MANAGEMENT OF BITTER GOURD MOSAIC BY ENHANCING HOST RESISTANCE

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THESIS
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DECLARATION

I hereby declare that the thesis entitled **"Management of bitter gourd mosaic by enhancing host resistance"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me any degree, diploma, fellowship or other similar title of any other University or Society.

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Introduction

1. INTRODUCTION

Bitter gourd (*Momordica charantia* L.) a native of Indo-Burma is one of the important cucurbit vegetable crops that occupies pivotal position among fruit vegetables, in South India. It is also known as bitter melon, bitter squash or balsam-pear. The fruits of this crop which have a high commercial value are being used as supplementary food as they contain large quantities of minerals, vitamins and essential amino acids and also in various medicinal preparations as it is having anti diabetic property.

In spite of the economic importance of the vegetable in our country, one limiting factor in its cultivation is its low productivity due to diseases of diverse etiology. Among the various diseases of bitter gourd, mosaic is the most important one and is caused by different viruses. In Kerala, 100 per cent mosaic incidence and substantial economic loss has been reported when virus infects in early stage of crop growth. The major viruses causing bitter gourd mosaic in Kerala are cucumber mosaic virus (CMV), potyvirus and bitter gourd distortion mosaic virus (BDMV). As these viruses cause mixed infection, it is quite difficult to study the symptomatology of individual viruses from field.

As insect vectors spread the virus, bitter gourd mosaic is prevalent throughout the year and is very severe during summer season. The conditions which are congenial for the growth of bitter gourd are also found to be ideal for the multiplication of insect vector. Management of vectors through insecticides is one of the methods adopted by the farmers to overcome the disease even though it is not accepted ecologically. Use of resistant varieties is the best way to prevent viral diseases. As limited numbers of resistant varieties are available and breakdown of resistance occurs rapidly, management of bitter gourd mosaic is a very difficult task.

In view of the serious nature of the disease, the studies were undertaken on symptomatology of bitter gourd viruses and screening of accessions against each virus. The screening of bitter gourd accessions was attempted to identify the promising ones with resistance reaction to the virus.

Defense inducers are useful for management of plant diseases especially for viral diseases. Plants can be induced to develop enhanced resistance to pathogen infection by treatment with a variety of defense inducers. Hence, the role of defense inducers in management of bitter gourd mosaic was also evaluated.

The present investigation was carried out on the following aspects of the bitter gourd mosaic.

- Maintenance of pure cultures of bitter gourd virus
- Standardization of buffers for sap transmission of CMV and potyvirus
- Screening of bitter gourd accessions individually against CMV, potyvirus and BDMV
- Management of bitter gourd mosaic by plant defense inducers

Review of Literature

2. REVIEW OF LITERATURE

Bitter gourd is one of the most important cucurbitaceous vegetable crops cultivated in Kerala. During the last decade, mosaic disease has become the major limiting factor for the cultivation of the crop, especially during summer season. Cucumber mosaic virus, bitter gourd distortion mosaic virus and potyvirus were the major virus diseases of bitter gourd in Kerala. Yield loss up to 100 per cent has been reported if the infection occurs in early stages of crop growth (Giri and Mishra, 1986). So the better way for the management of viral disease is to develop resistant varieties, which is a time consuming process. Breakdown of resistance of the varieties due to rapid development of virus strains is also a problem. Hence, different approaches are to be followed for the management of viral diseases. Use of defense inducers is one such method for the management of viral diseases which is environmentally safe. Hence, a study was conducted for the management of bitter gourd mosaic by enhancing host resistance using defense inducers.

2.1 BITTER GOURD MOSAIC

Mosaic on bitter gourd is caused by different viruses. Nagarajan and Ramkrishanan (1971) reported cucumber mosaic virus for the first time from Coimbatore, India. The virus was transmitted by five different species of aphid vectors and the virus was tentatively named as bitter gourd mosaic virus. Khan *et al.* (2002) reported whitefly (*Bemisia tabaci*) transmitted begomovirus for first time in Lucknow, Uttar Pradesh, India. The symptoms consist of upward curling, shortening and distortion of leaves. Infected bitter melon fruits were stunted and deformed and disease incidence as high as 100 per cent. Occurrence of papaya ring spot virus in bitter gourd was reported by Chin and Ahamad (2007).

