EVALUATION OF PLANT EXTRACTS AGAINST COWPEA (VIGNA UNGUICULATA L.) APHID-BORNE MOSAIC VIRUS

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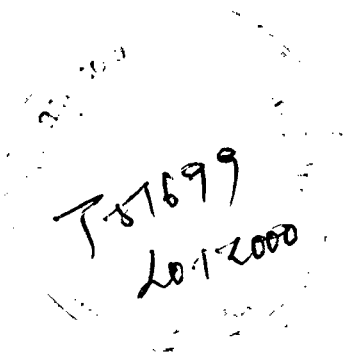
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ABSTRACT

EVALUATION OF PLANT EXTRACTS AGAINST COWPEA (*VIGNA UNGUICULATA* L.) APHID-BORNE MOSAIC VIRUS

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ABSTRACT

The plants affected by cowpea aphid-borne mosaic virus (CABMV) showed severe mosaic, dark green vein-banding, veinal chlorosis, inter-veinal chlorosis, blistering and stunting in glass-house conditions. The virus culture was maintained in glass-house by frequent inoculations. The symptoms appeared in 5-8 days.

The management of CABMV by plant extract given as Pre-inoculation treatment (PrIT) with phytoextracts of *Clerodendrum inerme*, *Jatropha curcas* and *Ocimum sanctum* had 100% inhibitory effect upto 16 days in glass-house condition. The Mix-inoculation treatment (MIT) with phytoextract of *Boerhaavia diffusa* (leaf), *Datura metal* (flower), *Duranta plumerie*, *Eucalyptus globulus*, *Phyllanthus fraternus*, *Phasalis minima*, *Solanum nigrum* and *Tribulus tenestris* had 90% inhibitory effect upto 6 days. Whereas in Post-inoculation treatment (PoIT) with phytoextract of

Clerodendrum inerme and *Ocimum sanctum* had 90% inhibitory effect upto 6 days. The ELISA results showed no reaction upto 6 days and within 12 and 18 days mild and strong reactions respectively, were observed in PrTT but visible symptoms were not observed. The repeated number of sprays (more than one) of phytoextract of *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* had 100% inhibition upto 24, 20 and 22 days, respectively.

The physical properties of phytoextracts viz., dilution, solvents, pH and temperature were studied. The dilution of *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* 1:100, 1:100 and 1:500 respectively were inhibitory. The solvents for *Jatropha curcas*, *Ocimum sanctum* were Water, Ethanol and Methanol and for *Clerodendrum inerme* Water and Ethanol were used. The extracts of *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* were found to be active at pH 6, 7 and 8, respectively. Similarly, *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* were active upto 55, 55 and 60 °C temperature, respectively. The percentage of protein present in leaf of *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* were 6.90, 3.85 and 5.95, respectively.

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CERTIFICATE

This is to certify that the thesis entitled **EVALUATION OF PLANT EXTRACTS AGAINST COWPEA (*VIGNA UNGUICULATA* L.) APHID-BORNE MOSAIC VIRUS** submitted by **Shri Dilipkumar Jayantibhai Patel** in partial fulfilment of the requirement for the award of the degree of **Master of Science (Agriculture)** in **Plant Pathology** of the Gujarat Agricultural University is a record of bonafide research work carried out by him under my personal guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

PLACE : ANAND
DATE : 20 March, 1999

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INTRODUCTION

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) belonging to the order Fabales, family Leguminosae, genus *Vigna* is one of the principal pulses in common use in India. Cowpea or Choli is reported to have originated in Southernmost region of Africa. Worldwide area under cowpea was 5.6 million ha with an annual grain production of 2.7 million tonnes during 1996. (Quim, 1997). In Gujarat, the area, production and productivity of cowpea was 23,600 hectares, 19,900 metric tonnes and 845 kg/ha, respectively (Anonymous, 1997-98).

Cowpea is of major importance to the livelihood of millions of relatively poor people in developing and less developed countries as food, animal feed and income from the production of this crop. In fresh form the young leaves, immature pods and pea are used as vegetable, while several snacks and main meal dishes are prepared from the grain (Quim, 1997).

Fresh green pod contains (per 100 gm) about 24.6% protein and 500 mg of vitamin B. Cowpea is considered as hot, dry, diuretic seed and is difficult to digest, but seed possess high nutritive value. The nutritive content of cowpea (per 100 gm edible portion) are moisture (84.6 gm),

fat (0.2 gm), fibre (2.0 gm), calories (51), phosphorus (74 mg), vitamin (941 IU), riboflavin (0.09 mg), protein (4.3 gm), mineral (0.99 gm), other carbohydrates (8 gm), calcium (80 mg), iron (2.5 mg), thiamine (0.07 mg), nicotinic acid (0.9 mg) and vitamin-C (13 mg) (Kumar and Singh, 1998).

Cowpea is cultivated as a sole crop, as an intercrop, as cover crop to reduce weed population. In these production systems the spreading, indeterminate or semi-determinate bush of growth of cowpea provides ground cover, thus suppressing weeds and providing some protection against soil erosion (Quim, 1997).

Another important feature of cowpea is that it fixes atmospheric nitrogen through symbiosis with nodulating bacteria (*Brachyrhizobium* spp.). Therefore, the crop does not deplete the natural reserves of soil nitrogen and many experimental findings illustrate that the soil nitrogen level increases following cowpea cultivation. A contribution in the range of 40-80 kg N/ha is commonly obtained, while the total amount of nitrogen fixation is 70-350 kg/ha (Quim, 1997).

The crop suffers from different abiotic and biotic stresses. Diseases caused by fungi, bacteria,nematode and viruses are the limiting factors of production. Among diseases, viruses are known to infect cowpea and to be major

constraint in the production wherever cowpeas are grown. World-wide more than 20 viruses have been identified as naturally infecting cowpea. In India, cowpeas are known to be infected by atleast 11 viruses belonging to different taxonomic groups viz., Alfalfa mosaic, Carla, Cucumo, Gemini, Poty, Tobamo, Sobemo and Nepo viruses (Mali, 1996).

Among the viral diseases Cowpea Aphid Borne Mosaic Virus (CABMV) and Black Eye Common Mosaic Virus (BICMV) have been considered as synonymous in literature. However, peptide profiling (McKern *et al.*, 1994) and a panel of monoclonal antibodies (Huguenot *et al.*, 1993) can differentiate the two viruses. Shukla *et al.* (1994) considered CABMV and BICMV as two distinct viruses. In absence of these techniques in our lab, we have considered BICMV and CABMV as similar virus for the present. CABMV was first described by Lovisolo and Conti in 1966. It is now widely disseminated in the world through infected cowpea seedlots and causes severe crop damage (Thottappilly and Rossel, 1992).

Therefore, management of the virus, becomes very important.. No viricide have been reported in the literature and only preventive measures like use of virus free seeds, adjustment of planting dates and vector management are being used to minimize losses caused by viruses.

Plants as sources of potential antiviral substances, are gaining considerable attention throughout the world. Experimental work in this direction has revealed a wide occurrence of substances with antiviral properties in various plants. Antiviral substance present in higher plant tissues have long been regarded as important factor in resistance of higher plants to various viral diseases.

Plants are the richest source of organic chemical. The naturally occurring phytochemicals offer great potential for safe and effective control of viral pathogens. The chemical characteristic of the inhibitor from various plants and the mechanism by which they reduce virus infection, show that the problem of inhibitors present in different plant extracts is a complex one and the inhibitors present in the different plants may display individuality. Purification of the inhibitor from different plants involve different techniques, depending upon the chemical and physical nature of the inhibitor present in the plant extracts (Verma *et al.*, 1986).

The present investigation was, therefore, undertaken in order to generate information on plant extracts as inhibitors of CARMV, its physical properties like Dilution, Solvent, pH, Temperature and Purified plant extract. Results of these studies are presented and discussed herein.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

During the past few decades much work has been done on inactivation of plant viruses by plant extract and attempts have been made to obtain inhibitors of plant viruses from higher plants.

2.1 PLANT EXTRACTS AS INHIBITORS OF VIRUSES

According to Raychaudhuri and Chadha (1965) Deodar fruit extract was an inhibitor of chilli mosaic virus. Extracts of many plants have been screened for their inhibitory properties against potato virus-Y. The extracts of *Callistemon lanceolatus* and *Syzygium cumini* inhibited potato virus-Y (Raychaudhuri and Gupta, 1972; Sharma and Chauhan, 1973). The cucumber mosaic virus-1 was inhibited by the crude leaf extracts of *Syzygium cumini* and *Callistemon lanceolatus* (Sharma and Chauhan, 1973).

Kulshreshtha and Sarkar (1977) tested inhibitory preparation mixed with inoculum (Bean common mosaic virus-BCMV), applied to *Chenopodium amaranticolor*. They found inhibition in the range of 55.5 to 87.4 per cent with maximum in *Crassula falcata*.

Plant material from 29 spp. belonging to 15 families was screened for inhibition of cucumber mosaic virus (CMV). The most active components were detected in expanded leaves of *Chenopodium album*, *Chenopodium amaranticolor*, *Curriya murale*, *Hibiscus sabdariffa*, guava and pomegranate (Allam *et al.*, 1978).

Verma and Mukherjee (1979) found *Datura metel* leaf extract inhibited Tobacco mosaic virus (TMV) in hypersensitive hosts and Tobacco ring spot virus (TRSV) in both local and systemic hosts when applied 24 hrs. before inoculation in *Nicotiana glutinosa*.

Taniguchi (1980) reported that the seed extracts of *Dianthus barbatus* inhibit the development of local lesion induced by potato virus-X (PV-X) potexvirus on *Chenopodium amaranticolor* when applied mixed with the inocula. The extract prevents infection of Frenchbean (*Phaseolus vulgaris*) by PV-X when applied within 3 days before or within 3 hrs. after application of inoculum.

Foliar spray of *Mirabilis jalapa* leaf extract caused marked symptom suppression, improved growth and flowering and considerably reduced the virus multiplication rate in cucumber green mild mottle virus (CGMV) and CMV (Verma and Kumar, 1982).

