INDUCTION OF RESISTANCE AGAINST COWPEA APHID-BORNE MOSAIC VIRUS IN *Vigna unguiculata* var.*sesquipedalis* (L.)Verdcourt

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DECLARATION

I hereby declare that this thesis entitled **"Induction of resistance against cowpea aphid-borne mosaic virus in *Vigna unguiculata* var.*sesquipedalis* (L.)Verdcourt"** 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 of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled **"Induction of resistance against cowpea aphid-borne mosaic virus in *Vigna unguiculata* var.*sesquipedalis* (L.)Verdcourt"** is a record of research work done independently by Miss. Veena, I.V. (2005-11-120) 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

Vegetable cowpea, also known as yard long bean [*Vigna unguiculata* var. *sesquipedalis* (L.) Verdcourt] is an important leguminous vegetable crop grown in different parts of the world. This crop has come to occupy a prime position among the vegetable crops raised in Kerala in coverage and popular preference. The tender green pods used as vegetable are rich in protein, minerals, vitamins and dietary fibre.

Cowpeas are susceptible to a wide range of pests and pathogens that attack the crop at all stages of growth. These include insects, bacteria, fungi and viruses. Viruses are known to occur and infect cowpea at all stages of the plant growth and its effects can be devastating and are a major constraint to increase production. More than twenty viruses are reported in cowpea from different parts of the world (Thottappilly and Rossel, 1992). Among the viruses infecting cowpea, cowpea aphid-borne mosaic virus (CABMV) is a serious one causing heavy losses in yield. Cowpea aphid-borne mosaic potyvirus (CABMV) is a cosmopolitan, economically significant seed-borne virus of cowpea. It can cause a yield loss of 13 - 87% under field conditions depending upon crop susceptibility, virus strain and the environmental conditions (Bashir et al., 2002). CABMV was first described from Italy by Lovisolo and Conti (1966) and was referred to as the European strain of the virus. It was reported to cause severe distorting mosaic in cowpea. Umamaheswaran (1996) described that first visible symptom of CABMV infection appeared as vein clearing followed by conspicuous mottling, chlorosis of leaflets, cupping, arching and inward curling of margins of leaflets, stunting, shortening of internodes, excessive branching and deformed pods.

Management of virus diseases is more difficult than that of diseases caused by other pathogens. Integration of various approaches like the

avoidance of sources of infection, control of vectors using chemicals, cultural practices and use of resistant host plants are used. The use of disease resistant varieties is the most important method of plant disease control as it puts no economic burden on farmers and possesses no hazard to the environment. Unfortunately almost all cultivable and popular cowpea varieties are susceptible to CABMV. A broad and often over use of pesticides is ecologically harmful, toxic to many vertebrates and may lead to development of pesticide resistance in pathogen. To produce pesticide free produce and improve the environment, the most important plant protection technique that can be used is induced resistance. This method is based not on direct pathogen suppression as it occurs in the application of toxic chemicals, but on stimulating natural defence mechanism in plant tissues. In some cases, the toxic chemicals that are applied to protect the plant may suppress plant immunity when the adaptation threshold is exceeded. There are certain cases when the plant immunity itself requires protection. Therefore, a development of various methods for immune-correction is presently of vital importance for controlling and enhancing the immune response in plants.

Induced resistance is also referred to as immunization, vaccination, acquired immunity and sometimes cross protection. Induced resistance is heightened resistance in plant towards pathogen as a result of a previous treatment with a pathogen, an attenuated pathogen or a chemical that is not itself a pesticide (Deverall and Dann, 1995). Induced resistance may be localized at the site of the inducing treatment or it may be systemic in all or some parts of the plant distant from the site of induction. Induced resistance is distinguished from conventional chemical as well as biological procedures in plant protection by the lack of toxicity of the inducing agents towards the pathogens. The basic idea behind induced resistance is that genes for resistance or defense reactions exist in all plants and these genes are not expressed until after a resistance inducing treatment activates or enhances their expression, or that changes in the plant metabolism modify the activity of such genes. Induced resistance can be elicited by the application of living organisms or by abiotic treatments. Plant immunization may find its niche in sustainable agriculture as one of a variety of practices that

promote plant health and reduce plant disease. Immunization of plant with biotic or abiotic inducers effectively controls disease in field.

The present study was undertaken to exploit the use of biotic agents and abiotic factors for the management of CABMV in cowpea. For this study, various abiotic factors like chemicals inducing resistance *viz.*, salicylic acid, ethrel and benzoic acid; indigeneous materials inducing resistance *viz.*, panchagavya, neem seed oil emulsion, turmeric powder-baking soda mixture, fresh cowdung solution and vermiwash; chemicals having antiviral properties *viz.*, carbendazim, naphthalene acetic acid, manganese chloride, sodium phosphonate and betadiene and ten crude extracts of plants having antiviral properties and various beneficial / endophytic bacteria were screened to find their efficiency in local lesion host against CABMV. The best candidates were further evaluated in cowpea against CABMV and assessed the induction and dynamics of biochemicals, defence compounds and pathogenesis related proteins against CABMV.

Review of Literature

2. REVIEW OF LITERATURE

Induced resistance is a physiological "state of enhanced defensive capacity" elicited by specific environmental stimuli, whereby the plant's innate defenses are potentiated against subsequent biotic challenges (van Loon et al., 1998). This enhanced state of resistance is effective against a broad range of pathogens. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance; in both SAR and ISR, plant defenses are preconditioned by prior infection or treatment that results in resistance (or tolerance) against subsequent challenge by a pathogen or parasite. Inducing agents can be biotic or abiotic.

2.1 ABIOTIC FACTORS

2.1.1 Chemicals inducing resistance

White (1979) reported that pathogenesis related (PR) protein accumulation and resistance to tobacco mosaic virus (TMV) could be induced by treatment of tobacco with salicylic acid (SA), aspirin (acetyl SA) or benzoic acid (BA). Conti et al. (1988) found that a very low concentration of 0.01 per cent equivalent to 50 μM acetyl salicylic acid (ASA) was effective in inducing resistance in *Datura* against TMV. The effect was greater when ASA was applied after the inoculation of virus.

Exogenous application of SA or the synthetic resistance-inducing chemical benzo (1,2,3) thiadiazole-7-carbothioic acid methyl ester (BTH) can induce some degree of resistance to viruses even in plants that do not possess a corresponding resistance gene (White et al., 1983; Hooft van Huijsduijnen et al., 1986; Friedrich et al., 1996).

Brederode et al. (1991) found that ethephon treatment on TMV infected tobacco results in the induction of acidic PR proteins and thereby inducing

resistance. Ryals et al. (1994) found that chemical inducers such as SA and its analogues, BTH and dichloroisonicotinic acid (INA) could elicit systemic acquired resistance (SAR).

Treatment with SA caused reduced accumulation of TMV RNA in directly inoculated TMV-susceptible tobacco leaf tissue (Chivasa et al., 1997). It was also found that the ratio of genomic RNA to coat protein (CP) mRNA and the ratio of plus- to minus-sense RNAs were affected by SA treatment. In SA-treated tobacco, the accumulation of at least two viruses, TMV and *Potato virus X (PVX)* was inhibited at the site of inoculation (Chivasa et al., 1997; Naylor et al., 1998). Salicylic acid plays a critical signaling role in the activation of disease resistance in plants (Dempsey et al., 1999; Durrant and Dong, 2004).

Murphy et al. (2001) observed that a state of enhanced disease resistance can be induced by treatment with solutions of SA or its synthetic functional analogues. Murphy and Carr (2002) reported that the replication of TMV was greatly decreased in the leaf mesophyll cells of SA treated plants. It also induced resistance to movement of virus between the epidermal cells of host plant.

Wong et al. (2002) found that SA, nonlethal concentrations of cyanide and Antimycin A (AA) could induce resistance to Turnip vein clearing virus (TVCV). They observed inhibition of TVCV in SA treated *Arabidopsis.*

Gruner et al. (2003) reported that pathogenesis-related protein 1a (PR-1a) is induced in tobacco plants during the hypersensitive response (HR) after exposure of plants to SA. Singh et al. (2004) reported multiple antiviral defence mechanism of SA. He observed that SA triggers resistance to viral infection process viz. replication, cell to cell movement and long distance movement.

Carl et al. (2005) found that SA induced resistance against CMV in tobacco *(Nicotiana tabacum)* results from inhibition of systemic virus movement. This could be by signal transduction pathway that also can be triggered by antimycin A, an inducer of the mitochondrial enzyme alternative oxidase (AOX).

They found that inhibition of CMV systemic movement is also induced by SA and antimycin A in *Arabidopsis thaliana.*

2.1.2 Indigenous materials inducing resistance

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

Neem seed oil and neem leaf extracts had been reported to inhibit lesion production by mechanically transmitted viruses when mixed with the inoculum or when applied to test plants (Verma, 1974; Chowdhuri and Saha, 1985; Zaidi et al., 1988).

Aiyanathan and Narayanaswamy (1988) studied the effect of neem oil on rice tungro virus infection and observed that the pre inoculation as well as post-inoculation spray of neem oil (5 per cent) reduced RTV infection. Mariappan et al. (1988) recorded significant reduction in the transmission of rice tungro virus (RTV) by *Nephotettix virescens* in neem oil-sprayed plants..

Pretreatment with neem oil reduced local lesion production by TMV on *Nicotiana glutinosa, N tabaccum*,var.samsun NN, *Chenopodium amaranticolor* and *Datura stramonium* (Roy choudhary and Jain, 1993).

Rajappan et al. (2000) found that lowest population of the vector, *N. virescens* was recorded with application of neem cake at 5 kg per 0.032 ha of nursery, followed by foliar spray of neem seed kernel extract at 5 percent in the main field. This results in reduction of RTV incidence.

2.1.3 Chemicals having antiviral properties

Prakash and Joshi (1979) reported maximum inhibition of cowpea banding mosaic virus by 92 per cent when six sprays of gallic acid was given after virus inoculation. Caner et al. (1984) examined activity of triazofurin (2-beta-D-ribo furanosyl thiazole-4-carboxamide) against tomato-spotted wilt virus (TSWV) in tomato plants. They found that 100 and 200 mg l^{-1} of triazofurin were the most

efficient concentrations to suppress TSWV infection, thereby delaying the appearance of systemic symptoms. The drug was more effective when applied after than before virus inoculation. Caner et al. (1985) reported that antiviral activity of chemicals like polyacrylic acid, distamycin A, EHNA, foscarnet and a pyrazoino-pyrazine derivative for the control of bean golden mosaic virus on *Phaseolus lunatus* .L.

Hayati and Varma (1985) reported that 0.02 per cent validamycin, 0.01 per cent abomycin A, 0.2 per cent guanidine carbonate, 0.1 per cent 8-azaguanine and 0.8 per cent azaadenine suppressed symptom expression of tomato leaf curl virus on tomato. White et al. (1986) reported that the chemicals manganese chloride and barium chloride induced resistance in Xanthi-nc tobacco leaves to TMV infection. Antiphytoviral activity of ribavirin, 2,4-dioxohexahydro-1,3,5 triazine (DHT) compounds against tobacco viruses has been reported by Schuster (1986).

Bauer et al. (1993) observed that the chemical 1-(K-carboxyalkyl)-4,5-dimethyl imidazol-3-oxide was capable of restricting the activity of red clover mottle virus (RCMV) and alfalfa mosaic virus in systemically infected host plants. Kovalenko et al. (1993) reported the induction of resistance with mannan sulphate in hypersensitive host plants against TMV infection.

Ghoshroy et al. (1998) found that exposure of tobacco plants to non toxic concentration of cadmium completely blocked viral disease caused by Turnip vein clearing virus. Cadmium mediated viral protection was due to inhibition of systemic movement of virus. Manickam and Rajappan (1999) studied the effect of chemicals and certain plant extracts on green gram leaf curl disease under pot culture. Copper sulphate and copper acetate (1000 ppm) were found to suppress the virus symptoms. Bioassay of chemicals, viz., SA, manganese chloride and barium chloride to evaluate their efficiency in reducing the symptoms caused by cowpea aphid borne mosaic virus (CABMV) in *Chenopodium amaranticolor* and cowpea revealed that the post inoculation

treatment of manganese chloride gave maximum inhibition of symptoms (Radhika, 1999).

Pappu et al. (2000) reported that acibenzolar-*S*-methyl (Novartis Crop Protection, Inc., Raleigh, NC) plant activator induced a defense mechanism and was shown to have antiviral activity across several different plant species. Pun et al. (2000) reported that barium chloride was most effective in reducing the symptoms of bhindi yellow vein mosaic virus when sprayed exogenously on bhindi plants.

Csinos et al. (2001) studied the effect of acibenzolar-*S*-methyl and imidacloprid on the management of TSWV in flue-cured tobacco. They reported that acibenzolar-*S*-methyl alone and along with imidacloprid significantly reduced TSWV incidence. They also found that pre transplant applications of acibenzolar-*S*-methyl were critical to the suppression of TSWV.

Herms et al. (2002) reported that tobacco cv xanthi nc plants treated with the synthetic strobilurin derivative F 500 showed reduction in infection by TMV. They found that F 500 enhances TMV resistance in tobacco either by acting downstream of SA in the SA signaling mechanism or by functioning independently of SA. Nakashita et al. (2003) reported that brassinolide (BL) the most important brassinosteroid (BR) which play role in the hormonal regulation of plant growth and development, was found to induce disease resistance in plants. Wild-type tobacco treated with BL exhibited enhanced resistance to TMV.

2.1.4 Crude extract of plants having antiviral proteins / principles

Verma and Kumar (1980) found that leaf extracts of *Datura* sp, *Azadirachta indica* and *Mirabilis jalapa* contain antiviral proteins which inhibited infection by CMV, TMV and yellow vein mosaic virus in black gram. Verma et al. (1996) reported that *Clerodendron aculeatum* leaf extract induced local and systemic resistance in host plant against TMV infection. Sreelekha (1987) reported that cowpea mosaic virus infection could be effectively controlled by pre inoculation spraying of *Bougainvillea* sp. and *Eupatorium odoratum*. Habuka et

al. (1991) found that AVP isolated from roots of *Mirabilis jalapa* inhibited mechanical transmission of plant viruses. Pre-inoculation application of plant extracts was found to be more effective than post inoculation application in reducing incidence of cowpea mosaic virus (Mallika, 1990). Pre-inoculation application of plant extracts of medicinal plants such as *Phyllanthus fraternus, Plumbago roseas* and *Thespesia populnea* was better than post inoculation application in inhibiting TMV infection. (Vimi louis and Balakrishnan, 1996). Foliar spraying of *Bougainvillea spectabulis* was effective in inhibiting infection of TSWV, TMV, CMV and CaMV (Balasaraswathy et al., 1998).

Vivanco et al. (1999) evaluated extracts of *Mirabilis jalapa* containing a ribosome inactivating protein (RIP) called mirabilis antiviral protein (MAP), against infection by potato virus X, potatovirus Y, potato leaf roll virus, and potato spindle tuber viroid and found that there were significant reduction in viral incidence. Mandal and Singh (2001) reported that tender guava leaf extract had inhibitory effect on the sap transmission of chilli mosaic virus. A greater inhibitory activity of virus transmission by guava leaf extract was observed when it was mixed with the virus inoculum.

Mistry et al. (2003) found that plant extracts from *Clerodendron inerme* and *Ocimum sanctum* efficiently reduced systemic infection of tobacco chlorotic mottle virus in cowpea. The plant extracts were found to induce systemic resistance in the host, as highest retardation in infection was noticed when extracts were sprayed 24 h prior to inoculation.

Bhatia (2004) tested the antiviral proteins from *Bougainvillea* along with virus and also before virus inoculation. When AVPs are applied prior to virus inoculation or in combination with virus, there was suppression of necrotic lesion formation in local lesion host. Renukadevi et al. (2004) found that the antiviral protein from *M. jalapa* (MAP) and *Herpula cupanioides* (HAP) were highly effective in inhibiting TSWV. Pre-application of MAP and HAP induced the activity of phenols, peroxidase (PO), polyphenol oxidase(PPO) and

phenylalanine ammonialyase (PAL) leading to the suppression of TSWV on local lesion and systemic host.

Gholizadeh et al. (2004) observed that leaf extract of *Celosia cristata,* imparted good level of resistance against different viruses. The purified proteins could reduce number of local lesions caused by different viruses like TMV, sunhemp rosette virus (SRV) and potato virus x (PVX). An antiviral protein (AVP), imparting high level of resistance against SRV was purified from the dried leaves of *Amaranthus tricolor.* The purified protein (AAP-27) exhibited 98 per cent inhibition of local lesion formation at a concentration range of 30 $\mu g\ ml^{-1}$ (Roy et al., 2006).

Prasad et al. (2007) evaluated the efficacy of certain plant extracts in reducing *Bean common mosaic potyvirus* strain *blackeye cowpea mosaic* (BCMV-BlCMV) disease in cowpea. They found that when plant extracts were mixed with BCMV-BlCMV inoculum and young seedlings were inoculated, *B. spectabilis*, *C. inerme* and *M. jalapa* extracts reduced the disease incidence up to 42, 40 and 48 per cent respectively under greenhouse conditions when compared to control.

2.2 BIOTIC AGENTS

Mann (1965) reported that culture of *Bacillus uniflagellatus* and extracts from such cultures when applied to soil could significantly reduce TMV infection.

PGPR mediated systemic resistance is often associated with onset of defence mechanism including the early and increased expression of defence enzymes such as chitinase, glucanase, peroxidase and phenyl alanine ammonia lyase and accumulation of phenolics, phytoalexins and lignins (Mosch et al., 1993; Schneider and Ullrich 1994; Chen et al., 2000; Nandakumar et al., 2001). Maurhofer et al. (1994) evaluated the root-colonizing bacterium *Pseudomonas fluorescens* as an inducing agent against the lesion-inducing tobacco necrosis virus (TNV) in tobacco. They observed a reduction in TNV induced lesion number in *P. fluorescens* treated plants. They reported that ISR by *P. fluorescens*

strain CHAO against TNV in tobacco was associated with accumulation of PR proteins namely β-1,3 glucanase and endo chitinases.

Raupach et al. (1996) reported that seed-treatment with *P. fluorescens* strain 89B-27 and *Serratia marcescens* strain 90-166 has consistently reduced the number of CMV infected plants and delayed the development of symptoms in cucumber and tomato. Induced systemic resistance (ISR) elicited by plant growth-promoting rhizobacteria (PGPR) has shown promise in managing a wide spectrum of plant pathogens, including virus, in several plant species under greenhouse and field environments (Murphy et al., 2000; 2003; Raupach et al., 1996; Wei et al., 1996; Zehnder et al., 2000).

Maurhofer et al. (1998) showed that soil drenched with *Pseudomonas fluorescens* CHA0 strain induced systemic protection against TNV in tobacco. de Meyer et al. (1999) observed that *Pseudomonas aeruginosa* 7NSK2 elicited ISR against TMV.

Murphy et al. (2000) studied the effect of PGPR under field conditions for induced resistance to Tomato mottle virus (ToMoV). They showed that under natural conditions of high levels of vector–virus pressure, some PGPR treatments resulted in reduced ToMoV disease severity. Zehnder et al. (2000) evaluated specific strains of PGPR for induced resistance against CMV in tomato. They identified PGPR strains that protected tomato against systemic infection by CMV under greenhouse and field conditions. In greenhouse experiments where plants were challenged by mechanical inoculation of CMV, the percentage of symptomatic plants in the most effective PGPR treatments ranged from 32 to 58 percent, compared with 88 to 98 percent in the non bacterized, challenged disease control treatment.

Pseudomonas fluorescens was effective in suppresing the infection of TSWV in tomato under glasshouse and field conditions (Kanden et al., 2002). Kanchalee et al. (2003) showed that four mixtures of PGPR strains (all *Bacillus* spp.) elicited induced systemic resistance in several plants against different plant

pathogens. They tested these mixtures against CMV and observed reduction in disease incidence.

Ryu et al. (2004) found that *Arabidopsis thaliana* ecotype Columbia plants (Col-0) treated with PGPR, *Serattia marcescens* strain 90-166 and *Bacillus pumilus* strain SE34 had significantly reduced symptom severity of CMV infection.

2.3 BIOCHEMICAL CHANGES OF HOST PATHOGEN INTERACTION

2.3.1 Protein

Singh et al. (1978) found that southern bean mosaic virus infection in cowpea resulted in higher total nitrogen, total protein, nitrate and nitrite nitrogen than in healthy leaves of cowpea. Singh and Singh (1981) while investigating the changes in nitrogenous constituents of cowpea pods due to cowpea mosaic virus infection found that there was an increase in total nitrogen, protein and nitrate nitrogen. Johri and Pandhi (1985) while investigating the effect of yellow vein mosaic virus on the physiology of okra reported that the total protein declined in diseased tissues but its insoluble fraction increased in diseased tissues as against soluble fraction. Ahmed et al. (1992) reported that total protein and soluble proteins were found high in virus free resistant varieties. Mali et al. (2000) reported that free aminoacids and soluble protein content increased with increasing levels of yellow mosaic virus infection in susceptible variety of moth bean. Manickam et al. (2000) studied the impact of application of a foliar spray of AVPs from *Cocos nucifera, Sorghum vulgare, Sorghum bicolor* and *Croton sparsiflorus* leaves and inoculation of TSWV on the non-reducing sugar and total soluble protein contents of cowpea plants. It was found that AVP treated cowpea plants showed marginal increase in protein contents compared to significant increase in TSWV inoculated plants. They also studied the effect of foliar spray of AVPs and inoculation of TSWV on the RNA content of *Vigna unguiculata* and *Vigna radiata* plants. TSWV inoculation showed a significant increase in the RNA content while there was no change in RNA content in AVPs treated cowpea plants inoculated with TSWV. Sindhu (2001) studied total soluble protein content

in the susceptible cowpea plants inoculated with BICMV. The total soluble protein was found higher in the case of inoculated susceptible plants.

2.3.2 Total and reducing sugars

An increase in total sugar content was reported in virus infected plants (Prasad et al., 1995). Khatri and Chenulu (1969) reported that reducing sugar content was not appreciably affected by cowpea mosaic virus in resistant and susceptible cowpea cultivars. Ramiah (1978) found that there was decrease in synthesis of total carbohydrates in infected leaves of susceptible cowpea. He also observed that the trifoliate leaves showed reduction in the level of carbohydrates commencing from the 10^{th} day after inoculation. Singh and Singh (1979) studied the changes in carbohydrate fractions of black gram due to severe mosaic infection. They observed that there was reduction in reducing and non-reducing sugars, total sugar and starch in infected leaves, stems and roots at every stage of infection. Rajendrasingh and Kumar Singh (1980) reported a decrease of total sugar and starch in leaf tissues of sunhemp infected with bean mosaic virus. Singh and Singh (1984) observed that the virus infection decreased total sugar and starch in cowpea cultivars infected with southern bean mosaic virus and cowpea mosaic virus. Johri and Pandhi (1985) reported that the carbohydrate level declined positively with severity of disease symptoms in case of yellow vein mosaic of okra. Sastry and Nayudu (1988) recorded a higher quantity of carbohydrate in hypersensitive cowpea cultivars infected with tobacco ring spot NEPO virus and suggested that the infected area could act as a metabolic sink. Bensal et al. (1990) found that there was significant increase in amount of total sugars, amino acid and phenol in virus infected plants sprayed with sorghum leaf extract.

Thind et al. (1996) reported that the amount of reducing sugars, non - reducing sugars, total sugars and starch decreased in black gram infected with yellow mosaic virus when compared to healthy plants. Umamaheswaran (1996) found that the level of carbohydrate was significantly lower in susceptible varieties of cowpea when inoculated with CABMV.

Bhagat and Yadav (1997) reported that healthy leaves of susceptible and highly susceptible cultivars showed higher content of reducing, non reducing and total sugar than resistant cultivars of bhindi when inoculated with bhindi yellow vein mosaic virus. It was also reported that increased sugar content in inoculated leaves of bhindi was due to their accumulation, as a result of the disruption of normal phloem transport. Mali et al. (2000) reported that infection of yellow mosaic virus in moth bean resulted in reduction of total soluble carbohydrate in susceptible when compared to resistant genotypes. Manickam et al. (2000) reported that foliar spray of AVPs from *Cocos nucifera, Sorghum vulgare, Sorghum bicolor* and *Croton sparsiflorus* leaves increased the non reducing sugar content in cowpea plants while TSWV inoculation decreased its concentration. Sutha et al. (1998 a) studied the changes in concentrations of chemical constituents in tomato caused by TSWV infection and revealed that there was accumulation of carbohydrate in infected plants. Total, reducing and non-reducing sugars decreased in infected leaves and the reduction was more in the initial stages compared to later stages of infection. In contrast to sugar concentration, the starch increased in infected plants at all stages of infection. Sutha et al. (1998 b) reported that TSWV infection reduced the concentration of total, non-reducing and reducing sugars of tomato fruits.

2.3.4 Phenol

Ramiah (1978) found that there was no difference in phenol content between healthy and inoculated leaves of MS 9804 and CO-1. It was reported that in the variety CO-2 the inoculated leaves had higher content of phenol than that of healthy leaves at 40 days after inoculation with cowpea aphid-borne mosaic virus. Ando et al. (1984) reported that fungitoxic phenolic compounds were released from CMV infected cowpea protoplast. Sharma et al. (1984) studied the effect of virus and fungus infection in muskmelon and showed an increasing trend of the enzyme activity and phenol component as compared to healthy control irrespective of the nature of infection. Rathi et al. (1986) assayed total phenol and other biochemical parameters in pigeonpea cultivars resistant and

susceptible to sterility mosaic virus and reported that there was no difference between varieties with respect to total phenol content. Sastry and Nayadu (1988) recorded higher quantities of phenolic compounds in hypersensitive cowpea leaves infected with tobacco ring spot virus. Kato et al. (1993) extracted and characterized two phenolic compounds from cowpea leaves infected with cucumber mosaic virus. Sutha et al. (1997) found that both total phenol and *ortho*-dihydroxy phenol increased in TSWV infected plants. Resistant cultivars had higher content of phenol, OD- phenol and flavanol due to cotton leaf curl virus infection when compared to susceptible varieties (Gurdeep Kaur et al., 1998). Radhika (1999) reported that there was not much change in phenol content in both resistant and susceptible varieties of cowpea infected with black eye cowpea mosaic virus. Mali et al. (2000) reported that *ortho*-dihydroxy phenol was higher in healthy leaves than diseased leaves in case of yellow mosaic virus infected moth bean. Sutha et al. (1998 b) reported that TWSV infection reduced the concentration of total and *ortho*-dihydroxy phenol contents of tomato fruits.

2.3.5 Defence Related Enzymes

Khatri and Chenulu (1970) studied the changes in the peroxidase enzyme activity in leaves of resistant and susceptible cowpea varieties and observed that the peroxidase activity increased in both resistant and susceptible variety but was higher in susceptible variety. Batra and Kuhn (1975) found that when primary leaves of hypersensitive soybean plants were infected with cowpea chlorotic mottle virus, the enzymes polyphenol oxidase and peroxidase increased 2-3 times over healthy plants. They also found that the increase was concomitant with the development of acquired resistance.

Wagih and Coutts (1982) reported that tobacco necrosis virus infected cowpea and cucumber showed increase in the amount of extractable peroxidase and polyphenol oxidase activity. Sharma et al. (1984) while studying the effect of virus and fungus infection in musk melon found an increasing trend of enzyme activity when compared to healthy control. Verma and Prasad (1984) found that

spraying aqueous leaf extract of *Clerodendron aculeatum* prevented infection of sunhemp rosette virus on cluster beans.The resistance thus induced was due to increased activity of catalase, peroxidase and polyphenol oxidase.

Rathi et al. (1986) assayed peroxidase, polyphenol oxidase and isozyme of peroxidase in pigeon pea resistant and susceptible cultivars to sterility mosaic virus and noted that there was not much difference between two varieties with respect to peroxidase and polyphenol oxidase activities. Resistance was characterized by the presence of specific isoperoxidase and proteins. Zaidi et al. (1992) reported the changes in phenolic content and phenylalanine ammonialyase in response to infection by carnation etch ring virus. The results suggested the existence of a positive correlation between the elevated levels of phenolics and phenylalanine ammonialyase with disease resistance. Ahmed et al. (1992) found that peroxidase and polyphenol oxidase showed no significant difference in virus free susceptible and resistant plants while studying biochemical basis of resistance to yellow vein mosaic virus in okra. Umamaheswaran (1996) reported that there was progressive increase in peroxidase, polyphenol oxidase and phenylalanine ammonialyase activity in CABMV inoculated and susceptible varieties of cowpea. Mali et al. (2000) reported that the activity of catalase, peroxidase and nitrate reductase was found to reduce with increased intensity of disease in the case of yellow mosaic disease of moth bean (*Vigna aconitifolia*). Radhika and Umamaheswaran (2000) reported higher activity of peroxidase, polyphenol oxidase and phenylalanine ammonialyase in resistant variety when compared to susceptible variety of cowpea infected with BICMV. Muthulakshmi and Renukadevi (2001) found that reduction in percentage of infection by rice tungro virus may be due to induction of defence related proteins (PR 1 and PR 2) by application of AVP.

Sindhu (2001) investigated on changes of defence related enzymes *viz.*, peroxidase, polyphenol oxidase and phenylalanine ammonialyase and indicated that there was significant increase in activities of these enzymes in inoculated cowpea plants.

Bhatia et al. (2004) found that the activities of enzymes superoxide dismutase and peroxidase were increased as against decreased activities of catalase in TMV infected tobacco leaves. The trend was reversed when the leaves were treated with AVP alone. However, the activities of all the three enzymes decreased in TMV plus AVP treated leaves and it was midway between TMV alone and AVP alone treatments.

2.4 ELECTROPHORESIS OF SOLUBLE PROTEINS

Gianinazzi and Kassanis (1973) studied the effect of polyacrylic acid (PA) in inducing resistance in tobacco cv. Xanthi-nc plants infected with TMV. Disc-electrophoresis in 10 percent polyacrylamide gels showed three additional soluble proteins in PA injected leaves.

