MANAGEMENT STRATEGIES FOR TOSPOVIRUS IN TOMATO (*Lycopersicon esculentum* Mill.)

Thesis submitted in part fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY (AGRICULTURE) in PLANT PATHOLOGY to the TAMIL NADU AGRICULTURAL UNIVERSITY Coimbatore - 641 003.

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CERTIFICATE

This is to certify that the thesis entitled "**Management strategies for Tospovirus in Tomato (*Lycopersicon esculentum* Mill.)** " submitted in part fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **PLANT PATHOLOGY** to the Tamil Nadu Agricultural University, Coimbatore, is a record of *bonafide* research work carried out by **Mrs. P. RENUKADEVI** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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Abstract

ABSTRACT

MANAGEMENT STRATEGIES FOR TOSPOVIRUS IN TOMATO (*Lycopersicon esculentum* Mill.)

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Fifty-eight germplasm entries of tomato were screened for resistance against Tospovirus. The lowest incidence of 35.49 per cent and the highest incidence of 99.5 per cent of TSWV were recorded in LE31 and LE 231 respectively. Pre inoculation spray of *Mirabilis jalapa*, *Harpullia cupanioides*, *Adhatoda vesica, Phytolacca americana*, Bion and *Pseudomonas chlororaphis* were effective against Tospovirus under glass house conditions. Studies on thermal stability of AVPs indicated that all the AVPs were effective at room temperature (28 ± 2°C) and at 40°C. But, root extract of *M. jalapa*, leaf extract of *B. spectabilis* and seed extract of *H. cupanioides* retained their antiviral effect even at 70°C. *B. spectabilis*, *M. jalapa*, *H. cupanioides*, *P. americana*,

and *A. vesica* were effective at alkaline pH. Storage of *M. jalapa* and *H. cupanioides* at room temperature retained their antiviral effect upto 75 days after storage.

Protein profile of AVPs showed the presence of 91, 74, 41, 24, 29 and 17kDa proteins in *M. jalapa,* 97, 68, 41, 29, 20 and 17kDa proteins in *H. cupanioides* and 68, 43, 42, 40, 38, 33, 31, 22 and 12kDa proteins in *A. vesica*. Eluted antiviral fractions of *M. jalapa* consists of 41, 29, 22.5 and 17kDa proteins and *H. cupanioides* consists of 68, 55.5 and 29kDa proteins. The purified antiviral proteins were basic in nature with *pI* values ranging from 8.4 to 9.5. TLC analysis of *H. cupanioides* revealed the presence of terpenoids similar to desacetyl nimbin and isomelidinin. The Western blot analysis of *M. jalapa* and *H. cupanioides* proteins confirmed the constitutive expression of 41kDa chitinase and 29kDa RIP. Serodiagnosis of *A. vesica*, *P. americana*, *S. vulgare*, *B. spectabilis*, *C. nucifera, G. sylvestris* and *P. chilensis* showed positive reaction for the presence of HAP like proteins indicating the non specific nature of HAP. The concentration of 600µg/ml of *Harpullia* antiviral protein (HAP) and 400µg/ml of *Mirabilis* antiviral protein (MAP) was found to inhibit Tospovirus.

Studies on the induction of defense mechanisms revealed that, the pre application of AVPs challenged with Tospovirus induced 41kDa protein. Foliar application of *M. jalapa, H. cupanioides*, *A. vesica,* Bion and *P. chlororaphis* increased the accumulation of PAL, PO, PPO and phenols. Studies on the bio-efficacy of AVPs showed that foliar application of *M. jalapa, H. cupanioides* and Bion at fortnightly intervals were effective in reducing the incidence of Tospovirus and increased the yield under field conditions.

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CHAPTER I

INTRODUCTION

Tospoviruses are well-established viruses with increasing economic importance as pathogen. This virus has one of the widest host range of any other plant virus, infecting over 900 species of plants in more than 70 families including both monocots and dicots. (Peters, 1998). It affects the productivity of the crops grown in both temperate and tropical regions (Francki and Hatta, 1981). The virus has become a major constraint in the production of pepper, pea, potato, tobacco and tomato (Betrand, 1997) with an annual loss of 100 million $ in Georgia. The loss of marketable tomato yield due to TSWV epidemics was accounted for millions of dollars and reduced the tomato production by 50-90% in the Hawaiian Islands (Cho *et al.*, 1987).

The virus is exclusively transmitted by thrips (Thysanoptera). Thrips include tobacco thrips (*Frankliniella fusca*), western flower thrips (*F. occidentalis*), common blossom thrips (*F. schultzei*), chilli thrips (*Scirtothrips dorsalis*), *Thrips setosus* and onion thrips (*T. tabaci*). Nymphs alone can acquire the virus (Sakimura, 1963) and transmission is effected by adults, which serve as the primary inoculum in initiating epidemics (German *et al.*, 1992).

Viral parasitism is unique, in contrast to fungi and bacteria. Viruses do not attack the structural integrity of their host tissues, but instead they subvert the synthetic machinery of host cell, acting as molecular pirates. The recent resurgence of Tospovirus is mainly due to it's broad host range and it's efficient transmission by the vector western flower thrips (*Frankliniella occidentalis* Pergande) (Wijkamp *et al.*, 1993), which is an important insect pest having expanded geographic range to almost all climatic zones of the continents and makes management of Tospovirus as a challenging task.

No safe viricidal chemical that can eliminate viruses without adversely affecting their hosts has yet been found. Hence, there is no direct chemical control for viruses. This

problem has become a serious challenge to plant pathologists, biochemists and molecular biologists to develop a long-term and a sustainable management strategy for Tospovirus.

A large number of substances originating from different sources of plants, animals, microorganisms, chemicals and synthetic compounds affect the ability of plant viruses to infect and multiply in plants either by inactivation of the virus or through induced systemic resistance. After the first report of tobacco mosaic virus (TMV) inhibitor in the leaves of *Phytolacca* sp. by Allard (1914), many scientists in India and abroad have reported virus inhibitors in several plant species.

However, the chemical nature of the inhibitory principle is known only in a relatively few instances. Those inhibitors that have been characterized are found to vary chemically. It is obvious that purification and identification of the inhibitor are necessary to put them for practical use in the management of plant virus diseases. Hence, it was felt necessary to test their efficacy in reducing the spread of virus disease in the field. A large number of plants, which are not affected by viruses, remain as an unexploited potential to be tapped, as sources of antiviral principles. Therefore, the present investigations on the following lines were taken up:

1. Screening of tomato germplasm entries against Tospovirus.
2. Screening different non-host species as sources of antiviral principles (AVP) against Tospovirus.
3. Identification and characterization of AVP's.
4. To study the mechanism of resistance.
5. Field-testing of potential AVP's / Chemicals against Tospovirus.

Review of Literature

CHAPTER – II

REVIEW OF LITERATURE

Plant virus diseases have always been of great concern to farmers and researchers since they cause enormous yield loss in many cereals, vegetables, fruits and legumes. Tospovirus causes enormous yield loss in both varieties and hybrids of tomato. Therefore management of virus diseases remains as a difficult and challenging task. Management of virus diseases can be achieved through exploitation of host plant resistance *via* conventional breeding programme and through induced resistance mediated through anti viral phytoproteins. The possibility of using plant extracts having antiviral properties for the management of many diseases have been suggested by many researchers (Narayanasamy and Ramiah, 1983; Ganapathy, 1985; Kurucheve, 1988; Shanker, 1995; Baranwal and Verma, 2000). The literature available on the various antiviral plant products, chemicals and biocontrol agents used for the management of the viral diseases are reviewed hereunder.

2.1 Occurrence

The first evidence of "spotted wilt" was reported in Australia in 1915 (Brittlebank, 1919). The infectious nature of the viral disease was confirmed by Samuel *et al.* (1930), who coined the name, Tomato spotted wilt virus, TSWV has been reported under different names on several plant species in many countries. e.g. Tomato bronzing virus, Kromnek virus, Pineapple yellow poty virus, Marchoka virus and others. (Best, 1968; Smith, 1944; Sakimura, 1962). The disease was also recorded in United Kingdom, Eastern and Western Europe, United States, South America and South Africa. (Smith, 1944).

Todd and his coworkers from Nilgiris observed TSWV in 1964 and reported during 1975. Subsequently, the virus was observed in groundnut from Punjab and Andhra pradesh (Chohan, 1974; Ghanekar *et al.*, 1979a), in mungbean and urdbean from Andhra Pradesh (Ghanekar *et al.,* 1979b), in chillies from Karnataka (Bidari and Reddy, 1984), in tomato

from Southern states (Rao *et al.*, 1981; Sastry, 1982; Sabitha *et al.*, 1984) and in peas from Andhra Pradesh (Rao *et al.*, 1985).

2.2 Symptomatology

The symptoms on tomato have been fully described by Samuel *et al.* (1930) and Gardner *et al.* (1935). The symptoms vary with plant age, nutritional level and environmental conditions. Symptom expression varies with different strains of the virus (Best, 1968). The field symptoms on the infected plants are bronzing of the leaves with brown necrotic lesions, which are found to spread systemically followed by wilting of the plants with the advancement of the disease. Young-growing bud is also necrotized. Curling of leaves and leaflets, flattening of leaves and marked shortening of internodes and formation of purple pigments along petioles and veins are also noticed, (Best and Gallus, 1953; Todd *et al.*, 1975). Fruits exhibit spots with circular markings about one cm in diameter as concentric bands of red and yellow broken rings (Plate1- 8). The centre of these spots is raised and has a roughened appearance (Rana *et al.*, 1993).

2.3 Yield loss

TSWV ranks one among the ten economically most important plant viruses causing crop loss of more than 1 billion dollars worldwide (Goldbach and Peters, 1994). Greenough *et al.* (1985) reported that TSWV infection was found to be 60 per cent in the fields and 100 per cent in home gardens, causing severe yield loss in tomato, pepper and tobacco. German *et al.* (1992) reported that the Hawaii growers lost millions of dollars in fresh marketable tomato (*Lycopersicon esculentum* Mill) due to TSWV infection and it led to 50-90 per cent drastic reductions in tomato production. In Georgia the annual loss due to TSWV was estimated as 100 million dollars (Betrand, 1997).

The yield loss by TSWV varies depending on the stage of infection of the crop. In Hawaii 50-90 per cent loss in tomato production was reported (Yudin, 1984). Singh and Tripathi (1991) reported that the maximum occurrence of the disease was observed in the

Symptoms of tospovirus infecting tomato

Plate 1. Brown necrotic lesion – Initial stage

Plate 2. Brown necrotic lesion – Advanced stage

Symptoms of tospovirus infecting tomato

Plate 3. Advanced level of infection

Plate 4. Stem necrosis

Symptoms of tospovirus infecting tomato

Plate 5. Bronzing and stunting

Plate 6. Death of the plant

Symptoms of tospovirus infecting tomato

Plate 7. Chlorotic lesions on cowpea

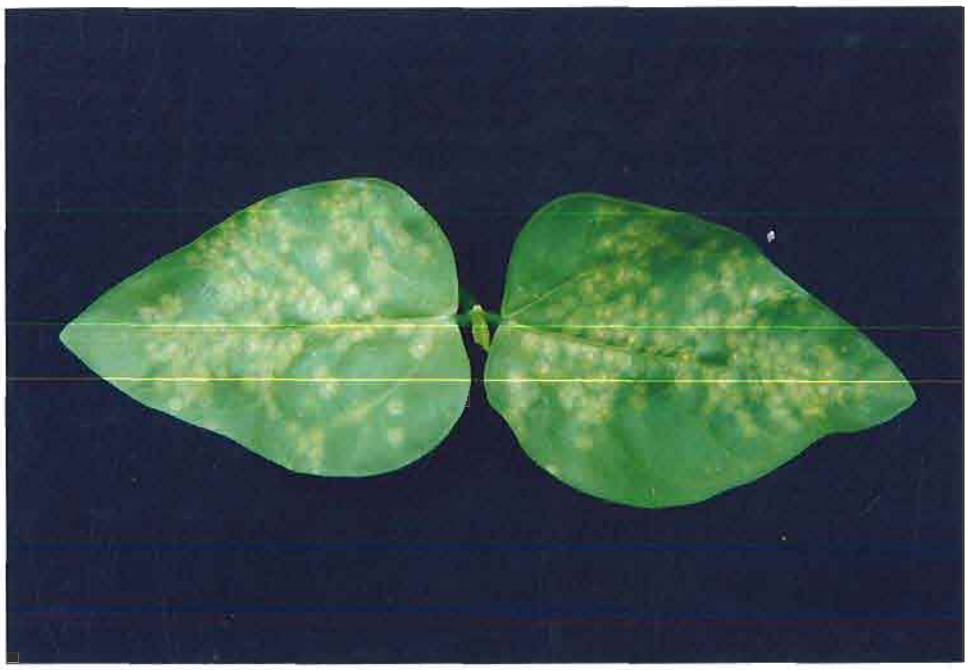


Plate 8. Chlorotic lesions on cowpea

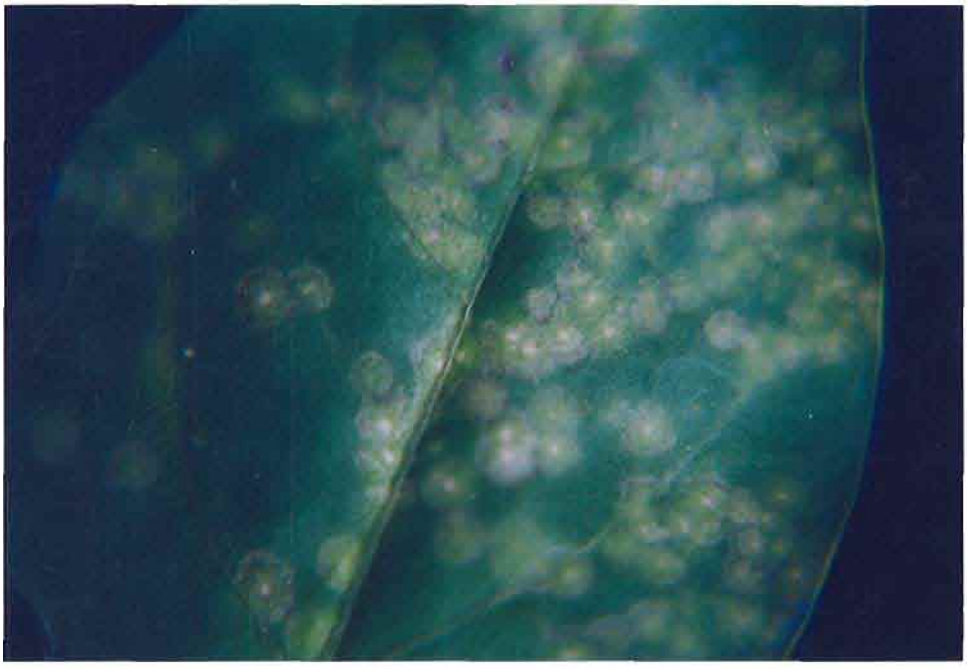


Plate 6a. Symptoms of Tospovirus on fruit

month of March. He also stated that the plant height was reduced by 19.12 per cent, number of fruits by 74.63 per cent and weight of fruits by 80.69 per cent. Kumar and Irulappan (1991) reported that the infection by TSWV at 15, 30, 45, 60 and 75 days after transplanting had a negative correlation with number of fruit clusters, number of fruits and yield.

2.4.Transmission

Tomato spotted wilt virus is transmitted by thrips belonging to the family Thripidae (Kobatake, 1984). Virus is acquired only during the nymphal stages. Nymph can transmit the virus before they pupate, but adults transmit the virus more commonly. Adults remain infective throughout their lifespan. But transmission is variable (Sakimura, 1962). TSWV is transmitted in a circulative manner. Thrips not only serve as vector but also it remains as a virus host. Thrips cannot transmit the virus unless they acquire the virus during their immature stages.

TSWV was also transmitted mechanically (Samuel *et al.*, 1930). The virus was transmitted by sap inoculation to groundnut (Chohan, 1967; Sabitha *et al.*, 1984). TSWV is transmitted by different species of thrips belonging to the family, Thripidae (Best, 1968). *Frankliniella schultzei* and *Scirtothrips dorsalis* transmit the disease to groundnut (Ghanekar *et al.*, 1979a). TSWV in tomato was transmitted by *Thrips tabaci* (Sabitha *et al.*, 1984) and *F. occidentalis* (Adam and Kegler, 1994).

2.5 Host range

TSWV infects more than 900 dicotyledonous species worldwide (Peters, 1998). Typical chlorotic and necrotic local lesions were observed in *Chenopodium amaranticolor (L.), C. quinoa (L.), Crotalaria juncea (L.)* , *Cucumis sativus (L.), Gomphrena globosa (L.), Nicotiana rustica (L.)* and *Petunia hybrida* (Vilm.). Systemic infection was observed in *Datura stramonium (L.), Dolichos uniflorus* (L.), *Glycine max (L.)* Merr., *Nicotiana clevelandii* (Gray), *N. glutinosa (L.)* and *Phaseolus vulgaris (L.)* (Ghanekar *et al.*, 1979b).

Host Plants of TSWV

Family	*Plant species*	Reference
Paplionaceae	*Arachis hypogaea (L.)*	Sabitha *et al.* (1984)
	Crotalaria juncea (L.)	Reddy *et al.* (1983)
	Desmodium triflorum L.)	Reddy *et al.* (1983)
	Glycine max (L.Merr.)	Reddy *et al.* (1983)
	Lablab niger (L.)	Sabitha *et al.* (1984)
	Phaseolus vulgaris (L.)	Nene (1972)
	Vigna radiata (L.)	Ghanekar *et al.* (1979b)
	Vigna unguiculata (L. Walp.)	Chohan (1967)
Compositae	*Ageratum conyzoides (L.)*	Reddy *et al.* (1983)
	Aster sp. (L.)	Sabitha *et al.* (1984)
	Zinnia elegans (L.)	Sabitha *et al.* (1984)
Solanaceae	*Capsicum annuum (L.)*	Sabitha *et al.* (1984)
	Nicotiana glutinosa (L.)	Sabitha *et al.* (1984)
	Nicotiana rustica (L.)	Sabitha *et al.* (1984)
	Nicotiana sylvestris (L.)	Sabitha *et al.* (1984)
	Solanum tuberosum (L.)	Reddy *et al.* (1983)
Cucurbitaceae	*Cucumis melo (L.)*	Chohan (1967)
	Momordica charantia (L.)	Chohan (1967)
Liliaceae	*Allium cepa (L.)*	Reddy *et al.* (1983)
Convolvulaceae	*Datura metel (L.)*	Sabitha *et al.* (1984)
	Datura stramonium (L.)	Sabitha *et al.* (1984)

Several weeds such as *Amaranthus viridis (L.), Chenopodium album (L.), D. stramonium (L.), Portulaca oleracea (L.),* and *Stellaria media (L.)* were reported to be the reservoirs of TSWV in Hawaii (Cho *et al.,* 1986). *Ageratum conyzoides (L.)* in India (Reddy *et al.,* 1983), *Sonchus oleraceus* and *Youngia japonica* in the Nara prefecture in Japan (Kobatake *et al.,* 1984). Apart from these reservoirs of TSWV, some of the new

host species are *Valeriana officinalis* (L.) (Bellardi *et al.,* 1999), *Dimorphotheca sinuata* (DC.) (Manoussopoulos *et al.,* 1999) and *Eustoma grandiflorum* (Raf. Shinn) (Vovlas and Potere, 1997).

2.6 Properties of the virus

Tomato spotted wilt virus (TSWV) belongs to the family Bunyaviridae, comprising mostly of animal viruses. It is the type member of tospovirus group (Francki and Hatta, 1981; Elliot, 1990). Groundnut bud necrosis virus has also been suggested as a distinct one among the tospovirus group (Reddy *et al.,* 1992). The virus has a thermal inactivation point of 40°C. The dilution end point was between $10^{-2.5}$ to $10^{-3.0}$, longevity *in vitro* was 3 to 4 hrs; half-life period of this virus is 20 minutes at 35°C and is inactivated by a 10-minute exposure to 46°C and pH values below 6 (Best, 1968). TSWV in tomato had a dilution end point of 1: 10,000 and thermal inactivation point between 40°C and 45°C for 10 minutes exposure. The longevity *in vitro* of this virus isolate was one hour at room temperature (28 ±2°C) (Todd *et al.,* 1975). Best and Gallus (1953) characterized six strains based on symptom expression. They were designated as A, B, C1, C2, D and E. Ghanekar (1979a) observed membrane bound spherical particles in the cytoplasm of infected leaves of tomato and groundnut. TSWV is a roughly spherical, enveloped virus of 80-100nm in size. The virus contains three major proteins, two glycoproteins (G1, G2) associated with the viral envelope and a nucleocapsid N protein (Mohamad *et al.,* 1973; Tas *et al.,* 1977).

2.7 Management of the disease

2.7.1 Antiphytoviral chemicals and symptom suppressors

Antiphytoviral chemicals are now widely used for the management of virus diseases. Several promising chemicals have been described. Triazofurin at 0.01 and 0.02% was effective against TSWV in tomato (Jayme Caner *et al.,* 1984). Srinivasalu and Narayanasamy (1990) screened 13 chemicals and found that acridine orange, copper sulphate and silver nitrate reduced local lesion formation of TSWV by 96-100 per cent. Shanker (1995) reported that the chemicals like copper sulphate, phosphates, copper, copper acetate and barium chloride were effective in reducing TSWV. Kroell (1988)

reported that application of 6-azauracil and 8-azaguanine reduced infections by TMV, PVX and CMV.

Ravinder Reddy (1988) showed that application of 0.1 % DHT (2,4–dioxohexahydro –1, 3, 5 – triazine) and 2 % carbendazim recorded 48.1 and 57.3 % incidence of urdbean leaf crinkle virus (ULCV) respectively as against 80 % in control. Krishnamoorthy (1994) reported that benzoic acid, barium chloride and ammonium sulphate at 1000 ppm reduced ULCV infection significantly and increased the incubation period of virus significantly. Acetyl salicylic acid (Aspirin) and salicylic acid reduced the multiplication of TMV in the systemically infected Samsun NN Tobacco through the induction of new PR-proteins (White *et al.,* 1983; Vanloon and Antoniw, 1982). Manickam and Rajappan (1999) reported that copper acetate recorded the lowest infection of green gram leaf curl virus (2.5 %) under field conditions.

2.7.2 Development of resistant cultivars

Current research on viral disease management is centered on to confer resistance to host plants against virus infection. Development of host plant resistance through breeding plays a vital role in disease management. Among 32 *Lycopersicon* accessions tested for resistance to TSWV under natural conditions. *L. peruvianum* line LA-444-1, *L. hirsutum* line PI 127826, *L. hirsutum* var. *glabrum* line PI (34417) and *L. pimpinellifolium* line PI 1732293-2v showed less infection (Maluf *et al.;* 1991). Three *Lycopersicon* accessions belonging to *L. peruvianum* var. *hirsutum showed* less infection (Kumar and Irulappan, 1991). Jaya jasmine and Seemanthini (1993) recorded the least incidence of TSWV in the hybrid ARTH-4 and variety Co-3. *L. pennellii, L. chilensis* and *L. peruvianum* were least susceptible to TSWV with both mechanical and thrips inoculations (Kumar *et al.,* 1993). Aramburu and Rodriguez (1999) reported that the presence of sw-5 gene in tomato hybrids conferred resistance to TSWV infection in 10 out of 12 tomato hybrids.

2.7.3 Antiphytoviral principles for disease management

Duggar and Armstrong first reported virus inhibition by plant products as early as 1925. They demonstrated that the sap of pokeweed, *Phytolacca decandra (L.)* mixed with TMV prevents infection of tobacco. It is well recognized that a number of higher plants contain substances acting against plant virus infection. It may be profitable to explore natural plant products when all other methods fail. The phytoproteins or their smaller peptides may serve as "lead structures" for the development of synthetic compounds. The value of these antiviral proteins is unlimited since they are quite safe, nontoxic even after repeated and prolonged use. It also enhances plant growth and yield against viral diseases. The different aspects of inhibitors from plants have been reviewed by many workers (Verma *et al.*, 1985; Awasthi *et al.*, 1985; Narayanasamy, 1991; Baranwal and Verma, 2000).

2.7.3.1 Distribution of anti phytoviral principles and their mode of action

Presence of viral inhibitors in different plant species have been reported by many researchers (Yoshii and Sako, 1967; Verma and Kumar,1980; Murthy, 1982; Sumathi, 1996). Plant species belonging to Chenopodiaceae, Amaranthaceae and Basellaceae have inhibitors of virus infection (Smookler, 1971).

2.7.3.2 Leaf

Verma and Mukherjee (1975) reported that pre inoculation with brinjal leaf extract induced local and systemic resistance in *Nicotiana glutinosa (L.)* against TMV and against tobacco ring spot virus in *N. tabacum (L.)*. Homogenates of tobacco leaf tissues contain anti viral principles capable of inhibiting TMV on several hosts (Zaitlin and Siegel, 1963). Velazhahan and Narayanasamy (1991) studied the inhibitory effect of the extracts of 71 different plants on TSWV and found that the extracts of 4-plant species *viz., Crotalaria juncea (L.), Morus alba, Delonix regia* (Bou.ex.Hook) Rafin *and Tectona grandis (L.)* showed high per cent inhibition. Kurucheve (1988) found that the extracts of *Ocimum sanctum (L.), Abutilon indicum* (G.Don.), *Carica papaya (L.), Prosopis chilensis*

(D.C.), *Nerium odorum* (Soland), *Poliyalthia longifolia* (Sonner) Thw., *Chenopodium murale (L.), Piper nigrum (L.) , Cannabinus sativa (L.) and Bougainvillea spectabilis* Wild inhibited TSWV infection in cowpea by more than 90 per cent .

Pre inoculation spray of systemic resistance inducers from *Clerodendrum aculeatum* (Gaertn.), *C. inerme (Gaertn.), Boerhavia diffusa* (L.), *B. spectabilis* and *Pseudoranthemum bicolor* Rodalk., have been shown to modify the susceptibility of several host plants like tomato, tobacco, mungbean, urdbean, bhendi and sunhemp against viruses producing local lesions and systemic infections (Verma and Dwivedi, 1984). Verma *et al.* (1985) reported that the extracts from the leaves of *Pseudoranthemum atropurpureum* Rodlk. and *B. spectabilis* showed high antiviral activity against sunnhemp rosette virus on *Cyamopsis tetragonaloba* (L.) and TMV on *N. glutinosa* (L.). Verma and Kumar (1980) and Kubo *et al.* (1990) reported the antiviral activity of the leaf extracts of *M. jalapa* (L.). *Mirabilis* antiviral protein (MAP) showed highly potent activity against mechanical transmission of tobacco mosaic virus, cucumber green mottle mosaic virus, potato virus, turnip mosaic virus, urdbean mosaic virus, tomato yellow mosaic virus and cucumber mosaic virus.

Narayanasamy and Ramiah (1983) reported the presence of AVPs in the leaves of sorghum, maize, pearl millet, finger millet, coconut, royal palm and neem leaves. Extracts of nerium and yellow oleander inhibited TSWV (Ganapathy ,1985). The antiviral property of *B. diffusa, B. spectabilis, C. aculeatum* and *Sorghum vulgare* was reported against tomato spotted wilt virus, cowpea aphid borne mosaic virus and tobacco mosaic virus (Sadasivam *et al.,* 1991). Bougainvillea leaf extract was effective in the control of TSWV both on its hypersensitive and systemic hosts (Balasaraswathi, 1995).

2.7.3.3 Seeds, roots and other plant parts

Antiviral principles are found at varying levels in different plant parts. Inhibitory effects of plant viruses have been identified in the extracts of germinating seeds of flowering plants. (Murty and Nagarajan, 1980). Mirabilis antiviral protein (MAP) inhibitory to TMV on *N. tabacum* was identified in petals, leaves, stalks and roots of *Mirabilis jalapa*

(Kubo *et al.,* 1990). Pokeweed antiviral proteins were found in leaves, seeds and roots (Irvin *et al.,* 1980; Barbieri and Stirpe, 1982; Bolognesi *et al.,* 1990). Ribosome inactivating protein (RIP) from the pokeweed seed was found effective against the infection of PVX, PVY, TMV, CMV, ACMV and CaMV.

Pokeweed antiviral protein (PAP) is one of the best-characterized RIP, which has been purified from seeds and leaves of poke weed (Chen *et al.,* 1991). A similar antiviral protein has been purified from barley grains (Roberts and Selitrennikoff, 1986). The effect of MAP against PVX and PVY indicated that it penetrated the upper layer of the epidermis and was localized in the intercellular spaces. MAP penetrated the epidermal and other leaf cells through the wound developed during the virus infection. It deglycosylates the 28S rRNA and prevents viral replication at an early stage by deactivating the cell protein synthesis machinery (Vivanco, 1999). MAP may have a direct nuclease activity against potato spindle tuber viroid (Stirpe *et al.,* 1996; Mehta and Boston, 1998). Further they reported the ability of RIPs to inhibit viral replication without damaging host cell protein chemistry. It could behave as a signal molecule that turns on cascade response, activating a series of defense mechanisms before viral infection occurs (Zoubenko *et al.,* 1997).