Bitter gourd is a natural host of watermelon silver mottle virus (Tokashiki and Yasuda, 1991), zucchini yellow mosaic potyvirus (Ullman *et al.,* 1991), tomato leaf curl New Delhi virus infecting bitter gourd was observed in Pakistan. Plants showing yellow blotch symptoms were reported from fields, with an average incidence of 60-70 per cent (Tahir and Haider, 2005), Tomar and Jitendra (2005) reported potyvirus in bitter gourd for the first time from western U. P. and association with severe mosaic and the isolate was identified as a strain of watermelon mosaic virus- 1, the association of Indian cassava mosaic virus (ICMV) with yellow mosaic disease of bitter gourd has been reported from Tamil Nadu, South India (Rajinimala and Rabindran, 2007) and squash vein yellowing virus and cucurbit leaf crumble virus (Adkins *et al.,* 2008).

2.2 CMV INFECTION IN BITTER GOURD

Cucumoviruses are icosahedral particles of 29 nm in diameter with 180 capsid protein subunits. The molecular weight of CMV is in the range of 5.8 to 6.7 million which consists of about 18% RNA and 82 per cent protein (CMI, 1979). The RNA is tightly packed by the protein shell, leaving a hollow core of about 110 A^{o} along the threshold axes (Smith *et al.,* 2000).

Nagarajan and Ramakrishanan (1971), reported the symptoms of CMV in naturally and artificially infected bitter gourd plants. In naturally infected plants symptoms were confined mostly to leaves. Infected plants were somewhat difficult to locate in the field as no severe damage was caused to the vines. The leaves in the secondary branches produced at the apical end of the plant showed symptoms of the disease. Symptoms on the leaves consisted of scattered small irregular yellowish patches. Some leaves showed vein clearing in one of two lobes on the leaf. Severely infected leaves showed reduction in the size with normal flowers and fruits.

On the artificially inoculated bitter gourd plants the symptoms reported was vein clearing starting from the top end of the lobes and progressed inwards. Leaves produced subsequent to primary infection showed filiformity and yellowing. Young developing leaves were completely destroyed and malformed with considerable reduction in their size. The number of lobes in a severely diseased leaf was reduced. Some of the leaves showed marked reduction in the development of lamina resulting in a shoestring effect. Flower production was normal but flowers were shed early. Very few fruits were produced which were normal without any symptom on them.

2.2.1 Host range

According to (Zitter and Murphy, 2009) CMV causes systemic infection in most host plants, but may remain symptomless in some crops like alfalfa. Symptoms of cucumber mosaic can vary greatly depending on the crop infected and the age of the plant when infection occurs. Singh *et al.* (1999) and Raj *et al.* (2002), has reported that *Chenopodium amaranticolar* produces chlorotic local lesions and it can be used as indicator host for CMV bio assay.

According to Parvin *et al.* (2007) cosmos (*Cosmos sulphureus*) is systemic indicator host of CMV. The mechanical inoculation of the virus was carried out using sodium phosphate buffer at pH 7.1. The inoculated plants developed mosaic accompanied with yellowing which was eventually visible as yellow mosaic symptom. The symptomatic leaves produced vein chlorosis along with mild curling. In many infected plants leaf distortion accompanied with shoestring were found as prominent symptoms. Necrosis of the infected leaves was also observed in severely infected plants. Leaf size of the infected plants was drastically reduced as compared to the healthy plants. The symptomatic plants produced small, twisted and deformed flowers.