Parent and Asselin (1987) described the pattern of proteins that accumulated in the intercellular fluids (IF) of potato leaves on TMV infection and found that these IF proteins contained chitinase, β -1, 3 glucanase and peroxidase.

Maurhofer et al. (1994) performed polyacrylamide gel electrophoresis of TNV affected *Nicotiana glutinosa* plants which were previously inoculated with *P. fluoroscens* strain CHAO. Several clearly visible protein bands appeared on the gel. The PR proteins 1a, 1b and 1c were identified together with the β- 1, 3 glucanase and the endochitinase P and Q.

Ayisha (2005) performed polyacrylamide gel electrophoresis to analyse the protein profile of cowpea plants under inoculated and uninoculated conditions. The experiment revealed three newly induced virus related proteins in the inoculated cowpea plants compared to healthy control. The new induced proteins obtained were with molecular weight 28 kDa, 15 kDa and 6.2 kDa.

Materials and Methods

3. MATERIALS AND METHODS

3.1 ABIOTIC FACTORS

Different chemicals, plant extracts and indigenous materials possessing antiviral property are known to induce resistance against viral infection in plants. This study was undertaken to evaluate the above for the management of cowpea aphid borne mosaic virus (CABMV) in cowpea (Plate 1a and 1b). All the studies were conducted in an insect proof glass house.

3.1.1 Chemicals inducing resistance

Chemicals like salicylic acid (w/v), ethrel (ethephon) (v/v) and benzoic acid (w/v) were taken to induce resistance against CABMV. Aqueous solutions of these chemicals were prepared at 100 ppm, 250 ppm, 500 ppm and 1000 ppm.

3.1.2 Indigenous materials inducing resistance

Indigenous materials like vermi-wash (v/v), fresh cowdung solution (w/v), panchagavya (v/v), neem seed oil emulsion (v/v) and turmeric powder-baking soda mixture (w/v) (4:1) were used in this study at 1 per cent, 5 per cent and 10 per cent concentrations to evaluate the same for the management of CABMV.

3.1.3 Chemicals having antiviral properties

Different chemicals are known to possess antiviral property. The following chemicals at different concentrations were used in this investigation to find out their antiviral property.

1a. Vein banding symptom

1b. Mosaic symptom

Plate 1. CABMV infected cowpea

Plate 2. Local lesion host, *Chenopodium amaranticolor*

Sl No.	Chemicals having antiviral properties	Concentration
1	Carbendazim	0.05 %, 0.1% & 0.2% (w/v)
2	Betadiene	0.1%, 0.2 % & 0.5% (v/v)
3	Sodium/Potassium phosphonate	0.05%, 0.2% & 0.4 % (v/v)
4	Naphthalene acetic acid	100, 250 & 500 ppm (w/v)
5	Manganese chloride	100, 250 & 500 ppm (w/v)

3.1.4 Crude extract of plants having antiviral proteins / principles

Plants possessing antiviral proteins were screened for their efficiency against CABMV. Leaf extracts of such plants were prepared at 5%, 10% and 15% concentrations. The plants mentioned below are widely used in Ayurvedic medicines against various human diseases. The following are the list of plants having antiviral proteins

Sl.No.	Plants having antiviral proteins	Common name
1	*Adathoda vasica*	Adalodakom
2	*Boerhavia diffusa*	Thazhuthama
3	*Bougainvillea* spp	Bougainvillea
4	*Catharanthus roseus*	Shavamnaari
5	*Datura stramonium*	Ummam
6	*Hemidesmus indicus*	Naruthandi
7	*Ocimum sanctum*	Thulasi
8	*Mirabilis jalapa*	4 O' clock plant
9	*Phyllanthus niruri*	Keezharnelli
10.	*Jathropha curcus*	Jatropha

3.2 BIOTIC AGENTS

Biotic agents like beneficial and endophytic bacteria were used in this study to evaluate their efficiency in inducing resistance against CABMV.

3.2.1 Beneficial / Endophytic bacteria

Endophytic and Rhizobacteria isolated from cowpea, *Pseudomonas fluorescence* and *Bacillus* sp from TNAU and KAU were used for the study. These were evaluated at different concentration viz., 10^7, 10^8, 10^9 & 10^{10}cfu ml^{-1}.

3.2.1.1 Bioassay in local lesion host

The efficacy of biotic and abiotic factors mentioned above was evaluated in the local lesion host of the virus, *Chenopodium amaranticolor* (Plate 2). Pre-inoculation, post-inoculation and simultaneous inoculation treatments were done.

In pre-inoculation, materials were sprayed 24 h prior to mechanical inoculation of virus. For mechanical transmission of virus, sap was extracted from young leaves showing severe mosaic symptoms. One part of the leaf tissue was homogenized with one part of ice cold 0.01M phosphate buffer (pH 7.2) using a chilled mortar and pestle. The homogenate was strained through a thin layer of cotton. The supernatant was maintained in an icebox and immediately used for inoculation. Inoculation was done on the leaves of local lesion host, *Chenopodium amaranticolor*. Plants at 8-10 leaf stage were chosen. Leaves at the middle portion of the plant (Fourth to eighth leaves) were inoculated. Prior to inoculation leaves were uniformly dusted with celite powder. Test plants were inoculated with the pestle moistened with the inoculum by gently rubbing on the upper surface of the fully opened leaves. The surface was rinsed off after 5 minutes with distilled water using a wash bottle.

In post-inoculation, materials were sprayed 24 h after inoculation of virus. Materials and virus inoculum were mixed together and applied in simultaneous inoculation treatment. The plants were kept for 6-7 days for the development of symptoms. Control leaves were maintained without any treatment.

Plants treated with biotic and abiotic factors were kept in glass house. Local lesions were recorded for evaluating the efficiency. From this per cent inhibition was calculated based on the formula.

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

C=Number of lesions on control leaves

T= Number of lesions on treated leaves

3.2.1.2 Bioassay in Cowpea plants

Induction of resistance was also done on cowpea plants cv. Sharika. A pot culture experiment was laid out in CRD with three replications. Treatments of

biotic and abiotic factors which showed high inhibition percentage in local lesion assay were selected for the bioassay in cowpea plants. Among the abiotic factors, chemicals like salicylic acid (250 ppm), ethrel (250 ppm), betadiene (0.1 percent) and plant extracts viz., *Phyllanthus niruri* (10 percent) and *Boerhavia diffusa* (10 percent) were used for evaluation. *Pseudomonas fluorescence* (TNAU culture, 10^8cfu ml^{-1}), the biotic agent was also selected for the assay. Pre, post and simultaneous inoculation treatments were done as that of previous experiment. Control plant was also maintained. Treatments were imposed at primary leaf stage. Effects of materials on expression of symptoms were recorded. Based on the severity of symptoms, vulnerability index was calculated. This was in accordance with the 0-5 scale developed by Bos (1982) as mentioned below.

0 = no symptom

1 = slight vein clearing, very little mottling of light and dark green colour in younger leaves - Resistant (R)

2 = mottling of leaves with light and dark green colour – Medium resistant (MR)

3 = blisters and raised surface on the leaves - Medium susceptible (MS)

4 = distortion of leaves - Susceptible(S)

5 = stunting of the plant with negligible or no flowering and fruiting - Highly susceptible (HS)

Based on the rating, Vulnerability Index (VI) was calculated using the following equation,

$$VI = \frac{(0n_1 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5) \times 100}{n_t\ (n_c - 1)}$$

VI = Vulnerability Index

$n_0, n_1, \ldots\ldots n_5$ = number of plants in the category 0,1,2,3,4,5

n_t = Total number of plants

n_c = Total number of categories

3.3 BIOCHEMICAL CHANGES OF HOST PATHOGEN INTERACTION

Biochemical analysis of healthy, diseased and treated plants was carried out. Cowpea variety Sharika was selected for the study. Seeds were sown and mechanically inoculated at primary leaf stage. Treatments were done as pre, post and simultaneous application. Samples were taken at one day, three day, five day, fifteen day and thirty day after inoculation.

Biochemical analysis was conducted to estimate the changes in total sugars, reducing sugar, phenol and protein. Analysis of defence related enzymes such as peroxidase, polyphenol oxidase, and phenylalanine ammonialyase were also carried out. Protein profile study was performed using SDS-PAGE.

3.3.1 Estimation of Protein

Total soluble protein content was estimated as per the procedure described by Bradford (1976). One gram of leaf sample was homogenized in 10 ml of 0.1 M sodium acetate buffer (pH 4.7) and centrifuged at 5000 g for 15 min at 4° C. The supernatant was collected for estimation of soluble protein. The reaction mixture consisted of 0.5 ml enzyme extract, 0.5 ml distilled water and 5 ml of diluted (5 times) dye solution. Blank was prepared with 1 ml distilled water and 5 ml of diluted (5 times) dye solution. The absorbance was read at 595 nm in a spectrophotometer against reagent blank. Bovine serum albumin was used as the protein standard. The protein content was expressed as microgram albumin equivalent of soluble protein per gram on fresh weight basis.

3.3.2 Estimation of Total Sugars

Total sugar content was estimated by Anthrone method (Hedge and Hofreiter, 1962). Samples of 100 mg each were weighed out and hydrolyzed with 5 ml of 2.5 N hydrochloric acid (HCl) in a boiling water bath. The hydrolyzate was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged at 5000 rpm for 15 min. From the

supernatant 0.5 ml aliquot was taken and made up to one ml by adding distilled water. To this 4 ml anthrone reagent was added and heated for eight minutes in a boiling water bath. This was cooled rapidly and absorbance was measured at 630 nm in a spectrophotometer (Systronics UV-VIS Spectrophotometer 118). Blank was prepared with 1 ml distilled water and 4 ml anthrone reagent. Amount of carbohydrate present was calculated from standard graph prepared using glucose and expressed in terms of milligrams of glucose equivalent per gram of leaf tissue on fresh weight basis.

3.3.3 Estimation of Reducing Sugars

Reducing sugar content was estimated by dinitrosalicylic acid method. One hundred milligram leaf sample was ground in 10 ml of 80 percent ethanol and centrifuged at 5000 rpm for 15 min. The supernatant was collected and evaporated by keeping it on a water bath at 80° C. The sugars were dissolved in 10 ml distilled water. An aliquot of 0.5-3 ml was pipetted out in test tubes and volume was equalized to three ml with distilled water in all the tubes. To this 3 ml of dinitrosalicylic acid reagent (DNS) was added. Contents were heated in a boiling water bath for 5 min and to this 1 ml of 40 percent rochelle salt solution was added. After cooling the absorbance was measured at 510 nm against reagent blank. Amount of reducing sugar was calculated from standard graph prepared using glucose and expressed in terms of milligrams of glucose equivalent per gram of leaf tissue on fresh weight basis.

3.3.4 Estimation of Phenol

The phenol content was estimated following the procedure described by Bray and Thorpe (1954). One gram leaf sample was ground in 10 ml of 80 per cent ethanol. The homogenate was, centrifuged at 10000 rpm for 20 min and the supernatant was saved. The residue was extracted with five times the volume of 80 per cent ethanol and again centrifuged. The supernatant was saved and evaporated to dryness. The residue was dissolved in 5 ml distilled water. An aliquot of 0.3 ml was pipetted out and made up to 3 ml with distilled water. Folin - Ciocalteau reagent (0.5 ml) and 2 ml of 20 per cent sodium carbonate solution

was added to each tube after three minutes. This was mixed thoroughly and kept in boiling water for one min. After cooling absorbance was measured at 650 nm against reagent blank. Blank was prepared with 3 ml distilled water, 0.5 ml Folin - Ciocalteau reagent and 2 ml of 20 per cent sodium carbonate solution. Standard curve was prepared using different concentrations of catechol. The phenol content was expressed in catechol equivalents as microgram per gram leaf tissue on fresh weight basis.

3.3.5 Estimation of Defence Related Enzymes

3.3.5.1 Estimation of Phenylalanine ammonialyase (PAL)

PAL activity was analysed based on the procedure described by Dickerson et al. (1984). The enzyme extract was prepared by homogenizing one gram leaf sample in 5 ml of 0.1 M sodium borate buffer (pH 8.8) containing a pinch of PVP using chilled mortar and pestle. The homogenate was centrifuged at 10000 rpm for 10 minutes at 4^0C. The supernatant was used for the assay of PAL activity. The reaction mixture contained 3 ml of 0.1 M sodium borate buffer (pH 8.8), 0.2 ml enzyme extract and 0.1 ml of 12 mM *l*-phenylalanine prepared in the same buffer. The blank contained 3 ml of 0.1 M sodium borate buffer (pH 8.8) and 0.2 ml enzyme extract. The reaction mixture and blank was incubated at 40^0C for 30 min and reaction was stopped by adding 0.2 ml of 3 N hydrochloric acid. The absorbance was read at 290 nm in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). PAL activity was expressed as micrograms of cinnamic acid produced per min per gram on fresh weight basis.

3.3.5.2 Estimation of Polyphenol Oxidase (PPO)

Polyphenol oxidase activity was determined as per the procedure given by Mayer et al. (1965). The enzyme extract was prepared as per the procedure given for the estimation of peroxidase.

The reaction mixture contained one ml of 0.1 M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract. The reaction was initiated

after adding one ml of 0.01 M catechol. The observations were recorded in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). The change in absorbance was recorded at 495 nm and PPO activity was expressed as change in the absorbance of the reaction mixture per minute per gram on fresh weight basis.

3.3.5.3 Estimation of Peroxidase (PO)

Peroxidase activity was determined as per the procedure described by Srivastava (1987). Leaf sample of 200 mg was homogenized in one ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 4^0C using a mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 min at 4^0C. The supernatant was used as the enzyme extract for the assay of PO activity.

The reaction mixture consisting of 1 ml 0.05 M pyrogallol and 50 µl of enzyme extract was taken in both reference and sample cuvettes mixed well and kept in a spectrophotometer (Systronics UV-VIS spectrophotometer 118). The reading was adjusted to zero at 420 nm. The enzyme reaction was started by adding one ml of one per cent hydrogen peroxide (H_2O_2) (v/v) into sample cuvettes and change in absorbance was measured at 30 seconds interval.

3.4 ELECTROPHORETIC ANALYSIS OF PROTEINS

Characterization of proteins by SDS-PAGE

Electrophoretic separation of soluble protein of cowpea leaves were carried out as per the procedure described by Laemelli (1970). Leaf sample of healthy, diseased and treated plants were taken for analysis.

Five hundred milligram each of healthy infected and treated leaf samples were homogenized in 200 µl of cold denaturing solution at 4°C. The supernatant was mixed with chilled acetone in the ratio 1:4 and the protein was allowed to precipitate by keeping the mixture at 4°C for 30 min. The sample was centrifuged

at 5000 rpm for 15 min at 4°C. The precipitate was resuspended in 20 µl of denaturing solution and vortexed. The homogenate was centrifuged at 5000 rpm for 15 min. The supernatant was mixed with equal volume of sample buffer and kept in a boiling water bath for 3 min. These samples were used for SDS-PAGE. Ten µl of medium range molecular weight markers (Genei, Bangalore) mixed in 10 µl of sample buffer were also loaded. The protein concentration was adjusted in each sample to strength of 100 µg of protein following Bradford method.

Reagents

a) Acrylamide stock (30 %)

Acrylamide	- 29.2 g
Bis-acrylamide	- 0.8 g
Double distilled water	- 100.0 ml

b) Separating (resolving) gel buffer stock (1.5 M Tris-HCl p^H 8.8)

Tris base (18.15 g) was dissolved in approximately 50 ml of double distilled water. The p^H was adjusted to 8.8 with 6 N HCl and made up the volume to 100 ml with double distilled water and stored at 4°C.

c) Stacking gel buffer stock (0.5 M Tris-HCl p^H 6.8)

Tris base (6.0 g) was dissolved in approximately 60 ml of double distilled water and adjusted the p^H to 6.8 with 6 N HCl and the volume was made upto 100 ml with double distilled water and stored at 4°C.

d) Polymerising agents

Ammonium persulphate (APS) 10 per cent prepared freshly before use.

TEMED – Fresh from the refrigeration.

e) Electrode buffer p^H 8.3

Tris base	- 6.0 g
Glycine	- 28.8 g

Double distilled water - 2 litre

f) Sample buffer

Double distilled water - 2.6 ml

0.5 M Tris HCl pH 6.8 - 1.0 ml

2-mercaptoethanol - 0.8 ml

Glycerol - 1.6 ml

0.5 % Bromophenol blue - 0.4 ml

g) Staining solution

Comassie brilliant blue R 250 - 0.1 g

Methanol - 40.0 ml

Glacial acetic acid - 10.0 ml

Double distilled water - 50.0 ml

h) Destaining solution

As above without Coomassie brilliant blue R 250

Procedure

Separating gel was first casted followed by stacking gel by mixing the various solutions as indicated below.

a) Preparation of separating gel (12%)

Double distilled water - 6.7 ml

Tris HCl, pH 8.8 - 5.0 ml

Acrylamide stock - 8.0 ml

The above solution was mixed well and degassed for three min and then the following were added immediately.

10 per cent Ammonium persulphate (APS)

Freshly prepared - 0.10 ml

Tetra methyl ethylene diamine (TEMED) - 0.01 ml

The separating gel was mixed well and poured immediately between glass plates and a layer of water was added above the polymerizing solution to quicken the polymerization process.

b) Preparation of stacking gel

Double distilled water	- 6.1 ml
Tris HCl, pH 6.8	- 2.5 ml
SDS	- 1.0 ml
Acrylamide stock	- 1.3 ml

The solution was mixed well, degassed and the following were added.

APS 10 %	- 0.05 ml
TEMED	- 0.1 ml

The water layered over the separating gel was removed and washed with a little electrode buffer and then the stacking gel was poured over the polymerized separating gel, after keeping the comb in position.

After polymerization the samples were loaded into the wells. The electrophoresis was performed at 100 V till the tracking dye reached the separating gel. Then the voltage was increased in 200 V and continued till the dye reached the bottom of the gel. The gel was removed immediately after electrophoresis between the glass plates and incubated in the staining solution for overnight with uniform shaking. Then the gel was transferred to the destaining solution. The protein appeared as bands and the gel was photographed after placing it on a transilluminator (Appligene Model White / UV TMW-20). The molecular weights of the new and induced polypeptides were calculated from the standard graph prepared for a 12 per cent gel using standard markers.

Results

4. RESULTS

4.1 ABIOTIC FACTORS

Various abiotic factors like, chemicals inducing resistance *viz.*,salicylicacid, ethrel and benzoic acid, indigenous materials inducing resistance viz. panchagavya, neem seed oil emulsion, turmeric powder- baking soda mixture, fresh cowdung solution and vermiwash, chemicals having antiviral properties *viz.*, carbendazim, naphthalene acetic acid, manganese chloride, sodium phosphonate and betadiene, ten crude extracts of *Adathoda vasica, Boerhavia diffusa*, *Bougainvillea* spp, *Catharanthus roseus*, *Datura stramonium, Hemidesmus indicus, Ocimum sanctum, Mirabilis jalapa, Phyllanthus niruri, and Jathropha curcus* having antiviral properties were screened to find out their efficiency in local lesion host and cowpea.

4.1.1 Chemicals inducing resistance

Efficacy of various chemicals inducing resistance were assessed against CABMV on the local lesion host, *C. amaranticolor*. It was observed that all the three chemicals tested showed inhibition of virus. Per cent inhibition of the virus in local lesion host was found in the range of 20 per cent to 89.60 per cent (Table 1). Among the chemicals, ethrel and salicylic acid were found to be effective in inhibition of virus (Plate 3, 4). Ethrel recorded 89.60 per cent inhibition at 1000pppm followed by salicylic acid (86.46 per cent). Per cent inhibition was found to be increased as the concentration increased in almost all the treatments (Fig.1). Ethrel showed maximum virus inhibition in pre, post and simultaneous inoculation treatments at higher concentration (1000 ppm) (Fig. 2). Salicylic acid significantly inhibited the virus even at lower concentration of 100 ppm in pre-inoculation treatment. Per cent inhibition was found to be on par in the case of SA in pre and simultaneous inoculation treatment. In the case of treatment with ethrel per cent inhibition was found to be on par when applied at all the concentrations.

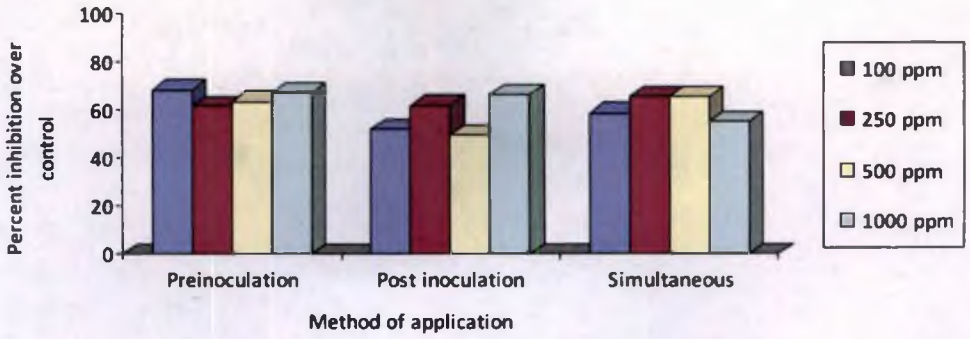
Table 1 Effect of chemicals inducing resistance on CABMV in local lesion host, *C. amaranticolor*

Chemicals	Method of application	Per cent inhibition over control			
		100 ppm	250 ppm	500 ppm	1000 ppm
Salicylic acid	Pre-inoculation	86.54 (45.00)	77.78 (61.85)	80.33 (63.65)	85.23 (67.37)
	Post-inoculation	62.14 (52.01)	78.11 (62.08)	58.06 (49.62)	83.88 (66.31)
	Simultâneous	73.34 (58.89)	83.3 (65.85)	83.3 (65.85)	67.41 (55.1)
Ethrel	Pre-inoculation	80.17 (63.53)	82.94 (65.58)	86.54 (68.45)	89.60 (71.16)
	Post-inoculation	21.71 (27.76)	83.90 (66.32)	63.87 (53.03)	86.33 (68.27)
	Simultaneous	26.65 (31.06)	67.35 (55.13)	61.18 (51.44)	85.34 (67.46)
Benzoic acid	Pre-inoculation	81.12 (64.22)	61.66 (51.72)	79.53 (63.17)	85.34 (67.46)
	Post-inoculation	20.02 (26.57)	61.40 (51.57)	72.67 (58.45)	84.93 (67.13)
	Simultaneous	28.63 (32.33)	75.23 (60.13)	68.88 (56.10)	78.93 (62.65)

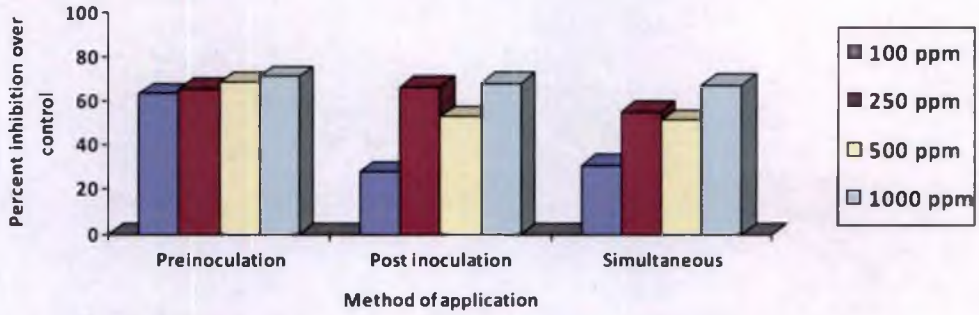
CD values : A - 11.29 B - 5.65

A- Concentration, B- Treatment X Method of application

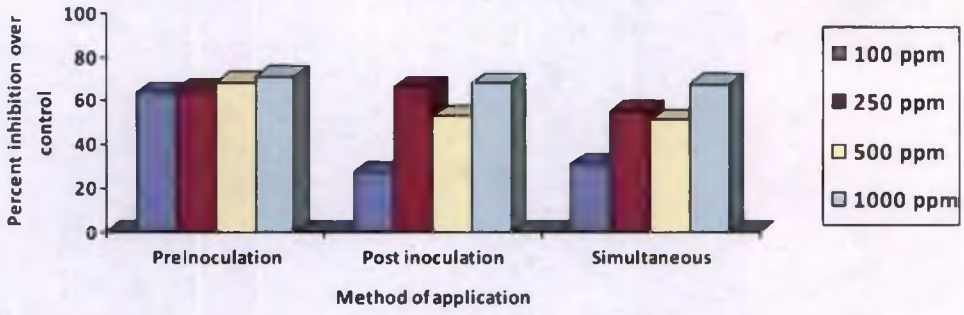
- Values are the mean of three replications and are expressed as percent inhibition over control.
- Values in parentheses are transformed values.



1a Salicylic acid

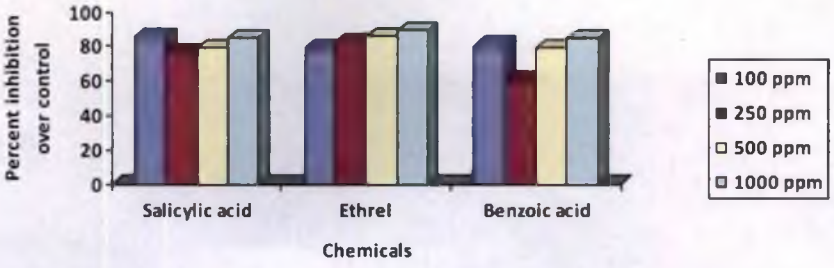


1 b Ethrel

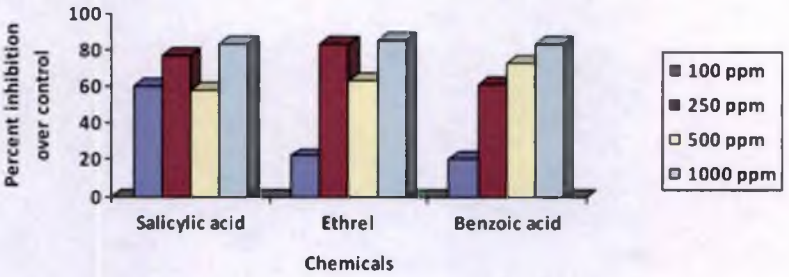


1c Benzoic acid

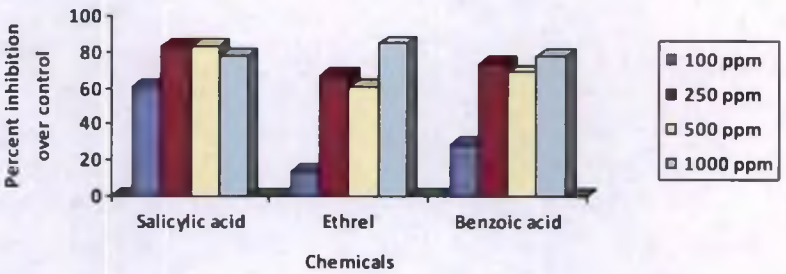
Fig. 1 Effect of chemicals inducing resistance on CABMV in local lesion host, *C. amaranticolor*



2a. Pre-inoculation



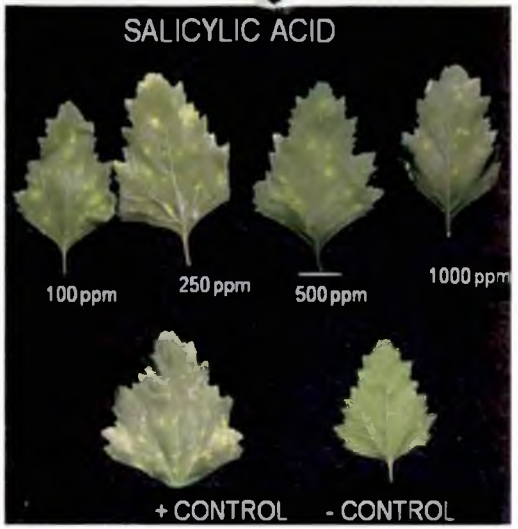
2b. Post-inoculation



2c. Simultaneous-inoculation

Fig. 2 Effect of different methods of application of chemicals inducing resistance on CABMV in local lesion host, *C. amaranticolor*

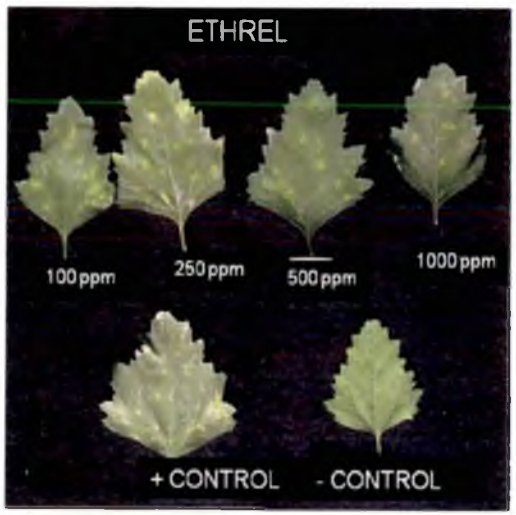
3a. Pre-inoculation



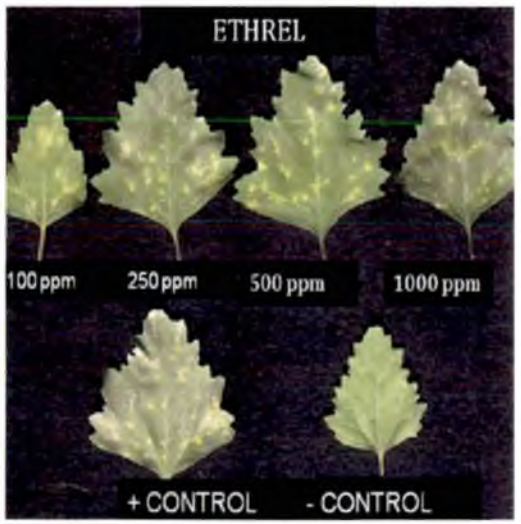
3b. Post-inoculation

3c. Simultaneous-inoculation

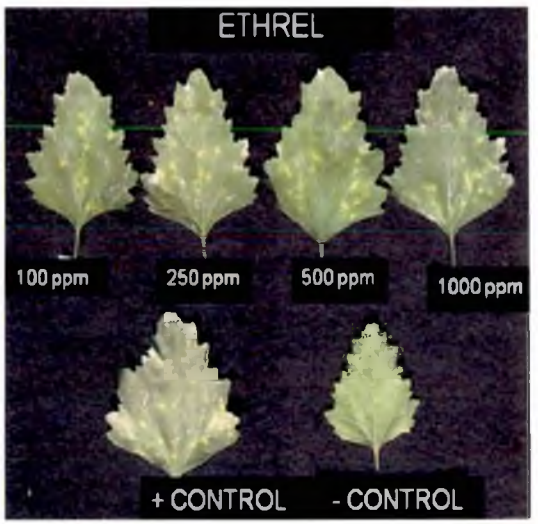
Plate 3. Effect Salicylic acid on CABMV in local lesion host, *C. amaranticolor*



4a. Pre-inoculation



4b. Post-inoculation



4c. Simultaneous-inoculation

Plate 4. Effect of Ethrel on CABMV in local lesion host, *C. amaranticolor*

These data indicated that ethrel and salicylic acid could be applied even at lower concentration to inhibit the virus. Among the three treatments, benzoic acid showed comparatively low inhibition percentage but it could inhibit the virus significantly at higher concentrations. The data revealed that there was significant difference between pre-inoculation, post-inoculation and simultaneous method of applications. Among the different method of application pre-inoculation spray was found to show better inhibition in all the three treatments. Post-inoculation and simultaneous spray were on par with each other.