Fresh latex from fig, mulberry and *Calotropis procera* completely inhibited the development of local lesions by radish mosaic, zinnia mosaic and petunia mottle viruses (Rafig *et al.,* 1985). Fischer and Nienhaus (1973) reported that the extracts of different parts of *Capsicum annuum* differed in their inhibitory effect. El-kandelgy and Wilcoxon (1966) have studied the effect of red clover flower extract on red clover vein mosaic virus. Aqueous flower extracts of *Argemone mexicana, Azadirachta indica, Euphorbia milli, Jasminum sambac, Lantana indica, Nerium indicum* and *Vinca rosea* contained AVPs capable of reducing the number of local lesions or systemic infection against PVX (Rao *et al.,* 1985).

2.7.4 Rhizobacteria in viral disease management

Rhizosphere bacteria are present in large numbers on root surfaces, where the root exudates provide nutrients for the survival of rhizobacteria (Lynch and Whipps, 1991). Plant growth promoting rhizobacteria (PGPR) are subset of rhizosphere bacteria colonizing plant root and it exerts beneficial effects on crop development including plant growth promotion and biological control of pests and diseases (Kloepper *et al.*, 1992). Mann (1965) first attempted by applying cultures of *Bacillus uniflagellatus* and extracts from such cultures to tobacco roots as soil drench to induce systemic resistance against tobacco mosaic virus (TMV), which resulted in a significant reduction in the number of lesions in tobacco caused by TMV infection. Maurhofer *et al.* (1994) observed that root colonizing bacterium, *Pseudomonas fluorescens* strain CHAO induced systemic resistance against the lesion inducing Tobacco Necrosis Virus (TNV) in tobacco. They found a significant reduction in lesion number and lesion size in PGPR treated tobacco plants. Raupach *et al.* (1996) reported that PGPR treatment to cucumber or tomato plants induced systemic resistance against cucumber mosaic virus (CMV) under greenhouse conditions.

Zehnder *et al.* (2000) evaluated specific strains of PGPR for induced resistance against cucumber mosaic virus (CMV) in tomato under field conditions. The incidence of CMV infection was lower in PGPR treated plants (*Bacillus* strains IN 9379, IN 937 b and SE 34) that were mechanically challenged with virus before transplanting in the field. Kandan *et al.* (2002) reported that challenge inoculation of TSWV with *P. fluorescens* as seed treatment, seedling dip, soil and foliage application led to the triggering of pathogenesis related proteins like chitinase, glucanase, PAL, PO and PPO. Induction of a new protein of 18kDa was observed in *P. fluorescens* treated tomato plants challenged with TSWV.

2.7.5 Biological properties of AVPs

2.7.5.1 Effect of temperature

The AVPs were sensitive to temperature. Their activity was reduced with increase in temperature from 40°C to 60°C (Shanker, 1995). AVPs from coconut and sorghum were

less sensitive to higher temperature among different AVPs tested. Sumathi (1996) tested the temperature sensitivity of *Bougainvillea* antiviral principles (BAVP), *Prosopis* antiviral principle (PAVP), Sorghum antiviral principle (SAVP) and Coconut antiviral principle (CAVP). BAVP was least sensitive for the temperature than other AVPs. It was found effective up to 40°C.

The inhibitory property was not lost when several plant extracts were exposed to 100°C for 10 minutes. They are dodder (Miyakawa and Yoshi, 1951); sugarbeet (Ruppel, 1967); guava (Singh, 1969; Singh and Gupta, 1970); French bean (Taniguchi, 1974); *Prunus persica* (Singh and Singh, 1975) and *P. americana* (Fukaya and Taniguchi, 1979). Heat stable viral inhibitors were also reported from wheat (Verma and Verma, 1965); *Datura* sp. (Lal *et al.*,1973); *B. spectabilis* and *M. jalapa* (Noronha *et al.*, 1980).

2.7.5.2 Effect of pH

Verma *et al.* (1985) reported that *P. atropurpureum* lost its inhibiting effect at pH 4.0 and 10.0 where as pH of *B. spectabilis* was not affected at pH 4.0 but destroyed at pH 10.0. Shanker (1995) reported that all AVPs were effective at their neutral pH levels and with increase in acidity to alkalinity the AVPs lost their effectiveness indicating that the proteins exert its antiviral activity at neutral to acidic conditions.

2.7.5.3 Effect of dilution

Antiviral principles are in high concentrations in non-host plants. Due to these, several plant extracts were found to inhibit virus infection even at very low concentrations. On the other hand, in some plants the inhibitory effect was lost even at low dilutions. *B. spectabilis* and *M . jalapa* retained their inhibitory effect up to a dilution of 1:500 (Noronha *et al.*, 1980). But *Datura metel* lost its activity even at 1:3 dilution (Singh and Varma, 1981). The AVP from *B. spectabilis* was effective even at 1 % concentration. Sorghum and coconut AVPs also inhibited local lesion formation in cowpea at 1% concentration. *P. chilensis* AVP was effective at 5 per cent concentration against groundnut bud necrosis virus (Shanker, 1995).

2.7.5.4 Effect of aging under *in vitro*

Several workers have investigated the retention of antiviral activity in crude extracts of leaves against virus infection. Ramakrishnan *et al.* (1964) found that the leaf extracts of *Basella alba* and *B. rubra* were inhibitory for six weeks. Brinjal extracts withstood storage for one day (Lal *et al.*, 1973). Noronha *et al.* (1980) reported that *B. spectabilis* and *M. jalapa* retained their antiviral activity for a period of one year. Manickam (1991) reported that the AVP from *S. vulgare* and *Croton sparsiflorus* inhibited the local lesion of TSWV by 90 per cent upto 7 days after application. Sumathi (1996) observed that AVP of *B. spectabilis* was stable and its antiviral activity persisted for a period of 8 days when compared to prosopis AVP, sorghum AVP and coconut AVP whose antiviral activity persisted for 6 days.

2.8 Purification and characterization of AVPs

Though the studies on antiviral property of non-host plants dates back to 1918, it is only during the last 20 years some very interesting and valuable informations on molecular level characterization, mode of action and their possible use in management of plant viruses have been generated. However phyto antiviral substances are characterized only from very few plants. Most of the characterized antiviral substances are basic proteins. Their molecular weight ranges between 20 kDa to 32 kDa. A few of the antiviral substances have been also characterized as polysaccharides, phenolics, alkaloids, quinones and salts. However detailed studies have been carried out only on antiviral proteins (Verma *et al.*, 1995; 1998).

2.8.1 Proteins

AVP from *P. americana* was found to be a basic protein with a molecular weight of 27kDa (Irvin, 1975). This protein was referred as pokeweed antiviral protein (PAP). PAP is the best characterized AVP (Irvin, 1980). It inhibited the infection of both RNA and DNA viruses from a number of plant virus groups (Chen *et al.*, 1991). AVPs from

C. amaranticolor, C. album, Atriplex nites, A. caudatus (Smookler, 1971), *Cocos nucifera* , *S. vulgare* (Narayanasamy and Ramiah, 1983), *B. spectabilis* (Verma and Dwivedi, 1984), *P. atropurpureum* (Verma and Khan, 1985) and *Spinacia oleraceae* (Straub *et al.,* 1986) were also found to be proteinaceous in nature.

Among plant derived antiviral proteins, RIPs are widely distributed in higher plants. RIPs exist either as single-chain (Type I) or double-chain (Type-II) proteins. Both of them are basic proteins with iso electric points above 9. Type II RIPs possess a biologically active polypeptide (A chain and B chain), which includes a galactose-binding domain (Mehta and Boston, 1998). It enables to bind the cell wall galactose receptors and to internalize A chain in to the cell. Penetration of Type I RIPs into plant cells are enabled by the wounds on plant cell walls caused by insects, mechanical contact of leaves or previous viral infection (Reddy *et al.,* 1986). Recently, an acidic Type III RIP typified by maize B-32, has been identified (Mundy *et al.,* 1994). All RIPs inactivate ribosomes by modifying the 28S rRNA through its N glycosidase activity, which is manifested by cleavage of the N- glycosidic bond at a specific adenine residue (Stirpe *et al.,* 1992).

M. jalapa was found to contain an antiviral protein active against the mechanical transmission of certain plant viruses (Kubo *et al.,* 1990; Takanami *et al.,* 1990) . This Type I RIP was named as *Mirabilis* antiviral protein (MAP) (Habuka *et al.,* 1991). MAP was purified to homogeneity and was revealed to be lysine rich and basic protein of pI 9.8, with a molecular weight close to 24.2kDa (Takanami *et al.,* 1990). Purified MAP has been shown to inhibit the mechanical transmission of Tomato mosaic virus (ToMV) in tobacco, tomato and pepper plants, and cucumber green mottle mosaic virus in cucumber plants (Kubo *et al.,* 1990). More over it also had repellent properties against aphids and white flies (Verma and Kumar, 1979). Verma *et al.* (1996) purified a basic protein from *Clerodendrum aculeatum* leaves with a molecular mass of 34kDa. Balasaraswathi (1995) reported two inhibitory proteins *viz., Bougainvillea* antiviral protein I (BAPI) and *Bougainvillea* antiviral protein II (BAPII). BAPI was found effective in controlling TSWV, which is a basic protein with a molecular weight of 28kDa.

2.8.2 Glycoproteins

The AVPs from *N. glutinosa* (Palm, 1967), *Punica granatum* (Sabitha, 1969), *Boerhaaevia diffusa* (Verma *et al.*, 1979), *Dianthus caryophyllus* (Stirpe *et al.*, 1981) and *Basella alba* (Ushari *et al.*, 1982) were identified as glycoproteins. Stirpe *et al.* (1986) isolated a glycoprotein inhibitory to TMV from the roots of *Bryonia dioica*. Prasad *et al.* (1995) purified two basic glycoproteins from leaves of *Clerodendrum inerme viz.*, CIP 29 and CIP 34 with molecular masses of 29 and 34kDa respectively. They induced an actinomycin – D sensitive systemic resistance against TMV. Two antiviral glycoproteins, active against mechanical transmission of two tobamo viruses, TMV and sunnhemp rosette virus and citrus ring spot virus were purified from the dried leaves of *Celosia crystata*. These proteins known as CCP- 25 and CCP- 27 have molecular weight of 25 and 27kDa respectively. Their concentration was found to vary between the pre-flowering and post flowering stages of the plant. Either of these proteins obtained at different growth stages inhibited more than 90% lesions at a concentration of 20 to 30μg/ml. Application of glycoproteins individually also inhibited the systemic infection (Balasubramanian *et al.*, 2000).

2.8.3 Polysaccharides

Polysaccharides, which reduced virus infection, were found in *Cetaria islandica* (Gubanski, 1965), *Trifolium pratense* (El-Kandelgy and Wilcoxon, 1966), *Physarum polycephalum* (Dennis and Ford, 1971), *Beta vulgaris* (Ebrahim – Nesbat and Nienhaus, 1972), *Brassica oleracea* (Verma, 1973) and *Abutilon striatum* (Moraes *et al.*, 1974).

2.8.4 Alkaloids and glycoalkaloids

Doepke . *et al.* (1975) isolated antiviral glycoalkaloids from the leaves of *Solanum torvum*. Other antiviral glycoalkaloids were isolated from leaves of *S. nigrum* and *S. khasianum* (Roychoudhury and Basu, 1983). The leaf, bark and seed kernal extracts of

Azadirachta indica contain some alkaloids, which inhibited virus infection (Singh and Singh, 1988).

2.8.5 Flavones

AVPs from leaf extracts of *Capsicum annuum* were found to be flavones and sterols (Fischer and Ninehaus, 1973). A steroidal virus inhibitor was identified in *Artemisia annua* containing sitosterol and stigmasterol (Khan and Verma, 1990).

2.9 Induction of resistance against viruses by AVPs

Application of AVPs may induce either systemic or localized resistance against virus infection in host plants, by activation of host defense mechanisms (Verma and Khan 1985; Narayanasamy and Ganapathy, 1986). The leaf extracts from *B. spectabilis* induced systemic resistance against virus infection in several plants (Mukherjee *et al.*, 1982). Similarly *Celosia cristata* leaf extract induced localized resistance against TMV and Sunnhemp rossette virus in different test hosts (Baranwal and Verma, 1992). The leaf extracts from *B. spectabilis* induced systemic resistance against virus infection in several plants. The treated plants developed a highly active virus-inhibiting agent (VIA) systemically (Verma and Dwivedi, 1984). Rao *et al.* (1985) reported that aqueous flower extract of neem induced resistance against PVX infection in *C. amaranticolor.* The resistance varied from 79.2 to 99.2 per cent and remained active up to 96 h. after foliar spray.

Krishnamoorthy, (1994) observed increased activities of peroxidase (PO), catalase and phenyl alanine ammonia lyase (PAL) in black gram plants due to treatment with *M. jalapa*, *Vitex negundo* and *Leucaena leucocephala* leaf extracts and it resulted in resistance to leaf crinckle virus. Similar trend in increased enzyme activities was also observed in AVP treated groundnut plants resistant to bud necrosis virus (Shanker, 1995). Ragupathi (1995) observed increased PO, polyphenol oxidase (PPO) and PAL activities in tomato plants treated with AVPs from *M. jalapa* and *Catharanthus roseus* and resulted in resistance to leaf curl virus.

Similar changes in the activities of PO, PPO, PAL, chitinase and ß-1, 3 glucanase in chilli plants treated with *M. jalapa* were observed by Aiyanathan (1995). Verma *et al.* (1996) isolated a basic protein from *C. aculeatum* leaves, which induced systemic resistance against TMV and sunnhemp rosette virus on *C. tetragonoloba*. The treated plant accumulated 34kDa protein and was consistently observed in the treated plants confirming resistance against the virus infection. Muthulakshmi and Renuka Devi (2001) found that the activities of PO and PAL increased in seed sprout extracts of pigeonpea (P-AVP) and mungbean (M-AVP) treated rice plants against rice tungro virus (RTV), when compared to healthy and RTV inoculated control plants. Accumulation of chitinases and peroxidases with the onset of ISR by PGPR has been observed in some plants. Challenge inoculation of TSWV with *P. fluorescens* triggered pathogenesis related proteins like chitinase, glucanase, PAL, PO and PPO. Induction of a new protein of 18kDa was observed in *P. fluorescens* treated tomato plants challenged with TSWV (Kandan *et al.*, 2002).

2.10 Efficacy of AVPs under field conditions

Only few antiviral substances are practically used to prevent TMV infection since 1974. But their efficacy was not quite satisfactory (Ko and Misanto, 1979). Murthy *et al.* (1981) found that three sprays with *Basella alba* leaf extract reduced both the intensity and incidence of TMV on tobacco and increased the yield. Narayanasamy and Ramaiah (1983) reported that spraying sorghum or coconut leaf extracts on 10 and 20 days after sowing reduced the spread of groundnut ring mosaic strain of TSWV. Awasthi *et al.* (1985) reported that dried roots of *B. diffusa* prevented virus infection in tomato, potato, french bean and pea under field conditions when applied twice a week for one month from seedling stage onwards. Maximum protection (60 - 90 per cent) was obtained when the inhibitor was applied for two months. Sasirekha (1998) reported that application of *P. fluorescens* (soil 2.5 kg/ha + seed 10g/kg + foliar spray 0.5%) and two foliar sprays on 15th and 25th day after sowing with leaf extract of *C. roseum* (5%) + barium chloride (1000 ppm), significantly reduced urdbean leaf crinkle virus incidence and increased the yield of urdbean.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1. Maintenance of Tospovirus Culture

3.1.1 Local lesion host

Young tomato leaves exhibiting typical yellow chlorotic rings characteristic of the tospovirus infection was taken and ice tray technique was followed to establish pure culture of Tospovirus in glass house on local lesion host (Cowpea *cv.* C152) using Phosphate buffer 0.1M at pH 7.0 (Subramanian and Narayanasamy, 1973).

Young infected tomato leaves were macerated with chilled phosphate buffer at the rate of one ml per gram of infected tissue. The sap was squeezed through a thin layer of cotton wool and inoculated on the primary leaves of cowpea (*Vigna unguiculata* L. Walpi) *cv.* C152 previously dusted with carborundum (600 mesh) as an abrasive. Chlorotic local lesions developed on the inoculated cowpea plants after four days of inoculation and the leaf tissues containing local lesions were used for preparing inoculum for subsequent inoculations using one ml of chilled phosphate buffer for 10 lesions. This served as a standard inoculum and the virus was maintained in cowpea by inoculating the plants at regular intervals.

3.1.2 Systemic host (Tospovirus)

The virus-infected scion was grafted to 45 days old tomato cultivar, PKM1 which is highly susceptible to Tospovirus. The grafted plants were immediately covered with polythene bags for five days to protect the graft union from drying.

3.2 Screening of germplasm entries of tomato against Tospovirus

Fifty-eight accessions of tomato were screened for resistance against Tospovirus. The crop was raised following the recommended package of practices, but without any plant protection measures. Incidence of tomato spotted wilt was recorded at 30, 60 and 90 days after planting. In order to bombard the test plants, the highly susceptible Co3 variety

was planted 15 days ahead to planting on all four sides of the plot. Further for every four rows of test entries, one row of Co3 was transplanted 15 days earlier.

3.3 Screening of AVPs

Non-host plants were screened for their efficacy against Tospovirus. One gram of fresh leaf or root or seed or fruit flesh obtained from the non host plant species were homogenized with 10 ml of distilled water using a pestle and mortar .The macerate was squeezed through a thin layer of cotton wool or cheese cloth. The resulting extract was made up to 10 ml with distilled water to obtain a dilution of 1:10 (w/v).

The extracts were sprayed at the rate of one ml per plant using a hand sprayer (Misty, Varun Industries, Bombay-28) on the primary leaves of one-week old *cv.*C152 cowpea plants. The control plants were sprayed with distilled water. The AVP treated and control cowpea plants were inoculated with the standard inoculum, 24 hours after (pre inoculation) AVP application. Observations on development of local lesions were recorded on fourth day after inoculation. Each treatment having five plants was replicated three times. The percentage of inhibition of local lesion formation by each treatment over the control was calculated based on the number of local lesions produced using the formula,

$$I = (C - T) \times 100 / C$$

Where I = Per cent inhibition of lesion formation over control

C = Number of local lesions in control

T = Number of local lesions in plants treated with AVP.

List of non-host plants screened for antiviral activity against Tospovirus

Nerium odorum Soland .	*Bougainvillea spectabilis Wild.*	*Mirabilis jalapa L. (seed)*
Vitex negundo L.	*Azadirachta indica A.Juss .*	*Solanum viarum L.*
Casuarina equisettifolia Forst.	*Leucaena leucocephala L.*	*Phyllanthus niruri L.*
Ipomoea batatus Jacq.	*Cocos nucifera L.*	*Peltophorum ferrugineum Benth.*
Parthenium hysterophorus Adans.	*Sorghum vulgare L.*	*Tagetes erecta L.*

Acacia nilotica	*Achras sapota L.*	*Bacopa monnieri (L.) Wettstein.*
Allium cepa L.	*Abutilon indicum G.Don.*	*Adhatoda vesica Nees.*
Capsicum annuum L.	*Cynodon dactylon (L.) Pers.*	*Harpullia cupanioides Roxb.*
Basella rubra L.	*Acorus calamus L.*	*Achyranthes aspera L.*
Datura metel L.	*Mirabilis jalapa L. (leaf)*	*Gymnima sylvestris (Retz.) R.Br.ex.Schultes.*
Prosophis chilensis D.C.	*Mirabilis jalapa L. (root)*	*Phytolacca americana* (10%)
Anona squamosa L. (Fruit flesh-10%, Fruit peel- 10%))	*Anona squamosa L.* (Seed extract-10%)	*Erythrina indica Lam.* (Seed extract- 10%)

3.4 Screening of chemicals and biocontrol agents

The antiviral efficacy of chemicals such as Bion (100, 200 ppm), salicylic acid (50, 100 ppm), carbendazim (1000, 2000 ppm), tricyclazole (1000, 2000 ppm), copper sulphate (1000 ppm) and barium chloride (1000 ppm) and biocontrol agents like *Pseudomonas chlororaphis* and *Bacillus subtilis* were screened at the rate of @10^8 cfu/ ml. Efficacy was assessed as mentioned above.

3.5 Effect of antiviral principles, chemicals and biocontrol agents at different stages of virus inoculation

Effective non–host plant species (*B. spectabilis, M. jalapa, A. vesica, P. americana* and *H. cupanioides*) were selected and studied for their ability to control Tospovirus at three different stages of inoculation. Chemicals such as salicylic acid (50 ppm), Bion (100 and 200 ppm) and biocontrol agent *P. chlororaphis* (10^8 cfu/ml) were tested for their efficacy at pre, post and simultaneous inoculation of virus. AVPs, chemicals and biocontrol agents were applied 24 hours prior to virus inoculation, 24 hours after virus inoculation and by simultaneous inoculation. For simultaneous inoculation, one ml of AVP extract / chemical/ bio control agent was added to one ml of the virus inoculum in the chilled mortar and both were mixed thoroughly. The mixture was inoculated on carborundum dusted primary leaves

of *cv.*C152 cowpea plants and development of local lesions was recorded on fourth day. Suitable control was maintained. Three replications were maintained for each treatment. Per cent inhibition of lesion formation for each treatment over control was calculated.

3.6 Effect of AVPs on Tospovirus titre in Cowpea *cv.* C152 as determined by ELISA

The indirect DAS-ELISA was carried out for the detection of Tospovirus titre in AVP treated and untreated local lesion hosts. One gram of leaf sample from each treatment was taken and was ground with 10 ml of 0.01M sodium-potassium phosphate buffer and the extracts were centrifuged at 10000 rpm for 10 minutes. 50µl of supernatant (antigen) per well was added to the ELISA plate previously coated with 200µl of Tospovirus immunoglobulin and incubated overnight at 4°C. Three wells were filled with ELISA extraction buffer. Alkaline phosphatase conjugated antirabbit immunoglobulin was added at 1/2000 dilution and incubated for 3h at 37°C. Plates were washed four times between each step with phosphate buffered saline containing 0.05 per cent Tween 20, later *p*-nitrophenyl phosphate was added at 1 mg/ml and incubated for 1 hr at room temperature. The reaction was terminated with addition of 3M NaOH. ELISA reactions were measured spectrophotometrically at 405 nm using ELISA reader and positive readings were confirmed by visual observations. The A405 nm values presented were obtained by substracting the buffer control absorbance values (average of three wells) from sample values. The same technique was also followed to detect the presence of *Harpullia* antiviral proteins in other non-host plants.

3.7 Biological properties of selected AVPs

3.7.1 Effect of temperature on the antiviral activity of AVPs

The best performing AVPs such as *B. spectabilis, M. jalapa, H. cupanioides, P. americana* and *A. vesica* at 10 per cent concentration were taken in test tubes and exposed to different temperatures *viz.*, 28 ±2°C, 40°C, 50°C, 60°C and 70°C for 10 minutes in a water bath and tubes were immediately plunged in ice-cold water. The extracts cooled to room temperature were sprayed on the assay host and inoculated with

Tospovirus after 24 h of AVP spray. Plants sprayed with distilled water served as control. Per cent inhibition of lesion formation by each treatment over control was calculated.

3.7.2 Effect of different pH levels on inhibition of antiviral activity of AVPs

Phosphate buffer at different pH levels *viz.*, 4.0, 5.0, 6.0, 7.0 and 8.0 were prepared. One gram of selected plant product containing AVP was ground with 10 ml of the buffer at different pH. The pH of the *B. spectabilis, M. jalapa, H. cupanioides, P. americana* and *A. vesica* at 10 per cent concentration was adjusted using 1N HCL or 1N NaOH. The AVPs at different pH levels were sprayed on the assay host and the virus was challenged after 24 hours. Suitable controls were maintained with distilled water. Each treatment was replicated three times. The per cent reduction of local lesion formation in each treatment over control was determined.

3.7.3 Persistence of antiviral activity of selected AVPs

The persistence of antiviral activity of the effective plant extracts was assayed by spraying the extracts, 24 h prior to graft inoculation of tomato plants (Avinash 2) with Tospovirus. Controls were sprayed with distilled water. The per cent infection and reduction over control were assessed on 8 and 14 days after graft inoculation.

3.7.4 Effect of storage on the antiviral activity of AVPs

The effective plant extracts containing AVPs were taken and extracts were prepared to obtain 10 per cent concentration using distilled water and stored at room temperature of 28±2°C and the extracts were sprayed on the primary leaves of cowpea *cv.* C152 on 15, 30, 45 and 75 days after storage. Suitable controls were maintained with distilled water. The per cent inhibition of lesion formation by different treatments over control was calculated.

3.8 Effect of AVPs extracted using different organic solvents against Tospovirus

The effective plant extracts (AVPs) were extracted with ethanol, acetone, petroleum ether, diethyl ether and chloroform @ 5 ml/g of material and vaccum dried. Then they were redissolved in 5 ml of distilled water and tested for their inhibitory effect.

3.9 Characterization of AVPs

3.9.1 Fractionation of AVP from plant materials and study of their bioefficacy

The chemical nature of the antiviral principle was analysed by the protein extracted through ammonium sulphate fractionation method. Antiviral efficacy was assessed on cowpea plants that served as the local lesion host. Necessary control was maintained by spraying water and three replications were maintained for each test. All the analytical procedures were carried out in a cold room maintained at 4°C. Centrifugations were done in a HITACHI 18 PR-52 AUTOMATIC high-speed refrigerated centrifuge.

Preparation of the extract

Extract was prepared from the fresh roots of *M. jalapa,* seeds of *H. cupanioides* and leaves of *A. vesica,* following the procedure of Takanami *et al.* (1990) with some modifications.

The root, seed or leaves of AVPs were ground in a pre-chilled blender with 10 volumes of cold phosphate buffer (PB), pH 7.2 containing 0.1 per cent 2–mercaptoethanol. The extract was filtered through muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected in a separating funnel. To this, equal volume of chloroform was added, mixed and allowed to settle. The clarified aqueous and chloroform layers were collected separately and used in the subsequent steps.

Differential precipitation of AVPs with ammonium sulphate

The protein present in the clarified (aqueous layer) root extract of *M. jalapa,* seed extract of *H. cupanioides* and leaf extract of *A. vesica* were precipitated with different per cent saturation of ammonium sulphate.

20% saturation

To the root extract of *M. jalapa,* seed extract of *H. cupanioides* and leaf extract of *A. vesica*, ammonium sulphate (107g/lit) was added to get 20% saturation by stirring at 2- 4°C in a cold room. The extracts were incubated for 12h at 5°C.The precipitate was collected by centrifugation at 10,000×g for 15 min. It was dissolved in phosphate buffer pH- 7.2.This solution was dialysed against pH 7.2 on a magnetic stirrer at 5°C with frequent changes of the buffer for 10-12 h. Precipitates formed during dialysis were removed by centrifugation at 10,000g for 15 min. Remaining supernatant was lyophilized and stored at -70°C.

40% saturation

The supernatants collected from 20% ammonium sulphate saturation were used for the succeeding step. To this supernatant, ammonium sulphate was added (150g / lit) with stirring to bring them to 40% saturation. After 12h of incubation at 5°C, the suspensions were centrifuged. The pellets and supernatant were collected separately. The supernatant was used in the next step. The pellets were dissolved in Phosphate buffer and dialysed.

60% saturation

The supernatants collected from 40% ammonium sulphate saturation were used for the succeeding step. To this supernatant, ammonium sulphate was added (122g/lit) by stirring to bring them to 60% saturation. After 12h of incubation at 5°C, the suspensions were centrifuged. The pellet and supernatant were collected separately. The supernatants were used in the next step. The pellets were dissolved in Phosphate buffer and dialysed .

80% saturation

To each of the supernatant's ammonium sulphate was added to bring to 80% saturation (131g/lit). After 12h of incubation at 5°C, the suspensions were centrifuged and pellets were collected and used for bioassay as described above. The purified proteins

from different levels of saturation were subjected to SDS analysis to study their protein profile.

3.9.2 Bioassay of antiviral protein

Proteins from each level of saturation were tested for the inhibitory efficacy against Tospo by bioassay on local lesion host. In addition, the antiviral action of the purified *Mirabilis* antiviral protein (MAP) and *Harpullia* antiviral protein (HAP) were assessed for their efficacy at 25, 50, 100, 200, 400, 600 and 800 µg/ml of the proteins obtained at 80 per cent saturation. Crude extract and total protein of 80 per cent saturation were used to compare the efficacy of purified proteins at different concentrations.

3.10 Purification of antiviral protein

Antiviral proteins of *H.cupanioides* and *M. jalapa* obtained through differential ammonium sulphate precipitation were purified through Bio-Rad Biologic Chromatographic System. The samples were filtered through 0.22µm filter and then used for elution through Bio-Scale pre packed DEAE-5 column supplied through Bio- Rad laboratories. Protein was eluted through two different buffers (Buffer A and Buffer B) of low and high ionic strength. Buffer A consists of 20mM sodium phosphate with pH 7 and Buffer B consists of 20mM sodium phosphate with 0.5 M NaCl of pH 7. Protein sample of 1ml per run was eluted at a flow rate of 1ml per minute with a maximum pressure of 700 psi and with a minimum pressure of 50 psi. Eluted protein fractions were collected through fraction collector, which was programmed to collect 5ml per minute. After the complete run, different protein fractions were collected separately and each fraction was tested for its antiviral activity on the local lesion host. Fractions with antiviral activity were lyophilized and subjected for SDS-PAGE to ascertain their molecular weight. Protein was purified as per the steps given below. Protocols were followed as per the instructions given in the user manual supplied by Biologic HR Chromatographic System with slight modifications.