Almost all cucurbits are susceptible to CMV, with symptoms varying in severity. Plants infected early in the season are severely stunted and leaves are malformed, and fruit are unmarketable because of pronounced rugosity (roughness) on the fruit surface. Infection of crops, such as muskmelon, show severely stunted growing tips and poor quality fruits. The yellow squash variety without precocious gene develop color breaking on the fruit, causing the fruit to show green blotchy patterns and these symptoms are absent in yellow squash varieties with the precocious gene. Pumpkin is another cucurbit, that when infected at any early stage, will express severe foliar mosaic and the fruit will show a mosaic pattern and would be unmarketable (Zitter, 2009). CMV infected pumpkin plants show light green mottled crinkled foliage, smaller than normal size (Zitikaite, 2011).

Zitter and Marphy (2009) had reported a detailed account on host range of CMV. According to him, almost all cucurbits are susceptible to CMV, with symptoms varying in severity. Plants infected early in the season were severely stunted and leaves were malformed and fruits were unmarketable because of pronounced rugosity on the fruit surface. Infection of veining crops, such as muskmelon, showed severely stunted growing tips and poor quality fruits.

Symptoms of CMV infection in pepper were varied with stage of infection. The initial symptoms include chlorosis of young leaves that may occur over the basal portion of the leaf or over the entire leaf. Oak leaf and ring spot patterns may develop on these leaves as the plant ages. As the new leaves emerge, these leaves develop a chlorotic mosaic pattern that tends to encompass the entire leaf. Leaves that develop subsequent to those expressing the chlorosis and chlorotic mosaic symptoms may have varied degrees of deformation including sunken inter venal lamina with protruding primary veins. These leaves also have a dull light green appearance as opposed to the dark green, rather shiny leaves of healthy pepper plants. Those plants

infected by CMV early in development express severe stunting, whereas plants infected at later stages of development may have little stunting. Pepper fruit may develop ring spotting and roughness leading to unmarketable fruit.

Tomato plants infected with CMV in the early stages are yellow, bushy and considerably stunted. The most characteristic symptom of CMV was filiformity or shoestring-like leaf blades. The symptoms caused by CMV can be transitory, with the bottom leaves or newly developed top leaves showing severe symptoms, while the middle leaves may appear almost normal. Severely affected plants produced few fruit, which were usually small, often mottled or necrotic, with delayed maturity.

CMV infection of spinach is often referred to as spinach blight. Typical symptoms include leaf chlorosis, which progress to cause severe blighting of the growing point and eventual plant death. In addition to chlorotic mottle, leaves can show narrowing, crinkling with vein distortion, and inward leaf roll.

Symptoms of CMV infection in bean consist of leaf curl, green mottle and blistering, and a zipper like roughness along the main veins involving only a few leaves. Foliar symptoms are most obvious and pod infection and loss is greatest when plants are infected before bloom. Early infected plants may yield no or few pods because CMV caused flower abortion and abnormal development. The pods are mostly curved, mottled and reduced in size. Plants may recover and resume normal growth with limited yield loss if plants are infected after bloom.

Chandrakar *et al.* (2013) reported that artificially inoculated CMV shows solid grey local lesion in *Gomphrena globosa*, necrotic local lesion in *Nicotiana glutinosa* L, *Nicotiana rustica* L, *Chenopodium murale* L, and brown local lesion in *Phaseolus vulgaris*, and distortions of leaves in *Carthamus tinctori*.

2.2.2 TRANSMISSION

According to Shukla and Govinda (2000) and Palukaitis and Garcia- Arenal (2003), *Myzus persicae* and *Aphis gossypii* are more efficient vectors for this virus.

Parvin *et al.* (2007) used 0.02M sodium phosphate buffer (pH 7.1) to transmit CMV from cosmos to *Gomphorina globosa*, marigold, pumpkin, datura, physalis, tobacco, pea and cowpea. In all the cases the inoculated plants developed symptoms within 7-10 days of inoculation.

Chandrakar *et al.* (2013) reported the mechanical transmission of CMV from cucumber to cucumber. The leaf samples from naturally infected cucumber plants showing mosaic, leaf distortion, leaf puckering, vein clearing symptoms were collected from field. These samples were macerated in 0.1M potassium phosphate buffer (pH 7.5) supplemented with 0.1% sodium sulphite in a ratio of 1:10 (w/v) using a sterile pestle and mortar. The filtrate was inoculated on healthy young cucumber var. Pune Khira at cotyledon stage by leaf rub method using carborundum. The inoculated plants developed symptom with 10 to 14 days after inoculation. CMV inoculated cucumber plants manifest yellowing, mosaic, leaf distortion, leaf puckering, vein banding and stunted growth.