Tripathi and Tripathi (1982) found that the extract of *Azadirachta indica* was most potent in reducing CABMV infection. The crude extracts from aerial parts of flowering and fruiting parts of *Solanum nigrum* and *Solanum khasianum* inhibited sunhemp rosette virus (SRV) in inoculated plants (Raychaudhuri and Basu, 1983).

Awasthi *et al.* (1984) reported that extract from dried roots of mature plants of *Boerhaavia diffusa*, when sprayed on the leaves of *Nicotiana tabacum*, *Lycopersicon esculentum*, *Cucumis melo*, *Crotolaria juncea* plants, prevented the infection of TMV in *N. tabacum*, CMV and TMV in *L. esculantum*, CGMV in *C. melo*, SRV in *C. juncea*. The yellow mosaic disease on mungbean and urdbean under natural conditions was suppressed by aqueous leaf extracts of *Clerodendrum fragrans* and *Aerva sanguinolenta* and root extract of *Boerhaavia diffusa* (Verma *et al.*, 1985).

Molina *et al.* (1986) injected the extract of *Syzygium cumini*, *Callistemon lanceolatus* and *Phytolacca americana* 24 hrs. before, at the time and after inoculation with soybean mosaic potyvirus (SMPV) in sorghum. Results were evaluated by symptoms and double antibody ELISA. All extracts were highly inhibitory against infection, crude and seed extracts were most effective (75 - 100% inhibition), when applied before or at the time of inoculation.

Zaidi *et al.* (1988) reported that the extracts of all 10 plants tested inhibited SMV. The maximum inhibition was by extracts of *Ocimum sanctum* followed by *Glycyrrhiza glabra* and *Anagallis arvensis* leaves. The extract from plant of *Crinum augustum* was inhibitory to potato-X potexvirus and potato-Y potyvirus (Fahmy and Mohamed, 1989).

Patel (1990) reported the efficacy of leaf extract of *Clerodendrum inerme* for controlling contact transmission of TMV in tobacco when applied before inoculation with virus. However, the extract failed to inhibit infection when applied after infection.

Extracts from *Bougainvillea spectabilis*, *Mirabilis jalapa* and *Phytolacca thirstiflora* prevented potato virus-X and potato virus-Y symptom development in single or double inoculated *Chenopodium amaranticolor*. Plant extracts inhibited both, systemic infection by PV-X and PV-Y and the interaction of two viruses (Duarte *et al.*, 1990, 1996; Noronha 1995).

Extracts from *Bougainvillea spectabilis*, *Capsicum annum* and *Datura metal* were mechanically inoculated on sorghum variety Tx41 2 hrs. before or simultaneously with sugarcane mosaic potyvirus (SCMV). The results were evaluated by symptoms observed on double antibody ELISA

after 7, 14 and 21 days of inoculation. All extracts had a highly inhibitory activity (Molina and Leon, 1991; Molina and Sanchez, 1993).

The pre-inoculation treatment with *Celosia cristata* leaf extract prevented lesion production by potato virus-X potexvirus in several local lesion hosts (Baranwal and Verma, 1992).

Kannan and Doraiswamy (1993) reported that the plant extract from *Azadirachta indica*, *Prosopis chilensis*, *Vitex negundo* and *Madhuca longifolia* inhibited 50%, 65%, 50% and 65% CABMV, respectively.

The root extract of *Boerhaavia diffusa* plant exhibited broad spectrum and high antiviral activity against tomato leaf enation mosaic virus (TLEMV) in tomatoes in the hypersensitive as well as systemic hosts (Awasthi and Rizvi, 1998).

2.2 EFFECT OF PHYSICAL PROPERTIES

2.2.1 Effect of Dilution

Verma and Mukherjee (1979) reported that the leaf extract of *Datura metal* inhibited the TMV and SRV and the extract was active upto a dilution of 1:10. The extract from *Pseuderanthemum atropurpureum* leaves produced some

strong virus interfering agent (VIA) against SRV and CGMMV. The extract was active upto a dilution of 1:40 (Verma and Abid Alikhan, 1985).

Barakat (1988) found that the aqueous extract from sinai-flora inhibited the infection of PV-X and were active upto a dilution of 1:1000. An aqueous leaf extract of *Clerodendrum inerme* (1:10 dilution) was highly inhibitory to TMV infection (Patel, 1990).

Othman *et al.* (1991) reported that extract of *Allium aestivum* (garlic) inhibited local lesion produced by TMV and the extract was active upto a dilution of 1:100. The *Bougainvillea spectabilis*, *Mirabilis jalapa* and *Phytolacca thirstifolia* inhibited PV-X upto 1:2000, 1:3000 and 1:5000 dilution, respectively (Duarte *et al.*, 1996).

2.2.2 Effect of solvents

Verma *et al.* (1973) reported that the virus inhibitor from cabbage leaves against TMV in *Nicotiana glutinosa* using different solvent like Water, Chloroform, Ethanol, Methanol and Petroleum products. Among all solvents water gave maximum inhibition. The leaf extract of *Hauttunia cordata* prepared in phosphate buffer with 0.1% triton X-100 enhanced the infectivity of TMV. The extract prepared with calcium salt lost this inhibitory effect (Matsushita and Sanada, 1978).

2.2.3 Effect of pH

Raychaudhuri and Gupta (1972) reported the effect of adjusting pH of plant extract of *Callistemon lanceolatus* and *Syzygium cumini* on potato. Among these, maximum inhibition of virus was recorded at pH 5 to 6. The leaf extract of *Hauttunia cordata* enhanced the infectivity of TMV and the extract was active at pH 7.2 (Matsushita and Sanada, 1978).

Verma and Abid Alikhan (1985) found that the extract from *Pseuderanthemum atropurpureum* leave inhibited SRV and CGMMV. The extract remained active between pH 5 to 8. The water extracts from fresh leaves of *Pseuderanthemum atropurpureum* and *Bougainvillea spectabilis* inhibited SRV and TMV and extract was active upto a pH 6 to 8 (Verma *et al.*, 1985).

2.2.4 Effect of temperature

Singh and Varma (1981) reported that the *Datura metal* completely inhibited TMV on *Chenopodium amaranticolor* upto 75 °C. The *Clerodendrum inerme* extract completely inhibited SRV upto 60 °C (Verma *et al.*, 1984).

Verma and Mukherjee (1989) reported that the leaf extract of *Datura metal* inhibited the TMV and SRV and the extract was inactivated on heating at 60 °C. The water extracts from fresh leaves of *Pseuderanthemum atropurpureum*

and *Bougainvillea spectabilis* inhibited SRV in *Cyamopsis tetragonoloba* and TMV in *Nicotiana glutinosa*. Both extracts were inactivated by heating at 90 °C and 65 °C, respectively.

2.3 ISOLATED PROTEIN

Kassanis and Kleczkowski (1948) identified the inhibitor from pokeweed as glycoprotein. The extracts of *Syzygium cumini* and *Callistemon lanceolatus* inhibited cucumis virus-1. The leaf and seed extracts of these plants are known to contain protein (Sharma and Chauhan, 1973).

Matsushita and Sanada (1978) reported the enhancing substance in extract of *Hauttuyaria cordata*. It was analysed by high pressure liquid chromatography and identified as protein.

A naturally occurring glycoprotein present in *Boerhaavia diffusa* root extract causes plant cells to produce a high Antiviral agent (AVA). AVA had protein characteristic (Verma and Awasthi, 1980; Awasthi and Rizvi, 1998).

Taniguchi (1980) found inhibitory activity of leaf extract of *Dianthus harbatus*. It could be separated by chromatography on a column of Sephadex G-25. Strong inhibitory activity was observed in a high molecular weight

substance. The leaf extract of *Datura metal* inhibited TMV. The inhibitor may be proteinaceous and not a sugar or phenol (Singh and Varma, 1981). The AVA obtained from leaf extract of *Turnera ulmifolia* purified by filteration of leaf extract through Sephadex G-25 column was characteristic protein with high molecular weight (Figueria *et al.*, 1994).

A non-phytotoxic systemic resistance inducing agent present in *Clerodendrum aculeatum* leaves was found to be *Clerodendrum aculeatum* systemic resistance inducing (CA-SRI) protein (Verma *et al.*, 1996 and Kumar *et al.*, 1997).

MATERIALS AND METHODS

III. MATERIALS AND METHODS

To carry out the present investigations, following Materials and Methods were adopted.

3.1 MATERIALS

3.1.1 Seeds

Genetically pure seeds of cowpea, variety Pusa falguni were obtained from Vegetable Research Station, GAU, Anand.

3.1.2 Miscellaneous

All required glasswares used in the experimental work were of Borosil grade, while chemicals were of B.D.H. or E. Merck and Biorads (USA).

3.2 RAISING OF SEEDLINGS

The seedlings of cowpea used for the present investigations were raised in an insect proof glass-house located behind the building of B.A. College of Agriculture, GAU, Anand. All experimental test plants were raised from seeds in earthern pots (12 cm x 10 cm) filled with a mixture of sand, medium black soil and well decomposed FYM. Seeds

were treated with 0.3 % thiram to prevent them from rotting. The seedlings in glass-house were sprayed with systemic insecticide, Dimethoate (Rogar 30 EC) @ 0.03% concentration as a routine at an interval of 10-15 days to keep them free from insects which might enter accidentally.

3.3 MAINTENANCE OF VIRUS CULTURE

The CABMV isolate maintained by the Plant Virus Laboratory, Department of Plant Pathology, B.A. College of Agriculture, Anand was further multiplied and maintained on cowpea (cv. Pusa falguni) by mechanical inoculation periodically. The virus inoculum was prepared by macerating young leaves, petioles and terminal portion of stem of diseased cowpea plants in a sterilized mortar with pestle using 0.1 M phosphate butter (pH - 7.6). The sap was then clarified by straining through a thin pad of sterile absorbent cotton. Cotton swab method was used to inoculate the primary leaves of healthy cowpea, after dusting of carborundum powder (400 mesh) as on abrasive as described by Holmes, 1929 and by rubbing with the cotton swab. Immediately after inoculation, the inoculated leaves were washed with tap water to remove excess inoculum sap and the abrasive. The seedlings were labelled properly for observation. Inoculated plants were recorded after repeated transfer of the virus on cowpea plants for confirmation on cowpea throughout the course of

investigation. It was multiplied on healthy cowpea seedlings at frequent interval to provide a constant sources of fresh inoculum.