Step Number	Step
1	Isocratic flow with 100% Buffer A at 1ml/ min for 5ml
2	Set UV base line to zero at 280 nm
3	Set alarm to inject sample after the completion of step 1 and 2
4	Inject the sample (1ml) after receiving the alarm signal through the static loop. Before injecting the sample, it was loaded with syringe in the load position of the AV7-3 inject valve before the initiation of run.
5	Linear gradient flow with 50% Buffer A and 50% Buffer B at the rate of 1ml / min for 50ml.
6	Isocratic flow with 100% Buffer A for 5ml at the rate of 1ml / min
7	End of the protocol

3.11 Estimation of proteins (Bradford, 1976)

Ammonium sulphate fractionated proteins of root extract of *M. jalapa*, seed extract of *H. cupanioides* and leaf extract of *A. vesica* were estimated for their protein contents using this method.

Protein stock standard:

50mg of bovine serum albumin was weighed accurately and dissolved in distilled water and made up to 50 ml in a standard flask.

Working standard:

10 ml of the stock solution diluted to 50 ml with distilled water in a standard flask. One ml of this solution contained 200-µg proteins.

Bradford's Dye Concentrates

100 mg of Coomassie brilliant blue G-250 was dissolved in 50 ml of 50% ethanol and to this 100 ml of orthophosphoric acid was added. The final volume was made upto 200 ml with distilled water. It was mixed and stored in the refrigerator until use.

Working dye solution

The concentrated dye was diluted from one to five with distilled water, filtered and used fresh to assess the protein concentration using Beckman spectrophotometer. Aliquots

of sample to be estimated were taken in replicates in clean test tubes and the volume was made upto 1 ml with distilled water. The contents were mixed and 5 ml of Bradford's working dye solution was added. The contents were mixed and allowed for the colour to develop for at least 5 min but not longer than 30 min. The absorbance was recorded at 595 nm in the spectrophotometer. Suitable blank was also conducted taking 1.0 ml distilled water in the place of the sample. The protein standards were prepared by taking different aliquots of working standard solution ranging from 20–100 µg and developing colour with Bradford's working dye solution. The contents of protein in the samples were estimated by comparing their absorbance values with protein standards.

3.12 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

One g of powdered leaf sample was extracted with one ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 15000 g. The supernatant was used for the SDS-PAGE (Laemmli, 1970). The protein content of the sample was determined by Bradford method (Bradford, 1976).

Hundred µg of protein from different treatments was taken and mixed with 10 µl of sample buffer in microfuge tube, boiled for 4 min and incubated at 4°C for 30 min. Then the samples containing equal amount of proteins were loaded into the wells of polyacrylamide gels. (Sigma-Aldrich Techware System, Sigma, USA). The medium range molecular weight markers (Bangalore Genei, India) were used. Electrophoresis was carried out at constant voltage of 75 volts. The gels were stained with 0.2% Coomassie brilliant blue (R250) solution. Based on the Rf value of each protein band, the molecular weight was calculated. The induction of new proteins by the foliar spraying of antiviral principles was assessed.

Acrylamide solution

Thirty gram acrylamide and 0.8g bisacrylamide (Sigma, USA) were dissolved in 60 ml of distilled water. The final volume was made up to 100 ml and stored at 4°C in an amber coloured bottle.

Resolving gel buffer

A quantity of 18.15 g of trizma base (Tris-Hcl) was dissolved in 60-ml deionized water, the pH was adjusted to 8.8 with HCl and the volume was made upto 100 ml.

Stacking gel buffer

Six g of Trizma base was dissolved in 60 ml of deionized water and the pH was adjusted to 6.8 with HCl and volume was made upto 100 ml.

Sodium dodecyl sulphate (SDS, 10%)

Ten g of SDS was dissolved in 100 ml of distilled water.

Ammonium persulphate (10% APS)

One hundred mg of ammonium per sulphate was dissolved in one ml of distilled water (prepared fresh). N, N, N, N – Tetramethyl ethylene diamine (TEMED).

Electrode buffer

Electrode buffer was prepared by dissolving 4.32g of glycine, 300mg of SDS and 900 mg of Trizma base in 200 ml of distilled water. Final volume was made upto 300ml.

Staining solution

One hundred mg of Coomassie Brilliant Blue R250 was dissolved in 100 ml acetic acid: methanol: water (10:40:50 v/v).

Destaining solution

Acetic acid: methanol: water (10: 40: 50 v/v)

Resolving gel (10%) (Volume 10 ml)

Stock Solutions (ml)	Acrylamide Concentration (10%)
Acrylamide mixture	5.00
1:5 M Tris HCl (pH 8.8)	3.75
10% SDS	0.10
Distilled water	6.25
10% APS	0.05
TEMED	0.01

Stacking gel (4%)

Acrylamide mixture	0.065 ml
0.5 M Tris-HCl (pH 6.8)	1.250 ml
10% SDS	0.050 ml
Distilled water	3.050 ml
10% APS	0.025 ml
TEMED	0.005 ml

Sample buffer

Glycerol	20%
Tris-HCl (pH 6.8)	0.125 M
Na_2EDTA	5 mM
SDS	20%
Bromophenol blue	0.1% (W/V)
2-mercapto ethanol	1% v/v

3.13 Iso Electric Focussing

The isoelectric focusing point (*pI*) of the anti viral protein was estimated by isoelectric focusing (IEF) two dimensionally on a Phast gel IEF 3 – 9 with the Phast system (Pharmacia – LKB Biotechnology Inc., Uppsala, Sweden) using broad *pI* calibration kit as standard proteins. Pharmacia broad range *pI* standards were used. The markers used and their *pI* value are as follows: amyloglucosidase (3.50), soybean trypsin inhibitor (4.55), β- lactoglobulin (5.20), bovine carbonic anhydrase (5.85), human carbonic anhydrase (6.55), horse myoglobin – acidic (6.85), horse myoglobin – basic (7.35), lentil lectin – acidic (8.15), lentil – lectin middle (8.45), lentil – lectin basic (8.65) and trypsinogen (9.30). Three steps *viz*., pre focusing step, Sample application step and a focusing step were followed for isoelectric focusing of the protein. In the pre focusing step of the dimension, the pH gradient was formed and the sample applicators were loaded. In the sample application

step of the second dimension, the gel was rotated clockwise to 90° and about 30ng protein /µl was applied with the sample applicators perpendicular to the pH gradient across the middle of the gel for 15v / h. In the focusing step, the applicators were raised and the proteins charged negatively or positively started to migrate depending on their *pI* value from the centre to the anode or cathode. The separation of protein was followed by silver staining to observe the *pI* values of the proteins.

3.14 Native gel electrophoresis

3.14.1 Peroxidase

To study the expression pattern of different isoforms of peroxidases in different AVPs treatments, discontinuous native polyacrylamide gel electrophoresis (native PAGE) was carried out. One gram of the leaf sample was collected on fourth day from the local lesion host after challenge inoculation with Tospovirus. The samples were homogenized with 1ml of phosphate buffer (pH 7.0) under 4°C. It was centrifuged at 16,000g (4°C) for 20 min. The supernatant was used as the enzyme source to study the expression of different isoforms of peroxidase. For native anionic polyacrylamide gel electrophoresis, resolving gel of 8% acrylamide concentration and stacking gel of 4% acrylamide concentration were prepared. The protein content of the sample was determined by the Bradford method (Bradford, 1976). After electrophoresis, the gels were incubated in the solution containing 100mg benzidine (dissolved in 0.5 ml of acetone) in 50ml of acetate buffer. The gel was incubated for a period of 30 min. in darkness. Then drops of 30% H_2O_2 were added with constant shaking till the bands appear. After staining, the gel was washed with distilled water and photographed (Nadlony and Sequira, 1980).

3.14.2 Polyphenol oxidase

After native gel electrophoresis the gel was equilibrated for 30 min in 0.1M phosphate buffer (pH 7.0) containing 0.1% p-Phenylene diamine followed by 10 mM catechol in the same buffer. A gentle shaking followed by the addition of catechol, which resulted in appearance of dark brown discrete protein bands (Jayaraman *et al.*, 1987).

3.15 Antiserum production of *Harpullia* antiviral protein (HAP)

Antiserum of HAP was produced for detecting the presence of HAP like proteins in the other non-host plants effective against Tospovirus. Purified HAP protein was injected intramuscularly in the hind leg of three month old NewZealand White rabbit (3.5 Kg) with 0.5 ml of partially purified protein + 0.5 ml of Freund's incomplete adjuvant + 0.5 ml of saline. Four injections were given at an interval of seven days alternating with Freund's incomplete adjuvant and complete adjuvant each time, followed by a booster injection 12 days after the fourth injection. The rabbit was bled by giving a deep incision in the mid ear vein, after shaving the hair in the ear region, with a help of a sharp sterile blade and the blood collected in a vial was kept in a slanting position for an hour at room temperature and then kept in refrigerator overnight at 4 °C. The serum, which was separated as white thick fluid, was transferred to sterile eppendorf tubes under sterile conditions and the precipitate was removed by centrifugation at 5,000 rpm for 10 minutes. After discarding the RBC pellets, the supernatant was transferred to sterile eppendorf tubes and stored in deep freezer (-70°C) by adding preservative. The developed antiserum of HAP was tested for its presence in the other effective AVPs used in the study through ELISA.

3.16 Western blotting

Western blotting was carried out to confirm the presence of chitinase and ribosome inactivating proteins in *H. cupanioides and M. jalapa*. The purified antiviral proteins of *H. cupanioides* (HAP) and *M. jalapa* (MAP) were subjected to SDS – PAGE. After electrophoresis on to 0.45 µm PVDF (millipore) membranes (Sigma, USA) as per Gallagher *et al.* (1995). The electrophoretic transfer of proteins was carried out from gel to membrane in a Bio Rad Semidry transblot apparatus (140 mA, 30 min). The membranes were then stained with Ponceau S stain (Sigma, USA) for 2 min to check the resolution and transfer quality. Ponceau S stain was destained with TBST for 2 min and the membrane was

blocked for 1.5 h at room temperature (28 ± 2°C) in TBST containing 2.5% (w/v) gelatin. The membrane was then soaked in diluted primary antibody of barley chitinase / RIP of *Mirabilis expansa* obtained from Dr. Vivanco, Department of Horticulture and Landscape Architecture, Colorado, U.S.A. at 1:3000 dilution for overnight in TBST. After incubating with the primary antibody, the membrane was washed with TBST thrice for 10 to 15 min each time to remove the unbound antibody. The membrane was then incubated in secondary antibody for 3h. Affinity purified goat anti-rabbit immunoglobulin (IgG) alkaline phosphate conjugate (Sigma, USA) was used as secondary antibody at a dilution of 1:7000. The membrane was then washed thrice with TBST and thrice in TBS for 10 to 15 min each time. Immunological reaction was visualized by soaking the membranes in alkaline phosphatase colour development reagents (Bangalore Genei, India). Immediately after colour development the membranes were washed in distilled water and dried.

3.17 Assay of terpenoids through TLC

Literature survey for the presence of various secondary metabolites in *H. cupanioides* revealed that only the bark and stem consists of terpenoids. But, so far no work has been carried out to elucidate the compounds associated with the seeds. As terpenoids inhibit viral infection, the seeds of *H. cupanioides* were assayed for the presence of terpenoids through thin layer chromatography (TLC).

For preparation of the extract, the seeds were powdered and the sample (100g) was exhaustively extracted with methanol (750ml) in a Soxhlet apparatus. The hexane extracts were evaporated to dryness in vacuum flash evaporator withlow pressure under vacuum. The dried extract was dissolved in methanol in the ratio of 25mg / ml of methanol. The reduced methanol extract was filtered under acrodisc syringe filter of 0.5 µm super membranes, non-pyrogenic manufactured by Pall Gelman laboratory. 20µl of the filtrate was dotted in TLC aluminum plate of silica gel 60F 254 manufactured by Emerck, Germany. The plates were placed in the saturated chamber-containing methanol: hexane of ratio 60:40 as mobile phase. The chromatography was run until the solvent front reached

80% of the TLC plate. The plate was dried under hot air oven at 120°C and the chromatogram was visualized under infrared cabinet at wavelength of 254ηm. The visualized band was documented through photography. The standard terpenoids like salanin, nimbin, isomelidin, desacetyl nimbin and switenine with Rf value of 0.327, 0.545, 0.527, 0.4545 and 0.40 respectively were used as markers to confirm the presence of terpenoids in the test sample (Bhutani *et al.*, 1984).

3.18 Induced systemic resistance

Enzyme extraction

Tomato and cowpea leaf tissues obtained after AVPs treatment (with and without inoculation of Tospovirus) were homogenized immediately with liquid nitrogen. One g of powdered sample was extracted with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4°C. The homogenate was centrifuged for 20 minutes at 15000g. The supernatant was used as crude enzyme extract for assaying PO, PPO and PAL enzymes. For all enzyme assays, the samples were collected at 24h interval upto 4 days after the pathogen inoculation from cowpea cv. C- 152 plants, and on 4^{th}, 8^{th}, 12^{th} and 16^{th} day after graft inoculation of the virus in 45 days old tomato plants.

3.18.1 Assay of peroxidase (PO)

Both the tomato and cowpea homogenates were centrifuged at 15,000g for 10 min at 4°C and the supernatant was immediately used for enzyme assay. Peroxidase activity was assayed using guaiacol as a hydrogen donor as previously reported by Rathmell and Sequiera (1974). Exactly 1.5ml of sodium phosphate buffer, 1.5ml of guaiacol, 100µl of supernatant was taken as a reaction mixture. Finally, 100µl of hydrogen peroxide was added and the change in absorbance was read at 470ηm. The peroxidase activity was expressed as change in absorbance / min / g of fresh tissue.

3.18.2 Assay of phenyl alanine ammonia lyase (PAL)

PAL activity was determined as the rate of conversion of L- phenylalanine to trans–cinnamic acid at 290nm as described by Dickerson *et al.* (1984). One gram of leaf was homogenized in 5ml of 0.1M sodium borate buffer, pH 7.0 containing 0.1g of insoluble poly vinyl pyrolidone (PVP). The homogenate was centrifuged at 15,000g for 20min. The supernatant was used as the enzyme source for assay. Samples containing 0.4ml of enzyme extract were incubated with 0.5ml of 0.1M borate buffer, pH 8.8 and 0.5ml of 12mM L- phenylalanine in the same buffer for 30min at 30°C. The reaction was arrested by adding 0.5 ml of 1 M trichloroacetic acid and incubated at 37°C for 5 min. The blank contains 0.4ml of crude enzyme extract and 2.7 ml of 0.1 M borate buffer (pH 8.8) and absorbance was measured at 290nm and used an extinction coefficient of 9630 per minute cm^{-1} for transcinnamic acid in 0.1 M borate buffer (pH 8.8). The absorbance 9630 is equal to 1 mol/1 min or the absorbance is 0.963, the product formed is 100 ηmol/ml/min. The enzyme activity was expressed on the fresh weight basis of amount of trans-cinnamic acid as ήmol $min^{-1}g^{-1}$.

3.18.3 Assay of phenol

The phenolic assay was conducted as per the method of Zieslin and Ben-zaken (1993). The leaf tissues (tomato and cowpea) were homogenized at the rate of 1g per 10ml of 80% methanol and the methanolic extract was kept in water bath at 70°C for 15 min with frequent agitation. One ml of methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1 N) was added and the solution was kept at 25°C for 30 min. Finally, one ml of saturated solution of Na_2CO_3 and 1 ml of distilled water was added and the reaction mixture was incubated for 1h at 25°C. After the blue colour development, the absorbance was recorded at 725ηm. The contents of total soluble phenols were calculated according to a standard curve obtained from a Folin-Ciocalteau reaction with a catechol solution. The phenol content was expressed as phenol equivalents in µg g^{-1} fresh weight of plant tissue.

3.18.4 Assay of polyphenol oxidase (PPO)

The PPO activity was determined as per the procedure given by Mayer *et al.* (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. Two hundred µl of catechol of 0.01 M was added to initiate the experiment and the activity was expressed as changes in absorbance at 495ηm min^{-1} g^{-1} fresh tissues.

3.19 Efficacy of AVPs, chemicals and Biocontrol agents under field condition

Three field trials were conducted at Alandurai, Thenamanallur and Mathvarayapuram village of Coimbatore district. The plot size was 5mx4m. The treatments were replicated three times in a randomised block design. Extracts of AVPs *viz. M. jalapa* (10%), *H. cupanioides* (10%), *A. vesica* (10%), neem oil (1%), monocrotophos (0.1%), Bion 200ppm and *Pseudomonas chlororaphis* (2%) were applied as foliar spray at fortnightly intervals. The per cent-infection and yield were recorded. The per cent infection of wilt by Tospovirus was recorded on 30, 60 and 90 days after planting. Population of thrips was recorded at monthly intervals (30, 60 and 90 DAP) from 10 plants per replication. The statistical analysis was carried out as per the Irristat version 3 / 93.

Experimental Results

CHAPTER – IV

EXPERIMENTAL RESULTS

4.0 Screening of tomato germplasm

A total of fifty-eight germplasm entries obtained from Department of Olericulture, Tamil .Nadu Agricultural University, Coimbatore were screened for resistance against Tospovirus under field conditions. The reaction of various genotypes revealed that the per cent incidence of wilt by Tospo virus was found to increase from 30 to 90 days after planting. Among the accessions, the lowest incidence of 35.49% and the highest incidence of 99.5% were recorded in LE31 and LE231 respectively after 90 days of planting. None of the entries were resistant to Tospovirus.(Table 1).

4.1 Screening of non host plants against Tospovirus

Thirty-six non-host plants were screened for their antiviral activity against Tospovirus on the local lesion host (Cowpea *cv.* C152). The efficacy was assessed in terms of per cent reduction in number of local lesions over control. Among the various non-host plants screened, pre inoculation spray (24h prior to virus inoculation) of *B. spectabilis* and the root extract of *M. jalapa* were highly effective against Tospovirus. Leaf extract of *B. spectabilis* and the root extract of *M. jalapa* recorded 99.7 and 99.3 per cent reduction of local lesions over control respectively (Table 2). The next in order was 10 per cent seed extract of *M. jalapa,* which was followed by the seed extract of *H. cupanioides,* leaf extract of *A. vesica* and *P. americana.*

4.2 Screening of chemicals and biocontrol agents against Tospovirus

Efficacy of various chemicals and biocontrol agents were assessed against Tospovirus on the local lesion host and it was observed that pre inoculation spray of Bion at 200 ppm recorded 89.15 per cent reduction over control followed by foliar

Table 1. Screening of tomato germplasm entries against Tospovirus

S.No.	Per cent infection of Tospovirus *			
	Entries	30 DAP	60 DAP	90 DAP
1.	LE 1231	19.23 (26.01)	42.49 (40.68)	56.50 (48.73)
2.	LE 1220	24.99 (30.00)	53.00 (46.72)	64.01 (53.13)
3.	LE 231	51.00 (45.51)	80.00 (63.46)	99.50 (85.93)
4.	LE 823	20.99 (27.27)	34.99 (36.26)	60.50 (51.06)
5.	LE 1172	53.00 (46.72)	61.00 (51.35)	71.00 (57.42)
6.	MTL 115	16.99 (24.34)	26.99 (31.30)	58.00 (49.60)
7.	LE 945	33.00 (35.06)	46.99 (43.27)	61.00 (51.35)
8.	LE 1184	18.99 (25.83)	30.99 (33.83)	54.00 (47.29)
9.	LE 1212	20.99 (27.27)	57.00 (49.02)	90.55 (72.10)
10.	LE 228	14.00 (23.21)	26.99 (31.30)	50.00 (44.99)
11.	LE 96	20.99 (27.27)	51.00 (45.57)	63.00 (52.53)
12.	LE 85	16.99 (24.34)	28.99 (32.57)	55.00 (47.87)
13.	LE 406	43.00 (40.98)	64.01 (53.13)	83.01 (65.65)
14.	LE 118	18.99 (25.83)	28.99 (32.57)	63.51 (52.84)
15.	LE 1029	26.99 (31.30)	70.00 (56.73)	71.00 (57.42)
16.	LE 3	57.00 (49.02)	79.00 (62.73)	98.00 (83.02)
17.	LE 1228	28.99 (32.58)	49.00 (44.42)	75.54 (60.36)
18.	LE 931	21.97 (27.95)	43.99 (41.55)	57.00 (49.02)
19.	LE 971	22.00 (28.21)	46.99 (43.27)	60.00 (50.77)
20.	LE 123	21.48 (27.61)	38.99 (38.64)	54.00 (47.29)
21.	LE 1216	16.99 (24.34)	36.99 (37.46)	54.00 (47.29)
22.	LE 980	16.42 (23.91)	30.98 (33.82)	43.47 (41.24)
23.	LE 618	26.99 (31.30)	49.00 (44.42)	59.00 (50.18)
24.	LE 726	33.99 (35.66)	50.00 (44.99)	74.00 (59.34)
25.	LE 113	57.00 (49.02)	80.03 (63.46)	98.13 (82.14)
26.	LE 335	24.99 (30.00)	52.00 (46.14)	61.00 (51.35)
27.	LE 165	20.99 (27.27)	50.00 (44.99)	63.51 (52.84)
28.	LE 1001	16.99 (24.34)	26.99 (31.30)	50.50 (45.28)
29.	LE 1057	43.00 (40.98)	94.00 (76.21)	95.04 (77.14)
30.	LE 881	51.00 (45.51)	73.00 (58.69)	98.54 (83.06)
31.	LE 328	18.33 (25.21)	50.00 (44.99)	71.00 (57.42)
32.	LE 627	29.50 (32.90)	50.50 (45.28)	69.50 (56.47)
33.	LE 828	48.66 (43.21)	73.00 (58.69)	93.03 (74.69)
34.	LE 23	54.00 (47.30)	77.00 (61.34)	98.13 (82.14)
35.	LE 1215	26.88 (31.13)	41.99 (40.39)	54.00 (47.29)
36.	LE 470	12.98 (28.21)	28.99 (32.57)	40.00 (40.12)
37.	LE 128	22.00 (21.12)	38.49 (38.35)	58.00 (49.60)
38.	LE 31	12.98 (21.12)	24.99 (29.99)	35.49 (36.57)
39.	LE 1223	8.98 (17.42)	29.98 (33.19)	48.00 (43.85)
40.	LE 525	14.66 (23.26)	34.99 (36.26)	56.00 (48.44)

41.	LE 100	19.99 (26.53)	30.49 (33.51)	60.00 (50.76)
42.	LE 115	20.99 (27.27)	32.99 (35.05)	50.50 (47.87)
43.	LE 281	24.33 (29.86)	54.33 (47.80)	55.00 (47.87)
44.	LE 561	9.98 (18.41)	29.98 (33.19)	50.00 (44.99)
45.	LE 1528	12.98 (21.12)	41.99 (40.39)	59.00 (51.06)
46.	LE 2109	10.98 (19.35)	33.98 (35.65)	48.50 (44.14)
47.	LE 985	18.99 (25.83)	32.99 (35.05)	51.50 (45.85)
48.	LE 267	16.99 (24.34)	32.96 (35.04)	49.49 (44.71)
49.	LE 1204	14.99 (22.78)	32.99 (35.05)	44.48 (41.83)
50.	LE 598	16.99 (24.34)	36.99 (37.46)	48.49 (44.13)
51.	LE 358	18.99 (25.83)	39.99 (39.22)	56.52 (48.75)
52.	LE 931	19.96 (26.54)	29.98 (33.19)	53.10 (46.78)
53.	LE 1159	12.98 (21.12)	27.99 (31.94)	42.50 (40.68)
54.	LE 411	13.94 (21.92)	30.49 (33.51)	49.49 (44.71)
55.	LE 158	64.01 (53.14)	73.00 (58.69)	88.07 (69.80)
56.	LE 514	31.00 (33.83)	42.99 (40.97)	64.04 (53.15)
57.	LE 543	53.00 (46.72)	79.00 (62.73)	97.00 (80.04)
58.	LE 1170	49.00 (44.43)	61.00 (51.35)	75.54 (60.36)

LSD (5%) 1.23 1.23 9.42

Values in the parentheses are arcsine-transformed values.

Values are the mean of two replications.

Table 2. Screening of non-host plants against Tospovirus on local lesion host cowpea *cv.* C152

S.No.	Non-host plants	NOLL*	Per cent reduction over control
1.	*Abutilon indicum*	68.00^{q}	52.45
2.	*Acacia nilotica*	108.00^{t}	24.47
3.	*Achras sapota*	46.00^{l}	67.83
4.	*Achyranthus aspera*	25.00^{g}	82.52
5.	*Acorus calamus*	41.00^{k}	71.33
6.	*Adhatoda vesica*	18.33^{de}	87.18
7.	*Allium cepa*	122.67^{r}	14.22
8.	*Anona squamosa (fruit flesh extract)*	50.00^{m}	65.03
9.	*Anona squamosa (seed extract*	30.67^{hi}	78.55
10.	*Azadirachta indica*	24.00^{fg}	83.22
11.	*Bacopa monneri*	30.00^{h}	79.02
12.	*Basella rubra*	120.00^{u}	16.08
13.	*Bougainvillea spectabilis*	0.33^{a}	99.77
14.	*Capsicum annuum*	120.00^{u}	16.08
15.	*Casuarina equisettifolia*	69.67^{q}	51.28
16.	*Cocos nucifera*	16.00^{cd}	88.81
17.	*Cynodon dactylon*	55.00^{n}	61.54
18.	*Datura metel*	110.00^{t}	23.08
19.	*Erythrina indica (seed extract)*	43.00^{k}	69.93
20.	*Gymnema sylvestrie*	18.00^{de}	87.41
21.	*Hurpullia cupanioides (seed)*	14.67^{c}	89.74
22.	*Ipomoea batatus*	73.00^{v}	48.95
23.	*Leucaena leucocephala*	93.00^{s}	34.96
24.	*Mirabilis jalapa (leaf)*	19.00^{e}	86.71
25.	*Mirabilis jalapa (root)*	1.00^{a}	99.30
26.	*Mirabilis jalapa (seed)*	11.67^{b}	91.84
27.	*Nerium odorum*	38.00^{j}	73.42
28.	*Parthenium hysterophorus*	33.00^{i}	76.92
29.	*Peltophorum ferrugineum*	64.00^{p}	55.24
30.	*Phyllanthus niruri*	61.00^{o}	57.34
31.	*Phytolacca americana*	16.33^{cd}	88.58
32.	*Prosopis chilensis*	22.00^{f}	84.62
33.	*Sorghum vulgare*	33.00^{i}	76.92
34.	*Solanum viarum*	48.00^{lm}	66.43
35.	*Tagetes erecta*	59.00^{o}	58.74
36.	*Vitex negundo*	43.00^{k}	69.93
37.	*Untreated control*	143.00^{w}	-
	LSD (5%)	2.40	

- Mean of three replications, NOLL – Number of local lesions
- Means followed by a common letter are not significantly different at the 5% level by DMRT.

spray of Bion (100ppm). Among the biocontrol agents, foliar application *P. chlororaphis* at the rate of 10^8 cfu / ml recorded 70.85 per cent reduction in local lesions over control (Table 3).

4.3 Antiviral efficacy of non-host plants, biocontrol agents and chemicals on Tospovirus

Effective non-host plants, biocontrol agents and chemicals were tested for their efficacy by spraying 24h prior to virus inoculation, 24h after virus inoculation and simultaneous inoculation of both antiviral substances and the Tospovirus. Results indicated that the antiviral efficacy was experienced to a greater level as pre inoculation followed by simultaneous inoculation, irrespective of various AVPs tested. Pre inoculation spray of *M. jalapa* (root extracts) recorded 98.46 per cent reduction in the number of local lesions. It was on par with the seed extract of *M. jalapa* and leaf extract of *B. spectabilis*. Post inoculation spray was not found to be effective in inhibiting Tospovirus (Table 4).

4.4 Effect of temperature on the antiviral activity of AVPs against Tospovirus

The antiviral activity of effective non-host plants were tested at different temperatures *viz.*, 28±2°C, 40°C, 50°C, 60°C and 70°C on its local lesion host. Results revealed that the antiviral efficacy of all the AVPs tested was more at room temperature (28±2°C) and 40°C. As the temperature increased beyond 40°C, there was significant increase in the number of local lesions, indicating the loss of antiviral activity of AVPs. Among various extracts tried, root extract *of M. jalapa*, leaf extract of *B. spectabilis* and seed extract of *H. cupanioides* were more tolerant to higher temperatures (60°C and 70°C). The inhibition of local lesion by *M. jalapa* was 97.64% at 28±2°C. But the exposure of the same to 70°C resulted in 85.71% inhibition of local lesions, followed by *B. spectabilis* and *H. cupanioides* (Table 5).

Table 3. Effect of pre inoculation spray of chemicals and biocontrol agents against Tospovirus on cowpea *cv.* C152

Sl.No.	Treatments	NOLL*	Per cent reduction over control
1.	Salicylic acid (50 ppm)	20.33^{cd}	65.15
2.	Salicyclic acid (100 ppm)	Phytotoxic	Phytotoxic
3.	Carbendazim (1000 ppm)	31.33^{e}	46.29
4.	Carbendazim (2000 ppm)	22.33^{d}	61.71
5.	Tricyclazole (1000 ppm)	45.33^{g}	22.28
6.	Tricyclazole (2000 ppm)	39.67^{f}	31.99
7.	Bion (100 ppm)	11.67^{b}	79.99
8.	Bion (200 ppm)	6.33^{a}	89.15
9.	*P. chlororaphis*	17.00^{c}	70.85
10.	*B. subtilis*	21.67^{d}	62.85
11.	Copper sulphate	45.33^{g}	22.29
12.	Barium chloride	42.67^{fg}	26.85
13.	Control	58.33^{h}	-
	LSD (5%)	3.35	

- Values are the mean of three replications
- Means followed by a common letter are not significantly different at the 5% level by DMRT.