For transmission and host range studies of banana CMV, mechanical inoculations were carried out by extracting banana tissues infected with CMV in 0.1 M phosphate buffer, pH 7.0 (1:2 W/V) containing 1% sodium sulphite. The infectious sap was applied to healthy *Commelina* sp, *Nicotiana glutinosa, Vigna radiata, Vigna mungo* and *Chenopodium* spp in addition to banana. Inoculated plants were maintained in the green house at 25-30°C for 30 days and the plants were inspected daily for symptom development. The results revealed that tobacco plants expressed the CMV symptom

after three months of inoculation. Transmission of CMV causes severe mosaic and leaf deformations in inoculated plants (Dheepa and Paranjothi, 2010)

CMV can transmit through planting material (Hsu *et al.*, 2000). Seed as a source of CMV has been reported in common chickweed (*Stellaria media*) and in 19 other plant species. In chickweed, the rate of transmission was as high as 40% in plants grown from infected seed. Other plant families with seed borne CMV (including crop plants) are amaranthaceae, brassicaceae and fabaceae. This seed transmission feature has proven to be important for the annual occurrence of CMV-infected legumes in commercial fields, and for the potential infection of green house crops growing close to chickweed plants. The seed borne characteristic increases the probability of survival of the virus in nature (Zitter and Marphy, 2009).

2.3 POTYVIRUS INFECTION IN BITTER GOURD

This is the largest genera of plant viruses (91species and 88 tentative species) and contains economically important viruses such as potato virus Y, bitter gourd yellow mosaic virus, plum pox virus, papaya ring spot virus, zucchini yellow mosaic virus and watermelon mosaic virus. Potyviruses have a worldwide distribution and are responsible for significant yield losses to cucurbit crops (Oanal *et al.*, 1999).

The members of the Potyvirus genus have non-enveloped rod shaped flexuous particles 680-900 nm long and 11- 13 nm in diameter, helix pitch 3.4-3.5 nm, encapsidating a genome of about 9.7 kb with multiple copies of a single protein species of 30-47 kDa. The genome of potyviruses is positive sense single stranded RNA of approximately 10000 nucleotides (Oanal *et al.*, 1999).

All members of the Potyviridae family form cylindrical inclusion bodies in infected cells, but they are unique in the diversity of inclusion bodies. The cylindrical inclusion bodies are formed by a virus-encoded protein and can be considered as the most important phenotypic criterion for assigning viruses to the Potyvirus group (Shukla *et al.,* 1991). Most of the Potyviruses induce cytoplasmic amorphous inclusion bodies and some form nuclear inclusions (Oana *et al.*, 1999).

2.3.1 Host range

According to Verma *et al.* (2006) bottle gourd plants were infected when inoculated mechanically with ZYMV and produced chlorotic spots, prominent veinal chlorosis followed by mosaic, vein banding and leaf distortion such as blistering and shoestring appearance. Under field condition, the characteristic symptoms of the disease are severe mosaic, blisters, enation and filiformism on leaves. It also causes mosaic and distortion on fruits making them unmarketable.

In addition to papaya, PRSV-W and P strain can also affect some cucurbit species/varieties and susceptibility according to the virus isolate (Capoor and Varma, 1958; Sureka *et al.*, 1977; Yeh *et al.*, 1984). Isolates of PRSV-W and ZYMV cause the common and yellow mosaic diseases, respectively, in many cucurbit species, dramatically reducing the yield, particularly for the most susceptible species/varieties, including zucchini squash (*Cucurbita pepo* L) (Rezende and Pacheco, 1998; Lecoq *et al.,* 2009). PRSV-P was geographically widespread but has a narrow host range within the plant families of Caricaceae, Chenopodiaceae and Cucurbitaceae. In the summers of 1999 and 2000, prominent vein clearing symptoms were observed on leaves of a common weed, cerasee (*Momordica charantia* L.), in papaya orchards of western Jamaica. Up on mechanical inoculation, vein clearing symptoms were

observed on cerasee and symptoms of PRSV infection were obtained on papaya (Chin and Ahamad, 2007).