3.4 METHOD FOR VIRUS INHIBITION

3.4.1 Selection of plants

The present study was aimed at screening of plant species for their antiviral properties against cowpea aphid-borne mosaic virus (CABMV). Most of these plants are from a part of natural vegetation and some of them are not of agricultural importance but easily and abundantly available. Plants selected for the present studies are listed in Table 1.

3.4.2 Preparation of phytoextract

Fresh plant were washed with tap water and were air dried. Each sample was ground in mortar with pestle and was filtered through double layers of sterilized muslin cloth.

3.4.3 Application of phytoextract

Various plants (Table 1) were collected from GAU Farms, Anand campus. The water extract of plant was applied to 10 cowpea plants against CABMV at three different stages *viz.*, pre-inoculation (24 hrs before inoculation), at the time of inoculation (mixed with inoculum) and post-

Table 1 : List of plant species used for antiviral activity with their common name

Sr. No.	Plant Extract	Family	Local name	Part used for preparation of
1.	2.	3.	4.	5.
1.	*Adhatoda vasica* Lini	Agavaceae	Ardusi	leaves
2.	*Aegle marmeli* Lini	Aeropodeae	Billi	leaves
3.	*Allium aestivum* Lini	Alismataceae	Lasan	cloves
4.	*Allium cepa* Lini	Alismataceae	Dungri	bulb
5.	*Andrographis peniculata* Bef.	Agavaceae	Keriatu	leaves
6.	*Anacyclus pyrenthrum* Will	Anacardiceae	Akkalgaro	leaves
7.	*Azadirachta indica* Aer	Avicenniaceae	Limdo	leaves
8.	*Boerhaavia diffusa* Lini	Bignoniaceae	Satodi	leaves, roots, seeds
9.	*Bougainvillea spectabilis* Wef	Bombaceae	Bougainvillea	leaves
10.	*Cassia absus* Lini	Caryuphyllaceae	Chimed	leaves
11.	*Cassia tora* Lini	Caryuphyllaceae	Kuvadio	leaves
12.	*Catharanthus roseus* Gen	Chenopodiceae	Barmasi	leaves
13.	*Clerodendrum inerme* Lar	Chenopodiceae	Kadvi Mahendi	leaves
14.	*Clerodendrum multiflorum* Oce	Chenopodiceae	Mahendi	leaves
15.	*Coleus aromaticus* Bef	Compositae	Ajmapan	leaves
16.	*Connolus microphyllus* Bef	Convolvulaceae	Safed Shankhavali	leaves
17.	*Corcharus aestuans* Lini	Dilleniaceae	Chhunch	leaves
18.	*Datura innoxia* Mat	Dilleniaceae	Kalo Dhaturo	flowers leaves
19.	*Desmolium sangeticum* Lini	Dilleniaceae	Shaliparni	leaves
20.	*Digera muricata* Lar	Dilleniaceae	Kanjro	leaves
21.	*Duranto plumarie* Jam	Dioserenceae	--	leaves
22.	*Eucalyptus globulus* Lini	Eriocaulaceae	Asopalav	leaves

1.	2.	3.	4.	5.
23.	*Euphorbia dracunculoides* Lini	Eriocaulaceae	Dhbi Dhudheli	leaves
27.	*Indigofera tinctoria* Lini	Fabaceae	Gali, Gudi	leaves
24.	*Jetropha curcas* Lini	Juncaceae	Ratan Jyot	leaves
25	*Lantena camera* Lini	Lamiaceae	--	leaves
26.	*Launaea procumbens* Lam	Lamiaceae	Bhoi Pathri	leaves
27.	*Lawsonia inerme* Rad	Lauraceae	Heena Mahendi	leaves
28.	*Leucas aspera* Lini	Lauraceae	Kubi	leaves
29.	*Mimosa pudica* Sat	Melastomaceae	Lajvanti	leaves
30.	*Moringa oleifera* Lini	Moraceae	Saragavo	leaves, pods, seeds
31.	*Mentha spicata* Lam	Menispermaceae	Pudina	leaves
32.	*Ocimum american* Lini	Oleceae	Ajlo	leaves
33.	*Ocimum sanctum* Lini	Periplogaceae	Tulsi	leaves
34.	*Phyllanthus fraternus* Wef	Periplogaceae	Bhoy Amli	leaves
35.	*Physalis minima* Lini	Periplogaceae	Popti	leaves
36.	Piper longum Raf	Plantaginaceae	--	leaves
37.	*Plantago ovata* Pam	Plantaginaceae	Isabgol	leaves
38.	*Plumbago zeylachia* Lini	Proteaceae	Chitrak	leaves
39.	*Psoralea corylifolia* Lam	Proteaceae	Bavachi	leaves
40.	*Solanum nigrum* Lini	Solanaceae	Piludi	leaves
41.	*Spilanthes calva* Lini	Solanaceae	Akkalgaro	leaves
42.	*Thuja virensis* Dar	Taccaceae	Vidya	leaves
43.	*Tribulus tenestris* Lini	Trapaceae	Gokhru	leaves
44.	*Uralia picta* Lini	Ulinaceae	Krushnaparni	leaves
45.	*Vigna unguiculata* Lini	Verbenaceae	Kulthi	leaves
46.	Withania samnifera Lini	Proteaceae	Ashwagandha	leaves

inoculation (24 hrs after inoculation). The observations for appearance of symptoms or inhibitory action of plant extracts was taken periodically. The inoculated plants were screened by Enzyme Linked Immuno Sorbent Assay (ELISA) for confirmation of CABMV in all the cases whether it showed symptom or remained symptomless. Inhibition percentage was calculated by the following formula

$$\text{Percentage virus inhibition} = \frac{\text{Percentage disease on control plant} - \text{Percentage disease on treated plant}}{\text{Percentage disease on control plants}} \times 100$$

3.4.4 Procedure for ELISA

Following protocol for direct antigen coating (DAC) ELISA was followed.

(1) One gram of the leaf sample was taken, homogenized in a mortar and pestle and diluted upto 5-10 % with the ELISA coating buffer. It was filtered with the help of 4 layered muslin cloth and centrifuged in eppendorf tubes for 30 seconds to 1 min.

(2) 100 µl of the antigen (sample supernatant) was added to each well of the ELISA plate and kept for overnight in the refrigerator.

(3) Washed with PBS-tween for 3 times at an interval of 3 min. The plate was tapped on a piece of paper towel to remove the remaining liquid.

(4) 100 µl of blotto (Non fat milk) 1-5 % prepared in PBS was loaded in each well and left for 30 min, then washed 3 times with PBS-Tween, the plate was tapped on paper towel to remove excess liquid.

(5) 100 µl of antibody (CABMV and Potyviridae probe) diluted (1:1000) in blotto was loaded in each well and incubated for 45 min-1 hr at room temperature.

(6) Washed with PBS- tween for 3 times at the interval of 3 min.

(7) 100 µl of second antibody conjugate (Horse-radish peroxidase) diluted (1:1000) in blotto was added in each well and kept for 45 min-1 hr at room temperature.

(8) Washed with PBS-tween 3 times at the interval of 3 min.

(9) Substrate solution was prepared using ABTS 0.5 mg/ml in substrate buffer, H_2O_2, 2 µl/ml was added just before the use.

(10) 50 µl of the substrate solution was added to each well. After about 10-15 mins, reaction (O.D) was read at 490 nm in ELISA plate Reader.

Buffers for ELISA

1) Coating buffer pH - 9.6

Na_2CO_3	- 1.50 gm
$NaHCO_3$	- 2.93 gm

Add H_2O to 1 Lt.

2) PBS (Phosphate buffer saline) pH - 7.4

Kcl	- 0.2 gm
NaCl	- 8.0 gm
KH_2PO_4	- 0.2 gm
$Na_2HPO_4.12H_2O$/	- 2.9 gm
$Na_2HPO_4.2H_2O$	- 1.44 gm

Add H_2O to 1 Lt.

3) PBS-T (PBS-Tween 20) : pH-7.4

PBS (1000 ml) + 0.8 ml Tween 20.

3.5 PHYSICAL PROPERTIES OF PHYTOEXTRACT

3.5.1 Dilution of phytoextracts

The extract (1 gm sample in 1 ml water) prepared were designated to be of 100 % concentration. These were further diluted to 1:1, 1:10, 1:100, 1:500, 1:1000 and 1:2000 (v/v) by adding required amount of sterilized distilled water. All dilutions of each extract were tested on 10 plants before 24 hrs of inoculation to find out best effective dilution.

3.5.2 Solvents for phytoextracts

Each leaf sample (5 gm) was weighed and 10 ml of each solvent like Water, Ethanol, Methanol, Acetone and Benzene were used for extraction. Each extracts were tested on at least 10 cowpea plants for pre-inoculation treatment of CABMV and observations were periodically recorded.

3.5.3 pH of phytoextract

The extract of each sample was prepared in distilled water and the pH was adjusted in a range of 5 to 9 using 1 N NaOH and 1N HCl. For each extract and each pH 10 cowpea plants were used for spraying before mechanical inoculation of CABMV and observations were periodically recorded.

3.5.4 Exposure of phytoextract to different temperature

The extract of every plant species was prepared in distilled water and taken in thin walled tubes as about 10 cm in diameter at rate of 5 ml per tube. Each test tube was then individually exposed to temperature at 50, 52, 55, 60, 62 and 65 °C for 10 minutes in a Metallic constant temperature water bath. The heated sap from each test tube was applied on 10 cowpea plants 24 hrs before mechanical inoculation of CABMV and periodically observations were recorded.

3.6 DETERMINATION OF TOTAL PROTEIN CONTENT FROM LEAF BY MICRO-KJELDAL METHOD

(1) 100 mg sample of each plant species was weighed and transferred to a digestion flask. 1 gm of catalyst mixture and 2 ml of conc. sulphuric acid was added.

(2) It was digested until the solution becomes colourless (approx. 40 min at 370 °C).

(3) After cooling, minimum quantity of water was added to dissolve solids and allowed to cool.

