp rosette virus. Selvaraj and Narayanasamy (1991) reported that the contents of total and reducing sugar, phenolics and proteins were increased in rice plants inoculated with rice tungro virus after treatment with barium chloride, ammonium ferrous sulfate, acridine orange and leaf extracts of *Tribulus terrestris* and *Vitex negundo.*

**2.7c Effect of Plant Extract on Vector**

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.* Kulat *et al.* (1997) conducted field trials to determine efficacy of six plant extracts and two insecticides for the control of *A. gossypii* and *Amrasca devastans* on okra. Aqueous leaf extract of tobacco (2%) *Ipomea carnea* (5%) and a seed extract of *Azadirachta indica* and *Pongamia glabra* (*P. pinnata*) both at five

per cent gave a similar level of control to endosulfan (0.06%) and monocrotophos (0.05%).

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-emulsiable oils (2.5 and 3%) completely prevented transmission of the virus by *A. craccivora*. Devi (1990) got 100 per cent inhibition of insect transmission of cowpea mosaic virus with extracts of *Azadirachta indica, C. infortunatum, Phyllanthus niruri* and *Vitex negundo*.

Mariappan *et al.* (1982) observed that many seed oils such as custard apple oil and neem oil possessed inhibitory action against rice tungro 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 derivatives 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 incubation period in the plants. A pre-inoculation spray with *M. jalapa* leaf extract reduced transmission upto five days after spraying and maximum reduction in transmission was observed when the plants were inoculated one day after the treatment.

Ponniah *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 ionophyllum*) were most effective than the seed oil of neem in reducing the survival of green leaf hopper, *N. virescens*. Insect mortality was 100 per cent, four days after spraying compared to 60 per cent survival on control. Rice tungro virus infection was 17-35 per cent in treated plants compared to 51 per cent in control.

Hunter and Ullman (1992) showed the repellent property of neem product RD-Repelin on settling behaviour of aphid *Acyrthosiphon pisum* and transmission of zucchini yellow mosaic virus. In 81 per cent plants treated with one per cent concentration of RD-Repelin, symptom expression was delayed eventhough virus transmission was not prevented.

Louis and Balakrishnan (1997) studied the effect of *B. alba, G. glabra, P. fraternus, P.rosea* and *T. populnea* on transmission of PMV at three stages viz., before acquisition, before inoculation and after inoculation feeding of vector *A. gossypii.* They reported that *P. rosea* showed maximum effect when applied before inoculation feeding of vector, and the effect decreased with time after application of extract.

Pun *et al.* (2000) studied effects of virus inhibitory chemicals and neem products against okra yellow vein mosaic virus (OYVMV) and reported that all the chemicals and neem products significantly reduced OYVMV infection. The effectiveness of neem derivatives was explained as direct interference with the vector, *Bemisia tabaci.*

## 2.8 ISOLATION OF VIRUS INHIBITORY PRINCIPLES OF PLANT EXTRACT

Substances have been extracted from plants which when applied to other plant species make them resistant to virus infection in various ways. They may interact directly with virus or act indirectly through an effect on host and or vector. Verma and Prasad (1983) showed that in many cases where inhibition is host mediated, the increased resistance of the host is due to development of virus inhibitory agents (VIA). Verma and Baranwal (1987) reported that VIAs are low molecular weight proteins. These proteins inactivated the virus *in vitro* and their production commences soon after application of antiviral agents.

Different methods for isolation of virus inhibitory agents from plants have been reported. Khan and Verma (1990) isolated VIA from the diethyl ether extract of *Cyamopsis tetragonoloba* which was treated with *Pseuderanthemum bicolor* and VIA was precipitated with ammonium sulphate and further purified by ion-exchange

chromatography and gel chromatography. Valsaraj *et al.* (1997) fractionated fruit rind extract of *Terminalia bellerica* over silica gel and collected 12 fractions which led to the isolation of four compounds possessing anti-HIV-1, antimalarial and antifungal activity.

Active principles of most of antiviral agents reported from plants were proteins. These included plants *Dianthus caryophyllus*, *Spinacea oleracia*, *Amaranthus caudatus* and *Solanum tuberosum* (Verma *et al.*, 1981). Ushari *et al.* (1982) partially purified virus inhibitor of *Basella alba* against TMV and identified as glycoprotein containing 20 per cent carbohydrate and 80 per cent protein. Vivek-Prasad *et al.* (1995) isolated two basic glycoproteins from leaves of *Clerodendrum inerme* namely CIP 29 and CIP 34 against TMV. An antiviral protein, BAP-I was isolated from *Bougainvillea spectabilis* by Balasaraswathi *et al.* (1998).

Antiviral principles of non-proteinaceous nature was also reported from plants. Verma and Raychaudhuri (1970) isolated ellagic acid and ascorbic acid from *Emblica officinalis* as inhibitors of potatovirus X. Crude tannins of *Acacia arabica* leaf extract reported to be inhibitory to potato virus Y (Gupta and Raychaudhuri, 1971a). Roychoudhury and Basu (1983) reported that crude steroidal glycoalkaloid extracts from aerial parts of *Solanum nigrum* and fruits of *S. khasianum* inhibited TMV and sunnhemp rosette virus. Khan *et al.* (1991) isolated inhibitory agents of TMV from *Artemisia annua* and identified as sterols especially sitosterol and stigma sterol.

## 2.9 MANAGEMENT OF VIRAL DISEASES OF PLANTS BY PLANT EXTRACTS

Eventhough there are many reports on antiviral properties of plant extracts, field level studies were conducted in few cases. Verma and Kumar (1980) reported that foliar application of *M. jalapa* leaf extract caused marked suppression of disease symptoms, improved growth and flowering and considerably reduced virus multiplication rate in the treated plants of tomato against tomato yellow mottle and tomato yellow mosaic viruses, cucumbers against cucumber mosaic and

cucumber green mottle viruses and urd against urd mosaic virus. Awasthi and Mukerjee (1980) reported that *B. diffusa* root extract and *Cuscuta reflexa* stem extract protected potato against PVX. Fortnightly spraying of potato plants in the field prevented natural infection by viruses and growth and yield of tubers were improved.

Application of dried roots of *B. diffusa* prevented virus infection under field conditions in crops including tomato, potato, auberigine, french bean, pea and snake gourd, twice weekly for one month from the seedling stage. Maximum protection was obtained when the inhibitor was applied for two months (Awasthi *et al.*, 1984). Verma *et al.* (1985) studied control of natural viral infection of *Vigna radiata* and *V. mungo* by plant extracts namely aqueous partially clarified leaf extracts of *C. fragrans, Aerva sanguinolenta* and root extract of *B. diffusa* when applied at four per cent at every 3-4 days, from the seedling stage. *Clerodendrum fragrans* was the most effective one and reduced infection by 60 per cent whereas other extracts only delayed appearance of disease symptom. The yield of plants treated with *C. fragrans* extract was considerably enhanced. In tobacco when plant extracts were sprayed to check TMV incidence, *Pithecolobium dulce* was found to be most effective followed by leaf extracts of *Peltophorum ferrugenium,* tannic acid and milk. The net benefit from spraying plant inhibitors would be Rs.1000 $ha^{-1}$ (Murthy and Nagarajan, 1986).

Patil and Joy (1992) reported that ten per cent extract of *Capsicum annuum* and *Acacia arabica* when applied as root dipping to tomato seedlings, protected tomato from spotted wilt virus infection upto 30-40 days after transplanting. When six pre inoculation sprays of partially purified leaf extract of *C. aculeatum* was given to soybean plants which were inoculated with soybean mosaic virus 24 h after last spray, there was reduction in infection, early flowering and fruiting, higher nodulation and good yield (Verma *et al.*, 1992). Kannan and Doraiswamy (1993) reported that application of one per cent emulsion of *A. indica* was very effective in checking cowpea aphid borne mosaic virus infection on cowpea. A significant increase in yield was also produced (890 kg $ha^{-1}$) compared with (540 kg $ha^{-1}$) for the control. Verma and Singh (1994) reported that application of

*C. aculeatum* on *V. radiata* in field, reduced the incidence of mungbean yellow mosaic bigeminivirus. Spraying of leaf extract together with soil amendment with dry leaf powder or fresh extract reduced disease incidence where as control plants showed severe symptom. Sandhu *et al.* (1996) studied efficacy of antiviral substances against summer squash mosaic diseases (squash mosaic comovirus) and reported that sorghum and thuja extracts were effective and increased yield of plants compared to control. Baranwal and Ahmed (1997) also tested effect of *C. aculeatum* (CA) on tomato against tomato leaf curl virus. Tomato plants which received CA as soil application, spray or both showed delayed incidence of virus infection and produced comparatively higher yield. Symptoms of CA treated plants were less severe compared with untreated plants.

# *Materials and Methods*

# 3. MATERIALS AND METHODS

The present investigation on the management of pumpkin mosaic disease caused by pumpkin mosaic virus (PMV) using medicinal plant extract was carried out during 1998-2002 at the Department of Plant Pathology and Biochemistry Laboratory of College of Horticulture, Vellanikkara, Thrissur. The field experiments were conducted in the experimental plots attached to the Plant Pathology Department. The soil of the experimental field was laterite having a pH of 5.8.

The works were undertaken in the following heads

- Symptomatology
- Transmission
- Host range of the virus
- Electron microscopy
- Serology
- Biochemical changes due to host pathogen interaction
- Effect of dilution and extraction media on PMV inhibitory property of medicinal plants
- Effect of different parts and temperature on PMV inhibitory property of *Plumbago rosea*
- Effect of *P. rosea* on acquisition feeding and inoculation feeding of the vector *Aphis gossypii*
- Isolation of PMV inhibitory principles of *P. rosea*
- Management of pumpkin mosaic using *P. rosea*
- Effect of *P. rosea* on PMV
- Biochemical changes due to *P. rosea* spray

## 3.1. SYMPTOMATOLOGY

Symptomatology was studied by observing the development of symptoms in naturally infected as well as artificially inoculated pumpkin plants.

The symptoms of pumpkin mosaic virus (PMV) infection was recorded under natural conditions from pumpkin plants grown in experimental plots of the Department of Olericulture, College of Horticulture, Vellanikkara during September-December, 2000.

To study the symptomatology under artificial conditions, seedlings of *Cucurbita moschata* variety Ambili were raised in pots (25 x 20 cm) filled with sterile potting mixture of sand, soil and cowdung (1:1:1) and kept in insect proof net house which was carried out during the same period.

Young symptomatic leaves of pumpkin were collected from the field. These were washed and dried by blotting paper. The leaves were weighed and crushed using sterile pestle and mortar at 4°C. The sap was extracted after adding one ml of the potassium phosphate buffer (0.1 M and pH 7.2) to every gram of infected leaf tissue. The resulting pulp was strained through sterilized cotton wool and immediately used for inoculation.

Inoculation was carried out on nine days old pumpkin seedlings in insect proof net house. Carborundum powder (600 mesh) was dusted uniformly on the upper surface of cotyledonary leaves and inoculum was applied by gently rubbing on the upper surface of leaves with a piece of adsorbent cotton wool soaked in the inoculum. Care was taken not to cause excess injury to the leaves during inoculation. Soon after inoculation, the leaf surface was washed with sterile distilled water. The development of symptoms was observed daily for a period of four weeks.

### Maintenance of PMV culture

The culture of PMV was maintained on pumpkin seedlings by periodical sap transmission and these cultures were kept in insect proof net house.

## 3.2. TRANSMISSION

### 3.2.1 Sap Transmission

Young symptomatic leaves of pumpkin were collected from the culture maintained in the insect proof net house. The inoculum of the virus was prepared as described in 3.1

Inoculation was carried out on nine days old pumpkin seedlings as described in 3.1. Two sets of inoculations were carried out and for each set, 20 pumpkin seedlings were used and transmission was recorded after four weeks.

#### 3.2.1.a Effect of Age of Seedling on Sap Transmission of PMV

An experiment was laid out to study the effect of age of seedling on the transmission of PMV. Seeds of pumpkin were sown in polybags (18 x 12 cm) at different intervals in order to get seedlings of age 6, 9, 12 and 15 days . These seedlings were inoculated by sap transmission as described in 3.1.There were three replications and 10 plants per replication. These plants were kept in insect proof net house and observed for the development of symptoms daily for a period of four weeks. The mean incubation period was also calculated based on the result of the study.

### 3.2.2 Insect Transmission

Maintenance of pure culture of *Aphis gossypii*

Single apterous adult aphid, *A. gossypii* was collected from the field grown bhindi (*Abelmoschus esculentus*) plant and released on healthy bhindi seedlings grown in plastic pots of size 20 x 10 cm, and kept inside the insect proof aphid rearing cages. The pure culture was maintained by weekly transfer of aphids to healthy plants of bhindi ( Plate 1).

Aphid transmission

The aphid colonies were disturbed with camel hair brush by touching the antennae or cornicles. After ensuring the withdrawal of their stylets from plant

**Plate 1.** ***Aphis gossypii*** **- vector of PMV**

tissue, a gentle tap was given on the plant, subsequently the moving aphids were picked up with moistened brush into the petri plate. The petri plates were covered with black paper to provide dark condition to avoid movement and the aphids were subjected to pre-acquisition fasting period for one hour in the petri plates.

The starved aphids were then allowed to feed on young detached leaves of pumpkin plants infected with PMV for an acquisition access period of 30 min.The viruliferous aphids were then released to nine days old healthy pumpkin seedlings at the rate of 10 aphids per plant. After 24 h of inoculation access period, the aphids were killed by spraying quinalphos (0.05%) and plants were kept in the insect proof net house for symptom expression upto a period of four weeks.

Two sets of inoculations were carried out and for each set, 20 pumpkin seedlings were used and transmission was recorded after four weeks.

### 3. 2.3 Seed Transmission

Seed transmission was studied using 130 no. of seeds extracted from the artificially inoculated and 118 no. of seeds from naturally infected pumpkin plants during November-December 2000. Seeds were sown in poly bags (18 x 12 cm) filled with potting mixture at the rate of one seed per bag and kept in insect-proof net house without touching each other. Observations on symptom development were recorded at weekly interval upto six weeks.

## 3.3 HOST RANGE

The transmission of PMV to different hosts belonging to family cucurbitaceae, solanaceae, fabaceae and caricaceae was studied by sap inoculation during December 2000-January 2001. One week old seedlings of legumes, two weeks old seedlings of cucurbits and papaya and one month old seedlings of solanaceous crops were inoculated. Hosts inoculated in each family are given below.

| Sl.No | Scientific name | Common name |
|---|---|---|
| **Family cucurbitaceae** | | |
| 1 | *Benincasa hispida* (Thunb ) | Ash gourd |
| 2 | *B. hispida* (Thunb ) | Ash gourd, wild |
| 3 | *Citrullus vulgaris* Schrad | Water melon |
| 4 | *Cucumis melo* L. | Musk melon |
| 5 | *C. melo* var. *conomon* Mak. | Cucumber |
| 6 | *C. sativus* L. | Salad cucumber |
| 7 | *Cucurbita maxima* Duch | Winter squash |
| 8 | *Lagenaria siceraria* (Mol ) Standl. | Bottle gourd |
| 9 | *Luffa acutangula* (Roxb.) L. | Ridge gourd |
| 10 | *Momordica charantia* L. | Bitter gourd |
| 11 | *Trichosanthes anguina* L. | Snake gourd |
| **Family solanaceae** | | |
| 1 | *Capsicum annuum* L. | Chilli |
| 2 | *Datura metel* L. | Datura |
| 3 | *Lycopersicon esculentum* L. | Tomato |
| 4 | *Solanum melongena* L. | Brinjal |
| **Family fabaceae** | | |
| 1 | *Cyamopsis tetragonoloba* (L.) Taub | Cluster bean |
| 2 | *Glycine max* L. Merril | Soybean |
| 3 | *Vigna unguiculata* (L.) Walp. | Cowpea |
| **Family caricaceae** | | |
| | *Carica papaya* L. | Papaya |

The inoculated plants were kept in the insect proof net house and the observations on symptom development was taken at weekly interval upto six weeks. Those plants which do not produce any symptoms by sixth week, were indexed back to healthy pumpkin seedlings and observed for the development of symptoms, if any.

## 3.4 ELECTRON MICROSCOPIC STUDY

The morphology of the virus particles associated with infected pumpkin leaf samples was studied by examining the leaf dip preparations (Gibbs *et al.*, 1966) under electron microscope (EM) (JEOL 100C X 11) at the Advanced Centre for Plant Virology, Indian Agricultural Research Institute, New Delhi.

a. Grid preparation

Copper grids (3 mm diameter, 400 mesh) were cleaned with acetic acid and loaded on a filter paper placed at the bottom of a clean petri dish with sterile distilled water. A clean slide was then coated with a thin film of carbon in a vacuum coating unit (BIORAD, E 6440,Evaporation PSU) and gently brought in contact with water in the petri dish containing grids. The carbon film was gently allowed to float off on the water surface and was adsorbed on the grids when the filter paper was lifted. The carbon coated grids were finally air dried in the dark at room temperature for 12-24 h.

b. Virus extraction

Diseased leaf bits were macerated on a clean glass slide with a flat ended glass rod in few drops of phosphate buffer (0.1 M, pH 6.5) and left the finally homogenized material for few seconds. The supernatant was then taken for mounting the grids.

c. Mounting

A fresh carbon coated grid was picked up with a fine clean forceps. A drop of homogenized virus material was put on the coated side of the grid and excess was washed off from the grid using few drops of distilled water.

d. Staining

The carbon coated grid was treated with 2-3 drops of uranyl acetate (aqueous 2%, pH 4.2) for few seconds. Excess stain was removed by touching the edge of the grid on a filter paper. The grid was air dried for one to two minutes. The negatively stained grid was finally examined under EM.

3.5 SEROLOGY

**3.5.1 Purification of PMV**

The young leaves from artificially inoculated pumpkin plants were homogenized in a mixer with equal volume of 0.5M borate buffer pH 8.0

containing 0.2M urea and 10 m M ethylene diamine tetra acetic acid (EDTA). The homogenate was passed through double layered muslin cloth and emulsified with one half volume of 1:1 mixture of chloroform and carbon tetra chloride by stirring for 15 min. The mixture was centrifuged at 5000 g for 10 min and the supernatant was passed through Whatman No.1 filter paper. To the filtrate added polyethylene glycol (PEG) 6000 and sodium chloride to a final concentration of 4 and 1.75 per cent respectively. The mixture, after stirring for 30 min was allowed to stand at 4°C for one hour. This was further centrifuged at 8000 g for 12 min. The precipitate was resuspended in $1/5^{th}$ of the original volume of 0.5 M borate buffer pH 8.0, with overnight slow agitation at 4°C. On the next day, this was again centrifuged at 5000 g for 10 min and pellets were discarded. Supernatant was layered over 0.5 ml of 30 per cent sucrose (w/v) in 0.5M borate buffer per centrifuge tube and centrifuged at 65,000 g ( Beckman ; T1 28 rotor ) for 2½ h. The pellet obtained was resuspended in 0.05 M borate buffer containing 5m M EDTA and kept at 4°C and was used for antiserum production.

Infectivity of partially purified virus

Pumpkin seedlings were inoculated with partially purified virus to test infectivity.

EM of partially purified virus

Partially purified virus preparation was observed under electron microscope.

Estimation of protein content of purified virus

Protein content of the purified virus preparation was estimated using Spectrophotometric method ( Whitaker, 1972 ).

Protein (mg $ml^{-1}$) = 1.55 x A 280nm – 0.76 x A 260 nm

### 3.5.2 Antiserum Production

One year old New Zealand White female rabbits were used for the production of antiserum. The purified virus preparation was emulsified with

Freund's incomplete adjuvant (Difco) in the ratio 1:1 (v/v). Two ml of this emulsion was injected intramuscularly to the thigh muscles. Four injections were given at an interval of six days. Three weeks after the final injection, a booster dose was given. One week after booster injection, rabbits were bled by cardiac puncture.

The blood was collected aseptically in screw-capped tubes and were allowed to coagulate at room temperature for two hours. The coagulated blood clot was loosened with the help of a sterilized glass rod and samples were kept overnight at 4°C. The clear serum was decanted and centrifuged at 5000 g for 30 min at 4°C. The purified serum was pipetted out using sterile pipette and dispensed into 5 ml vials. Sodium azide was added to the clarified serum as a preservative so as to make a final concentration of 0.02 per cent. Vials were then sealed, labelled and kept in freezer.

### 3.5.3 Serodiagnosis

#### 3.5.3.1 Ouchterlony's Agar Double Diffusion Test

Ouchterlony's agar double diffusion tests were conducted to study the serological relationship of PMV with other potyviruses (Ouchterlony, 1968 ).

Preparation of antigens

Young leaves of plants suspected to be infected with poty viruses were collected. Partial purification of the virus was done as explained in section 3.5.1. The host plants collected were snake gourd (*T. anguina*), bitter gourd (*M. charantia*), wild ash gourd (*B. hispida*), coccinia (*C. grandis*), cowpea (*V. unguiculata*), soybean (*G. max*), cluster bean (*C. tetragonoloba*), french bean (*P. vulgaris*), chilli (*C. annuum*), brinjal (*S. melongena*), tomato (*L. esculentum*) and papaya (*C. papaya*).

Cross absorption of antiserum

The leaves of healthy pumpkin plant was extracted with potassium phosphate buffer (0.1 M, pH 7.2) @ one gram leaf with 10 ml buffer. Antiserum was mixed with the healthy sap in equal volume. The serum sap mixture was

stored at 5°C for 12 h and centrifuged at 2000 rpm for 30 min. The supernatant antiserum was used for serodiagnosis work.

The test was done on microscopic slides. A thin layer of 0.9 per cent agarose (prepared in 0.85 per cent sodium chloride) was smeared on a clean slide and allowed to dry. Above this layer 5 ml of agarose (0.9 per cent) was added carefully and allowed to dry. After one hour wells were cut with a sterilized gel cutter, one well in the center and four wells around the central well. Each well was 3 mm deep, 4 mm diameter and distance between the central well and periphery wells was 8 mm. Cross absorbed antiserum was added to the central well and antigens in the periphery wells at the rate of 30 µl per well.

The slides were kept inside petri dish and moistened cotton was placed inside the petri dish to prevent drying. This was incubated at room temperature and examined periodically for the appearance of characteristic precipitin bands and it was documented by gel documentation system (Alpha Imager 1200).

Ouchterlonys double diffusion test was performed with cross absorbed antiserum and purified sap of infected and healthy pumpkin and purified antigens from different plants as mentioned above.

### 3.5.3.2 Direct Antigen Coating - Enzyme Linked Immunosorbent Assay (DAC-ELISA)

#### 3.5.3.2a Standardisation of DAC-ELISA for PMV Detection

DAC-ELISA procedure (Barbara and Clark, 1982) was standardized with the PMV antiserum raised in rabbit and Horse radish peroxidase conjugated antirabbit immmunoglobulin (Genei, Bangalore). Following solutions were prepared for ELISA.

1. Stock buffer ( Phosphate buffer- saline 1X PBS, pH 7.4 )

| | | |
|---|---|---|
| NaCl | - | 8.00 g |
| $Na_2HPO_4.2H_2O$ | - | 1.44 g |
| or | | |
| $Na_2HPO_4.12H_2O$ | - | 2.90 g |

or

| | | |
|---|---|---|
| $Na_2HPO_4$ | - | 1.50 g |
| $KH_2PO_4$ | - | 0.20 g |
| KCl | - | 0.20 g |
| Distilled water | - | 1 litre |

2. Wash buffer (PBS – Tween, PBS-T)
   Added 0.5 ml Tween 20 to 1 litre PBS

3. Coating buffer (Carbonate buffer, pH 9.6)

| | | |
|---|---|---|
| $Na_2CO_3$ | - | 1.59 g |
| $NaHCO_3$ | - | 2.93 g |
| Distilled water | - | 1 litre |

4. Enzyme conjugate diluent/buffer ( PBS-T polyvinyl-pyrrolidone ovalbumin, PBS-TPO)
   Added 20 g polyvinyl pyrrolidone (PVP) and 2.0 g egg ovalbumin to 1 litre PBS-T.

5. Antibody diluent/buffer
   Same as PBS-TPO

6. Substrate buffer (citrate phosphate buffer, pH 5.0)

   Solution A – 0.1 M citric acid (19.21 g in 1000 ml)

   Solution B – 0.2 M dibasic sodium phosphate (28.39 of $Na_2PHO_4$ or 71.7 g $Na_2 HPO_4\ 12H_2O$ in 1000 ml).

   Mixed 24.3 ml of citric acid and 25.7 ml dibasic sodium phosphate and made up to 100 ml.

   To 10 ml of substrate buffer 4 mg ortho phenylenediamine (OPD) and 5 µl of 30 volume hydrogen peroxide were added.

7. Blocking solution – Added 5 g bovine serum albumin (BSA) per one litre PBS

Cross absorption of antiserum

Since antiserum developed may contain antibodies against plant proteins, cross absorption was done to eliminate plant protein antibodies and to get antiserum specific to PMV antigen. Extracted one gram healthy pumpkin leaf with 10 ml of PBS-TPO and the extract was taken by sieving through cotton. Mixed required amount of antiserum to it so as to get 1:250 dilution. Incubated this mixture at 37°C for two hours and centrifuged at 10,000 rpm for 30 min and the supernatant was taken.

Dilutions of antiserum and conjugate

Antiserum dilutions – The cross absorbed antiserum was further diluted to prepare other dilutions, 1:500 and 1:1000, using PBS-TPO as the diluent.

Conjugate dilutions - Four dilutions of conjugate viz., 1:2000, 1:4000, 1:8000 and 1:16,000 were prepared using PBS-TPO as the diluent.

Preparation of plant sample

Infected and healthy leaves of pumpkin were collected and extracted with carbonate buffer at the rate of one gram leaf with five ml of buffer. Centrifuged the extracts at 10,000 rpm for 30 min at 4°C and supernatant was taken for loading ELISA plate.

Steps of ELISA

1. Added 200 µl of healthy and infected leaf extracts to each well of microtitre plate. Covered the plate and incubated at 4°C overnight.
2. Emptied and washed with PBS-T by filling three fourth of each well. Care was taken to prevent overflow from one well to nearby wells. Repeated the procedure thrice and the residual liquid was shaked off by draining on a paper towel.

3. Added 300 µl of blocking solution to each well. Incubated at 37°C for one hour to block polystyrene well reactive surfaces.

4. Drained the blocking solution and the residual liquid was shaked off on a paper towel.

5. Added 200 µl cross absorbed antiserum dilutions to each well and incubated at 37°C for two hours.

6. Washed the plate as explained in Step.2.

7. Added 200 µl of conjugate dilutions to each well and incubated at 37°C for two hours.

8. Washed the plate as explained in Step 2.

9. Added 200 µl of freshly prepared substrate (chromogen plus $H_2O_2$) and incubated at room temperature for 15 min.

10. The absorbance was measured at 450 nm using ELISA reader (Lab Systems multiskan MS).

**3.5.3.2b Detection of PMV by DAC-ELISA**

Leaf samples were collected from artificially inoculated plants in the net house, with no symptom, mild symptom (vein chlorosis and mottling) and severe symptom (blistering and malformation) and DAC-ELISA was carried out as described in section 3.5.3.2a using 1:250 and 1:4000 dilutions of antiserum and conjugate respectively. Negative control samples were taken from healthy pumpkin seedlings.

The results were interpreted based on a cut off value which is the mean OD values of all negative control samples plus 2 times standard deviation (Clark,1981).

## 3.6 BIOCHEMICAL CHANGES DUE TO HOST PATHOGEN INTERACTION

Healthy and PMV infected plants were analysed for the biochemical changes in protein, phenol, chlorophyll and activity of enzymes peroxidase, catalase and phenylalanine ammonia lyase (PAL), at one month and two months after sowing.

Pumpkin seeds were sown in pots of size (20 x 15 cm) and one healthy plant was maintained in each pot . On the ninth day they were inoculated with PMV and kept apart from un inoculated control plants. The biochemical analysis of infected and healthy plants were carried out when these seedlings were one months and two months old.

### 3.6.1 Influence on Soluble Protein Content

The soluble protein was estimated as per the procedure described by Lowry *et al.* (1951). Five hundred milligram of leaf sample was homogenized with 2 ml of 0.1 M phosphate buffer pH 7.0 and centrifuged at 10,000 rpm for 20 min in refrigerated centrifuge. The protein was precipitated from the supernatant with one millilitre of 10 per cent trichloroacetic acid (TCA) and centrifuged at 5000 rpm for 15 min and the TCA free precipitate was dissolved in 2 ml of 0.1 N sodium hydroxide.

The reaction mixture consisted of 0.1 ml of protein, 0.9 ml distilled water and 5 ml alkaline copper solution. After mixing, kept it for 10 min. Then 0.5 ml of Folin-ciocalteau reagent was added and incubated in dark for 30 min at room temperature. The blue colour developed was read at 660 nm in spectrophometer (Spectronic 20 Genesys). Bovine serum albumin was used as the standard protein and estimated protein content of sample and expressed as mg $g^{-1}$ on fresh weight of sample.