PRSV induces variable symptoms in papaya and cucurbit cultivars, including vein clearing, mottling, malformed leaves, filimorphism, ring spots and streaks on fruits, stem and petioles, and stunting (CMI, 1984). The most commonly observed symptoms infected with PRSV on squash (*Cucurbita pepo*) were mosaic, malformation such as blisters and narrow leaf blades and malformed fruits (Omar *et al.*, 2011). PRSV from snake gourd produced chlorotic local lesions on leaves of *C. amaranticolor,* mosaic and chlorosis in *Luffa acutangula,* mosaic and leaf blisters in *Trichosanthes cucumerina* and *Lagenaria siceraria,* mosaic and leaf distortion in *Cucumis sativus* and *Cucurbita moschata* (Kumar *et al.*, 2014).

2.3.2 Transmission

ZYMV isolate of bottle gourd was mechanically transmitted to cucurbitaceae, chenapodeceae and solanaceous species. Sap inoculation was done by macerating leaf tissues with 0.01 M potassium phosphate buffer (pH 7.3), adding a pinch of celite powder to the extract and rubbing the extract on leaves of above mentioned species. The inoculated plants produced symptoms like, chlorotic local lesion in *C. amaranticolar,* mosaic and shoe-string in *C. sativus* and mosaic in *L. cylindrica* (Verma *et al.*, 2006).

The virus sap from field-grown squash tissue infected with ZYMV-Ca was diluted (1:1, w/v) in 0.02 M potassium phosphate buffer, pH 7.0, containing 1% celite (w/v) and inoculated onto 10 day old seedlings of yellow squash (*C. pepo* L.) and cantaloupe (*C.melo* L.), *C. amarnaticolor* and *L. acutalgula.* Inoculated plants had

systemic mosaic on *C. melo, C. pepo* and *L. acutalgula* and local lesion on *C. amarnaticolor* after 14 days of inoculation (Nametha *et al.,* 1985).

Young symptomatic squash leaves were ground in 0.2 M potassium phosphate buffer pH 7, containing 0.02 M sodium sulfite and carborundum was rubbed onto squash cotyledons and true leaves. The symptoms observed were vein clearing, mottling, malformed leaves, filimorphism, ring spots and streaks on fruits, stem and petioles and stunting (Omar *et al.,* 2011).

According to Owolabi *et al.* (2011) young symptomatic leaves from *C. moschata* were macerated in cold (0.03 ML^{-1} sodium phosphate buffer pH 8.0) and inoculated on cucurbitaceous host. General symptoms observed were mosaic, green vein banding, leaf malformation, rugosity, and reduced leaf size.

Potyviruses are transmitted mechanically by aphid's mouth parts in a non-persistent, non-circulative, stylet borne manner using a helper component protein (HC-Pro) which facilitates binding of virus particles to the aphid's maxillary stylet (Oanal *et al.,* 1999). Potyviruses infecting bitter gourd (PRSV and ZYMV) are transmitted by several species of aphids in a non-persistent manner (CMI, 1984; Desbiez and Lecoq, 1997). According to Chin and Ahamad (2007), high rate of potyvirus transmission by *A. gossypii* was obtained from *M. charantia* to papaya (77-83%), papaya to *M. charantia* (90-93%), and *M. charantia* to *M. charantia* (60-70%).

Out of 1400 squash seedlings tested for seed transmission of ZYMV-Ca, none gave a positive reaction for ZYMV-Ca in ELISA (Nametha *et al.,* 1985). ZYMV infecting cucumber was not seed transmitted (Glasa and Kollerova, 2007). PRSV in melon is not seed transmitted but transmit mechanically and also by aphids (Tripathi *et al.,* 2008).