(4) 10 ml of boric acid solution was pipetted into 100 ml erlenmeyer flask. 2-3 drops of indicator solution was added and placed the flask under condenser extending below surface of the solution.

(5) Digest was transferred to distillation apparatus and the flask was rinsed 4 times with 8.3 ml portions of distilled water.

(6) 10 ml sodium hydroxide sodium thiosulphate solution was added to still and steam distilled and about 20 ml of distillate was collected.

(7) The receiving flask was lowered and continue distillation for more minutes. The tip of the condenser was washed with a few drops of water and removed.

(8) The contents of receiving flask was titrated with HCL to the point or first appearance of violet colour.

(9) Blank determination (without sample), some quantity of reagent was weighed and some digested with same distillation period as for sample determination.

Calculation :

$$\% N = \frac{(\text{ml of HCl for sample} - \text{ml of HCl for blank}) \times \text{Normality} \times 100 \times 1.926}{\text{mg sample}}$$

% Protein = % N x 6.25

RESULTS

IV. RESULTS

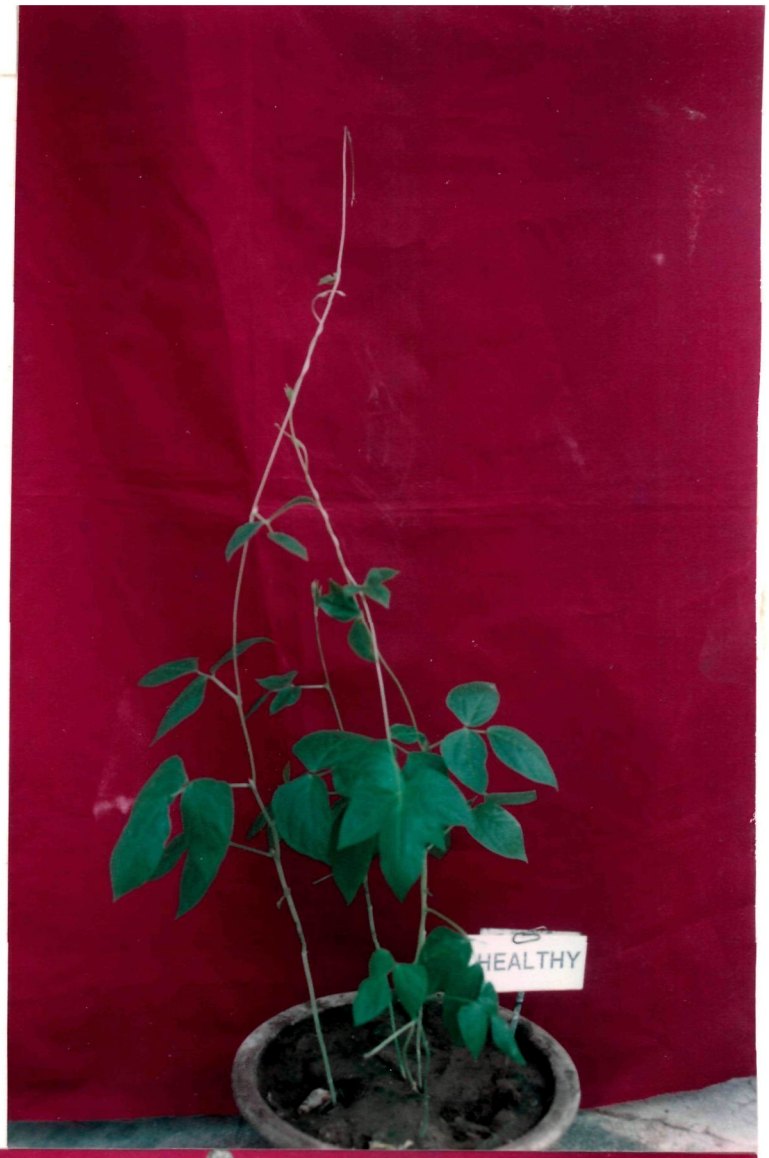
4.1 SYMPTOMATOLOGY

Cowpea aphid-borne mosaic virus (CABMV) was first observed in Italy by Lovisolo and Conti (1966). The plants affected by CABMV show severe mosaic, with the severity dependent on host cultivar and virus strain. Typical symptoms of dark green vein-banding, veinal chlorosis, interveinal chlorosis, leaf distoration, blistering and stunting (Plate II) was observed in field as well as glass-house condition.

4.2 MAINTENANCE OF VIRUS CULTURE

The CABMV was maintained in the glass-house by frequent inoculation of the cowpea plants with CABMV. The healthy cowpea (Var. Pusa falguni) seedlings (Plate I) were inoculated at two leaf stage after germination and the symptoms appeared after 5-8 days of inoculation. With each inoculated pot (containing 10 plants), one plant was kept as control (without inoculation). The symptoms (systemic mosaic) appeared only in the inoculated plants while the healthy (without inoculation) did not show any symptoms.

I Healthy plant of cowpea



II Cowpea aphid-borne mosaic virus (CABMV) symptoms on cowpea plants

The glass-house was sprayed with the Dimethoate (Rogar 30 EC), so that the insect vectors mainly aphids which are the suitable vector carriers of the CABMV can not contaminate the plant. The virus culture was tested and confirmed by Enzyme Linked Immuno Sorbent Assay (ELISA). In ELISA, the positive result was obtained with CABMV and a potyviridae antisera.

4.3 EFFECT OF PHYTOEXTRACTS

The result of phytoextracts on CABMV as pre-inoculation treatment (PrIT) (Plates III, IV & V) are presented in Table 2. It is clear from the data that the phytoextracts of *Clerodendrum inerme* (Plate VI), *Jatropha curcas* (Plate VI) and *Ocimum sanctum* (Plate VI) gave 100% inhibitory effect on the virus upto a 16 days of application but the effect gradually decreased over a period of time. The extracts of *Azadirachta indica*, *Boerhaavia diffusa* (root) and *Phyllanthus fraternus* had 100% inhibition upto 8 days, 50% inhibition upto 12 days and that of *Boerhaavia diffusa* (leaf), *Bougainvillea spectabilis*, *Catharanthus roseus*, *Connulus microphyllus*, *Clerodendrum multiflorum*, *Datura innoxia* (leaf), *Duranta plumarie*, *Lawsonia inerme*, *Leacus aspera*, *Moringa oleifera* (pod), *Ocimum americanum*, *Physalis minima*, *Solanum nigrum* and Thuja-30 X upto 6 days of extract application, respectively.

Table 2 : Induction of systemic resistance by pre-inoculation treatment with plant extracts against CABMV

No.	Plant extract	Per cent inhibition in virus infectivity							
		Days after extract application							
		6	8	10	12	14	16	18	20
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.	*Adhatoda vasica*	50	40	30	10	-	-	-	-
2.	*Allium aestivum*	60	50	30	-	-	-	-	-
3.	*Allium cepa*	90	70	60	30	-	-	-	-
4.	*Anacyclus pyrenthrum*	80	70	50	40	20	-	-	-
5.	*Andrographis peniculata*	100	70	50	30	20	10	-	-
6.	*Azadirchta indica*	100	100	80	60	20	20	-	-
7.	*Boerhaavia diffusa*	100	80	50	30	20	10	-	-
8.	*Boerhaavia diffusa* (root)	80	60	50	30	20	10	-	-
9.	*Boerhaavia diffusa* (seed)	80	60	40	20	10	-	-	-
10.	*Bougainvillea spectabilis*	100	80	60	50	30	10	-	-
11.	*Catharanthus roseus*	100	80	50	30	10	-	-	-
12.	*Cassia absus*	90	70	50	30	20	10	-	-
13.	*Clerodendrum inerme*	100	100	100	100	100	100	90	80
14.	*Clerodendrum multiflorum*	100	90	90	50	30	10	-	-
15.	*Coleus aromaticus*	60	30	10	-	-	-	-	-
16.	*Connolus microphyllus*	100	80	50	30	20	10	-	-
17.	*Corchorus aestuans*	90	60	50	30	10	-	-	-
18.	*Datura innoxia*	100	70	60	50	30	10	-	-

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
19.	*Datura innoxia* (Flower)	80	70	50	30	10	-	-	-
20.	*Desmodium sangeticum*	90	60	40	20	-	-	-	-
21.	*Digera muricuta*	70	60	50	20	10	-	-	-
22.	*Duranta plumarie*	100	70	40	30	20	10	-	-
23.	*Eucalyptus alobulus*	70	50	30	10	-	-	-	-
24.	*Euphorbia dracunculoides*	80	70	40	20	10	-	-	-
25.	*Indigofera tinctoria*	70	50	40	10	-	-	-	-
26.	*Jatropha curcas*	100	100	100	100	100	100	80	60
27.	*Launaea procumbens*	70	40	30	10	-	-	-	-
28.	*Lawsonia inerme*	100	90	90	70	50	30	20	-
29.	*Leacus aspera*	100	70	50	40	30	-	-	-
30.	*Lantana cemera*	70	60	50	30	10	-	-	-
31.	*Mimosa pudica*	90	10	40	10	-	-	-	-
32.	*Moringa oleifera*	50	30	10	-	-	-	-	-
33.	*Moringa oleifera* (pod)	100	90	60	40	20	-	-	-
34.	*Moringa oleifera* (seed)	90	90	70	50	30	10	-	-
35.	*Mentha spicata*	70	30	10	-	-	-	-	-
36.	*Ocimum american*	100	90	70	50	40	30	10	-
37.	*Ocimum sanctum*	100	100	100	100	100	100	90	70
38.	*Phyllanthus franternus*	100	100	70	60	50	40	20	-
39.	*Physalis minima*	100	90	60	40	30	10	-	-
40.	*Piper longum*	30	20	10	-	-	-	-	-

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
41.	*Plumbago zeylachia*	80	50	30	10	-	-	-	-
42.	*Psoralea corylifoliya*	80	70	30	10	10	-	-	-
43.	*Sorghum bicolor*	90	70	70	60	30	10	-	-
44.	*Solanum nigrum*	100	80	70	50	40	30	10	-
45.	*Spilanthes calva*	70	60	30	10	10	-	-	-
46.	*Thuja virensis*	70	60	40	10	-	-	-	-
47.	*Thuja - 30 X*	100	90	70	50	40	10	-	-
48.	*Tribulus tenestris*	90	80	60	40	10	-	-	-
49.	*Vigna unguiculata*	70	50	40	10	-	-	-	-
50.	*Withania somnifera*	80	60	50	30	20	10	-	-
51.	*Uralia picta*	80	80	90	30	20	10	-	-
52.	Control	-	-	-	-	-	-	-	-