### 3.6.2 Influence on Phenol

The phenol content was estimated as per Malik and Singh (1980). Five hundred milligram of leaf sample was ground in distilled water and made up to 100 ml with distilled water in a volumetric flask.

. Two millilitre of 20 per cent sodium carbonate solution was added to a mixture of 2.4 ml distilled water and 0.6 ml of the extracted sample. To this 0.5 ml of Folin-ciocalteau reagent was added and read absorbance after 20 min at 650 nm in spectrophotometer (Spectronic 20 Genesys) against reagent blank. Standard curve was prepared using different concentrations of catechol and expressed the phenol content as catechol equivalents as mg $g^{-1}$ on fresh weight of sample.

### 3.6.3 Influence on Chlorophyll

The chlorophyll was estimated by the method described by Arnon (1949). Two hundred and fifty milligram of leaf sample was ground in mortar with addition of 80 per cent acetone. The homogenate was centrifuged at 5000 rpm for five min and the supernatant was transferred to 50 ml volumetric flask. This procedure was continued till the extract became colourless. The final volume was made upto 50 ml. Read the absorbance at 645 and 663 nm in spectrophotometer (Spectronic 20 Genesys) against solvent blank. The quantity of chlorophyll was calculated using the following equation.

Milligram chlorophyll a/gram tissue

$$= 12.7\,(A\ 663) - 2.69\,(A\ 645) \times \frac{V}{1000 \times W}$$

Milligram chlorophyll b/gram tissue

$$= 22.9\,(A\ 645) - 4.68\,(A\ 663) \times \frac{V}{1000 \times W}$$

Milligram total chlorophyll/gram tissue

$$= 20.2\,(A\ 645) + 8.02\,(A\ 663) \times \frac{V}{1000 \times W}$$

Where,

A = Absorbance of sample at specific wavelength

V = Final volume of chlorophyll extract in 80 per cent acetone

W = Weight of tissue extracted

### 3.6.4 Influence on Peroxidase Activity

Peroxidase activity was carried out as per the procedure of Sadasivam and Manickam (1992).

Enzyme extract – Five hundred milligram of leaf sample was extracted with 2 ml of 0.1 M phosphate buffer, pH 7.0 with a pre-cooled mortar and pestle. Centrifuged the homogenate at 10,000 rpm for 20 min in refrigerated centrifuge at 4°C and used the supernatant as enzyme source.

Activity studies - The reaction mixture consists of 3 ml of 0.1 M phosphate buffer, pH 7.0, 50 µl of 20 mM Guaicol solution and 30 µl of 12.3 mM $H_2O_2$ (Diluted 0.14 ml of 30% $H_2O_2$ to 100 ml with distilled water and absorbance at 240 nm was 0.485) and 20 µl of enzyme. Mixed well and immediately placed in spectrophotometer (Spectronic 20 Genesys) set at 436 nm. When absorbance was increased by 0.05, started a stop watch and noted the time required to increase the absorbance by 0.1 and the enzyme activity per litre of extract was calculated.

### 3.6.5 Influence on Catalase Activity

The catalase activity was studied as per the method of Sadasivam and Manickam (1992).

Enzyme extract – One gram of leaf tissue was homogenized with 5 ml of 0.01 M phosphate buffer pH 7.0 and centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used as enzyme source.

Activity studies - Hydrogen peroxide solution was prepared by mixing 0.16 ml of hydrogen peroxide to 100 ml phosphate buffer, 0.1 M, pH 7.0. To 3 ml hydrogen peroxide solution 50 µl of enzyme was added and noted the time to decrease the absorbance from 0.45 to 0.4, at 240 nm in spectrophotometer (Spectronic Genesys 5). Calculated the catalase activity and expressed as units per gram tissue.

### 3.6.6 Influence on Phenylalanine Ammonia Lyase (PAL) Activity

PAL activity was studied as per the procedure described by Dickerson *et al.* (1984).

Enzyme extract – One gram leaf sample was homogenized with 5 ml of 0.1 M sodium borate buffer pH 8.8. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used for the assay.

Activity studies - The reaction mixture contained 3 ml borate buffer, 0.1 ml enzyme extract and 0.1 ml 12 mM L-phenylalanine prepared in the same buffer. The blank contained 3.1 ml borate buffer and 0.1 ml L-phenylalanine. The reaction mixture and the blank were incubated at 40°C for 30 min and stopped the reaction by adding 0.2 ml of 3N hydrochloric acid. The absorbance was read at 290 nm in spectrophotometer (Spectronic Genesys 5).

## 3.7 ANTIVIRAL PROPERTY OF PLANT EXTRACTS

Widely accepted and therapeutic agents have not yet been developed for the control of viral diseases of plants. Many biological products including medicinal plant extracts are reported to be effective for controlling viral diseases of plants. The present study is aimed to throw light on the use of medicinal plants to manage pumpkin mosaic. The following medicinal plants were selected for the study (Plate 2).

| **Botanical name** | **Common name** | **Family** | **Part used** |
|---|---|---|---|
| *Basella alba* Linn. | Spinach, Indian *(Vallikkeera) | Basellaceae | Leaf |
| *Glycyrrhiza glabra* Linn. | Liquorice (Erattimadhuram) | Papilionaceae | Dried stem |
| *Phyllanthus fraternus* Webst. | Jarmala (Keezharnelli) | Euphorbiaceae | Whole plant |
| *Plumbago rosea* Linn. | Lead wort (Chethikoduveli) | Plumbaginaceae | Root |
| *Thespesia populnea* Soland ex. Correa | Bhindi tree (Poovarasu) | Malvaceae | Leaf |

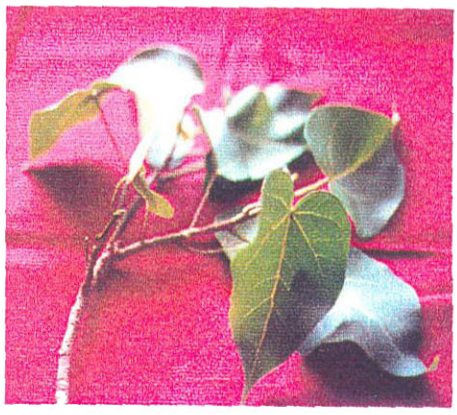
*Names given in parenthesis are Malayalam names

A

B

C

D



E

**Plate 2. Medicinal plants selected for PMV inhibitory properties**

A - *Basella alba* B - *Glycyrrhiza glabra* C - *Plumbago rosea*
D - *Phyllanthus fraternus* E - *Thespesia populnea*

### 3.7.1 Effect of Dilution and Extraction Media on the Virus Inhibitory Property of Medicinal Plant Extracts

Inhibitory effect of the extract from the above five selected medicinal plants were tested by spraying before mechanical inoculation of virus on eight days old pumpkin seedlings. Extracts were prepared in four solvents at different dilution levels.

Since the extract of fresh roots of *P. rosea* showed severe phytotoxicity( leaf scorching), the dry powder was used for the study. Dry stem bits of *G. glabra* was also used for the study purpose. Both of these plant's parts were sundried well and powdered in a dry grinder and sieved through 0.1 mm sieve. The powder was kept in screw- capped bottles at 4°C to prevent microbial infection and used for the study.

Preparation of medicinal plant extracts

Solvents selected for the extraction of medicinal plants were chloroform, distilled water, ethyl acetate and petroleum ether. One hundred gram of plant sample was extracted with each solvent separately and made up to one litre. The clear extract was decanted and evaporated the solvent by keeping in a hood fixed with exhaust fan and dry residue was obtained. Dissolved the residue in minimum quantity of ethanol and made up with distilled water to one litre to get 10 per cent of the extract. Similarly extracted 50 g and 20 g of medicinal plants with one litre of each solvents so as to get five per cent and two per cent extracts respectively.

Distilled water extract was prepared directly in one litre water. In the case of Plumbago and Glycyrrhiza, dry powder kept overnight in one litre of the solvent and followed the procedures as described above. For Plumbago, even 10 per cent was found to cause phytotoxicity and so 5, 2, 1 and 0.1 per cent were used for the study.

Application of plant extract

The plant extract was applied as fine spray using an atomizer on eight days old seedling. Each extract was applied on 20 pumpkin seedlings at the rate of ten

plants per replication. The details of treatments used in the experiment are listed in the Table 1.

Table 1. Combination of extraction media and dilution of medicinal plants for testing PMV inhibitory property

| Sl. No. | Treatment details | Abbreviations used |
|---|---|---|
| 1 | 10% chloroform extract of *Basella alba* | BI DI |
| 2 | 5% -do- | BI D2 |
| 3 | 2% -do- | BI D3 |
| 4 | 10% distilled water extract of *B. alba* | BII D1 |
| 5 | 5% -do- | BII D2 |
| 6 | 2% -do- | BII D3 |
| 7 | 10% ethyl acetate extract of *B. alba* | BIII D1 |
| 8 | 5% -do- | BIII D2 |
| 9 | 2% -do- | BIII D3 |
| 10 | 10% petroleum ether extract of *B. alba* | BIV D1 |
| 11 | 5% -do- | BIV D2 |
| 12 | 2% -do- | BIV D3 |
| 13 | 10% chloroform extract of *Glycyrrhiza glabra* | GI DI |
| 14 | 5% -do- | GI D2 |
| 15 | 2% -do- | GI D3 |
| 16 | 10% distilled water extract of *G. glabra* | GII D1 |
| 17 | 5% -do- | GII D2 |
| 18 | 2% -do- | GII D3 |
| 19 | 10% ethyl acetate extract of *G. glabra* | GIII D1 |
| 20 | 5% -do- | GIII D2 |
| 21 | 2% -do- | GIII D3 |
| 22 | 10% petroleum ether extract of *G. glabra* | GIV D1 |
| 23 | 5% -do- | GIV D2 |
| 24 | 2% -do- | GIV D3 |

Table contd.

| Sl. No. | Treatment details | Abbreviations used |
|---|---|---|
| 25 | 10% chloroform extract of *Phyllanthus fraternus* | PI DI |
| 26 | 5% -do- | PI D2 |
| 27 | 2% -do- | PI D3 |
| 28 | 10% distilled water extract of *P. fraternus* | PII D1 |
| 29 | 5% -do- | PII D2 |
| 30 | 2% -do- | PII D3 |
| 31 | 10% ethyl acetate extract of *P. fraternus* | PIII D1 |
| 32 | 5% -do- | PIII D2 |
| 33 | 2% -do- | PIII D3 |
| 34 | 10% petroleum ether extract of *P. fraternus* | PIV D1 |
| 35 | 5% -do- | PIV D2 |
| 36 | 2% -do- | PIV D3 |
| 37 | 10% chloroform extract of *Thespesia populnea* | TI DI |
| 38 | 5% -do- | TI D2 |
| 39 | 2% -do- | TI D3 |
| 40 | 10% distilled water extract of *T. populnea* | TII D1 |
| 41 | 5% -do- | TII D2 |
| 42 | 2% -do- | TII D3 |
| 43 | 10% ethyl acetate extract of *T. populnea* | TIII D1 |
| 44 | 5% -do- | TIII D2 |
| 45 | 2% -do- | TIII D3 |
| 46 | 10% petroleum ether extract of *T. populnea* | TIV D1 |
| 47 | 5% -do- | TIV D2 |
| 48 | 2% -do- | TIV D3 |
| 49 | 5% chloroform extract of *Plumbago rosea* | PGI DI |
| 50 | 2% -do- | PGI D2 |
| 51 | 1% -do- | PGI D3 |
| 52 | 0.1% -do- | PGI D4 |
| 53 | 5% distilled water extract of *P. rosea* | PGII D1 |
| 54 | 2% -do- | PGII D2 |
| 55 | 1% -do- | PGII D3 |
| 56 | 0.1% -do- | PGII D4 |
| 57 | 5% ethyl acetate extract of *P. rosea* | PGIII D1 |
| 58 | 2% -do- | PGIII D2 |
| 59 | 1% -do- | PGIII D3 |
| 60 | 0.1% -do- | PGIII D4 |
| 61 | 5% petroleum ether extract of *P. rosea* | PGIV D1 |
| 62 | 2% -do- | PGIV D2 |
| 63 | 1% -do- | PGIV D3 |
| 64 | 0.1% -do- | PGIV D4 |
| 65 | Control – distilled water spray | Control |

Inoculation of PMV

Inoculum was prepared using infected leaves maintained at the net house as explained in 3.1 Inoculation was carried out 24 h after the spray and the plants were maintained in the insect proof net house for observation.

After four weeks of inoculation, symptoms were scored using the following scale

0 = No symptoms

1 = Slight vein clearing, very light mottling of light and dark green colour in younger leaves

2 = Mottling of the leaves with light and dark green colour

3 = Blisters and raised surface on the leaves

4 = Distortion of leaves

5 = Stunting of the plants with negligible or no flowering and fruiting

(Rajamony *et al.*, 1990)

The disease severity index was calculated as per Silbernagel and Jafri (1974).

Severity Index or Vulnerability Index

$$= \frac{(0n_o + 1n_1+2n_2+3n_3+4n_4 + 5n_5) \times 100}{nt\,(nc-1)}$$

no,n1 ..... n5 = Number of plants in category 0, 1, ..... 5

nt = total number of plants

nc = Total number of categories

Among the different medicinal plants, *P. rosea* showed maximum PMV inhibitory effect with distilled water extract. Other plants were less effective or showed effectiveness at higher concentration of distilled water extract. So *P. rosea* at one per cent dilution was selected for further studies.

### 3.7.2 Effect of Different Parts and Temperature on Inhibitory Property of *Plumbago rosea* on PMV

Effect of different parts of *P. rosea* viz., tender leaf, mature leaf, tender stem, mature stem and roots on inhibition of PMV was studied (Plate 3). Thermostability of plant extract was also tested by heating the extracts to different temperatures.

The details of treatments used in the experiment are listed in Table 2.

Table 2. Combination of different parts and temperature of *Plumbago rosea* for testing PMV inhibitory property

| Sl. No. | Treatment details | Abbreviations used |
|---|---|---|
| 1 | Root extract at 30°C | RT1 |
| 2 | -do- 40°C | RT2 |
| 3 | -do- 50°C | RT3 |
| 4 | -do- 60°C | RT4 |
| 5 | -do- 70°C | RT5 |
| 6 | -do- 80°C | RT6 |
| 7 | Tender leaf extract at 30°C | TL T1 |
| 8 | -do- 40°C | TL T2 |
| 9 | -do- 50°C | TL T3 |
| 10 | -do- 60°C | TL T4 |
| 11 | -do- 70°C | TL T5 |
| 12 | -do- 80°C | TL T6 |
| 13 | Mature leaf extract at 30°C | ML T1 |
| 14 | -do- 40°C | ML T2 |
| 15 | -do- 50°C | ML T3 |
| 16 | -do- 60°C | ML T4 |
| 17 | -do- 70°C | MLT5 |
| 18 | -do- 80°C | ML T6 |
| 19 | Tender stem extract at 30°C | TS T1 |
| 20 | -do- 40°C | TS T2 |
| 21 | -do- 50°C | TS T3 |
| 22 | -do- 60°C | TS T4 |
| 23 | -do- 70°C | TS T5 |
| 24 | -do- 80°C | TS T6 |
| 25 | Mature stem extract at 30°C | MS T1 |
| 26 | -do- 40°C | MS T2 |
| 27 | -do- 50°C | MS T3 |
| 28 | -do- 60°C | MS T4 |
| 29 | -do- 70°C | MS T5 |
| 30 | -do- 80°C | MS T6 |
| 31 | Control | |

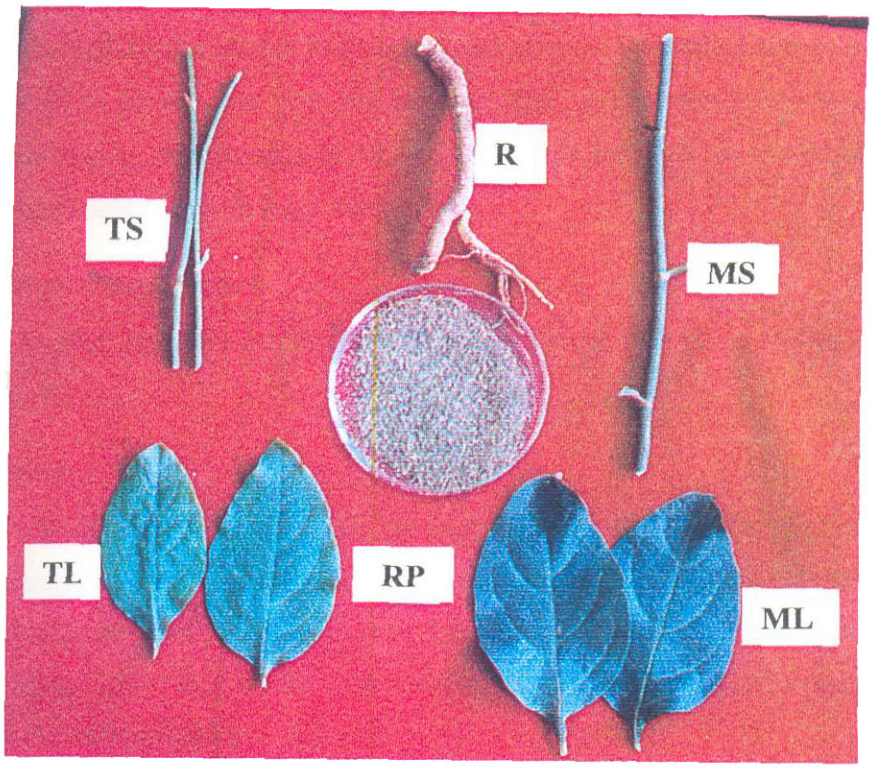



**Plate 3. Different parts of *Plumbago rosea* tested for PMV inhibitory property**

**TS - Tender stem** **MS - Mature stem** **TL - Tender leaf**
**ML - Mature leaf** **R - Root** **RP - Root powder**

Preparation of extracts

One per cent extract of tender leaf (light green), mature leaf (dark green), tender stem (light green) and mature stem (dark green) of *P. rosea* were separately prepared by crushing in pestle and mortar after adding distilled water. Filtered the extract through muslin cloth. Soaked dry root powder in distilled water overnight and filtered to prepare one per cent extract. The extract was taken in separate bottle and kept at temperatures of 30, 40, 50, 60, 70 and 80°C in a water bath for 10 min and cooled to room temperature.

Application of plant extract

Applied the plant extracts as fine spray on eight days old pumpkin seedling using an atomizer.

Inoculation

Inoculation was carried out as explained in 3.1, 24 h after application of plant extract. There were 20 seedlings at the rate of 10 seedlings per replication for each treatment and the plants were maintained in the insect proof net house for observation. After four weeks of inoculation, disease incidence and severity were worked out as described in section 3.7.1.

### 3.7.3 Effect of *Plumbago rosea* on the Vector *Aphis gossypii*

Effect of Plumbago on *A. gossypii* was studied by applying one per cent root extract before acquisition and inoculation feeding of the vector.

Non viruliferous aphids maintained in the laboratory were used for the study with the following treatments.

T1 - 24 h before acquisition feeding

T2 - 48 h before acquisition feeding

T3 - 24 h before inoculation feeding

T4 - 48 h before inoculation feeding

T5 - Control (application of distilled water before acquisition feeding and before inoculation feeding)

Application of plant extract

In the case of treatment one and two, Plumbago spray (1%) was given to 30 days old infected pumpkin plant maintained by artificial inoculation in the net house. Acquisition was given 24 h and 48 h after spray. For T3 and T4 the spray was given to eight days old healthy pumpkin seedlings. Inoculation was conducted 24 hours and 48 hours after the spray. In the case of T5, spray of distilled water was given to infected pumpkin plant and healthy pumpkin seedlings of the same age as described above.

Transmission of PMV

Non viruliferous aphids were collected from the culture maintained in the laboratory. They were starved for one hour and gave acquisition feeding on sprayed infected leaf for T1 and T2 and non-sprayed infected leaf for T3 and T4 and water sprayed infected leaf for T5 for 30 min. After acquisition, the viruliferous aphids were released on 9 days old healthy pumpkin seedlings for T1 and T2, sprayed healthy seedlings for T3 and T4 (24 and 48 h after the spray respectively) and water sprayed healthy seedlings for T5 @ 10 aphids per plant. The seedlings were kept under insect proof cages for 24 h and then sprayed quinalphos 0.05 per cent to kill the aphids. Then seedlings were transferred to the net house .

Each treatment was applied on 30 seedlings @ 10 plants per replication. Disease incidence and severity were calculated after four weeks as described in section 3.7.1

## 3.8 ISOLATION OF PMV INHIBITORY PRINCIPLES OF *PLUMBAGO ROSEA*

Fractions were collected from Plumbago water extract by passing through silica gel column (60-120 mesh) and each fraction was tested for PMV inhibitory property on pumpkin seedlings.

Preparation of the sample

Soaked 100 g of dry root powder of Plumbago in distilled water overnight. Filtered and the residue and repeatedly extracted with water and made upto one litre .

Preparation of silica gel column

Silica gel (60-120 mesh) column of 30 x 1.6 cm size was used for fraction collection.

Collection of fractions

Saturated the silica gel column with distilled water and added 5 ml of the prepared sample at the top without disturbing silica gel. Twelve fractions at the rate of 5 ml per fraction were collected separately.

Qualitative Tests

Qualitative tests were conducted to identify the presence of alkaloids, carbohydrates, saponins, phenols and terpenes, in each fraction.

| Components | Test performed |
|---|---|
| 1. Alkaloids | Dragendroff's reagent test |
| 2. Carbohydrates | Fehling's solution test |
| 3. Saponins | Vanillin – sulphuric acid reagent test |
| 4. Phenol | Folin – ciocalteau reagent test |
| 5. Terpenes | Aqueous potassium permanganate test |

Bioassay

Each fraction of Plumbago water extract was applied on cotyledonary and true leaves of eight days old pumpkin seedlings in poly bags by swabbing with cotton wool. The seedlings were inoculated 24 h after application of plumbago fraction as explained in section 3.1, and kept in insect proof net house and symptom development was observed for four weeks. Disease incidence and severity were worked out as described in section 3.7.1.

## 3.9 MANAGEMENT OF PUMPKIN MOSAIC USING *PLUMBAGO ROSEA*

Field experiments were conducted with the following objectives.

1. (a) Evaluation of the effect of Plumbago on pumpkin mosaic and vegetative and yield attributes under artificial inoculation.

(b) Evaluation of the effect of Plumbago on incidence of pumpkin mosaic and population of aphids under natural condition.

2. To study the effect of Plumbago on PMV

3. To study biochemical changes due to Plumbago spray

### 3.9.1 Effect of Plumbago Spray on Pumpkin Mosaic

Two field experiments were conducted one with artificial inoculation of PMV and other without artificial inoculation of PMV. Following treatments were applied in each experiment.

$T_1$ - Weekly spray of Plumbago

$T_2$ - Fortnightly spray of Plumbago

$T_3$ - Monthly spray of Plumbago

$T_4$ - Bimonthly spray of Plumbago

$T_5$ - Single spray of Plumbago

$T_6$ - Weekly spray of insecticide

$T_7$ - Control

Preparation of Plumbago extract

Dried Plumbago root powder was soaked overnight in water, sieved and made upto one per cent for application.

Spray of Plumbago extract

The spray of Plumbago extract was begun when the seedling was eight days old. The spraying was given at different intervals for a period of two and half months.

Spray of insecticide

Weekly spray of insecticides was given to treatment $T_6$. Quinalphos (Ekalux 25EC) carbaryl (Sevin 50 WP) and mercaptothion (Malathion 50 EC) at the recommended dosages were applied alternatively.

Raising of seedlings and planting

Pumpkin seeds (var. Ambili) were sown in polythene covers (10 x 8 cm) filled with potting mixture. First spray of Plumbago was given to eight days old seedlings. In the experiment with artificial inoculation of PMV, pumpkin seedlings were inoculated 24 h after the first spray of Plumbago and transplanted to experimental plot on the next day. In the experiment without inoculation of PMV, seedlings were transplanted to experimental plot two days after first Plumbago spray. Availability of inoculum was assured in this case by growing PMV inoculated pumpkin plants around the experimental plot.

The study was conducted in the experimental plots of Plant Pathology Department, College of Horticulture, Vellanikkara during September-December, 2001 and September-November, 2002, the experiments with artificially inoculated and healthy seedlings of pumpkin respectively. The pumpkin was raised with a plot size of 32 x 10 m in Randomized Block Design with three replications. The seedlings were planted in furrows of two meters long and 30 cm deep and at a distance of 60 cm. There were four seedlings per replication. All crop management operations were carried out as per the package of practices recommendations of Kerala Agricultural University (1996).

The following observations were recorded.

a) Field experiment with artificial inoculation

1. Weekly disease scoring
2. Vegetative characters – Length of vine, number of branches, average length of internodes.

3. Yield characters – Days to first female flower anthesis, number of female flowers, days to first fruit set, fruits per plant, yield per plant.

b) Field experiment without artificial inoculation

1. Weekly population of aphid (*A. gossypii*)

2. Time for appearance of the disease

3. Disease scoring after the appearance of disease in control plants.

### 3.9.2 Effect of Plumbago on PMV

The plants which showed symptoms of PMV infection 45 days after planting were labelled. Leaf samples were collected from these labelled plants 60 days after planting after imposing treatments as explained in Section 3.9.1.

DAC-ELISA was performed as per the procedure described in 3.5.3.2a using the dilutions of antiserum and conjugate standardized for PMV detection.

### 3.9.3 Biochemical Changes by Plumbago Spray

Biochemical analysis of weekly Plumbago treated plants and control plants were recorded to establish the effect of Plumbago in the biochemical constituents namely protein, phenol, chlorophyll of the plant and in the activity of peroxidase, catalase and phenylalanine ammonia lyase. Samples were collected from infected and healthy plants after nine sprays of Plumbago. The procedure adopted for analysis was same as described in section 3.6.

# *Results*

# 4. RESULTS

## 4.1 SYMPTOMATOLOGY

Symptoms of pumpkin mosaic virus (PMV) disease was studied under natural and artificial condition.

Under natural condition, mottling of the leaves with light green and dark green patches and vein banding were the initial symptoms (Plate 4B). Blisters and raised surfaces were also appeared in leaves (Plate 4C). This was followed by disfiguration of leaf into filiform shape or some other shapes and also resulted in reduction of leaf area (Plate 4D). Occasionally irregular chlorotic spots appeared on the leaf lamina which later coalesced and turned to large yellow patches (Plate 4E). The infected seedlings were stunted, flowered very sparingly with less number of female flowers and reduced fruit setting. The fruits were reduced in size and often malformed (Plate 4F).

First symptom on artificial inoculation was the chlorosis of veins and veinlets (Plate 4A). This was evident six to seven days after inoculation on newly emerged leaves of pumpkin. Thereafter mottling of the leaves with light and dark green patches were observed. Leaves exhibited vein banding along the midrib and lateral veins. Older leaves had prominent dark green blisters or raised surfaces. The leaf lamina was very often distorted and the veins and veinlets usually extended beyond the margins as narrow projections of varying sizes resulting in filiform leaves. Infected plant showed stunted growth with weak branches. Flowering was delayed and size of the flowers was reduced. The infected plant produced less number of fruits which were often malformed with dome shaped protuberances and irregular fruit surface. Flesh colour of fruit of the infected plant was yellowish-white instead of yellowish orange colour of fruits of healthy plant.