*NOLL – Number of Local Lesions.

Table 4. Effect of pre, post and simultaneous inoculation of antiviral principles, biocontrol agent and chemicals on the incidence of Tospovirus on cowpea (*cv.* C152)

Treatments	Number of local lesions*		
	Pre inoculation	Post inoculation	Simultaneous inoculation
B. spectabilis (L)	3.33^{g} (92.37)	32.67^{c} (34.66)	7.00^{h} (84.56)
M. jalapa (S)	1.33^{g} (96.95)	36.67^{b} (26.66)	10.67^{g} (76.46)
M. jalapa (R)	0.67^{g} (98.46)	21.33^{e} (57.34)	4.00^{h} (91.18)
A. vesica (L)	7.67^{f} (82.44)	33.33^{c} (33.34)	19.67^{e} (56.61)
H. cupanioides (S)	13.67^{cd} (68.69)	38.00^{b} (24. 00)	21.00^{de} (53.67)
P. americana (L)	16.33^{c} (62.60)	26.67^{d} (46.66)	24.00^{cd} (47.05)
Salicylic acid (50ppm)	24.00^{b} (45.04)	39.67^{b} (20.66)	25.67^{c} (43.37)
Bion (100 ppm)	11.67^{de} (73.28)	26.67^{d} (46.66)	40.00^{b} (11.76)
Bion (200 ppm)	9.00^{ef} (79.39)	28.00^{d} (44.00)	24.67^{c} (45.58)
P. chlororaphis	9.67^{ef} (77.86)	29.00^{d} (42.00)	15.67^{f} (65.43)
Untreated control	43.67^{a}	50.00^{a}	45.33^{a}
Mean	12.82	33.09	21.61

LSD (5%) :
Treatment (T) : 2.22
Stages of inoculation (I) : 4.30
T x I : 3.03

*Values are the mean of three replications
Values in parentheses are per cent reduction over control
In a column, means followed by common letter do not differ significantly for interaction effect at 5% level by DMRT.
L – Leaf; S – Seed; R – Root.

Table 5. Effect of temperature on the antiviral activity of AVPs against Tospovirus on cowpea (*cv.* C152)

Treatment	Number of local lesions at different temperatures*					
	28 ± 2°C	40°C	50°C	60°C	70°C	Mean
B. spectabilis (L)	4.00^{d} (90.55)	7.33^{d} (82.68)	9.00^{d} (79.07)	10.00^{e} (77.27)	12.0^{e} (75.51)	8.46
M. jalapa (R)	1.00^{e} (97.64)	3.00^{e} (92.91)	4.00^{e} (90.69)	6.67^{f} (84.84)	7.0^{f} (85.71)	4.33
H. cupanioides (S)	10.00^{c} (76.38)	11.00^{c} (74.01)	13.67^{c} (68.21)	16.67^{b} (62.11)	17.33^{d} (64.63)	13.73
P. americana (L)	14.00^{b} (66.93)	14.33^{b} (66.15)	19.67^{b} (54.26)	22.33^{d} (49.25)	25.66^{b} (47.63)	19.20
A. vesica (L)	5.67^{d} (86.60)	9.00^{cd} (78.74)	13.00^{c} (69.76)	19.67^{c} (55.29)	22.33^{c} (54.43)	13.93
Untreated control	42.33^{a}	42.33^{a}	43.00^{a}	44.00^{a}	49.00^{a}	44.13
Mean	12.83	14.50	17.06	19.89	22.22	17.29

LSD (5%)
Treatments (T) : 2.09
Temperature(t) : 2.26
T x t : 2.22
*Values are the mean of three replications
Values in parentheses are percent reduction over control
In a column, means followed by common letters do not differ significantly for the interaction effect of 5% level by DMRT.
L – Leaf, S – Seed; R – Root.

4.5 Effect of pH on the antiviral activity of AVPs against Tospovirus

The effect of different pH levels on the antiviral activity of non-host plants on Tospovirus were studied. The results (Table 6) revealed that among the five pH levels tested, *B.spectabilis*, *M.jalapa* and *H. cupanioides*, showed maximum level of inhibition of lesion formation at pH 8.0 followed by 7.0 pH. But A. *vesica* and *P.americana* were found effective at 7.0 pH than 8.0 pH . The antiviral activity of *B. spectabilis*, *M.jalapa, H. cupanioides, P. americana* and *A. vesica* was reduced at acidic pH levels (4.0 to 6.0 pH). The inhibition of local lesions at 8.0 pH was 90.16, 75.75 and 82.57 per cent by *M. jalapa, H. cupanioides* and *B. spectabilis* respectively.

4.6 Persistence of antiviral activity of AVPs against Tospovirus

The persistence of antiviral activity of *B. spectabilis, M. jalapa, H. cupanioides, P. americana, A. vesica,* Bion (200ppm) and *P. chlororaphis* was studied on Avinash-2 tomato hybrid and the results are presented in Table 7. Application of *M. jalapa, H. cupanioides* and Bion (200ppm), 24h prior to grafting with Tospovirus infected tomato scion showed 100 per cent decrease of wilting on 8 days after grafting. Antiviral activity decreased beyond 8 DAG. After 14 days of grafting *M. jalapa* and *H. cupanioides* treated plants recorded 86.84 per cent, and 76.55 per cent reduction of wilt incidence by Tospovirus respectively. The antiviral activity of the AVP's declined after 14 DAG, indicating that antiviral effect started to decline after 14 days of grafting.

4.7 Longevity *in vitro* of the antiviral activity of AVPs

The effect of storage on the selected AVPs at room temperature on the antiviral activity against Tospovirus was studied. The results (Table 8) showed that the antiviral activity of *M. jalapa* and *H. cupanioides* were retained upto 75 days after storage. The inhibition of local lesions by *M. jalapa* and *H. cupanioides* after 15 days of storage was 95.65 and 83.33 per cent respectively. But after 75 days of

Table 6. Effect of pH on the antiviral activity of AVPs against Tospovirus on cowpea (*cv.* C152)

Treatment	Number of local lesions in different pH*					
	4 pH	5 pH	6 pH	7 pH	8 pH	Mean
B. spectabilis (L)	20.00^{c} (50.00)	25.67^{c} (34.73)	13.00^{d} (69.05)	9.00^{bc} (78.57)	7.67^{d} 82.57)	15.07
M. jalapa (R)	11.67^{e} (70.82)	15.00^{e} (61.86)	13.33^{d} (68.26)	7.67^{c} (81.74)	4.33^{a} (90.16)	10.40
H. cupanioides (S)	16.33^{d} (59.17)	17.67^{d} (55.07)	14.67^{d} (65.07)	11.00^{b} (73.80)	10.67^{c} (75.75)	14.07
P. americana (L)	21.67^{c} (45.82)	26.00^{c} (33.89)	18.33^{c} (56.36)	10.67^{b} (74.59)	13.00^{c} (70.45)	17.93
A. vesica (L)	27.67^{b} (30.82)	29.67^{b} (24.56)	22.67^{b} (46.02)	11.00^{b} (73.81)	17.00^{b} (61.37)	21.60
Untreated control	40.00^{a}	39.33^{a}	42.00^{a}	42.00^{a}	44.00^{a}	41.47
Mean	22.89	25.56	20.67	15.22	16.11	20.09

LSD (5%)
Treatment : 2.57
pH : 2.77
Treatment x pH : 2.59
*Values are the mean of three replications
Values in parentheses are percent reduction over control
In a column, means followed by common letters do not differ significantly for the interaction effect of 5% level by DMRT.
L – Leaf, S – Seed; R – Root.

Table 7. Persistence of antiviral activity of antiviral principles against Tospovirus on Tomato (Avinash-2)

Treatment	Per cent infection*			
	8 DAG	% decrease over control	14 DAG	% decrease over control
B. spectabilis	13.01[ab] (21.14)	(83.88)	58.21[b] (49.73)	41.11
M. jalapa	0.0 (0.0)	100	13.01[a] (21.14)	86.84
H. cupanioides	0.0 (0.0)	100	23.18[ab] (28.78)	76.55
P. americana	23.18[b] (28.78)	71.27	60.14[b] (50.85)	39.16
A. vesica	4.53[a] (12.29)	94.38	26.52[ab] (31.00)	73.17
Bion (200 ppm)	0.0 (0.0)	100	23.18[ab] (28.78)	76.55
P. chlororaphis	13.01[ab] (21.14)	83.87	29.67[ab] (33.00)	69.98
Control	80.69[c] (63.93)	-	98.85[c] (83.85)	-
LSD (5%)	16.03		16.77	

- Values in parentheses are arcsine transformed values
- T2 : T3 : T6 are excluded from the analysis.
- DAG : Days after grafting
- In a column means followed by the same letters do not differ significantly at 5% level by DMRT.

Table 8. Longevity *in vitro* of antiviral principles against Tospovirus on cowpea *cv.* C152

Treatment	Number of local lesions in different days after storage **					
	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Mean
B. spectabilis (L)	6.00^{c} (86.96)	7.33^{c} (85.04)	8.67^{d} (83.54)	20.67^{c} (63.10)	32.00^{c} (44.44)	14.93
M. jalapa (R)	2.00^{d} (95.65)	3.00^{d} (93.88)	4.33^{e} (91.78)	6.33^{e} (88.70)	11.00^{f} (80.90)	5.33
H. cupanioides (S)	7.67^{c} (83.33)	13.00^{b} (73.47)	13.33^{c} (74.69)	15.33^{d} (72.62)	18.3^{e} (68.22)	13.53
P. americana (L)	11.67^{b} (74.63)	13.33^{b} (72.79)	17.00^{b} (67.72)	30.00^{b} (46.43)	43.3^{b} (24.83)	23.00
A. vesica (L)	7.67^{c} (83.33)	10.00^{bc} (79.59)	14.00^{bc} (73.42)	21.33^{c} (61.91)	24.3^{d} (57.81)	15.40
Control	46.00^{a}	49.00^{a}	52.67^{a}	56.00^{a}	57.6^{a}	52.20
Mean	13.50	15.94	18.33	24.94	31.11	

LSD (5%)
Treatment : 3.07
Fractions : 3.29
Interaction : 3.40

Values (mean of 3 replications) in parentheses are per cent decrease over control.

In a column, means followed by common letter do not differ significantly for interaction effect at 5% level by DMRT.

DAS : Days after storage; L – Leaf; S – Seed; R – Root.

storage the per cent inhibition of local lesions by *M. jalapa* and *H. cupanioides* was 80.90 and 68.22 per cent respectively.

4.8 Effect of AVPs extracted with different organic solvents against Tospovirus

The antiviral effect of selected AVPs as mentioned in 4.7 were extracted in different organic solvents *viz.*, ethanol, acetone, petroleum ether, diethyl ether and chloroform and assessed for their efficacy on the local lesion host. Among the various extracts tested (Table 9; Fig 2), acetone extract of *M. jalapa, H. cupanioides and B. spectabilis* recorded 100.00, 91.13 and 82.91 per cent inhibition of local lesions respectively. It was followed by ethanol extraction. The number of local lesions was increased in all the AVPs extracted in diethyl ether and petroleum ether except in *B. spectabilis* where the number of local lesions was found to be more in ethanol extract treatment (Fig 1).

4.9 Protein profile of *M. jalapa, H. cupanioides* and *A. vesica*

Results of protein profile revealed that *M. jalapa* consists 91, 74, 41, 24, 29 and 17kDa proteins. *H. cupanioides* consists of 97, 68, 41, 29, 20 and 17kDa proteins. *A. vesica* contains 68, 43, 42, 40, 38, 33, 31, 22 and 12kDa proteins (Plate 9,10). Protein profile of the eluted fractions (F2 and F3) of *M. jalapa* effective against Tospovirus consists 41, 29, 22.5 and 17kDa protein (Plate 9a). Similarly the protein profile of the fractions F4 and F5 of *H. cupanioides* consists 68, 55.5 and 29kDa proteins with antiviral activity against Tospovirus. But fraction F2 consists only 55.5kDa protein with minimum antiviral activity (Plate 10a).

4.10 Iso Electric Focusing (IEF)

The *pI* value of the purified antiviral proteins of *H. cupanioides* and *M. jalapa* estimated through IEF using an Ampholine PAG plate (pH 3.5-9.5 Pharmacia) revealed that 68, 55.5 and 29kDa proteins of *H. cupanioides* and 41,

Table 9. Effect of different organic solvent extracts of AVPs on the inhibition of Tospovirus on cowpea *cv.* C152.

Treatment	Number of local lesions in different extractions **				
	Ethanol	Acetone	Petroleum ether	Diethyl ether	Mean
B. spectabilis (L)	16.67[b] (66.88)	9.00[d] (82.91)	10.67[c] (79.74)	11.00[c] (77.55)	11.83
M. jalapa (R)	7.33[d] (85.44)	0.00[f] (100.00)	11.67[c] (77.84)	12.33[c] (74.84)	7.86
H. cupanioides (S)	11.00[c] (78.14)	4.67[e] (91.13)	16.00[b] (69.62)	16.67[b] (65.98)	12.08
P. americana (L)	14.00[b] (72.18)	15.67[b] (70.25)	16.67[b] (68.35)	18.00[b] (63.26)	16.08
A. vesica (L)	10.00[cd] (80.13)	12.33[c] (76.59)	15.00[b] (71.52)	17.67[b] (63.94)	13.75
Control	50.33[a]	52.67[a]	52.67[a]	49.00[a]	51.17
Mean	18.22	15.74	20.44	20.78	18.80

LSD (5%)
Treatment : 2.57
Fractions : 3.18
Interaction : 2.86

Values (mean of 3 replications) in parentheses are per cent decrease over control.

In a column, means followed by common letter do not differ significantly for interaction effect at 5% level by DMRT.

DAS : Days after storage; L–Leaf; S–Seed; R–Root.

Fig 1. Effect of different organic solvent fractions of AVP on the number of local lesions of TSWV

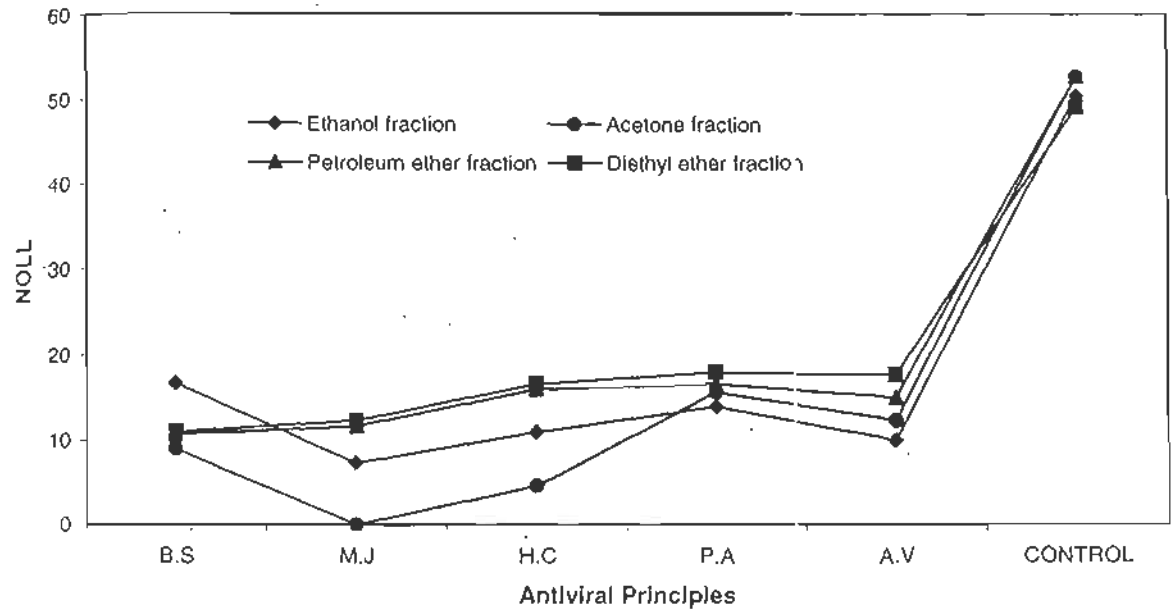
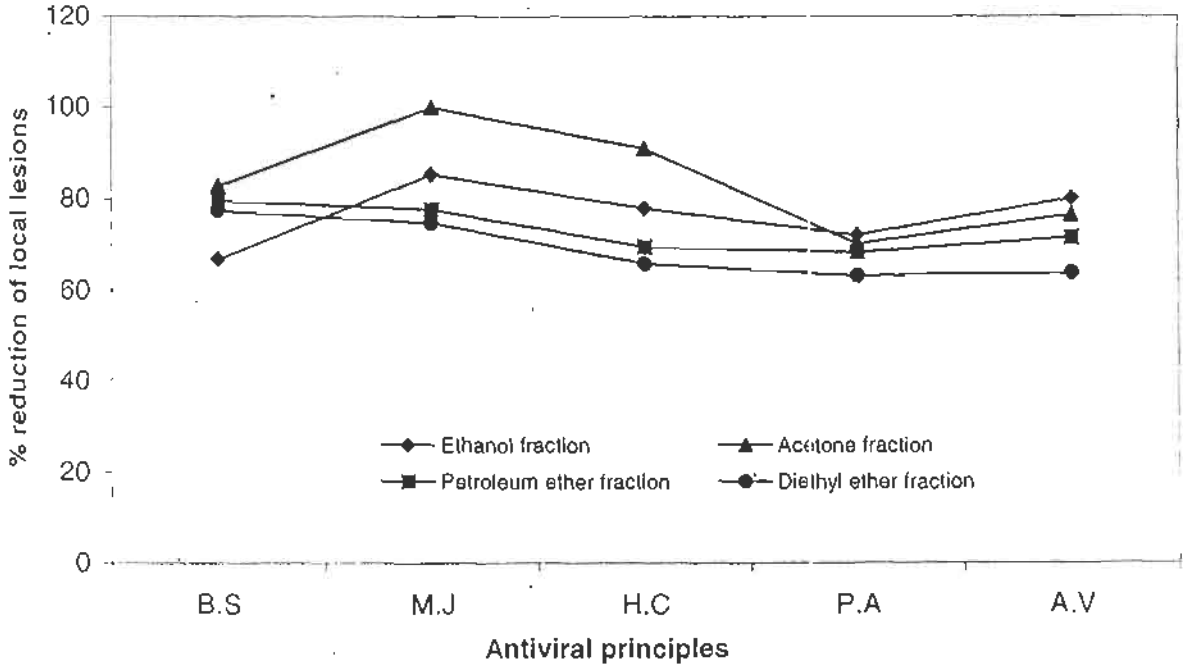
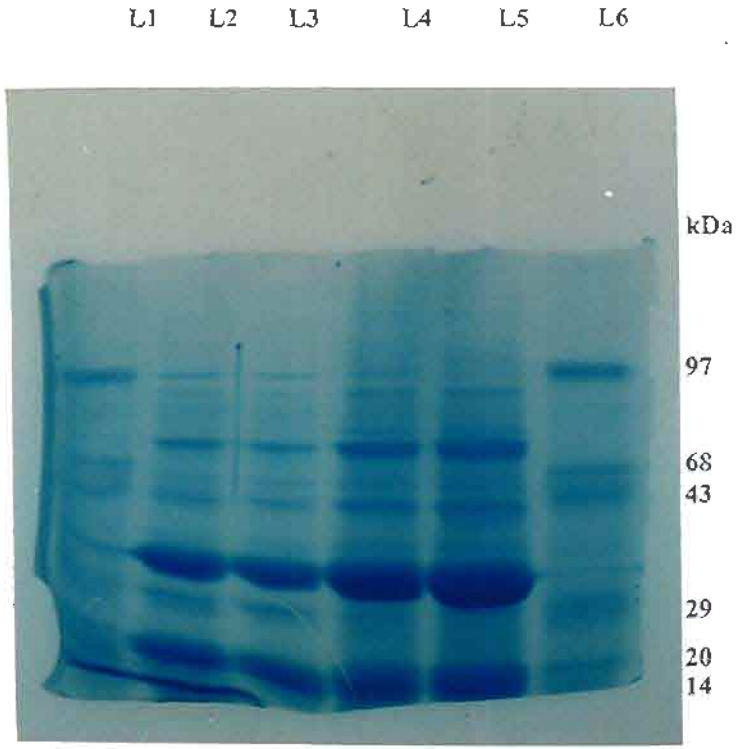


Fig 2. Efficacy of different organic solvent fractions of antiviral principles on the inhibition of local lesions of TSWV on Cowpea



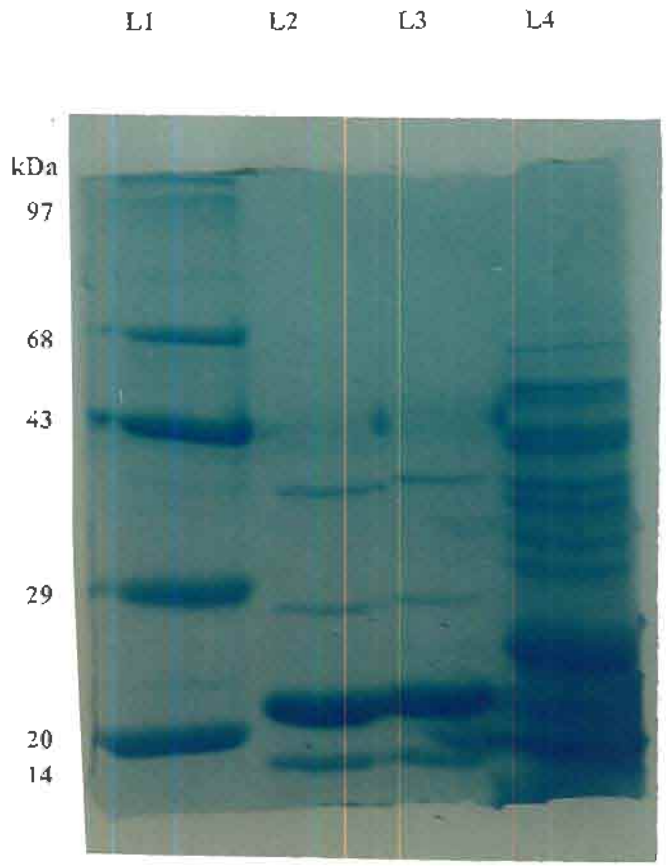
B.S : *B. spectabilis*; M.J: *M. jalapa;* H.C: *H. cupanioides* ; P.A: *P. americana*; A.V: *A. vesica*

Plate 9. Protein profile of *Mirabilis jalapa*



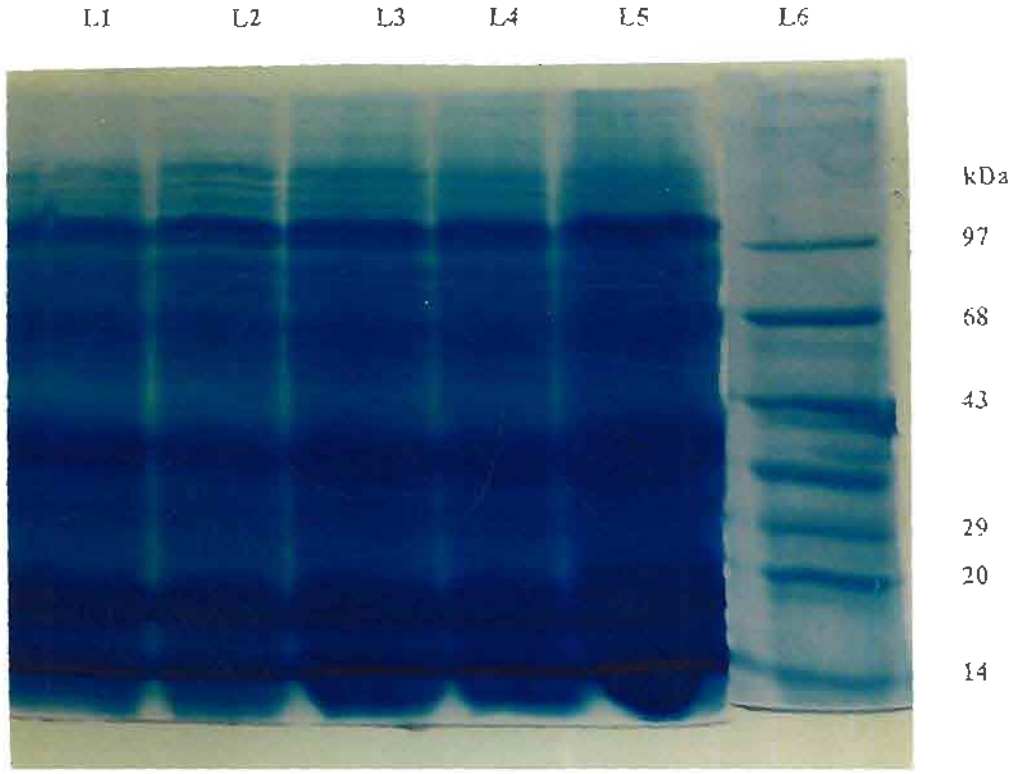
L1 - Marker; L2, L3 - Profile at 60% saturation
L4, L5 - Profile at 80% saturation; L6 - Marker

Plate 9a. Protein profile of eluted fractions of *M. jalapa* and Protein profile of *A. vesica*



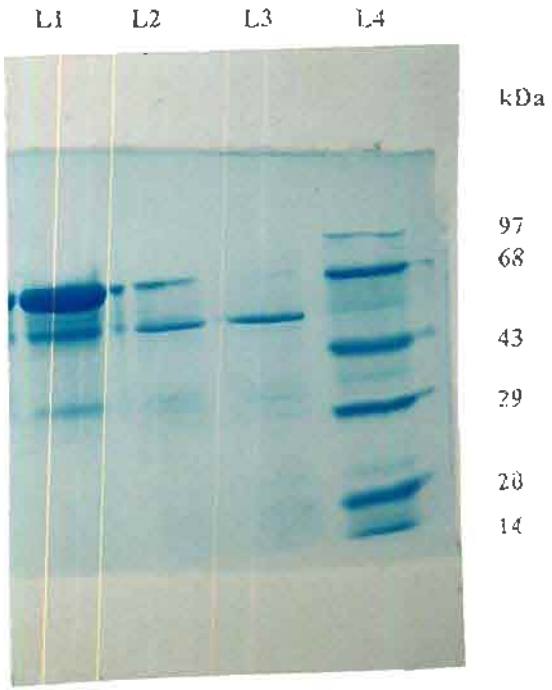
L1:Marker
L2:Fraction 2
L3:Fraction 3
L4 - Profile of *A. vesica* at 80% saturation

Plate 10. Protein profile of *Harpulia cupanioides*



L1 – L3:- Profile at 60% saturation
L4, L5 - Profile at 80% saturation; L6 - Marker

Plate 10a. Protein profile of eluted fractions of *Harpulia cupanioides*



L1: Fraction 4; L2:Fraction 5
L3:Fraction 2; L4 - Marker

29 kDa proteins of *M. jalapa* were basic proteins with pI values ranging from 8.4 – 8.6. But 22 and 17kDa proteins of *M. jalapa* were strongly basic in nature with *pI* values of more than 9.5 (Plate 11).

4.11 Western blot analysis of *M. jalapa and H. cupanioides.*

Western blot analysis of *M. jalapa* and *H. cupanioides* proteins revealed that the presence of 41kDa protein cross reacted with barley chitinase antiserum (Plate12). Antiserum of ribosome inactivating protein (RIP) of *M. expansa* cross reacted with 29kDa protein of *M. jalapa* and *H. cupanioides* (Plate 13).

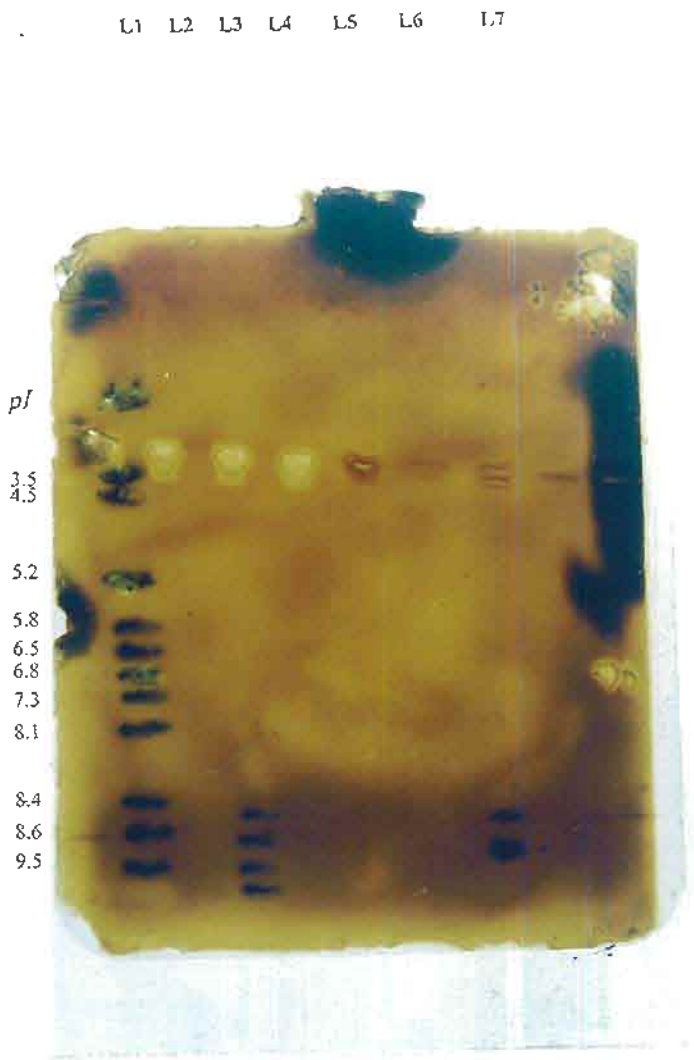
4.12 Thin layer chromatography (TLC) analysis for terpenoids in *H. cupanioides*

TLC analysis confirmed the presence of terpenoids similar to isomelidinin and desacetyl nimbin with Rf values of 0.527 and 0.4545 respectively (Plate 14).