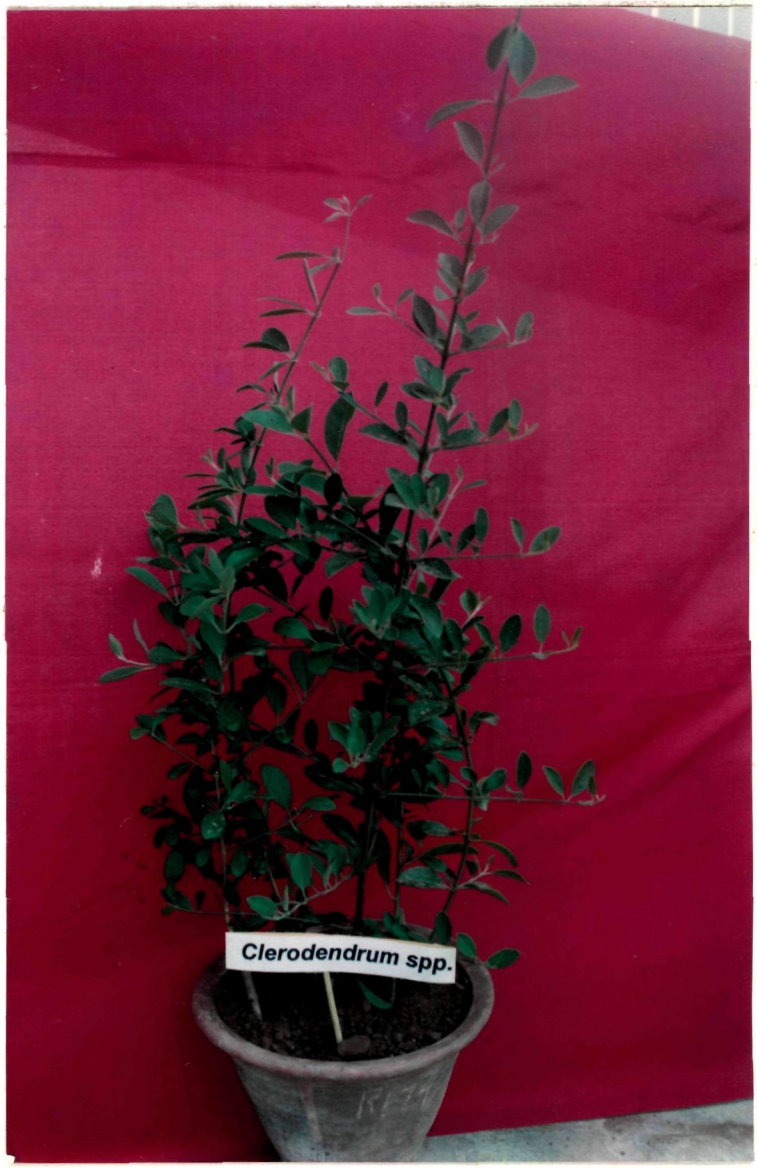
Jatropha curcas leaf

IV Effect of *Ocimum sanctum* leaf extract on cowpea aphid-borne mosaic virus

V Effect of *Clerodendrum inerme* leaf extract on cowpea aphid-borne mosaic virus

V/ *Jatropha curcas*

VII *Clerodendrum inerme*



VIII *Ocimum sanctum*

Rest all phytoextracts were also better over control with their inhibition ranging from 10 to 90% when applied as pre-inoculation treatment in glass-house condition at 28 - 35°C temperature.

The influence of different phytoextracts on CABMV due to their mixed inoculation treatment (MTT) with virus is presented in Table 3. It can be inferred from the data that the phytoextracts of *Boerhaavia diffusa* (leaf), *Datura innoxia* (Flower), *Duranta plumerie*, *Eucalyptus globulus*, *Phyllanthus fraternus*, *Physalis minima*, *Solanum nigrum* and *Tribulus tenestris* had 90% inhibitory effect on CABMV over control upto 6 days which further gradually decreases. Rest of the phytoextracts were of course better than the control for inhibiting this virus with their inhibitory effect ranging between 10 to 80% over control, when applied as mixed inoculation treatment in glass-house (temperature ranged between 28 - 35 °C).

The results on the effect of post-inoculation treatment (PoIT) of phytoextracts on CABMV are presented in Table 4. It is clear from the data that the phytoextracts of *Clerodendrum inerme*, *Ocimum sanctum*, and Thuja-30x had 90, 90 and 80 per cent inhibitory effect upto 6 days on CABMV which gradually decreased. While rest of phytoextracts were good for inhibition (10-70%) of CABMV.

Table 3 : Induction of systemic resistance by mix-inoculation treatment with plant extracts against CABMV

No.	Plant extract	Per cent inhibition in virus infectivity					
		Days after extract application					
		6	8	10	12	14	16
1.	2.	3.	4.	5.	6.	7.	8.
1.	*Adhatoda vasica*	80	50	30	10	-	-
2.	*Allium aestivum*	30	10	-	-	-	-
3.	*Allium cepa*	20	10	10	-	-	-
4.	*Anacyclus pyrenthrum*	50	20	10	-	-	-
5.	*Andrographis peniculata*	60	40	20	10	-	-
6.	*Azadirchta indica*	40	30	10	-	-	-
7.	*Boerhaavia diffusa*	90	70	40	10	-	-
8.	*Boerhaavia diffusa* (root)	70	70	50	30	10	-
9.	*Boerhaavia diffusa* (seed)	40	20	10	-	-	-
10.	*Bougainvillea spectabilis*	50	40	20	-	-	-
11.	*Catharanthus roseus*	40	20	-	-	-	-
12.	*Cassia absus*	70	60	40	30	10	-
13.	*Clerodendrum inerme*	70	70	60	30	10	-
14.	*Clerodendrum multiflorum*	50	20	10	-	-	-
15.	*Coleus aromaticus*	40	10	-	-	-	-
16.	*Connolus microphyllus*	60	50	20	10	-	-
17.	*Corchorus aestuans*	70	50	20	10	-	-
18.	*Datura innoxia*	60	40	20	10	-	-

1.	2.	3.	4.	5.	6.	7.	8.
19.	*Datura innoxia* (Flower)	90	70	50	10	-	-
20.	*Desmodium sangeticum*	80	50	30	10	-	-
21.	*Digera muricuta*	40	30	10	-	-	-
22.	*Duranta plumarie*	90	70	40	10	-	-
23.	*Eucalyptus alobulus*	90	60	30	20	-	-
24.	*Euphorbia dracunculoides*	30	20	-	-	-	-
25.	*Indigofera tinctoria*	40	30	20	10	-	-
26.	*Jatropha curcas*	70	50	40	10	-	-
27.	*Launaea procumbens*	70	50	20	10	-	-
28.	*Lawsonia inerme*	70	50	30	10	-	-
29.	*Leacus aspera*	70	60	40	30	10	-
30.	*Lantana cemera*	60	40	20	-	-	-
31.	*Mimosa pudica*	70	50	30	10	-	-
32.	*Moringa oleifera*	60	40	10	-	-	-
33.	*Moringa oleifera* (pod)	40	30	10	-	-	-
34.	*Moringa oleifera* (seed)	40	20	10	-	-	-
35.	*Mentha spicata*	80	50	40	20	-	-
36.	*Ocimum american*	80	70	30	10	-	-
37.	*Ocimum sanctum*	80	70	30	10	-	-
38.	*Phyllanthus franternus*	90	50	30	10	-	-
39.	*Physalis minima*	90	70	50	40	10	-
40.	*Piper longum*	70	30	10	-	-	-

1.	2.	3.	4.	5.	6.	7.	8.
41.	*Plumbago zeylanchia*	50	30	10	-	-	-
42.	*Psoralea corylifoliya*	70	50	30	10	-	-
43.	*Sorghum bicolor*	70	50	40	20	10	-
44.	*Solanum nigrum*	90	70	50	30	10	-
45.	*Spilanthes calva*	90	40	10	-	-	-
46.	*Thuja virensis*	70	60	40	10	-	-
47.	*Thuja - 30 X*	70	50	30	-	-	-
48.	*Tribulus tenestris*	90	60	40	10	-	-
49.	*Vigna unguiculata*	50	30	10	-	-	-
50.	*Withania somnifera*	30	10	-	-	-	-
51.	*Uralia picta*	80	50	30	10	-	-
52.	Control	-	-	-	-	-	-

Table 4 Introduction of systemic resistance by post-inoculation treatment with plant extracts against CABMV

No.	Plant extract	Per cent inhibition in virus infectivity						
		Days after extract application						
		6	8	10	12	14	16	18
1.	2.	3.	4.	5.	6.	7.	8.	9.
1.	*Adhatoda vasica*	40	30	10	-	-	-	-
2.	*Allium aestivum*	70	30	10	-	-	-	-
3.	*Allium cepa*	60	40	10	-	-	-	-
4.	*Anacyclus pyrenthrum*	50	20	10	-	-	-	-
5.	*Andrographis peniculata*	20	10	-	-	-	-	-
6.	*Azadirchta indica*	40	20	10	-	-	-	-
7.	*Boerhaavia diffusa*	50	30	10	-	-	-	-
8.	*Boerhaavia diffusa* (root)	70	50	30	10	-	-	-
9.	*Boerhaavia diffusa* (seed)	80	60	40	20	10	-	-
10.	*Bougainvillea spectabilis*	50	40	20	10	-	-	-
11.	*Catharanthus roseus*	30	10	10	-	-	-	-
12.	*Cassia absus*	70	60	50	40	20	-	-
13.	*Clerodendrum inerme*	90	60	30	10	-	-	-
14.	*Clerodendrum multiflorum*	30	20	10	-	-	-	-
15.	*Coleus aromaticus*	40	10	-	-	-	-	-
16.	*Connolus microphyllus*	60	40	20	-	-	-	-
17.	*Corchorus aestuans*	70	50	20	10	-	-	-
18.	*Datura innoxia*	40	20	10	-	-	-	-