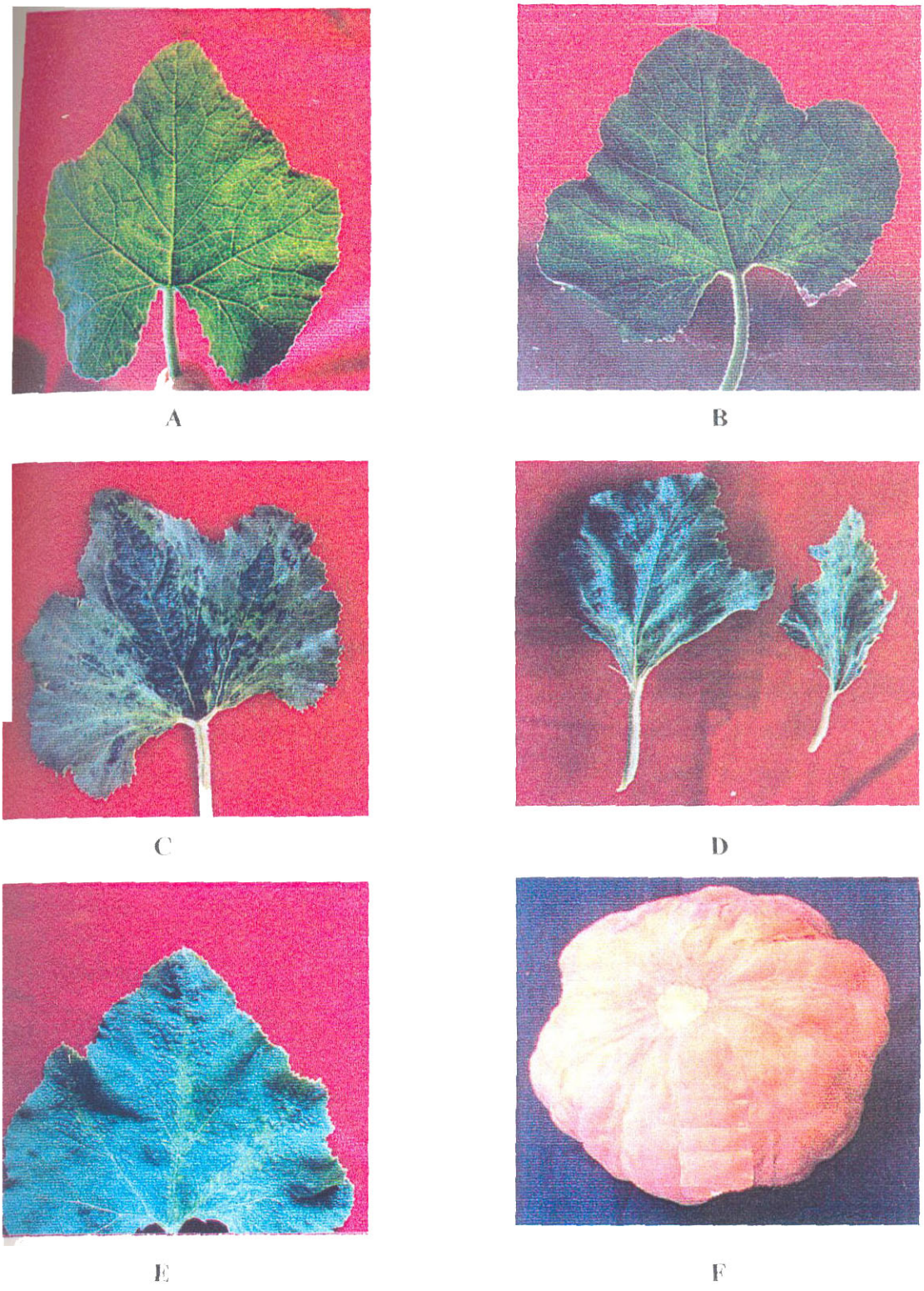



**Plate 4. Symptoms of PMV infection on pumpkin**

A - Vein chlorosis B - Mottling C - Blistering D - Malformation
E - Yellow patches F - Fruit malformation

## 4.2 TRANSMISSION

### 4.2.1 Sap Transmission

The virus transmission was successfully carried out by mechanical inoculation. The success of transmission ranged from 85-90 per cent, after four weeks of inoculation (Table 3 ).

Table 3. Sap transmission percentage of PMV

| Inoculation | Plants infected/plants inoculated | Per cent transmission |
|---|---|---|
| I | 17/20 | 85 |
| II | 18/20 | 90 |
| Mean | | 87.5 |

### 4.2.1a Effect of Age of Seedling on Sap Transmission of PMV

Sap transmission on pumpkin seedlings of different ages, viz., six, nine, twelve and fifteen days were conducted. During the first week of inoculation the symptoms were recorded only on six and nine days old seedlings (56.67 and 46.67 per cent respectively). During the second week also, six and nine days old seedlings showed high percentage infection (76.67% and 73.33% respectively). From third week onwards all the seedlings irrespective of age showed high rate of transmission (Table 4).

Table 4. Effect of age of seedling on sap transmission of PMV

| Days after sowing | Per cent transmission | | | | Mean |
|---|---|---|---|---|---|
| | 1st week | 2nd week | 3rd week | 4th week | |
| 6 | 56.67 (0.85) | 76.67 (1.07) | 83.33 (1.15) | 83.33 (1.15) | 75.00 (1.06) |
| 9 | 46.67 (0.75) | 73.33 (1.03) | 86.67 (1.20) | 86.67 (1.20) | 73.33 (1.05) |
| 12 | 0 (0.16) | 33.33 (0.63) | 76.67 (1.07) | 83.33 (1.15) | 48.33 (0.75) |
| 15 | 0 (0.16) | 36.67 (0.65) | 83.33 (1.15) | 86.67 (1.20) | 51.65 (0.79) |
| Mean | 25.84 (0.48) | 55.00 (0.84) | 82.50 (1.14) | 85.00 (1.18) | |

CD ($P< 0.05$) Days = 0.048, Week = 0.056, Days x weeks = 0.112
*Values in parenthesis are Arc Sin transformed

The mean incubation period of six and nine days old seedlings was observed to be 9 to 10 days whereas 12 and 15 days old seedlings recorded the incubation period of 14-15 days (Table 5, Fig.1).

Table 5. Effect of seedling age on incubation period of PMV during sap transmission

| Days after sowing | Mean incubation period (days) |
|---|---|
| 6 | 9.00 |
| 9 | 9.65 |
| 12 | 14.43 |
| 15 | 14.74 |
| CD ( P< 0.05 ) | 1.66 |

### 4.2.2 Insect Transmission

The PMV was found to be transmitted by the aphid, *Aphis gossypii*. Insect transmission was carried out as described in 3.2.2. Transmission percentage was found to be 62.5 ( Table 6 ).

Table 6 Transmission of PMV by *Aphis gossypii*

| Inoculation | Plants infected/plants inoculated | Per cent transmission |
|---|---|---|
| I | 12/20 | . 60 |
| II | 13/20 | 65 |
| Mean | | 62.5 |

### 4.2.3 Seed Transmission

Seeds collected from artificially inoculated plant were sown in poly bags and kept in the insect proof net house. The percentage germination was 90.76. First infection appeared only in the fourth week and seed transmission was found to be 5.08 per cent . The seedlings raised from seeds of naturally infected plant

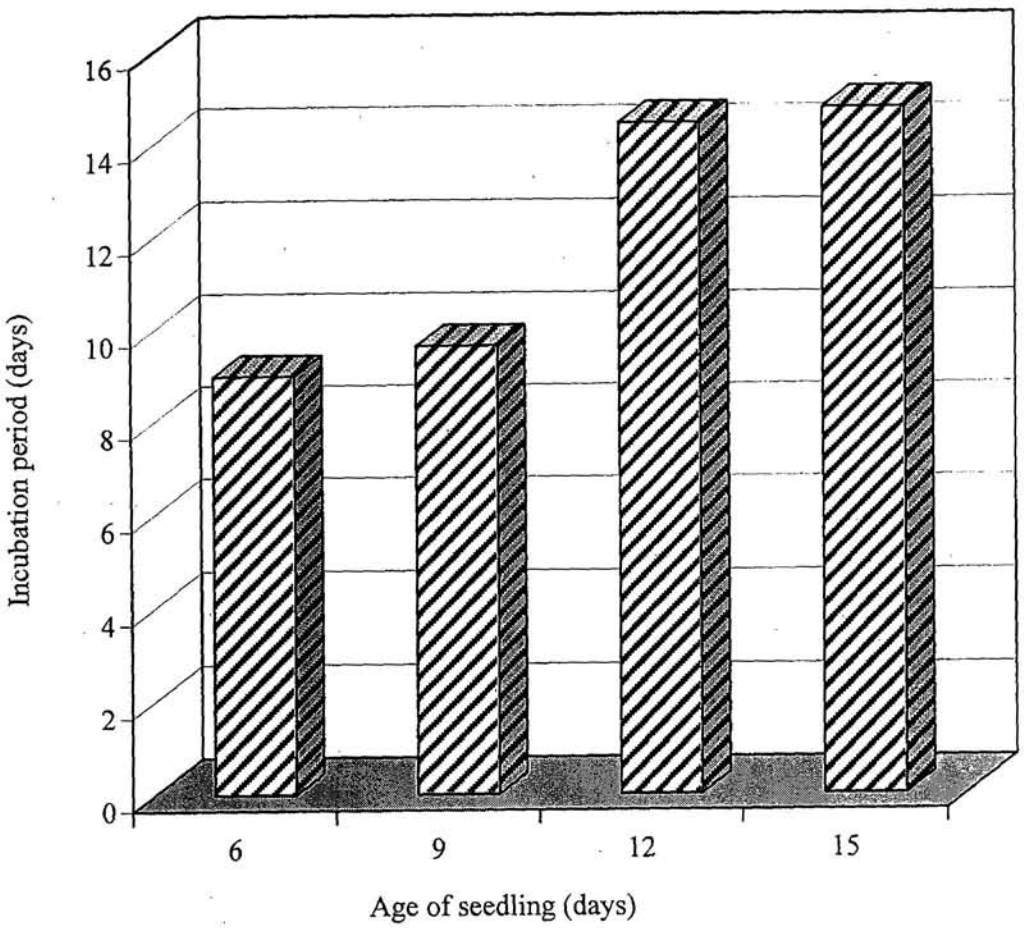



**Fig. 1. Effect of age of seedling on incubation period of PMV**

showed one per cent transmission and symptoms appeared only in the sixth week (Table 7).

Table 7. Seed transmission of PMV

| Experiment details | Per cent germination | Plants infected/plants germinated | Per cent transmission | Mean incubation period (weeks) |
|---|---|---|---|---|
| Seeds from artificially inoculated plant | 90.76 | 6/118 | 5.08 | 5 |
| Seeds from naturally infected plant | 83.33 | 1/100 | 1.00 | 6 |

From the results it could be inferred that PMV might also be transmitted through seeds to a certain extent.

## 4.3 HOST RANGE

Host range studies of PMV was conducted by sap inoculation on a total of 19 host species belonging to four different families viz., cucurbitaceae (11 no.), solanaceae (4 no.), fabaceae (3 no.) and caricaceae (1 no.) and results are presented in Table 8.

In cucurbitaceae, out of 11 plant species inoculated, symptoms appeared on five species namely *Benincasa hispida* (wild), *Citrullus vulgaris, Cucurbita maxima, Momordica charantia* and *Trichosanthes anguina.* Severe mosaic mottling with light and dark green patches appeared in *T. anguina* (Plate 5A), *B. hispida*, wild (Plate 5B) and *C. maxima* (Plate 5D). The symptoms appeared by the second week of inoculation of the virus. Mosaic mottling with light and dark green patches and leaf distortion was seen in *C. vulgaris* (Plate 5C) and *M. charantia* (Plate 5E) by third week.

However, *B. hispida, L. siceraria, L. acutangula, C. sativus, C. melo* and *C. melo* var. *conomon* did not produce any symptom. The host plants which did not show symptoms on mechanical transmission were indexed back to healthy

pumpkin seedlings. The sap from ridge gourd and bottle gourd produced symptoms on pumpkin seedlings.

In solanaceous hosts, *C. annuum* and *D. metel* showed symptoms by the third week of inoculation. Leaf distortion was observed in *C. annuum* (Plate 6A) and chlorotic spots were developed on the leaf of *D. metel* (Plate 6B).

Among leguminous plants, cowpea and soybean were infected by PMV. Mosaic mottling was produced in soybean (Plate 6D) within one week while in the case of cowpea this symptom appeared only in the second week of inoculation (Plate 6E).

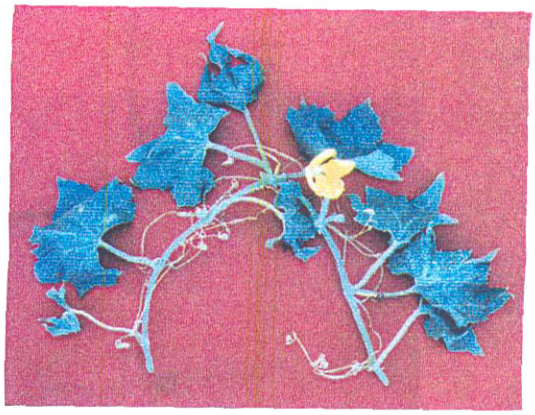
On papaya which belongs to family caricaceae, chlorotic rings and leaf distortion were appeared by second week of inoculation (Plate 6C).

Table 8. Symptoms of PMV infection in different host plants

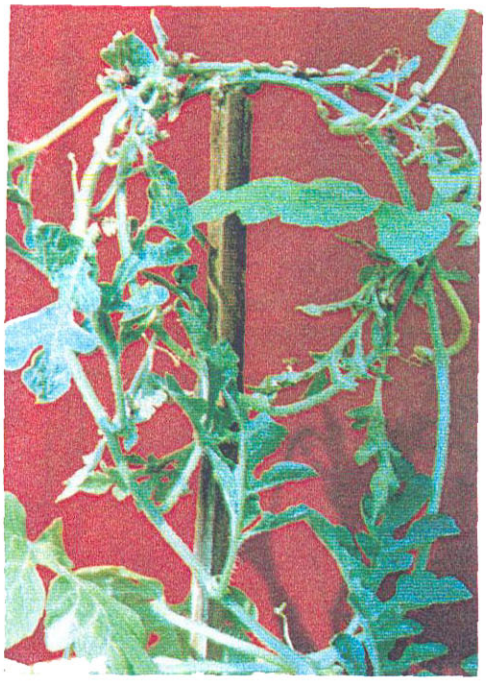
| Host Plant | Symptoms |
|---|---|
| **Family cucurbitaceae** | |
| *Benincasa hispida* | NS |
| *B. hispida* (wild) | Severe mosaic mottle |
| *Citrullus vulgaris* | Mosaic mottle and leaf distortion |
| *Cucumis melo* | NS |
| *C. melo* var. *conomon* | NS |
| *C. sativus* | NS |
| *Cucurbita maxima* | Severe mosaic mottle |
| *Lagenaria siceraria* | NS |
| *Luffa acutangula* | NS |
| *Momordica charantia* | Mosaic mottle and leaf distortion |
| *Trichosanthes anguina* | Severe mosaic mottle |
| **Family solanaceae** | |
| *Capsicum annuum* | Leaf distortion |
| *Datura metel* | Chlorotic spots |
| *Lycopersicon esculentum* | NS |
| *Solanum melongena* | NS |
| **Family fabaceae** | |
| *Cyamopsis tetragonoloba* | NS |
| *Glycine max* | Mosaic mottle |
| *Vigna unguiculata* | Mosaic mottle |
| **Family caricaceae** | |
| *Carica papaya* | Chlorotic rings and leaf distortion |

NS – No symptom

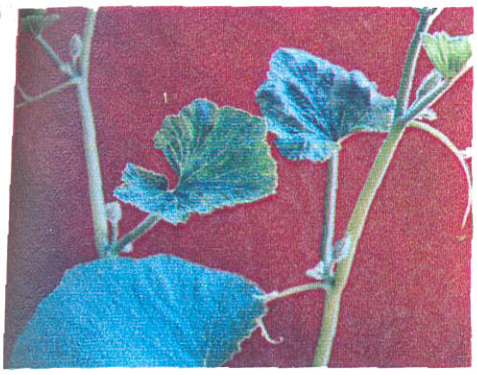
A



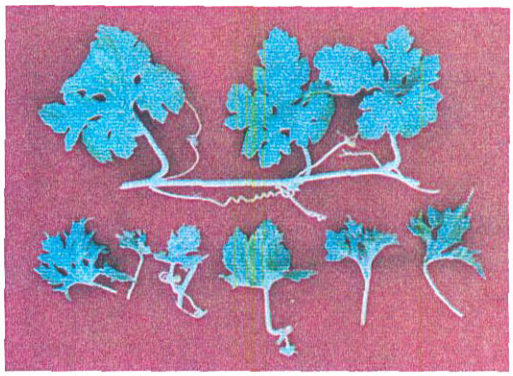
B



C



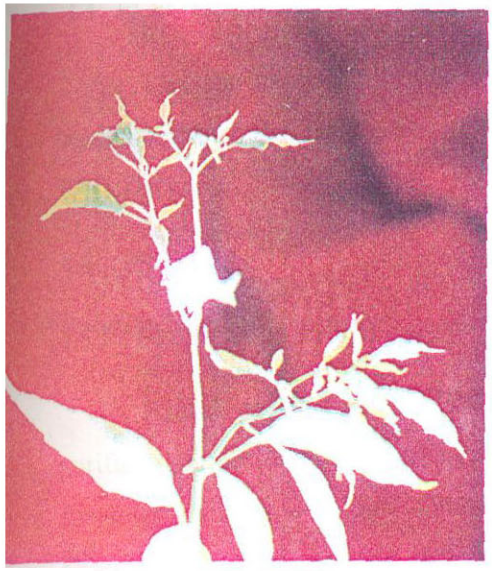
D



E

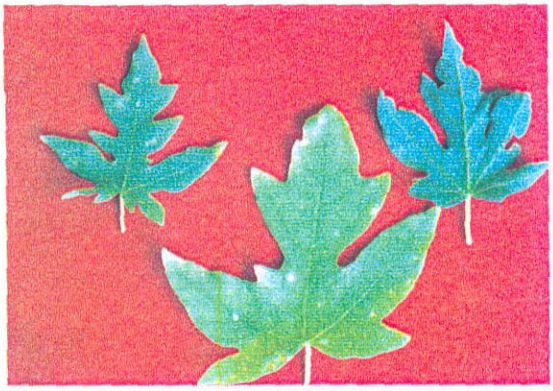
**Plate 5. Symptoms of PMV infection on cucurbitaceous crops**

A - Snake gourd (*Trichosanthes anguina*)
B - Wild ash gourd (*Benincasa hispida*)
C - Water melon (*Citrullus vulgaris*)
D - Winter squash (*Cucurbita maxima*)
E - Bitter gourd (*Momordica charantia*)

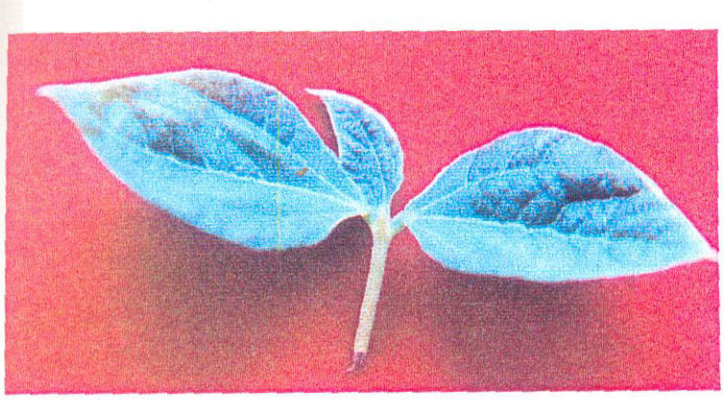


A

B



C



D

E

**Plate 6. Symptoms of PMV infection on crops other than cucurbitaceous crops**

**A - Chilli (Family solanaceae) B - Datura (Family solanaceae) C - Papaya (Family caricaceae) D - Soybean (Family fabaceae) E - Cowpea (Family fabaceae)**

## 4.4 ELECTRON MICROSCOPIC STUDY

Electron microscopy was done by leaf dip method as described in 3.4. The results revealed the association of flexuous virus particles of size 700-800 x 11 nm (Plate 7A).

## 4.5 SEROLOGY

### 4.5.1 Purification of PMV

Partial purification of PMV was done as explained in 3.5.1. When the purified preparation was observed under electron microscope, characteristic flexuous particles were seen (Plate 7B). Infectivity of the virus was confirmed by observing the symptoms on inoculated pumpkin seedlings. The protein content of the partially purified virus extract was 4.93 mg $ml^{-1}$ of the extract.

### 4.5.2 Antiserum Production

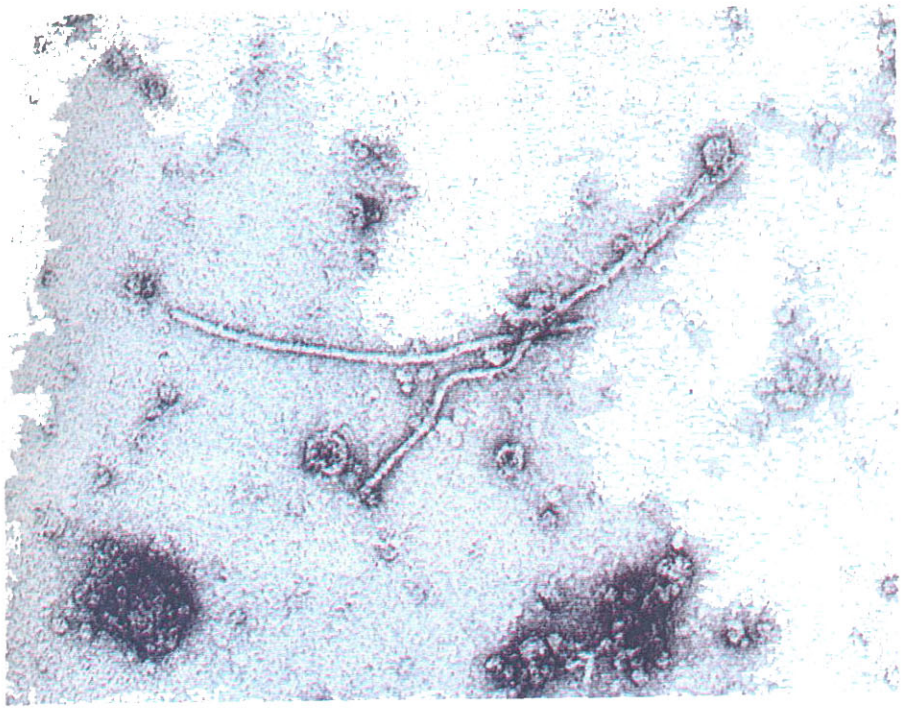
Antiserum of PMV was successfully produced from one year old New Zealand White female rabbit following the procedure described in section 3.5.2.

### 4.5.3 Serodiagnosis

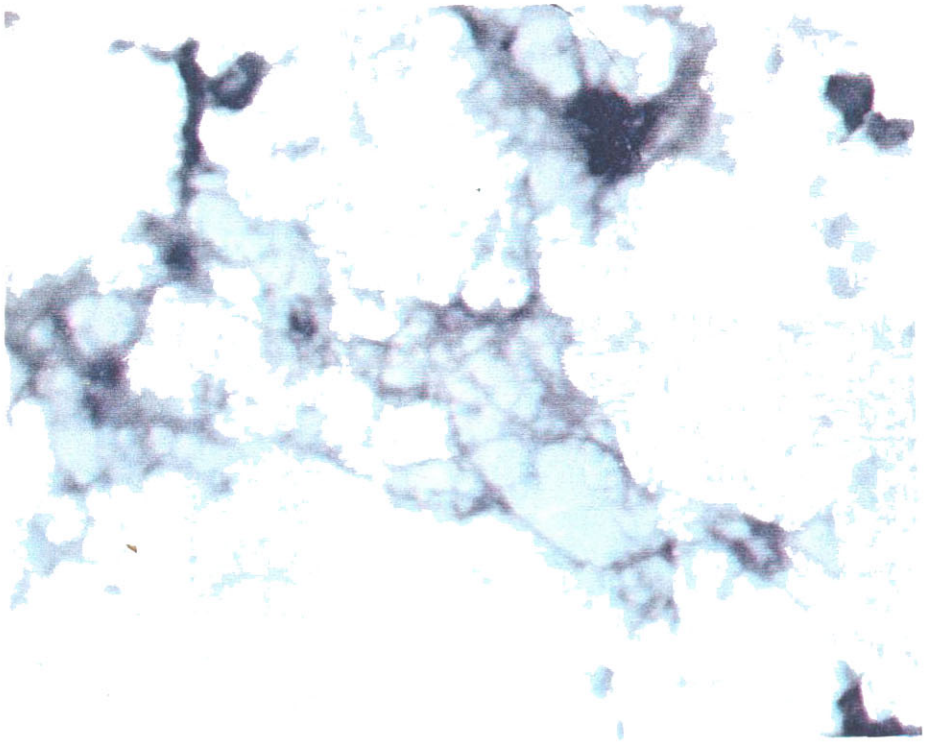
#### 4.5.3.1. Ouchterlony's Double Diffusion Test

Ouchterlony's double diffusion test of cross absorbed antiserum with purified sap of healthy and infected pumpkin plants produced characteristic precipitin bands with infected pumpkin sap and not with healthy pumpkin sap (Plate 8A).

Antiserum of PMV showed relation with poty viruses infecting snake gourd (*T. anguina*), bitter gourd (*M. charantia*), wild ash gourd (*B. hispida*) (Plate 8B), cowpea (*V. unguiculata*), soybean (*G. max*) (Plate 8D), chilli (*C. annuum)* (Plate 8E) and papaya (*C. papaya*) (Plate 8C).



A

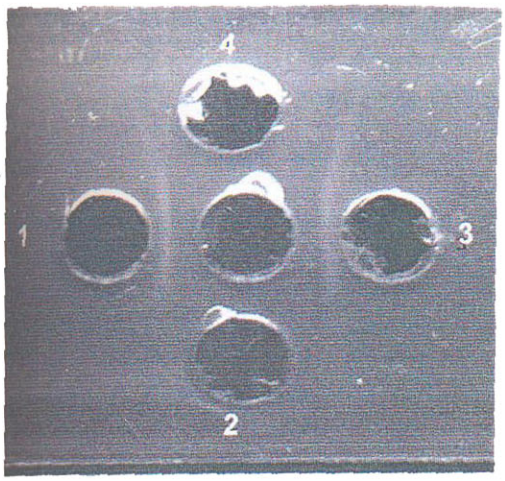


B

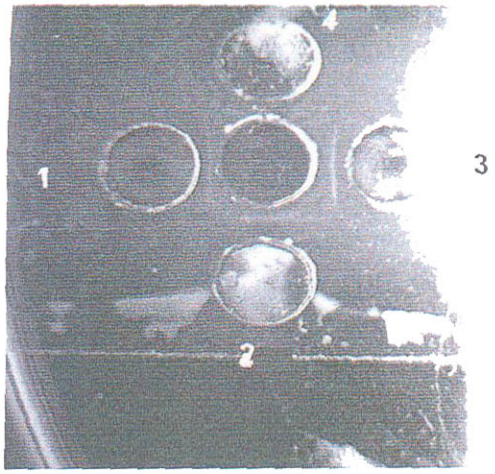
**Plate 7. Electron micrograph of PMV**

**A - Leaf dip preparation [X 1,00,000]**

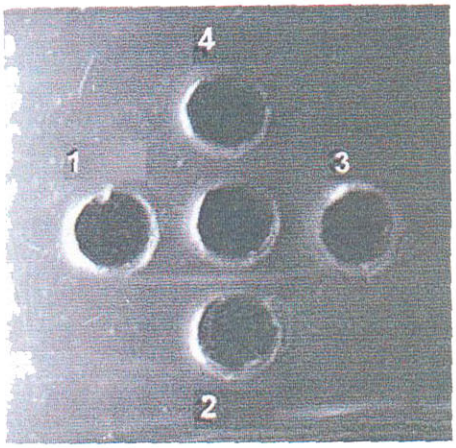
**B - Purified virus preparation [X 21,000]**



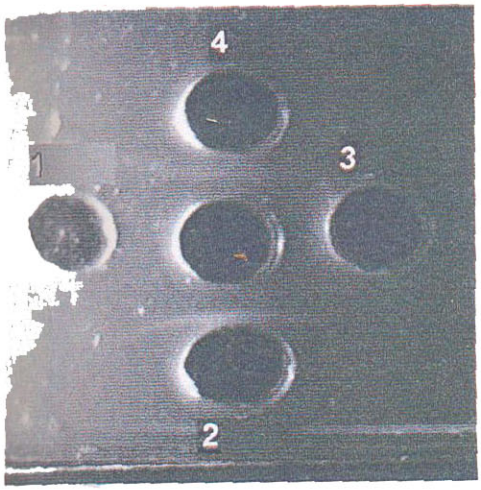

A) 1,3 – Infected pumpkin
2,4 - Healthy pumpkin



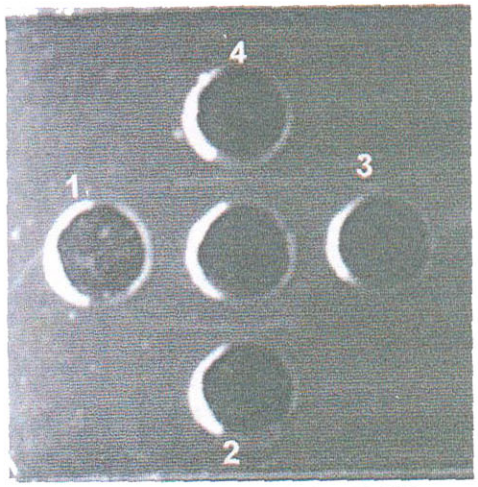

B) 1-Ash gourd 2-Bitter gourd
3-Snake gourd 4- Coccinia




C) 1,3,4- Buffer 2- Papaya




D) 1 - Cluster Bean 2 - Cowpea
3 - French Bean 4 - Soya Bean




E) 1 - Brinjal 2,4- Chilli
3 - Tomato

**Plate 8. Ouchterlony's double diffusion test**
(PMV antiserum in central well)

### 4.5.3.2 Direct Antigen Coating - Enzyme Linked Immunosorbent Assay (DAC-ELISA)

#### 4.5.3.2a Standardisation of DAC-ELISA for PMV Detection

Different dilutions of antiserum viz., 1:250, 1:500 and 1:1000 and that of conjugate viz., 1:2000, 1:4000, 1:8000 and 1:16,000 were used for standardisation of DAC-ELISA for PMV detection.