4.13 Effect of purified antiviral proteins against Tospovirus

The proteins from effective non-host plants such as *M. jalapa* (Root), *H. cupanioides* (Seed) and *A. vesica* (leaf) were precipitated by saturated ammonium sulphate method and were assessed for their antiviral efficacy at 40, 60 and 80 per cent saturation. The antiviral protein from *M. jalapa*, *H. cupanioides* and *A. vesica* were designated as MAP, HAP and AAP proteins respectively.The efficacy of MAP, HAP and AAP proteins obtained from different levels of saturation revealed that (Table 10) the above proteins at 60 and 80per cent saturation were highly effective in inhibiting Tospovirus infection compared to 40 per cent saturation. The purified proteins at 40% level were even inferior to crude extracts. MAP, HAP and AAP at 60 per cent saturation recorded 98.17, 91.57 and 83.87 per cent decrease in the number of local lesions respectively over control. There was no significant difference between antiviral proteins of MAP, HAP and AAP obtained at 80% saturation level (Plate 15,16).

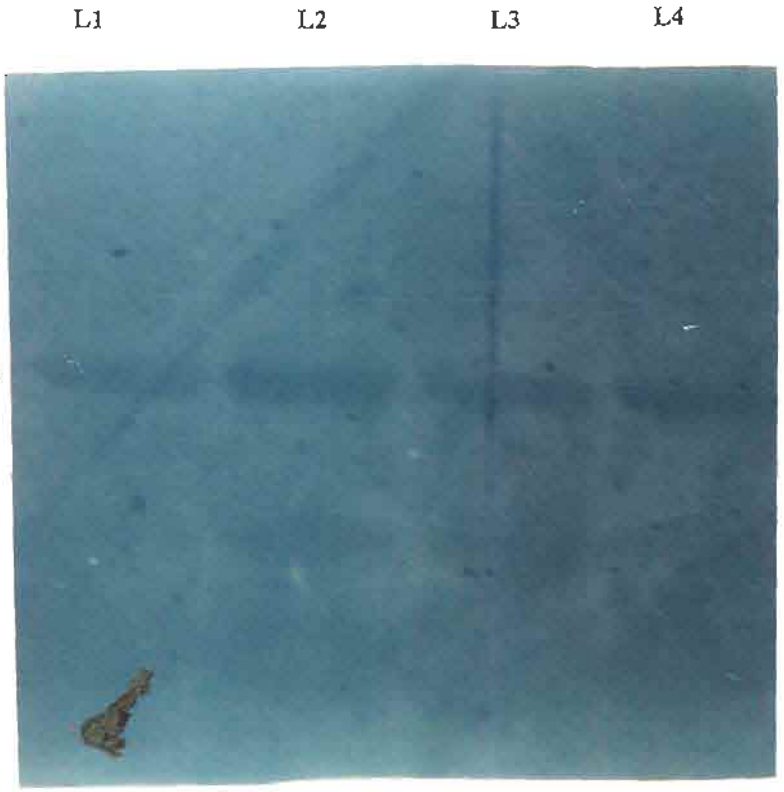
Plate 11. Iso electric focusing of antiviral proteins of *H. cupanioides* and *M. jalapa*



L1 – Marker; L3 – *M. Jalapa*; L7 – *H. Cupanioides*

Western blot analysis of chitinase and RIP in *M. jalapa* and *H. cupanioides*

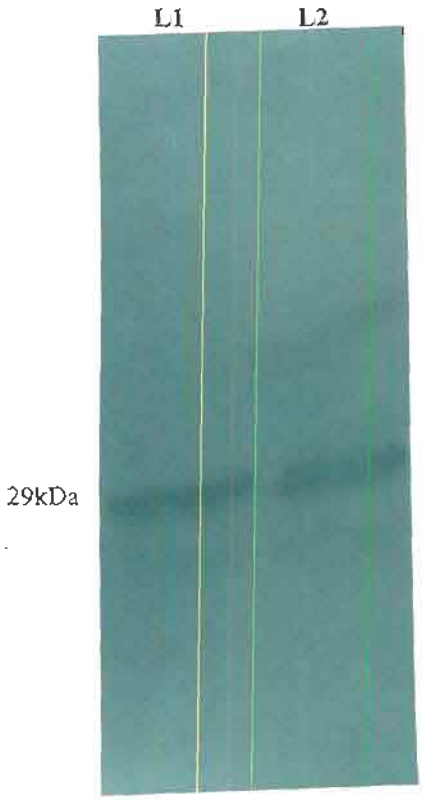
Plate 12. Expression of chitinase



L1, L2 : *M. jalapa*

L3, L4 : *H. cupanioides*

Plate 13. Expression of RIP



L1 : *M. jalapa*

L2 : *H. cupanioides*

Plate 14. TLC analysis of terpenoids in *H. cupanioides*

L1: Salanin

L2: Nimbin

L3; Isomeldinin

L4: *H. cupanioides*

L5: Desacetyl nimbin

L6: Switenine

Table 10. Effect of purified antiviral proteins against Tospovirus on cowpea *cv.* C152

Pre inoculation of anti viral proteins **	Number of local lesions*					
	MAP	PDC	HAP	PDC	AAP	PDC
40%	22.0[c]	59.71	21.00[c]	64.59	24.0[c]	61.29
60%	1.00[a]	98.17	5.00[a]	91.57	10.0[a]	83.87
80%	1.00[a]	98.17	3.3[a]	94.43	7.0[a]	88.71
Crude extract	6.00[b]	89.90	14.3[b]	75.88	16.3[b]	73.71
Sterile water	52.60[d]	3.66	58.0[e]	2.19	52.0[e]	16.13
Control	54.60[d]	-	59.3[e]	-	62.0[f]	-
LSD (5%)	4.026	-	3.46	-	3.33	-

- Values are the mean of three replications
- Proteins purified at different levels of saturation with ammonium sulphate
- PDC : Per cent decrease over control
- In a column means followed by the common letter do not differ significantly at 5% level by DMRT
- MAP - *Mirabilis* antiviral protein; HAP- *Harpullia* anti viral protein; AAP - *Adhatoda* antiviral protein.

Plate 15. Effect of MAP purified at 80% saturation against tospovirus on local lesion host

Plate 16. Effect of HAP purified at 80% saturation against tospovirus on local lesion host

4.14 Effect of different concentration of MAP and HAP against Tospovirus

MAP and HAP proteins obtained at 80 per cent $(NH_4)_2 SO_4$ saturation were tested at various concentrations ranging from 25, 50, 100, 200, 400, 600 and 800 µg/ ml (Bradford method). Total protein of *Mirabilis* root extract , *Harpullia* seed extract and *A. vesica* per 50 g of the material was 104.16 ,152.26 and 98.32 mg respectively. Total proteins of both the AVPs were used to compare the antiviral efficacy of purified proteins at different concentrations by applying on local lesion host. MAP inhibited lesion formation by 98.95 per cent at a concentration of 800 µg/ml. But the efficacy was similar at 400 and 600 µg/ml of MAP and total protein at 80 per cent saturation, indicating that a minimum concentration of 400 µg/ml of MAP is sufficient to inhibit the infection of Tospovirus (Table 11). HAP at 800 µg/ml recorded 98.41 per cent inhibition of lesion formation as against 95.77 per cent reduction at 600 µg/ml of HAP.

4.15 Antiviral efficacy of MAP and HAP protein fractions against Tospovirus

The MAP and HAP proteins were purified by DEAE ion exchange chromatography (Fig 3 & 4). The purified fractions were tested for their antiviral action by applying on local lesion host. The results (Table 12) revealed that fraction 2 (F2) of MAP inhibited Tospovirus infection to a tune of 92.42 per cent. The per cent inhibition of Tospovirus was lowest in F7 (6.06%). With respect to HAP, F4 and F5 fractions recorded 84.83 and 76.96 per cent reduction of Tospovirus infection respectively over control. Rest of the fractions was not effective against Tospovirus.

4.16 Induction of proteins

Studies on the induction of proteins on the local lesion host treated with AVPs 24h prior to challenge inoculation of Tospovirus revealed the presence of

Table 11. Antiviral action of purified antiviral protein of different concentrations against Tospovirus on cowpea *cv.* C152

Test sample concentration (µg/ml)	Pre inoculation		Pre inoculation	
	MAP	PDC	HAP	PDC
Total protein * at 80% saturation	0.61[a] (1.05)	98.95	0.89[a] (1.18)	98.37
25	33.25[e] (5.81)	42.66	45.29[h] (6.76)	17.10
50	23.57[d] (4.91)	59.35	33.98[g] (5.87)	54.63
100	8.98[c] (3.08)	84.51	22.60[f] (4.80)	58.63
200	5.32[b] (2.41)	90.83	11.94[d] (3.52)	78.14
400	1.64[a] (1.46)	97.17	8.64[c] (3.02)	84.18
600	1.31[a] (1.34)	97.74	2.31[b] (1.67)	95.77
800	0.61[a] (1.05)	98.95	0.87[a] (1.17)	98.41
Crude extract **	8.98[c] (3.08)	84.51	16.65[e] (4.14)	69.52
Control	57.99[f] (7.65)		54.63[i] (7.42)	
LSD (5%	0.56		0.41	

PDC – Per cent decrease over control
Values are the mean of three replications
**180.33 mg/50g of *Mirabilis* root extract
**246.42 mg/50g of *Harpullia* seed extract
*104.16 mg/50g of *Mirabilis* root extract at 80% saturation
*152.26 mg/50g of *Harpullia* seed extract at 80% saturation
In a column, mean followed by common letter do not differ significantly at 5% level by DMRT.

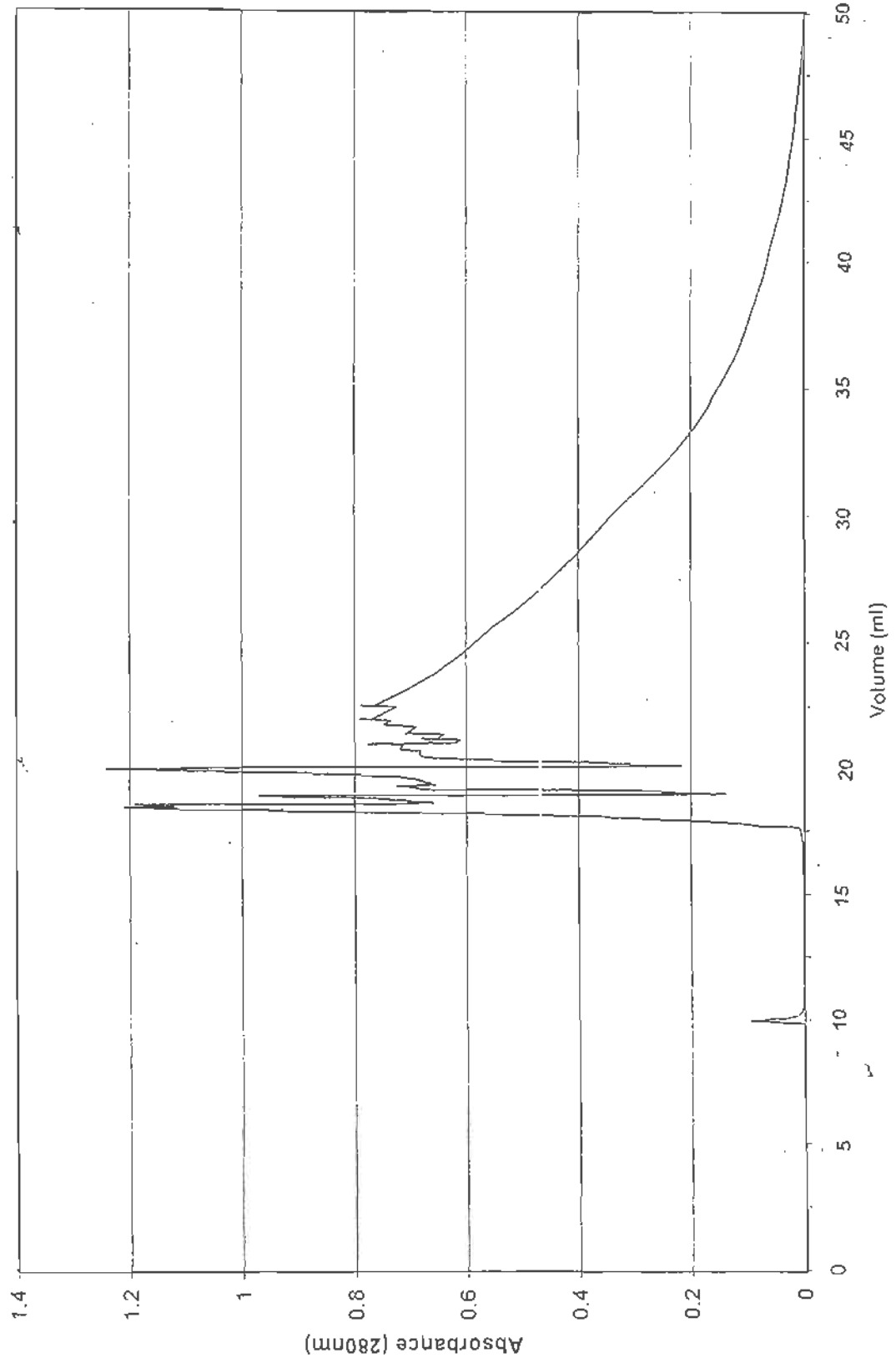


Fig3: ELUTION OF ANTI VIRAL PROTEIN FROM HARPULIA CUPANIOIDES (80%)

Fig4: ELUTION OF ANTI VIRAL PROTEIN FROM MIRABILIS JALAPA (80%)

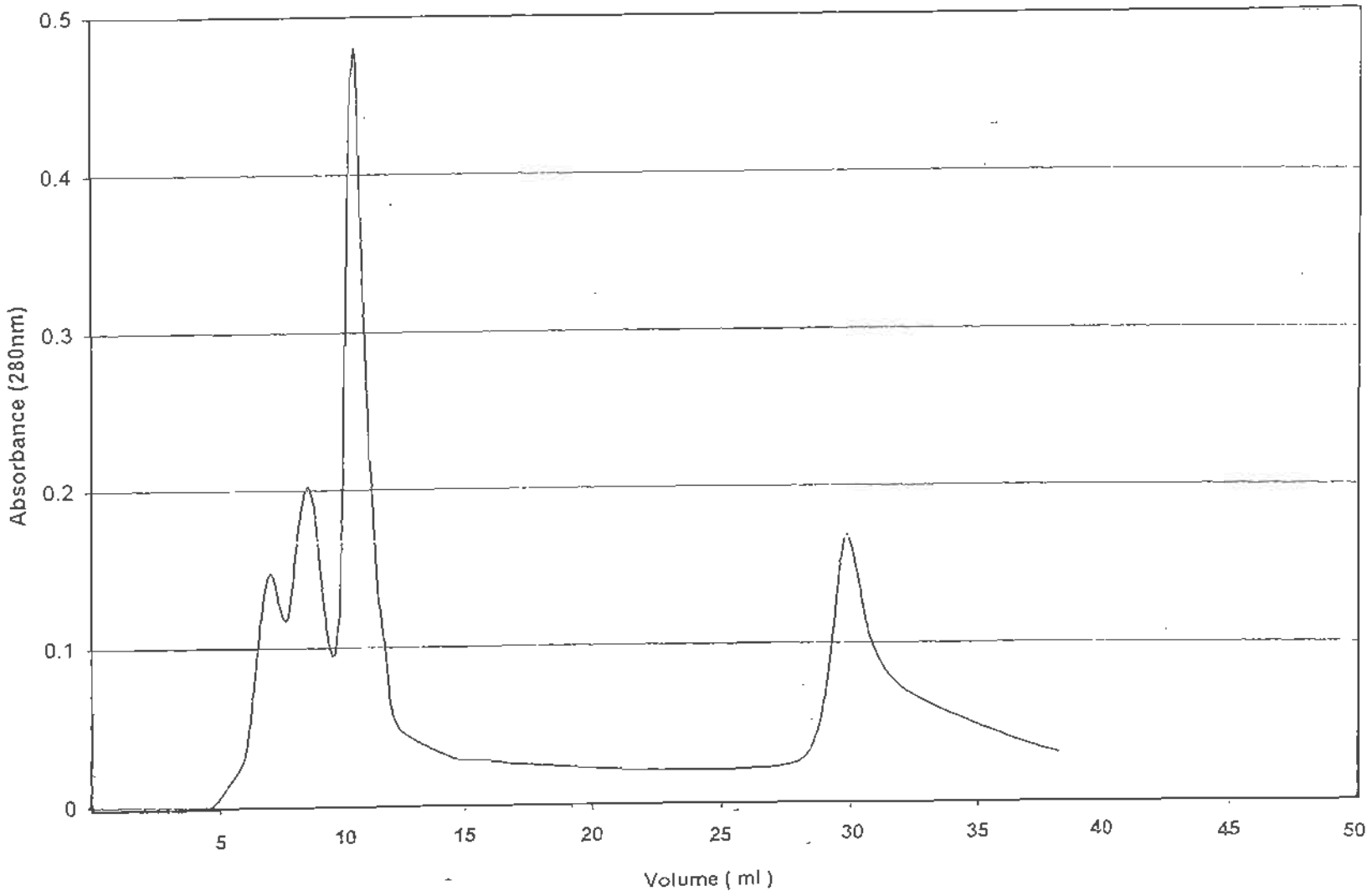


Table 12. Effect of eluted antiviral protein fractions of MAP and HAP at 80% saturation against Tospovirus on cowpea (*cv.* C152)

Protein fractions	Number of local lesions*			
	Pre inoculation		Pre inoculation	
	MAP**	PDC	HAP**	PDC
F1	52.00^{e}	21.21	50.00^{bc}	15.72
F2	5.00^{a}	92.42	49.33^{bc}	16.85
F3	9.00^{b}	86.36	44.67^{b}	24.71
F4	40.00^{c}	39.39	9.00^{a}	84.83
F5	48.00^{d}	27.27	13.67^{a}	76.96
F6	56.00^{f}	15.15	55.33^{cde}	6.74
F7	62.00^{g}	6.06	53.33^{cd}	10.11
F8	40.00^{c}	39.39	60.00^{e}	1.13***
F9	42.00^{c}	36.36	59.33^{de}	0.00
F10	48.00^{d}	27.27	46.00^{b}	22.47
Control	66.00^{h}	-	59.33^{de}	-
LSD (5%)	3.39		5.85	

*Values are the mean of three replications

** Protein fractions (MAP, HAP) were sprayed 24h prior to virus inoculation

***Per cent increase over control

In a column mean followed by a common letter do not differ significantly at the 5% level by DMRT.

41kDa protein in all the treatments. But the expression of 41k Da was more in all the AVP treatments compared to virus alone-inoculated control. The comparative analysis of the same protein between the AVPs treatment revealed that the expression was more in *M. jalapa*, followed by *H. cupanioides, A. vesica, B. spectabilis* and *P. americana*. In addition high molecular weight proteins were also induced in all the AVPs treatment, compared to virus-inoculated control (Plate 17).

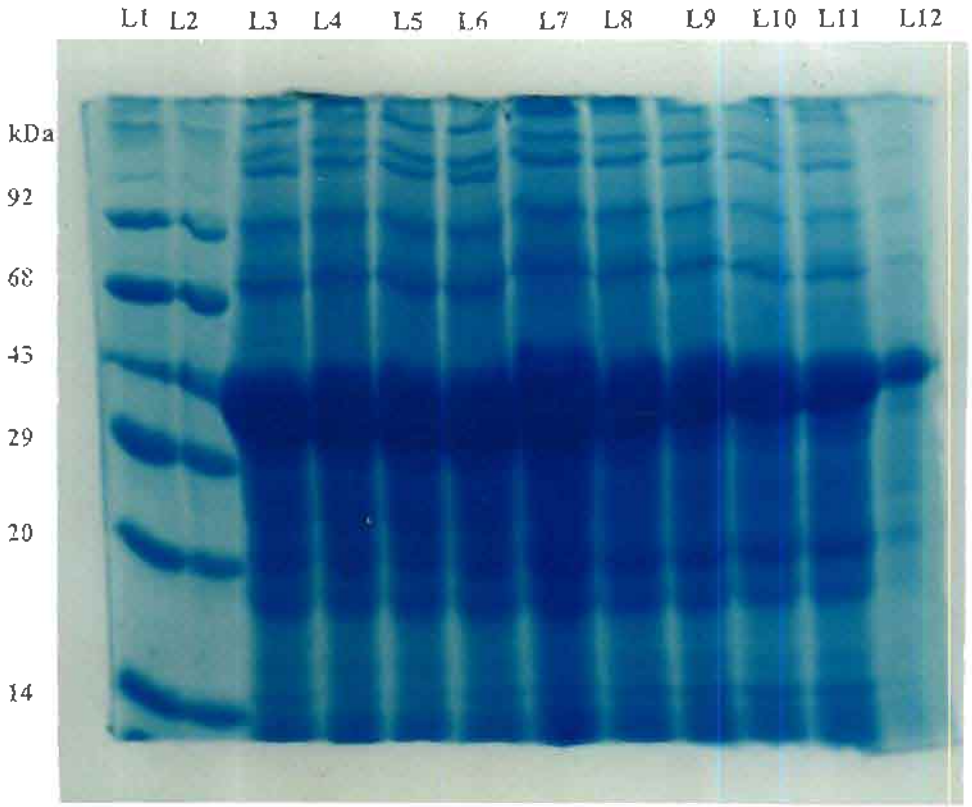
Since *M. jalapa* showed high level of protein induction, its ability to induce protein in local lesion host and tomato (Avinash 2) were studied at pre, post and simultaneous inoculation of Tospovirus after four days of virus inoculation. Pre inoculation of *M. jalapa* challenged with Tospovirus in local lesion host induced higher level of 41kDa protein than post and simultaneous inoculation. Similar level of induction of 41kDa protein was also observed in tomato after four days of challenge inoculation with Tospovirus (Plate 18 &1 9).

4.17 Induction of peroxidase (PO) activity against Tospovirus

Studies on the mode of action of antiviral principles and chemicals against Tospovirus infection revealed that they induced defense mechanisms in plants challenged with Tospovirus. Accumulation of PO was observed from first day after challenge inoculation with Tospovirus on cowpea. Though peroxidase was observed in healthy, virus infected, AVP and Bion treated plants without challenge inoculation, no significant difference in the induction of PO was observed over a period of time.

Among the AVPs tested, AVP from *M. jalapa* induced hundred per cent increase in PO activity compared with *H. cupanioides* treatment after 24h of inoculation. There was a steady increase upto 3 days after challenge inoculation and declined there after from 4th day. The induction of PO activity was greater in Bion treated plants challenged with Tospovirus than *M. jalapa*. The PO activity on 3rd DAI was 7.10 absorbance at 420nm as against 1.48 in plants treated with Tospovirus alone, which was accounted for 379 per cent increase over virus

Plate 17. Induction of proteins on local lesion host by AVPs challenged with tospovirus

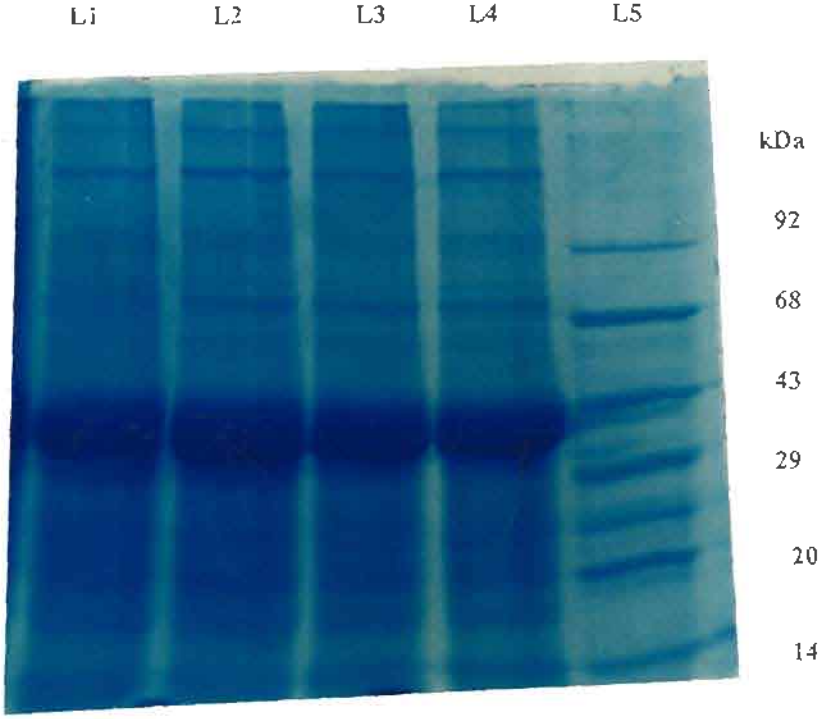


L1, L2: Marker ; L3: *P. americanum* ; L4: *B. spectabilis;* L5: *H. cupanioides*

L6: *A. vesica;* L7: *M. jalapa;* L8: *Punica granatum*; L9: *B. diffusa*

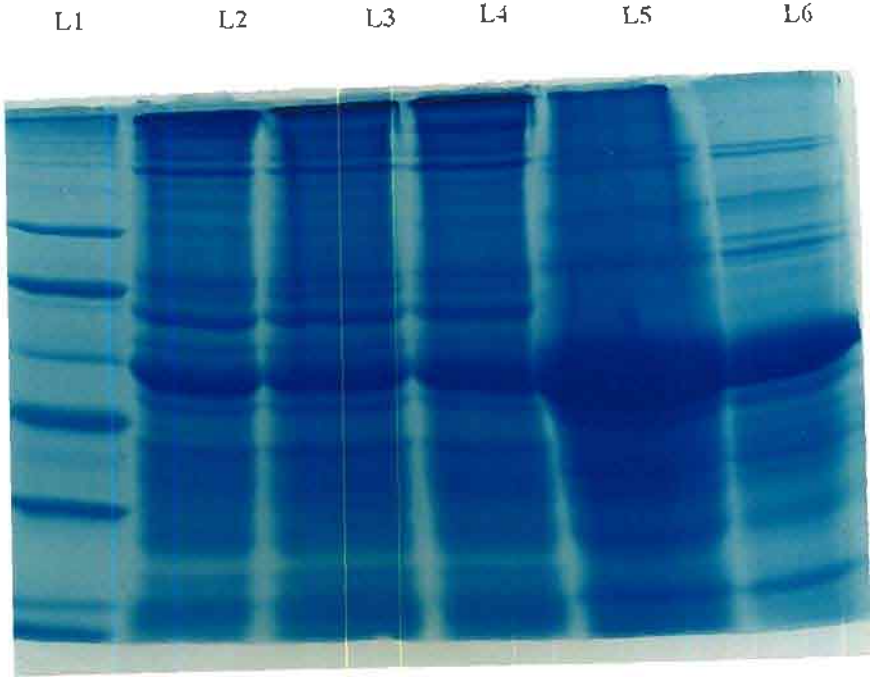
L10: *C. nucifera;* L11: *S. vulgare;* L12: *TSWV alone*

Plate 18. Induction of proteins on local lesion host by *M. Jalapa* challenged with tospovirus



L1: Simultaneous inoculation of *M. jalapa*; L2: Pre inoculation of *M. jalapa*
L3: Virus inoculated control; L4: Healthy control; L5: Protein marker

Plate 19. Induction of proteins on tomato by *M. Jalapa* challenged with tospovirus



L1: Marker; L2: Healthy control; L3: Virus inoculated control
L4: Post inoculation of *M. jalapa*; L5: Pre inoculation of *M. jalapa*
L6: Simultaneous inoculation of *M. jalapa*

inoculated control. It was followed by *M. jalapa, H. cupanioides* and *A. vesica* challenged with Tospovirus (Table 13).

Similarly, induction of PO activity was also observed in Avinash - 2 tomato hybrid treated with *M. jalapa* and Bion challenge inoculated with tomato spotted wilt virus through grafting. Increased activity was observed from four days after grafting (DAG) to 12 DAG and declined after 16 days. PO activity was 7.55 absorbance at 420 nm on 16 DAG and was only 2.65 in virus treated control. *M. jalapa* and Bion were followed by *H. cupanioides* and *A. vesica* challenged with TSWV (Table 14 ; Fig 5, 6).

4.18 Induction of polyphenol oxidase (PPO) against Tospovirus

The increase in PPO in local lesion host was observed one day after challenge inoculation (DACI) of virus. The activity of this enzyme was increased upto 3 DACI, and slowly declined from 4 DACI except in *A. vesica*. The activity was increased by several fold compared with healthy and virus inoculated control. Among the AVPs, *M. jalapa* treatment challenged with Tospovirus recorded the maximum absorbance of 2.95 on 1DACI and it increased up to 4.95 on 3 DACI and declined there after. It was not found to differ significantly from bion challenged with Tospovirus. Above treatments showed multifold increase of PPO activity, than healthy and virus-inoculated control, which recorded an absorbance value of 1.17, 1.50 on first DACI and 1.20, 1.50 on 3DACI respectively. But significant difference was not observed in the PPO activity between AVPs, Bion (without challenge inoculation), virus inoculated and healthy control over a period of time (Table 15).