1.	2.	3.	4.	5.	6.	7.	8.	9.
19.	*Datura innoxia* (Flower)	30	20	10	-	-	-	-
20.	*Desmodium sangeticum*	70	50	20	10	-	-	-
21.	*Digera muricuta*	40	30	10	-	-	-	-
22.	*Duranta plumarie*	50	30	20	10	-	-	-
23.	*Eucalyptus alobulus*	70	60	20	-	-	-	-
24.	*Euphorbia dracunculoides*	30	20	10	-	-	-	-
25.	*Indigofera tinctoria*	50	20	-	-	-	-	-
26.	*Jatropha curcas*	40	20	10	-	-	-	-
27.	*Launaea procumbens*	20	10	-	-	-	-	-
28.	*Lawsonia inerme*	50	30	10	-	-	-	-
29.	*Leacus aspera*	60	30	10	-	-	-	-
30.	*Lantana cemera*	70	50	30	10	-	-	-
31.	*Mimosa pudica*	70	50	40	20	-	-	-
32.	*Moringa oleifera*	20	10	-	-	-	-	-
33.	*Moringa oleifera* (pod)	20	10	10	-	-	-	-
34.	*Moringa oleifera* (seed)	80	60	40	20	10	-	-
35.	*Mentha spicata*	80	50	30	20	10	-	-
36.	*Ocimum american*	50	30	10	-	-	-	-
37.	*Ocimum sanctum*	90	40	10	-	-	-	-
38.	*Phyllanthus franternus*	50	20	10	-	-	-	-
39.	*Physalis minima*	80	70	50	30	10	-	-
40.	*Piper longum*	30	10	-	-	-	-	-

1.	2.	3.	4.	5.	6.	7.	8.	9.
41.	*Plumbago zeylanchia*	30	10	-	-	-	-	-
42.	*Psoralea corylifoliya*	70	30	10	-	-	-	-
43.	*Sorghum bicolor*	70	30	10	-	-	-	-
44.	*Solanum nigrum*	80	70	50	30	20	-	-
45.	*Spilanthes calva*	60	50	20	10	-	-	-
46.	*Thuja virensis*	40	30	20	10	-	-	-
47.	*Thuja - 30 X*	80	60	40	10	-	-	-
48.	*Tribulus tenestris*	80	60	30	20	-	-	-
49.	*Vigna unguiculata*	40	30	20	-	-	-	-
50.	*Withania somnifera*	50	30	10	-	-	-	-
51.	*Uralia picta*	40	20	10	-	-	-	-
52.	Control	-	-	-	-	-	-	-

These extracts were applied after virus inoculation treatment in the glass-house (temperature ranging between 28 - 35 °C).

The absence or presence of virus was confirmed by ELISA (Table 5). In ELISA, there was no (negative) reaction observed at 6th day after *Clerodendrum inerme*, *Jetropha curcas* and *Ocimum sanctum* extract application as PrIT. While there was mild and strong reaction with both the antisera (CABMV and potyviridae) at 12th and 18th day after inoculation, in all three extracts. These results indicate that the virus does not multiply before 6th day in plants treated with extract as it also does not show any visible symptoms.

The results of repeated application of phytoextracts on CABMV are presented in Table 6. It is clear from the data that the phytoextract of *Clerodendrum inerme* with 1 spray (PrIT) had 100 % inhibition upto 18 days and 2 spray (PrIT + 6 days PoIT) and 3 spray (PrIT + 6 day PoIT + 12 day PoIT) gave 100 % inhibition upto 22 days. The phytoextract of *Ocimum sanctum* with 1 spray (PrIT), 2 spray (PrIT + 6 day PoIT) and 3 spray (PrIT + 6 day PoIT + 12 day PoIT) had 100 % inhibition upto 18, 20 and 20 days, respectively. The phytoextract of *Jatropha curcas* with 1 spray (PrIT), 2 spray (PrIT + 6 day PoIT) and 3

Table 5 : Reactivity of CABMV to antisera of CABMV and potyviridae probe in ELISA

Extracts	Days after ELISA done	Antisera Reaction	
		CABMV	Poty
Jatropha curcas	6	-	-
	12	+	+
	18	++	+++
Ocimum sanctum	6	-	-
	12	+	++
	18	++	+++
Clerodendrum inerme	6	-	-
	12	+	++
	18	++	++

\- No reaction

\+ Mild reaction

++ ┐
   ├ Strong reaction
+++ ┘

Table 6 : Effect of repeated spray of plant extracts on CABMV

No	Plant exctracts	No. of spray*	Per cent inhibition in virus infectivity									
			Days after extract application									
			6	10	12	14	16	18	20	22	24	26
1.	*Clerodendrum inerme*	1	100	100	100	100	100	100	90	80	80	60
		2	100	100	100	100	100	100	100	100	90	80
		3	100	100	100	100	100	100	100	100	90	70
2.	*Ocimum sanctum*	1	100	100	100	100	100	100	90	70	60	50
		2	100	100	100	100	100	100	100	90	80	50
		3	100	100	100	100	100	100	100	90	70	50
3.	*Jatropha curcas*	1	100	100	100	100	100	100	90	80	70	60
		2	100	100	100	100	100	100	100	100	100	70
		3	100	100	100	100	100	100	100	100	100	70

* 1 = PrIT
2 = PrIT + 6 day PoIT
3 = PrIT + 6 day PoIT + 12 day PoIT

PrIT = Pre-Inoculation Treatment
PoIT = Post-Inoculation Treatment

spray (PrTT + 6 day PoTT + 12 day PoTT) had 100 % inhibition upto 18, 24 and 24 days, respectively. These results indicate that the plant can be protected from virus by repeated application of phytoextracts.

4.4 PHYSICAL PROPERTY OF PHYTOEXTRACTS

4.4.1 Effect of Dilution

The results (Table 7) showed that the crude extract of *Jatropha curcas* diluted to a range between 1:1 to 1:100 produced inhibition of CABMV on all the tested plants. While extract diluted to 1:500 and more failed to provide any inhibition of disease in test plants. The results thus indicated that the Dilution end point (DEP) of the *Jatropha curcas* lies upto 1:100 in glass-house condition.

The crude extract of *Ocimum sanctum* diluted to range of 1:1 to 1:100 produced inhibition on CABMV (Table 8). The extract when diluted to 1:500 and above do not inhibit the disease in test plants. The results thus indicated that the DEP of the *Ocimum sanctum* lies around 1:100 in glass-house conditions.

With the data presented in Table 9 it can be interpreted that the extract of *Clerodendrum inerme* diluted to a range of 1:1 to 1:500 caused inhibition of CABMV on all

Table 7 : Effect of dilution on the inhibitory property of leaf extract of *Jatropha curcas* against CABMV

No	Dilution	Percentage decrease in virus infectivity								
		Days after extract application								
		6	8	10	12	14	16	18	20	22
1	1:1	100	100	100	100	100	100	80	80	80
2	1:10	100	100	100	100	100	80	80	70	70
3	1:100	100	90	80	70	70	60	60	30	20
4	1:500	90	90	80	80	60	50	30	10	-
5	1:1000	70	60	40	40	20	-	-	-	-
6	1:2000	50	30	10	-	-	-	-	-	-
7	Control	-	-	-	-	-	-	-	-	-

Table 8 : Effect of dilution on the inhibitory property of leaf extract of *Ocimum sanctum* against CABMV

No	Dilution	Percentage decrease in virus infectivity								
		Days after extract application								
		6	8	10	12	14	16	18	20	22
1	1:1	100	100	100	100	100	100	90	90	90
2	1:10	100	100	100	100	100	90	70	70	60
3	1:100	100	100	100	100	90	90	70	60	60
4	1:500	90	90	80	80	70	70	60	50	50
5	1:1000	60	50	40	20	20	10	-	-	-
6	1:2000	50	30	30	20	10	-	-	-	-
7	Control	-	-	-	-	-	-	-	-	-

Table 9 : Effect of dilution on the inhibitory property of leaf extract of *Clerodendrum inerme* against CABMV

No	Dilution	Percentage decrease in virus infectivity								
		Days after extract application								
		6	8	10	12	14	16	18	20	22
1	1:1	100	100	100	100	100	100	100	90	90
2	1:10	100	100	100	100	100	100	100	90	90
3	1:100	100	100	100	90	90	90	80	80	70
4	1:500	100	100	80	80	70	60	60	50	40
5	1:1000	90	80	70	60	50	40	30	-	-
6	1:2000	60	40	30	10	-	-	-	-	-
7	Control	-	-	-	-	-	-	-	-	-

Table 10 : Effect of solvents on the inhibitory property of leaf extract of *Jatropha curcas* against CABMV

No	Solvent	Percentage decrease in virus infectivity								
		Days after extract application								
		6	8	10	12	14	16	18	20	22
1	Water	100	100	100	100	100	100	100	90	70
2	Ethanol	100	100	100	90	80	70	69	30	10
3	Methanol	100	90	80	70	50	30	20	-	-
4	Acetone	50	40	10	-	-	-	-	-	-
5	Benzene	40	30	-	-	-	-	-	-	-
6	Control	-	-	-	-	-	-	-	-	-

tested plants. While extract diluted to 1:100 and more failed to inhibit the disease on tested plants. The results indicated that the DEP of leaf extract of *Clerodendrum inerme* lies around 1:500.

4.4.2 Effect of solvents

The results (Table 10) showed that the extract of *Jatropha curcas* with different solvent viz., Water, Ethanol and Methanol produced 100% inhibition of CABMV upto 18, 10 and 8 days, respectively. The extract with Acetone and Benzene as solvents failed to produce inhibition of disease on any plant under test. It may be concluded that the *Jatropha curcas* extract in water had better and prolonged (days) inhibition of cowpea aphid-borne mosaic virus than all other solvents.

The data presented in Table 11 indicated that the extract of *Ocimum sanctum* with solvents, water, Ethanol and Methanol produced 100% inhibition of CABMV upto 18, 8 and 8 days, respectively. While Acetone and Benzene extracts did not produce inhibition in tested plants. The results indicated that the water solvent for the *Ocimum sanctum* when applied against CABMV was best both in terms of inhibition percentage and its long lasting effect.