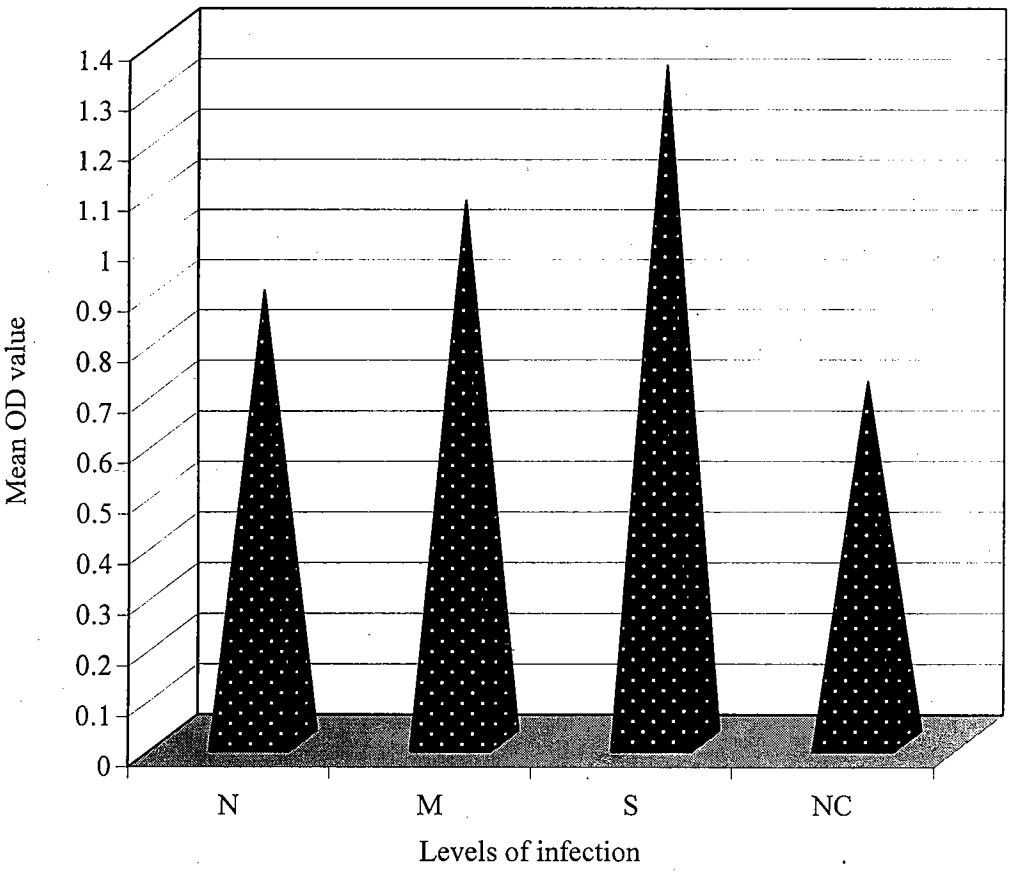
Maximum increase in absorbance value of infected sample over healthy control (101%) was obtained when the antiserum at 1:250 dilution and conjugate at 1:4000 dilution were used (Table 9).

Table 9. Standardisation of DAC-ELISA for PMV detection

| Per cent increase in absorbance over healthy | | | | | |
|---|---|---|---|---|---|
| | | Conjugate dilutions | | | |
| | | 1:2000 | 1:4000 | 1:8000 | 1:16000 |
| Antiserum dilutions | 1:250 | 81.96 | 101 | 80.32 | 57.89 |
| | 1:500 | 32.25 | 72.41 | 55.62 | 51.77 |
| | 1:1000 | 29.03 | 27.09 | 26.64 | 31.75 |

#### 4.5.3.2b Detection of PMV by DAC- ELISA

Results of DAC-ELISA (Table 10, Fig.2, Plate 9) showed that the infection, severity of symptoms and OD values were positively correlated. The OD values of both mild and severely infected samples were more than the cut off value (0.94) but that of apparently healthy sample was less than the cut off value.




N - No infection (Score - 0) M - Mild infection (Score - 1,2)
S - Severe infection (Score - 3,4) NC - Negative Control (Healthy)

**Fig. 2. DAC-ELISA test for PMV detection**

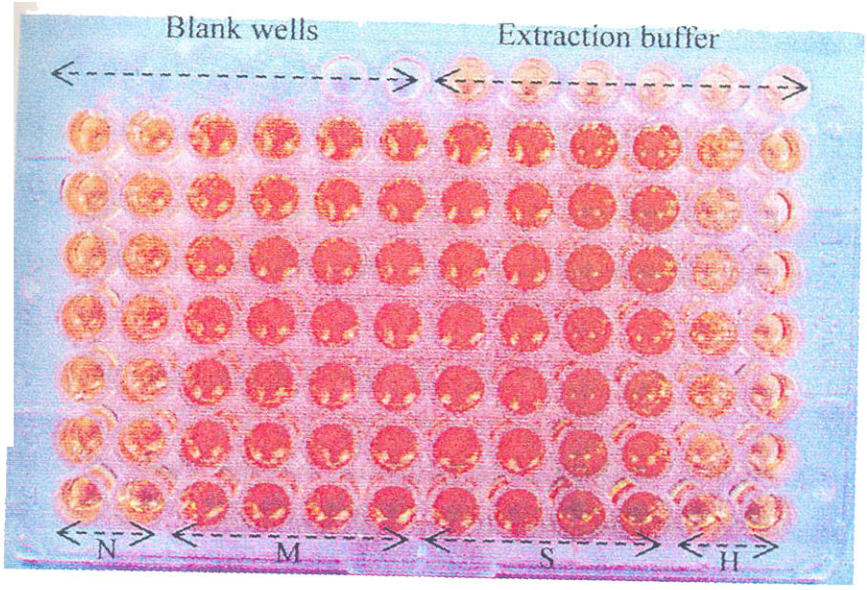



**Plate 9. DAC - ELISA test for PMV detection**

**Samples**

**N - No symptom (Score - 0)**
**M - Mild symptom (Score - 1, 2)**
**S - Severe symptom (Score 3, 4)**
**H - Healthy**

Table 10. Detection of PMV by DAC - ELISA

| Leaf Sample | Mean OD value at 450 nm |
|---|---|
| No symptom | 0.89 |
| Mild symptom | 1.07 |
| Severe symptom | 1.34 |
| Negative control | 0.71 |
| Cut off value | 0.94 |

## 4.6. BIOCHEMICAL CHANGES DUE TO HOST PATHOGEN INTERACTION

The effect of infection on protein, phenol, chlorophyll and activity of peroxidase, catalase and phenylalanine ammonia lyase (PAL) enzymes was studied (Fig.3).

### 4.6.1 Influence on Soluble Protein Content

Infected plant showed a significant reduction in protein content than healthy (Table 11). Percentage decrease in protein content of infected plant was 33.61 in one month old plants and 15.21 in two months old plants. The protein content was found to increase with age of plant and it was 22.39 per cent for healthy plant and 39.23 per cent for infected plant.

Table 11. Effect of age and infection on the protein content of pumpkin

| Age of plant | Mean protein content (mg $g^{-1}$) | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | |
| One month old | 4.74 | 7.14 | 33.61 | t value - 5.79** |
| Two months old | 7.80 | 9.20 | 15.21 | |
| Per cent variation over 1 month | 39.23 | 22.39 | | |
| t value - 5.78** | | | | |

** Significant at 1% level

### 4.6.2 Influence on Phenol

The phenol content of PMV infected plant was lower than healthy plant (Table 12 ). The per cent decrease was 10.5 for one month old plants and 21.86 for two months old plants. Due to aging the phenol content showed a significant

increase and per cent increase was more for healthy plant (37.92) than for infected plant (20.42).

Table 12. Effect of age and infection on the phenol content of pumpkin

| Age of plant | Mean phenol content (mg $g^{-1}$) | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | |
| One month old | 15.67 | 17.51 | 10.50 | t value - $2.97^{NS}$ |
| Two months old | 18.87 | 24.15 | 21.86 | |
| Per cent variation over 1 month | 20.42 | 37.92 | | |
| t value - 3.98** | | | | |

** Significant at 1% level
NS - Non significant

### 4.6.3 Influence on Chlorophyll

The PMV infected plants showed high chlorophyll (chlorophyll a, b and total) content and per cent increase in total chlorophyll was 18.48 for one month old plants and 10.58 for two months old plants. But chlorophyll a : b ratio was low for infected plants eventhough not significantly statistically (Table 13). The per cent decrease was 10.8 for one month old plants and 5.6 for two month old plants. Due to aging chlorophyll a, b and total showed significant increase whereas a : b ratio showed a decrease.

Table 13. Effect of age and infection on the chlorophyll content of pumpkin

| Chlorophyll content (mg $g^{-1}$) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age of plant | Infected | | | | Healthy | | | | Per cent variation over healthy | | | |
| | Ch. a | Ch. b | Total ch | a/b | Ch.a | Ch.b. | Total ch | a/b | a | b | Total ch | a/b |
| One month old | 0.78 | 0.32 | 1.09 | 2.47 | 0.68 | 0.24 | 0.92 | 2.77 | 14.70 | 33.33 | 18.48 | 10.80 |
| Two months old | 1.47 | 0.62 | 2.09 | 2.36 | 1.28 | 0.51 | 1.89 | 2.50 | 14.84 | 21.57 | 10.58 | 5.60 |
| Per cent variation over 1 month | 88.46 | 93.75 | 91.74 | 4.45 | 88.23 | 112.50 | 105.43 | 9.74 | t value (for comparison of infected and healthy) | | | |
| | | | | | | | | | 4.12 ** | 2.62 NS | 4.18 * | 2.03 NS |
| T value (for comparison of age of plant) | | | | | 7.58 ** | 9.29 ** | 17.71 ** | 1.38 NS | | | | |

** Significant at 1% level; * Significant at 5% level; NS - Non significant

### 4.6.4 Influence on Peroxidase Activity

Peroxidase activity of PMV infected plant was significantly higher than healthy plant (Table 14). Per cent increase in peroxidae activity was 100 for one month old plant and 122 for two months old plant. Due to aging also, peroxidase activity showed an increase and it was higher for infected (33.34%) than healthy (19.88%).

Table 14. Effect of age and infection on the peroxidase activity of pumpkin

| Age of plant | Peroxidase activity (units litre$^{-1}$) | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | t value - 7.21** |
| One month old | 199.06 | 99.53 | 100 | |
| Two months old | 265.44 | 119.32 | 122 | |
| Per cent variation over 1 month | 33.34 | 19.88 | | |
| t value - 2.93$^{NS}$ | | | | |

** Significant at 1% level; NS - Non significant

### 4.6.5 Influence on Catalase Activity

The catalase activity showed a decrease in trend due to infection (Table 15, Fig.13). Per cent decrease of one month old plant was 30.39 whereas that of two months old plant was 14.54. Due to aging the activity showed a significant decrease both in infected (46.33%) and healthy (56.29%).

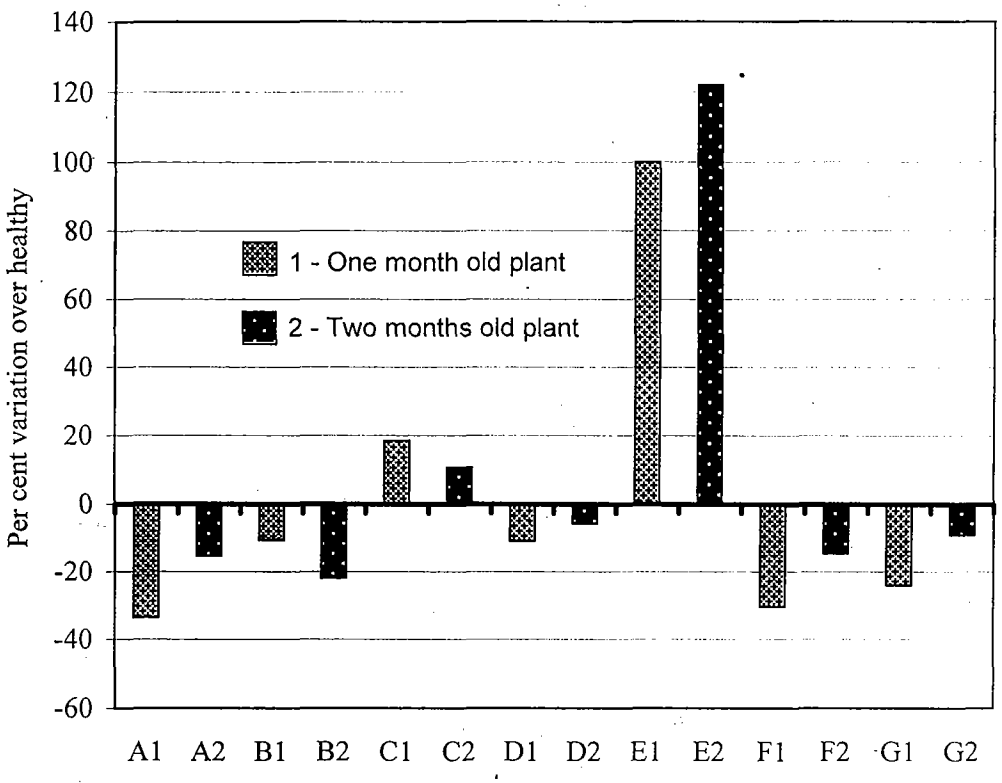
Table 15. Effect of age and infection on the catalase activity of pumpkin

| Age of plant | Catalase activity (g$^{-1}$) | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | t value - 2.17$^{NS}$ |
| One month old | 136.22 | 195.71 | 30.39 | |
| Two months old | 73.11 | 85.55 | 14.54 | |
| Per cent variation over 1 month | 46.33 | 56.29 | | |
| t value - 5.75** | | | | |

** Significant at 1% level; NS - Non significan

### 4.6.6 Influence on Phenylalanine Ammonia Lyase (PAL) Activity

The activity of PAL showed significant reduction due to infection. Per cent decrease was 24.08 for one month old plant and 9.04 for two months old plant




A - Protein
B - Phenol
C - Total chlorophyll
D - Chlorophyll a:b
E - Peroxidase activity
F - Catalase activity
G - PAL activity

**Fig. 3. Biochemical changes of host pathogen interaction in pumpkin**

(Table 16). Due to aging PAL showed significant increase and it was more pronounced for infected (84.98%) than healthy (54.41%).

Table 16. Effect of age and infection on the PAL activity of pumpkin

| Age of plant | PAL - Absorbance value | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | |
| One month old | 0.413 | 0.544 | 24.08 | t value - 3.26* |
| Two months old | 0.764 | 0.840 | 9.04 | |
| Per cent variation over 1 month | 84.98 | 54.41 | | |
| t value - 20.21** | | | | |

** Significant at 1% level; * Significant at 5% level

## 4.7 ANTIVIRAL PROPERTY OF PLANT EXTRACTS

### 4.7.1 Effect of Dilution and Extraction Media on the Virus Inhibitory Property of Medicinal Plant Extracts

Antiviral property of five medicinal plants namely *Basella alba*, *Glycyrrhiza glabra*, *Phyllanthus fraternus*, *Plumbago rosea* and *Thespesia populnea* was studied against PMV on pumpkin plants. Medicinal plant extracts were prepared in different solvents viz., chloroform, distilled water, ethyl acetate and petroleum ether and effect of the extract in each solvent at different dilutions as explained in section 3.7.1 was studied. Disease incidence and disease severity were calculated after four weeks of inoculation of PMV on pumpkin plants and virus inhibitory property of each medicinal plant was studied.

#### 4.7.1.1 *Basella alba*

The treatments showed significant difference in disease incidence and severity (Table 17). Ten per cent ethyl acetate extract was the most effective treatment. This was followed by two per cent chloroform and five per cent petroleum ether extracts. Ten per cent water extract showed least effect on disease incidence and severity.

When compared dilutions of extraction media, higher dilutions were more effective for chloroform and distilled water extracts, and lower dilutions showed

more effectiveness for ethyl acetate extract. When extracted in petroleum ether, D2 level was more effective than D1 and D3 levels.

Table 17. Effect of dilution and extraction media on inhibitory property of *Basella alba*

| Sl. No. | Treatment | Disease incidence * | Disease severity |
|---|---|---|---|
| 1 | Chloroform D1 | 50 (0.785) cde | 20 bcd |
| 2 | Chloroform D2 | 65 (0.938) ef | 27 d |
| 3 | Chloroform D3 | 30 (0.580) b | 11 ab |
| 4 | Distilled water D1 | 70 (0.991) f | 30 d |
| 5 | Distilled water D2 | 50 (0.785) cde | 20 bcd |
| 6 | Distilled water D3 | 45 (0.735) bcd | 20 bcd |
| 7 | Ethyl acetate D1 | 10 (0.322) a | 2 a |
| 8 | Ethyl acetate D2 | 45 (0.733) bcd | 20 bcd |
| 9 | Ethyl acetate D3 | 50 (0.785) cde | 22 bcd |
| 10 | Petroleum ether D1 | 60 (0.886) def | 24 cd |
| 11 | Petroleum ether D2 | 35 (0.632) bc | 13 abc |
| 12 | Petroleum ether D3 | 60 (0.886) def | 26 d |
| 13 | Control | 80 (1.107) f | 49 e |

*Values in parenthesis are Arc Sin transformed

D1 – 10% (100 g plant sample per litre solvent)
D2 – 5% (50 g plant sample per litre solvent)
D3 – 2% (20 g plant sample per litre solvent)

### *4.7.1.2 Glycyrrhiza glabra*

The high rate of PMV inhibition was expressed by D3 level of ethyl acetate extract (Table 18). Distilled water extract at D2 level also inhibited disease incidence and severity at a higher level. Petroleum ether and ethyl acetate extracts of D2 level and distilled water extract at D3 level were the least effective treatments.

When dilutions of extraction media were compared, higher dilutions were found to be more effective for chloroform and ethyl acetate. The distilled water extract showed more effect at D1 and D2 levels and petroleum ether extract at D1 and D3 levels.

Table 18. Effect of dilution and extraction media on inhibitory property of *Glycyrrhiza glabra*

| Sl. No. | Treatment | Disease incidence * | Disease severity |
|---|---|---|---|
| 1 | Chloroform D1 | 30 (0.58) cd | 14 bc |
| 2 | Chloroform D2 | 20 (0.464) bc | 8 ab |
| 3 | Chloroform D3 | 25 (0.522) bc | 7 ab |
| 4 | Distilled water D1 | 25 (0.522) bc | 11 abc |
| 5 | Distilled water D2 | 10 (0.322) ab | 4 a |
| 6 | Distilled water D3 | 60 (0.886) e | 24 d |
| 7 | Ethyl acetate D1 | 30 (0.580) cd | 16c |
| 8 | Ethyl acetate D2 | 45 (0.735) de | 28 d |
| 9 | Ethyl acetate D3 | 5 (0.161) a | 4 a |
| 10 | Petroleum ether D1 | 20 (0.451) bc | 8 ab |
| 11 | Petroleum ether D2 | 55 (0.838) e | 24 d |
| 12 | Petroleum ether D3 | 20 (0.464) bc | 8 ab |
| 13 | Control | 80 (1.107) f | 49 e |

*Values in parenthesis are Arc Sin transformed

D1 – 10% (100 g dry sample per litre solvent)
D2 – 5% (50 g dry sample per litre solvent)
D3 – 2% (20 g dry sample per litre solvent)

### *4.7.1.3 Phyllanthus fraternus*

*Phyllanthus fraternus* exhibited high rate of inhibition of disease incidence and severity with distilled water extract at D3 level. The extract at D2 level of distilled water, D2 and D3 levels of chloroform and petroleum ether and D1 and D2 levels of ethyl acetate extracts were also found to be equally effective in checking disease incidence. These treatments showed very low disease severity which was on par with D3 level of distilled water extract (Table 19). The least inhibitory property was expressed by the extracts of D3 level of ethyl acetate and D1 level of petroleum ether.

When the dilutions of extraction media were compared, higher dilutions were found to be effective for the extracts of all solvents except ethyl acetate which was more effective at lower dilutions.

Table 19. Effect of dilution and extraction media on inhibitory property of *Phyllanthus fraternus*

| Sl. No. | Treatment | Disease incidence * | Disease severity |
|---|---|---|---|
| 1 | Chloroform D1 | 40 (0.685) b | 15 d |
| 2 | Chloroform D2 | 10 (0.322) a | 3 ab |
| 3 | Chloroform D3 | 15 (0.393) a | 5 ab |
| 4 | Distilled water D1 | 35 (0.632) b | 11 c |
| 5 | Distilled water D2 | 20 (0.464) a | 6 b |
| 6 | Distilled water D3 | 10 (0.322) a | 2 a |
| 7 | Ethyl acetate D1 | 10 (0.322)a | 4 ab |
| 8 | Ethyl acetate D2 | 20 (0.451) a | 6 b |
| 9 | Ethyl acetate D3 | 60 (0.886) c | 25 e |
| 10 | Petroleum ether D1 | 60 (0.886) c | 18 d |
| 11 | Petroleum ether D2 | 15 (0.393) a | 4 ab |
| 12 | Petroleum ether D3 | 10 (0.322) a | 3 ab |
| 13 | Control | 80 (1.107) d | 49 f |

*Values in parenthesis are Arc Sin transformed

D1 – 10% (100 g plant sample per litre solvent)
D2 – 5% (50 g plant sample per litre solvent)
D3 – 2% (20 g plant sample per litre solvent)

#### 4.7.1.4 *Thespesia populnea*

Among the treatments of *T. populnea* extract, D3 levels of ethyl acetate, petroleum ether and D1 and D2 levels of distilled water showed high rate of inhibition of disease incidence and severity (Table 20). Ethyl acetate extract at D1 level was the least effective treatment.

Variation in inhibitory property was observed in the dilution of extraction media. The lower dilution of distilled water extract (D1 and D2 levels) and higher dilutions of ethyl acetate (D2 and D3 levels) extracts were more effective. The chloroform extract showed more effectiveness at D2 level. But D2 level of petroleum ether extract was least effective compared to other dilutions.

Table 20. Effect of dilution and extraction media on inhibitory property of *Thespesia populnea*

| Sl. No. | Treatment | Disease incidence * | Disease severity |
|---|---|---|---|
| 1 | Chloroform D1 | 60 (0.888) c | 31 d |
| 2 | Chloroform D2 | 30 (0.580) ab | 12 ab |
| 3 | Chloroform D3 | 60 (0.886) c | 24 cd |
| 4 | Distilled water D1 | 25 (0.522) ab | 7 a |
| 5 | Distilled water D2 | 20 (0.464) ab | 8 a |
| 6 | Distilled water D3 | 35 (0.632) b | 19bc |
| 7 | Ethyl acetate D1 | 70 (0.991) c | 26 cd |
| 8 | Ethyl acetate D2 | 60 (0.886) c | 26 cd |
| 9 | Ethyl acetate D3 | 20 (0.464) ab | 5 a |
| 10 | Petroleum ether D1 | 35 (0.632) b | 13ab |
| 11 | Petroleum ether D2 | 55 (0.836) c | 24 cd |
| 12 | Petroleum ether D3 | 20 (0.451) a | 10 a |
| 13 | Control | 80 (1.107) c | 49 e |

*Values in parenthesis are Arc Sin transformations
D1 – 10% (100 g plant sample per litre solvent)
D2 – 5% (50 g plant sample per litre solvent)
D3 – 2% (20 g plant sample per litre solvent)

### *4.7.1.5 Plumbago rosea*

Higher concentration of *P. rosea* showed toxicity on pumpkin seedlings. So concentrations of *P. rosea* extract were reduced to five, two, one and 0.1 per cent as explained in section 3.7.1.

The treatments varied significantly in disease incidence and severity (Table 21). The extracts at D3 level of distilled water, D1 level of petroleum ether and D1 and D3 levels of ethyl acetate showed highest rate of inhibition of disease incidence and severity. The least effect in disease incidence was noticed in the extract of D2 level of chloroform and severity by D1 and D2 levels of chloroform and D2 levels of ethyl acetate and petroleum ether.

The inhibitory properties varied with dilution of extraction media. The chloroform extract showed more inhibitory property at higher dilutions (D3 and D4 levels) and distilled water at D3 and D2 levels, than D1 and D4 levels. The

extracts of ethyl acetate and petroleum ether were least effective at D2 level than other dilutions.

Table 21. Effect of dilution and extraction media on inhibitory property of *Plumbago rosea*

| Sl. No. | Treatment | Disease incidence * | Disease severity |
|---|---|---|---|
| 1 | Chloroform D1 | 65 (0.938) fg | 28 e |
| 2 | Chloroform D2 | 70 (0.991) g | 26 e |
| 3 | Chloroform D3 | 20 (0.464) bc | 6 abc |
| 4 | Chloroform D4 | 25 (0.522) c | 8 bc |
| 5 | Distilled water D1 | 60 (0.886) f | 26 e |
| 6 | Distilled water D2 | 15 (0.393) ab | 4 ab |
| 7 | Distilled water D3 | 10 (0.322) a | 2 a |
| 8 | Distilled water D4 | 35 (0.632) d | 10 c |
| 9 | Ethyl acetate D1 | 10 (0.322)a | 4 ab |
| 10 | Ethyl acetate D2 | 65 (0.938) fg | 27 e |
| 11 | Ethyl acetate D3 | 10 (0.322) a | 4 ab |
| 12 | Ethyl acetate D4 | 25 (0.522) c | 8 bc |
| 13 | Petroleum ether D1 | 10 (0.322) a | 2 a |
| 14 | Petroleum ether D2 | 50 (0.785) e | 24e |
| 15 | Petroleum ether D3 | 40 (0.685) d | 18d |
| 16 | Petroleum ether D4 | 35 (0.632) d | 15 d |
| 17 | Control | 80 (1.107) h | 49 f |

*Values in parenthesis are Arc Sin transformed

D1 – 5% (50 g dry sample per litre solvent)
D2 – 2% (20 g dry sample per litre solvent)
D3- 1% (10 g dry sample per litre solvent)
D4 – 0.1% (1 g dry sample per litre solvent)

A comparison of virus inhibitory properties of combinations of dilutions and extraction media of all medicinal plants were made (Table 22) and those showed 50 per cent or more reduction in disease incidence and 75 per cent or more reduction in disease severity over control were selected (Table 23). Various concentrations of petroleum ether extracts of *B. alba, G. glabra, T. populnea and P. rosea,* ethyl acetate extract of *P. rosea* and chloroform extract of *T. populnea* included in the study were expressing inconsistent results which may be due to uneven disbursement of active principles while diluting with water for making spray volume.