4.1.2. Indigenous materials inducing resistance

Studies on the indigenous materials against CABMV on the local lesion host revealed that they did not show significant level of inhibition of the virus at various concentrations (1, 5 and 10 per cent) tested. Per cent inhibition varied from 3.5 to 67.33 (Table 2, Fig.3). Among the different methods of application, panchagavya and neem seed oil emulsion recorded higher inhibition of CABMV in pre and post inoculation (Plate 5, 6) In simultaneous inoculation treatment turmeric powder- baking soda mixture was found better in inhibition of the virus. In the case of panchagavya, pre-inoculation spray was found better than other methods of application and per cent inhibition was found to be on par as the concentration increased. Significant difference in post and simultaneous inoculation treatments were observed in panchagavya treatment. This was followed by neem seed oil emulsion which also showed similar trend of per cent inhibition as observed in panchagavya treatment. Neem seed oil treatment showed about 67 percent inhibition over control in post-inoculation treatment. As the concentration increased, per cent inhibition of the virus was found to increase in treatment with fresh cowdung solution in pre-inoculation. Pre-inoculation spray was found to be significant in the treatments viz., panchagavya, neem seed oil emulsion and fresh cow dung solutions (Fig. 4). Treatment with vermiwash showed minimum percent inhibition in all three methods of application when compared to other indigenous materials used for the study.

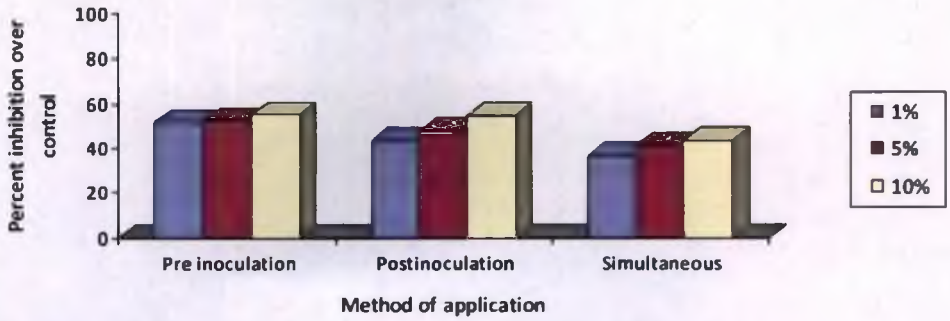
Table 2 Effect of indigenous materials inducing resistance on CABMV in local lesion host, *C. amaranticolor*

Indigenous materials	Method of application	Per cent inhibition over control		
		1 %	5 %	10 %
Panchagavya	Pre-inoculation	61.47 (51.61)	62.93(52.47)	67.40(55.16)
	Post-inoculation	47.23 (43.40)	54.90(47.80)	66.34(54.52)
	Simultaneous	35.52 (36.57)	43.99(41.53)	47.69(43.66)
Neem seed oil emulsion	Pre-inoculation	61.47(51.61)	66.60(54.67)	45.14(42.19)
	Post-inoculation	38.09(38.09)	44.26(41.70)	67.74(55.37)
	Simultaneous	44.73(41.95)	55.90(48.37)	56.67(48.81)
Turmeric powder–baking soda mixture	Pre-inoculation	55.90(48.37)	48.79(44.30)	57.82(49.48)
	Post-inoculation	21.92 (27.90)	43.88(41.47)	30.44(33.47)
	Simultaneous	47.59(43.60)	60.45(51.01)	66.24(54.45)
Fresh cowdung solution	Pre-inoculation	15.56(23.22)	46.37(42.90)	59.03(50.18)
	Post-inoculation	15.33 (23.04)	14.46(22.34)	43.23(41.09)
	Simultaneous	28.96 (32.54)	31.26(33.98)	37.29(37.62)
Vermi-wash	Pre-inoculation	3.59 (10.93)	14.37(22.27)	29.30(32.76)
	Post-inoculation	6.93 (15.25)	13.53(21.57)	29.30(32.76)
	Simultaneous	18.73(25.63)	22.12(28.04)	43.3(41.13)

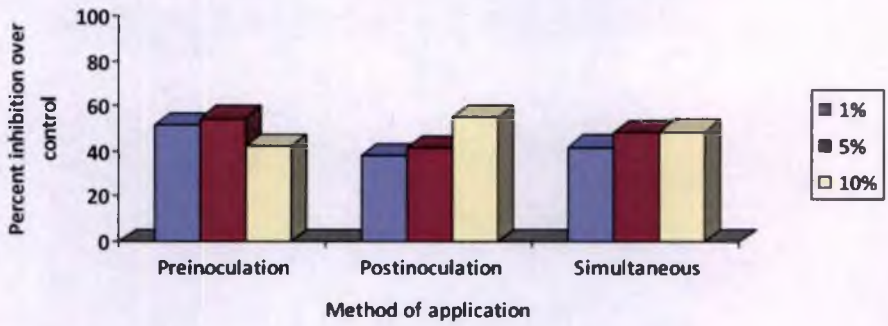
CD values : A- 7.07 B- 4.08

A - Concentration, B - Treatment X Method of application

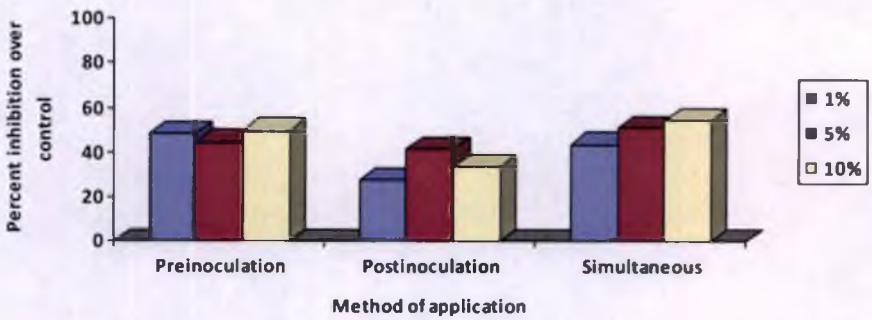
- Values are the mean of three replications and are expressed as percent inhibition over control.
- Values in parentheses are transformed values.



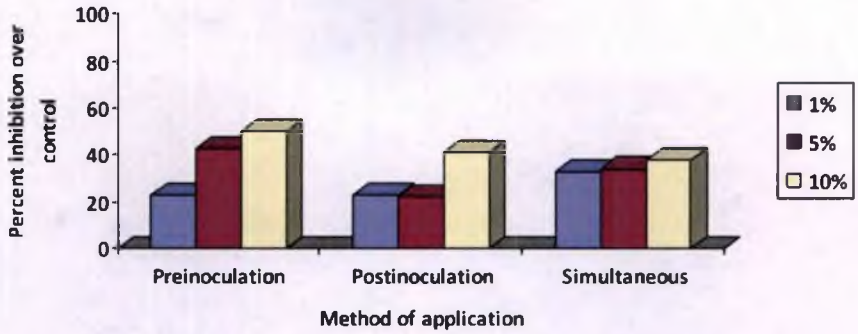
3a Panchagavya



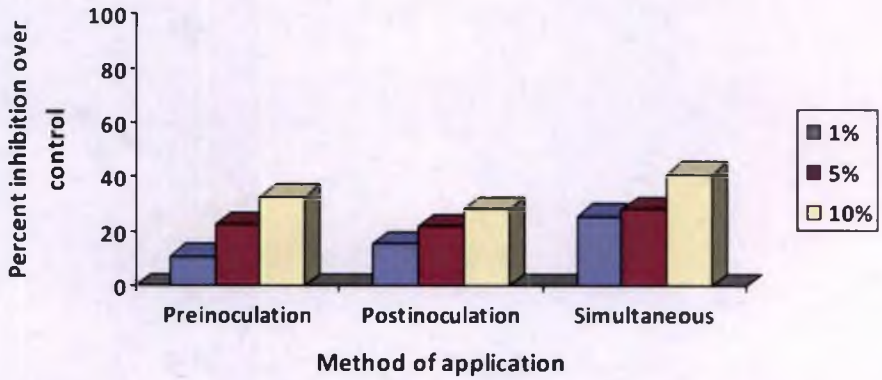
3b Neem seed oil emulsion



3c Turmeric powder– baking soda mixture

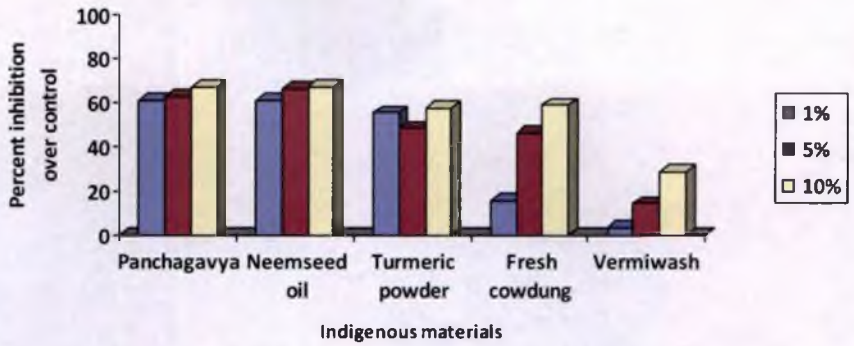


3d Fresh cowdung solution

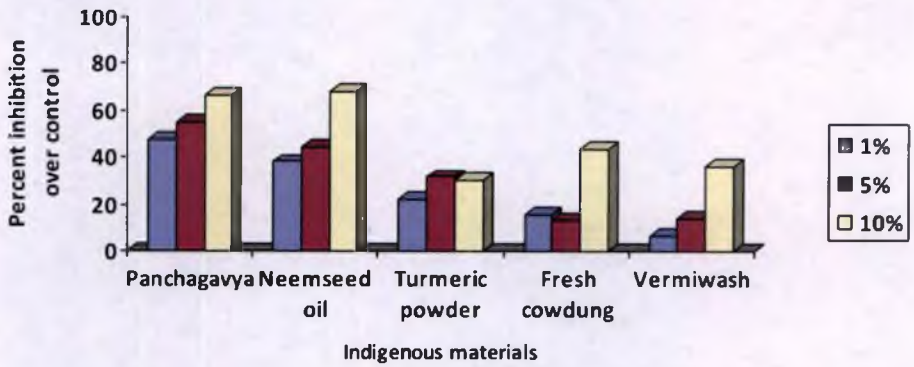


3e Vermi-wash

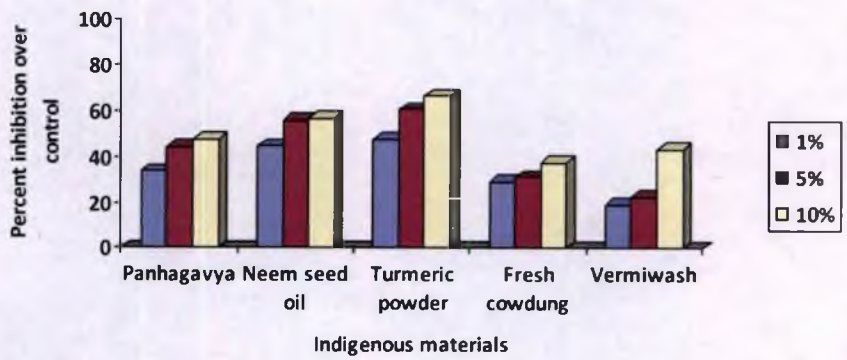
Fig. 3 Effect of indigenous materials inducing resistance on CABMV in local lesion host, *C. amaranticolor*



4a Pre-inoculation

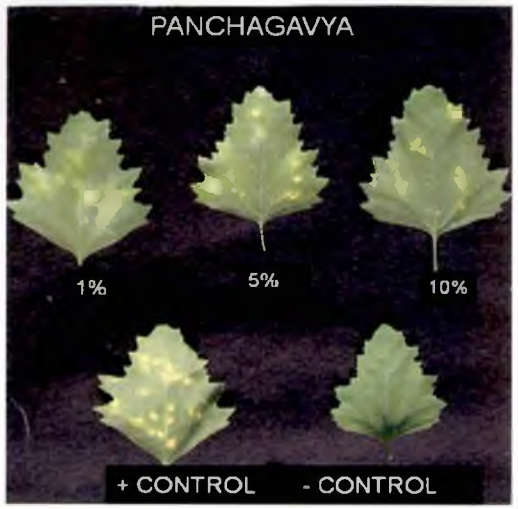


4b Post-inoculation

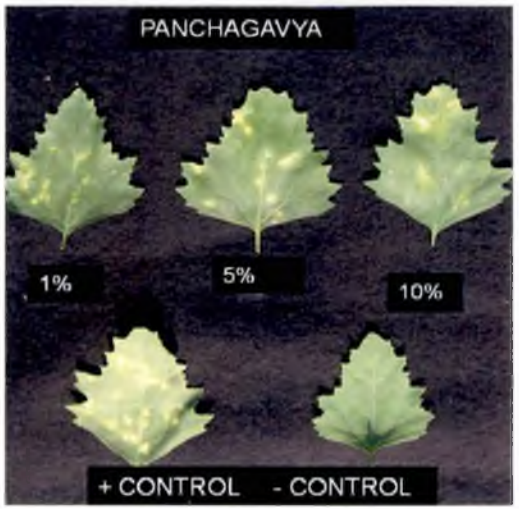


4c Simultaneous-inoculation

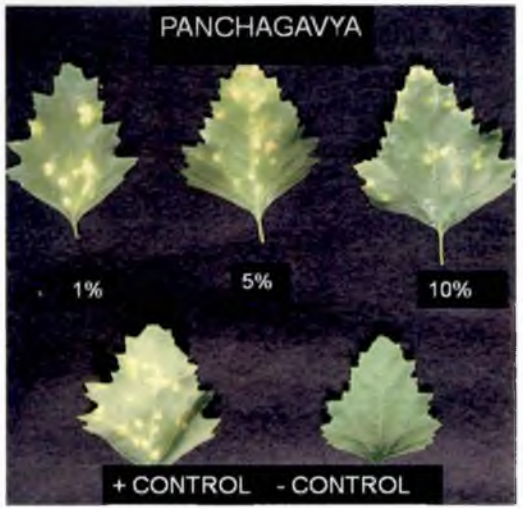
Fig. 4 Effect of different methods of application of indigenous materials inducing resistance on CABMV in local lesion host, *C. amaranticolor*



5a. Pre-inoculation

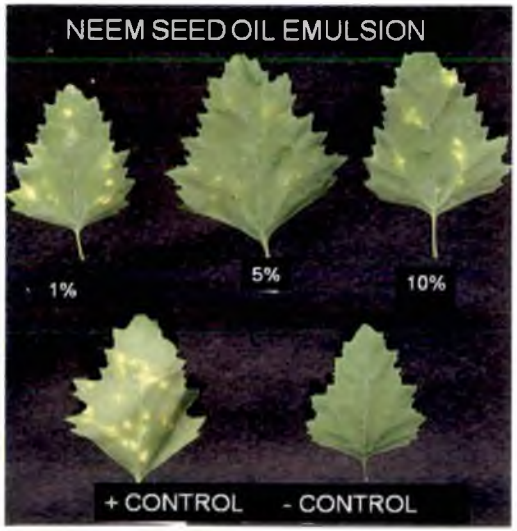


5b. Post-inoculation

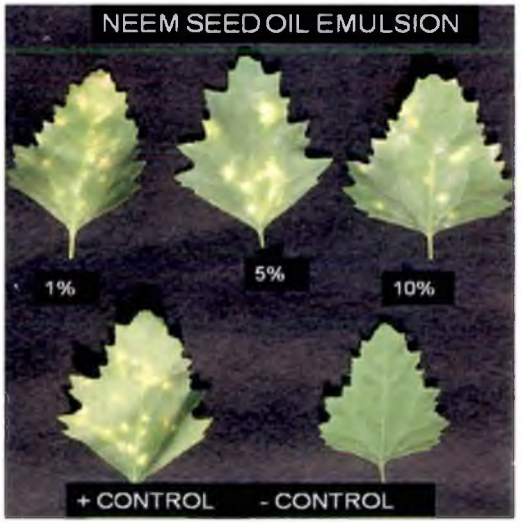


5c. Simultaneous-inoculation

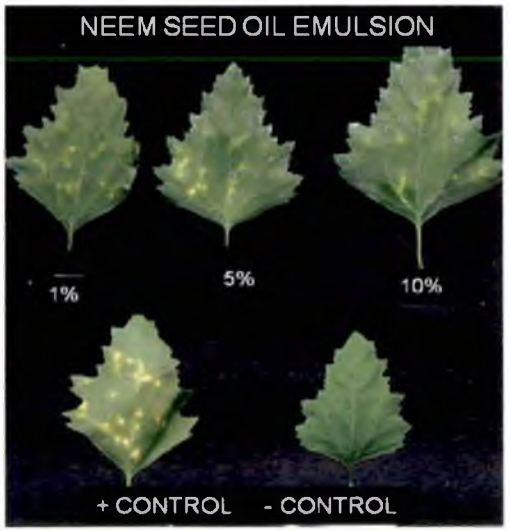
Plate 5. Effect of Panchagavya on CABMV in local lesion host, *C. amaranticolor*



6a. Pre-inoculation



6b. Post-inoculation



6c. Simultaneous-inoculation

Plate 6. Effect of Neem seed oil emulsion on CABMV in local lesion host, *C .amaranticolor*

4.1.3. Chemicals having antiviral properties

Different chemicals having antiviral properties were tested by pre, post and simultaneous methods of application at various concentrations. Per cent inhibition was found to increase with increase in concentration in most of the treatments (Table 3, Fig.5). Sodium phosphonate showed maximum inhibition percentage of 56.36 at 0.4 per cent concentration in pre-inoculation treatment followed by betadine (51.96 per cent) at 0.1 per cent (Fig 6). A significant difference in different concentrations was observed in pre-inoculation treatment of sodium phosphonate and per cent inhibition varied from 8.9 to 56.36 per cent. The trend got reversed in post-inoculation and recorded 51.4 per cent inhibition over control at low concentration of 0.05 per cent. Treatment with manganese chloride also showed significant difference at three concentrations tested and drastic increase in per cent inhibition was noticed. This was true for its post and simultaneous treatment (Plate 8). Betadine treatment showed better inhibition of the virus in all the three methods of application (Plate 7). It was observed that betadine was effective even at low concentration and about 70 per cent inhibition was noticed in post-inoculation treatment. Among the various chemicals tested, treatment with betadine reduced the virus infection to a maximum level in pre, post and simultaneous methods of application .

4.1.4. Crude extracts of plants having antiviral properties

Studies on the mode of action of AVPs against CABMV infection revealed that treatments with different plant extracts inhibited the virus ranging from 4.4 per cent to 100 per cent at different concentrations in different methods of application. Per cent inhibition was found to be increased with increase in the concentration. Among the ten plant extracts tested, more than 90 per cent inhibition of the virus could be noticed in *P. niruri* , *B. diffusa, Bougainvillea spp., O. sanctum* and *C. roseus* (Table 4).

P. niruri extract recorded 100 per cent inhibition in post and simultaneous inoculation treatment (Fig.7, Plate 9). In post-inoculation treatment with *P. niruri,* inhibition over control was found to be maximum (93.39 per cent)

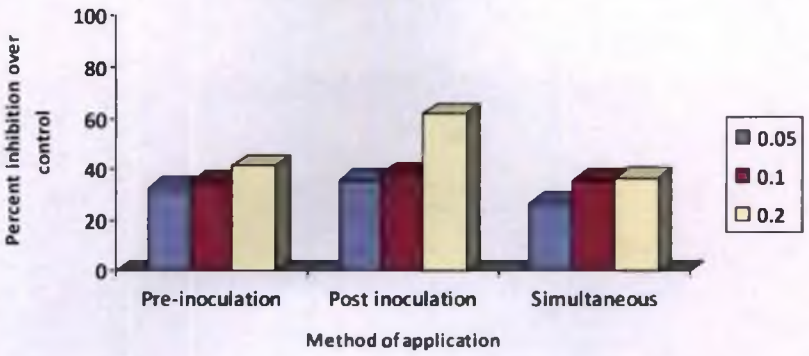
Table 3 Effect of chemicals having antiviral properties on CABMV in local lesion host, *C. amaranticolor*

Chemicals	Methodof application	Per cent inhibition over control		
		0.05 %	0.1 %	0.2 %
Carbendazim	Pre-inoculation	32.49 (34.70)	34.88 (36.19)	41.39 (40.03)
	Post-inoculation	35.95 (36.83)	38.39 (38.27)	61.60 (51.69)
	Simultaneous	26.95 (31.26)	35.70 (36.67)	36.49 (37.15)
		100 ppm	250 ppm	500 ppm
Naphthalene acetic acid	Pre-inoculation	13.48(21.53)	27.01 (31.30)	31.67(34.23)
	Post-inoculation	8.65 (17.10)	21.92 (27.90)	16.58(24.01)
	Simultaneous	2.60 (9.27)	27.19 (31.41)	35.06 (36.29)
		0.1%	0.2%	0.4%
Sodium phosphonate	Pre-inoculation	25.65(30.42)	32.63 (34.82)	56.36 (48.63)
	Post-inoculation	44.53(41.84)	14.72 (22.55)	9.65 (18.09)
	Simultaneous	48.99 (44.40)	20.81(27.13)	43.96 (41.50)
		100 ppm	250 ppm	500 ppm
Manganese chloride	Pre-inoculation	3.80 (11.20)	4.82 (12.69)	34.09 (35.71)
	Post-inoculation	3.65 (11.02)	40.93(39.75)	68.50 (55.83)
	Simultaneous	14.26 (22.18)	66.00 (54.30)	78.66 (62.46)
		0.1%	0.2%	0.5%
Betadiene	Pre-inoculation	55.01 (47.86)	41.39 (40.03)	48.66 (44.21)
	Post-inoculation	56.36 (48.63)	70.47 (57.06)	48.33 (44.02)
	Simultaneous	60.90 (51.20)	31.74 (34.27)	55.67(48.23)

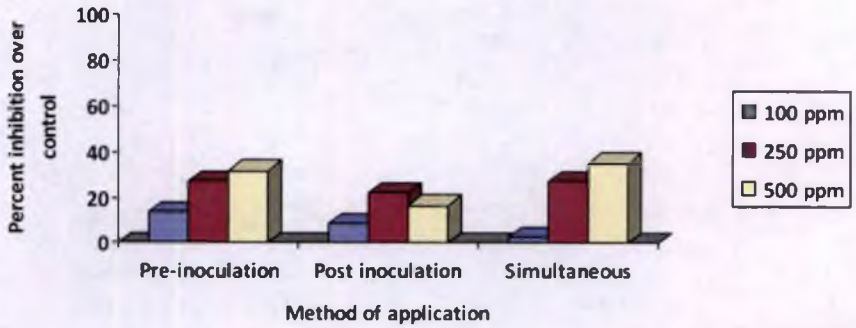
CD values : A - 3.98 B - 2.29

A - Concentration, B - Treatment X Method of application

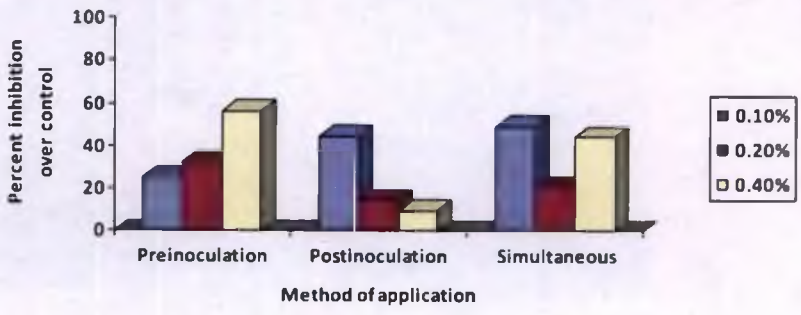
- Values are the mean of three replications and are expressed as percent inhibition over control.
- Values in parentheses are transformed values.