Table 11 : Effect of solvents on the inhibitory property of extract of *Ocimum sanctum* against CABMV

No	Solvent	Percentage decrease in virus infectivity								
		Days after extract application								
		6	8	10	12	14	16	18	20	22
1	Water	100	100	100	100	100	100	100	90	80
2	Ethanol	100	100	90	70	50	30	10	-	-
3	Methanol	100	100	90	70	40	20	10	-	-
4	Acetone	30	20	10	-	-	-	-	-	-
5	Benzene	40	20	10	-	-	-	-	-	-
6	Control	-	-	-	-	-	-	-	-	-

Table 12 : Effect of solvents on the inhibitory property of leaf extract of *Clerodendrum inerme* against CABMV

No	Solvent	Percentage decrease in virus infectivity								
		Days after extract application								
		6	8	10	12	14	16	18	20	22
1	Water	100	100	100	100	100	100	90	80	80
2	Ethanol	100	90	80	60	50	40	30	10	-
3	Methanol	90	80	60	40	30	10	-	-	-
4	Acetone	90	80	60	40	30	10	-	-	-
5	Benzene	40	20	-	-	-	-	-	-	-
6	Control	-	-	-	-	-	-	-	-	-

The results (Table 12) showed that the extract of *Clerodendrum inerme* with solvent viz., water and Ethanol produced 100% inhibition on CABMV upto 16 and 6 days, respectively. Extract with Methanol, Acetone and Benzene failed to produce inhibition against CABMV. The results indicated that water solvent for extraction of *Clerodendrum inerme* produced good inhibition out of all other solvents tried.

4.4.3 Effect of pH

The data presented in Table 13 revealed that the extract of *Jatropha curcas* with pH at 6,7 and 8 produced 100% inhibition of CABMV on all tested plants upto 10, 14 and 12 days, respectively. While extract at pH 5 and 9 failed to produce much inhibition on CABMV in tested plants. The results thus indicated that the extract at pH 7 produced good inhibition as compared to all other pH levels tried and control treatment in glass-house condition.

The results (Table 14) showed that the extract of *Ocimum sanctum* with pH at 6,7 and 8 produced inhibition on CABMV in all tested plants upto 12, 16 and 12 days, respectively. The pH at 5 and 9 failed to produce 100% inhibition in CABMV of tested plants. The results indicate that the pH level at 7 produced good inhibition against CABMV which was long lasting than all other pH levels tried.

Table 13 : Effect of pH on the inhibitory property of leaf extract of *Jatropha curcas* against CABMV

No	pH	Percentage decrease in virus infectivity								
		Days after extract appliction								
		6	8	10	12	14	16	18	20	22
1	5	70	60	40	20	10	-	-	-	-
2	6	100	100	100	80	70	50	50	30	20
3	7	100	100	100	100	100	90	80	70	60
4	8	100	100	100	100	90	70	50	40	40
5	9	60	50	40	20	10	-	-	-	-
6	Control	-	-	-	-	-	-	-	-	-

Table 14 : Effect of pH on the inhibitory property of leaf extract of *Ocimum sanctum* against CABMV

No	pH	Percentage decrease in virus infectivity								
		Days after extract application								
		6	8	10	12	14	16	18	20	22
1	5	70	60	40	30	10	-	-	-	-
2	6	100	100	100	100	80	70	50	30	20
3	7	100	100	100	100	100	100	90	80	60
4	8	100	100	100	100	90	70	60	50	40
5	9	70	50	30	10	-	-	-	-	-
6	Control	-	-	-	-	-	-	-	-	-

From the data presented in Table 15 it can be seen that the extract of *Clerodendrum inerme* with pH 6, 7 and 8 produced inhibition on cowpea aphid-borne mosaic virus (CABMV) upto 12, 16 and 10 days, respectively. While extract at pH 5 and 9 failed to produce inhibition in the tested plants. The results indicated that the extract at pH 7 produced good inhibition against CABMV and had long lasting effect than the other two.

4.4.4 Exposure of phytoextracts to different temperature

The data presented in Table 16 indicate that the extract of *Jatropha curcas* with exposure at 50, 52 and 55 °C produced inhibition on CABMV in all tested plants upto 20, 18 and 18 days, respectively. The temperature at 60, 62 and 64 °C were not able to produce 100% inhibition on CABMV of tested plants. The results indicated that the temperature at 50, 52 and 55 °C produced good inhibition in glass-house which was long lasting than other higher temperature.

The data presented in Table 17 showed that the extract of *Ocimum sanctum* with exposure at 50, 52, 55 and 60 °C produced 100% inhibition on all tested plants upto 18, 18 and 16 and 12 days, respectively. While extract at temperature at 62 and 65 °C was unable to produce 100% inhibition in the tested plants. The results indicated that the extracts exposure at 50 and 52 °C produced good inhibition against CABMV which has long lasting effect than other higher temperatures.

Table 15 : Effect of pH on the inhibitory property of leaf extract of *Clerodendrum inerme* against CABMV

No	pH	Percentage decrease in virus infectivity									
		Days after extract application									
		6	8	10	12	14	16	18	20	22	24
1	5	70	60	40	30	20	20	10	-	-	-
2	6	100	100	100	100	90	80	70	60	40	30
3	7	100	100	100	100	100	100	80	60	50	40
4	8	100	100	100	90	80	70	60	40	30	20
5	9	70	60	50	30	10	-	-	-	-	-
6	Control	-	-	-	-	-	-	-	-	-	-

Table 16 : Effect of temperatures on the inhibitory property of extract of *Jatropha curcas* against CABMV

No	Temperature	Percentage decrease in virus infectivity								
		Days after exttract application								
		6	8	10	12	14	16	18	20	22
1	50	100	100	100	100	100	100	100	100	90
2	52	100	100	100	100	100	100	100	90	80
3	55	100	100	100	100	100	100	100	90	90
4	60	90	80	70	70	60	50	30	20	10
5	62	70	50	40	30	10	-	-	-	-
6	65	50	30	10	-	-	-	-	-	-
7	Control	-	-	-	-	-	-	-	-	-

Table 17 : Effect of temperatures on the inhibitory property of extract of *Ocimum sanctum* against CABMV

No	Temperature	Percentage decrease in virus infectivity								
		Days after extract application								
		6	8	10	12	14	16	18	20	22
1	50	100	100	100	100	100	100	100	90	80
2	52	100	100	100	100	100	100	100	80	80
3	55	100	100	100	100	100	100	90	80	60
4	60	100	100	100	100	90	80	60	50	30
5	62	90	70	60	40	20	-	-	-	-
6	65	70	50	40	20	-	-	-	-	-
7	Control	-	-	-	-	-	-	-	-	-

Table 18 : Effect of tempreatures on the inhibitory property of extract of *Clerodendrum inerme* against CABMV

No	Temperature	Percentage decrease in virus infectivity								
		Days afterextract application								
		6	8	10	12	14	16	18	20	22
1	50	100	100	100	100	100	100	100	100	90
2	52	100	100	100	100	100	100	100	90	80
3	55	100	100	100	100	100	100	100	90	90
4	60	90	70	60	40	20	10	-	-	-
5	62	70	50	40	30	10	-	-	-	-
6	65	60	40	30	20	-	-	-	-	-
7	Control	-	-	-	-	-	-	-	-	-

Table 19 : Per cent protein present in inhibitory leaf

Plant leaf	Percentage protein
Jatropha curcas	6.90
Ocimum sanctum	3.85
Clerodendrum inerme	5.95

The data presented in Table 18 revealed that the extract of *Clerodendrum inerme* with temperatures viz., 50, 52 and 55 °C produced 100% inhibition on CABMV in all tested plants upto 18 days. Extract with temperature at 60, 62 and 65 °C failed to produce inhibition on CABMV. The results indicate that temperature up to 55 °C produced good inhibition out of all other temperatures tried for the *Clerodendrum inerme* in glass-house condition.

4.5 TOTAL PROTEIN OF INHIBITORY LEAF

The data presented in Table 19 showed that the maximum percentage of protein (6.90) was present in *Jatropha curcas* whereas in *Ocimum sanctum* and *Clerodendrum inerme* percentage of protein was 3.85 and 5.95, respectively.

DISCUSSION

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V. DISCUSSION

Cowpea (*Vigna unguiculata*) belonging to the leguminoseae family is one of the most important pulse crops in common use in India. It is grown since ancient time. The productivity of cowpea is low due to incidence of various diseases i.e., fungal, bacterial, nematodal and viral. Fungal, bacterial and nematodal diseases are controlled by fungicide, bactericide and nematicide, respectively but management of viral diseases of plants is difficult as no viricide has been found till now. Therefore, studies were taken up on plant extracts as inhibitors of cowpea aphid-borne mosaic virus (CABMV), the physical properties and protein percentage of some plants.

In literature, CABMV and BICMV have been considered as synonymous. However, peptide profiling (McKern *et al.*, 1994) and a panel of monoclonal antibodies (Huguenot *et al.*, 1993) can differentiate the two viruses. Shukla *et al.* (1994) considered CABMV and BICMV as two distinct viruses. In absence of these techniques in our lab, we have considered BICMV and CABMV as similar virus for the present. The plants affected by CABMV showed severe mosaic, with the severity dependent on host cultivar and virus strain. Typical symptoms of dark green vein banding, veinal

chlorosis, interveinal chlorosis, leaf destortion, blistering and stunting was observed, which was in confirmation with the observations by Levisolo and Conti (1966).

The virus culture was maintained in the glass-house and frequent inoculation of the cowpea plants was done with CABMV. The healthy cowpea seedlings were inoculated at two leaf stage after germination and symptoms appeared after 5-8 days. Also the glass-house was sprayed with the Dimethoate (Rogar 30 EC), so that the insect vectors mainly aphids which are the suitable vector/carriers of CABMV can not contaminate other plants.