Table 22. Effect of dilution and extraction media on PMV inhibitory property of medicinal plant extracts

| Sl. No. | Treatment | Disease incidence (DI)* | Percent reduction in DI | Disease severity (DS) | Per cent reduction in DS |
|---|---|---|---|---|---|
| 1 | GIII D3 | 5 (0.161) a | 93.75 | 4 abc | 91.84 |
| 2 | PIVD3 | 10 (0.322) ab | 87.50 | 3 ab | 93.88 |
| 3 | PIII D1 | 10 (0.322) ab | 87.50 | 4 abc | 91.84 |
| 4 | PII D3 | 10 (0.322) ab | 87.50 | 2 a | 95.92 |
| 5 | PGIV D1 | 10 (0.322) ab | 87.50 | 2 a | 95.92 |
| 6 | PI D2 | 10 (0.322) ab | 87.50 | 3 ab | 93.88 |
| 7 | PGII D3 | 10 (0.322) ab | 87.50 | 2 a | 95.92 |
| 8 | GII D2 | 10 (0.322) ab | 87.50 | 4 abc | 91.84 |
| 9 | BIII D1 | 10 (0.322) ab | 87.50 | 2 a | 95.92 |
| 10 | PGIII D1 | 10 (0.322) ab | 87.50 | 4 abc | 91.84 |
| 11 | PGIII D3 | 10 (0.322) ab | 87.50 | 4 abc | 91.84 |
| 12 | PGII D2 | 15 (0.393) bc | 81.25 | 4 abc | 91.84 |
| 13 | PIV D2 | 15 (0.393) bc | 81.25 | 4 abc | 91.84 |
| 14 | PI D3 | 15 (0.393) bc | 81.25 | 5 abcd | 89.80 |
| 15 | TIV D3 | 20 (0.451) bcd | 75.00 | 10 abcdefg | 79.59 |
| 16 | GIV D3 | 20 (0.464) bcd | 75.00 | 8 abcdef | 83.67 |
| 17 | PIII D2 | 20 (0.451) bcd | 75.00 | 6 abcde | 87.76 |
| 18 | GIV D1 | 20 (0.451) bcd | 75.00 | 8 abcdef | 83.67 |
| 19 | GI D2 | 20 (0.464) bcd | 75.00 | 8 abcdef | 83.67 |
| 20 | TII D2 | 20 (0.464) bcd | 75.00 | 8 abcdef | 83.67 |
| 21 | TIII D3 | 20 (0.464) bcd | 75.00 | 5 abcd | 89.80 |
| 22 | PII D2 | 20 (0.464) bcd | 75.00 | 6 abcde | 87.76 |
| 23 | PGI D3 | 20 (0.464) bcd | 75.00 | 6 abcde | 87.76 |
| 24 | PGI D4 | 25 (0.522) cde | 68.75 | 8 abcdef | 83.67 |
| 25 | PGIII D4 | 25 (0.522) cde | 68.75 | 8 abcdef | 83.67 |
| 26 | TII D1 | 25 (0.522) cde | 68.75 | 7 abcdef | 85.71 |
| 27 | GII D1 | 25 (0.522) cde | 68.75 | 11 bcdefgh | 77.55 |
| 28 | GI D3 | 25 (0.522) cde | 68.75 | 7 abcdef | 85.71 |
| 29 | TI D2 | 30 (0.580) cdef | 62.50 | 12 cdefghi | 75.51 |
| 30 | GI D1 | 30 (0.580) cdef | 62.50 | 14 efghi | 71.43 |
| 31 | GIII D1 | 30 (0.580) cdef | 62.50 | 16 ghijk | 67.35 |
| 32 | BI D3 | 30 (0.580) cdef | 62.50 | 11 bcdefgh | 77.55 |
| 33 | TII D3 | 35 (0.632) defg | 56.25 | 19 ijklm | 66.22 |
| 34 | TIV D1 | 35 (0.632) defg | 56.25 | 13 defghi | 73.47 |
| 35 | PII D1 | 35 (0.632) defg | 56.25 | 11 bcdefgh | 77.55 |
| 36 | PGII D4 | 35 (0.632) defg | 56.25 | 10 abcdefg | 79.59 |
| 37 | BIV D2 | 35 (0.632) defg | 56.25 | 13 defghi | 73.47 |
| 38 | PGIV D4 | 35 (0.632) defg | 56.25 | 15 fghij | 69.39 |
| 39 | PI D1 | 40 (0.685) efgh | 50.00 | 15 fghij | 69.39 |
| 40 | PGIV D3 | 40 (0.685) efgh | 50.00 | 18 hijkl | 63.27 |

| Sl. No. | Treatment | Disease incidence (DI)* | Percent reduction in DI | Disease severity (DS) | Per cent reduction in DS |
|---|---|---|---|---|---|
| 41 | BII D3 | 45 (0.735) fghi | 43.75 | 20 ijklmn | 59.18 |
| 42 | GIII D2 | 45 (0.735) fghi | 43.75 | 28 nop | 42.86 |
| 43 | BIII D2 | 45 (0.735) fghi | 43.75 | 20 ijklmn | 59.18 |
| 44 | BI D1 | 50 (0.785) ghij | 37.50 | 20 ijklmn | 59.18 |
| 45 | BIII D3 | 50 (0.785) ghij | 37.50 | 22 jklmno | 55.10 |
| 46 | PGIV D2 | 50 (0.785) ghij | 37.50 | 24 klmnop | 51.02 |
| 47 | BII D2 | 50 (0.785) ghij | 37.50 | 20 ijklmn | 59.18 |
| 48 | GIV D2 | 55 (0.838) hijk | 31.25 | 24 klmnop | 51.02 |
| 49 | TIV D2 | 55 (0.838) hijk | 31.25 | 24 klmnop | 51.02 |
| 50 | PIV D1 | 60 (0.886) ijk | 25.00 | 18 hijkl | 63.27 |
| 51 | GII D3 | 60 (0.886) ijk | 25.00 | 24 klmnop | 51.02 |
| 52 | BIV D1 | 60 (0.886) ijk | 25.00 | 24 klmnop | 51.02 |
| 53 | PIII D3 | 60 (0.886) ijk | 25.00 | 25 lmnop | 48.98 |
| 54 | TIII D2 | 60 (0.886) ijk | 25.00 | 26 lmnop | 46.94 |
| 55 | TI D1 | 60 (0.886) ijk | 25.00 | 31 p | 36.73 |
| 56 | BIV D3 | 60 (0.886) ijk | 25.00 | 26 lmnop | 46.94 |
| 57 | PGII D1 | 60 (0.886) ijk | 25.00 | 26 lmnop | 46.94 |
| 58 | TI D3 | 60 (0.886) ijk | 25.00 | 24 klmnop | 51.02 |
| 59 | PGIII D2 | 65 (0.938) jkl | 18.75 | 27 mnop | 44.90 |
| 60 | BI D2 | 65 (0.938) jkl | 18.75 | 27 mnop | 44.90 |
| 61 | PGI D1 | 65 (0.938) jkl | 18.75 | 28 nop | 42.86 |
| 62 | BII D1 | 70 (0.991) kl | 12.50 | 30 op | 38.78 |
| 63 | TIII D1 | 70 (0.991) kl | 12.50 | 26 lmnop | 46.94 |
| 64 | PGI D2 | 70 (0.991) kl | 12.50 | 26 lmnop | 46.94 |
| 65 | Control | 80 (1.107) l | 0 | 49 q | 0 |

* Values in parenthesis are Arc Sin transformed

Abbreviations

B - *Basella alba*
G - *Glycyrrhiza glabra*
P - *Phyllanthus fraternus*
T - *Thespesia populnea*
PG - *Plumbago rosea*

I - Chloroform
II - Distilled water
III - Ethyl acetate
IV - Petroleum ether

D1: 100 g of plant sample extracted with 1 litre solvent or 50 g plumbago dry root powder extracted with 1 litre solvent

D2: 50 g of plant sample extracted with 1 litre solvent or 20 g plumbago dry root powder extracted with 1 litre solvent

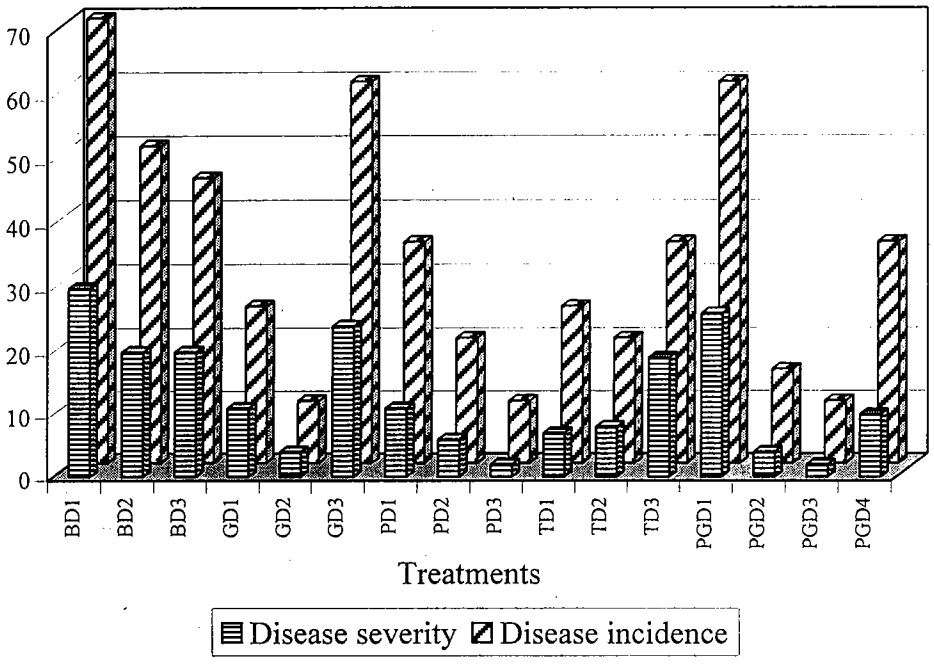
D3 : 20 g of plant sample extracted with1 litre solvent or 10 g plumbago dry root powder extracted with 1 litre solvent

D 4 : 1 g of plumbago dry root powder extracted with 1 litre solvent

Table 23. Solvent dilution combinations of medicinal plants with high PMV inhibitory property

| Sl. No. | Treatment details |
|---|---|
| 1 | 2% chloroform extract of *Basella alba* |
| 2 | 10% ethyl acetate extract of *B. alba* |
| 3 | 5% chloroform extract of *Glycyrrhiza glabra* |
| 4 | 2% chloroform extract of *Glycyrrhiza glabra* |
| 5 | 10% distilled water extract of *G. glabra* |
| 6 | 5% distilled water extract of *G. glabra* |
| 7 | 2% ethyl acetate extract of *G. glabra* |
| 8 | 5% chloroform extract of *Phyllanthus fraternus* |
| 9 | 2% chloroform extract of *Phyllanthus fraternus* |
| 10 | 10% distilled water extract of *P. fraternus* |
| 11 | 5% distilled water extract of *P. fraternus* |
| 12 | 2% distilled water extract of *P. fraternus* |
| 13 | 10% ethyl acetate extract of *P. fraternus* |
| 14 | 5% ethyl acetate extract of *P. fraternus* |
| 15 | 5% petroleum ether extract of *P. fraternus* |
| 16 | 2% petroleum ether extract of *P. fraternus* |
| 17 | 10% distilled water extract of *T. populnea* |
| 18 | 5% distilled water extract of *T. populnea* |
| 19 | 2% ethyl acetate extract of *T. populnea* |
| 20 | 1% chloroform extract of *Plumbago rosea* |
| 21 | 0.1% chloroform extract of *Plumbago rosea* |
| 22 | 2% distilled water extract of *P. rosea* |
| 23 | 1% distilled water extract of *P. rosea* |
| 24 | 0.1% distilled water extract of *P. rosea* |

Distilled water extract showed moderate rate of PMV inhibition at lower dilution in the case of *G. glabra* and *T. populnea* and higher dilution in the case of *P. rosea* . *Phyllanthus fraternus* showed inhibitory property at lower and higher dilutions (Fig.4). The inhibitory effect exhibited by one per cent water extract of *P. rosea* was same as that of the two per cent water extract of *P. fraternus*. Because of the higher PMV inhibitory property of *P. rosea* at higher dilutions, it was selected for further studies.




BD1 - *Basella alba* 10%
BD2 - *Basella alba* 5%
BD3 - *Basella alba* 2%
GD1 - *Glycyrrhiza glabra* 10%
GD2 - *Glycyrrhiza glabra* 5%
GD3 - *Glycyrrhiza glabra* 2%
PD1 - *Phyllanthus fraternus* 10%
PD2 - *Phyllanthus fraternus* 5%
PD3 - *Phyllanthus fraternus* 2%
TD1 - *Thespesia populnea* 10%
TD2 - *Thespesia populnea* 5%
TD3 - *Thespesia populnea* 2%

PGD1 - *Plumbago rosea* 5%
PGD2 - *Plumbago rosea* 2%
PGD3 - *Plumbago rosea* 1%
PGD4 - *Plumbago rosea* 0.1%

**Fig. 4. Inhibitory effect of distilled water extract of medicinal plants on PMV**

### 4.7.2 Effect of Different Parts and Temperature on Inhibitory Property of *Plumbago rosea* on PMV

The effect of plant parts of *P. rosea* was tested at different temperatures against PMV on pumpkin seedlings. Artificial inoculation of the virus was done 24 h after application of plant extracts. Observation was recorded four weeks after inoculation of the virus and disease incidence and disease severity were estimated as explained in 3.7.1. The observations revealed that there was significant difference in disease severity and no significant difference in disease incidence (Table 24).

Table 24. Inhibitory effect of parts of Plumbago at different temperatures against PMV

| Sl. No. | Treatment | Disease incidence* (DI) | Per cent reduction in DI | Disease severity** (DS) | Per cent reduction in DS |
|---|---|---|---|---|---|
| 1 | Root T1 | 20 (0.451) | 76.44 | 5 (2.121) ab | 81.41 |
| 2 | T2 | 40 (0.685) | 52.94 | 10 (3.146) abcd | 62.91 |
| 3 | T3 | 40 (0.685) | 52.94 | 9 (2.995) abcd | 66.64 |
| 4 | T4 | 40 (0.685) | 52.94 | 9 (2.995) abcd | 66.64 |
| 5 | T5 | 40 (0.685) | 52.94 | 9 (2.995) abcd | 66.64 |
| 6 | T6 | 60 (0.886) | 29.41 | 14 (3.732) bcde | 48.15 |
| 7 | Tender Leaf T1 | 65 (0.996) | 23.54 | 23 (4.795) de | 14.81 |
| 8 | T2 | 70 (1.017) | 17.65 | 19 (4.32) cde | 29.64 |
| 9 | T3 | 60 (0.886) | 29.41 | 17 (4.107) bcde | 37.11 |
| 10 | T4 | 35 (0.632) | 58.84 | 8 (2.828) abcd | 70.34 |
| 11 | T5 | 25 (0.503) | 70.51 | 7 (2.581) abc | 74.11 |
| 12 | T6 | 40 (0.685) | 52.94 | 10 (2.828) abcd | 62.91 |
| 13 | Mature Leaf T1 | 40 (0.685) | 52.94 | 9 (2.995) abcd | 66.69 |
| 14 | T2 | 30 (0.580) | 64.71 | 9 (2.995) abcd | 66.69 |
| 15 | T3 | 25 (0.503) | 70.51 | 5 (2.121) ab | 81.41 |
| 16 | T4 | 35 (0.632) | 58.84 | 8 (2.806) abcd | 70.34 |
| 17 | T5 | 50 (0.785) | 41.14 | 10 (3.146) abcd | 62.91 |
| 18 | T6 | 30 (0.580) | 64.71 | 6 (2.449) abc | 77.74 |
| 19 | Tender Stem T1 | 50 (0.785) | 41.14 | 16 (3.864) bcde | 40.74 |
| 20 | T2 | 45 (0.733) | 47.04 | 13 (3.536) bcde | 51.84 |
| 21 | T3 | 40 (0656) | 52.94 | 13 (3.461) bcde | 51.84 |
| 22 | T4 | 35 (0.632) | 58.84 | 8 (2.806) abcd | 70.34 |
| 23 | T5 | 35 (0.604) | 58.84 | 10 (3.096) abcd | 62.91 |

| Sl. No. | Treatment | Disease incidence* (DI) | Per cent reduction in DI | Disease severity** (DS) | Per cent reduction in DS |
|---|---|---|---|---|---|
| 24 | T6 | 20 (0.451) | 76.44 | 7 (2.639) abc | 74.11 |
| 25 | Mature stem T1 | 35 (0.604) | 58.84 | 11 (2.943) abcd | 59.24 |
| 26 | T2 | 30 (0.580) | 64.71 | 9 (2.995) abcd | 66.64 |
| 27 | T3 | 45 (0.733) | 47.04 | 14 (3.650) bcde | 48.15 |
| 28 | T4 | 10 (0.322) | 88.22 | 2 (1.414) a | 92.54 |
| 29 | T5 | 40 (0.685) | 52.94 | 8 (2.828) abcd | 70.34 |
| 30 | T6 | 60 (0.888) | 29.41 | 18 (4.236) cde | 33.31 |
| 31 | Control | 85 (1.178) | 0 | 27 (5.188) e | 0 |

*Values in parenthesis are Arc Sin transformed
**Values in parenthesis are square root transformed

T1 - 30°C
T2- 40°C
T3 - 50°C
T4 - 60°C
T5 - 70°C
T6 - 80°C

Among different treatments, mature stem extract at 60°C, showed the maximum reduction in disease incidence (88.22%) and severity (92.54%). This was followed by root extract at 30°C with 76.44 and 81.41 per cent reduction in disease incidence and severity. Mature leaf extract at 50°C also showed a similar lower trend in disease incidence and severity. Tender leaf extract at room temperature showed least effect which was on par with control.

Based on the disease severity rankings as shown in the table 24, treatments were grouped into four which can be compared easily.

Group 1 – Disease severity upto eleven per cent (rankings a, ab, abc and abcd) .

Group 2 –Disease severity of 12 to17 per cent (rankings bcde).

Group 3 –Disease severity of 18 to19 per cent (rankings cde) .

Group 4 –Disease severity of 23 per cent (ranking de) .

Treatments included in each group are given in Table 25.

Table 25. Combinations of plant part, temperature and PMV inhibitory property of *Plumbago rosea*

| Plant part | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| Roots | T1,T2,T3.T4,T5 | T6 | | |
| Tender leaf | T4,T5,T6 | T3 | T2 | T1 |
| Tender stem | T4,T5,T6 | T1,T2,T3 | | |
| Mature leaf | T1,T2,T3,T4,T5,T6 | | | |
| Mature stem | T1,T2,T4,T5 | T3 | T6 | |

T1 – 30°C
T2 – 40°C
T3 – 50°C
T4 – 60°C
T5 – 70°C
T6 – 80°C

From the table 25, it is evident that the root extract showed low disease severity at temperatures ranging from 30°C to 70°C whereas highest severity at 80°C (Fig. 5A).

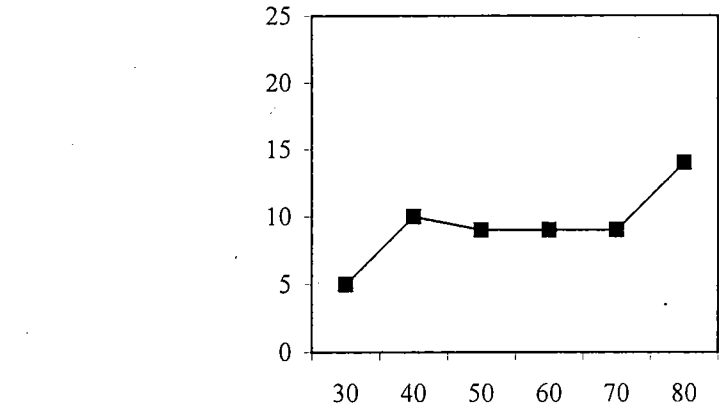
Tender leaf extract exhibited high PMV inhibitory property at 60-80°C with the maximum at 70°C. The effect was minimum at 30°C which showed significant increase at higher temperature of extraction (Fig.5B).

The inhibitory property expressed by mature leaf extract was on par with all temperatures tried with less percentage of disease severity (Fig.5C).

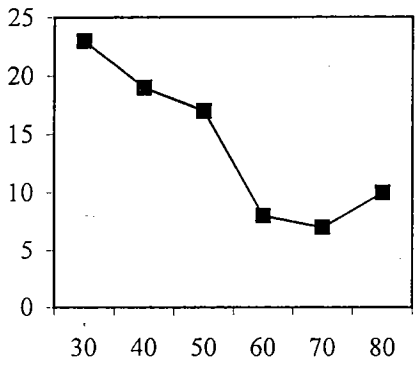
The tender stem exhibited high inhibitory property of the virus at 60-80°C with the maximum effect at 80°C. The minimum inhibitory property was at 30°C which was on par with the effect of 40°C and 50°C (Fig.5D).

For mature stem, extract at 60°C showed higher PMV inhibitory property as evidenced by low disease severity and which was on par with that of 30, 40 and 70°C (Fig.5E).

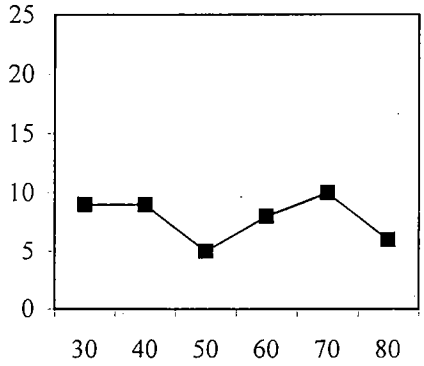
At 30°C root extract recorded the highest rate of inhibition and so the study was proceeded with only root extract of Plumbago.



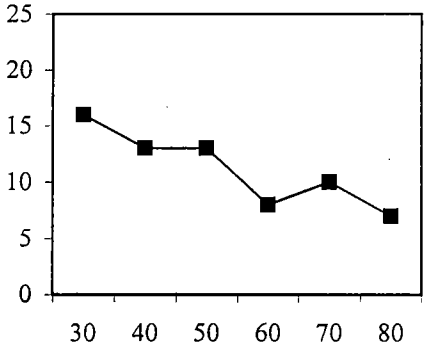

**A. Root**



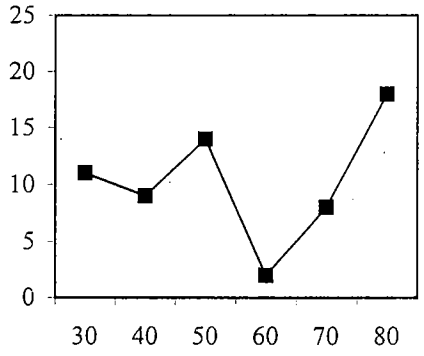

**B. Tender leaf**




**C. Mature leaf**




**D. Tender stem**




**E. Mature stem**

X axis - Temperature

Y axis - Disease severity

**Fig. 5. Effect of different parts of *Plumbago rosea* on PMV infection**

### 4.7.3 Effect of *Plumbago rosea* on the Vector *Aphis gossypii*

The effect of Plumbago water extract one per cent before acquisition feeding and inoculation feeding on *Aphis gossypii* was studied. Significant reduction in disease incidence and disease severity were observed due to the application of the extract.

Reduction in disease symptoms was more when Plumbago extract was applied before inoculation feeding than before acquisition feeding.

The Plumbago spray was more effective when the interval of spray and acquisition or inoculation of the virus was less i.e., 24 h treatment was more effective than 48 h treatment (Table 26, Fig.6).

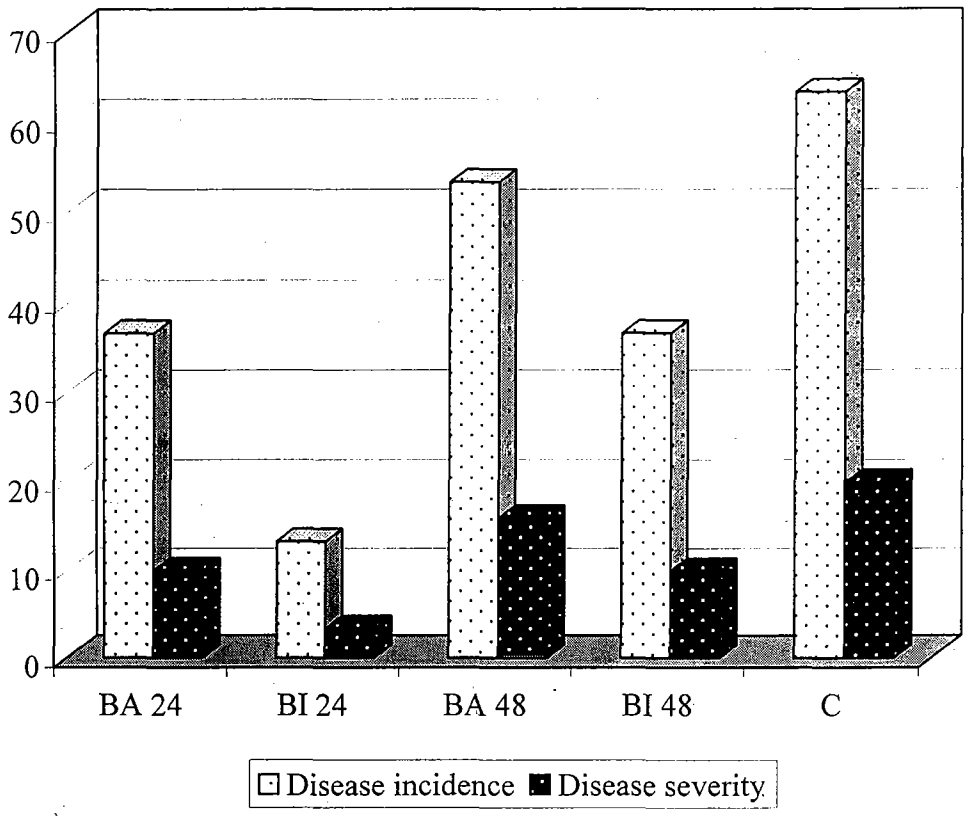
Table 26. Effect of Plumbago on acquisition and inoculation of PMV by vector, *Aphis gossypii*

| Treatment No | Treatment details | Disease incidence *(DI) | Per cent reduction in DI | Disease severity ( DS) | Per cent reduction in DS |
|---|---|---|---|---|---|
| T1 | 24 BAF | 36.667 (0.650) | 42.10 | 10.00 | 50.00 |
| T2 | 48 BAF | 53.333 (0.819) | 15.79 | 16.00 | 20.00 |
| T3 | 24 BIF | 13.333 (0.369) | 78.94 | 3.33 | 83.35 |
| T4 | 48 BIF | 36.667 (0.650) | 42.10 | 10.00 | 50.00 |
| T5 | Control | 63.333 (0.921) | 0 | 20.00 | 0 |
| CD | | 0.126 | | 3.37 | |

* Values in parenthesis are Arc Sin transformed

BAF - Before acquisition feeding
BIF - Before inoculation feeding

Among the four treatments, T3 was the most effective one. It reduced disease incidence by 78.94 per cent and disease severity by 83.35 per cent over control. The treatment T4 showed the same effect of T1. There was 42.1 per cent reduction in disease incidence and 50 per cent reduction in disease severity over




BA - Before acquisition BI - Before inoculation
C - Control

**Fig. 6. Effect of *Plumbago rosea* on acquisition and inoculation of PMV by vector *Aphis gossypii***

control. In the case of T2 , eventhough there was reduction in disease incidence and severity, the reduction was 15.79 and 20 per cent respectively.

## 4.8 ISOLATION OF PMV INHIBITORY PRINCIPLES OF *PLUMBAGO ROSEA*

Water extract of Plumbago was fractionated through silica gel column as explained in section 3.8. Qualitative tests were performed to observe the presence of active principles in each fraction (Table 27).

Table 27. Qualitative analysis of fractions of water extract of *Plumbago rosea*

| Fraction No. | Tests performed | | | | | |
|---|---|---|---|---|---|---|
| | Qualitative tests | | | | | Visual observation of fractions |
| | DRT | FT | VST | FCR | PPT | |
| F1 | - | - | - | - | - | No colour |
| F2 | - | - | - | - | - | Do |
| F3 | - | - | - | - | - | Do |
| F4 | - | - | - | - | - | Light brown |
| F5 | + | + | + | + | + | Do |
| F6 | - | - | + | + | + | Do |
| F7 | - | - | + | - | + | Yellow |
| F8 | - | - | + | - | - | Do |
| F9 | - | - | + | - | - | Do |
| F10 | - | - | + | - | - | Do |
| F11 | - | - | - | - | - | Light yellow |
| F12 | - | - | - | - | - | Do |

DRT - Dragendroff's reagent test
FT - Fehlings test
VST - Vanillin sulphuric acid test
FCR - Folin-ciocalteau reagent
PPT - Potassium permanganate test

\+ - Positive reaction
\- - Negative reaction

The alkaloid (Dragendroff's reagent) and carbohydrate (Fehlings test) were positive in only fifth fraction which produced orange red and brick red precipitates respectively. The fractions five to ten showed yellowish precipitate in vanillin sulphuric acid test indicated the presence of saponin. Folin - ciocalteau reagent showed positive test (blue colour) on fractions 5 and 6. Presence of

terpenes in fractions 5, 6 and 7 was recorded by aqueous potassium permanganate test which produced reddish brown precipitate.

Bioassay of the fractions

Each fraction of Plumbago was applied on ten pumpkin seedlings and disease was scored after four weeks of inoculation. The fractions 9 and 10 caused phytotoxicity (Table 28).

Table 28. PMV inhibitory property of fractions of *Plumbago rosea*

| Fraction No. | Disease incidence | Disease severity |
|---|---|---|
| 1 | 80 | 24 |
| 2 | 70 | 24 |
| 3 | 80 | 24 |
| 4 | 80 | 24 |
| 5 | 80 | 24 |
| 6 | 90 | 24 |
| 7 | 100 | 30 |
| 8 | 100 | 30 |
| 9<br>10 | Plants were damaged due to phytotoxicity | |
| 11 | 80 | 24 |
| 12 | 90 | 24 |
| Control | 100 | 34 |

From the above results it can be inferred that the individual fractions of Plumbago were not effective in inhibiting the disease just as in the case of total extract. It is seen that the fractions 9 and 10 at higher concentration was phytotoxic.