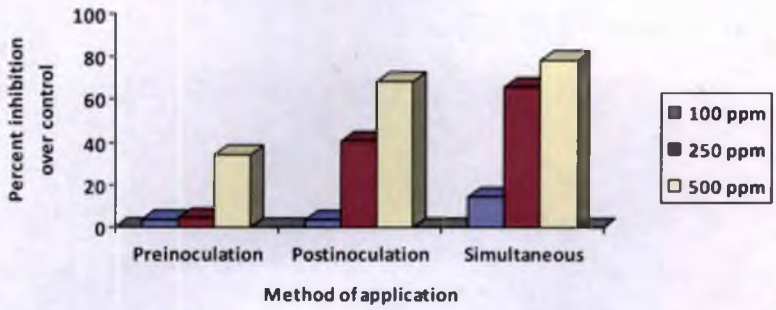
5a. Carbendazim



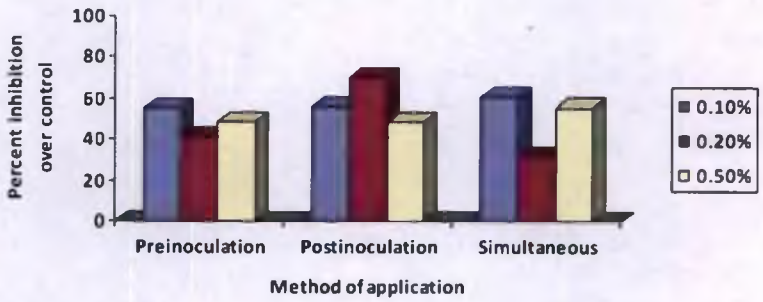
5b. Naphthalene acetic acid



5c. Sodium phosphonate

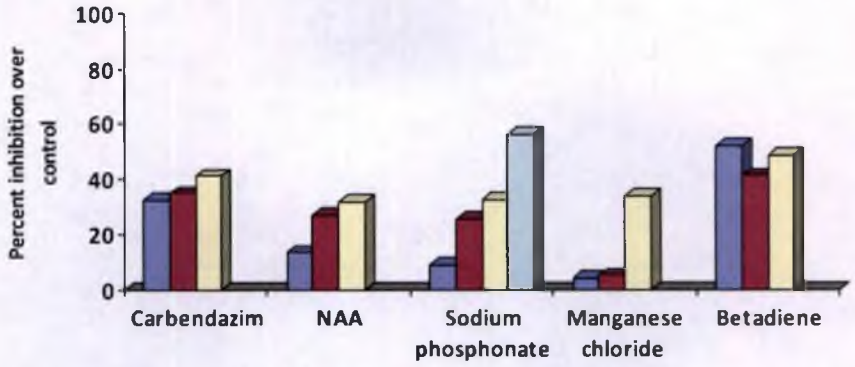


5d. Manganese chloride

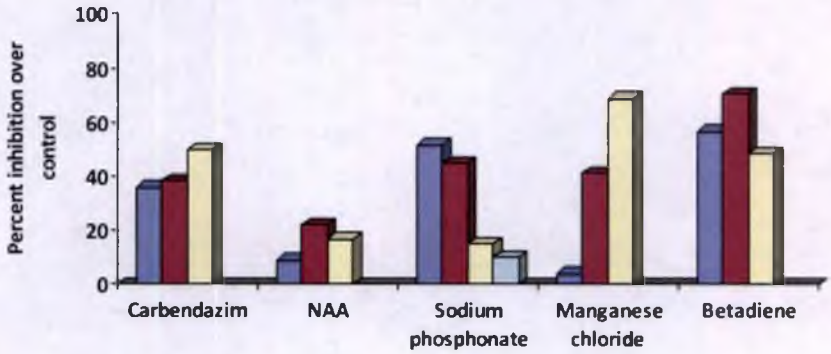


5e. Betadiene

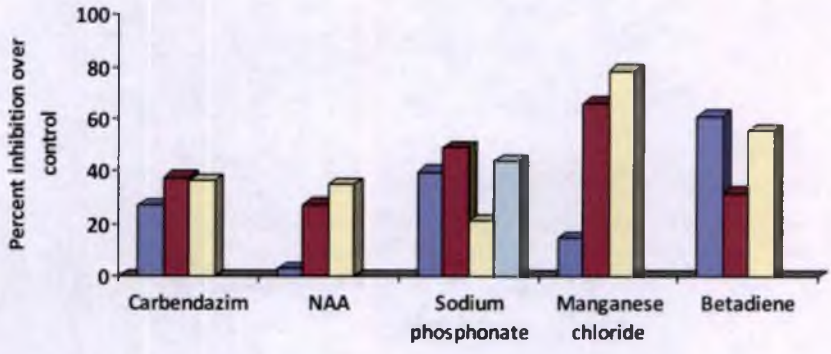
Fig. 5 Effect of chemicals having antiviral properties on CABMV in local lesion host, *C. amaranticolor*



6a Pre-inoculation

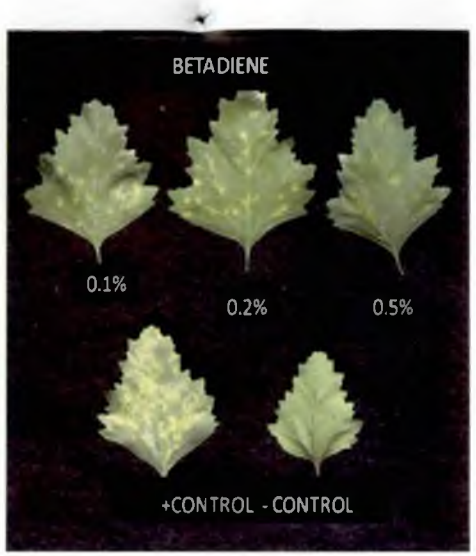


6b Post-inoculation

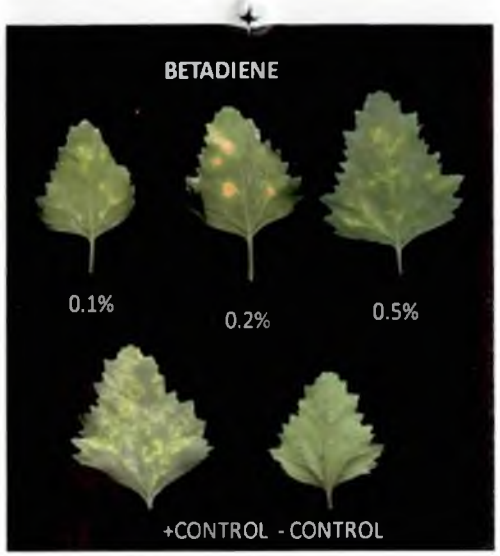


6c. Simultaneous-inoculation

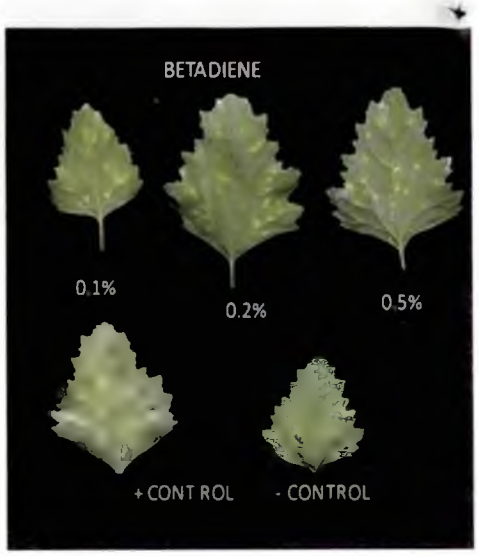
Fig. 6 Effect of different methods of application of chemicals having antiviral properties on CABMV in local lesion host, *C. amaranticolor*



7a. Pre-inoculation

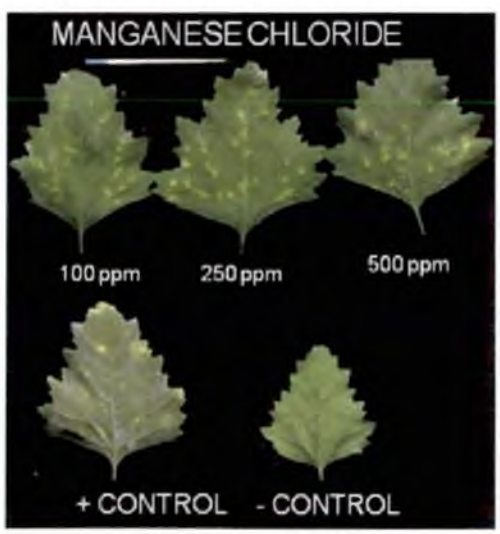


7b. Post-inoculation

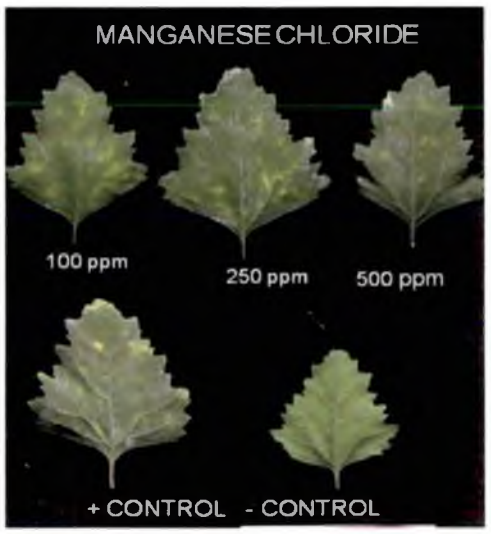


7c. Simultaneous-inoculation

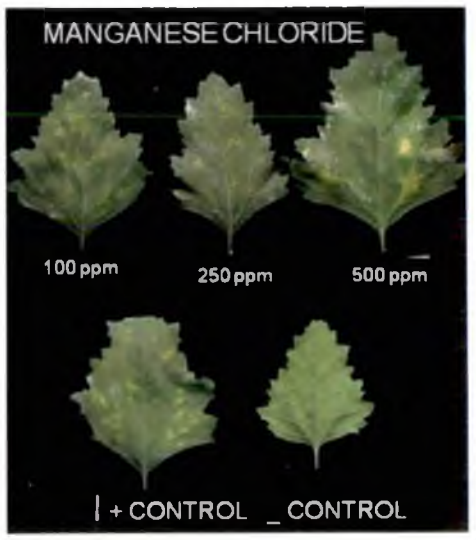
Plate 7. Effect of Betadiene on CABMV in local lesion host,*C. amaranticolor*



8a. Pre-inoculation



8b. Post-inoculation



8c. Simultaneous-inoculation

Plate 8. Effect of Manganese chloride on CABMV in local lesion host, *C. amaranticolor*

Table 4 Effect of crude extracts of plants having antiviral properties on CABMV in local lesion host, *C. amaranticolor*

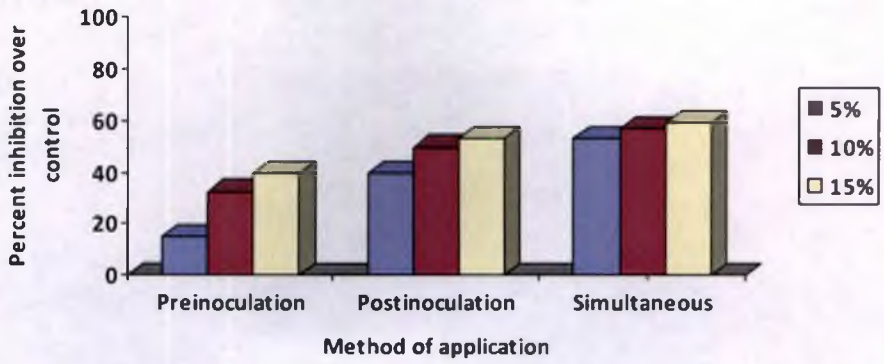
Plant extracts	Method of application	Per cent inhibition over control		
		5%	10 %	15 %
Hemidesmus indicus	Pre-inoculation	6.58 (14.86)	28.75 (32.41)	40.69 (39.62)
	Post-inoculation	40.88 (39.73)	58.21 (49.71)	63.67 (52.91)
	Simultaneous	63.34 (52.72)	70.00 (56.76)	73.48 (58.98)
Mirabilis jalapa	Pre-inoculation	4.28 (11.93)	12.40 (20.61)	66.95 (54.88)
	Post-inoculation	50.56 (45.30)	76.51(60.98)	66.23 (54.45)
	Simultaneous	4.28 (11.93)	8.60 (17.04)	13.30 (21.38)
Datura stramonium	Pre-inoculation	37.66 (37.84)	39.71(39.05)	42.83 (40.86)
	Post-inoculation	61.54 (51.65)	69.17(56.25)	73.07 (58.72)
	Simultaneous	20.06 (26.60)	66.60 (54.67)	70.44 (57.04)
Bougainvillea spp	Pre-inoculation	45.55 (42.43)	71.24 (57.54)	92.37(73.94)
	Post-inoculation	88.78 (70.40)	93.13 (74.78)	93.13 (74.78)
	Simultaneous	46.45 (42.95)	53.65 (47.07)	88.15 (69.84)
Catharanthus roseus	Pre-inoculation	20.83(27.14)	72.94 (58.63)	78.77 (62.54)
	Post-inoculation	79.53(63.07)	85.34 (67.46)	91.08 (72.59)
	Simultaneous	25.91(30.59)	46.64 (43.05)	65.59 (54.06)
Ocimum sanctum	Pre-inoculation	78.60 (62.42)	92.33 (73.90)	94.10 (75.91)
	Post-inoculation	71.44 (57.67)	79.27 (62.89)	92.26 (73.82)
	Simultaneous	20.83 (27.14)	61.34 (51.53)	71.12 (57.47)
Adathoda vasica	Pre-inoculation	54.85 (47.76)	51.82 (46.02)	87.49 (69.26)
	Post-inoculation	68.22 (55.66)	77.32 (61.54)	88.93 (70.54)
	Simultaneous	47.10 (43.32)	49.96 (44.96)	64.14 (53.19)

Phyllanthus niruri	Pre-inoculation	72.45 (58.32)	95.47 (77.69)	95.20 (77.320
	Post-inoculation	93.39 (75.08)	100.00(90.00)	100.00 (90.00)
	Simultaneous	88.85 (70.46)	91.11 (72.62)	100.00 (90.00)
Boerhavia diffusa	Pre-inoculation	94.40 (76.28)	95.62 (77.88)	95.62 (77.88)
	Post-inoculation	86.98 (68.82)	90.00 (71.53)	86.98 (68.82)
	Simultaneous	79.83 (63.29)	94.28 (76.13)	93.90 (75.67)
Jathropha curcus	Pre-inoculation	38.08 (38.09)	41.37 (40.01)	40.45 (39.48)
	Post-inoculation	35.47 (36.54)	58.88 (50.10)	62.79 (52.39)
	Simultaneous	61.54 (51.65)	69.17 (56.25)	72.97 (58.65)

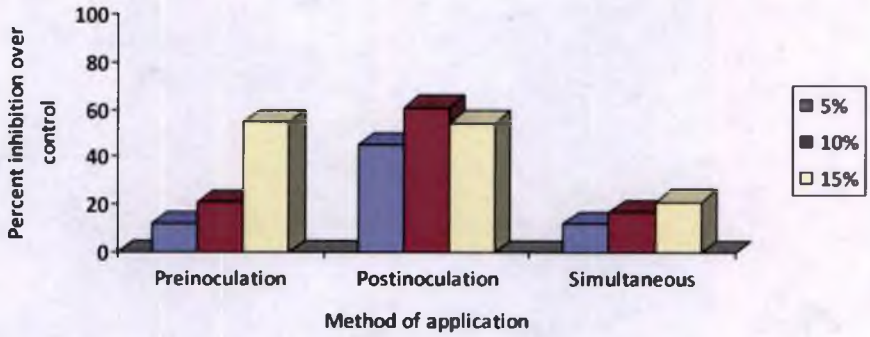
CD values : A- 10.50 B- 6.06

A - Concentration, B - Treatment X Method of application

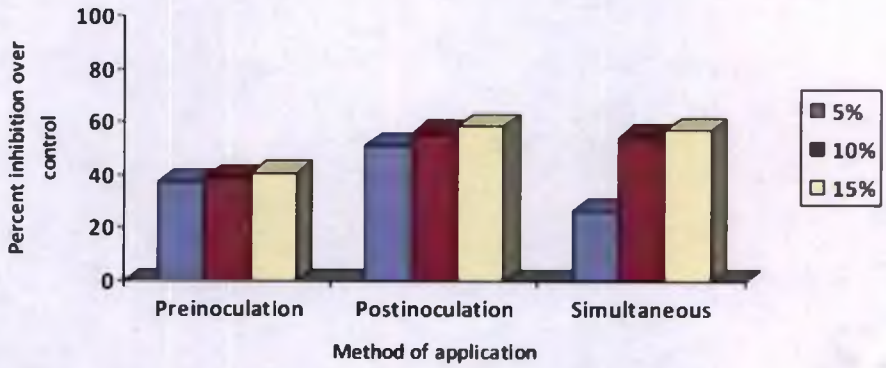
- Values are the mean of three replications and are expressed as percent inhibition over control.
- Values in parentheses are transformed values.



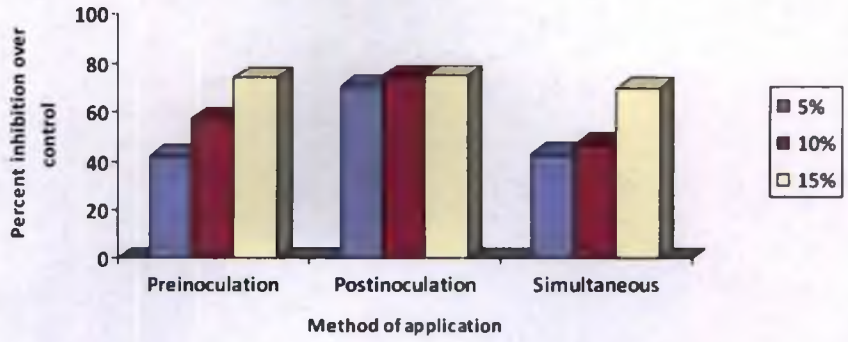
7a *Hemidesmus indicus*



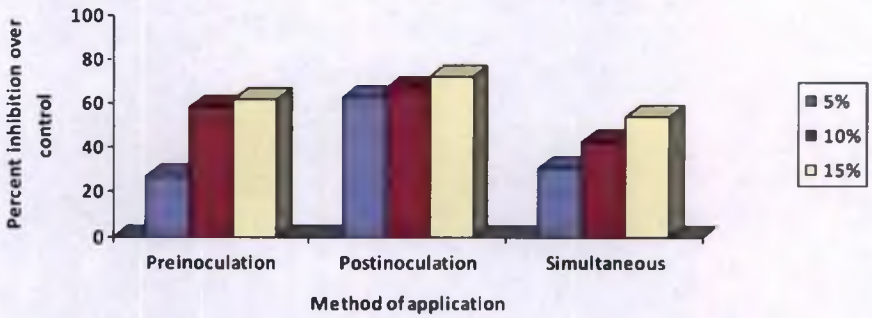
7b *Mirabilis jalapa*



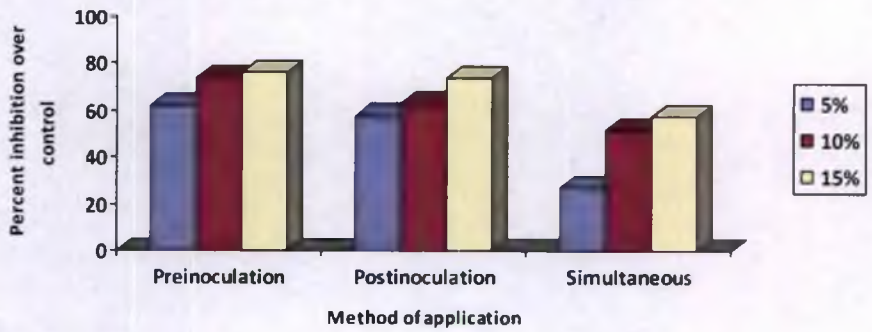
7c *Datura stramonium*



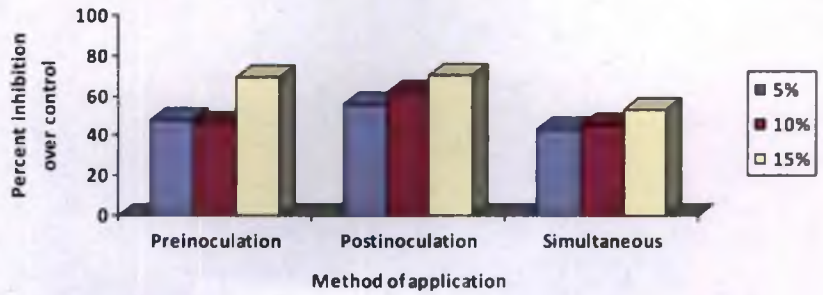
7d *Bougainvillea* spp



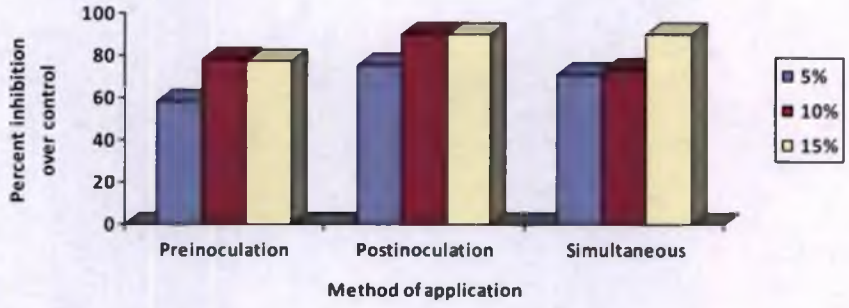
7e *Catharanthus roseus*



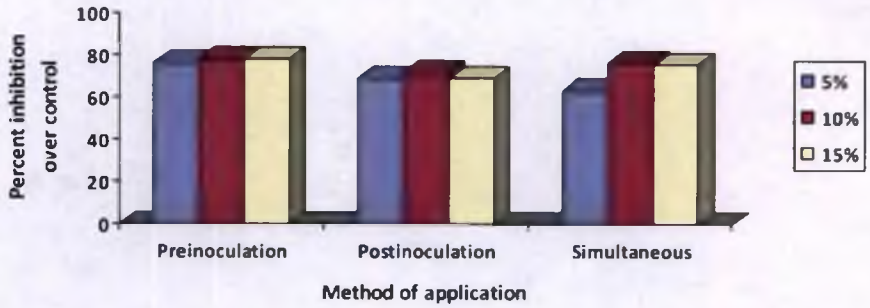
7f *Ocimum sanctum*



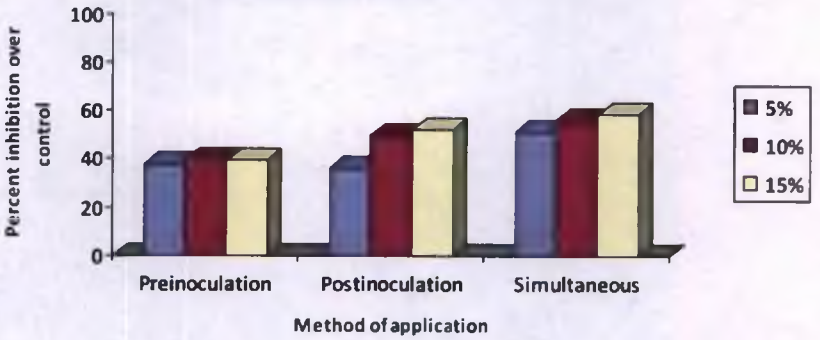
7g *Adathoda vasica*



7h *Phyllanthus niruri*

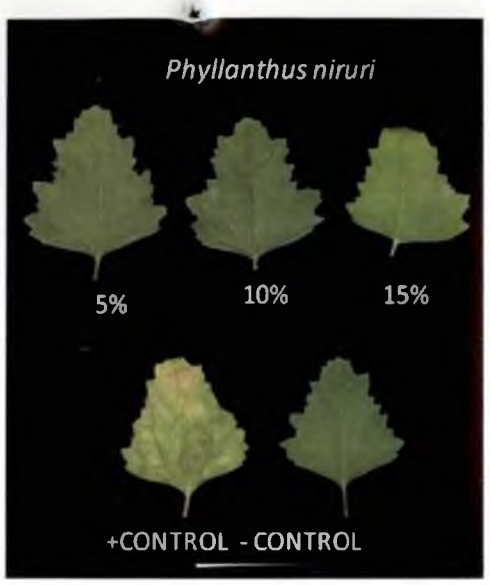


7i *Boerhavia diffusa* on CABMV in local lesion host

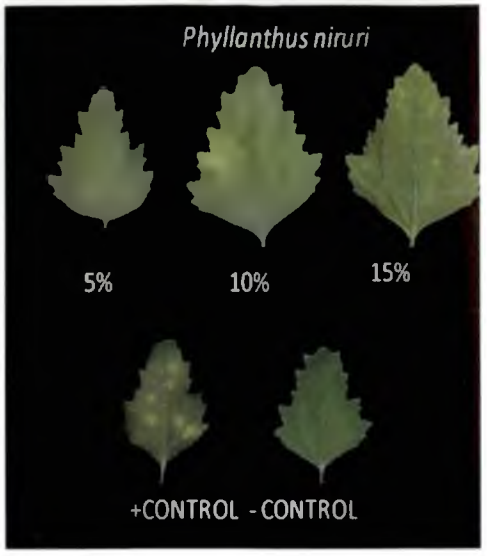


7j *Jathropha curcus*

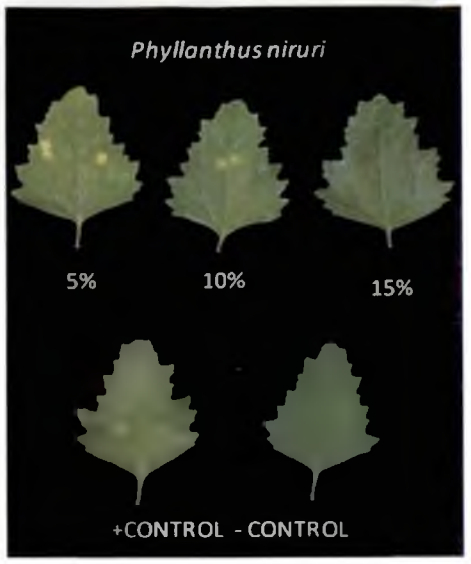
Fig. 7 Effect of crude extracts of plants having antiviral properties on local lesion host, *C. amaranticolor*



9a. Pre-inoculation

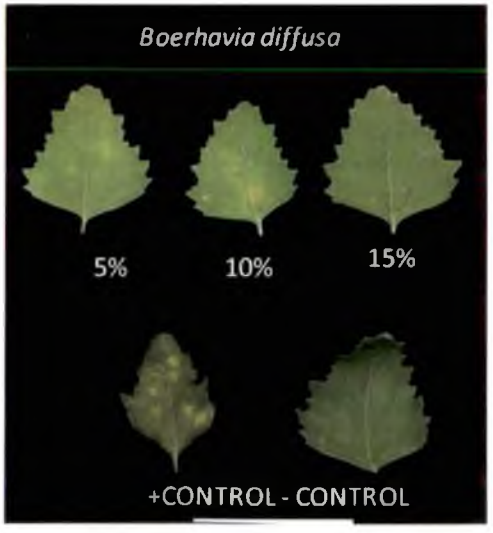


9b. Post-inoculation

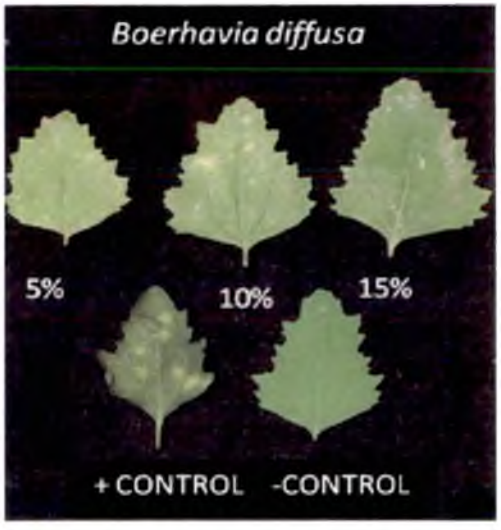


9c. Simultaneous-inoculation

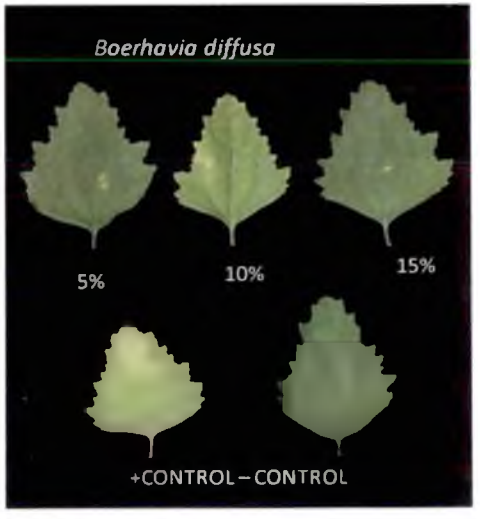
Plate 9. Effect of *Phyllanthus niruri* on CABMV in local lesion host, *C. amaranticolor*



10a. Pre-inoculation



10b. Post-inoculation



10c. Simultaneous-inoculation

Plate10. Effect of *Boerhavia diffusa* on CABMV in local lesion host, *C. amaranticolor*

even at 5 per cent concentration and the per cent inhibition increased with increase in concentration. But there were no statistical difference among these concentrations tested for inhibition of the virus. Significant difference in inhibition at low and high concentration was observed in pre and simultaneous inoculation treatment.

The next most effective treatment inhibited the virus was *B. diffusa*. It could inhibit the virus significantly even at 5 per cent concentration at different methods of treatments (Table 4). At 10 and 15 per cent concentrations, *B. diffusa* could inhibit the virus to an extend of more than 90 per cent over control irrespective of the method of applications. There were no significant differences in inhibition of virus among the different concentrations of the extract tried in different method of applications (Plate 10).

Bougainvillea spp., O. sanctum and *C. roseus* were also found to inhibit the virus significantly at higher concentrations in different methods of treatment. Per cent inhibition was more than 90 per cent with these plant extracts. All the above plants could inhibit the virus to an extend of more than 90 per cent in post-inoculation.

In pre-inoculation treatment, maximum percent inhibition was observed in *B. diffusa* followed by *Ocimum sanctum, Phyllanthus niruri* and *Bougainvillea* sp. Per cent inhibition was found to be on par in pre-inoculation treatment of *B. diffusa* at three different concentrations tested. The data on per cent inhibition of virus in pre-inoculation treatment revealed that almost all the treatments except *H. indicus, D. stramonium* and *J. curcas* showed good inhibition percentage over control (Table 5).

Data on post-inoculation treatment with plant extracts revealed that *P. niruri* extract was the best one to inhibit the virus at 10 and 15 per cent concentration. This was followed by *Bougainvillea sp, O. sanctum* and *C. roseus*. *J. curcas* and *H. indicus* did not show much inhibition compared to other treatments. Per cent inhibition of virus over control was found the same in all the

Table 5 Effect of pre-inoculation of crude extracts of plants having antiviral properties on CABMV in local lesion host, *C. amaranticolor*

Sl. No.	Plant extracts and its concentration		Percent inhibition over control
1	*Hemidesmus indicus*	5 %	6.75
		10 %	28.76
		15 %	40.70
2	*Mirabilis jalapa*	5 %	4.40
		10 %	12.43
		15 %	66.93
3	*Datura stramonium*	5 %	37.80
		10 %	39.90
		15 %	42.86
4	*Bougainvillea* sp	5 %	48.59
		10 %	69.50
		15 %	91.80
5	*Catharanthus roseus*	5 %	22.42
		10 %	72.86
		15 %	78.66
6	*Ocimum sanctum*	5 %	78.36
		10 %	92.13
		15 %	94.10
7	*Adathoda vasica*	5 %	54.83
		10 %	51.80
		15 %	87.20
8	*Phyllanthus niruri*	5 %	71.83
		10 %	93.33
		15 %	93.00
9	*Boerhavia diffusa*	5 %	94.30
		10 %	95.53
		15 %	95.50
10	*Jathropha curcas*	5 %	38.10
		10 %	41.60
		15 %	40.46

CD values (0.05): A- 12.90 B – 7.45; A - Concentration, B- Chemicals

*Values are the mean of three replications and expressed as percent inhibition over control

three concentrations tested in case of *B. diffusa* when applied 24 h after virus inoculation (Table 6).

Simultaneous inoculation of virus and plant extracts showed not much reduction in the number of local lesions in various treatments and it was maximum in the case of *P. niruri* and *B. diffusa* (Table 7). The per cent inhibition of the virus increased with increase in concentrations of plant extracts. Reduction in the number of lesions on *C. amaranticolor* was found to increased with increase in concentration. It was recorded that *P. niruri* extract reduced the virus accumulation even at low and higher concentrations when applied along with the virus. About 100 percent inhibition over control was noticed at 15 percent concentration.

Post-inoculation treatment of *P. niruri* was done at lower concentrations of 1 and 0.5 per cent. It was found that maximum inhibition of the virus was observed even at lower concentrations. About 90 percent inhibition was recorded at 0.5 per cent and 93 per cent at 1 per cent concentration (Table 8). *P. niruri* extracts were also applied as post-inoculation treatment at different time intervals viz. one day, two day, three day, four day and five days after inoculation. Hundred per cent inhibition was noticed at 1,2 and 3 DAI and more than 90 per cent at 4 and 5 DAI (Table 9).

The data on effect of plant extracts on CABMV infecton in local lesion host revealed that *P. niruri* and *B. diffusa* leaf extracts when applied as pre, post and simultaneous inoculation were the best in reducing the virus accumulation at different concentrations (Plate 9, 10).