In the literature there are no reports of inhibition studies on CABMV with the plant extracts and, therefore relevant literature about other viruses have been quoted here for results presented. In the present investigation, CABMV was inhibited by pre-treatment inoculation (PrIT) (24 hrs before virus challenge) by extracts of *Clerodendrum inerme*, *Jatropha curcas* and *Ocimum sanctum*. 100 % Inhibition effect lasted upto 18 days. These results are in confirmity with the earlier reports on water extract of *Pseuderanthemum atropurpureum* and *Bougainvillea spectabilis* inhibiting tobacco mosaic virus and sunnhemp rosette virus infection, when sprayed prior to virus inoculation (Verma *et al.*, 1985).

The mix-inoculation treatment with phytoextract of *Boerhaavia diffusa*, *Datura innoxia* (flower), *Duranta plumerie*, *Eucalyptus globulus*, *Phyllanthus fraternus*, *Physalis minima*, *Solanum nigrum* and *Tribulus tenestris* gave 90 % inhibitory effect on CABMV virus upto 6 days. Almost similar results were obtained by Verma *et al.* (1984) with the extract of *Clerodendrum* spp. which inhibited tobacco mosaic virus between 70 to 90 %.

The effect of post-inoculation treatment (PoTT) (24 hrs after virus challenge) with phytoextract of *Clerodendrum inerme* and *Ocimum sanctum* had 90 % inhibitory effect upto 6 days on CABMV. This results are in conformity with the earlier reports of *Clerodendrum spp.* inhibiting tobacco mosaic virus, tomato yellow mosaic virus, gomphrena mosaic virus, when extract treatment was given after 24 hrs. of inoculation (Verma *et al.*, 1984). The PrTT was superior over MxTT and PoTT. Similar results were observed by Verma *et al.* (1984, 1985).

The PrTT was superior because the occurrence of some antiviral agent in virus infected plants is well established and systemic resistant to virus infection following treatment with certain plant extracts or viruses was presumably due to production of some virus interfering agent (VIA) or antiviral factor in resistant factor

(Verma and Abid Alikhan 1985). The inhibitor on being introduced in to plant system goes surrounding cell and stimulate them and produce other substances. These effectively inhibit virus multiplication and transmit virus resistance to whole of plant (Awasthi *et al.*, 1998). Baranwal and Verma (1992) reported that the three possible mechanisms of plant virus inhibitor's may operate i.e. by acting directly on virus, by acting on the establishment phase of the virus infection process and by affecting the susceptibility of the plant by altering cell metabolism. It has been suggested that these inhibitor's alter the metabolism of the hosts in such a way that introduced virus particles are unable to multiply (Verma *et al.*, 1984).

The results on inhibition was evaluated by Enzyme Linked Immuno Sorbent Assay (ELISA) at 6, 12 and 18 days. The serological techniques are most important, and very sensitive for detection and identification of viruses, In *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* no reaction was observed at 6th day while at 12th and 18th day mild and strong reaction was observed with the antisera CABMV and potyviridae probe (Mishra *et al.*, 1997) antisera respectively. These results indicate that the plant extracts are able to provide protection by inhibiting the virus or by producing an antiviral agent upto 6 days. After that its effect is gradually reduced or diluted in the developing plant which might require further doses of extracts.

There is no review available on repeated application of phytoextracts in the literature. However, it would be worthwhile exploring this aspect for the protection of plants from virus infection. The repeated application of phytoextracts of *Clerodendrum inerme*, *Jatropha curcas* and *Ocimum sanctum* with one spray gave 100 % inhibition upto 18 days. While two spray (PrIT + 6 day PoIT) and three spray (PrIT +6 day PoIT + 12 day PoIT) gave 100 % inhibition upto 22, 20 and 24 days, respectively. There is a clear indication that period of protection from virus can be enhanced by the repeated application of phytoextracts. However, more research is required on the interval, frequency and concentration of a particular plant extract to manage CABMV.

Information on physical properties such as dilution, solvents, pH and temperature *in vitro* shows a better understanding of inhibitors. The physical property studied in this investigation on inhibitors dilution end point (DEP) of *Jatropha spectabilis*, *Ocimum sanctum* and *Clerodendrum inerme* ranged between 1:10 1:100, 1:1 to 1:100 and 1:1 to 1:500 which gave 100 % inhibition upto 6, 12 and 18 days, respectively. These results are in confirmity with the earlier reports of Verma and Mukherjee (1979) on leaf extract of *Datura metal* which inhibited TMV and SRV upto dilution of 1:10. Barakat (1988) found that the extract from sinai-flora inhibit potato-X and were active upto a

dilution of 1:1000. The leaf extract of *Clerodendrum inerme* (1:100) was highly inhibitory to TMV infection (Patel,1990). *Bougainvillea spectabilis*, *Mirabilis jalapa* and *Phytolacca thirstifolia* inhibit potato virus-X upto 1:2000, 1:3000 and 1:5000 dilution, respectively (Duarte *et al.*, 1996).

Water, Ethanol and Methanol as solvents of *Jatropha curcas* and *Ocimum sanctum* and water and ethanol for *Clerodendrum inerme* produced good inhibition. Almost similar results were obtained by Verma *et al.* (1973) in case of virus inhibitor from cabbage leaves against TMV by using different solvents like Water, Chloroform, Ethanol, Methanol and Petroleum products. Among all solvents Water gave maximum inhibition. The leaf extract of *Hauttunia cordata* prepared in phosphate buffer enhanced the activity of TMV (Matsushita and Sanada, 1978).

Jatropha curcas, *Ocimum sanctum* and *Clerodendrum inerme* extract at pH 6, 7 and 8 produced 100% inhibition upto 12 days. These results are confirmatory with earlier reports of Raychaudhary and Gupta (1972) on the extract of *Callistemon lanceolatus* and *Syzygium cumini* inhibiting PV-X, with maximum inhibition of virus recorded at pH 5 to 6. The leaf extract from *Pseuderanthemum atropurpureum* inhibited SRV. The extract remained active between pH 5 to 8. Verma *et*

al. (1986) reported the leaf extract of *Bougainvillea spectabilis* inhibited SRV and TMV and extract was active upto pH 6 to 8.

Jatropha curcas and *Ocimum sanctum* extract were active upto exposure at 55 °C and *Clerodendrum inerme* extract were active upto exposure at 60 °C. In the studies by Singh and Varma (1981) *Datura metal* inhibited TMV upto 75 °C. The *Clerodendrum inerme* completely inhibited SRV upto 60 °C (Verma *et al.*, 1984).

The percentage of protein in leaves of *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* were 6.90, 3.85 and 5.95, respectively. Almost similar results were obtained by Kassanis and Kleczkowski (1948) and they identified the inhibitor from pokeweed as glycoprotein. The extracts of *Syzygium cumini* and *Callistemon lanceolatus* inhibit cucumis virus-1. The leaf and seed extracts of these plants are known to contain protein (Sharma and Chauhan, 1973). A naturally occurring glycoprotein present in *Boerhaavia diffusa* root extract cause plant cell to produce a high antiviral agent (AVA) (Verma *et al.*, 1980; Awasthi and Rizvi, 1998). The antiviral agent (AVA) obtained from leaf extract of *Turnera ulmifolia* had characteristic of protein with high molecular weight (Figueria *et al.*, 1994).

A non-phytotoxic systemic resistance inducing agent present is *Clerodendrum aculeatum* systemic resistance inducing (CASRT) protein (Verma *et al.*, 1996 and Kumar *et al.*, 1997).

SUMMARY

VI. SUMMARY

Cowpea (*Vigna unguiculata*), one of the important commercial pulse crops of Gujarat has been observed to be infected by cowpea aphid-borne mosaic virus (CABMV). Investigations were undertaken to study the effect of different plant extracts given as pre-inoculation treatment (PrIT), mixed inoculation treatment (MIT) and post inoculation treatment (PoIT) for inhibition of this virus and the influence of dilution, solvent, pH and temperature on the effectiveness of plant extracts against CABMV.

Out of all the three times of application viz., PrIT, MIT and PoIT of plant extracts, PrIT i.e., 24 hrs. before virus inoculation was found to be more efficient over the MIT and PoIT.

Phytoextracts of *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* had 100% inhibitory effect on virus upto 16 days when given as PrIT. Whereas in case of treatments given with mixed inoculation *Solanum nigrum*, *Physalis minima*, *Tribulus tenestris*, *Phyllanthus fraternus*, *Duranta plumerie*, *Datura innoxa*, *Boerhaavia diffusa* and *Eucalyptus globulus* had 90% inhibitory effect upto 6 days.

Whereas PoIT of Thuja-30 X (Homeopathic chemical) had 80 to 90% inhibition upto 6 days. The inhibitory effect of CABMV was also confirmed in ELISA. After 6th day of treatment the virus was not detected and after 12th and 18th day, mild and strong reactions were observed in ELISA.

There is clear indication that period of protection from virus can be enhanced by the repeated applications of *Jatropha curcas*, *Ocimum sanctum* and *Clerodendrum inerme* up to 22, 20 and 24 days, respectively.

Phytoextracts of *Jatropha curcas* and *Ocimum sanctum* were inihibitory when diluted upto 1:100 whereas *Clerodendrum inerme* was efficient upto 1:500 in glass-house conditions with temperature ranging from 28-35°C

In different solvent studies, it was found that water was better solvent for all the three phytoextracts tried, whereas Acetone and Benzene failed to produce any inhibition. Ethanol and Methanol solvents had inhibition effect on virus extract of *J. curcas* and *O. sanctum* had less time inhibition as compared to water as solvent.

pH of solution was also found to have considerable effect on the inhibitory character of phytoextract solution on CABMV. pH 7.0 was the best for solution to be more effective against virus as compared to pH 6.0 and 8.0 while pH 5.0 and 9.0 for extracts were totally ineffective for all the phytoextracts tried.

The exposure of extracts to various temperatures before application had influence on inhibitory effect of the solutions. Temperature ranging from 50 to 55 °C was found to have no effect on inhibition activity. Temperature of solution ranging from 60 to 65 °C failed to produce considerable inhibition on CABMV for all the three phytoextracts tried indicating that the inhibitors might be denatured or inactivated.

The total proteins in phytoextracts of *Jatropha curcas*, *Ocimum santum* and *Clerodendrum inerme* was estimated. In these three phytoextracts the protein content was 6.90, 3.65 and 5.95 per cent, respectively.

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