## 4.9 MANAGEMENT OF PUMPKIN MOSAIC USING *PLUMBAGO ROSEA*

### 4.9.1 Effect of Plumbago Spray on Pumpkin Mosaic

Field experiments were conducted to study the effect of Plumbago spray on artificially inoculated and naturally infected pumpkin seedlings.

### 4.9.1a On Artificially Inoculated Plants

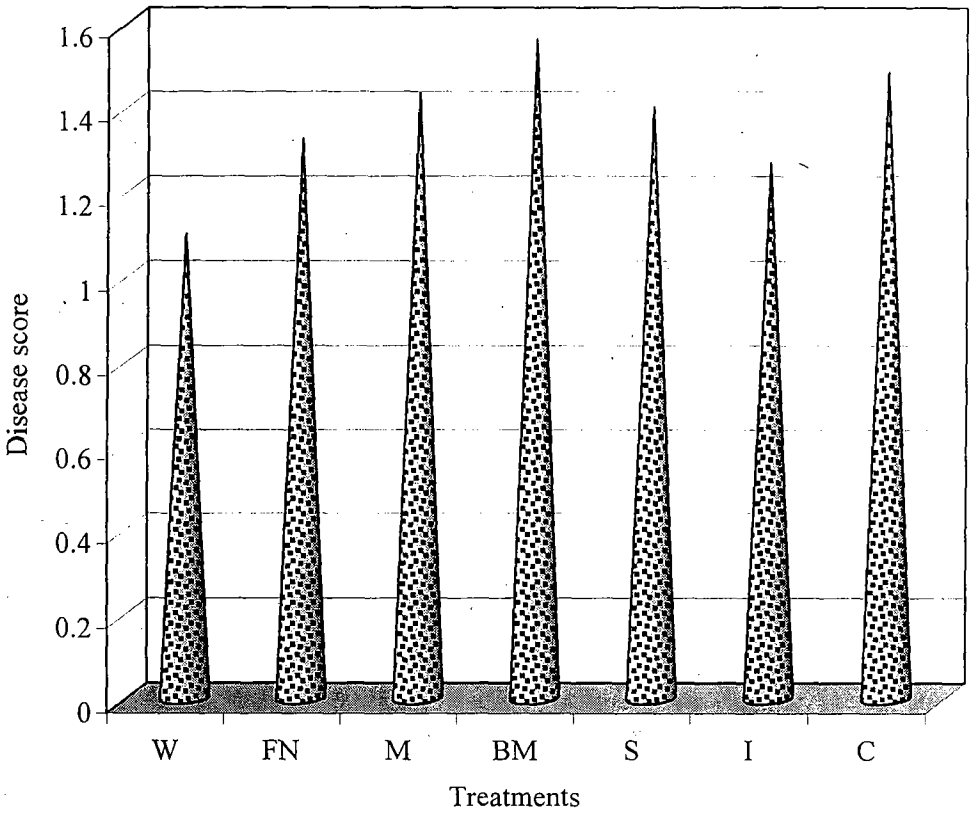
Effect of Plumbago on artificially inoculated plants was studied. The extract was applied as pre-inoculation spray and post inoculation sprays as described in section 3.9.1. Observations on the disease score was taken at weekly intervals for a period of 10 weeks and results are presented in Table 29 and Fig.7. The treatments T1 and T6 showed significant reduction in disease intensity over control which recorded mean disease scores of 1.1 and 1.27 respectively. The least effect was showed by treatment T4, which recorded a disease score of 1.56 and it was on par with control. Other treatments were not very effective and on par with control.

Table 29. Effect of Plumbago spray on disease intensity of artificially inoculated field grown pumpkin plants

| Treatment No. | Treatment details ( Spray) | Mean disease score over 10 weeks |
|---|---|---|
| T1 | Weekly | 1.10 (1.011)* |
| T2 | Fortnightly | 1.33 (1.135) |
| T3 | Monthly | 1.43 (1.172) |
| T4 | Bimonthly | 1.56 (1.218) |
| T5 | Single | 1.40 (1.157) |
| T6 | Insecticide | 1.27 (1.097) |
| T7 | Control | 1.48 (1.183) |
| CD ( P<0.05) | | 0.082 |

* Values in parenthesis are square root transformed

The effect of Plumbago application on the vegetative characters of the plants were recorded and presented in table 30 and Fig.8. At 30 DAP, the treatment T1 recorded a vine length of 1.5 m, which was significantly lower than other treatments which were on par with control. The maximum vine length of 2.33 m was showed by treatment T4. There was no significant difference in the number of branches and it was maximum for T2 (3.17) and minimum for control (2.5). Treatment T1 showed an internodal length of 6.87 cm which was significantly lower than all other treatments except T2 and they were on par. The




W - Weekly FN - Fortnightly M - Monthly BM - Bimonthly
S - Single I - Insecticide C - Control

**Fig. 7. Effect of *Plumbago rosea* on artificially inoculated pumpkin plants**

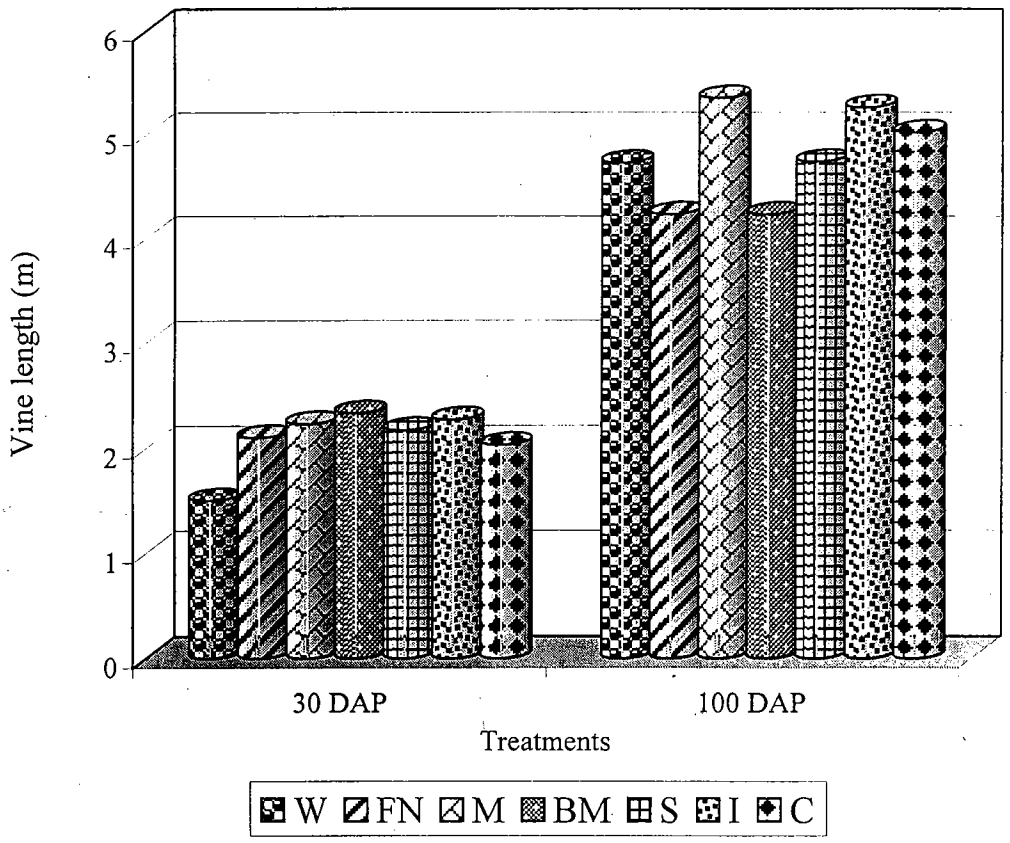
maximum internodal length was showed by T6 (10.6 cm) and it was on par with control. At 100 DAP, there was no significant difference between treatments in vine length, internodal length or number of branches.

Table 30. Effect of Plumbago on vegetative characters of pumpkin

| Treat-ment No | Treatment details ( sprays) | At 30 DAP | | | At 100 DAP | | |
|---|---|---|---|---|---|---|---|
| | | Length of vine (m) | Number of branches | Internodal length (cm) | Length of vine (m) | Number of branches | Internodal length (cm) |
| T1 | Weekly | 1.50 | 3.00 | 6.87 | 4.73 | 3.83 | 7.03 |
| T2 | Fortnightly | 2.10 | 3.17 | 7.33 | 4.23 | 4.50 | 9.07 |
| T3 | Monthly | 2.23 | 2.83 | 10.47 | 5.35 | 4.00 | 9.60 |
| T4 | Bimonthly | 2.33 | 3.00 | 10.27 | 4.23 | 3.33 | 10.50 |
| T5 | Single | 2.17 | 2.67 | 9.93 | 4.74 | 4.17 | 7.67 |
| T6 | Insecticide | 2.27 | 2.83 | 10.60 | 5.26 | 4.33 | 10.40 |
| T7 | Control | 2.03 | 2.50 | 9.00 | 5.02 | 4.50 | 7.73 |
| CD( P<0.05) | | 0.369 | NS | 2.040 | NS | NS | NS |

NS – Non significant

Weekly application of Plumbago produced significantly higher yield (1.77 kg/pl) and it was followed by T5 (1.56 kg/pl) which was on par with it. The yield was lowest for T6 (1.22 kg/pl) and it was on par with control (Table 31). Significant difference was also observed in the case of average fruit weight. T1 showed the maximum fruit weight (1.59 kg) followed by T5 (1.56 kg) and they were on par. There was no significant difference in other characters like number of fruits per plant, number of female flower per plant, per cent fruit set, days to first female flower anthesis and days to first fruit set.




W - Weekly FN - Fortnightly M - Monthly BM - Bimonthly
S - Single I - Insecticide C - Control

**Fig. 8. Effect of *Plumbago rosea* on vine length of pumpkin**

**Plate 10. Field experiment on management of pumpkin mosaic**

Table 31. Effect of Plumbago on yield characters of pumpkin

| Treat-ment No | Treatment Details (sprays) | Yield per plant (kg) | Fruits per plant (no.) | Average fruit weight (kg) | Female flowers per plant (no.) | Per cent fruit set | Days to first female flower anthesis | Days to first fruit set |
|---|---|---|---|---|---|---|---|---|
| T1 | Weekly | 1.77 | 1.11 | 1.59 | 8.67 | 11.57 | 89.00 | 93.00 |
| T2 | Fortnightly | 1.28 | 1.00 | 1.28 | 9.67 | 10.44 | 87.67 | 92.17 |
| T3 | Monthly | 1.42 | 1.19 | 1.19 | 9.00 | 11.20 | 87.50 | 91.67 |
| T4 | Bimonthly | 1.50 | 1.25 | 1.20 | 9.67 | 11.11 | 82.50 | 87.33 |
| T5 | Single | 1.56 | 1.00 | 1.56 | 10.00 | 10.28 | 78.00 | 88.83 |
| T6 | Insecticide | 1.22 | 1.11 | 1.10 | 9.33 | 11.11 | 80.83 | 87.67 |
| T7 | Control | 1.37 | 1.19 | 1.15 | 8.67 | 11.20 | 88.50 | 91.67 |
| CD (P<0.05) | | 0.237 | NS | 0.262 | NS | NS | NS | NS |

NS – Non significant

### 4.9.1b On naturally infected plants

Pumpkin seedlings were raised in net house and were transplanted to experimental plot and Plumbago sprays were given as described in section 3.9.1. The observations were taken on aphid population, mean incubation period and disease intensity and data are presented in table 32.

Table 32 Effect of Plumbago spray on disease intensity, aphid population and incubation period of naturally infected pumpkin plants

| Treatment | Treatment Details ( sprays) | Aphid* | Incubation period (days) | Disease score |
|---|---|---|---|---|
| T1 | Weekly | 20.97 (4.50)** | 60.22 | 1.31 (1.14) |
| T2 | Fortnightly | 15.65 (3.70) | 42.33 | 1.63 (1.28) |
| T3 | Monthly | 23.12 (4.77) | 41.00 | 1.80 (1.34) |
| T4 | Bimonthly | 14.27 (3.70) | 50.00 | 1.89 (1.37) |
| T5 | Single | 12.03 (3.46) | 41.33 | 2.08 (1.44) |
| T6 | Insecticide | 1.20 (1.41) | 57.00 | 2.11 (1.45) |
| T7 | Control | 19.24 (4.22) | 48.00 | 2.21 (1.49) |
| CD (P < 0.05) | | 1.810 | 7.078 | 0.115 |

* Mean aphid population over 10 weeks from 12 pumpkin plants @ three leaves per plant

** Values in parenthesis are square root transformed

The mean aphid population over ten weeks showed significant difference. Aphid population was least in T6 (1.20) and other treatments were not effective and they were on par with control. The maximum incubation period was shown by T1 (60.22 days) followed by T6 (57 days) and they were on par. All other treatments were on par with control and the minimum incubation period was recorded in T3 (Fig.9).

Plumbago spray significantly reduced disease intensity. T1 was significantly superior to all treatments. It showed a mean disease score of 1.31. It was followed by T2, T3 and T4. All these treatments were significantly superior to control, whereas other treatments viz., T5 and T6 were less effective and were on par with control (Fig.10).

### 4.9.2 Effect of Plumbago on PMV

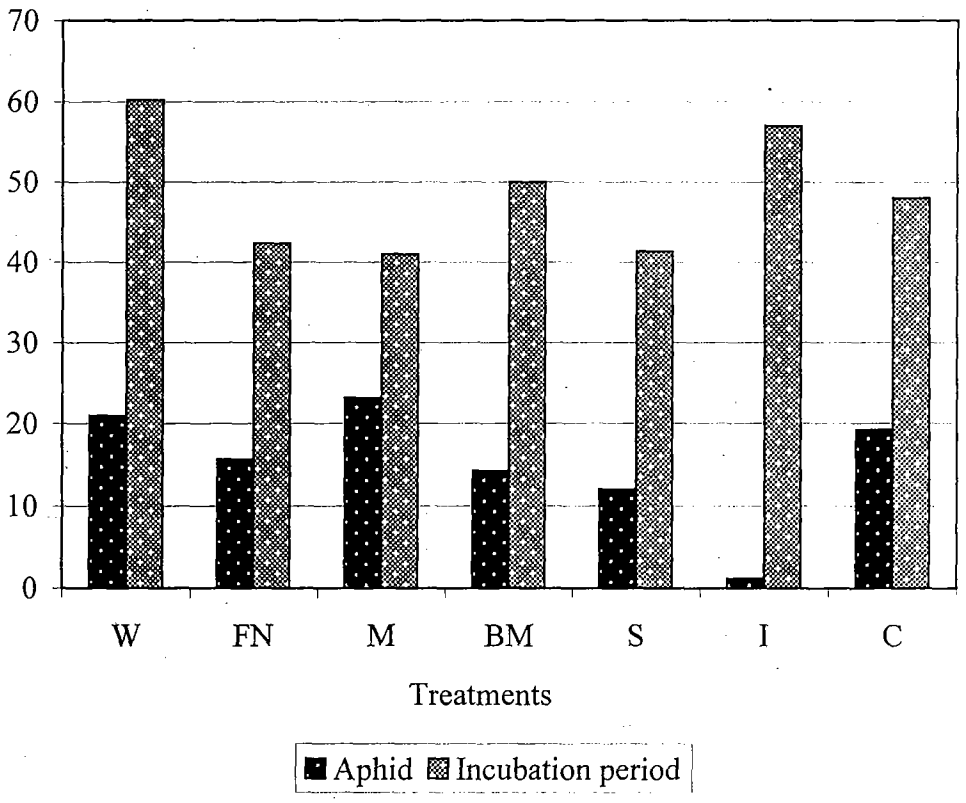
DAC – ELISA was performed using field samples of as explained in section 3.9.2, to compare virus concentration of treatments. The result is presented in table 33 and Fig.11.

The weekly Plumbago treated plant showed lowest O.D. value and so the lowest virus concentration. All the samples were found to be positive since the O.D. value is greater than the cut off value (0.94).

Table 33. Effect of Plumbago on PMV (DAC-ELISA test)

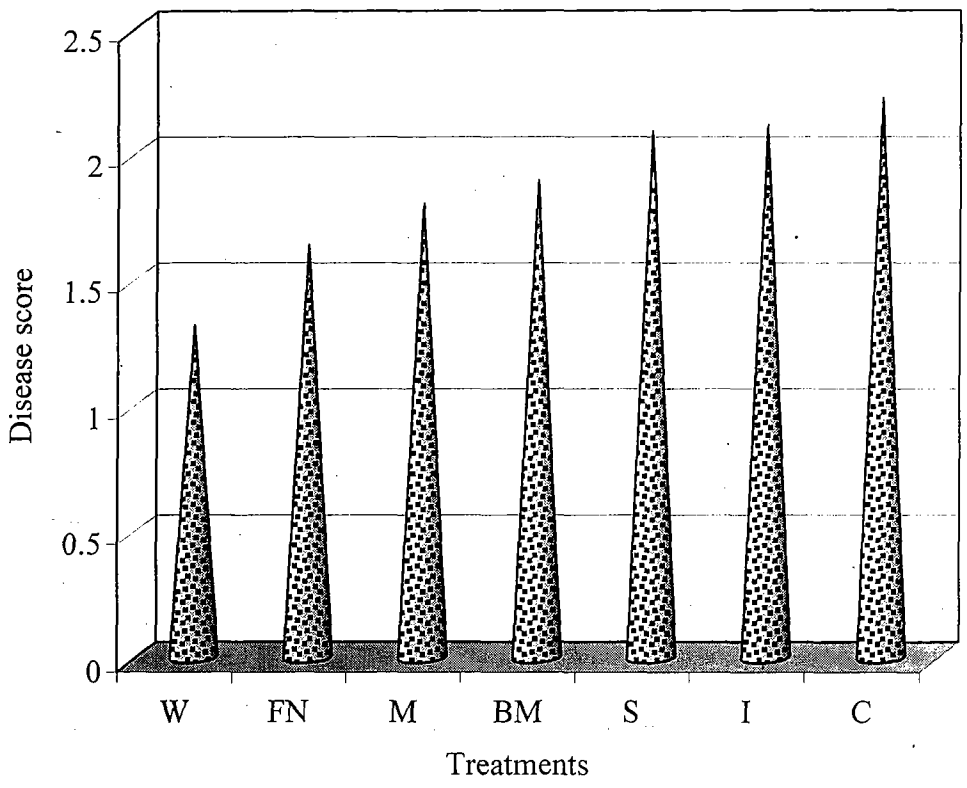
| Sl. No. | Treatment details / sprays | * OD Value at 450 nm |
|---|---|---|
| 1 | Weekly | 1.10 |
| 2 | Fortnightly | 1.20 |
| 3 | Monthly | 1.24 |
| 4 | Bimonthly | 1.22 |
| 5 | Single | 1.25 |
| 6 | Insecticide | 1.23 |
| 7 | Control | 1.23 |
| 8 | Negative Control | 0.71 |
| | Cut off value | 0.94 |

* Mean value of nine wells



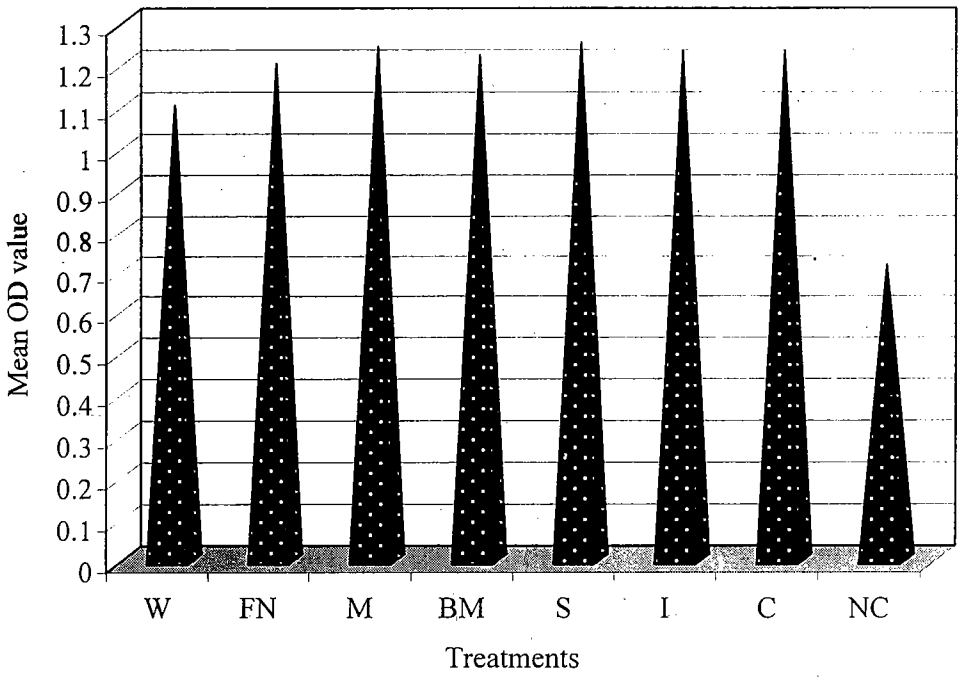

W - Weekly FN - Fortnightly M - Monthly BM - Bimonthly
S - Single I - Insecticide C - Control

**Fig. 9. Effect of *Plumbago rosea* on aphid population and incubation period**




W - Weekly FN - Fortnightly M - Monthly BM - Bimonthly
S - Single I - Insecticide C - Control

**Fig. 10. Effect of *Plumbago rosea* on naturally infected pumpkin plants**




W - Weekly FN - Fortnightly M - Monthly BM - Bimonthly
S - Single I - Insecticide C - Control NC - Negative control

**Fig. 11. Effect of *Plumbago rosea* on PMV (DAC-ELISA test)**

### 4.9.3 Biochemical Changes by Plumbago Spray

The effect of Plumbago on protein, phenol and chlorophyll contents and activity of enzymes viz., peroxidase, catalase, phenylalanine ammonia lyase (PAL) was studied (Fig.12).

Plumbago application significantly reduced protein content of pumpkin plants (Table 34) and reduction was more in infected plant (37.6%) than in healthy (20.6%). A significant reduction in protein content due to infection was also observed in treated and control plants.

Table 34. Influence of Plumbago extract on the protein content of pumpkin

| Treatment | Mean value of protein (mg $g^{-1}$) | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | |
| Plumbago applied | 5.16 | 7.16 | 27.93 | t value - 3.72* |
| Control | 8.27 | 9.02 | 8.30 | |
| Per cent variation over control | 37.60 | 20.60 | | |
| t value - 6.69** | | | | |

** Significant at 1% level
* Significant at 5% level

Plumbago application also significantly reduced phenolics in the plant. The reduction was more in infected plants (40.01%) than healthy ones (24.35%). Due to infection also phenol content showed a significant reduction and it was 17.02 per cent (Table 35 ).

Table 35. Influence of Plumbago extract on the phenolics of pumpkin

| Treatment | Mean value of phenolics (mg $g^{-1}$) | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | |
| Plumbago applied | 12.02 | 18.27 | 34.20 | t value - 8.22** |
| Control | 20.04 | 24.15 | 17.02 | |
| Per cent variation over control | 40.01 | 24.35 | | |
| t value - 10.84** | | | | |

** Significant at 1% level

The chlorophyll content (a, b and total chlorophyll) showed a decrease in trend by Plumbago application whereas chlorophyll a : b ratio was increasing by Plumbago application (Table 36). There was significant decrease in chlorophyll a:b ratio of infected plant eventhough total chlorophyll was more.

Table 36. Influence of Plumbago extract on the chlorophyll content of pumpkin

| Treatment | Chlorophyll content (mg $g^{-1}$ tissue) | | | | | | | | Per cent variation over healthy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Infected | | | | Healthy | | | | | | | |
| | Ch. a | Ch. b | Total ch | a/b | Ch.a | Ch.b | Total ch | a/b | a | b | Total ch | a/b |
| Plumbago applied | 1.40 | 0.61 | 2.01 | 2.29 | 1.53 | 0.47 | 1.67 | 2.60 | 8.50 | 31.18 | 20.36 | 11.92 |
| Control | 1.77 | 0.82 | 2.59 | 2.15 | 1.32 | 0.51 | 1.88 | 2.58 | 34.09 | 60.78 | 41.53 | 16.67 |
| Per cent variation over control | 20.90 | 25.61 | 22.39 | 6.51 | 15.91 | 8.82 | 8.74 | 0.78 | t value (for comparison of infected over healthy) | | | |
| t value (for comparison of treatments) | | | | | 0.48 NS | 2.56 NS | 3.07 NS | 1.88 NS | 0.94 NS | 4.58 ** | 4.09 ** | 9.83 ** |

** Significant at 1% level
NS - Non significant

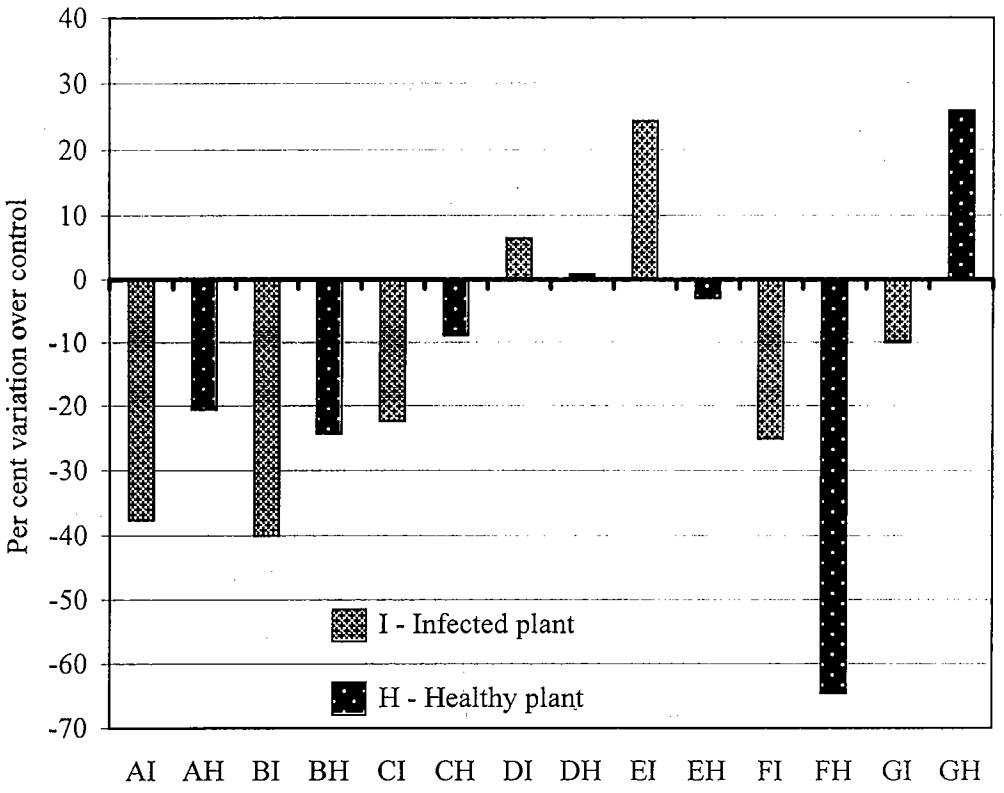
The peroxidase activity of Plumbago sprayed plant was higher than control plants (Table 37). The increase was more in infected plants (24.31%) than healthy ones (2.89%). A hike in peroxidase activity was also observed due to infection and it was significant.

Table 37. Influence of Plumbago extract on the peroxidase activity of pumpkin

| Treatment | Peroxidase activity (units $litre^{-1}$) | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | |
| Plumbago applied | 297.19 | 171.89 | 72.89 | t value - 5.10** |
| Control | 239.07 | 177.00 | 35.00 | |
| Per cent variation over control | 24.31 | 2.89 | | |
| t value - $1.44^{NS}$ | | | | |

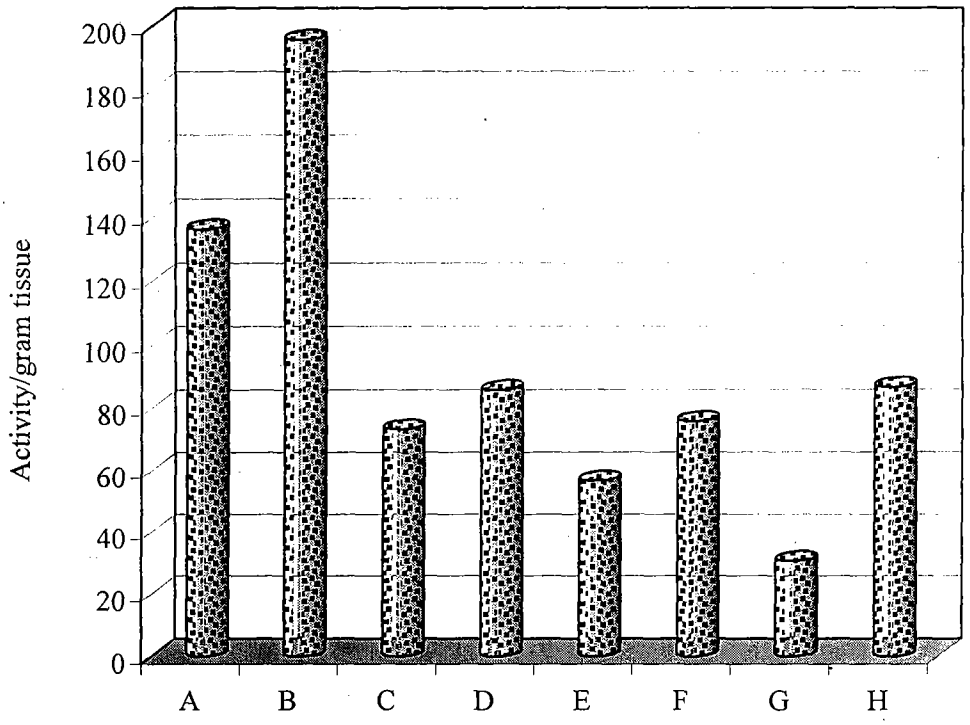
** Significant at 1% level
NS - Non significant

The catalase activity showed significant reduction due to Plumbago spray (Table 38 and Fig.13) and reduction was prominent in healthy plants (64.58%) than infected ones (25.13%).