4.2. BIOTIC AGENTS

Various biotic agents *viz.*, released cultures *of P. fluorescens* from KAU and TNAU, *Bacillus* sp., endophytic and rhizobacteria from cowpea were evaluated to test its efficiency against CABMV in local lesion host and cowpea by spraying 24 h prior to inoculation, 24 h after inoculation and simultaneous inoculation of CABMV. The data revealed that biotic agents showed no marked influence in reducing the virus infection in different methods of application except

Table 6 Effect of post-inoculation of crude extracts of plants having antiviral properties on CABMV in local lesion host, *C. amaranticolor*

Sl. No.	Plant extracts and its concentrations		Percent inhibition over control
1	*Hemidesmus indicus*	5 %	41.23
		10 %	58.03
		15 %	63.60
2	*Mirabilis jalapa*	5 %	50.56
		10 %	76.30
		15 %	66.23
3	*Datura stramonium*	5 %	61.53
		10 %	69.13
		15 %	73.03
4	*Bougainvillea* sp	5 %	88.53
		10 %	93.13
		15 %	93.13
5	*Catharanthus roseus*	5 %	79.43
		10 %	85.00
		15 %	91.06
6	*Ocimum sanctum*	5 %	71.10
		10 %	78.86
		15 %	92.20
7	*Adathoda vasica*	5 %	68.20
		10 %	77.26
		15 %	88.66
8	*Phyllanthus niruri*	5 %	93.20
		10 %	100.00
		15 %	100.00
9	*Boerhavia diffusa*	5 %	86.66
		10 %	86.66
		15 %	86.66
10	*Jathropha curcas*	5 %	36.36
		10 %	58.73
		15 %	62.50

CD values (0.05): A- 7.54 B – 4.35; A- Concentration, B- Chemicals

*Values are the mean of three replications and expressed as percent inhibition over control

Table 7 Effect of simultaneous-inoculation of crude extracts of plants having antiviral properties on CABMV in local lesion host, *C. amaranticolor*

Sl. No.	Plant extracts		Percent inhibition over control
1	*Hemidesmus indicus*	5 %	63.33
		10 %	70.00
		15 %	73.33
2	*Mirabilis jalapa*	5 %	4.40
		10 %	8.83
		15 %	13.30
3	*Datura stramonium*	5 %	20.30
		10 %	66.6
		15 %	70.3
4	*Bougainvillea* sp	5 %	46.70
		10 %	54.03
		15 %	87.20
5	*Catharanthus roseus*	5 %	27.20
		10 %	46.60
		15 %	65.53
6	*Ocimum sanctum*	5 %	22.42
		10 %	61.31
		15 %	71.03
7	*Adathoda vasica*	5 %	47.20
		10 %	49.86
		15 %	64.03
8	*Phyllanthus niruri*	5 %	88.66
		10 %	90.90
		15 %	100.00
9	*Boerhavia diffusa*	5 %	79.33
		10 %	94.00
		15 %	93.66
10	*Jathropha curcas*	5 %	61.53
		10 %	69.13
		15 %	72.90

CD values (0.05): A- 10.52 B – 6.07; A - Concentration, B - Chemicals

*Values are the mean of three replications and expressed as percent inhibition over control

Table 8 Effect of post-inoculation of *P. niruri* leaf extract on local lesion host, *C. amaranticolor*

Treatment	Concentration	Percent inhibition over control
Phyllanthus niruri	0.5%	90.00
	1%	93.00
	5%	93.40
	10%	100.00
	15%	100.00

Table 9 Effect of post-inoculation of *P. niruri* leaf extract on local lesion host at different time intervals

Treatment	Percent inhibition over control				
	1DAI	2DAI	3DAI	4DAI	5DAI
Phyllanthus niruri (10%)	100.00	100.00	100.00	94.00	94.00

P. fluorescens (TNAU culture) (Table 10, Fig.8). Results indicated that *P. fluorescens* at the rate of 10^8 cfu/ml recorded 84.06 per cent inhibition. Increase in per cent inhibition was noticed as the count increased from 10^7 cfu $ml^{-1}$to 10^8 cfu $ml^{-1,}$ thereafter found to be on par with each other. *P. fluorescens* recorded maximum per cent inhibition over control in all the three methods of application.

Pre-inoculation and post-inoculation treatment was found better in the case of *P. fluorescens* (Fig 9). Pre-inoculation spray of *P. fluorescens* found to be significant when compared to other treatments .*P. fluorescens* (KAU culture) did not showed much reduction in local lesions when compared to *P. fluorescens* from TNAU (Plate 11). It was observed that *Bacillus* sp recorded 56.83 percent inhibition when applied along with the virus. All other treatments were not as good as *P. fluorescens* in reducing the infection of CABMV in local lesion host (Plate 12).

4.2.1 Bioassay in cowpea

The treatments that showed best results in local lesion host were selected to find out its efficiency in cowpea. Vulnerability index was calculated and it was found that AVP treated plants showed less vulnerable to infection even at 30 DAI. In all the treatments, plants were found prone to infection with increase in plant age. *P. niruri* leaf extract showed minimum vulnerability index at 5 DAI of the virus. It was found less prone to virus infection even at the last stage of the crop. This was followed by *B. diffusa.* Plants treated with chemicals showed almost similar results in all the methods of application. Among the various treatments tested, betadine treated plants were found more prone to viral infection and they showed almost all the symptoms of CABMV even in pre-inoculation treatment.

Treatments that were applied before the challenge inoculation of the virus showed less symptom expression (Table 11, Plate 13). Post inoculation spray of treatments on plants were also less prone to infection (Table 12). It was found that cowpea plants were more vulnerable to infection by CABMV when the treatments were applied along with the virus (Table 13).

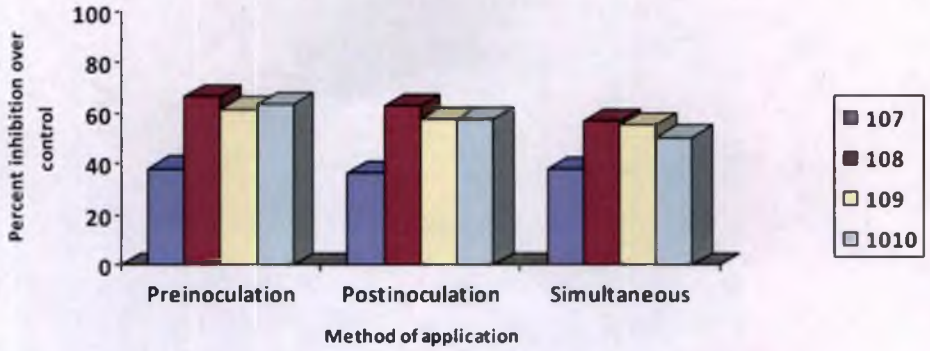
Table 10 Effect of biotic agents on CABMV in local lesion host *C. amaranticolor*

Biotic agents	Method of application	Per cent inhibition over control			
		10^7 cfu/ml	10^8 cfu/ml	10^9 cfu/ml	10^{10} cfu/ml
Pseudomonas fluorescens (TNAU culture)	Pre inoculation	38.35 (38.25)	84.06 (66.44)	77.46 (61.63)	80.00 (63.41)
	Post inoculation	36.26 (35.01)	79.27 (62.89)	7 1.44 (60.40)	71.44 (57.67)
	Simultaneous	37.88 (37.73)	69.80 (56.64)	67.47 (57.67)	59.45 (50.42)
Pseudomonas fluorescens (KAU culture)	Pre inoculation	15.68 (23.32)	33.28 (35.22)	39.75 (39.07)	37.04(37.47)
	Post inoculation	38.39 (38.27)	45.56 (42.43)	46.47 (42.96)	32.44(34.71)
	Simultaneous	36.49 (37.15)	20.03 (26.57)	22.13(28.05)	29.85(33.10)
Bacillus sp.	Pre inoculation	20.46 (26.88)	29.15(32.66)	33.85 (35.56)	38.46 (38.31)
	Post inoculation	20.95 (27.23)	31.03 (33.84)	36.88 (37.38)	47.89(43.77)
	Simultaneous	29.17 (32.68)	42.16 (40.47)	44.93 (42.07)	56.84(48.91)
Endophytic bacteria from cowpea	Pre inoculation	24.43 (29.61)	28.98(32.56)	27.68 (31.73)	30.02 (33.21)
	Post inoculation	21.68 (27.73)	31.01 (33.82)	35.80 (36.73)	39.30 (38.81)
	Simultaneous	11.00 (19.36)	15.01(22.78)	26.90 (31.23)	35.86 (36.77)
Rhizobacteria from cowpea	Pre inoculation	11.00 (22.87)	15.01 (25.87)	26.90(29.01)	35.86(33.23)
	Post inoculation	18.15 (25.21)	16.08 (23.63)	25.41 (30.25)	35.01(36.26)
	Simultaneous	9.42 (17.87)	14.82(22.63)	11.28(19.62)	22.47(28.28)

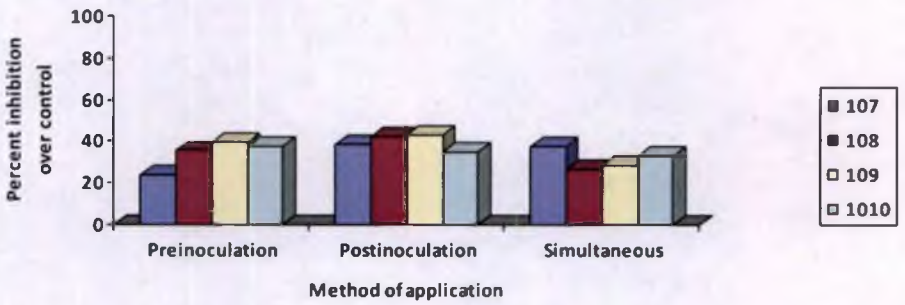
CD values : A- 11.32 B - 5.66

A - Concentration, B - Treatment X Method of application

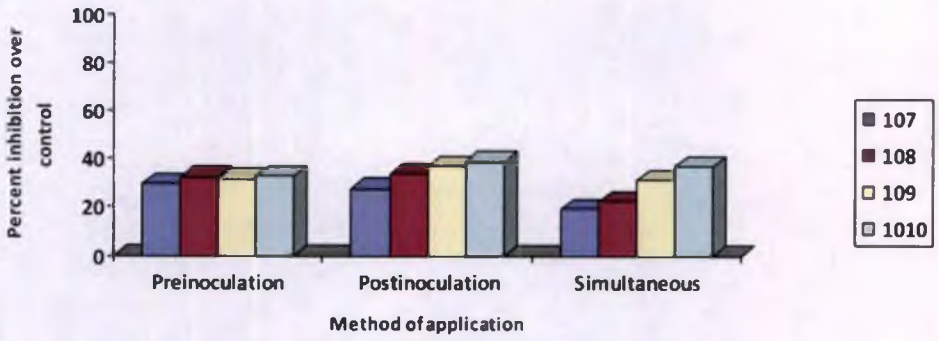
- Values are the mean of three replications and are expressed as percent inhibition over control.
- Values in parentheses are transformed values.



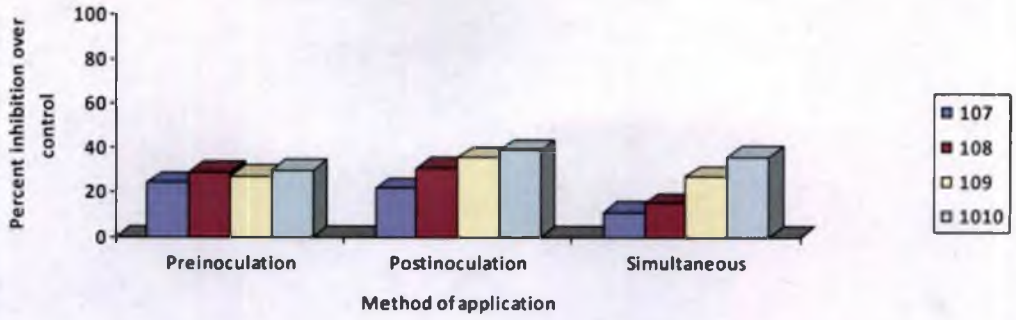
8a *Pseudomonas fluorescence* (TNAU culture)



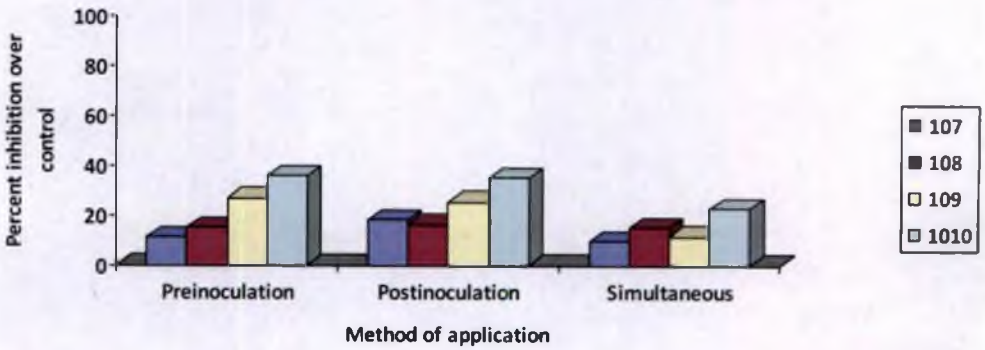
8b *Pseudomonas fluorescence* (KAU culture)



8c *Bacillus sp*

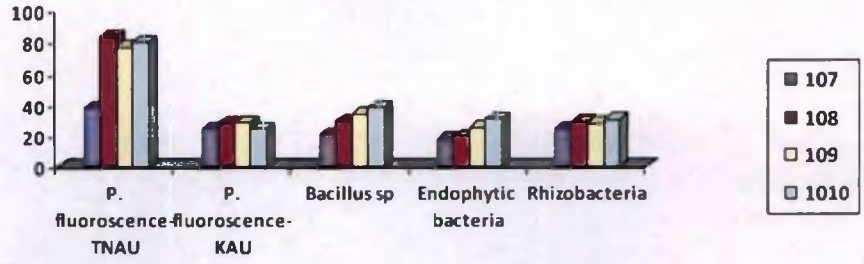


8d Endophytic bacteria

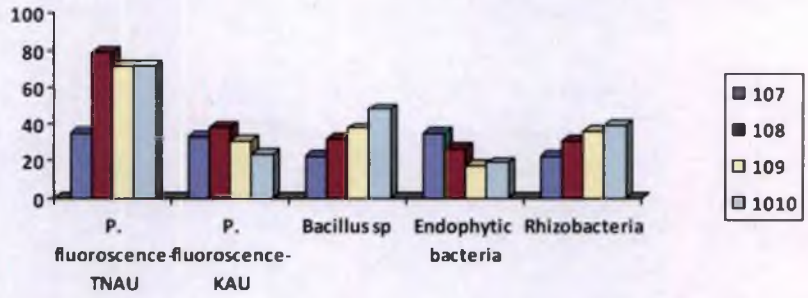


8e Rhizobacteria

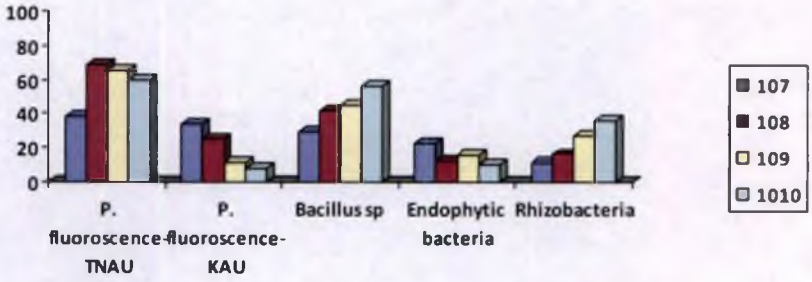
Fig. 8 Effect of biotic agents on CABMV on local lesion host, *C. amaranticolor*



9a Pre-inoculation

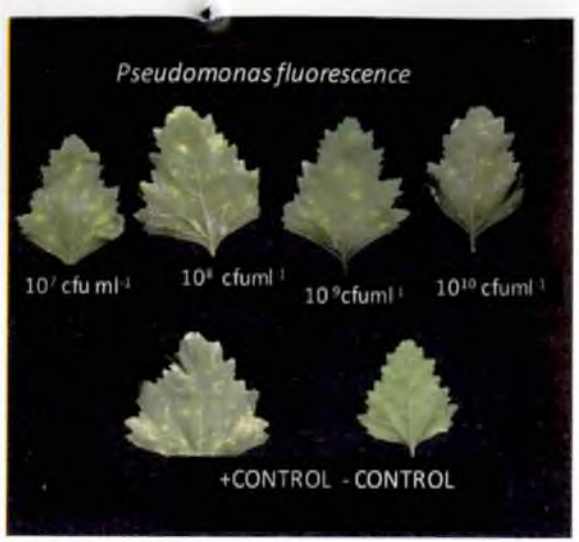


9b Post-inoculation

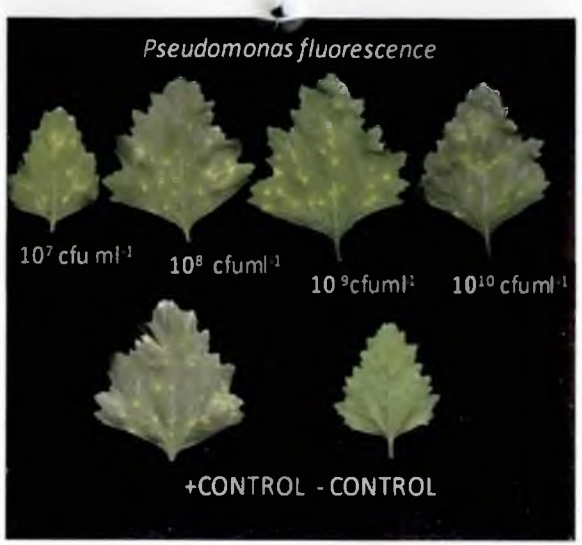


9c Simultaneous-inoculation

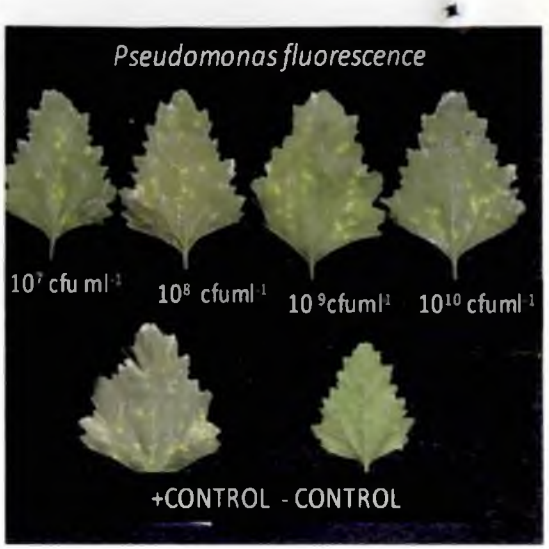
Fig. 9 Effect of different methods of application of biotic agents on CABMV on local lesion host, *C. amaranticolor*



11a. Pre-inoculation

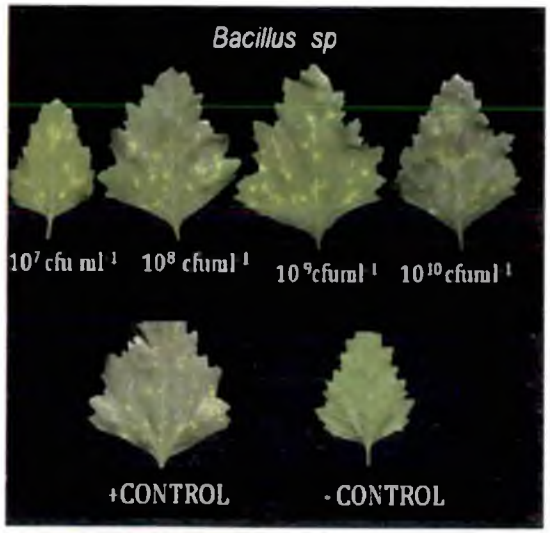


11b. Post-inoculation

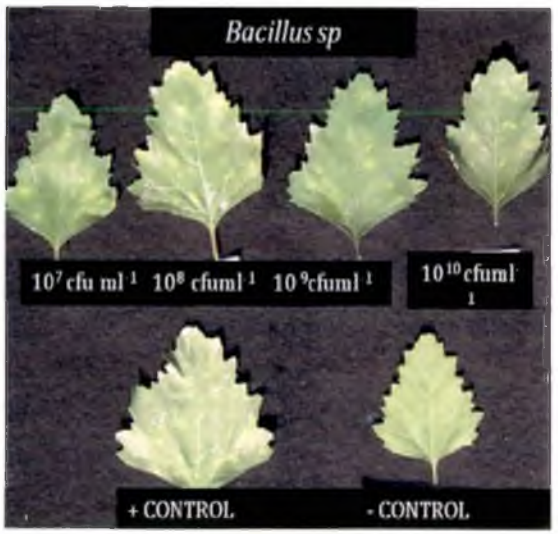


11c. Simultaneous-inoculation

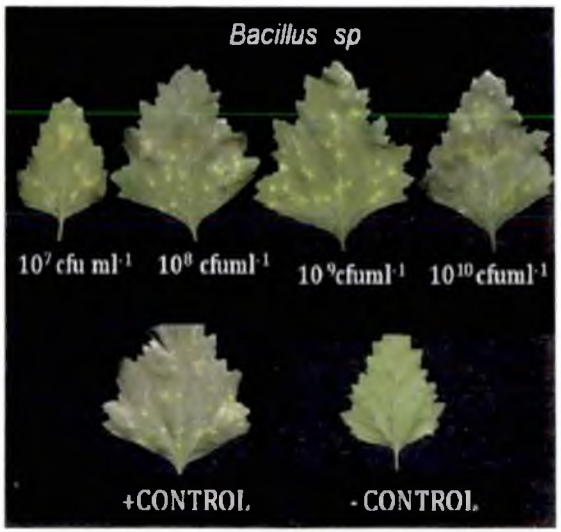
Plate 11. Effect of *Pseudomonas fluorescence* on CABMV in local lesion host, *C.amaranticolor*



12a. Pre-inoculation



12b. Post-inoculation



12c. Simultaneous-inoculation

Plate 12. Effect of *Bacillus* sp on CABMV in local lesion host, *C.amaranticolor*

Table 11 Vulnerability Index – Pre-inoculation

Treatments	Days after inoculation		
	5	15	30
P. niruri	8.27	23.20	27.54
B. diffusa	16.72	24.80	30.55
Salicylic acid	20.92	29.40	36.55
Ethrel	18.75	29.96	37.98
Betadiene	22.90	47.95	54.23
P. fluoroscens	27.53	43.41	46.94

CD values (0.05): A- 0.98, B - 0.77, AB - 1.88

A- Chemical, B - Period , AB- Interaction

* Values are the mean of three replications and expressed as percent inhibition over control

Table 12 Vulnerability Index – Post-inoculation

Treatments	Days after inoculation		
	5	15	30
P. niruri	8.88	25.05	30.99
B. diffusa	14.40	27.06	34.04
Salicylic acid	19.39	30.033	37.58
Ethrel	18.76	35.85	40.55
Betadiene	27.53	52.35	59.86
P. fluoroscens	30.33	45.19	48.75

CD values (0.05): A- 0.92, B - 0.49, AB - 1.21

A - Chemical, B - Period, AB- Interaction

* Values are the mean of three replications and expressed as percent inhibition over control

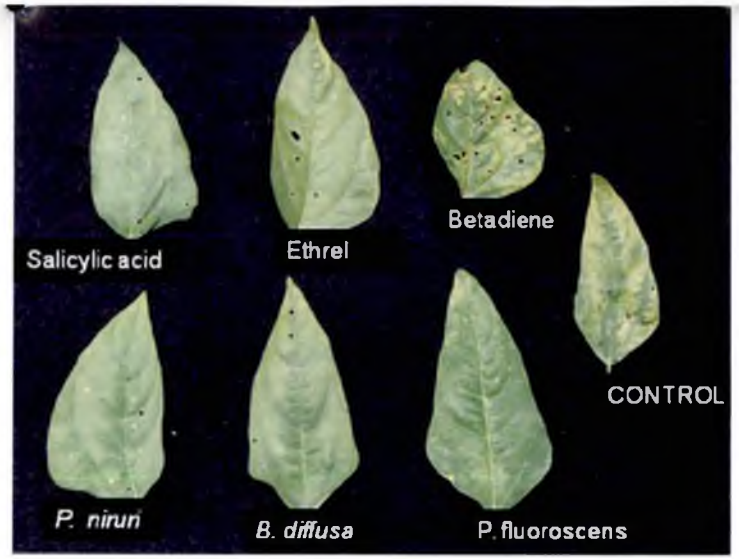
Table 13 Vulnerability Index –Simultaneous -inoculation

Treatments	Days after inoculation		
	5	15	30
P. niruri	8.27	29.84	31.28
B. diffusa	17.04	30.33	34.65
Salicylic acid	16.69	34.49	39.64
Ethrel	19.69	33.83	41.11
Betadiene	27.06	53.99	63.18
P. fluoroscens	29.66	43.88	50.94

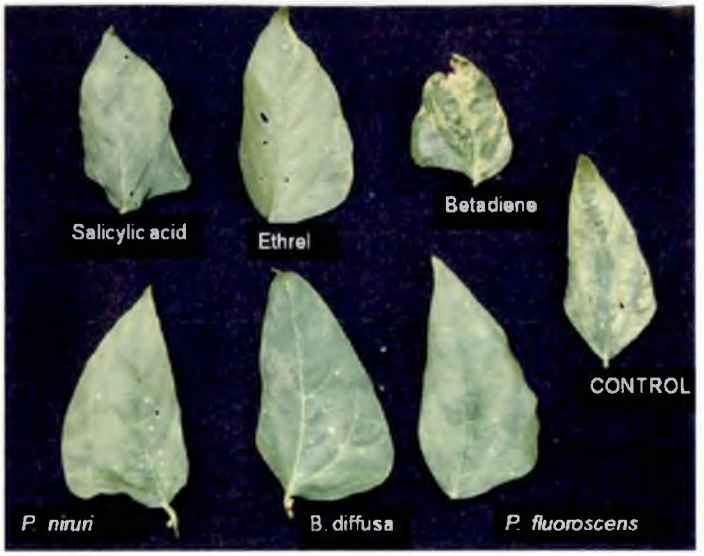
CD values (0.05): A- 1.14, B - 0.75, AB - 1.84

A- Chemical, B - Period, AB- Interaction

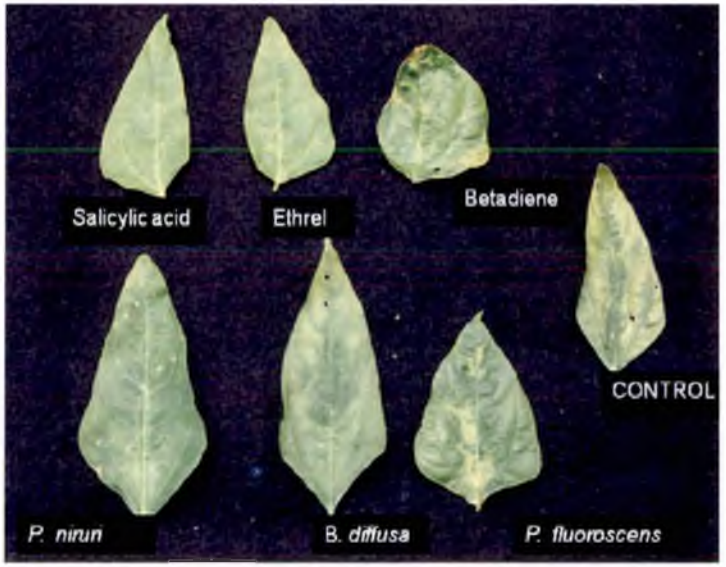
* Values are the mean of three replications and expressed as percent inhibition over control



13 a. Pre-inoculation



13 b. Post-inoculation



13 c. Simultaneous-inoculation

Plate 13. Vulnerability index of cowpea against CABMV

4.3 BIOCHEMICAL CHANGES OF HOST PATHOGEN INTERACTION

4.3.1. Estimation of protein

The result indicated that there was a progressive increase in the total protein content in both healthy and virus inoculated plants with increase in the age of plant. CABMV inoculated plants recorded maximum protein content of 3010 $\mu g\ g^{-1}$ as against 2250 $\mu g\ g^{-1}$ in healthy plants at 30 DAI (Table 14). Among the treatments *P. niruri* recorded maximum protein content (3003.3 $\mu g\ g^{-1}$) at 15 DAI. Thereafter it declined. The trend was the same for *B. diffusa* , salicylic acid, and ethrel. It was observed that there was significant difference between treatments and method of application. Pre-inoculation treatment was found superior than the post and simultaneous inoculation (Fig.10)

In pre-inoculation treatment, *P. niruri* and *B.diffusa* recorded maximum protein at 15 DAI. This was followed by ethrel. Several fold increase in protein content was observed in all the treatments when compared to healthy control. Protein content of betadine and *P. fluorescence* were low at one day after challenge inoculation with the virus and it was found to increase with the age of the plant. But remained low when compared with the plants treated with the virus alone.

In post-inoculation treatment, *P. niruri* treatment showed almost the same protein content at 15 DAI and 30 DAI of the virus. Among the treatments, betadine recorded low protein content. Significant increase in protein content was observed in AVP and chemicals treated plants at different time interval *viz.*, 1,3,5,15 and 30 DAI.

In simultaneous treatment, *P. niruri* recorded high protein at 15 DAI, thereafter drastic reduction in protein was observed. Protein content of *B. diffusa* and ethrel were on par with each other at 15 DAI.