Studies on the induction of PPO (Table 16) in tomato (Avinash-2) after challenge inoculation with Tospovirus through grafting revealed that the induction of PPO was observed from 4 DAG. The activity of PPO in *M. jalapa* was 3.70 on 4 DAG and increased upto 5.80 on 12 DAG and declined later. It was not found to differ significantly from Bion treatment challenged with Tospovirus. It was followed

Table 13. Induction of peroxidase in cowpea (*cv.* C152) against Tospovirus

Treatment	Peroxidase activity*				
	1 DAI	2 DAI	3 DAI	4 DAI	Mean
M. jalapa	2.30^{d}	2.33^{e}	2.41^{e}	2.36^{d}	2.35
M. jalapa + Virus	4.34^{a}	5.11^{b}	6.74^{b}	6.50^{a}	5.67
H. cupanioides	1.87^{e}	2.08^{f}	2.00^{f}	1.90^{e}	1.96
H. cupaniodes + virus	4.01^{b}	4.62^{c}	5.37^{c}	5.04^{b}	4.76
Bion	2.59^{c}	2.50^{e}	2.54^{e}	2.54^{d}	2.55
Bion + virus	4.53^{a}	5.78^{a}	7.10^{a}	6.70^{a}	6.03
A. vesica	1.52^{f}	1.63^{g}	1.78^{f}	1.68^{e}	1.65
A. vesica + virus	2.75^{c}	3.57^{d}	4.28^{d}	3.95^{c}	3.64
Healthy	1.43^{f}	1.53^{g}	1.44^{g}	1.44^{f}	1.46
Virus alone	1.60^{f}	1.56^{g}	1.48^{g}	1.32^{f}	1.49
Mean	2.70	3.07	3.51	3.34	3.16

LSD (5%)
Treatment : 0.24
DAI : 0.28
Interaction: 0.22

*Change in absorbance A420 min^{-1} g^{-1} fresh tissue

- DAI : Days after inoculation

In a column, means followed by common letter do not differ significantly at the 5% level by DMRT.

Fig.5 : Induction of peroxidase against TSWV in cowpea

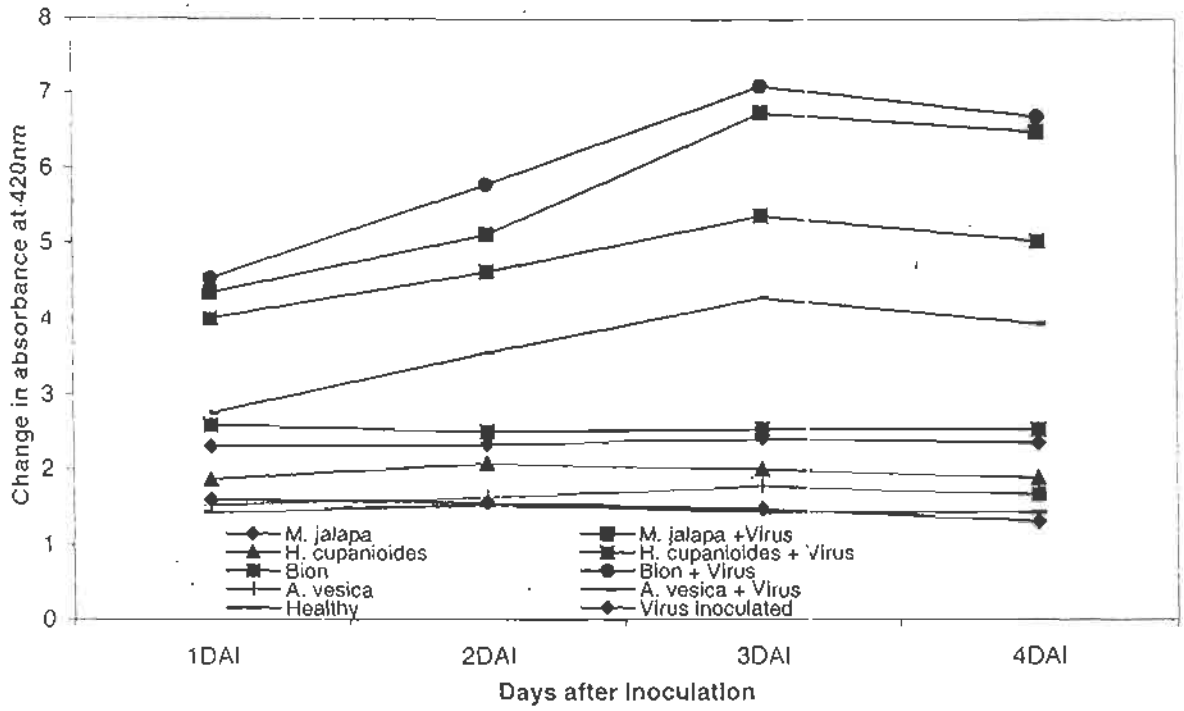


Fig 6 : Induction of peroxidase against TSWV in tomato

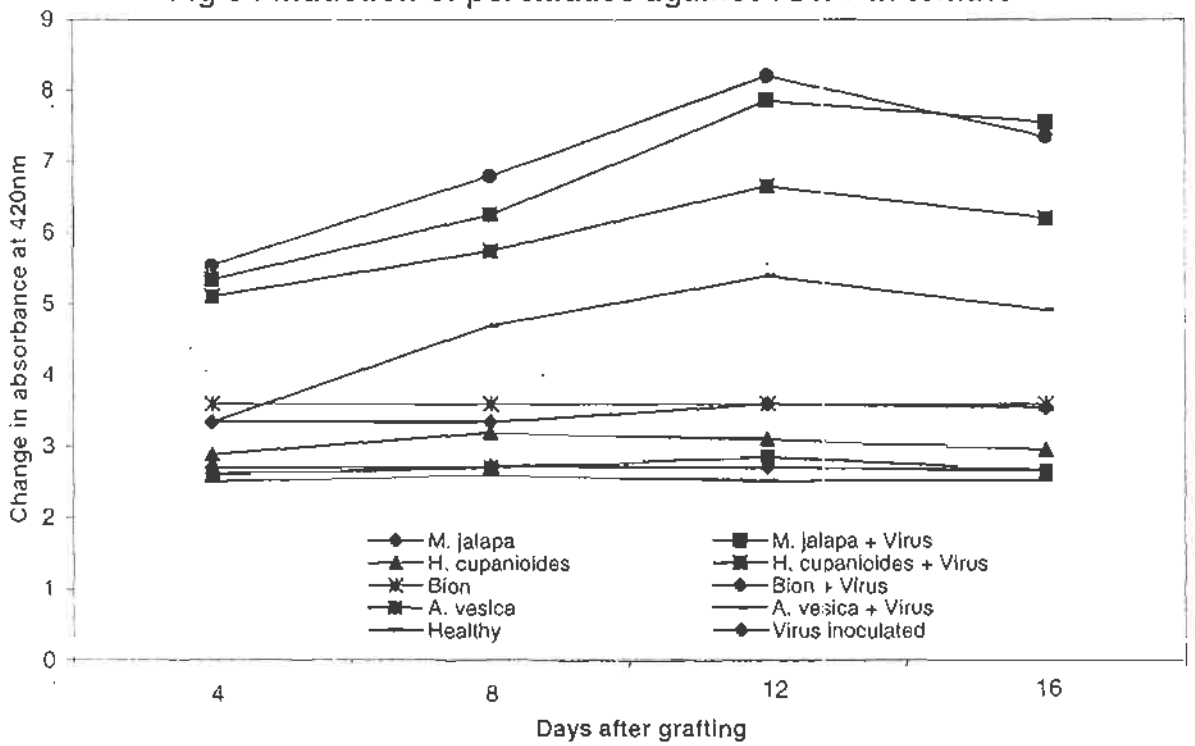


Table 14:- Induction of peroxidase in tomato (Avinash - 2) against Tospovirus infection

Treatments	Peroxidase activity *				
	4 DAG	8 DAG	12 DAG	16 DAG	Mean
M. jalapa	3.35^{c}	3.35^{ef}	3.60^{e}	3.55^{d}	3.46
M. jalapa + virus	5.35^{ab}	6.25^{b}	7.85^{b}	7.55^{a}	6.75
H. cupanioides	2.89^{d}	3.20^{f}	3.10^{f}	2.95^{e}	3.03
H. cupanioides + virus	5.11^{b}	5.75^{c}	6.65^{c}	6.20^{b}	5.95
Bion	3.60^{c}	3.60^{e}	3.60^{e}	3.60^{d}	3.60
Bion + virus	5.55^{a}	6.80^{a}	8.20^{a}	7.35^{a}	6.98
A. vesica	2.60^{de}	2.70^{g}	2.85^{fg}	2.65^{ef}	2.70
A. vesica + *virus*	3.35^{c}	4.70^{d}	5.40^{d}	4.92^{c}	4.59
Healthy	2.50^{e}	2.60^{g}	2.50^{h}	2.53^{f}	2.53
Virus alone	2.70^{de}	2.72^{g}	2.70^{gh}	2.65^{ef}	2.69
Mean	3.70	4.17	4.65	4.40	4.23

LSD (5%)
Treatment : 0.28
DAG : 0.29
Interaction : 0.33
Values are the mean of three replications
DAG : Days after grafting
Change in absorbance A420 min^{-1} g^{-1} fresh time
In a column, means followed by a common letter do not differ signfiicantly at the 5% level by DMRT.

Table 15. Induction of polyphenol oxidase in cowpea (*cv.*C152) against Tospovirus

Treatment	Polyphenol oxidase*				
	1 DAI	2 DAI	3 DAI	4 DAI	Mean
M. jalapa	2.03^{c}	2.28^{d}	2.58^{c}	2.37^{c}	2.32
M. jalapa + virus	2.95^{a}	3.43^{ab}	4.95^{a}	4.66^{a}	4.00
H. cupanioides	1.56^{d}	1.62^{e}	1.62^{d}	1.46^{e}	1.56
H. cupanioides + virus	2.82^{a}	3.24^{b}	3.97^{b}	3.94^{b}	3.49
Bion	2.16^{bc}	2.33^{d}	2.62^{c}	2.47^{c}	2.39
Bion + virus	2.75^{a}	3.64^{a}	5.00^{a}	4.89^{a}	4.07
A. vesica	1.50^{d}	1.77^{e}	1.68^{d}	1.76^{d}	1.68
A. vesica + virus	2.38^{b}	2.83^{c}	3.73^{b}	3.77^{b}	3.18
Healthy	1.17^{e}	1.33^{f}	1.20^{e}	1.27^{e}	1.24
Virus alone	1.50^{d}	1.65^{e}	1.50^{d}	1.80^{d}	1.61
Mean	2.08	2.41	2.89	2.84	2.55

LSD (5%)
Treatment : 0.29
DAI : 0.31
Interaction : 0.28

*Change in absorbance A495 min^{-1} g^{-1} fresh tissue

- DAI : Days after inoculation
- In a column, means followed by common letter do not differ significantly at the 5% level by DMRT.

Table 16. Induction of polyphenol oxidase in tomato (Avinash-2) against Tospovirus infection

Treatment	Polyphenol oxidase*				
	4 DAG	8 DAG	12 DAG	16 DAG	Mean
M. jalapa	3.45^{b}	3.40^{c}	3.80^{c}	2.59^{c}	3.31
M. jalapa + virus	3.70^{AB}	4.55^{AB}	5.80^{A}	4.88^{A}	4.73
H. cupanioxides	2.55^{CD}	2.70^{DE}	2.90^{d}	1.68^{de}	2.46
H. cupanioides + virus	3.90^{a}	4.35^{b}	4.94^{b}	4.10^{b}	4.32
Bion	3.40^{b}	3.40^{c}	3.80^{c}	2.65^{c}	3.31
Bion + virus	3.80^{ab}	4.75^{a}	6.00^{a}	4.90^{a}	4.86
A. vesica	2.30^{d}	2.80^{d}	2.70^{d}	1.95^{d}	2.44
A. vesica + virus	2.90^{c}	3.70^{c}	4.60^{b}	3.95^{b}	3.79
Healthy	2.58^{cd}	2.40^{e}	2.55^{d}	1.45^{e}	2.24
Virus alone	2.85^{c}	2.80^{d}	2.80^{d}	2.05^{d}	2.63
Mean	3.14	3.48	3.99	3.02	3.41

LSD (5%)
Treatment :0.21
- DAG :0.18
- Interaction :0.38

In a column, means followed by common letter do not differ significantly at the 5% level by DMRT.
DAG : Days after grafting

* Change in absorbance A 495 min^{-1} g^{-1} fresh tissue.

by *H. cupanioides*. Healthy and virus-inoculated control recorded an absorbance value of 2.58, 2.85 on fourth DACI and 2.55, 2.80 on 12 DACI respectively.

4.19 Induction of phenylalanine ammonia lyase (PAL) against Tospovirus

Studies on PAL activity in the local lesion host revealed that (Table 17) AVPs and Bion pre treated and challenged with Tospovirus increased the accumulation of PAL than healthy and virus inoculated control. The enzyme activity in the pretreated and virus challenged plants increased from the first day after inoculation and maintained at higher-level upto 4 DACI. While in healthy and virus inoculated control plants the activity was many folds lower than in challenge-inoculated plants. Among the AVPs, pre application of *M. jalapa* challenged with Tospovirus recorded the maximum PAL activity of 43.33 on 1 DACI and increased upto 3 DACI (65.33) followed by a slow decline on 4 DACI. It was not found to differ significantly from pre application of bion challenged with Tospovirus. But the activity of PAL in virus-inoculated control was only 17.33 on 1 DACI and 21.70 on 3 DACI. The induction in higher level of expression of PAL activity in *M. jalapa* challenged with Tospovirus than virus inoculated control might be responsible for the suppression of virus infection. Studies on the PAL activity on tomato hybrid (Avinash-2) revealed that pre application of *M. jalapa* challenged with Tospovirus recorded the maximum PAL activity of 55.0 on 4 DAG and increased upto 72.50 on 12 DAG followed by a slow decline on 16 DAG. The challenge inoculation of Bion was on par with *M. jalapa* (Table 18).

4.20 Induction of phenol against Tospovirus

Pre application of AVP and Bion on cowpea *cv.* C152 challenged with Tospovirus induced the higher accumulation of phenolics. Accumulation of phenolics started one day after challenge inoculation. The maximum accumulation was observed upto 3 DACI, and declined from 4 DACI. The total phenol content in *M. jalapa* challenged cowpea plants on 3 and 4 DACI was 164.67 and 159.33 $\mu g\ g^{-1}$

Table 17. Induction of phenylalanine ammonia lyase in cowpea (*cv.*C152) against Tospovirus

Treatment	PAL activity*				
	1 DAI	2 DAI	3 DAI	4 DAI	Mean
M. jalapa	26.33^{c}	30.00^{d}	32.00^{d}	30.00^{c}	29.58
M. jalapa + virus	43.33^{a}	51.33^{b}	65.33^{a}	62.00^{a}	55.50
H. cupanioxides	23.67^{cd}	27.00^{d}	24.00^{e}	21.33^{e}	24.00
H. cupanioides + virus	33.33^{b}	45.33^{c}	56.00^{b}	49.33^{b}	46.00
Bion	20.33^{de}	21.00^{e}	22.67^{e}	25.00^{d}	22.25
Bion + virus	45.33^{a}	55.67^{a}	66.67^{a}	62.67^{a}	57.58
A. vesica	20.33^{de}	23.00^{e}	24.67^{e}	18.33^{ef}	21.58
A. vesica + virus	36.00^{b}	45.33^{c}	52.00^{c}	48.00^{b}	45.33
Healthy	12.50^{f}	14.60^{f}	15.60^{f}	16.50^{f}	14.80
Virus alone	17.33^{e}	19.93^{e}	21.70^{e}	19.00^{ef}	19.49
Mean	27.85	33.32	38.06	35.22	33.61

LSD (5%)
Treatment : 4.12
DAI : 5.77
Interaction : 3.46

*nmol of trans cinnamic acid formed min^{-1} g^{-1} fresh tissue (A290 nm)

- DAI : Days after inoculation
- In a column, means followed by common letter do not differ significantly at the 5% level by DMRT.

Table 18. Induction of phenylalanine ammonia lyase in tomato (Avinash-2) against Tospovirus infection

Treatment	Phenyl alanine ammonia lyase *				
	4 DAG	8 DAG	12 DAG	16 DAG	Mean
M. jalapa	38.50^{c}	41.00^{c}	41.00^{c}	34.00^{c}	38.63
M. jalapa + virus	55.00^{a}	62.00^{a}	72.50^{a}	65.00^{a}	63.63
H. cupanioides	35.50^{cd}	39.00^{cd}	32.00^{de}	25.50^{d}	33.00
H. cupanioides + virus	45.00^{b}	56.00^{b}	66.00^{b}	53.00^{b}	55.00
Bion	31.50^{de}	32.50^{e}	32.00^{de}	28.50^{cd}	31.13
Bion + virus	56.00^{a}	65.50^{a}	74.00^{a}	65.00^{a}	65.13
A. vesica	31.00^{de}	33.50^{de}	35.00^{d}	24.00^{d}	30.88
A. vesica + virus	45.50^{b}	56.00^{b}	65.50^{b}	53.50^{b}	55.13
Healthy	24.00^{f}	27.50^{e}	26.50^{e}	23.50^{d}	25.38
Virus alone	28.00^{ef}	30.95^{e}	32.00^{de}	25.50^{d}	29.11
Mean	39.00	44.44	47.65	39.75	42.70

LSD (5%)
Treatment :5.22
DAG :4.03
Interaction :5.65

- *nmol of trans cinnamic acid formed min^{-1} g^{-1} fresh tissue (A 290 nm)
- DAG : Days after grafting
- In a column, means followed by common letter do not differ significantly at the 5% level by DMRT.

fresh tissue respectively. But, it was only 107.00 and 100.00 μg g^{-1} fresh tissue in virus inoculated control (Table 19). It clearly indicates that accumulation of phenolic compounds was comparatively very much lower in virus inoculated control when compared to *M. jalapa* treated plants challenged with Tospovirus. The phenol content in AVPs, Bion treated plants, virus inoculated control and healthy control plants were much lower than that of challenge inoculated plants (Table 19).

4.21 Induction of isoforms of Peroxidase (PO) and Polyphenol Oxidase (PPO)

Local lesion host pre treated with AVPs, Bion and *P.chlororaphis* challenged with Tospovirus and the virus inoculated control plants differed in the expression of isoforms of peroxidase and polyphenol oxidase. Pretreatment with Bion, *M. jalapa, H.cupanioides A.vesica and P.chlororaphis* expressed four isoforms of peroxidase (PO-1, PO-2, PO-3, PO-4). However, the intensity of expression of PO-3 was more in Bion followed by *H. cupanioides* and *M. jalapa* treated plants. The activity of the same isoform in *P. chlororaphis* and *A.vesica* treatments were comparatively very low. But the activity of PO-3 was completely absent in the virus inoculated control plants (Plate 20). Studies on the expression pattern of polyphenol oxidase reflect that, four isoforms of polyphenol oxidase (PPO-1, PPO-2, PPO-3, PPO-4) were induced in all the treatments. However, the expression of PPO-3 was higher in Bion treatment, followed by other AVPs and *P. chlororaphis.* But the activity of PPO-3 was much lower in virus inoculated control (Plate 21).

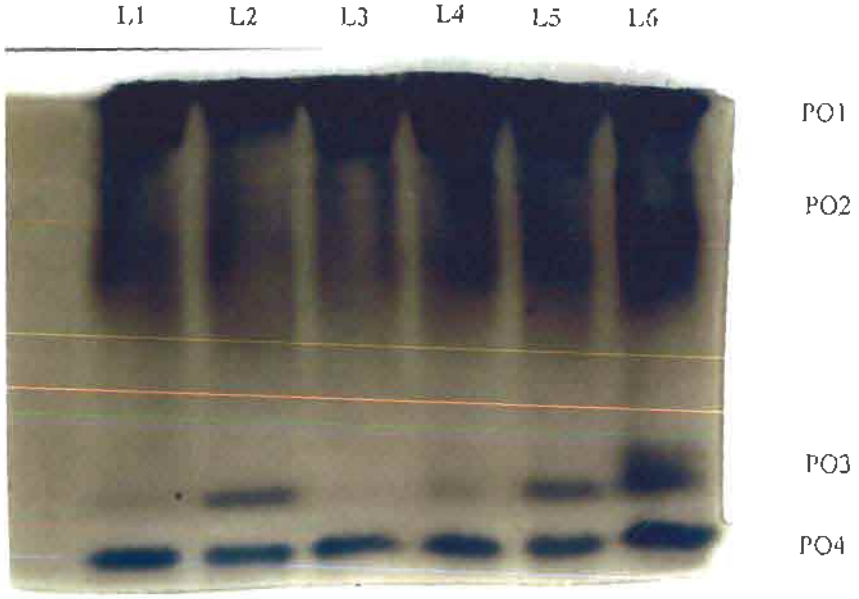
Similarly, the induction pattern of PPO in tomato hybrid Avinash-2, pretreated with AVPs challenged by Tospovirus indicated that the expression of PPO-1, PPO-2, PPO-3, PPO-4 and PPO-5 were greater than post and simultaneous inoculation of *M.jalapa, H.cupanioides* and *A.vesica* challenged with Tospovirus. However, the activity of PPO-3 and PPO-4 was of very low intensity compared to that of post and simultaneous inoculation. Hence, the results clearly explain that, pre inoculation of AVPs induce the maximum activity of PPO and there by reduced the virus infection (Plate 22).

Table 19. Assay of phenol content in cowpea (*cv.* C152) against Tospovirus infection

Treatment	Total Phenol (µg g^{-1} of fresh tissue)				
	1 DAI	2 DAI	3 DAI	4 DAI	Mean
M. jalapa	90.00^{d}	91.00^{ef}	86.33^{fg}	91.67^{e}	89.75
M. jalapa + virus	120.67^{b}	144.00^{b}	164.67^{b}	159.33^{a}	147.17
H. cupanioides	98.00^{c}	95.00^{def}	91.67^{efg}	87.33^{e}	93.00
H. cupanioides + virus	116.00^{b}	136.00^{c}	150.67^{c}	143.67^{b}	136.58
Bion	102.00^{c}	99.00^{de}	96.00^{e}	92.00^{e}	97.25
Bion + virus	138.67^{a}	165.33^{a}	174.67^{a}	166.00^{a}	161.17
A. vesica	88.67^{d}	87.67^{f}	84.00^{g}	83.33^{e}	85.92
A. vesica + virus	118.67^{b}	134.67^{c}	150.67^{c}	135.55^{c}	134.83
Healthy	87.33^{d}	90.33^{f}	93.33^{ef}	86.00^{e}	89.25
Virus alone	105.33^{c}	100.00^{d}	107.33^{d}	100.00^{d}	103.17
Mean	106.53	114.30	119.93	114.47	113.81

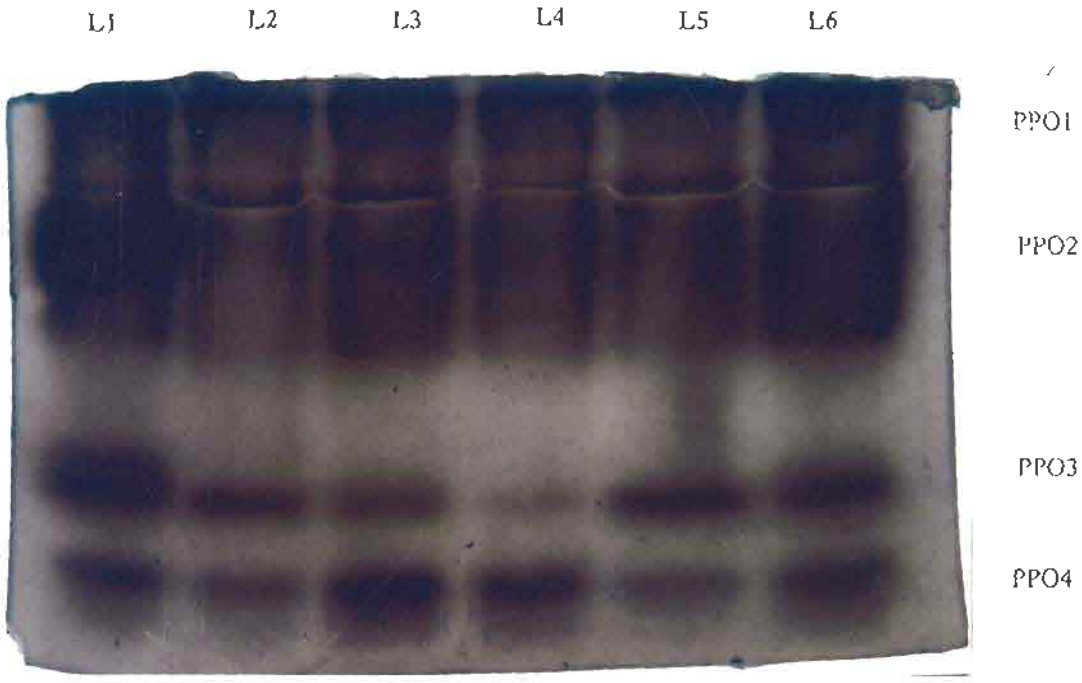
LSD (5%)
Treatment : 6.39
DAI : 8.91
Interaction : 7.88
Values are the mean of three replications
DAI : Days after inoculation
In a column, means followed by a common letter do not differ signfiicantly at the 5% level by DMRT.

Plate 20. Induction of PO isoforms by AVP, Bion and *P.chlororaphis* in local lesion host challenged with **tospovirus**

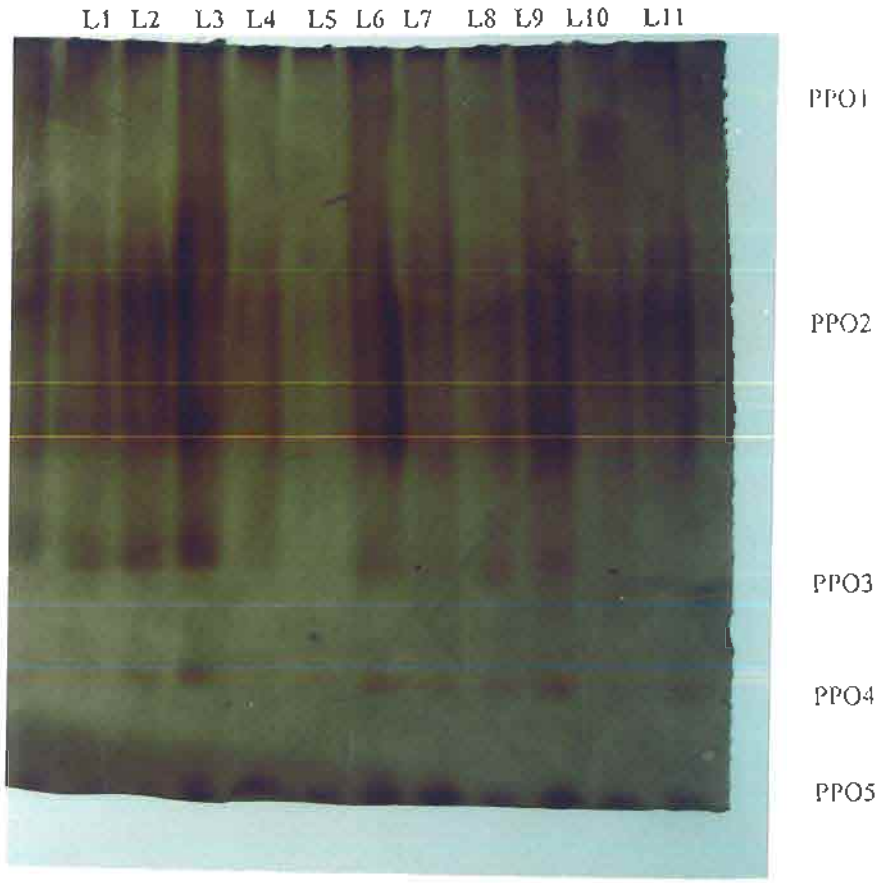


L1: *P. chlororaphis*; L2: *H. cupanioides*; L3: Virus inoculated control; L4: *A. vesica*; L5: *M. jalapa*; L6: Bion

Plate 21. Induction of PPO isoforms by AVP, Bion and *P.chlororaphis* in local lesio host challenged with **tospovirus**



L1: Bion; L2: *A. vesica*; L3: *M. jalapa*; L4: Virus inoculated control; L5: *H. cupanioides*; L6: *P. chlororaphis*



L1: Post application of *M. jalapa*; L2: Simultaneous application of *M. jalapa*

L3: Pre application of *M. jalapa;* L4: Post application of *H. cupanioides*

L5: Simultaneous application of *H. cupanioides;* L6: Pre application of *H. cupanioides*

L7: Post application of *A. vesica;* L8: Simultaneous application of *A. vesica*

L9: Pre application of *A. vesica;* L10: Virus inoculated control; L11: Healthy control

4.22 Production of polyclonal antibody for *Harpullia* antiviral protein (HAP) and serodiagnosis of HAP like proteins from non-host plants

The HAP protein was purified in DEAE column ion exchange chromatography. The purified HAP protein was injected intramuscularly into white Newzealand rabbit and the polyclonal antibody (PAb) was developed. Presence of HAP like proteins in other effective non-host AVPs was assayed by indirect DAS ELISA using HAP PAb (Table 20). The results revealed that the seed extract (10%) of *H. cupanioides* recorded the maximum absorbance value of 1.89. *M. jalapa, A. vesica, P. americana, S. vulgare, B. spectabilis, C. nucifera, G. sylvestris* and *P. chilensis* showed positive reaction to HAP like protein. But extracts from *A. cepa* and *B. rubra* showed negative reaction. The results in Table 2 also explained that the above two AVPs were not effective against Tospovirus, which indicates the absence of antiviral HAP like protein. Among the AVPs, *M. jalapa* (1.70) and *A. vesica* (1.61) showed maximum response for the presence of HAP like protein followed by *P. americana, S. vulgare* and *B. spectabilis* (Plate 23).