A - Protein
B - Phenol
C - Total chlorophyll
D - Chlorophyll a:b
E - Peroxidase activity
F - Catalase activity
G - PAL activity

**Fig. 12. Biochemical changes in pumpkin due to Plumbago spray**




A - One month old infected plant
B - One month old healthy plant
C - Two months old infected plant
D - Two months old healthy plant
E - Plumbago sprayed infected plant
F - Control infected plant
G - Plumbago sprayed healthy plant
H - Control healthy plant

**Fig. 13. Effect of age, infection and Plumbago spray on catalase activity of pumpkin**

Table 38. Influence of Plumbago extract on the catalase activity of pumpkin

| Treatment | Catalase activity ($g^{-1}$) | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | |
| Plumbago applied | 56.60 | 30.60 | 84.96 | t value - $0.70^{NS}$ |
| Control | 75.60 | 86.40 | 12.50 | |
| Per cent variation over control | 25.13 | 64.58 | | |
| t value - 3.52* | | | | |

* Significant at 5% level
NS - Non significant

Plumbago application increased PAL activity of healthy plants whereas infected plants showed a decrease in trend (Table 39).

Table 39. Influence of Plumbago extract on the PAL activity of pumpkin

| Treatment | PAL - Absorbance value | | | |
|---|---|---|---|---|
| | Infected | Healthy | Per cent variation over healthy | |
| Plumbago applied | 0.614 | 0.943 | 34.88 | t value - $2.14^{NS}$ |
| Control | 0.713 | 0.749 | 4.80 | |
| Per cent variation over control | 9.90 | 25.90 | | |
| t value - $0.56^{NS}$ | | | | |

NS - Non significant

# *Discussion*

# 5. DISCUSSION

Pumpkin mosaic caused by pumpkin mosaic virus (PMV) was first reported from India by Hariharasubramanian and Badami (1964) and since then, the disease has been observed wherever pumpkin is grown (Shankar *et al.*, 1972; Singh, 1981). Wide spread occurrence of mosaic diseases poses big threat to pumpkin cultivation in Kerala and the farmers are reluctant to take up its cultivation which resulted in a meagre area of 3300 ha under this crop. Effective plant protection chemicals have not yet been developed for the control of viral diseases of plants under field conditions. Unfortunately all the available pumpkin varieties are susceptible to this disease (Babu, 1989). Hence, management of the disease by reducing its severity below the economic threshold level is the only solution.

Many biological products including medicinal plant extracts are reported to be effective against viral diseases of plants (Verma, 1985). The present study was aimed to find out the utility of medicinal plants to manage pumpkin mosaic disease. Investigations on symptomatology, transmission, host range, EM studies, serology and biochemical changes due to host pathogen interaction were carried out as a part of this study.

Symptomatology of PMV infection was studied on naturally infected field grown pumpkin plants and artificially inoculated pumpkin seedlings. Mottling of leaves with light and dark green patches, vein banding along the midrib and lateral veins, leaf malformation and blistering were the prominent initial symptoms observed on both naturally infected and artificially inoculated plants. Older leaves showed dark green blisters or raised surfaces. The veins and veinlets usually extended beyond the margins as narrow projections of varying sizes and resulted in filiform shaped or malformed leaves. Stunted growth, delayed flowering, reduced fruit setting and fruit malformation were also observed. On artificial inoculation of nine days old pumpkin seedlings, it was observed that initial symptoms appeared on the leaf produced seven days after inoculation in the form of mild vein clearing. Typical mosaic mottling with dark green and light green patches appeared on the

leaves produced later on. The vein chlorosis observed under artificial inoculation was not clearly seen under natural infection. Jaganathan and Ramakrishnan (1971) reported that sap inoculation of pumpkin plants produced initial symptoms in the form of mild vein clearing. Typical mosaic mottling with dark and light green patches were evident on subsequent leaves. Similar observation was made in the present study also. As per Shankar *et al.* (1972) first visible symptom was mosaic mottling of the leaves. They reported that the younger leaves unfold very late and showed complete chlorosis followed by green vein banding whereas the older leaves exhibited prominent dark green raised blisters. The symptoms described on younger leaves i.e., late unfolding and complete chlorosis were not observed in the present study whereas blistering of older leaves was observed frequently.

Ragozzino and Stefanis (1977), Singh (1981) and Almeida and Borges (1983) also reported similar symptoms from pumpkin infected with watermelon mosaic virus (WMV). Umamaheswaran (1985) and Louis (1994) also reported similar symptoms of PMV infection. Hence by comparing the symptomatology of the virus under study with earlier reports on WMV, it can be inferred that the isolate under study might be a strain of WMV.

PMV was found to be easily transmitted through sap. The rate of transmission varied slightly with buffers used for extraction. In the present study, when 0.1 M potassium phosphate buffer was used as extraction buffer the rate of transmission was upto 90 per cent, while Shankar *et al.* (1972) and Roy and Mukhopadhyay (1979) used citrate phosphate buffer and recorded a tranmission of upto 100 per cent. Umamaheswaran (1985) reported 85 per cent transmission of PMV when extracted with sterile distilled water. Louis (1994) recorded a transmission of 75 per cent when sodium phosphate buffer was used for extraction.

Age of the host plant plays an important role in virus infection. An experiment was conducted to study the effect of age of seedlings on infection and incubation period and found that infection occurred early in young seedlings than older ones (Table 4). The mean incubation periods of young seedlings (six and nine days old) were observed to be 9-10 days while that of 12 and 15 days old

seedlings were 14-15 days (Table 5, fig.1). Roy and Mukhopadhyay (1979) observed symptoms within 15 days of inoculation when inoculated on 10-15 days old seedlings. Umamaheswaran (1985) also reported that symptoms appeared after 14 days of inoculation of 10 days old pumpkin seedlings. These reports are in conformity with above findings.

PMV was reported to be transmitted by a number of insect vectors viz., *Aphis gossypii, A. nerii* and *Myzus persicae* (Nagarajan and Ramakrishnan, 1971), *M. persicae* and *Sitobion rosaeformis* (Shankar *et al.*, 1972) and *A. gossypii* (Bhargava, 1977). Umamaheswaran (1985) reported that highest percentage of insect transmission of the virus is by the vector, *A. gossypii* (65%) when compared to *A. craccivora* (25%). *Bemisia tabaci* was unable to transmit the virus. Louis (1994) reported transmission of PMV to the tune of 62.5 per cent with *A. gossypii*. In this study, a pre-acquisition fasting period of one hour, acquisition feeding of 30 min and inoculation feeding of 24 h were given to *A. gossypii*. The result of the present study is in conformity with that of earlier reports (Table 6).

PMV was also found to be transmitted through seeds to an extent of 5.08 per cent (mean incubation period five weeks) and one per cent (mean incubation period six weeks) in the case of seeds from artificially and naturally infected plants respectively. Singh (1981) reported the seed transmission of pumpkin strain of WMV. Mukhopadhyay (1985) reported that WMV can be transmitted through seeds but the efficiency of transmission is low. But Shankar *et al.* (1972) and Umamaheswaran (1985) reported that PMV is not transmitted through seeds. In the present investigation, PMV showed seed transmission with a high incubation period upto six weeks.

Host range studies are an important step not only in the identification of the virus but also throws light on the survival of virus inoculum in the field. The vectors can retain the virus only for a very short period of time after acquisition. Hence the virus is perpetuated mostly through the succession of crops during different seasons. The secondary spread within the field is accomplished by aphids or by mechanical contact. Karmakar (1982) traced perpetuation of a strain of

cucumber green mottle mosaic virus in pumpkin, through the succession of susceptible crops in the fields. In the area under observation, pumpkin, chilli and brinjal were grown during March-April; chilli, brinjal and bottle gourd were grown during July-August while bitter gourd and ridge gourd were grown during December-January. The virus was found to perpetuate through pumpkin, bottle gourd and bitter gourd from March-April to December-January.

When host range of PMV was studied on crops of important vegetable families viz., cucurbitaceae, fabaceae and solanaceae it was found that PMV showed systemic infection on winter squash (*Cucurbita maxima*), snake gourd (*Trichosanthes anguina*), water melon (*Citrullus vulgaris*), bitter gourd (*Momordica charantia*) and wild ash gourd, (*Benincasa hispida*) of family cucurbitaceae, chilli (*Capsicum annuum*) and datura (*Datura metel*) of family solanaceae, cowpea (*Vigna unguiculata*) and soybean (*Glycine max*) of family fabaceae. Bottle gourd (*Lagenaria siceraria*) and ridge gourd (*Luffa acutangula*) were found to be symptomless carriers of the virus. So cultivation of these crops in plots near to pumpkin should be avoided. Shankar *et al.* (1972) reported that PMV produced systemic mosaic symptoms on *Cucurbita pepo, Cucumis melo, C. melo* var *utilissima, L. siceraria* var round and long, *L. acutangula, C. vulgaris, M. charantia, B. hispida* and *T. anguina*. Umamaheswaran (1985) also reported systemic infection of the virus on water melon (*C. vulgaris*), bitter gourd (*M. charantia*) and snake gourd (*T. anguina*).

Webb and Scott (1965) divided WMV into two groups, WMV-I and WMV-II. WMV-I has a restricted host range and cannot infect plants other than members of cucurbitaceae whereas WMV II can infect plants both belonging to cucurbitaceae as well as fabaceae. Mukhopadhyay (1985) reported that WMV infect family fabaceae apart from cucurbitaceae. Tripathi and Joshi (1985) found that a virus producing leaf distortion symptom on pumpkin also infects *Vigna sinensis* cv. black turtle and showed mosaic mottling. Kirde and Lokhande (1996) reported that WMV potyvirus showed systemic infection on soybean, cowpea and radish within 10-12 days of inoculation.

Papaya (*Carica papaya*) a fruit crop of family caricaceae, cultivated throughout Kerala is reported to be affected by various viruses and acts as collateral host for spread of viruses. Papaya ring spot virus (PRSV) is an important virus of papaya crop is found to be serologically related to WMV (Milne and Grogan, 1969). Yeh *et al.* (1984) studied the host range of nine PRSV isolates and reported that it included host plants belonging to cucurbitaceae and caricaceae. In the present investigation, PMV produced typical ring spot like symptoms on the leaves of papaya seedlings, which was similar to that of PRSV. Serologically also PMV showed relation with PRSV (Plate 8C). Hence based on the literature on the serological relation of PRSV and WMV, it may be inferred that PMV might be a strain of WMV.

In the present study, chilli and datura belong to family solanaceae are recorded to be hosts of the virus and produced symptoms upon artificial inoculation. Sastry (1982) reported PVY infections in these crops. None of the earlier workers reported any members of solanaceae as the host of WMV. But in this study, the isolate of PMV was found to infect members of solanaceae also. Hence it might be possible that this particular isolate might be a new strain of WMV.

The shape and size of the particle is the basic requirement for the identification of any viruses. In the present study, when the particles were observed under EM, the size ranged between 700-800 x 11 nm and shaped as long flexuous rods. Makkouk and Lesemann (1980) identified the particle from mosaic infected pumpkin as WMV-1 which were flexuous rods, measuring 750-800 nm. Mukhopadhyay (1985) reported that the size of WMV-I particles varied from 700-800 nm and that of WMV II usually varied from 700-760 nm. The reports of Meer *et al.* (1987) and Singh (1987) were also in conformity with the results of the present study. Hence based on the literature on the morphology of the virus also, pumpkin mosaic virus could be considered as a strain of water melon mosaic virus.

Purification of the virus was carried out by extracting the young infected pumpkin leaves with 0.5 M borate buffer of pH 8.0 containing 0.2 M urea and 10

mM EDTA. Addition of urea in the extraction buffer is reported to be useful for the release of virus particles from the host constituents and to prevent particle aggregation. The use of high molar buffer is justified as the virus is unstable in low molarity buffer (Walkey, 1991). Shankar *et al.* (1972) studied the effect of pH of extraction media and reducing agents for infectivity and stability of PMV and found that at pH 8.0, the virus showed maximum stability and infectivity and EDTA was an effective reducing agent for the extraction. They used 0.1 M citrate phosphate buffer at pH 8.0 as the extraction buffer for purification of PMV.

Organic solvents used in clarification cause the larger host constituents to coagulate and retains the virus in solution. Vigorous stirring of the sap with the mixture of chloroform and carbon tetra chloride improved clarification. On centrifugation, the homogenate separated into three layers, the heaviest layer contained the organic solvent and plant materials such as chlorophyll and waxes, the next layer contained the bulk of plant debris and the lightest, the aqueous phase contained the virus.

The precipitation of virus using polyethylene glycol (PEG 6000) is generally adopted for PVY purification. PEG was added to a concentration of four per cent along with 1.75 per cent sodium chloride and the mixture was stirred for 30 min and allowed to stand at 4°C for 1 h. Then it was centrifuged at 8000 g for 12 min. The PEG pellet (containing the precipitated virus) was then resuspended in 0.5 M borate buffer with overnight slow agitation at 4°C. Sako *et al.* (1980a) described purification of WMV from pumpkin and pointed out that PEG precipitation was not advisable as it caused close aggregation of WMV particles and difficulty in subsequent dispersion. To overcome this problem, in the present study, overnight slow agitation was adopted. Further low speed centrifugation removed denatured protein and coagulated debris resulting from PEG treatment and left concentrated virus in solution.

The virus was finally pelleted from the aqueous phase by high speed centrifugation and the pellets were resuspended in 0.05 M borate buffer containing 5 mM EDTA.

The purified preparation was infectious and produced symptoms on inoculated pumpkin seedlings and the presence of virus particles in the purified preparation was confirmed under EM (Plate 7B)..

The purified virus preparation was then used to produce antiserum against PMV by injecting into one year old New Zealand White female rabbits and collecting the serum as described in section 3.5.2. The titre of antiserum was checked by conducting Ouchterlony's double diffusion test which produced a very thick band. This confirmed that the antiserum could be used for serodiagnosis.

Since the antiserum was produced from partially purified virus preparation it may contain antibodies against host plant proteins. So cross absorption was adopted to minimize the quantity of antibodies produced against healthy plant proteins and to increase specificity against the antigen.

Serological tests are useful tools in the identification of any unknown virus and to study the relationship between related virus isolates. These are based on binding capacity of antibodies with their own specific antigens. In order to test the serological relationship of PMV with potyviruses infecting other crops, Ouchterlony's double diffusion tests were performed with cross absorbed antiserum of PMV and purified antigens from these crops. Precipitin bands were observed between PMV antiserum and antigens from papaya, snake gourd, bitter gourd, ash gourd (wild), soybean, cowpea and chilli. As described earlier, these crops showed symptoms when PMV was inoculated. So serodiagnosis studies confirmed the results of host range studies discussed earlier. Serological relationship of PMV with snake gourd and bitter gourd was described by Umamaheswaran (1985).

Ash gourd (*Benincasa hispida*) act as a host and non host of PMV. This is because, if the cultivated ash gourd do not produce symptom, the wild ash gourd, with very small fruit, and small leaves produce severe mosaic mottle, vein banding and leaf distortion on PMV inoculation. Wild ash gourd also showed serological relation with PMV.

WMV and PRSV are serologically related (Milne and Grogan, 1969; Purcifull and Hiebert, 1979; Yeh *et al.*, 1984). In the present study, PMV showed serological relationship with PRSV and poty viruses of cowpea and soybean. Makkouk and Lesemann (1980) reported that bean yellow mosaic virus is serologically related to WMV-1. Members of cucurbitaceae and fabaceae are reported as hosts of WMV (Mukhopadhyay, 1985). So based on these evidences, PMV can be considered as a strain of WMV.

Chilli, a member of solanaceae family showed serological relationship with PMV. Review of literature revealed that this virus is not reported earlier in any of the host plants belonging to solanaceae. But in this study the virus produced symptoms on chilli by artificial inoculation and serological tests conducted also confirmed this. Hence it might be possible to group this isolate of PMV as a different strain of WMV.

The DAC-ELISA was performed using standardized dilutions of antiserum (1:250) and conjugate (1:4000) and samples from artificially inoculated plants of nethouse showing different levels of disease severity. A progressive increase in OD values was noticed with levels of infection of the tested samples (Table 10, Fig.2). So with DAC-ELISA apart from disease incidence, disease severity could also be explained. The cut off value was calculated as per the method of Clark (1981) which is the arithmetic mean of negative control sample plus two times its standard deviation. All the symptomatic plants showed OD value more than 0.94, the cut off value while the asymptomatic plants showed OD value more than that of negative control but less than the cut off value.

Widely accepted therapeutic agents have not yet been developed for the control of viral diseases of plants. Since all cultivated varieties of pumpkin showed susceptibility to PMV (Babu, 1989), management of the disease and thereby reduce crop loss below the economic threshold level is the only solution. Destruction of infected plants, use of disease free seed, judicious selection of crops and cropping pattern and control of vector population are the basic steps to manage this disease. Application of insecticides is recommended to manage viral diseases

by reducing vector population. Indiscriminate use of chemical plant protectants could cause adverse impact on ecological balance and the health of human and animals. Many biological products including medicinal plant extracts are effective against viral diseases. So an attempt has made to find out the utility of medicinal plant extracts to manage pumpkin mosaic disease.

*Basella alba* (leaf), *Glycyrrhiza glabra* (dried stem), *Phyllanthus fraternus* (whole plant), *Plumbago rosea* (dry root) and *Thespesia populnea* (leaf) were the medicinal plants used in the present investigation. Screening of PMV inhibitory property of extracts of all plants except plumbago at 10, 5 and 2 per cent using four solvents viz., chloroform, distilled water, ethyl acetate and petroleum ether was done on pumpkin seedlings. In the case of *P. rosea,* concentrations of 5, 2, 1 and 0.1 percentages were used since high concentration caused phytotoxicity. As per the report of Louis and Balakrishnan (1996), plant extracts were applied on eight day old pumpkin seedlings 24 h before PMV inoculation. The inoculated plants were scored after four weeks using 0-5 scale (Rajamony *et al.*, 1990) and severity index was calculated as described by Silbernagel and Jafri (1974).

High rate of PMV inhibition (>50% reduction in disease incidence and >75% reduction in disease severity over control) was observed for 24 treatments (Table 23).

Among the different solvents tested for extraction, chloroform extract was found to be suitable for PMV inhibitory principles from *B. alba, G. glabra, P. fraternus* and *P. rosea* but not for *T. populnea.* The efficacy of chloroform in extracting TMV inhibitory principles from cabbage (Varma, 1973) and *B. alba* (Ushari *et al.*, 1982) was already reported.

Distilled water extract of *G. glabra, P. fraternus, P. rosea* and *T. populnea* showed high rate of PMV inhibition. Louis and Balakrishnan (1996) reported the PMV inhibitory effect of water extract of these plants. Pandey and Bhargava (1984) reported the effect of aqueous extract of the leaves of fern *Ampelopteris prolifera* against TMV and CMV. TMV inhibitory property of aqueous extract of *Helianthus annuus* was reported by Johari *et al.* (1983).

Ethyl acetate was found to be suitable for extracting PMV inhibitory principles from *B. alba* and *P. fraternus* at high concentration and *G. glabra* and *T. populnea* at low concentration. Use of ethyl acetate for extracting virus inhibitory principles was not reported earlier.

Petroleum ether was found to be suitable for extraction of PMV inhibitory principles from *P. fraternus* but unsuitable for *B. alba, G. glabra, P rosea* and *T. populnea.* The unsuitability of petroleum ether in extracting virus inhibitory principles against TMV were reported from cabbage (Varma, 1973), *Boerhaavia diffusa* (Verma *et al.*, 1979) and *Datura metel* (Singh and Varma, 1981).

Distilled water was effective for extracting PMV inhibitory principles from *G. glabra, P. fraternus, P. rosea* and *T. populnea.* Of these plants, *P. fraternus* and *P. rosea* showed more inhibitory property at lower concentrations and *T. populnea* and *G. glabra* showed more effect at higher concentrations. The water soluble virus inhibitory principles present in these extracts might be responsible for PMV inhibitory property.

Dilutions of plant extracts showed PMV inhibitory property either to increase or decrease or remains constant (Table 23). In general, decrease of PMV inhibitory property with increase of dilution ie., high inhibitory property was recorded at lower dilutions of ethyl acetate extract of *B. alba* and distilled water extract of *G. glabra.* Similar effect was reported by Habib *et al.* (1984) in the case of *Euphorbia pulcherrima* against TMV and Saigopal *et al.* (1986) in the case of *Phyllanthus fraternus* against TMV, peanut green mosaic virus and tobacco ring spot virus. Barakat and Ghanem (1996) reported that aqueous extract of legume seeds showed inhibition of tobacco necrosis virus and activity decreased on dilution; suggesting that they act as virus inhibitors.

The present study also showed an increased inhibition of PMV with increased dilution of plant extract . This observation was noted in the case of chloroform extract of *B. alba, G. glabra, P. fraternus, P. rosea,* ethyl acetate extract of *G. glabra, T. populnea,* distilled water extract of *P. rosea , P. fraternus* and petroleum ether extract of *P. fraternus.* Sawant and Ambekar (1990) reported

that when compared dilutions of 1:10, 1:100, 1:1000 of *Datura metel* against safflower mosaic virus on *Chenopodium amaranticolor*, maximum inhibition of the virus was shown by highest dilution.

Virus inhibitory property of plant extracts at higher dilution were reported by earlier workers. Simons *et al.* (1963) reported that freeze dried preparation of crude juice of five succulent plants namely *Aconitum arboreum, Crassula falcata, Kalanchoe somaliensis, Mesembryanthemum caprohetum* and *Sedum nussbaumerianum* were effective at dilutions of 1:250 against TMV. Thakur and Sastry (1971) observed that the extracts from *Boerhaavia diffusa, Bougainvillea spectabilis* and *Mirabilis jalapa* inhibited petunia distortion strain of TMV on *Datura innoxia* and PVX on *C. amaranticolor* even at 1:1000 dilution. Ushari *et al.* (1982) reported that *B. alba* leaf extract showed TMV inhibitory property upto a dilution of $10^{-5}$.

Among different treatments (combinations of medicinal plant and extraction media) screened against PMV inhibitory property, water extract of *P. rosea* was the best at one per cent dilution. Since water extract was the most effective treatment and because of the simplicity in preparation of the extract, it has practical importance to the farming community.

Effect of Plumbago extract at lower concentration could be interpreted on the basis of any of the following hypothesis or their combination.

1. Cumulative effect of constituents at lower concentration.
2. Effect of optimum concentration of a single constituent or constituents.
3. Effect of lowering concentrations of inhibitors.

Study was conducted to test the effect of temperature on PMV inhibitory property of different parts of Plumbago with different levels of maturity. The water extract of different parts (1%) were prepared and heated to temperatures of 30, 40, 50, 60, 70 and 80°C for 10 min. and applied on pumpkin seedlings after cooling. Inoculation of the virus and scoring of the disease were carried out as explained in the section 3.7.1.

The result revealed that the optimum temperature of extraction of root was 30°C which may be extended upto 70°C. It could be presumed that the temperature of 80°C has a negative effect in the extraction of roots because of the denaturation or the inactivation of the active principle.

Temperature had a direct influence on the inhibitory action of tender leaf extract. The extract heated at 60°C showed high inhibitory effect than lower temperature and the activity remained the same upto 80°C. Tender leaf could be considered as the active part of a plant, in which metabolic processes are governed at a higher rate. Temperature might have influenced the metabolism and resulted the production of antiviral constituents at a faster rate at higher temperature than at lower temperature. Factors including enzyme activity may have a role in this process. Root extracts showed higher inhibitory property at 30°C supporting the effect of leaf extract at 60°C because root is the part of storage whereas the tender leaf is the part of active growth in which complex metabolic process are taking place.

Extract of mature leaves showed a different picture. There was high inhibitory effect at all tested temperatures. When the leaf matured, the biochemical constituents might have obtained a stable nature, property and specificity. Some of the constituents available at this stage are different from tender leaf stage which might have happened due to the degradation or conversion of the constituents into different form while heating whereas the constituents of matured leaf was stable due to constant nature and properties of the same.

Extract of tender stem also showed the same trend of PMV inhibitory property as that of tender leaf. Barakat and Ghanem (1996) reported that the inhibitory property of seed extract of *Phaseolus vulgaris* against tobacco necrosis virus was increased by heating.

Mature stem also showed a similar trend of root extract with high inhibitory property at temperature of 30-40°C and low inhibitory property at 80°C. Heating of the extract to 60°C exhibited maximum effect which was similar to that shown by tender leaf and stem.

From the above results, it can be presumed that all parts of *P. rosea* viz., tender leaf, mature leaf, tender stem, mature stem and root showed PMV inhibitory property and it varied with temperature (Table 24 and 25). Allen and Kahn (1957) reported TMV inhibitors from leaf, flower, polish, roots, culm and kernels of rice. Different parts of *Datura metel* viz., root, stem, leaf, flower parts and seeds were tested for TMV inhibitory property and found that all parts except roots prevented TMV lesions on *C. amaranticolor* (Singh and Varma 1981). Saigopal *et al.* (1986) reported that both leaf and root of *P. fraternus* contains peanut green mosaic virus inhibitor. Gupta and Naqvi (1991) reported the effectiveness of different parts (leaves, roots, green stem, bark, seeds, green fruits and rhizomes) of seven flowering plants namely *Aconitum heterophyllum, Azadirachta indica, Capsicum annuum, Chenopodium amaranticolor, Datura metel, Glycyrrhiza glabra* and *Rauwolfia serpentina* against brinjal necrotic mosaic virus on auberigines.

From the study, it was also found that inhibitor of *P. rosea* is also stable upto 70-80°C. Thermostable virus inhibitors were reported from plants by earlier workers. Kuntz and Walker (1947) reported that spinach leaf extract when heated to 125°C for 15 min is still completely inhibited cabbage mosaic virus. Juice of *Pelargonium hortorum* did not lose its inhibitory activity against brownspot strain of potato virus X (PVX) when heated to 100°C for 10 min (Blaszezak *et al.*, 1959). A thermostable PVY inhibitor was reported from *Acacia arabica* by Gupta and Raychaudhuri (1971a). Singh and Singh (1988) reported a heat stable inhibitor from the leaf and bark extract of *Azadirachta indica* against cowpea mosaic comovirus.

At the temperature of 30°C, the inhibitory property was high in root extract followed by mature leaf, mature stem and tender stem. The lowest inhibitory property was shown by tender leaf extract at this temperature. The results of this study revealed the efficacy of root extract at 30°C for inhibiting PMV (Table 24).

In the field condition, pumpkin mosaic is transmitted mainly through the vector, *Aphis gossypii.* The effect of Plumbago spray on vector transmission was studied by applying the extract before acquisition feeding and inoculation feeding.

The infected pumpkin plants were sprayed with the root extract of Plumbago and after 24 h and 48 h the aphids were allowed to feed on the plants in order to acquire the virus. These viruliferous aphids were transferred to healthy pumpkin seedling for testing the efficacy of the extract on the acquisition of virus. Similarly the viruliferous aphids were allowed to feed (inoculation feeding) on healthy pumpkin seedlings 24 h and 48 h after the spray in order to test the efficacy of the extract on inoculation of virus. The study revealed that Plumbago spray was more effective when given before inoculation feeding than before acquisition feeding (Table 26).

The effect of spray of plant extracts on vector transmission may either be due to a direct action on the vector or indirect action through host plant or virus. Due to the deterency of plant extracts, insects (vectors) may not be able to acquire or transmit virus and thereby reducing the disease symptom. In indirect action, the antiviral principles of plant extract increases the resistance of host plants and thereby suppress disease symptoms.