4.3.2. Estimation of total sugars

The studies on total sugar content in cowpea revealed that there was increase in sugar content up to 15 DAI. In virus inoculated control, the sugar

Table 14 Changes in total soluble protein content of cowpea against CABMV

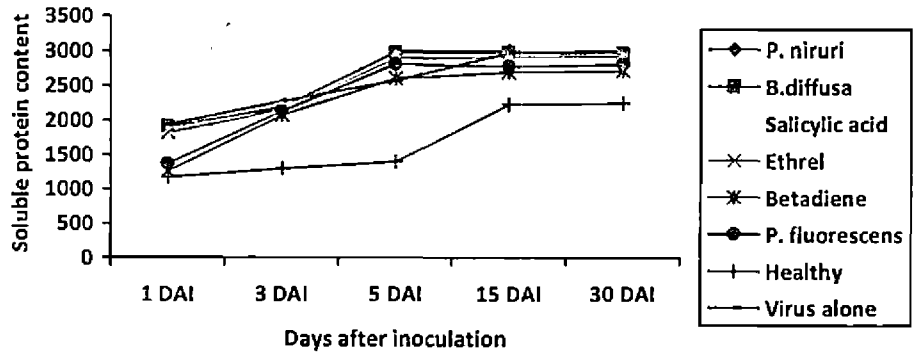
Treatments	Soluble protein content(μg g^{-1} fresh weight of tissue)														
	1DAI			3DAI			5DAI			15DAI			30DAI		
	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous
P. niruri	1910	1770	1746	2200	1990	1953	2993	2160	2140	3003	2980	2960	2930	2986	2870
B. diffusa	1920	1670	1720	2190	1960	1950	2980	2150	1480	2973	2920	2920	2960	2980	2950
Salicylic acid	1810	1640	1670	2160	1933	1910	2913	2130	2120	2890	2840	2860	2860	2880	2933
Ethrel	1833	1650	1660	2160	1920	1930	2900	2130	2150	2923	2900	2920	2930	2880	2900
Betadiene	1266	1620	1490	2083	1350	1610	2610	1960	2013	2700	2603	2680	2716	2750	2740
P. fluoroscens	1373	1673	1630	2130	1493	1720	2813	2060	2103	2780	2760	2790	2803	2860	2840
Healthy	1183	1183	1183	1300	1300	1300	1403	1403	1403	2230	2230	2230	2250	2250	2250
Virus alone	1943	1943	1943	2290	2290	2290	2580	2580	2580	2980	2980	2980	3010	3010	3010

DAI – Days after inoculation

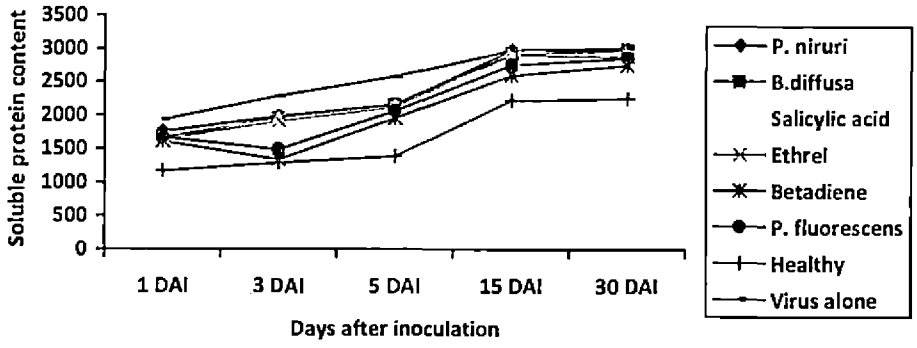
CD values (0.05): A – 31.76, B- 21.7, BC -37.58

A - Treatments, B - Time intervals, BC – Method of application X Time intervals

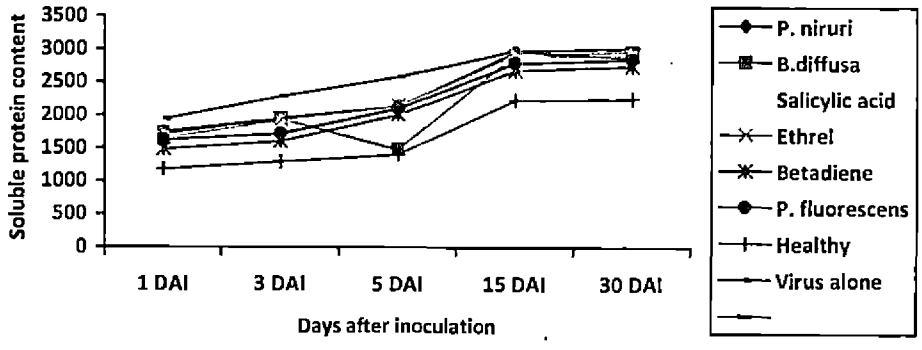
*Values are the mean of three replications



10a Pre-inoculation



10b Post inoculation



10c Simultaneous- inoculation

Fig. 10 Changes in total soluble protein content of cowpea leaves against CABMV

content reduced from 1DAI to 15 DAI of the virus, thereafter it gets increased at 30 DAI. Among the treatments tested AVP recorded the maximum total sugar content in pre, post and simultaneous inoculation. The result indicated that there was significant difference between treatments viz., chemicals, AVP and biotic agents at different time intervals (Table 15).

In pre-inoculation treatment, it was found that *B. diffusa* and *P. niruri* recorded high sugar content at 15 DAI. At 30 DAI, total sugar content was found to be reduced (Fig.11). The total sugar content was found to increase with increase in age of the plant and it was more than the healthy control. In the case of betadine, the sugar content was found higher at 1 DAI and thereafter it gets reduced .It was found to be lower than the virus inoculated control. Healthy plants showed increased total sugar content compared to virus inoculated ones

Post-inoculation treatment recorded low sugar when compared to pre inoculation in all the treatments except betadiene. Total sugar content of betadiene was found to be reduced at different time intervals. Treatments that were applied along with the virus recorded low sugar content when compared to 24 h before and 24 h after virus inoculation. In contrast, it was observed that cowpea plants treated with betadiene showed increased sugar in simultaneous application at 15 DAI and it was found in between virus inoculated and healthy control.

4.3.3. Estimation of reducing sugar

Healthy plants showed higher sugar content compared to virus alone in all the three methods of application (Table 16). Reducing sugar content was found to be increased from 1 DAI to 5 DAI in all the treatments tested. Thereafter the reducing sugar was found to declined at 15 and 30 DAI of the virus. Significant increase in reducing sugar content with different treatments were observed at different time intervals.

Among the different methods of application, pre-inoculation treatment recorded high reducing sugar content (Fig.12). *P. niruri* treated cowpea plants recorded increased reducing sugar content when compared with the healthy

Table 15 Changes in total sugar content of cowpea against CABMV

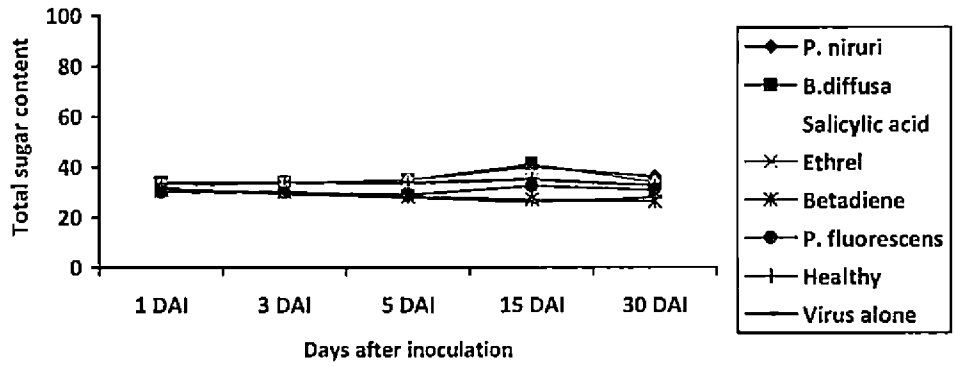
Treatments	Total sugar content(mg g^{-1} fresh weight of tissue)														
	1DAI			3DAI			5DAI			15DAI			30DAI		
	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous
P. niruri	33.6	33.3	33.0	33.3	33.3	33.1	35.0	34.6	34.0	40.3	38.0	35.7	36.0	35.3	34.6
B. diffusa	34.0	33.3	32.8	34.0	33.6	33.1	35.0	34.3	34.2	41.1	38.4	35.0	34.0	34.2	34.8
Salicylic acid	33.3	32.5	32.1	33.3	32.7	33.0	34.4	34.0	33.3	36.1	35.1	34.4	33.5	33.0	33.2
Ethrel	32.2	32.4	32.2	34.1	33.4	33.5	33.3	33.4	33.1	35.0	33.3	32.3	32.3	28.2	33.2
Betadiene	31.0	31.2	31.7	30.2	29.2	27.2	28.3	27.1	27.3	27.0	28.4	30.0	26.2	30.3	28.6
P. fluoroscens	30.3	30.1	30.2	30.0	29.4	27.8	29.1	30.0	30.4	32.4	31.4	30.5	31.0	28.2	29.5
Healthy	33.6	33.6	33.6	34.0	34.0	34.0	34.0	34.0	34.0	35.3	35.3	35.3	32.6	32.6	32.6
Virus alone	32.0	32.0	32.0	29.4	29.4	29.4	28.0	28.0	28.0	26.1	26.1	26.1	28.1	28.1	28.1

DAI – Days after inoculation

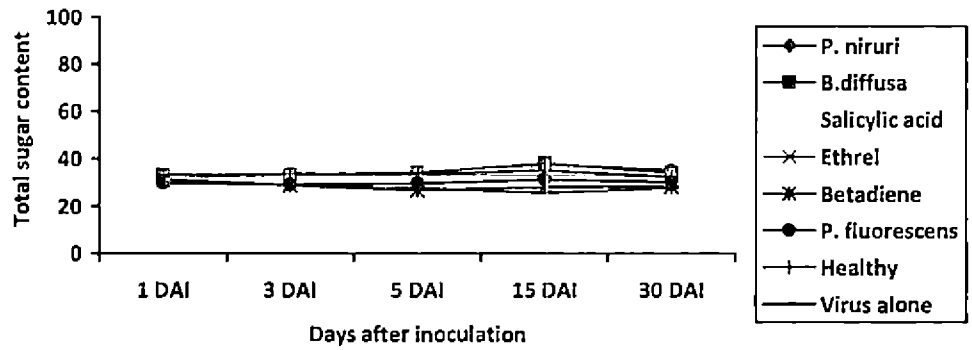
CD values (0.05): A – 0.56, B - 0.42 BC - 0.73

A - Treatments, B - Time intervals, BC – Method of application X Time intervals

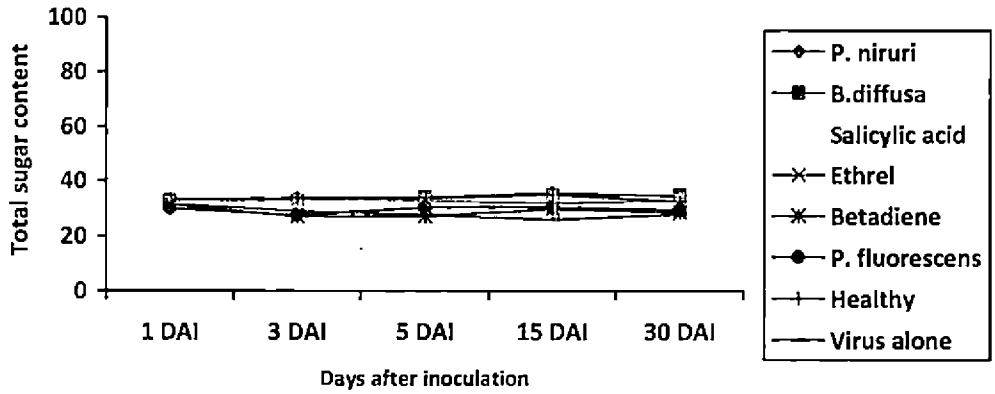
*Values are the mean of three replications



11a Pre-inoculation



11b Post-inoculation



11c Simultaneous- inoculation

Fig. 11 Changes in total sugar content of cowpea leaves against CABMV

Table 16 Changes in reducing sugar content of cowpea against CABMV

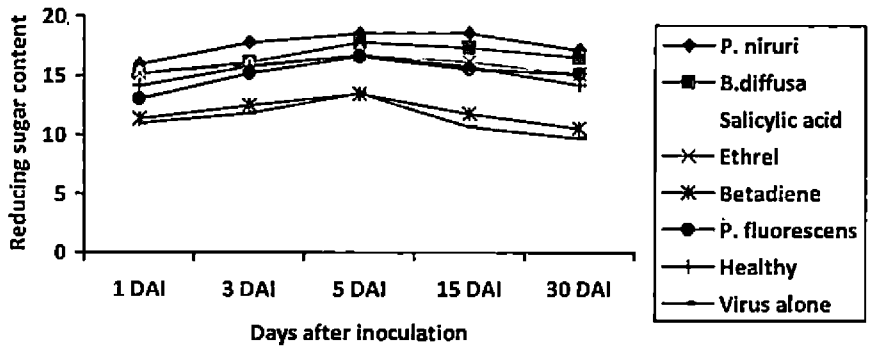
Treatments	Reducing sugar content(mg g^{-1} fresh weight of tissue)														
	1DAI			3DAI			5DAI			15DAI			30DAI		
	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous
P. niruri	16.00	15.60	15.10	17.83	17.66	16.46	19.83	18.80	17.83	18.50	18.23	16.50	17.16	16.50	15.50
B. diffusa	15.23	15.00	15.43	16.13	15.83	15.80	17.83	17.83	16.80	17.33	16.23	15.46	16.50	15.83	15.00
Salicylic acid	15.20	15.12	15.02	15.93	15.60	14.96	17.06	16.36	15.90	16.00	15.16	14.83	15.06	14.50	14.63
Ethrel	15.20	14.95	15.13	15.96	15.66	14.83	16.76	16.26	15.96	16.16	14.90	14.10	15.13	14.06	14.00
Betadiene	11.40	11.23	11.35	12.53	12.10	11.66	13.43	12.66	11.73	11.73	10.83	10.26	10.50	10.26	9.50
P. fluoroscens	13.06	12.76	13.72	15.23	14.90	14.83	16.63	15.96	15.16	15.50	14.50	13.66	15.10	14.26	12.66
Healthy	14.16	14.16	14.16	15.83	15.83	15.83	16.73	16.73	16.73	15.76	15.76	15.76	14.16	14.16	14.16
Virus alone	11.00	11.00	11.00	11.83	11.83	11.83	13.50	13.50	13.50	10.66	10.66	10.66	9.66	9.66	9.66

DAI – Days after inoculation

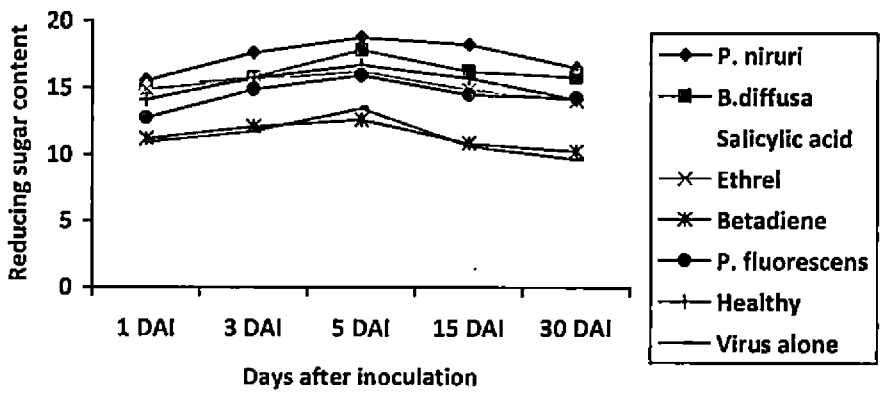
CD values (0.05): A – 0.284, B - 0.213, BC - 0.368

A - Treatments, B - Time intervals, BC – Method of application X Time intervals

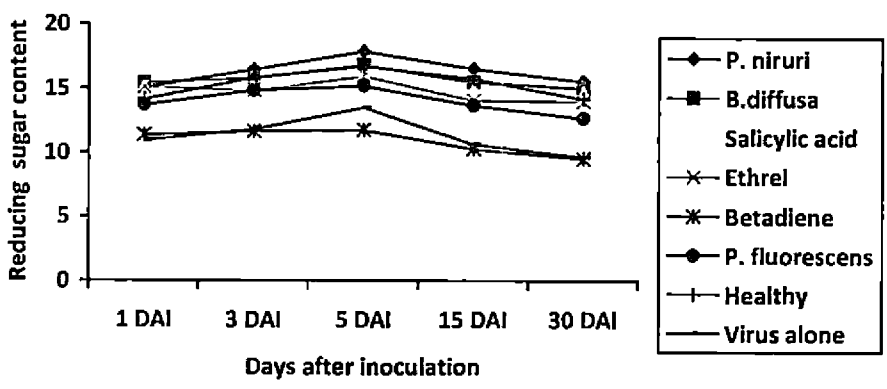
*Values are the mean of three replications



12a Pre-inoculation



12b Post-inoculation



12c Simultaneous-inoculation

Fig. 12 Changes in reducing sugar content of cowpea leaves against CABMV

control. On 5 DAI it was found to be 19.83 mg g^{-1} fresh tissue thereafter gradual decrease in reducing sugar content was observed on 15 DAI (18.50 mg g^{-1}) and 30 DAI (17.16 mg g^{-1}). Reducing sugar observed on treatment with ethrel and *P. flouoroscens* found on par with each other at 5 DAI in pre inoculation method of application. Betadiene recorded low reducing sugar when compared with other treatments and it was in between healthy and virus inoculated control.

Decrease in reducing sugar content was observed in post and simultaneous inoculation sprays compared to pre-inoculation. *P. niruri* was the one with high reducing sugar content in post-inoculation. It was found that the reducing sugar of chemicals inducing resistance like, SA and ethrel were on par with each other.

Simultaneous inoculation treatment showed lesser reducing sugar content. All the treatments showed lower reducing sugar when applied along with the virus. Only *P. niruri* and *B. diffusa* recorded more reducing sugar even in simultaneous inoculation when compared to healthy control.

4.3.4. Estimation of phenol

Application of selected biotic and abiotic factors on cowpea cv. Sharika challenged with CABMV induced higher accumulation of phenolics. Accumulation of phenolics started one day after challenge inoculation. The maximum accumulation was observed upto 5 DAI and declined thereafter. Healthy plants recorded lower phenol content when compared to virus alone control (Table 17). Significant increase in phenol contents of different treatments at different time intervals were noticed.

Among the treatments cowpea plants treated with *P. niruri* showed several fold increase in phenol content on 5 DAI as against healthy and virus alone in pre-inoculation spray. It was followed by *B. diffusa*. Phenol content of *P.fluorescens* and ethrel were the same at 5 DAI of the virus thereafter it declined. Betadine recorded lower accumulation of phenolics when compared with other treatments, but it was more than healthy and virus control.

Table 17 Changes in phenol content of cowpea against CABMV

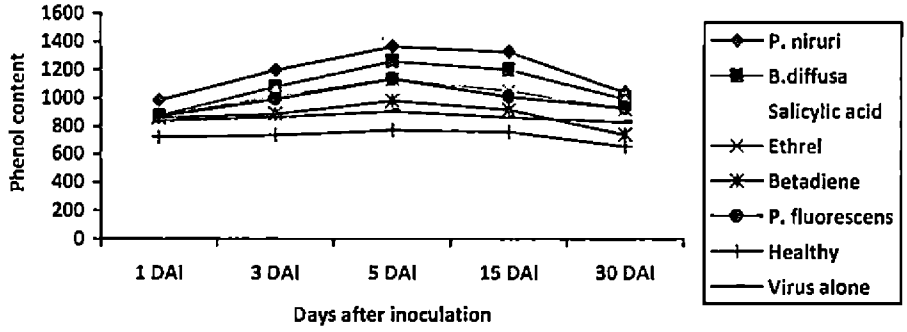
Treatments	Phenol content(µg g^{-1} fresh weight of tissue)														
	1DAI			3DAI			5DAI			15DAI			30DAI		
	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous
P. niruri	986	921	866	1199	1099	1016	1366	1343	1188	1327	1243	1160	1038	977	960
B. diffusa	877	899	855	1082	999	955	1260	1205	1071	1199	1132	1088	988	922	877
Salicylic acid	852	860	855	1032	988	944	1171	1121	1016	1082	994	933	960	883	849
Ethrel	874	844	827	1021	999	905	1132	1077	999	1049	977	899	920	860	821
Betadiene	863	822	810	888	855	822	982	960	910	916	860	822	738	721	744
P. fluoroscens	871	827	816	994	971	938	1132	1016	955	1005	949	905	927	894	821
Healthy	722	722	722	735	735	735	771	771	771	758	758	758	652	652	652
Virus alone	841	841	841	866	866	866	905	905	905	863	863	863	830	830	830

DAI – Days after inoculation

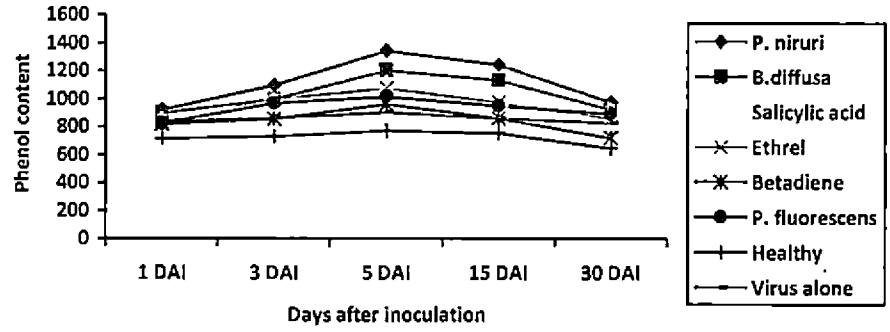
CD values (0.05) A – 16.49, B – 13.86, BC – 24.03

A - Treatments, B - Time intervals, BC – Method of application X Time intervals

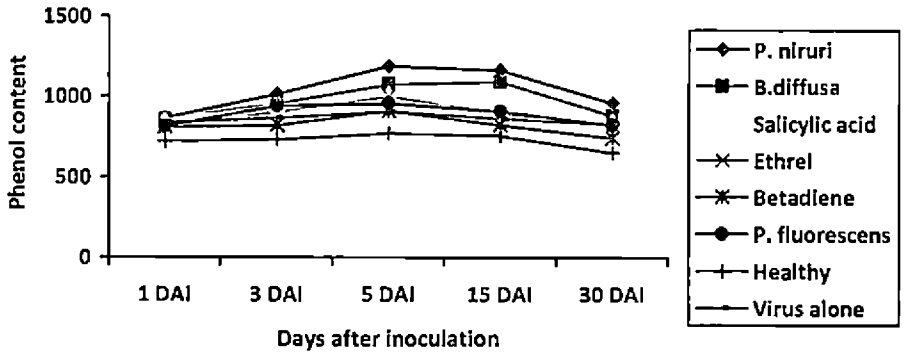
*Values are the mean of three replications



13a Pre-inoculation



13b Post-inoculation



13c Simultaneous-inoculation

Fig. 13 Changes in total phenol content of cowpea leaves against CABMV

Post-inoculation treatment was found to show low phenol content in all the treatments tested (Fig. 13). Increase in phenol was observed with increase in the age of plant, upto 5 DAI, later it reduced. AVPs and chemicals inducing resistance showed accumulation of phenol when compared to healthy plants.

Simultaneous inoculation of the virus and different treatments showed less phenol when compared to other method of application. But it was found still higher than virus alone and healthy control.

4.3.5 Estimation of Defence related enzymes

4.3.5.1 Estimation of Phenylalanine ammonialyase

Studies on PAL activity in cowpea revealed that AVPs and chemicals treated and challenged with CABMV increased the accumulation of PAL than healthy and virus inoculated control. Increased PAL activity was observed upto 5 DAI and thereafter it gets declined in plants inoculated with virus. Whereas in healthy control PAL increased upto 15 DAI and later declined. The data revealed that there was significant increase in PAL in different treatments (Table 18).

In pre-inoculation, *P .niruri* showed increased PAL activity and it was several fold more than healthy and plants inoculated with virus as control. This was followed by *B. diffusa* . PAL activity were on par in the case of SA and ethrel . *P. fluorescens* treated plants also showed increased PAL activity in pre inoculation. Among the various treatments, betadine treated plants showed reduced activity of this enzyme and it was found to be reduced from 1 DAI to 30 DAI of the virus

Post-inoculation also increased the PAL activity, but found to be lower when compared to pre inoculation in all the treatments except betadine (Fig 14). In betadine treated plants, initially PAL reduced upto 3 DAI and then gets increased. Thereafter it gets decreased from 15 to 30 DAI.. Simultaneous inoculation showed less PAL activity in all the treatments when compared with pre and post inoculation. It was found to be several fold more activity than healthy and virus inoculated control.

Table 18 Changes in Phenylalanine ammonialyase activity of cowpea against CABMV

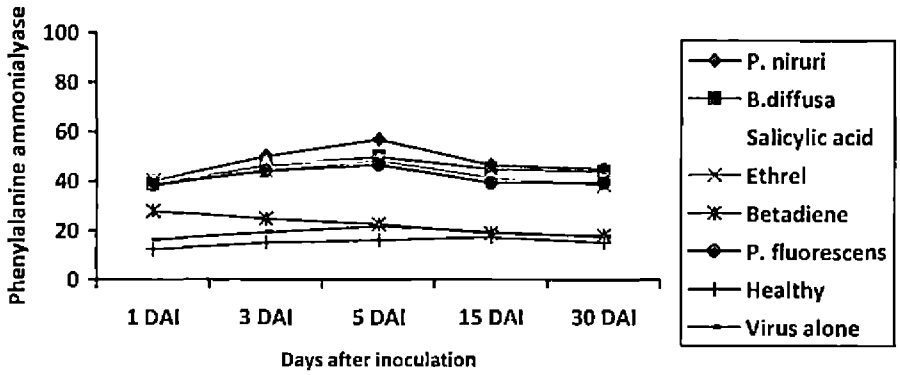
Treatments	Phenylalanine ammonialyase(µg g^{-1} min^{-1} fresh weight)														
	1DAI			3DAI			5DAI			15DAI			30DAI		
	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous
P. niruri	4041	38.88	38.90	50.21	45.40	41.20	57.06	47.36	44.20	46.40	45.55	41.86	45.13	39.00	37.00
B. diffusa	39.15	38.80	37.48	46.50	44.86	41.00	50.00	47.06	43.40	45.00	43.72	40.13	44.00	37.83	35.40
Salicylic acid	40.13	38.70	36.93	47.50	42.00	41.10	48.50	46.73	42.47	40.78	41.11	39.23	38.86	38.33	36.00
Ethrel	40.52	37.70	37.22	44.16	41.80	40.60	48.40	46.33	41.60	41.52	40.52	38.16	38.41	37.41	34.73
Betadiene	28.02	23.61	23.73	24.93	19.33	16.93	22.56	23.13	21.03	18.83	17.33	17.86	17.83	17.06	16.43
P. fluoroscens	38.66	37.40	38.03	44.31	38.80	34.33	46.73	44.06	40.25	39.41	37.75	37.31	39.40	35.73	37.40
Healthy	12.33	12.33	12.33	15.28	15.28	15.28	16.07	16.07	16.07	17.43	17.43	17.43	15.16	15.16	15.16
Virus alone	16.31	16.31	16.31	19.50	19.50	19.50	21.90	21.90	21.90	19.73	19.73	19.73	17.33	17.33	17.33

DAI – Days after inoculation

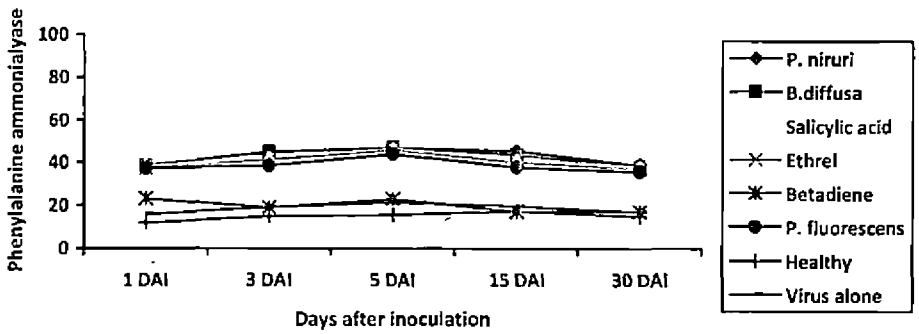
CD values (0.05) A – 0.69, B – 0.57, BC – 0.99

A - Treatments, B - Time intervals, BC – Method of application X Time intervals

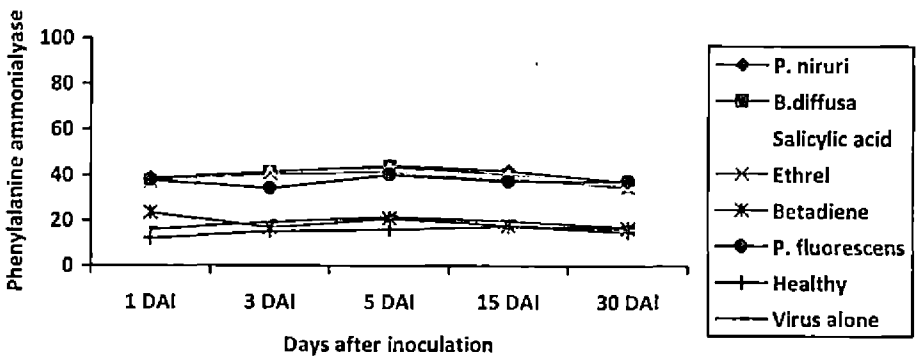
*Values are the mean of three replications



14a Pre- inoculation



14b Post- inoculation



14c Simultaneous- inoculation

Fig. 14 Changes in Phenylalanine ammonialyase activity of cowpea leaves against CABMV

4.3.5.2 Estimation of Polyphenol oxidase

Increase in Polyphenol oxidase (PPO) in cowpea was observed from 1 DAI with the virus. The activity of PPO increased upto 15 DAI and declined thereafter in all the treatments (Table 19). Virus inoculated control recorded more PPO activity when compared to healthy plants. Plants treated with *P. niruri* showed increase PPO activity in all the three methods of application. It was found to be increase with age of the plant, at 15 DAI, thereafter declined at 30 DAI. This was followed by *B. diffusa* and SA. Significant difference in PPO activity was observed in different treatments. All treatments except betadine showed increased PPO activity when compared to virus inoculated control

Pre-inoculation treatment was found to show increased PPO activity when compared to all other methods (Fig. 15). PPO activity was found more even at the initial stage of the crop in plants treated with AVPS and SA and ethrel., Betadiene treated plants showed reduced PPO activity and it was found in between healthy and virus inoculated control. It was observed that plants treated with *P. fluorescens* increase the activity of PPO but not more than other treatments.