4.23 Effect of AVPs on Tospovirus titre

Tospovirus titre in the local lesion host was assayed after 4 days of pre inoculation of AVPs challenged with Tospovirus through ELISA. Virus inoculated control was used to compare the efficacy of AVPs (Table 21). The plants treated with *M. jalapa, H. cupanioides* and *P. americana* challenged with Tospovirus did not show any significant variation in absorbance values when compared to uninoculated healthy plants, indicating the absence of any detectable virus. Pre treatment of local lesion host with *B. rubra* and *A. cepa* challenged with Tospovirus recorded the absorbance value as that of virus inoculated control, indicating the presence of virus equal to virus inoculated control.

Table 20. Serodiagnosis (ELISA) of non - host plants for the presence of HAP like proteins

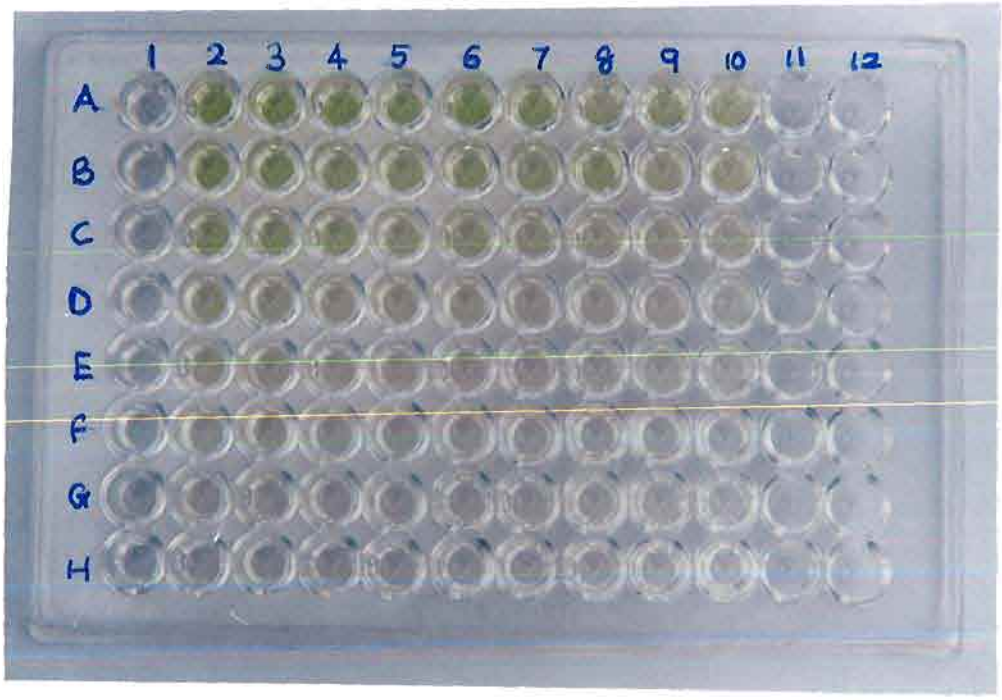
Non - host plants	Protein concentration (OD value at 405 nm)
H. cupanioides	1.89[h]
M. jalapa	1.70[g]
A. vesica	1.61[f]
P. americana	1.37[e]
S. vulgare	1.31[d]
B. spectabilis	1.34[de]
C. nucifera	0.66[b]
G. sylvestris	0.75[c]
P. chilensis	0.72[c]
A. cepa	0.06[a]
B. rubra	0.04[a]
Buffer control	0.05[a]

LSD (5%) : 0.04

Values are the mean of three replications

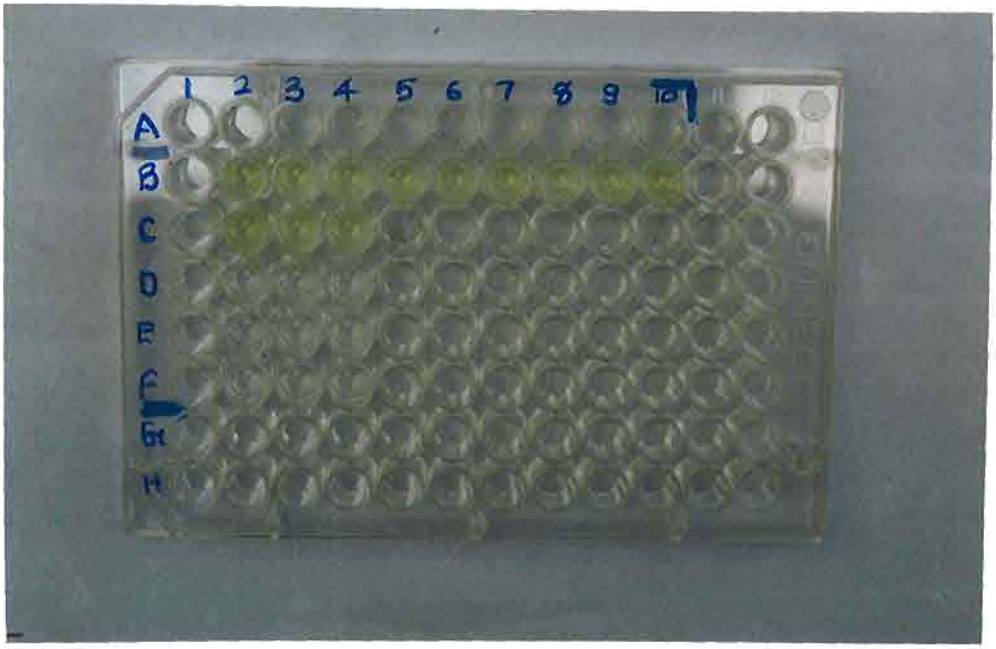
In a column, means followed by the same letter do not differ significantly at the 5% level by DMRT.

Plate 23. Serodiagnosis of HAP like proteins from non host plants through ELISA



A2- A5 : *H. cupanioides*; A6-A9: *M. jalapa;* B2- B5: *A. vesica;* B6-B9: *P. americana* C2-C5: *S. vulgare;* C6-C9: *B.spectabilis* ;D2- D5: *C. mucifera;* D6- D9: *G. sylvestris* E2-E5: *P. chilensis;* E6-E9: *A. cepa;* F2- F5: *B. rubra;* F6-F9: Buffer control

Plate 23a. Effect of AVPs on tospovirus titre in local lesion host



A2-A5: Blank; A6-A9: Healthy; B2-B4: Virus alone; B5-B7: Sterile water + Virus; B8-B10: *A. cepa* + Virus; C2-C4: *B. rubra* + virus; D2-D4: *M.jalapa* + virus; E2-E4: *H cupanioides*+Virus F2-F4: *P.americana* + Virus

Table 21. Effect of AVPs on Tospovirus titre in cowpea (*cv.* C152) as determined by ELISA

Treatments	Virus concentration at 4 DAI (OD value at 405 nm)
M. jalapa + virus	0.07[a]
H. cupanioides + virus	0.06[a]
P. americana + virus	0.09[a]
Virus alone	1.75[c]
St. Water + virus	1.77[c]
A. cepa + virus	1.59[b]
B. rubra + virus	1.57[b]
Healthy	0.07[a]
Buffer control	0.06[a]

LSD (5%) : 0.04
DAI : Days after inoculation
Values are the mean of three replications
In a column, means followed by common letter do not differ significantly at the 5% level by DMRT.

4.24 Effect of AVPs, chemicals and biocontrol agents against Tospovirus in tomato under *in vivo*

The efficacy of crude extracts of effective AVPs, chemicals and biocontrol agents were evaluated in tomato (PKM1) under field condition. The trial was conducted at Alandurai village during June to September 2001. Neem oil was used as standard check to compare the antiviral efficacy of *M. jalapa*, *H. cupanioides* and *A. vesica*. Disease incidence was recorded on 30, 60 and 90 days after planting. Results of the first trial emphasised that the foliar spraying of *M. jalapa* (10%) at fortnightly intervals recorded 13.23 per cent infection of wilt byTospovirus, followed by the foliar application of Bion (200ppm) and *Pseudomonas chlororaphis.* The disease was maximum to the tune of 38.28 per cent in untreated control plots.

The observation on the yield explained that the maximum yield of 15 t/ha was obtained in Bion treated plots. There was no significant difference in yield between *M. jalapa* and Bion treatments (Plate 24–27). Contrary to the above two treatments, the yield of 8 t/ha alone was recorded in untreated control plots. It was on par with the standard checks *viz.*, monochrotophos and neem oil (Table 22).

The results of the second field trial conducted at Mathuvarayapuram village during January to April 2002 with tomato hybrid Avinash-2 revealed that the disease incidence after 90 days of planting in the plots sprayed with *M. jalapa* was 11.64 per cent which was again on par with foliar application of Bion (13.31%). On the other hand the plots sprayed with neem oil recorded 19.15 per cent disease incidence and in control, it was 41.82 per cent.

The studies on the population dynamics of vector showed that the population of thrips was minimum in the plots treated with *M. jalapa* (22 No./ plant), *H. cupanioides* (28 No./ plant), and Bion (28No./ plant). But in untreated control plants 63 thrips per plant were observed (Table 23). The studies on the yield indicated that *M. jalapa* recorded the maximum yield of 24 t/ha, followed by Bion (21.3 t/ha) and *H. cupanioides* (19 t/ha), as against 13t / ha in the untreated control (Plate 28-31).

Plate 24. Control

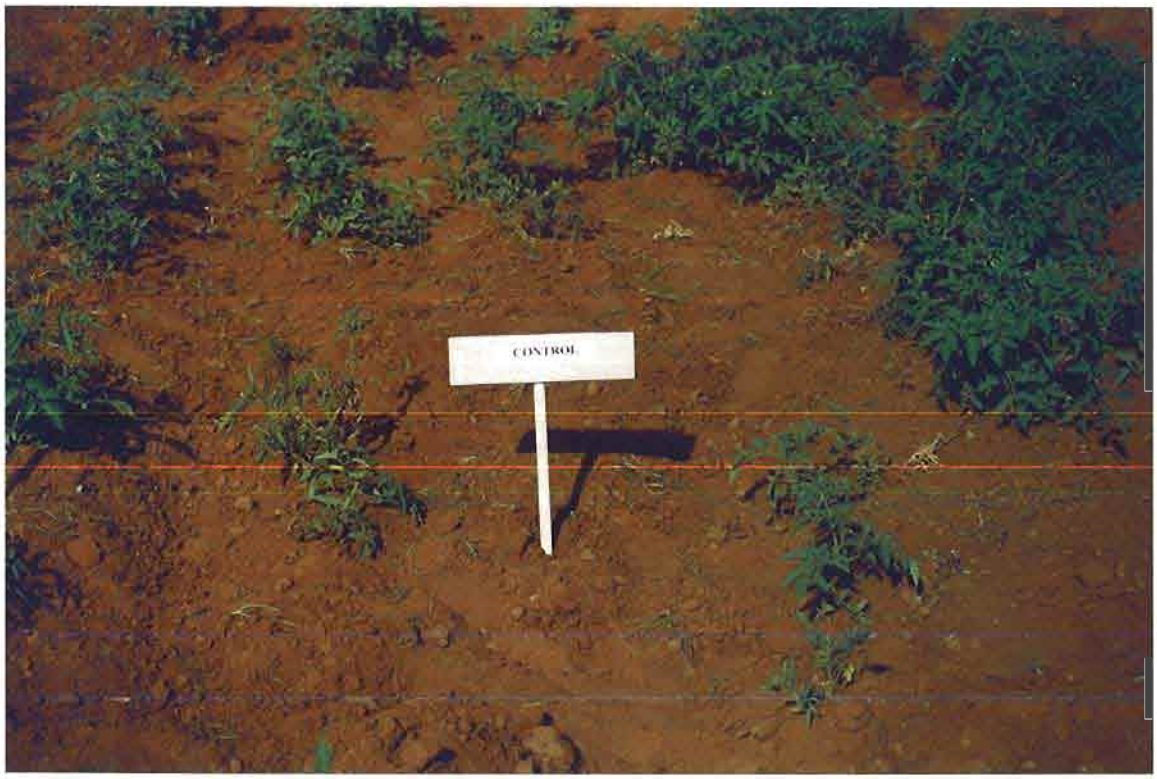


Plate 25: *M. Jalapa* (10% foliar spray)

Plate 26. *H. cupanioides* (10% Foliar spray)

Plate 27: Bion (200ppm foliar spray)

Table 22. Effect of AVPs, chemicals and Biocontrol agent against Tospovirus in Tomato PKM-1. (June-Sep. 2001)

Treatments	30 DAP		60 DAP		90 DAP		Yield tonnes/ha
	PI	PDC	PI	PDC	PI	PDC	
M. jalapa	6.61 (14.90)	33.21	11.47 (19.80)	51.82	13.24 (21.34)	65.42	14.33^{cd}
H. cupanioides	9.131 (17.58)	7.79	17.45 (24.69)	26.68	19.96 (26.54)	47.86	10.16^{ab}
A. vesica	11.64 (19.94)	-17.5	19.96 (26.54)	16.15	22.48 (28.29)	41.32	10.16^{ab}
Bion (200 ppm)	8.30 (16.74)	16.21	9.91 (18.35)	58.39	14.94 (22.74)	60.97	15.00^{d}
Monocrotophos (0.1%)	12.43 (20.64)	-25.5	18.13 (25.20)	23.84	25.77 (30.50)	32.68	8.00^{a}
P. chlororaphis	9.91 (18.34)	0.0	12.43 (20.64)	47.79	16.60 (24.05)	56.63	12.00^{bc}
Neem oil	9.07 (17.52)	8.40	10.81 (19.79)	54.61	19.32 (26.07)	49.55	9.33^{a}
Control	9.90 (18.34)	-	23.81 (28.84)	-	38.29 (38.23)	-	8.00^{a}
LSD (5%)	2.20		4.50		1.42		2.42

PI – Per cent infection, PDC – Per cent decrease over control,

- Mean number of thrips from 10 plants
- Values in parentheses are arcsine transformed values

ble 23: Effect of AVPs,Chemicals and Biocontrol agent against Tospovirus in tomato (Avinash-2) under field conditions (January – April 2002) .

Treatments	30 DAP			60 DAP			90 DAP			Yield t /ha
	PI	PDC	Thrips popln.	PI	PDC	Thirps Popln.	PI	PDC	Thrips Popln.	
jalapa	4.08 (11.65)	75.37	12.0	8.30 (16.74)	66.49	18.0	11.64 (19.95)	72.16	22.0	24.00^{f}
cupanioides	8.30 (16.74)	49.85	16.0	13.14 (21.25)	46.93	22.0	16.82 (24.21)	59.79	28.0	19.00^{d}
esica	17.64 (19.95)	-6.63	28.0	14.15 (22.10)	42.88	34.0	18.65 (25.59)	55.40	39.0	16.33^{bc}
n (200 ppm)	6.61 (14.90)	60.00	18.0	10.81 (19.19)	56.36	24.0	13.31 (21.39)	68.16	28.0	21.33^{e}
nocrotophos %)	13.31 (21.40)	19.63	32.0	22.11 (28.05)	10.68	38.0	29.98 (33.19)	28.31	44.0	14.66^{ab}
chlororaphis	11.64 (19.95)	29.64	34.0	16.48 (23.95)	33.43	38.0	19.96 (26.53)	52.27	42.0	17.00^{cd}
em oil %)	9.13 (17.59)	44.80	30.0	14.76 (22.59)	40.38	31.0	19.15 (40.29)	54.20	38.00	18.00^{a}
ntrol	16.54 (23.99)		44.0	24.76 (29.98)		52.0	41.81 (25.95)		63.0	13.00^{cd}
0 (5%)	3.25	4.32		4.52		5.32	2.33		63.0	1.67

PI – Per cent infection, PDC – Per cent decrease over control,

- Mean number of thrips from 10 plants
- Values in parentheses are arcsine transformed values

Plate 28. Control

Plate 29: *M. Jalapa* (10% foliar spray)

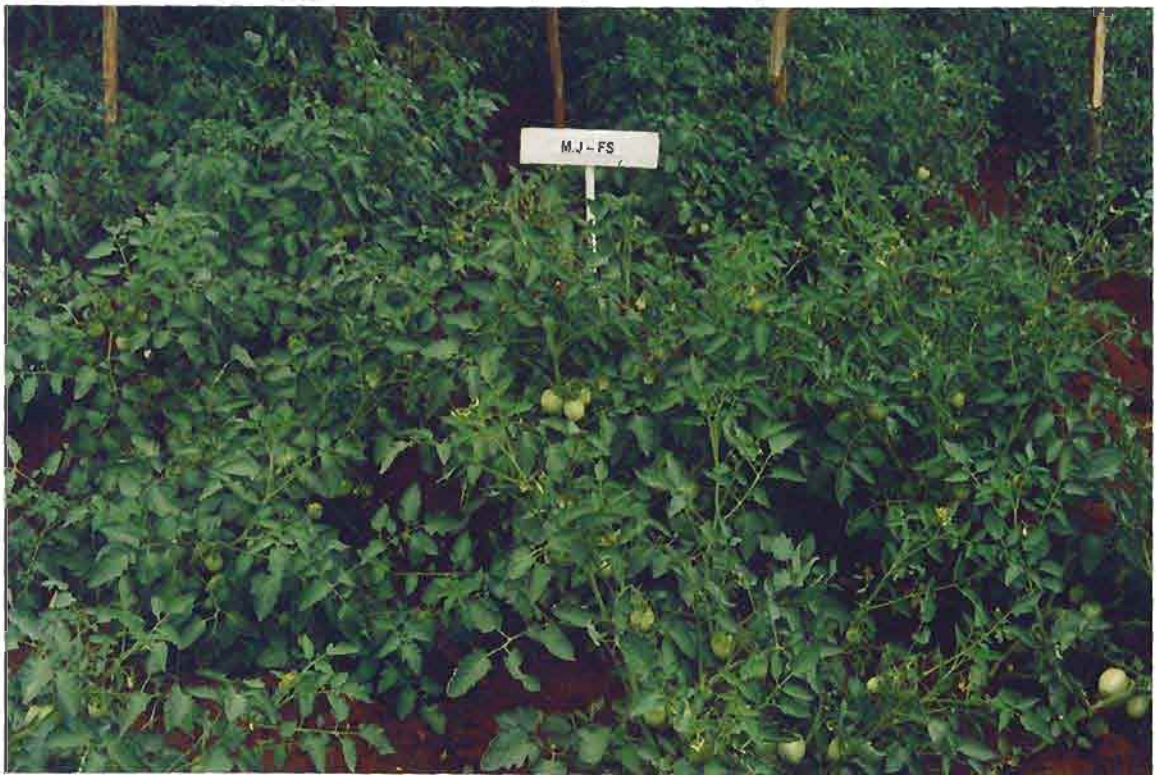


Plate 30. *H. cupanioides* (10% Foliar spray)

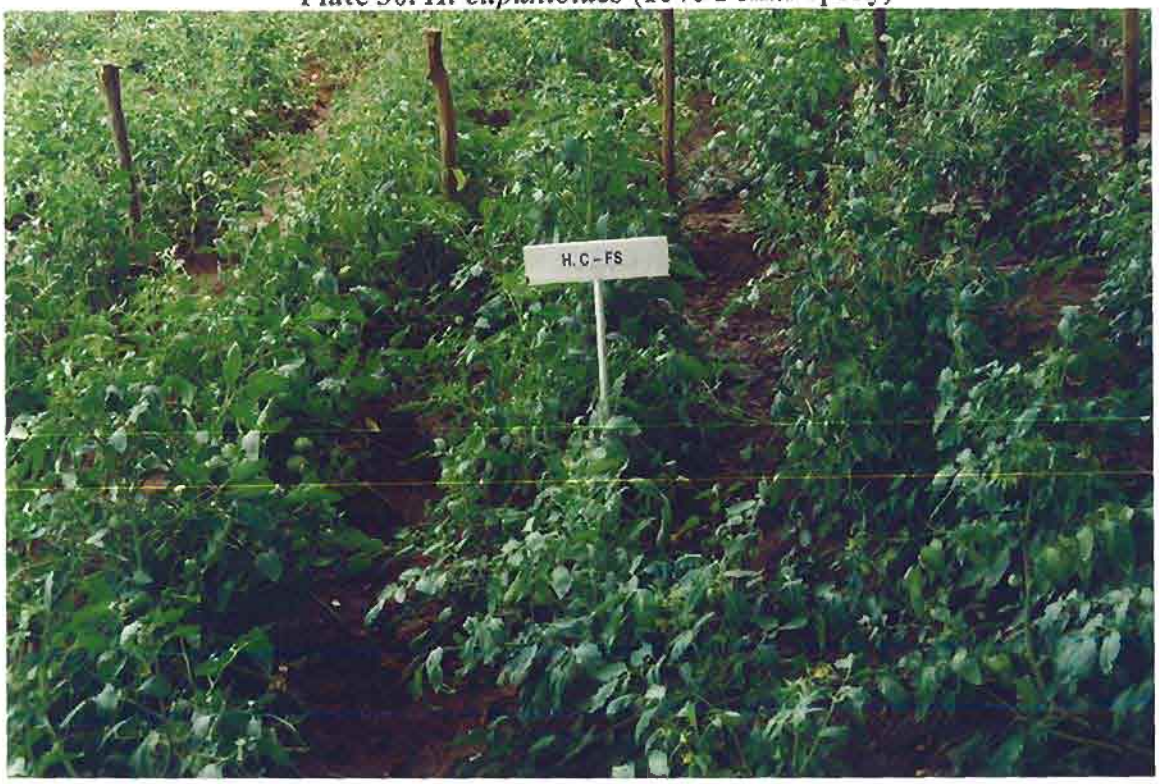
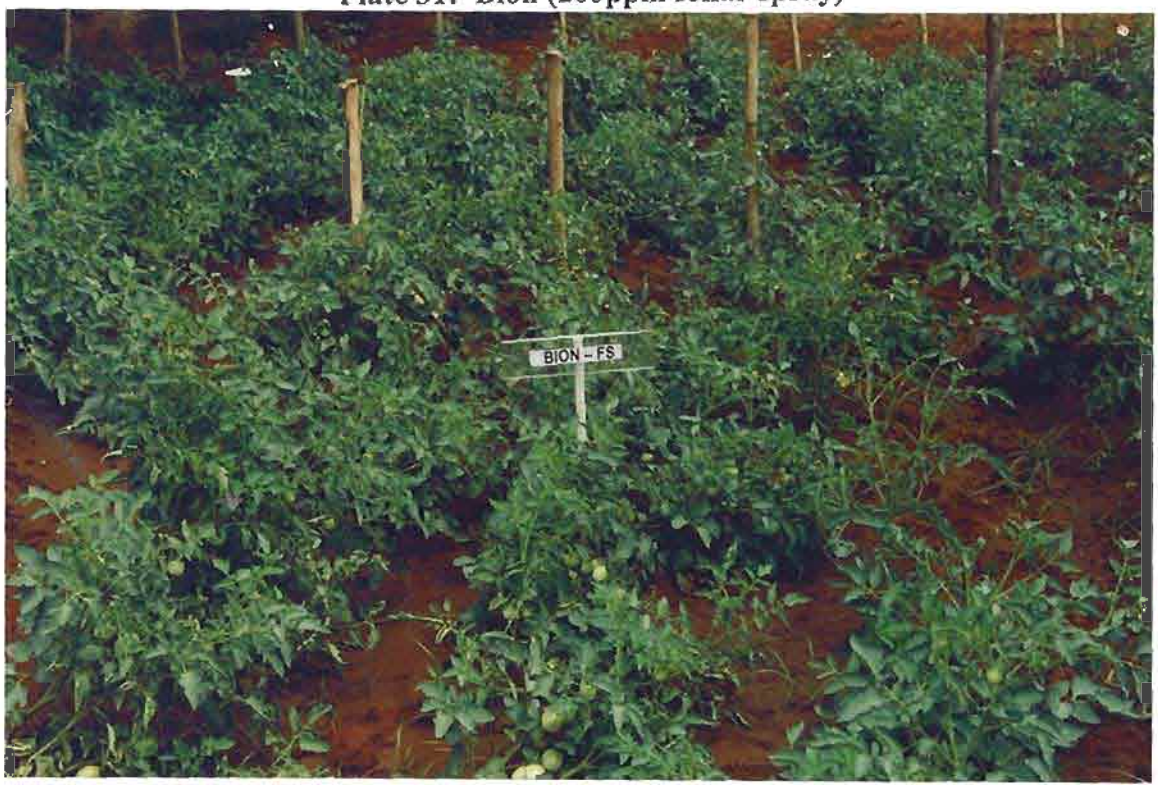


Plate 31: Bion (200ppm foliar spray)



The results on the third field trial conducted at Thennamanallur village of Coimbatore district conducted during April to July 2002 revealed that, there was 10.8 per cent disease incidence (72.98 per cent reduction over control) after 90 days of planting in *M. jalapa* treated plants with the yield of 25.3 t/ha. It was not found to differ significantly from *H. cupanioides, A. vesica*, Bion and *P. chlororaphis* treated plots, which were 12.43, 14.01, 11.64 and15.82 per cent respectively after 90 days of planting. However, significant difference in the yield was observed between Bion and *M.jalapa* treated plots. The yield of 19.0 t/ha was recorded in *P. chlororaphis* treated plots, which was on par with Bion. But in the untreated control plots 39.9 per cent disease incidence was observed with the yield of 12 t/ha (Table 24). An average of 20-24 thrips were recorded in all AVPs and Bion treatments as against 48 in-untreated controls (Plates 32-35).

Table 24. Effect of AVPs,Chemicals and Biocontrol agent against Tospovirus in tomato (April – July, 2002).

Treatments	30 DAP			60 DAP			90 DAP			Yield t /ha
	PI	PDC	Thrips popln.	PI	PDC	Thirps Popln.	PI	PDC	Thrips Popln.	
jalapa	3.24 (10.37)	67.28	18.0	6.61 (14.90)	73.45	18.0	10.81 (19.19)	72.98	20.0	25.33^{e}
cupanioides	5.78 (13.91)	41.64	16.0	8.29 (16.74)	66.69	22.0	12.43 (20.64)	68.92	22.0	18.00^{bc}
vesica	6.614 (14.90)	33.23	18.0	9.90 (18.35)	60.23	20.00	14.01 (21.98)	64.96	24.0	18.66
n (200 ppm)	3.241 (10.37)	67.28	20.0	6.614 (14.90)	73.45	20.0	11.64 (19.95)	70.89	22.0	21.66
nocrotophos 1%)	9.13 (17.59)	7.82	30.0	14.14 (22.09)	43.22	36.00	17.45 (24.69)	56.36	36.0	16.00^{bc}
chlororaphis	4.78 (12.63)	51.68	26.0	9.91 (18.35)	60.23	29.00	15.82 (23.43)	60.45	32.0	19.00^{cd}
em oil 1%)	8.29 (16.74)	16.31	22.0	14.15 (22.09)	43.22	28.00	23.42 (28.98)	41.45	38.0	15.33^{b}
ntrol	9.90 (18.344)	-	38.0	24.91 (28.94)	-	44.0	39.99 (39.23)	-	48.0	12.00^{a}
0 (5%)	4.29		2.23	6.29		3.32	4.72		2.92	3.32

PI – Per cent infection, PDC – Per cent decrease over control,

- Mean number of thrips from 10 plants
- Values in parentheses are arcsine transformed values

Plate 32. Control

Plate 33: *M. Jalapa* (10% foliar spray)

Plate 34. *H. cupanioides* (10% Foliar spray) 100

Plate 35: Bion (200ppm foliar spray)

Discussion

CHAPTER – V

DISCUSSION

5.0 Screening of tomato germplasm

The development of plants with resistance to Tospovirus is the most effective method for managing and preventing virus epidemics. In the present study fifty-eight germplasm entries of *Lycopersicon esculentum* were evaluated under field conditions. None of the entries were free from Tospovirus infection. The entry LE31 recorded the lowest incidence of 35.49%. Since vector population under field conditions are responsible for TSWV epidemics, identification of genetic source of resistance have relied upon natural field infection (Smith, 1944). The variation in per cent disease occurrence under field conditions could be attributed to plant factors that alter thrips response to plants by limiting TSWV transmission under field conditions (Jones, 1987). The results support the previous observations that all *Lycopersicon* species except *L. peruvianum* were susceptible to TSWV; however, it varied significantly among accessions (Krishnakumar and Ullman, 1993). The entry LE231 was highly susceptible to Tospovirus (99.5%). Kumar and Irulappan (1991) also reported that none of the germplasm entries belonging to *L. esculentum* were found resistant to TSWV under field conditions.