When Plumbago spray was given before acquisition feeding, there was reduction in mosaic symptom. Plumbago extract showed a repellent property to *A. gossypii* and this decreases with increase of time interval. So, Plumbago spray at 48 h before acquisition was less effective than 24 h.

When Plumbago spray was given on healthy pumpkin seedlings before inoculation feeding of *A. gossypii*, apart from repellency of the extract, indirect action through host plant may also be there and hence the application before inoculation was found to be more effective than application before acquisition. In this case also, with increase in time after application of plant extract, the effectiveness decreased. The loss of repellent property of plant extract with time may be the reason for this observation. Since the indirect action (through host plant) persist, there was disease suppression to a substantial level at 48 h also. Louis and Balakrishnan (1997) reported that *P. rosea* showed high rate of PMV inhibition when applied 3 h and 24 h before inoculation of the virus in pumpkin.

Hunter and Ullman (1992) studied the repellent property of RD-repelin to *Acyrthosiphon pisum* in which transmission of virus was not prevented but delayed symptom expression of zucchini yellow mosaic virus. According to Srinivasulu and Jeyarajan (1986) *Mirabilis jalapa* was more effective when applied one day before inoculation than five days before. As in the case of *Mirabilis jalapa*, the activity of Plumbago was also found to decrease with time after application. Mariappan *et al.* (1988) reported the mortality of *Nephotettix virescens* on plants sprayed with Karanj (*Pongamia pinnata*), Mahua (*Maduca longifolia* var *latifolia*) and Pinnai (*Calophyllum ionophyllum)* as 100 per cent by four days after spraying compared to 60 per cent survival on control. Host mediated resistance might be responsible for leaf hopper mortality and low per cent rice tungro infection, even after four days of application.

In all the above conditions, it was observed that spraying of plant extract at shorter intervals was the most effective. This highlights the importance of spraying plant extract in the field at shorter intervals.

Fractionation through silica gel column (60-120 mesh) was carried out to isolate inhibitory principle of water extract of Plumbago. Fractionation of Plumbago extract showed separation of components as evidenced by qualitative tests and in each fraction closely related components were seen in concentration. Individual fractions had no positive result as in the case of application of Plumbago extract.

The above observations throw light on the hypothesis that lower concentrations of plant extract have more inhibitory property. It is upholding the importance of cumulative effect of Plumbago extract at lower concentration, effect of optimum concentration of single constituent or constituents and effect of lowering concentration of inhibitors.

Field experiments were conducted to study the effect of Plumbago in managing pumpkin mosaic by applying one per cent aqueous extract on inoculated and healthy pumpkin seedlings at different intervals viz., weekly, fortnightly, monthly, bimonthly and single spray.

In the experiment with PMV inoculated plants, weekly spray showed significant reduction in disease intensity over control (Table 29). It can be inferred that the weekly spray of Plumbago is imparting resistance to pumpkin plants which check disease severity. Murthy *et al.* (1981) studied the effect of *B. alba, B. spectabilis* and *A. indica* on tobacco against TMV. They applied one per cent extract of these plants at 10 days interval on inoculated field grown tobacco seedlings. Among the plant extracts, *B. alba* and *B. spectabilis* showed low disease intensity than control eventhough disease incidence was 100 per cent.

Verma *et al.* (1992) reported the efficacy of *Clerodendrum aculeatum* against soybean mosaic virus when given as pre inoculation spray on soybean plants. There was significant suppression of infection when virus was inoculated after giving six sprays of *C. aculeatum*.

Weekly application of Plumbago extract showed reduction in growth of plant during the first month, which had no effect on growth and development during later stage (Table 30). This can be compared with farmer's practise of hardening of crop plants by limiting irrigation. Farmers adopt this practice to make the plants resistant to pests and diseases. In the case of present study also Plumbago extract might have influenced in a similar way which was unfavourable for the multiplication of virus.

The yield of plants sprayed with Plumbago at weekly intervals was more than that of other treatments (Table 31). Eventhough the insecticide treated plant showed low disease severity, its yield was low.

Murthy *et al.* (1981) reported that spray of all the three leaf extracts produced significantly superior yield compared to control and among these *B. alba* was found to be the best in inhibiting TMV and enhancing green leaf yield of tobacco. The beneficial effect of *C. aculeatum* spray in advancing flowering and enhancing yield and nodulation of inoculated soybean plants was also reported by Verma *et al.* (1992).

In the experiment with healthy pumpkin seedlings, weekly, fortnightly, monthly and bimonthly spray of Plumbago showed significant reduction in disease intensity over control, eventhough weekly spray was the best (Table 32).

Sandhu *et al.* (1996) reported the efficacy of sorghum and thuja extracts in inhibiting squash mosaic comovirus infection of summer squash. The application of *C. aculeatum* (CA) as soil amendment, as spray or both showed delayed incidence of tomato yellow leaf curl virus infection in tomato. Enhanced yield and reduced disease severity was also reported in CA treated plants (Baranwal and Ahmed; 1997).

Weekly application of insecticides showed low aphid population and high incubation period and high disease score. So it was evident that insecticides had effect on vector control and thereby delaying the onset of disease, it had no effect on suppression of disease. Eventhough the pumpkin plants infested with aphids, weekly spray of Plumbago extract showed minimum disease score and maximum incubation period. This might be an indication of the ability of Plumbago to make the plant resistant to PMV infection or symptom suppression.

Murthy and Nagarajan (1986) reported that *Pithecolobium dulce* twig extract and *Peltophorum ferrugenium* leaf extract when sprayed thrice in nursery and mainfield of tobacco, significantly reduced disease incidence and severity. The sprayed plants exhibited only mild symptoms at flowering stage by which time the majority of picking of green leaf were completed.

The Plumbago extract induced resistance in host plants and provided protection against PMV infection and showed low disease severity. The antiviral effect persisted only for short period after spray which was evident from the effect of spray interval studies.

The DAC-ELISA, the sensitive test for virus detection was performed using samples from field experiment with natural infection. The weekly Plumbago sprayed plants showed lowest virus concentration as indicated by lowest OD value. All the samples were positive for PMV as their OD value were greater than the cut off value (Table 33).

Biochemical changes due to infection, growth of plant and Plumbago application were analysed. Biochemical constituents viz., protein, phenol and chlorophyll and activity of peroxidase, catalase and phenylalanine ammonia lyase (PAL) were studied in the above situations.

PMV was inoculated on nine days old pumpkin seedling and these were analysed at one month and two months stage to study the changes due to infection. (Fig.3). Samples from weekly sprayed plants of field experiment with natural infection were analysed to study the effect of Plumbago spray (Fig.12).

The protein content of pumpkin plants showed a decrease in trend after inoculation of the virus (Table 11). Spraying of Plumbago extract also showed reduction in protein content both in healthy and infected plants (Table 34). The reduction in the protein content of sprayed healthy plant may be due to the effect of Plumbago which might have influenced the plant in the resistance/suppression of symptom by controlling the production of protein which may be favouring the multiplication of virus. Protein content of the infected plant which received Plumbago spray showed lower rate than the sprayed healthy plant. Ghosh and Mukhopadhyay (1979b) reported that the buffer soluble protein of WMV infected *C. moschata* plants showed an increase when analysed ten days after inoculation, decrease after 40 days of inoculation and further increase at 70 days after inoculation over uninoculated control plants. Singh and Suhag (1982) also reported reduction in protein content of mung and urd bean leaves infected with yellow mosaic virus, urd mosaic virus I and II and leaf crinkle virus.

The protein estimation studies revealed that the decrease in protein content favoured the control of symptom expression. The reduced growth of Plumbago sprayed plant at one month stage was an indication of lowering the protein content. It may be a similar process of hardening adopted by traditional farmers.

Chlorophyll a, b and total chlorophyll of PMV infected plant showed an increase and chlorophyll a : b ratio showed a decrease over healthy plants (Table 13). The virus may not be infecting the chloroplast proteins which resulted further growth and development of infected plant in an erratic manner. But

chlorophyll a : b ratio of infected plant is lower than healthy plant which revealed that overall photosynthetic efficiency was affected by infection. This gives an evidence of selectivity of the virus for the substrate protein of host plant. An increase in photosynethtic activity of TMV infected tobacco plants was reported (Owen, 1957; Zaitlin and Hesketh, 1965). Radhika (1999) reported an increase in total chlorophyll content in a resistant cowpea variety Co.6, due to infection of cowpea aphid borne mosaic virus. By Plumbago application decrease in total chlorophyll and increase in chlorophyll a : b ratio was observed and change was more in infected plant than healthy plant (Table 36). The increased chlorophyll a : b ratio of infected plants, showed better photosynthetic efficiency even in the infected state which may contribute to better yield in Plumbago sprayed plant.

The higher activity of catalase enzyme (195.71 $g^{-1}$) in one month old seedling than the two months old seedling (85.55 $g^{-1}$) may be related to general metabolism of the plant which contribute in growth and development (Table 15). Same trend was observed in infected plant at a lower rate, which was less than 50 per cent of healthy plant. So, rate of reduction in catalse activity was an indication of infection in accordance with stage of maturity. Khatri and Chenulu (1970) showed reduction in catalase activity in resistant variety of cowpea due to cowpea mosaic virus infection. Mali *et al.* (2000) also reported a significant decrease in the content of catalase enzyme with the increasing levels of yellow mosaic virus infection in moth bean (*Vigna aconitifolia*).

Interestingly, Plumbago extract spray showed very low catalase activity which may also be an indication of the influence of extract in minimizing the metabolic rate which in turn influence resistance/suppression of virus infection. Eventhough the catalase activity of Plumbago extract sprayed infected plant was more than healthy plant, it was less than control which again express the effect of Plumbago spray in controlling disease (Table 38). The reduction in catalase activity due to Plumbago spray in young plants brought under similar condition to that of mature ones may be unfavourable for virus multiplication and spread. This may be the principle behind the hardening of the crop by limiting irrigation

followed by traditional farmers. Prasad (1986) also made similar observation by *C. aculeatum* spray.

Due to infection, plant expressed high peroxidase activity (Table 14) and the same trend was observed by treatment of Plumbago also (Table 37). Here again, the cell disruption by the PMV followed by increase in activity of peroxidase was occurred which may be a struggle of the plant for protection from virus. In the study, mosaic symptom was recorded at a lower rate in plants sprayed with Plumbago at weekly intervals which may be due to the production of antipathogen compounds in advance for which the higher peroxidase activity was contributing.

An increase in peroxidase activity of cowpea due to infection of cowpea mosaic virus was reported by Khatri and Chenulu (1970). Verma *et al.* (1987), Verma and Prasad (1988) also reported higher peroxidase activity due to application of *C. aculeatum, C. fragrans* and *B. diffusa* on *Cyamopsis tetragonoloba* which induced antiviral state against sunhemp rosette virus. The role of peroxidase enzyme in virus resistance was reported by Rongle *et al.* (1998). They reported that WMV-2 resistant muskmelon cultivars exhibited twice peroxidase activity than susceptible cultivars.

PAL activity showed an increasing trend with age of plant which indicated the role of PAL in the metabolism of plant. Due to infection, the PAL activity showed a decrease in trend (Table 16). The same trend was observed in Plumbago sprayed plants. The influence of Plumbago extract on PAL activity was substantially high in the sprayed healthy plant (0.943 U) which was 25.9% greater than sprayed plant (Table 39). This showed the ability of the extract in increasing resistance of healthy plant by modifying its metabolism similar to that of mature plants.

Infected plant showed lower concentration of phenolics due to higher peroxidase activity as indicated in table 12. Due to infection of cowpea aphid borne mosaic virus, cowpea plants showed lower phenolics content (Radhika, 1999). Rajan (1985) observed a negative correlation between resistance and total

phenol content in tomato due to increased rate of oxidation of phenolics. Kosuge (1969) reported that peroxidases catalyse the oxidation of phenolics into more toxic quinones. PAL is one of the key enzyme involved in the metabolism of phenols (Vidyasekaran, 1993). Due to infection of PMV, decreasing trend was seen for the activity of PAL and so also the phenolics. The spray of Plumbago extract showed lower concentration of phenolics and higher peroxidase activity. In this context, it can be inferred that pumpkin plants contain higher concentration of phenolics which was the substrate for the enzyme to produce an end product to control/suppress the virus infection.

Further studies on active principles of *P. rosea* will be useful for commercial use of the extract for PMV control. Application of the Plumbago extract in various easier and economical ways can be tried so as to make it as a more farmer friendly method for management of pumpkin mosaic disease.

# *Summary*

# 6. SUMMARY

Studies were conducted on pumpkin mosaic virus disease commonly occurring in pumpkin plants (*Cucurbita moschata* Duch ex Poir.) in Kerala.

The symptoms appeared as typical mosaic mottling with light green and dark green patches. The leaves showed prominent vein banding and malformation. Occasionally dark green blisters appeared on the leaves. The infected plants produced weak branches with reduced flowering and fruiting. The fruits were reduced in size and often malformed.

Transmission studies showed that pumpkin mosaic virus (PMV) was transmitted mainly through sap (85-90%) and vector, *Aphis gossypii* (60-65%). The effect of age of pumpkin seedling viz., 6, 9, 12 and 15 days on sap transmission of PMV was studied and found that infection occurred early in young seedlings compared to older ones. The mean incubation period for symptom expression of six and nine days old seedlings were 9-10 days whereas 12-15 days old seedlings were 14-15 days.

PMV also showed seed transmission to an extent of 5.08 per cent (mean incubation period 5 weeks) and one per cent (mean incubation period 6 weeks) when tested the seeds of artificially inoculated and naturally infected plants respectively.

The results of host range studies showed that PMV produced systemic infection on water melon, snake gourd, bitter gourd, winter squash and wild ash gourd of family cucurbitaceae, chilli and datura of family solanaceae, soybean and cowpea of family fabaceae and papaya of family caricaceae. Bottle gourd and ridge gourd were found to be symptomless carriers of the virus.

Electron microscopic study showed that PMV particles were flexuous elongated rods of 700-800 x 11 nm in size.

PMV antiserum was raised in rabbit using partially purified virus and the antiserum was used for serodiagnosis studies..

Ouchterlony's double diffusion tests revealed serological relationship of PMV with potyviruses infecting snake gourd, bitter gourd, wild ash gourd, cowpea, soybean, chilli and papaya.

Based on symptomatology, particle morphology, host range and serological relations, it can be inferred that this isolate of PMV is a strain of water melon mosaic virus(WMV).

Direct antigen coating-enzyme linked immunosorbent assay technique was standardized for PMV detection using PMV antiserum and horse radish peroxidase conjugated anti rabbit immunoglobulins of goat.

Biochemical analysis of PMV infected plant showed that the level of protein and phenol were reduced whereas that of chlorophyll was increased due to infection. The peroxidase activity of infected plant showed an increase whereas that of catalase and phenylalanine ammonia lyase (PAL) showed a decrease.

The effect of extraction media and dilution on PMV inhibitory property of five medicinal plants viz., *Basella alba* (leaf), *Glycyrrhiza glabra* (dried stem), *Phyllanthus fraternus* (whole plant), *Plumbago rosea* (root) and *Thespesia populnea* (leaf) showed that chloroform was suitable for extracting PMV inhibitory principles from *B. alba, G. glabra, P. fraternus and P. rosea.* Distilled water was effective for all plants except *B. alba.* Ethyl acetate was unsuitable for *P. rosea* and suitable for other plants. Petroleum ether was found to be effective for extracting PMV inhibitory principles only from *P. fraternus*.

The effect of dilution on PMV inhibitory property of medicinal plants showed that higher dilution have more inhibitory effect than lower dilutions in the case of chloroform extract of *B. alba, G. glabra, P. fraternus* and *P. rosea* and ethyl acetate extract of *G. glabra, T. populnea*, distilled water extract of *P. rosea, P. fraternus* and petroleum ether extract of *P. fraternus* whereas lower dilutions

showed more inhibitory effect in the case of ethyl acetate extract of *B. alba* and *P. fraternus*, distilled water extract of *G. glabra, P. fraternus* and *T. populnea.*

The high PMV inhibitory effect of one per cent distilled water extract of *P. rosea* found out from the study has practical importance to the farming community because of the simplicity in preparation of the extract.

The effect of plant parts at different temperatures on PMV inhibitory property of *P. rosea* revealed that all parts showed inhibition of the virus and it varied with temperature. The high inhibitory effect was exhibited at temperature of 30-70°C and 30-80°C by roots and mature leaf extracts respectively. Extract of mature stem showed this effect at 30-40°C and 60-70°C. The inhibitory effect was high at 60-80°C than at 30-°C in the case of extracts of tender leaf and stem.

Studies on effect of Plumbago on vector showed that application before inoculation was more effective than before acquisition and effect was decreased with increase of time interval between spray and inoculation or acquisition.

The fractionation of Plumbago water extract through silica gel column to isolate active fractions with virus inhibitory property showed that individual fractions were not effective while plant extract as such showed the virus inhibitory property.

The effect of Plumbago water extract one per cent for managing pumpkin mosaic showed that the weekly spray decreased disease severity and increased yield of artificially inoculated pumpkin plants. This also delayed disease incidence and reduced disease severity of pumpkin seedlings.

The enzyme, protein, chlorophyll and phenolic estimation of plants treated with Plumbago extract revealed that Plumbago spray induced resistance in host plants by altering the host cell metabolism which is unfavourable for virus multiplication. The DAC-ELISA results also showed lower concentration of the virus in Plumbago treated plants.

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* Originals not seen

# *Appendices*

## Appendix – I

## BUFFERS

### 1. Potassium phosphate buffer 0.1Molar pH 7.2

Solution A – One Molar dipotassium hydrogen phosphate ($K_2HPO_4$).
Molecular weight (M.W) = 174.18 g. Dissolved 174.18 g $K_2HPO_4$ in one litre distilled water.

Solution B – One Molar dihydrogen potassium phosphate ($KH_2PO_4$).
M.W = 136.09 g. Dissolved 136.09 g $K_2HPO_4$ in one litre distilled water.

Mixed 71.7 ml of solution A and 28.3 ml of solution B and diluted to 1000 ml with distilled water.

### 2. Borate buffer 0.1 Molar, pH 8.8

Solution A – 0.2 Molar boric acid ($H_3BO_3$).
MW = 61.85 g. Dissolved 12.37 g boric acid in one litre distilled water.

Solution B – 0.05 Molar borax ($Na_2B_4O_710H_2O$)
MW = 381.38 g. Dissolved 19.069 g borax in one litre distilled water.

Mixed 50 ml of solution A and 30 ml of solution B and made upto 200 ml.

### 3. Borate buffer, 0.5 Molar, pH 8.0

Solution A - 2 Molar boric acid ($H_3BO_3$)
MW = 61.83 g . Dissolved 123.70 g boric acid in one litre distilled water.

Solution B - 0.5 Molar borax ($Na_2B_4O_710H_2O$)
MW = 381.38 g. Dissolved 190.69 g borax in one litre distilled water.

Mixed 25 ml A and 2.45 ml B and diluted to 200 ml.

### 4. Phosphate buffer 0.1 M, pH 7.0

Solution A – One Molar disodium hydrogen phosphate ($Na_2HPO_4$).
MW = 141.96 g. Dissolved 141.96 g $Na_2HPO_4$ in one litre distilled water.

Solution B – One Molar dihydrogen sodium phosphate ($NaH_2PO_4\ 2H_2O$).
MW = 156.01 g. Dissolved 156.01 g $NaH_2PO_4\ 2H_2O$ in one litre distilled water.

Mixed 57.7 ml of solution A and 42.30 ml of solution B and made upto one litre with distilled water.

## Appendix II

## REAGENTS FOR QUALITATIVE TESTS

### 1. Dragendroff's reagent

Solution A - Dissolved 8 g in Bismuth subnitrite [$Bi(No_3)3.5H_2O$] in 20 ml concentrated nitric acid

Solution B – Dissolved 27.2 g potassium iodide in 50 ml distilled water

Mixed solutions A and B and allowed to stand to precipitate $KNO_3$. Made the supernatant to 100 ml with distilled water.

This reagent was used for testing alkaloids.

### 2. Fehlings reagent

Solution A – Dissolved 34.65 g copper sulphate in distilled water and made up to 500 ml.

Solution B – Dissolved 125 of potassium hydroxide and 173 g potassium sodium tartrate (Rochelle salt) in distilled water and made upto 500 ml.

Mixed solution A and B in equal proportions. This reagent was used for testing carbohydrates.

### 3. Vanillin sulphuric acid reagent

To one gram vanillin, added 4 ml concentrated sulphuric acid and 16 ml ethanol with cooling. This was prepared fresh and used for testing saponin.

### 4. Aqueous potassium permanganate solution

Dissolved two gram potassium permanganate in one litre distilled water. This reagents was used for testing terpene.

## Appendix – III

## WEATHER DATA AT VELLANIKKARA, THRISSUR

Field Experiment I

| Period | Temperature (°C) | | Humidity (%) | | Wind $kmh^{-1}$ | Sunshine (h) | Rain (mm) | Evaporation (mm) |
|---|---|---|---|---|---|---|---|---|
| | Maximum | Minimum | Morning | Evening | | | | |
| 03.09.01 to 09.09.01 | 31.8 | 23.2 | 91 | 57 | 3.2 | 8.2 | 2.5 | 4.7 |
| 10.09.01 to 16.09.01 | 31.7 | 23.5 | 90 | 61 | 30 | 6.6 | 0 | 4.1 |
| 17.09.01 to 23.09.01 | 30.8 | 23.5 | 88 | 69 | 3.4 | 4.5 | 10.2 | 4.3 |
| 24.09.01 to 30.09.01 | 28.8 | 22.3 | 94 | 80 | 3.5 | 1.6 | 193.4 | 2.3 |
| 01.10.01 to 07.10.01 | 30.1 | 23.1 | 93 | 72 | 2.9 | 3.6 | 47.6 | 3.3 |
| 08.10.01 to 14.10.01 | 30.1 | 23.0 | 91 | 74 | 3.1 | 3.6 | 94.6 | 3.3 |
| 15.10.01 to 21.10.01 | 31.0 | 22.8 | 90 | 70 | 3.2 | 5.1 | 56.2 | 21.5 |
| 22.10.01 to 28.10.01 | 31.5 | 22.3 | 91 | 69 | 3.4 | 5.6 | 62.1 | 3.6 |
| 29.10.01 to 04.11.01 | 31.8 | 23.5 | 90 | 62 | 2.8 | 7.0 | 11.3 | 3.8 |
| 05.11.01 to 11.11.01 | 31.2 | 23.6 | 92 | 68 | 3.2 | 5.0 | 30.6 | 3.2 |
| 12.11.01 to 18.11.01 | 31.4 | 23.2 | 91 | 67 | 4.1 | 5.0 | 74.3 | 3.3 |
| 19.11.01 to 25.11.01 | 31.7 | 23.3 | 73 | 53 | 7.6 | 8.1 | 0 | 5.4 |
| 26.11.01 to 02.12.01 | 31.2 | 22.1 | 72 | 52 | 6.3 | 7.6 | 0 | 4.7 |
| 03.12.01 to 09.12.01 | 31.5 | 22.3 | 72 | 48 | 8.8 | 9.4 | 0 | 5.5 |
| 10.12.01 to 16.12.01 | 31.1 | 18.9 | 67 | 37 | 7 | 9.2 | 0 | 5.6 |
| 17.12.01 to 23.12.01 | 30.9 | 23.2 | 74 | 53 | 12.8 | 7.4 | 0 | 6.2 |
| 24.12.01 to 31.12.01 | 32.1 | 23.8 | 76 | 51 | 11.8 | 8.1 | 0 | 6.6 |

Field Experiment II

| Period | Temperature (°C) | | Humidity (%) | | Wind $kmh^{-1}$ | Sunshine (h) | Rain (mm) | Evaporation (mm) |
|---|---|---|---|---|---|---|---|---|
| | Maximum | Minimum | Morning | Evening | | | | |
| 10.09.02 to 17.09.02 | 30.7 | 22.9 | 92 | 63 | 3.7 | 8.7 | 0 | 4.4 |
| 18.09.02 to 23.09.02 | 31.3 | 22.8 | 91 | 59 | 3.6 | 8.3 | 0 | 4.3 |
| 24.09.02 to 30.09.02 | 32.5 | 22.7 | 90 | 55 | 3.6 | 8.2 | 21.5 | 4.4 |
| 01.10.02 to 07.10.02 | 32.2 | 23.3 | 89 | 67 | 3.2 | 5.7 | 51.0 | 3.7 |
| 08.10.02 to 14.10.02 | 29.3 | 23.1 | 93 | 89 | 3.5 | 2.1 | 0 | 1.8 |
| 15.10.02 to 21.10.02 | 30.1 | 23.0 | 92 | 74 | 2.8 | 4.3 | 25.1 | 3.5 |
| 22.10.02 to 28.10.02 | 31.5 | 23.5 | 92 | 66 | 3.1 | 6.0 | 9.9 | 3.3 |
| 29.10.02 to 04.11.02 | 31.6 | 23.3 | 84 | 61 | 5.1 | 5.7 | 33.4 | 4.4 |
| 05.11.02 to 11.11.02 | 31.8 | 23.5 | 90 | 66 | 3.4 | 4.7 | 8.7 | 3.1 |
| 12.11.02 to 18.11.02 | 31.2 | 23.2 | 83 | 59 | 5.1 | 4.7 | 9.4 | 4.2 |

# MANAGEMENT OF PUMPKIN MOSAIC USING SELECTED MEDICINAL PLANT EXTRACTS

By

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## ABSTRACT OF THE THESIS

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# ABSTRACT

"Management of pumpkin mosaic using selected medicinal plant extracts" was undertaken in the College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur during 1998-2002. Selection of suitable medicinal plant having antiviral property to pumpkin mosaic virus (PMV), isolation of the inhibitory principle present in the medicinal plant, management of pumpkin mosaic using the plant extract and partial purification and serological studies of PMV were the objectives of the study. Symptomatology, transmission, host range and electron microscopy of PMV were also studied.

The symptomatology of pumpkin mosaic was studied by observing the development of symptoms in naturally infected as well as artificially inoculated pumpkin plants. The symptoms appeared as typical mosaic mottling with light and dark green patches in the leaf lamina. This was followed by blistering and malformation of leaves into filiform or some other shapes and resulted in reduction of leaf area. The infected plants were stunted, flowered very sparingly with less number of female flowers and reduced fruit setting. The fruits were often malformed . The virus could be transmitted mainly through sap and vector, *Aphis gossypii.* The virus found to be weakly transmitted also through seeds. The inoculation of PMV on host plants of four families viz., cucurbitaceae, solanaceae, fabaceae and caricaceae showed systemic infection in water melon, snake gourd, bitter gourd, winter squash, wild ash gourd (cucurbitaceae) chilli, datura (solanaceae), soybean, cowpea (fabaceae) and papaya (caricaceae). Electron microscopic studies revealed the presence of flexuous virus particles (700-800 x 11 nm) in infected leaf sample.

Antiserum was raised against the virus and used for serodiagnostic work. The antiserum showed serological relationship with poty viruses infecting snake gourd, bitter gourd, wild ash gourd, cowpea, soybean, chilli and papaya. DAC–ELISA procedure was standardized and used for detection of PMV from pumpkin.

The inhibitory property of extracts of five medicinal plants namely *Basella alba, Glycyrrhiza glabra, Phyllanthus fraternus, Plumbago rosea* and *Thespesia populnea* were studied against PMV by pre-inoculation application on pumpkin seedlings. The medicinal plant extracts were prepared using different extraction media viz., chloroform, distilled water, ethyl acetate and petroleum ether at different dilutions. The inhibitory property varied with extraction media and dilution used.

The PMV inhibitory property of different parts of Plumbago viz., tender leaf, mature leaf, tender stem, mature stem and root were studied at different temperatures and found that all parts showed inhibitory property which varied with temperature. The root extract which showed the maximum inhibitory property at 30°C (near to room temperature) was used for further studies.

The effect of Plumbago on vector transmission was studied by applying the extract before acquisition feeding and inoculation feeding of *Aphis gossypii,* the vector of PMV. Application before inoculation feeding was found to be effective than acquisition feeding and the inhibitory effect decreased with time after application.

Distilled water extract of Plumbago was separated through silica gel column to isolate the inhibitory fraction and found that individual fractions were not effective as plant extract as such against PMV.

The inhibitory effect of Plumbago water extract one per cent, on artificially inoculated and healthy pumpkin seedlings was tested by weekly, fortnightly, monthly, bimonthly and single application. Weekly spray was effective to reduce disease severity of artificially inoculated and naturally infected pumpkin seedlings. Delayed incidence of the mosaic and enhanced yield of infected plants was also resulted due to weekly spray of the extract.

Enzyme, protein, chlorophyll and phenolics estimation revealed that Plumbago extract spray favoured the resistance and thereby suppression of symptoms. The DAC-ELISA of field samples showed the lower concentration of the virus in Plumbago treated plants.