Post-inoculation was found on par with pre inoculation in all treated plants. It was recorded that treatments when applied along with the virus showed less activity of the defence enzyme when compared with application at 24 h before and after virus inoculation. All the treatments except betadiene showed comparatively increased activity of the enzyme even in simultaneous application.

4.3.5.3 Estimation of Peroxidase

Studies on the mode of action of AVPs and chemicals against CABMV revealed that they induced defence mechanism in plants challenged with CABMV. Accumulation of peroxidase (PO) was observed from first day after treatment (Table 20) . Significant increase in peroxidase was found in plants treated with chemicals, AVPs and biotic agents at different time intervals. Progressive increase in PO activity was observed upto 15 DAI and thereafter it

Table 19 Changes in polyphenol oxidase activity of cowpea against CABMV

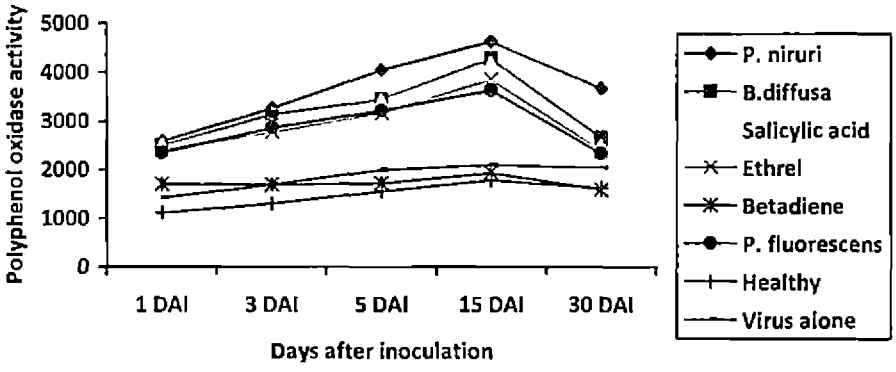
Treatments	Polyphenol oxidase (min^{-1} mg^{-1} fresh weight)														
	1DAI			3DAI			5DAI			15DAI			30DAI		
	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous
P. niruri	2600	2560	2340	3280	3000	2420	4050	3440	2740	4640	4650	2980	3680	3540	2380
B. diffusa	2510	2440	2300	3150	2650	2260	3450	3360	2820	4280	4120	2630	2660	3140	2310
Salicylic acid	2510	2310	2270	2870	2630	2110	3430	2920	2550	4190	4100	2950	2480	2910	1840
Ethrel	2400	2330	2200	2780	2430	2050	3180	2530	2580	3870	3820	2680	2383	2270	2220
Betadiene	1710	1670	1320	1700	1680	1630	1730	1690	1600	1940	1960	1830	1593	1833	1720
P. fluoroscens	2350	2240	2060	2870	2790	2040	3230	3010	2560	3640	3400	2930	2330	2500	1940
Healthy	1120	1120	1120	1310	1310	1310	1560	1560	1560	1790	1790	1790	1620	1620	1620
Virus alone	1430	1430	1430	1700	1700	1700	2000	2000	2000	2100	2100	2100	2040	2040	2040

DAI – Days after inoculation

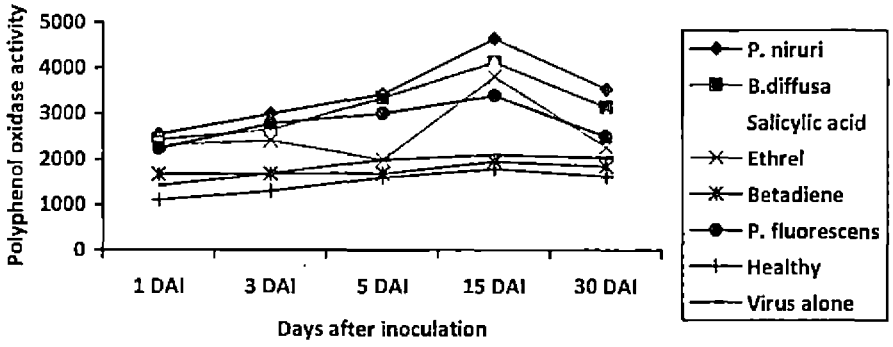
CD values (0.05) A – 171, B – 112, BC – 194

A - Treatments. B - Time intervals, BC – Method of application X Time intervals

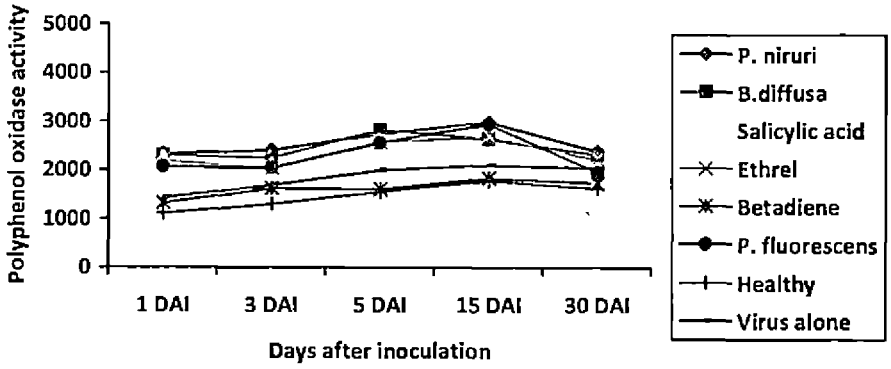
*Values are the mean of three replications



15a Pre-inoculation



15b Post-inoculation



15c Simultaneous inoculation

Fig. 15 Changes in Polyphenol oxidase activity of cowpea leaves against CABMV

Table 20 Changes in peroxidase activity of cowpea against CABMV

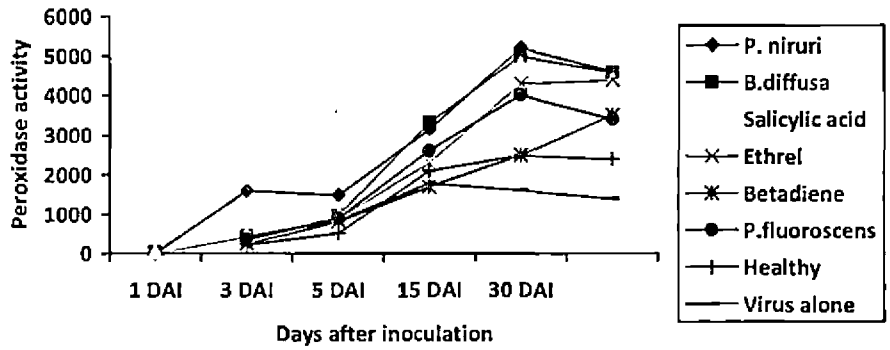
Treatments	Peroxidase (min^{-1} mg^{-1} fresh weight)														
	1DAI			3DAI			5DAI			15DAI			30DAI		
	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous	Pre	Post	Simultaneous
P. niruri	600	630	530	1500	1300	970	3150	2690	2000	5200	4100	4000	4600	4700	4400
B. diffusa	430	520	400	980	1000	920	3300	2600	2000	5000	4000	3600	4600	4300	4600
Salicylic acid	480	500	500	1000	960	800	2500	2000	2000	4800	3800	3400	4400	3600	4300
Ethrel	430	470	500	910	900	1000	2300	2000	1900	4300	3700	3400	4400	3500	4100
Betadiene	250	340	330	820	750	730	1700	1600	1500	2500	2500	2700	3500	3000	3700
P. fluoroscens	360	400	440	900	910	1000	2600	2200	1600	4000	3600	3100	3400	4000	4000
Healthy	230	230	230	530	530	530	2100	2100	2100	2500	2500	2500	2400	2400	2400
Virus alone	430	430	430	860	860	860	1800	1800	1800	1600	1600	1600	1400	1400	1400

DAI – Days after inoculation

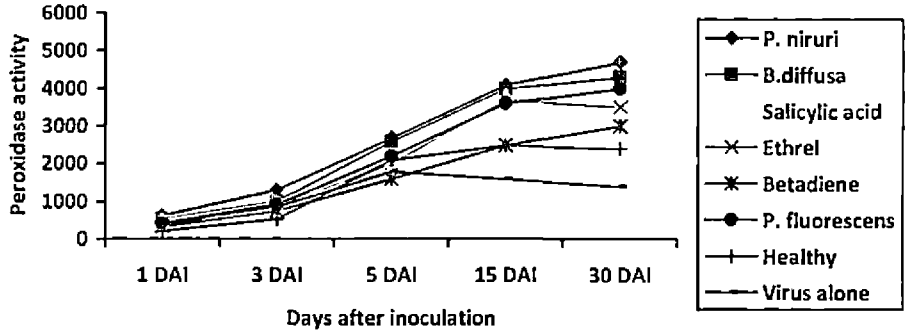
CD values (0.05) A – 902, B – 776. BC – 1340

A - Treatments, B - Time intervals, BC – Method of application X Time intervals

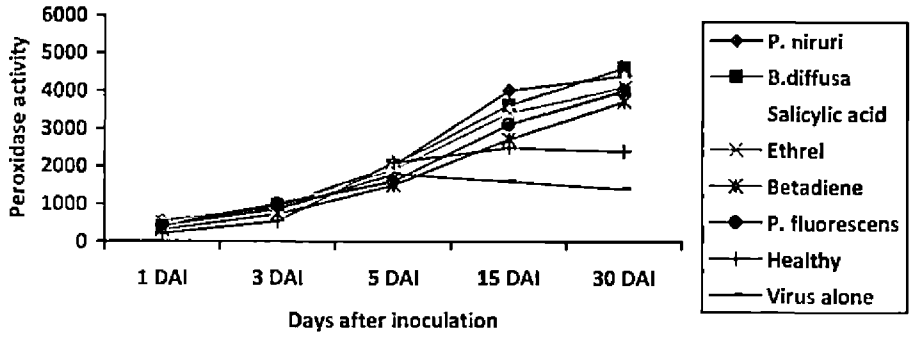
*Values are the mean of three replications



16a Pre-inoculation



16b Post-inoculation



16c Simultaneous-inoculation

Fig. 16 Changes in peroxidase activity of cowpea leaves against CABMV

gets declined. Healthy plants showed increased peroxidase activity than virus inoculated control. Among the various treatments, *P. niruri* extract showed high PO activity at 15 DAI followed by *B. diffusa*). These treatments showed several fold increase in PO activity compared to healthy and virus inoculated control. Significant difference between method of application and different time interval was observed. Pre-inoculation treatment showed increased activity of PO in all the treatments.

Pre-inoculation treatment revealed that all the treatments induced increased PO activity in cowpea (Fig.16). PO activity was found to be on par in treatments with *P. niruri* and *B. diffusa*. Similar trend was observed in the case of chemicals like SA and ethrel. In butadiene treated plants, PO was found to increase with increase in age of the plant but remained lower when compared to other treatments.

In post-inoculation treatment, peroxidase activity increased with increase in age of cowpea ie, upto 15 DAI in all the treated plants. Drastic increase in PO was seen from 1 DAI to 15 DAI in the case of AVPs treated plants. The results revealed that post inoculation treatment were less effective in inducing the defence mechanism in plants when compared to pre inoculation. In plants treated with *P. fluorescens*, increased PO was seen from 1 DAI to 30 DAI and was found to be more than healthy plants and plants challenged with virus alone.

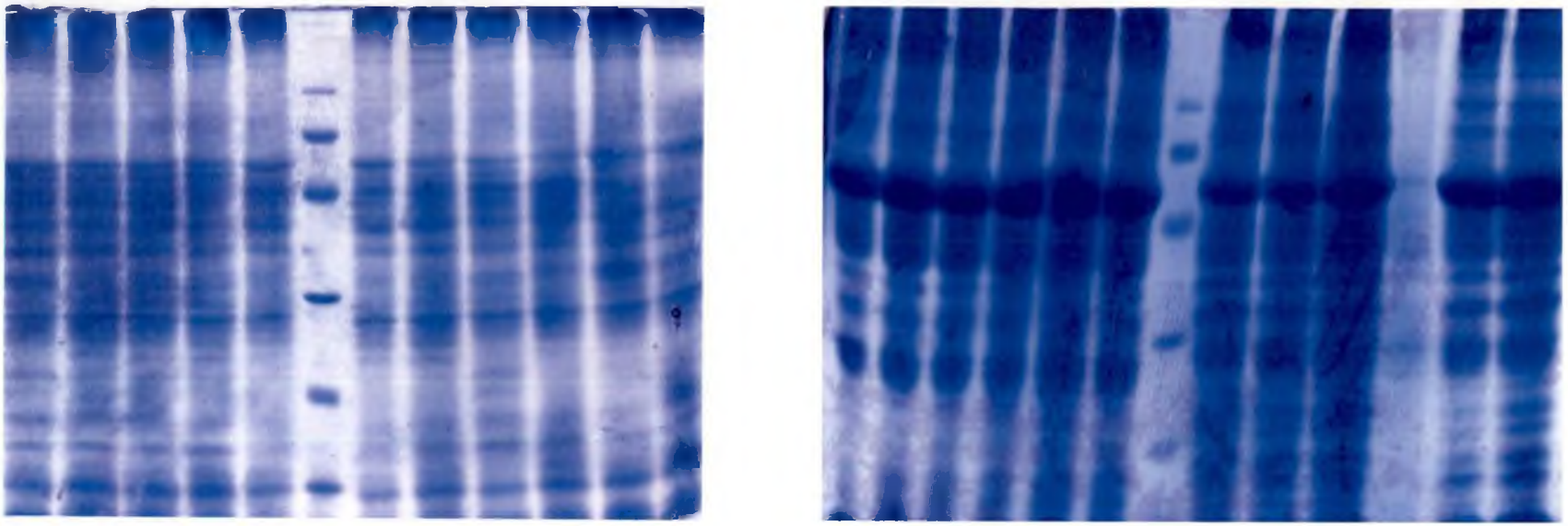
When compared with other methods of application, it was observed that PO activity increase upto the last stage of the crop. PO activity was found to be lower when treatments were applied along with the virus. SA and ethrel showed almost the same PO activity in cowpea.

4.4 ELECTROPHORETIC ANALYSIS OF PROTEINS

Electrophoretic analysis of proteins were conducted on cowpea against CABMV treated with different abiotic and biotic factors at different time intervals viz. one day, three day, five day, fifteen day and thirty days after inoculation. Healthy and diseased samples were analysed. Many proteins were induced in

response to various treatments. The induction of PR-proteins was more in leaves which were treated with biotic agent and also with plant extracts. This induction of PR-proteins was more prominent at 1 DAI in leaves. An extra band was noticed in diseased control when compared to healthy. Molecular weight of extra band in diseased were estimated using the molecular markers loaded along with the samples. It was found to be with molecular weight of 24.5 KDa. This protein were found to be absent in plants treated with *B. diffusa* (Simultaneous application), salicylic acid (Simultaneous), ethrel and butadiene at 1DAI (Plate 14.).

Plants treated with ethrel as post-inoculation, the intensity of bands with molecular weight of 20.1 KDa was high when compared with other treatments. Protein (24.5 K Da) which was found to present in diseased were absent in betadine (Post and simultaneous) and *P. fluorescens* treated plants at 3 DAI (Plate 15). The intensity of proteins of 24.0 K Da was less in plants treated with salicylic acid as pre and simultaneous at 5DAI. Protein with molecular weight of 27.5KDa which was present in diseased control at 5 DAI were absent in healthy and betadiene treated plants (Plate 16). Protein with 17.5 KDa molecular weight was observed in diseased and in almost all treatments plants except *B. diffusa* (Post and simultaneous), ethrel (Pre-inoculation), betadiene (Simultaneous) and *P. fluorescens* at 15 DAI (Plate 17). No new proteins were found to be induced at 30 DAI. Intensity of proteins were found to be reduced (Plate 18). The intensity of induction of PR-proteins were high during the early stages of treatment. But there were steady decrease in the intensity of PR-proteins as the time interval increased.



1 2 3 4 5 M 6 7 8 9 10 11 12 13 14 15 16 17 M 18 19 20 21 22

1. *P. niruri* (Pre-inoculation)
2. *P. niruri* (Post-inoculation)
3. *P. niruri* (Simultaneous)
4. *B. diffusa* (Pre-inoculation)
5. *B. diffusa* (Post-inoculation)

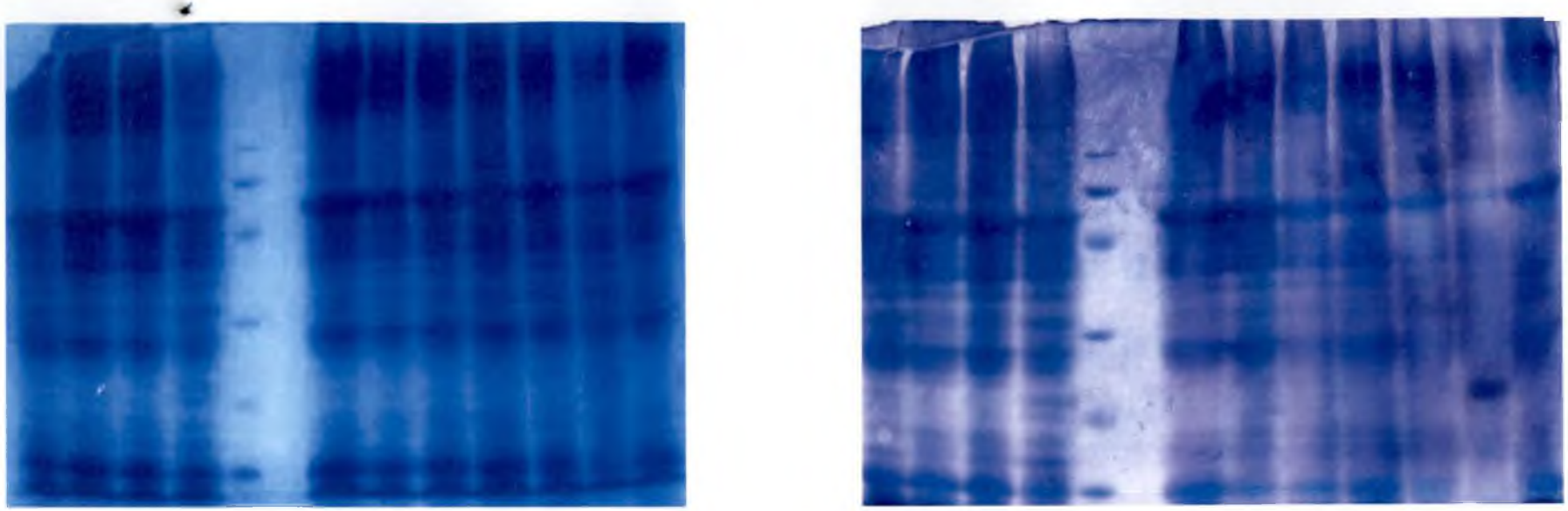
M. Marker.

6. *B. diffusa* (Simultaneous)
7. Salicylic acid (Preinoculation)
8. Salicylic acid (Post-inoculation)
9. Salicylic acid (Simultaneous)
10. Healthy control
11 Diseased control
12. Ethrel (Pre-inoculation)
13. Ethrel (Post-inoculation)
14. Ethrel (Simultaneous)
15. Betadiene (Pre-inoculation)
16. Betadiene (Post-inoculation)
17. Betadiene (Simultaneous)

M. Marker

18. *P. fluorescence* (Preinoculation)
19 *P. fluorescence* (Post-inoculation)
20. *P. fluorescence* (Simultaneous)
21. Healthy control
22. Diseased control

Plate 14. Appearance of PR proteins in cowpea leaves inoculated with CABMV and treated with different abiotic and biotic factors at 1 DAI.



1 2 3 4 M 5 6 7 8 9 10 11 12 13 14 15 M 16 17 18 19 20 21 22

1. *P. niruri* (Pre-inoculation)
2. *P. niruri* (Post-inoculation)
3. *P. niruri* (Simultaneous)
4. *B. diffusa* (Pre-inoculation)

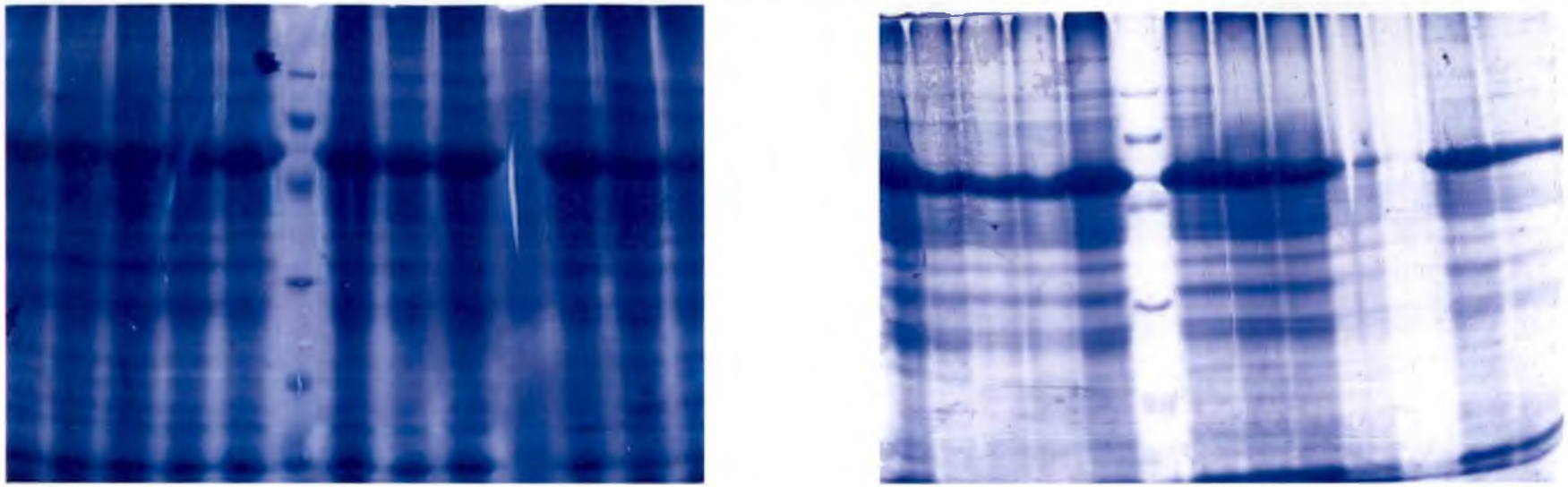
M. Marker.

5. *B. diffusa* (Post-inoculation)
6. *B. diffusa* (Simultaneous)
7. Salicylic acid (Pre-inoculation)
8. Salicylic acid (Post-inoculation)
9. Salicylic acid (Simultaneous)
10. Healthy control
11 Diseased control
12. Ethrel (Pre-inoculation)
13. Ethrel (Post-inoculation)
14. Ethrel (Simultaneous)
15. Betadiene (Pre-inoculation)

M. Marker

16. Betadiene (Post-inoculation)
17. Beatadiene (Simultaneous)
18. *P. fluorescence* (Pre-inoculation)
19 *P. fluorescence* (Post-inoculation)
20. *P. fluorescence* (Simultaneous))
21. Diseased control
22. Healthy control

Plate 15. Appearance of PR proteins in cowpea leaves inoculated with CABMV and treated with different abiotic and biotic factors at 3 DAI.



1 2 3 4 5 M 6 7 8 9 10 11 12 13 14 15 16 17 M 18 19 20 21 22

1. *P. niruri* (Pre-inoculation)
2. *P. niruri* (Post-inoculation)
3. *P. niruri* (Simultaneous)
4. *B. diffusa* (Pre-inoculation)
5. *B. diffusa* (Post-inoculation)

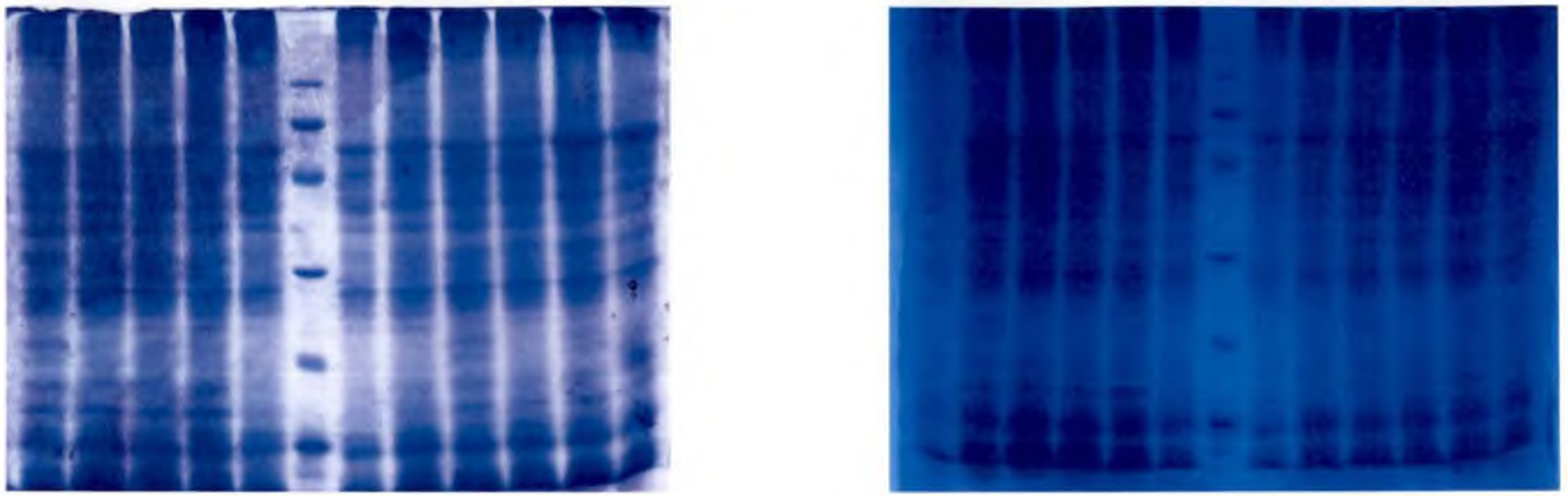
M. Marker.

6. *B. diffusa* (Simultaneous)
7. Salicylic acid (Preinoculation)
8. Salicylic acid (Post-inoculation)
9. Salicylic acid (Simultaneous)
10. Ethrel (Pre-inoculation)
11. Healthy control
12. Diseased control
13. Ethrel (Post-inoculation)
14. Ethrel (Simultaneous)
15. Betadiene (Pre-inoculation)
16. Betadiene (Post-inoculation)
17. Betadiene (Simultaneous)

M. Marker

18. *P. fluorescence* (Preinoculation)
19 *P. fluorescence* (Post-inoculation)
20. *P. fluorescence* (Simultaneous)
21. Healthy control
22. Diseased control

Plate 16. Appearance of PR proteins in cowpea leaves inoculated with CABMV and treated with different abiotic and biotic factors at 5 DAI.



1 2 3 4 5 M 6 7 8 9 10 11 12 13 14 15 16 17 M 18 19 20 21 22

1. *P. niruri* (Pre-inoculation)
2. *P. niruri* (Post-inoculation)
3. *P. niruri* (Simultaneous)
4. *B. diffusa* (Pre-inoculation)
5. *B. diffusa* (Post-inoculation)

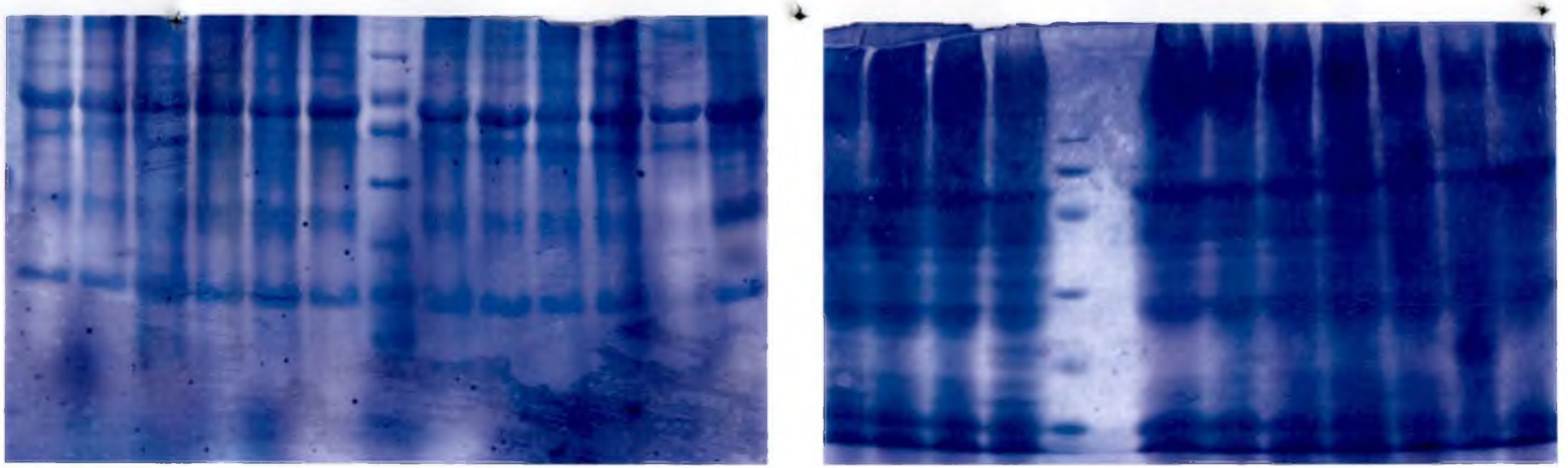
M. Marker.