5.1 Screening of non-host plants

Five of the thirty-six non-host plants tested showed high level of antiviral efficacy against Tospovirus on the local lesion host. Pre inoculation spray of *B. spectabilis, M. jalapa, H. cupanioides, A. vesica* and *P. americana* challenged with Tospovirus were highly effective against Tospovirus. The antiviral efficacy may be attributed to the presence of antiviral compounds such as furocoumarins, alkaloids, terpenoids, lignin and specific proteins (Zipf, 1995). Extracts of nerium and yellow oleander completely inhibited the TSWV lesion formation (Ganapathy, 1985). Extracts of *Ocimum sanctum, A. indica, Carica papaya, P. chilensis, N. odorum, P. longifolia, C. murale, S. nigrum* and *B. spectabilis* inhibited TSWV by more than 90% (Kurucheve, 1988). Sadasivam *et al.* (1991) reported

the anti viral effect of *B. diffusa*, *B. spectabilis*, *C. aculeatum*, and *S. vulgare* against TSWV. Chen *et al.* (1991) reported the antiviral action of pokeweed antiviral protein (PAP) against TMV. Velazhahan and Narayanasamy (1991) reported that the extracts of *C. juncea M. alba, D. regia and T. grandis* inhibited TSWV. Foliar spraying of *B. spectabilis* was effective in inhibiting the infection of TSWV, TMV, CMV and CaMV (Balasaraswathy *et al.*, 1998).

5.2 Antiviral action of chemicals and biocontrol agents and non-host plants.

Studies on the antiviral efficacy of chemicals and biocontrol agents reflected that foliar spraying of Bion and *P. chlororaphis* were found effective against Tospovirus. Triazofurin at 0.01 and 0.02% was effective against TSWV in tomato (Jayme Caner *et al.*, 1984). Srinivasalu and Narayanasamy (1990) found that the acridine orange and silver nitrate reduced local lesion formation of TSWV by 96-100 per cent. Antiviral efficacy of Bion against urdbean yellow mosaic virus was reported by Venkatesan *et al.* (2001)

Pre application of AVPs, Bion and *P. chlororaphis* was effective than simultaneous and post inoculation treatments against Tospovirus. Pre treatment was effective as it interferes with mechanical transmission (Kuntz and Walker, 1947; Habuka *et al.*, 1990) or due to the induction of systemic resistance (Prasad *et al.*, 1995). *P. fluorescens* was effective in suppressing the infection of TSWV in tomato under glass house and field conditions (Kandan *et al.*, 2002).

5.3 Effect of temperature on the antiviral activity of AVPs

M. jalapa, B. spectabilis and *H. cupanioides* retained their antiviral activity at 60 and 70°C indicating that the antiviral proteins were not sensitive to higher temperatures. But the antiviral action of bougainvillea, sorghum and pokeweed were sensitive to higher temperatures and the per cent inhibition of local lesion formation by groundnut bud necrosis virus was found to decline from 40 to 60°C (Shanker, 1995). Similarly, Jayakumar (1997) found that exposure of AVPs from 40 to 60°C resulted in the slow decline of antiviral action against groundnut bud necrosis virus. Antiviral activity of coconut, sorghum and *Prosopis*

lost their inhibitory effect against TSWV at high temperatures (Sumathi, 1996). The antiviral principle from spinach lost its activity at 70°C (heated for 30 minutes), indicating the sensitive nature (Straub *et al.*, 1986). But as in the present study, the heat stable nature of the antiviral principle of Chenopodium *murale, Casuarina equisettifolia* and *B. spectabilis* at 90°C was reported by Kurucheve in 1988. Similarly, the heat stable nature of antiviral protein of *P. americana* exposed to 100°C for 10 minutes was reported by Fukaya and Taniguchi during 1979. Balasubramaniyan *et al.* (2000) reported that two antiviral proteins purified from the dried leaves of *Celosia cristata* (CCP25 and CCP27) displayed tolerance to temperature and remained unaffected in inhibiting TMV, citrus ring spot virus, sunnhemp rosette virus and tobamoviruses even after 10 min of exposure to 90°C. However, at around 95°C they lost the antiviral efficacy. Comparative analysis of various antiviral principles towards temperature sensitivity explains that the stability of antiviral effect vary between the non-host plants. However, the antiviral proteins with thermal stability may have better shelf life under field conditions.

5.4 Influence of pH on the antiviral activity of AVPs

The experiments to determine the effect of pH on the antiviral activity of *B. spectabilis, M. jalapa, H. cupanioides, P. americana* and *A. vesica* revealed that they were not affected due to changes in pH from neutral to alkaline. But the efficacy was comparatively lost at acidic levels, indicating the sensitivity of AVPs at acidic levels. Similarly, Krishnamoorthy (1994) has also reported that the antiviral efficacy of AVPs from *M. jalapa, Vitex negundo* and *L. leucocephala* were reduced at acidic levels (< 6.5 pH). But he also reported that the antiviral efficacy of the above mentioned AVPs against black gram leaf crinckle virus was reduced at alkaline pH. But, Jayakumar (1997) reported that the antiviral activity of bougainvillea against PVY was more effective at acidic and alkaline pH.

In contrary to above, our studies indicated that *M. jalapa* was effective at pH 8 against Tospovirus in tomato but not at acidic pH. It indicates that AVPs may behave differently at different pH in different virus patho systems. The antiviral principles from

C. nucifera, *B. spectabilis*, *P. chilensis* and *S. vulgare* were highly effective at neutral pH when applied against TSWV (Shanker, 1995).

5.5 Persistence of antiviral activity against Tospovirus

The persistence of antiviral activity of various AVPs when treated on plants varies with respect to different viruses. The present investigation on the persistence of antiviral activity revealed that *M. jalapa* and *H. cupanioides* retained their activity upto 14 days after application. But their efficacy declined later and the decline in the activity may be due to inactivation of antiviral principles by various factors. Awasthi *et al.* (1987) reported that AVPs degraded in tissues of the host plant by the activity of intercellular enzymes. The AVPs from sorghum and Nerium showed antiviral activity for 10 days against TSWV in tomato (Narayanasamy and Ramiah, 1983; Narayanasamy and Ganapathy, 1986). Shanker (1995) reported the efficacy of BAVP for a maximum period of 12 days on peanut bud necrosis virus. Antiviral activity of BAVP was retained upto 8 days in tomato when applied against TSWV (Sumathi, 1996). The antiviral action of *M. jalapa* against PVY was retained upto 5 days after application (Aiyanathan, 1995).

5.6 Effect of storage on the antiviral activity of AVPs

Field level application of AVPs depends on the shelf life of the anti viral principles. Present study on aging *in vitro* revealed that *H. cupanioides* and *M. jalapa* retained their activity against Tospovirus upto 75 days after storage (DAS). But *B. spectabilis* and *P. americana* retained their antiviral activity upto 45 DAS. Retention of antiviral activity of *H. cupanioides* and *M. jalapa* even after 75 DAS may be due to the stable nature of the protein. Retention of antiviral activity upto 20 DAS was reported against viruses like TMV, sunnhemp rosette virus and tobacco ring spot viruses by the extract of *B. diffusa* (Verma and Awasthi, 1979). Antiviral principles from *B. alba* and *B. rubra* were found inhibitory to TMV upto 45 days after storage (Ramakrishnan *et al.*, 1964). The extracts of *B. spectabilis* and *M. jalapa* inhibited TMV even after one year of storage at room temperature (Nororiha *et al.*, 1980). Kurucheve (1988) opined that leaf extracts of *Chenopodium murale*,

P. chilensis and papain retained 100 per cent inhibition against TSWV upto 30 DAS. The results of the present study explained that, the antiviral action of *M. jalapa* and *H. cupanioides* against Tospovirus was retained upto 75 DAS. The increased shelf life of the extracts might be due to the thermo stable nature of the antiviral proteins.

5.7 Efficacy of organic solvent extracts of AVPs against Tospovirus

In the present study, extraction of antiviral principle in different organic solvents, revealed that *M. jalapa, H. cupanioides* and *A. vesica* extracted in acetone and ethanol showed higher level of antiviral action against Tospovirus, indicating the soluble nature of antiviral substance in the organic solvents. Similarly, soluble nature of extracts from non-host plants were reported by Demski and Chalkey (1977) in watermelon, Verma and Awasthi (1979) in *Euphorbia hirta,* Verma *et al.* (1979) in *B. diffusa* and Narayanasamy and Ramiah (1983) in sorghum. Similarly the soluble nature of antiviral principles from different non-hosts was reported by Kurucheve (1988) in *Chenopodium* spp., *Eucalyptus* spp., *Mentha* spp., *Prosopis chilensis, B. spectabilis* and *C. dactylon.*

5.8 Characterization of antiviral proteins

Antiviral proteins are generally basic proteins with or without a sugar moiety with size ranging from 24 to 32kDa (Kubo *et al.,* 1990; Lodge *et al.,* 1993). In the present study, purified antiviral protein of *M. jalapa* in DEAE column had a molecular weight of 41, 29, 22 and 17kDa. *H. cupanioides* consists of proteins of molecular weight 68, 55.5 and 29 kDa. The *pI* value of 41 and 29 kDa proteins of *M. jalapa* and 68, 55.5 and 29 kDa proteins of *H. cupanioides* were basic proteins with *pI* values ranging from 8.4 to 8.6. But the proteins of molecular weight 22 and 17kDa of *M. jalapa* were strongly basic in nature (*pI* > 9.5). MAP, the lysine rich and basic protein (*pI* 9.8) with a molecular mass of 24.2kDa inhibited the mechanical transmission of tomato mosaic virus in tobacco, tomato and pepper plants (Kubo *et al.,* 1990).

Irvin (1995) reported 29kDa antiviral protein (PAP I) from pokeweed. The pattern of total root soluble proteins of *M. jalapa* and *M. expansa* by SDS-PAGE revealed that MAP, a

type I RIP from *M. jalapa* migrated at the same protein range as ME1 (24kDa) and ME 2 (29kDa) from *M. expansa*. ME1 and ME2 are strongly basic proteins with *pI* values greater than 10.0 (Vivanco *et al.,* 1999). *M. jalapa* and *H. cupanioides* have a common antiviral protein of 29 kDa, as that of PAP I and ME 2 as reported by Irvin (1995) and Vivanco *et al.* (1999). The presence of 17kDa protein in *M. jalapa* may also have antiviral action against Tospovirus. Similarly, a basic antiviral protein of 13kDa was reported from pokeweed by Wyatt and Shepherd (1969).The low molecular weight protein of *H. cupanioides* may also have antiviral action.

Plant produces a diverse array of secondary metabolites, many of which have antifungal activity. Some of these compounds are constitutive and exists in their biologically active forms. In the present study, western blot analysis of the seed protein extract and storage root protein of *M. jalapa* were purified at 80 per cent ammonium sulphate saturation evidenced the presence of chitinase (41kDa) that cross reacted with barley chitinase antiserum. Similarly, the constitutive expression of defense related proteins like chitinases and β-1, 3-glucanases were observed in the roots of tobacco (Neale *et al.,* 1990). Savary and Flores (1994) reported the synthesis of class III chitinases in the storage roots of Chinese medicinal plant, *Trichosanthes kirilowii*, which presumably functions to protect juvenile roots against soil-borne fungal pathogens. Constitutive expression of two chitinases has been reported from the leaves of pokeweed (Ohta *et al.,* 1995). Seeds of wheat, barley and maize contain high concentrations of chitinases, glucanases and RIPs that protect seeds and seedlings from fungal invasion (Roberts and Selitrennikoff, 1988). Similarly the purified seed proteins of *H. cupanioides* revealed the presence of 41kDa chitinase. But the role of chitinase in inhibiting the infection of Tospovirus remains as an enigma. On the other hand, application of the plant extracts with chitinase activity may trigger some defense proteins in the host that inactivate the virus or arrest the virus replication or the chitinase may have a synergistic action with antiviral proteins.

Ribosome inactivating proteins (RIPs) are widely present in families such as Asparagaceae, Caryophyllaceae, Cucurbitaceae, Euphorbiaceae, Nyctaginaceae,

Phytolaccaceae and Poaceae (Mehta and Boston, 1998). RIPs are used by all plant species as a primary defense response (Stirpe *et al.*, 1992). In the present study, electro blotting of antiviral proteins of *M. jalapa* and *H. cupanioides* with the antiserum of RIP of *M. expansa* revealed the presence of 29kDa RIP. Similarly, the earlier works explained that the extracts of *M. jalapa* contains a ribosome inactivating protein (RIP) called *Mirabilis* antiviral protein (MAP) which was effective against PVX, PVY, Potato leaf roll virus and potato spindle tuber viroid (Kubo *et al.*, 1990; Kataoka *et al.*, 1991; Vivanco, 1999).

The exogenous application of purified antiviral protein of *M. jalapa* and *H. cupanioides* inhibited Tospovirus on the local lesion host. The antiviral action might be due to the RIPs as evidenced by the exogenous application of RIPs of PAP to the leaf surface, which completely prevented the mechanical transmission of unrelated viruses in several host plants (Chen *et al.*, 1991). Batelli and Stirpe (1995) reported that RIPs have broad spectrum of action against different RNA and DNA viruses. This apparent defense activity is usually coordinated with other defense proteins, such as chitinases (Leah *et al.*, 1990), β-1, 3-glucanases (Mauch *et al.*, 1988) and thaumatin like proteins (Hejgaard *et al.*, 1991). Hence, the maximum level of antiviral action exerted by *M. jalapa* and *H. cupanioides* might be due to the synergistic action of RIPs and chitinase in the antiviral principles.

5.9 Efficacy of antiviral proteins

Differential precipitation of proteins by ammonium sulphate is one of the most widely used purification procedure. It is based on the difference in the solubility of proteins in ammonium sulphate solution and results in a two to five fold increase in specific activity. Studies on the efficacy of purified antiviral proteins *viz.*, MAP, HAP and AAP explained that proteins obtained at 80 per cent saturation with ammonium sulphate were highly inhibitory to Tospovirus than at 40 per cent level. This indicates that the maximum recovery of antiviral protein was obtained at 80 per cent saturation level. The result was found in confirmation with Balaraswathi *et al.* (1998). Balasaraswathi and her coworkers reported that differential precipitation of *B. spectabilis* with ammonium sulphate at 70-90 per cent

yielded the maximum level of antiviral protein (BAPI), which inhibited Tospovirus upto 99.5 per cent on the local lesion host.

Similarly, the research findings of Takanami *et al.* (1990) were in line with the present findings. They explained that the antiviral activity of the root extract of *M. jalapa* was found in the precipitate with 90 per cent ammonium sulphate saturation. Similarly, Ushari *et al.* (1982) reported that the highest per cent inhibition of TMV was obtained with protein fractions between 70 to 100 per cent ammonium sulphate saturation in *B. alba*.

Concentration of 400 µg of purified *Mirabilis* antiviral protein (MAP)/ ml inhibited the local lesion of Tospovirus to an extent of 97.17 per cent. It was on par with the higher concentrations of 600 and 800 µg/ml of MAP, indicating that 400 µg is sufficient for inhibiting Tospovirus. Concentration of 600 µg of HAP inhibited local lesion to the tune of 95.00 per cent. The crude extract was inferior than purified antiviral proteins in inhibiting Tospovirus, indicating that the concentration of antiviral proteins in the crude extract was less than 200 µg of MAP and HAP. Chen *et al.* (1991) reported that 25 µg of purified PAP/ml completely inhibited TMV infection in tobacco. Antiviral protein of *Celosia cristata* referred as CCP-25 inhibited lesion formation of TMV by more than 90 per cent at a concentration as low as 20 µg per ml, whereas CCP-27 exhibited the same response at a concentration of 30 µg per ml (Balasubramanyan *et al.*, 2000).

5.10 Induction of systemic resistance

5.10.1 Induction of proteins

Exogenous application of potent inhibitors of plant viruses from non-host plants could behave as a signal molecule that turns on a cascade response, activating a series of defense mechanisms before viral infection occurs (Vivanco, 1999). In the present study pre application of *M. jalapa* on local lesion host and tomato induced a higher level of 41 kDa protein. But in virus inoculated control the expression was minimum than post and simultaneous inoculations, indicating that the higher-level expression of 41kDa protein

might inhibit the virus replication along with the antiviral proteins with synergistic action. Similar induction was also observed with *H. cupanioides* and *P. americana*. Van Loon *et al.* (1998) suggested that, the disease would be reduced if defense mechanisms were triggered by a stimulus prior to infection by a pathogen. *Phytolacca* antiviral protein triggers the host defense mechanism either by signal transduction or by increased synthesis of antiviral proteins in the host plants by systemic resistance inducers. Induced protein of 41 kDa by PAP might activate signal transduction or may increase the synthesis of antiviral proteins in the host by inactivating the virus (Smirnov *et al.*, 1997).

5.10.2 Induction of PO, PPO, PAL and Phenols

Application of the extracts of non-host plants to other plant species make them resistant to virus infection. These substances may interact either directly or indirectly with the virus. Recent evidences showed that in many cases, viral inhibition is due to the development of virus inhibiting substances within the tissues, but some induces systemic resistance (Verma and Prasad, 1983). Inspite of the detailed research on the ISR against viruses by non host plants, the knowledge on the role of PO, PPO, PAL and Phenols in inhibiting the viral infection is very limited.

PGPR mediated systemic resistance is often associated with the onset of defense mechanism including the early and increased expression of defense enzymes such as chitinase, glucanase, PO, PAL and accumulation of phenolics, phytoalexins and lignins (Chen *et al.*, 2000; Mosch *et al.*, 1993; Schneider and Ullrich, 1994; Nandakumar *et al.* , 2001). Similarly as PGPR mediated resistance, antiviral principles could also trigger similar defense mechanisms. Application of AVPs from non-host plants (*M. jalapa, H. cupanioides* and *A. vesica*) increased the accumulation of defense related enzymes such as PAL, PO, PPO and increased accumulation of phenols, when challenged with Tospovirus in both local lesion host and tomato in the present investigation.

Among these enzymes early triggering of PAL is more important as it is the principle enzyme involved in the phenylpropanoid pathway. It leads to the production of phytoalexins, terpenes and phenolic substances leading to formation of lignin with the help of peroxidases. Though the increased activity of PO, PPO, PAL and phenol was observed from 1 day after challenge inoculation with Tospovirus-, its expression was more from 1 to 3 days after challenge inoculation in local lesion host and from 4 to 12 days after challenge inoculation in tomato. Such increased activity was associated with the prior spraying of *M. jalapa, H. cupanioides* and *P. americana*. Increased level of above defense compounds was also observed in virus inoculated control, but it was several fold lower in AVPs treated tomato plants challenged with Tospovirus. Among the AVPs, *M. jalapa* was found to perform better than the 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 defense response against viral infection.

The presence of phenolic compounds in plants or their synthesis in response to infection has often been associated with resistance (Ingham, 1972). Resistant plants have more phenols than the susceptible ones. Multifold increase of phenols in local lesion host and tomato by AVPs after challenging with Tospovirus in the present study may be due to the excess production of H_2O_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 (Goodman *et al.*, 1967). So increase in PO and PAL content might have frequently enhanced the phenol content in challenge-inoculated plants.

In general plants contain a broad spectrum of peroxidase and polyphenol oxidase isoforms. These isoforms play an important role in lignification, leading to disease resistance (Lagrimini *et al.*, 1987). Present investigation resulted in the activation of PO3 isoform in the local lesion host by Bion and AVPs challenged with Tospovirus. But its expression was completely absent in the virus-inoculated control. A similar level of

expression of PPO3 isoform was also noticed with a very minimum level of expression in virus inoculated control. It indicates that PO3 and PPO3 might have initiated the defense reaction *via* diverse biochemical plant responses by the *de novo* synthesis of proteins, which directly or indirectly interfered with the replication of viral genome and would have suppressed the infection of Tospovirus. Activation of the defense reactions are always associated with key enzymes of general phenylpropanoid metabolism, such as PAL, 4 coumarate CoA ligase, phytoalexins, other secondary metabolites, hydroxyproline rich glycoproteins (HRGP), glycine rich proteins, PR-proteins including chitinase, β-1,3-glucanases, peroxidases, lipoxygenases, proteinase and antimicrobial proteins (Dixon and Harrison, 1990; Cutt and Klessig, 1992; Collinge *et al.*, 1994; Somssich, 1994; Kombrink and Somssich, 1995). As reported by the above mentioned researchers, 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 phenylpropanoid pathway.

Similarly, the previous research by several researchers explained that the pre application of AVPs challenged with plant viruses in different hosts results in the activation of key enzymes like PO, PPO, PAL, chitinase and glucanase leading to the suppression of viral pathogen (Aiyanathan, 1995; Ragupathi, 1995; Verma *et al.*, 1996; Muthulakshmi and Renukadevi, 2001). Though the key enzymes in phenylpropanoid pathway are activated by the application of AVPs from non-host plants, the knowledge of the mechanism of suppression of viral infection or the interaction of the enzymes with antiviral proteins remains to be elucidated.

5.11 Serodiagnosis of HAP like proteins from non-host plants

The antiserum developed against *Harpullia* antiviral protein was screened for its presence in other non-host plants found effective against Tospovirus. Antiviral principles from *M. jalapa, A. vesica, P. americana, S. vulgare, B. spectabilis, C. nucifera, G. sylvestris* and *P. chilensis* showed positive reaction to HAP like protein. They were also found effective against Tospovirus in reducing the number of local lesions. Similarly, agar gel diffusion tests with *Phytolacca* antiserum and purified proteins of different plant species

showed that 10 out of 14 plant species gave positive reactions indicating a serological relationship with *Phytolacca* AVP antiserum (Grasso and Shepherd, 1978). But Kubo *et al.* (1990) reported that the antiserum of MAP was specific to *M. jalapa* alone. Similarly, Balasaraswathy (1995) reported the specificity of BAP 1 of *B. spectabilis*. But in our study the HAP antiserum was found to be non specific.

5.12 Effect of AVPs on virus titre

Release of TMV coat protein involved the action of functional host ribosomes. Once uncoating and translation of viral RNA occurs, the infection by the virus may not be sensitive to pokeweed antiviral protein, a type I RIP, stating that pre inoculation or preoccupation of receptor sites by antiviral protein inhibits viral protein and enzyme synthesis (Chen *et al.*, 1991).

Pre application of MAP, a RIP reaches the active site of the ribosome first, preventing the viral infection at an early stage, probably before viral de-encapsidation (Vivanco, 1999). Similarly, pre inoculation with *M. jalapa, H. cupanioides* and *A. vesica* against Tospovirus, might have resulted due to the inhibition of viral protein and enzyme synthesis resulting in the reduction of virus titre as quantified by ELISA. The absorbance value was similar with uninoculated healthy plants. Hence we hypothesize that prevention of Tospovirus infection is due to the inhibition of protein synthesis, which reflects directly on the virus titre. Earlier workers have also reported the low TSWV titre in AVP treated groundnut and cowpea plants (Shanker, 1995; Sumathi, 1996; Jayakumar, 1997).

5.13 Effect of AVPs, chemicals and biocontrol agents against Tospovirus under field conditions

Potential agricultural applications of AVPs, RIPs from non-host plants has created avenues for their use as plant antiviral and fungicidal agents under field conditions. In the present study, foliar application of AVPs like *M. jalapa*, *H. cupanioides* and *A. vesica* under field conditions have suppressed the infection of Tospovirus in tomato besides increasing the yield. In addition it also suppressed the vector population (Thrips) when compared to

control. Reduction of Tospovirus infection under field conditions may be due to the presence of antiviral proteins and resistance induced by the application of AVPs by triggering the phenylpropanoid pathway. Antiviral proteins trigger the host defense mechanism in a specific manner either by signal transduction as in the case of PAP or by increased synthesis of antiviral proteins in the host plants treated by systemic resistance inducers like *C. aculeatum* (Smirnov *et al.*, 1997; Baranwal and Verma, 2000).

Application of AVPs of *C. nucifera*, *S. vulgare*, *P. chilensis*, *C.sparsiflorus* induced resistance by increasing PO and PAL activity resulting in the reduced incidence of TSWV in mungbean (Manickam and Narayanasamy, 1996). Earlier field studies reported the effectiveness of AVPs against Tospovirus (Narayanasamy and Ramiah, 1983; Kurucheve, 1988; Shanker, 1995), TMV on tobacco (Nagarajan *et al.*, 1984) and PVX and PVY in chilli (Jayakumar, 1997).

In the present study pre application of Bion was more effective than AVPs in inducing the systemic resistance against Tospovirus in tomato. Similarly Narusaka *et al.* (1999) reported the accumulation of chitinase in response to inoculation with *Cladosporium cucumerinum* in cucumber pretreated with acibenzolar-S-methyl (benzo[1,2,3] thiadiazole-7-carbothioic acid-S-methyl ester (BTH–Bion) indicating that Bion appears as a prospective signal in inducing systemic resistance. Foliar application of Bion has also induced systemic resistance against urdbean yellow mosaic virus under field conditions by the induction of PO, PPO and PAL activity (Venkatesan *et al.*, 2001).

Like AVPs and Bion, foliar application of *P. chlororaphis* also reduced the incidence of Tospovirus and increased the yield. Earlier reports explained that *P. fluorescens* (CHAO) induced resistance against tobacco necrosis virus. Cucumber and tomato plants were protected from cucumber mosaic virus through induced resistance mediated through PGPR (Raupach *et al.*, 1996; Zehnder, 2000). Kandan *et al.* (2002) reported that *P. fluorescens* when applied through seed, seedling dip and soil and as foliar spray significantly reduced the TSWV. Application of the same induced the phenylpropanoid pathway, which in turn led to significant reduction of TSWV.

In the present study, reduction of vector population by *M. jalapa* may be due to its repellent nature. Verma and Kumar (1979) reported the repellent action of MAP against aphids and white flies. The reduction of vector population by *H. cupanioides* may be due to its toxicity as reported by Gedeon and Kinel (1956).

Summary

SUMMARY

- Fifty-eight germplasm entries of tomato were screened for resistance against Tospovirus. The lowest incidence of 35.49 per cent and the highest incidence of 99.5 per cent of Tospovirus were recorded in LE31 and LE 231 respectively. None of the entries were found to be resistant.
- Screening of non-host plants against Tospovirus revealed that *M. jalapa*, *H. cupanioides*, *A. vesica* and *P. americana* were found effective against Tospovirus.
- Pre inoculation spray of Bion (200 ppm), non-host plants and *P. chlororaphis* were found effective against Tospovirus on the local lesion host. Post and simultaneous inoculations were inferior to pre inoculation treatment.
- The antiviral effects of tested AVPs were highly effective at room temperature (28 ± 2°C) and at 40°C. Among various AVPs, root extract of *M. jalapa*, leaf extract of *B. spectabilis* and seed extract of *H. cupanioides* retained their antiviral effect even at 70°C.
- The antiviral activity of *B. spectabilis*, *M. jalapa*, *H. cupanioides*, *P. americana,* and *A. vesica* were more effective at alkaline pH than at acidic pH.
- Pre inoculation of *M. jalapa* and *H. cupanioides* retained their antiviral activity upto 14 days after grafting in tomato.
- The antiviral activity of *M. jalapa* and *H. cupanioides* were maintained upto 75 days after storage at room temperature. But *B. spectabilis* and *P. americana* were found effective only upto 45 days of storage.
- Among various organic solvents, extraction of *M. jalapa* and *H. cupanioides* with acetone showed higher level of antiviral action against Tospovirus.
- *M. jalapa* consists of 91, 74, 41, 24, 29 and 17kDa proteins. *H. cupanioides* consists of 97, 68, 41, 29, 20 and 17kDa proteins. *A. vesica* contains 68, 43, 42, 40, 38, 33, 31, 22 and 12kDa proteins.

- Protein profile of the effective fractions of *M. jalapa* against Tospovirus consists 41, 29, 22.5 and 17kDa protein. The fractions of *H. cupanioides* consists of 68, 55.5 and 29 kDa proteins with antiviral activity against Tospovirus. These proteins were basic in nature with *pI* values ranging from 8.4 to 9.5.
- Western blot analysis of *M. jalapa* and *H. cupanioides* proteins showed the presence of 41kDa chitinase and 29 kDa RIP.
- TLC analysis of *H. cupanioides* revealed the presence of terpenoids similar to desacetyl nimbin and isomelidinin.
- Proteins of *M. jalapa*, *H. cupanioides* and *A. vesica* purified at 60 and 80 per cent ammonium sulphate saturation were highly effective in inhibiting the infection of Tospovirus.
- *Mirabilis* antiviral protein (MAP) was effective in inhibiting Tospovirus at 400 µg/ ml and *Harpullia* antiviral protein (HAP) was found effective at 600 and 800 µg ml^{-1}.
- Pre application of AVPs challenged with Tospovirus resulted in the induction of 41kDa protein.
- Foliar application of *M. jalapa, H. cupanioides*, *A. vesica,* Bion and *P. chlororaphis* increased the accumulation of PAL, PO, PPO and phenols, when challenged with Tospovirus.
- *M. jalapa*, *A. vesica*, *P. americana*, *S. vulgare*, *B. spectabilis*, *C. nucifera*, *G. sylvestris* and *P. chilensis* showed positive reaction for the presence of HAP like proteins.
- Pre treatment of local lesion host with the crude extracts of *M. jalapa*, *H. cupanioides*, and *P. americana* showed negative reaction for the presence of Tospovirus through ELISA.
- Foliar spraying of *M. jalapa, H. cupanioides* and Bion at fortnightly intervals were found effective in the management of Tospovirus under field conditions.
- *M. jalapa and H. cupanioides* reduced the vector population of tomato spotted wilt virus disease under field conditions.

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