6. *B. diffusa* (Simultaneous)
7. Salicylic acid (Preinoculation)
8. Salicylic acid (Post-inoculation)
9. Salicylic acid (Simultaneous)
10. Healthy control
11 Diseased control
12.Ethrel (Pre-inoculation)
13.Ethrel (Post-inoculation)
14. Ethrel (Simultaneous)
15. Betadiene (Pre-inoculation)
16. Betadiene (Post-inoculation)
17. Betadiene (Simultaneous)

M. Marker

18. *P. fluorescence* (Preinoculation)
19 *P. fluorescence* (Post-inoculation)
20. *P. fluorescence* (Simultaneous)
21. Healthy control
22. Diseased control

Plate 17. Appearance of PR proteins in cowpea leaves inoculated with CABMV and treated with different abiotic and biotic factors at 15 DAI



1 2 3 4 5 6 M 7 8 9 10 11 12 13 14 15 16 M 17 18 19 20 21 22

1. *P. niruri* (Pre-inoculation)
2. *P. niruri* (Post-inoculation)
3. *P. niruri* (Simultaneous)
4. *B. diffusa* (Pre-inoculation)
5. *B. diffusa* (Post-inoculation)
6. *B. diffusa* (Simultaneous)

M. Marker

7. Salicylic acid (Preinoculation)
8. Salicylic acid (Post-inoculation)
9. Salicylic acid (Simultaneous)
10. Ethrel (Pre-inoculation)
11. Healthy control
12. Diseased control
13. Ethrel (Post-inoculation)
14. Ethrel (Simultaneous)
15. Betadiene (Pre-inoculation)
16. Betadiene (Post-inoculation)

M. Marker

17. Betadiene(Simultaneous)
18. *P. fluoroscens*(Preinoculation)
19 *P. fluoroscens*(Post-inoculation)
20. *P. fluoroscens*(Simultaneous)
21. Healthy control
22. Diseased control

Plate 18. Appearance of PR proteins in cowpea leaves inoculated with CABMV and treated with different abiotic and biotic factors at 30 DAI.

Discussion

5. DISCUSSION

Cowpea is a major leguminous vegetable and grain crop cultivated in Kerala. Disease and pests are a major constraint in increasing the production of the crop. Among the pathogens, viruses are a threat to the cultivars. Diseases caused by viruses are often the most destructive and difficult to control. Management of viral disease has always been a problem to cultivators and research workers. Inducing resistance in plants is a good management practice against viral diseases. There are various elicitors or inducers which can induce resistance in these varieties. This study was undertaken to induce resistance against the virus by various abiotic and biotic factors.

Various chemicals inducing resistance like salicylic acid, ethrel and benzoic acid were tested for their effect on cowpea aphid-borne mosaic virus of cowpea. Pre, post and simultaneous inoculation treatments were done on the local lesion host of the virus, *C. amaranticolor*. These studies on the efficacy of chemicals in inducing resistance reflected that spraying of ethrel and SA were found effective against CABMV. Chemical inducers such as SA and its analogues BTH and INA could elicit systemic acquired resistance (Ryals et al., 1994). Murphy et al. (2001) found that SA or its synthetic functional analogues induced disease resistance. SA induced resistance against CMV in tobacco resulted in the inhibition of systemic virus movement (Carl et al., 2005). Pre-application of chemicals was effective than post and simultaneous inoculation treatments against CABMV. Pretreatment was effective as it interferes with mechanical transmission (Habuka et al., 1991) or due to the induction of systemic resistance (Prasad et al., 1995). Verma and Awasthi (1978) found that thio semicarbazone derivatives inhibited gomphrena mosaic virus in cowpea plants. They also found that the antiviral activity varied from host to host and was dependent on time of application. Application of chemicals at different concentrations on local lesion host revealed that SA and ethrel were best in inhibiting the virus even at low

concentration. Conti et al. (1988) reported that very low concentration of acetyl salicylic acid was effective in inducing resistance in *Datura* against TMV.

The experiment to determine the effect of indigenous materials on local lesion host, *C. amaranticolor* revealed that they were not as much effective as chemicals in inducing resistance against CABMV. Among the indigenous materials tested, neem seed oil emulsion and panchagavya were found effective in inhibition of local lesions. Neem seed oil and neem leaf extracts had been reported to inhibit local lesion production by mechanically transmitted viruses when mixed with the inoculum or when applied to test plants (Verma, 1974; Chowdhuri and Saha, 1985; Zaidi et al., 1985). Pre-inoculation treatment of neem oil recorded better inhibition of local lesion when compared to other treatments. This was in line with the findings of Roychoudhary and Jain, (1993). They reported that pretreatment with neem oil reduced local lesion production by TMV on *Nicotiana glutinosa, N tabaccum*,var.samsun NN, *Chenopodium amaranticolor* and *Datura stramonium.* Aiyanathan and Narayanasamy (1988) reported that pre-inoculation of neem oil reduced the infection of rice tungro virus in rice varieties.

Studies on the effect of chemicals having antiviral properties revealed that manganese chloride and betadiene showed significant inhibition of local lesions when compared to other treatments. Betadiene was found effective in pre, post and simultaneous method of application even at low concentration. White et al. (1986) reported that the chemicals manganese chloride and barium chloride induced resistance in Xanthi-nc tobacco leaves to TMV infection. Present findings revealed that post application of manganese chloride and betadiene were found effective in inhibiting the formation of local lesions. Inhibition of the virus in local lesion host may be due to the inhibition of systemic movement of the virus in the host plant. The research findings of Radhika (1999) were in line with the present findings. She reported that post inoculation treatment of manganese chloride gave maximum inhibition of symptoms caused by cowpea aphid borne mosaic virus (CABMV) in *Chenopodium amaranticolor* and cowpea. Ghoshroy et al. (1998) found that exposure of tobacco plants to non toxic concentration of

cadmium completely blocked viral disease caused by Turnip vein clearing virus. Cadmium mediated viral protection was due to inhibition of systemic movement of virus. Caner et al. (1984) found that triazofurin was more effective when applied after than before virus inoculation against TSWV in tomato plants. Manickam and Rajappan (1999) studied the effect of chemicals and certain plant extracts on green gram leaf curl disease under pot culture. copper sulphate and copper acetate (1000 ppm) were found to suppress the virus symptoms.

Two of the ten plants tested showed high level of antiviral efficacy against CABMV on the local lesion host. *P. niruri* and *B. diffusa* were shown to inhibit the virus in different methods of application and at different concentration. *P. niruri* was found to show complete inhibition of the virus (100 percent) when applied after inoculation of the virus or when applied along with the inoculum. Pre-inoculation spray was effective in the case of *B. diffusa*. This may be due to increased activity of phenols, peroxidase, polyphenol oxidase and phenylalanine ammonialyase (Renuka devi et al. 2004). They reported that pre application of Mirabilis Antiviral Protein (MAP) and Herpula Antiviral Protein (HAP) induced activity of defence related enzymes leading to the suppression of TSWV on local lesion and systemic host. Pre-inoculation application of plant extracts was found to be more effective than post inoculation application in reducing incidence of cowpea mosaic virus (Mallika, 1990). Among the various extracts tested, post inoculation spray of *P. niruri, B. diffusa, M.jalapa, Bougainvillea sp, C. roseus* and *A. vesica* challenged with CABMV were highly effective against CABMV. Bhatia (2004) tested the antiviral proteins from *Bougainvillea* along with virus and also before virus inoculation. When AVPs are applied prior to virus inoculation or in combination with virus, there was suppression of necrotic lesion formation in local lesion host. Prasad et al. (2007) evaluated the efficacy of certain plant extracts in reducing *Bean common mosaic potyvirus* strain *blackeye cowpea mosaic* (BCMV-BlCMV) disease in cowpea and found that when plant extracts were mixed with BCMV-BlCMV inoculum and young seedlings were inoculated, *B. spectabilis*, *C. inerme* and *M. jalapa* extracts reduced the disease incidence up to 42, 40 and 48 per cent respectively under greenhouse conditions

when compared to control. The antiviral efficacy may be attributed to the presence of antiviral compounds such as lignin, terpenoids, alkaloids and specific proteins. Application of extracts of AVPs make the plant resistant to virus infection by inhibiting either directly or indirectly. Some of the evidences showed that in many cases, viral inhibition is due to the development of virus inhibitory substances within the tissues, but some induces systemic resistance (Verma and Prasad, 1984).

Studies on the effect of biotic agents or CABMV in local lesion host recorded that *P. fluorescence* showed 83.66 per cent inhibition of virus. This may be due to the activities of defense mechanism in plants or due to accumulation of PR proteins namely β-1,3 glucanases and endochitinases (Maurhofer et al., 1994). PGPR mediated systemic resistance is often associated with onset of defence mechanism including the early and increased expression of defence enzymes such as chitinase, glucanase, peroxidase and phenylalanine ammonialyase and accumulation of phenolics, phytoalexins and lignins (Mosch et al., 1993; Schneider and Ullrich 1994; Chen et al., 2000; Nandakumar et al., 2001). Maurhofer et al., (1998) showed that soil drenched with *P. fluorescens* CHAO strain induced systemic protection against TNV in tobacco. Pre inoculation and post inoculation treatment was found better in the case of *P. fluorescens*.

Treatments that showed significant inhibition percentage of the virus was further selected for its assay in cowpea. Among the chemicals inducing resistance, salicylic acid and ethrel were selected for the assay. Betadine that showed inhibition of virus in local lesion host at low concentration was used to evaluate its efficiency in cowpea. *P. niruri* and *B. diffusa* were the selected plant extracts for bioassay in cowpea. Among the biotic agents, *P. fluoroscens* was good in inhibiting the virus and therefore used in bioassay in cowpea. Pot culture experiment was conducted on cowpea plants in completely randomized design. Treatments were applied as pre, post and simultaneous application. Vulnerability index was calculated and it was found that AVP treated plants showed less

vulnerable to infection even at 30 DAI in pre-inoculation. Mistry et al. (2003) found that plant extracts from *Clerodendron inerme* and *O. sanctum* efficiently reduced systemic infection of tobacco chlorotic mottle virus in cowpea. The plant extracts were found to induce systemic resistance in the host, as highest retardation in infection was noticed when extracts were sprayed 24 h prior to inoculation. Salicylic acid and ethrel treated plants were also found less prone to infection of the virus. This may be due to the inhibition of virus replication as mentioned by Singh et al. (2004).They reported multiple antiviral defence mechanism of SA and observed that SA triggers resistance to viral infection process viz. replication, cell -to -cell movement and long distance movement.

Biochemical changes due to virus infection were also studied. Estimation of protein indicated significant difference in the level of protein between healthy and inoculated plants. The total soluble protein was found higher in case of inoculated plants. In the inoculated plants protein content was found to increase with the plant age and recorded maximum at 30 DAI (3010 $\mu g\ g^{-1}$). In healthy control protein content increased at different time intervals and recorded maximum of 2250 $\mu g\ g^{-1}$ at 30 DAI, but not the level that of inoculated plants. Higher protein content due to virus infection has been reported by many authors (Singh et al., 1978., Singh & Singh ,1984). All these reports are in conformity with the results of the present investigation. The drastic increase in total protein content in the virus inoculated plants was due to increase in viral proteins and non-viral induced proteins occurring indirectly at the expense of normal host proteins directed by genes present in viral DNA . In pre inoculation treatment, *P. niruri* and *B.diffusa* recorded maximum protein at 15 DAI. This was followed by ethrel. Several fold increase in protein content was observed in all the treatments when compared to healthy control. Manickam et al. (2000) studied the impact of application of a foliar spray of AVPs from *Cocos nucifera, Sorghum vulgare, Sorghum bicolor* and *Croton sparsiflorus* leaves and inoculation of TSWV on the non-reducing sugar and total soluble protein contents of cowpea plants. It was found that AVP treated cowpea plants showed marginal increase in protein contents compared to significant increase in TSWV inoculated plants.The increase

in total soluble protein in TSWV inoculated plants might be due to the synthesis of virus coat proteins whereas in AVP treated plants, the increased protein content might be due to the formation of new proteins.

The present investigation revealed that there was significant difference in the total sugar content between healthy and inoculated plants of cowpea plant. The total sugar content in virus inoculated control was found to be 32 mg g^{-1} fresh weight of tissue at 1 DAI as against 33.6 mg g^{-1} fresh weight of tissue in healthy control. Many studies showed similar results regarding carbohydrate content due to virus infection. Decrease in total sugar concentration due to viral infection in susceptible cultivars of cowpea has been reported (Ramiah, 1978; Singh & Singh, 1984 and Mayoral et al., 1989). Sutha et al. (1998) reported that TSWV infection reduced the concentration of total, non reducing and reducing sugars and the reduction was more in initial stages of infection compared to later stages. It was also reported that in contrast to sugar concentration, the starch increased in infected plants at all stages of analysis. In the present study decrease in total sugar was noticed in inoculated plants. The reduction at the level of total sugar might be due to the breakdown of carbohydrate accelerated during respiration in virus infected plants as suggested by Narayanaswamy and Ramakrishnan (1966). AVP treated plants showed increased total sugar content when compared to control. It was found to be 41.1 mg g^{-1} fresh weight of tissue in pre-inoculation treatment of *B. diffusa*. Total sugar content was found to be increased with plant age in all the treatments tested. Manickam et al. (2000) reported that foliar spray of AVPs from *Cocos nucifera, Sorghum vulgare*, *Sorghum bicolor* and *Croton sparsiflorus* leaves increased the non reducing sugar content in cowpea plants while TSWV inoculation decreased its concentration.

Reducing sugar content was found to be more in healthy plants (16.73 mg g^{-1} fresh weight) when compared to virus inoculated plants (13.50 mg g^{-1} fresh weight). It was found to be increased with increase in plant age and reaches the maximum at 5 DAI. Thereafter it gets declined. Thind et al. (1996)

reported that the amount of reducing sugars, non -reducing sugars, total sugars and starch decreased in black gram infected with yellow mosaic virus when compared to healthy plants. *P. niruri* treated plants showed maximum reducing sugar content of 19.83 mg g^{-1} fresh weight at 5 DAI of the virus. Increased sugar content in treated plants might be due to its accumulation as a result of the disruption of normal phloem transport.

There was significant difference in phenol between the healthy and inoculated plants. Phenol content was significantly higher in inoculated plants. Due to CABMV infection phenol content increased from 841µg g^{-1} at one DAI to 905 µg g^{-1} at 5 DAI and then decreased to 830µg g^{-1} at 30 DAI. Maximum value of 771µg g^{-1} was recorded at 5 DAI in healthy plants and thereafter decreased to 652µg g^{-1} at 15 DAI . Ramiah (1978) observed that the total phenol content was increased in CABMV inoculated plants of susceptible cultivars of cowpea. Enhanced level of phenol content has been observed in hypersensitive cowpea leaves infected with tobacco ring spot virus. Sutha et al. (1997) found that both total and *ortho*-dihydroxy phenol increased in TSWV infected plants. Mali et al. (2000) reported that *ortho*-dihydroxy phenol was higher in healthy leaves than diseased leaves in case of yellow mosaic virus infected moth bean. Accumulation of phenol observed in virus infected plants may be due to excess production of hydrogen peroxide by increased respiration or due to activation of HMP- shunt pathway , acetate pathway and release of bound phenolics by hydrolytic enzyme as reported by Sutha et al. (1997). Among the treatments *P. niruri* treated cowpea plants showed several fold increase in phenol content on 5 DAI (1366 µg g^{-1})as against healthy and virus alone in pre inoculation spray. It was followed by *B. diffusa* (1260 µg g^{-1}). Resistant plants have more phenols than the susceptible ones. Multifold increase of phenols in cowpea by AVPs after challenging with CABMV in the present study may be due to the excess production of H_2 O_2 in infected plants via increased respiration (Farkas and Kiraly, 1962) or due to the activation of hexose- monophosphate pathway, acetate pathway and release of bound phenols by hydrolytic enzymes.

Investigation on changes in the activity of peroxidase, polyphenol oxidase and phenylalanine ammonialyase clearly indicated that there was difference in the activity of these defence related enzymes in healthy and inoculated plants of cowpea (cv. Sharika). Among these enzymes early triggering of PAL is more important as it is the principle enzyme involved in the phenyl propanoid pathway. It leads to the production of phytoalexins, terpenes and phenolic substances leading to the formation of lignin with the help of peroxidases. PAL activity was found higher in inoculated plants (21.90 $\mu g\ g^{-1}min^{-1}$fresh weight) at 5 DAI, but gradually decreased. The decrease of the enzyme activity may be due to the susceptibility of the plant to the virus. Application of AVPs, chemicals like, salicylic acid and ethrel, and the biotic agent *P. fluorescens* increased the accumulation of PAL, when challenged with CABMV in cowpea plants in the present investigation. PAL activity was found to be increased at 5 DAI and then declined. It was found to be several times more than healthy and virus inoculated control. Among the AVPs, *P. niruri* was found to perform better than other two, which might be due to the level of variation in the signal molecules that elicit a cascade response, *via* activating the series of defence response against viral infection. Pre-inoculation of the treatments showed increased PAL when compared to other method of application. Umamaheshwaran (1996) reported that there was progressive increase in peroxidase, polyphenol oxidase and phenylalanine ammonialyase activity in inoculated susceptible varieties of cowpea due to CABMV infection. Zaidi et al. (1992) reported the changes in phenolic content and phenylalanine ammonialyase in response to infection by carnation etch ring virus. The results suggested the existence of a positive correlation between the elevated levels of phenolics and phenylalanine ammonialyase with disease resistance.

Polyphenol oxidase was found maximum at 15 DAI in both healthy (1790 min^{-1} mg^{-1} fresh weight) and inoculated plants (2100 min^{-1} mg^{-1} fresh weight). *P. niruri* leaf extract recorded maximum PPO activity of 4640 min^{-1} mg^{-1} at 15 DAI. Verma and Prasad (1984) found that spraying aqueous leaf extract of

Clerodendron aculeatum prevented infection of sunhempe rosette virus on cluster beans. The resistance thus induced was due to increased activity of catalase, peroxidase and polyphenol oxidase. Salicylic acid and ethrel also showed increased PAL with increase in the age of the plant upto 15 DAI of the virus. PPO activity of betadine was in between healthy and virus inoculated control. This may be due to its reduced defense activity against the virus. Pre-inoculation was found to show increased activity of the enzyme in all the treatments.

Peroxidase activity was maximum (2500 min^{-1} mg^{-1}) at15 DAI in healthy control. In inoculated control, maximum enzyme activity recorded was only 1800 min^{-1} mg^{-1} at 5 DAI, thereafter it gets declined. Radhika and Umamaheswaran (2000) reported higher activity of peroxidase, polyphenol oxidase and phenylalanine ammonialyase in resistant variety when compared to susceptible variety of cowpea infected with BICMV. Mali et al (2000) reported that the activity of catalase, peroxidase and nitrate reductase enzymes decreased with increasing intensity of disease, in the case of yellow mosaic disease of moth bean. The decreasing trend in enzyme activity of the present study may be due to increase in intensity of disease or may be due to high susceptibility of plant to the disease. Application of AVPs, chemicals and biotic agents increased the peroxidase activity in cowpea plants. Pre-inoculation application recorded maximum peroxidase activity. Previous research by several researchers explained that the pre application of AVPs challenged with plant viruses indifferent hosts results in the activation of key enzymes like PAL, PPO, PO, chitinase and glucanase leading to the suppression of viral pathogen (Aiyanathan, 1995; Verma et al., 1996). All the treatments except betadiene showed several fold increase in peroxidase activity in all the three methods of application. Muthulakshmi and Renukadevi, (2001) found that reduction in percentage of infection by rice tungro virus may be due to induction of defence related proteins by application of AVP. The activity of enzymes like PAL, PO and PPO were found to be more in the present study indicating the induction of defense reaction by triggering phenyl propanoid pathway.

Several defence related proteins and enzymes seem to be involved in the inhibition of CABMV in cowpea as a result of various treatments. Many PR-proteins were induced during the initial stages of treatments and the induction of these PR-proteins decreased as the days advances. The induction of PR-proteins was more in leaves which were treated with biotic agent and also with plant extracts. This induction of PR-proteins was more prominent at 1 and 3 DAI in leaves. This may be due to the fact that the defence proteins or enzymes induced during the initial period could inhibit the virus and thereby prevented further development of symptoms in leaves. The new proteins induced could also inhibit the development of virus and symptoms in host plant. The induction of defence proteins and enzymes by various biotic and abiotic factors were well established in inhibition of viral diseases in plants (Deverall and Dann, 1995; Umamaheswaran, 1996; Mali et al. 2000). The present study has generated sufficient information on the use of biotic agents and abiotic factors for the management of CABMV. But the results should further be validated under field conditions.

Summary

6. SUMMARY

Studies were conducted to induce resistance in cowpea against CABMV using various abiotic and biotic factors. Various abiotic factors like, chemicals inducing resistance *viz.*, salicylic acid, ethrel and benzoic acid, indigenous materials inducing resistance *viz.*, panchagavya, neem seed oil emulsion, turmeric powder- baking soda mixture, fresh cowdung solution and vermiwash, chemicals having antiviral properties viz. carbendazim, naphthalene acetic acid, manganese chloride, sodium phosphonate and betadiene, ten crude extracts of plants having antiviral properties and various biotic agents *viz.*, released cultures *of P. fluorescence* from KAU and TNAU, *Bacillus* sp., endophytic and rhizobacteria from cowpea were screened to find out their efficiency in local lesion host of the virus, *Chenopodium amaranticolor* and cowpea. Pre-inoculation, post-inoculation and simultaneous inoculation treatments were done.

Among the chemicals inducing resistance, salicylic acid and ethrel inhibited the formation of local lesions in *C. amaranticolor.* Pre-inoculation spray was found to show better inhibition when compared with post and simultaneous method of application. Studies on the indigenous materials against CABMV on the local lesion host revealed that pre inoculation spray of panchagavya and neemseed oil emulsion reduced the number of local lesions .But they were not as much effective as chemicals.

Chemicals having antiviral properties were tested to find out their efficacy against CABMV and it was found that betadiene and manganese chloride showed better inhibition over control. Among the various chemicals tested, treatment with betadiene reduced the virus infection in pre, post and simultaneous methods of application. Ten plant extracts were tested to induce resistance in cowpea plants. *Phyllanthus niruri* and *Boerhavia diffusa* recorded 100 percent inhibition of local lesions in *C. amaranticolor.* It was found that in the case of post inoculation treatment of *P. niruri* maximum inhibition of the virus was

observed even at lower concentrations of 1 and 0.5 per cent. AVPs were found very much effective in inhibiting CABMV in the local lesion host.

Among the biotic agents, *Pseudomonas fluorescence* (TNAU culture) at the rate of 10^8 cfu ml^{-1} recorded 83.66 per cent inhibition over control. Pre inoculation spay of *P. fluorescence* found very much effective when compared to other treatments.

Biotic and abiotic factors that showed high inhibition percentage in local lesion host were selected for bioassay in cowpea plants. Among the abiotic factors, chemicals like salicylic acid (250 ppm), ethrel (250 ppm), betadiene (0.1 percent) and plant extracts viz., *Phyllanthus niruri* (10 percent) and *Boerhavia diffusa* (10 percent) were used for evaluation. *Pseudomonas fluorescens* (TNAU culture, 10^8cfu ml^{-1}), the biotic agent was also selected for the assay. Vulnerability index was calculated and it was found that AVP treated plants showed less vulnerable to infection even at 30 DAI. Treatments that were applied before the challenge inoculation of the virus showed less symptom expression. *P. niruri* leaf extract was found less prone to virus infection in all stages of crop growth.

The biochemical changes due to virus infection were studied in various selected treatments, healthy control and virus inoculated cowpea plants. There was a progressive increase in the total protein content in both healthy and virus inoculated plants with increase in the age of plant Among the treatments, *P. niruri* recorded maximum protein content. There was significant difference in total sugar content between various treatments. Among the treatments tested AVPs viz. *B. diffusa* and *P. niruri* recorded the maximum total sugar content in pre, post and simultaneous inoculation. Significant increase in reducing sugar content with different treatments were observed at different time intervals. Among the different methods of application, pre inoculation treatment recorded high reducing sugar content *P. niruri* treated cowpea plants recorded increased reducing sugar content. Application of selected biotic and abiotic factors on cowpea cv. Sharika challenged with CABMV induced higher accumulation of

phenolics. AVPs and chemicals inducing resistance showed accumulation of phenol when compared to healthy plants.

Studies on PAL activity in cowpea revealed that AVPs and chemicals treated and challenged with CABMV increased the accumulation of PAL than healthy and virus inoculated control. In pre inoculation, *P.niruri* showed increased PAL activity and it was several fold more than healthy and virus alone control. This was followed by *B. diffusa* . Studies on the mode of action of AVPs and chemicals against CABMV revealed that they induced defence mechanism in plants challenged with CABMV. Healthy plants showed increased peroxidase activity than virus alone control. Among the various treatments, *P. niruri* extract showed high PO activity at 15 DAI followed by *B. diffusa*). These treatments showed several fold increase in PO activity compared to healthy and virus inoculated control.

Electrophoretic analysis revealed that many defense proteins and PR-proteins were induced in response to various treatments. The induction of PR-proteins was more in leaves which were treated with biotic agent and also with plant extracts. This induction of PR-protein was more prominent during early stages of treatments and gradually decreased in due courses. New PR-proteins were also induced but no commom PR-proteins were induced in leaves due to different abiotic factors and biotic agents.

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Appendices

APPENDIX-I

Buffers for Sap extraction

1. 0.1M citrate-phosphate buffer (P^H 7.0) stock solution.

   A: 0.1M solution of citric acid (19.21g in 1000ml)

   B: 0.2M Solution of dibasic sodium phosphate (53.65g of $Na_2HPO+7H_2O$ in 1000 ml)

   6.5ml of A is mixed with 43.6 ml of B diluted to a total of 100 ml

2. A: 0.2M Solution of monobasic sodium phosphate (27.8 g in 1000 ml)

   B: 0.2M Solution of dibasic Sodium phosphate (53.65g of $Na_2HPO_4.12H_2O$ in 1000 ml)

   39.0 ml of A is mixed with 61.0 ml of B diluted to a total of 200 ml.

3. 0.1M Tris buffer (PH 7.2)

   A: 0.2M solution of Tris (24.2g in 1000ml)

   B: 0.2M HCl

   50 ml of A is mixed with 44.2 ml B diluted to a total of 200 ml.

4. 0.01M phosphate Mercaptoethanol buffer:- (P^H 7.2)

   KH_2PO_4 – 800 mg

   K_2HPO_4 – 2.2 g

   Mercaptoethanol – 100 µl

   Made up to 100 ml in distilled water.

APPENDIX II

Buffers for enzyme analysis

0.1 M sodium acetate (pH 4.7)

Stock solutions

A: 0.2 M solution of acetic acid (11.55 ml in 1000 ml)

B: 0.2 M solution of sodium acetate (16.4 g of $C_2H_3O_2$ Na or 27.2 g of $C_2H_3O_2$ Na $3H_2O$ in 1000 ml).

22.7 ml of A is mixed with 27 ml of B, diluted to a total of 100 ml.

0.1 M Borate Buffer (pH 8.8)

A: 0.2 M solution boric acid (12.4 g in 1000 ml)

B: 0.05 M solution of borax (19.05 g in 1000 ml)

50 ml of A is mixed with 30 ml of B, diluted to a total of 200 ml.

INDUCTION OF RESISTANCE AGAINST COWPEA APHID-BORNE MOSAIC VIRUS IN *Vigna unguiculata* var.*sesquipedalis* (L.)Verdcourt

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Abstract of the
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ABSTRACT

Studies were conducted to induce resistance in cowpea against CABMV using abiotic and biotic factors. Various abiotic and biotic factors were screened to find out their efficiency in local lesion host of the virus, *Chenopodium amaranticolor* and cowpea. Different chemicals and plant extracts were found to inhibit local lesions produced by CABMV. Salicylic acid and ethrel inhibited the formation of local lesions in *C. amaranticolor*. Pre inoculation spray of indigeneous materials viz. panchagavya and neem seed oil emulsion reduced the number of local lesions. But they were not as much effective as chemicals. Among the chemicals having antiviral properties, betadiene and manganese chloride showed better inhibition over control. Ten plant extracts were tested to induce resistance in cowpea plants. *Phyllanthus niruri* and *Boerhavia diffusa* recorded 100 percent inhibition of local lesions in *C. amaranticolor*. Pre inoculation spay of *P. fluorescens*, the biotic agent were found very much effective against CABMV.

Biotic and abiotic factors that showed high inhibition percentage in local lesion host also reduced the symptom development in cowpea. Among the abiotic factors, chemicals like salicylic acid (250 ppm), ethrel (250 ppm), betadiene (0.1 percent) and plant extracts viz., *Phyllanthus niruri* (10 percent) and *Boerhavia diffusa* (10 percent) were used for evaluation. *Pseudomonas fluorescens* (TNAU culture, 10^8cfu ml^{-1}), the biotic agent was also selected for the assay. AVPs were found very much effective in reducing the symptoms produced by CABMV in cowpea. Biochemical changes indicated a significant difference in protein in treated plants. *P. niruri* recorded maximum protein content. Among the treatments tested AVPs viz. *B. diffusa* and *P. niruri* recorded the maximum total sugar content in pre, post and simultaneous inoculation. *P. niruri* treated cowpea plants recorded increased reducing sugar content. Application of selected biotic and abiotic factors on cowpea cv. Sharika challenged with CABMV induced higher accumulation of phenolics. AVPs and chemicals inducing resistance showed accumulation of phenol when compared to healthy plants. Studies on the

mode of action of AVPs and chemicals against CABMV revealed that they induced defence mechanism in plants challenged with CABMV. AVPs and chemicals treated and challenged with CABMV increased the accumulation of phenyl alanine ammonialyase, polyphenol oxidase and peroxidase, than healthy and virus inoculated control. Many defence proteins and PR-proteins were induced in response to various treatments. The induction of PR-proteins was more in leaves which were treated with biotic agent and also with plant extracts. This induction of PR-protein was more prominent during early stages of treatments and gradually decreased in due courses.