2.4 BDMV INFECTION IN BITTER GOURD

Begomoviruses of family geminiviridae are emerging plant viral pathogens cause severe diseases in various crops in the tropical and subtropical regions (Brown, 2001). A number of serious diseases of cultivated crops of the fabaceae, malvaceae, solanaceae and cucurbitaceae families are caused by begomoviruses which are considered as a threat to their cultivation in India. Plants infected by begomoviruses do not recover, suffer serious yield losses and act as further source of inoculum, which is then picked up and spread by their vector whitefly (*B. tabaci*) to the crop and non crop species and thus continues their occurrence and spread year to year (Varma and Malathi, 2003; Jones and Usha, 2003).

Begomoviruses are transmitted by the *B. tabaci* and have two genomic components (bipartite) designated as DNA-A and DNA-B of ~2600–2800 nucleotides (Hanley-Bowdoin *et al.*, 1999). However, a number of begomoviruses isolated from tomato, cotton, chilli, okra and ageratum are monopartite consisting of single genomic DNA (DNA-A) (Navot *et al.*, 1991 and Dry *et al.*, 1993). Some monopartite as well as bipartite begomoviruses also carry satellite DNA molecules, called DNA-Beta and/or DNA-1, which are essential for the expression of disease symptoms (Saunders *et al.*, 2000; Jones and Usha, 2003; Briddon *et al.*, 2001 and Briddon *et al.*, 2004).

The association of begomovirus with bitter gourd was first reported in India from Kerala, by Mathew *et al.*, 1991. It was also reported from Uttar Pradesh by Khan *et al.* (2002). According to him the symptoms consists of upward curling, shortening, distortion of leaves, stunting of plants and deformation of fruits and the incidence was as high as 100 %. *B. tabaci* could transmit the BDMV associated virus from infected to healthy one (Khan *et al.*, 2002). The disease appeared in all stages of

the growth irrespective of crop season. The symptom first appeared in the newly formed leaves and rapidly spreads to other leaves on the same vine. The symptom caused by BDMV initially appeared as small chlorotic specks from the outer margins of leaves, which spreads rapidly to the whole leaf with typical mosaic symptom. As it progresses the infected leaves got distorted and caused reduction in leaf size. The shortening of internodes, clustering of leaves and reduction in number of flowers were appeared in severe infection. The early infection of BDMV led to stunted growth, clustering of leaves and sterility. However infection occurring after flowering resulted in small sized deformed fruits and more hairiness on vein (Arunachalam *et al.*, 2002).

. The details of begomoviruses occurring on bitter gourd reported from various places were summarized in table 1.

2.4.1 Host range

Mathew and Alice (2002) reported that *Melothria leiosperma,* a wild cucurbitaceous weed in Kerala, was easily infected by the BDMV through *B. tabaci* inoculation. Hence, *M. leiosperma* might act as an important collateral host of BDMV in Kerala. Field collected BDMV isolate was inoculated on different species of cucurbitaceae and solanaceae families and they produced characteristic symptoms. Leaf curl, stunting and mosaic on *Citrullus vulgaris , C. sativus, Melothria leiosperma* and chlorotic spot and mild mosaic in *Lagenaria siceraria, L. acutangula* were the symptoms produced in different host plants.

Table 1. Details of begomovirus occurring on bitter gourd

Locations	Disease symptom	Virus identified	Reference
Kerala	Mosaic	Bitter gourd distortion mosaic virus	Mathew *et al.*, 1991
Lucknow	Leaf curl	Tomato leaf curl virus	Khan *et al.*, 2002
Lucknow	Yellow mosaic	Gemini virus	Raj *et al.*, 2005
Tamilnadu	Yellow mosaic	Bitter gourd yellow mosaic virus	Rajinimala *et al.*, 2005
Tamilnadu	Mosaic	Indian cassava mosaic virus	Rajinimala and Rabindran, 2007
Lucknow	Mosaic	Pepper leaf curl Bangladesh virus	Raj *et al.*, 2010
Gorakhpur	Yellow mosaic	Tomato leaf curl New Delhi virus	Tiwari *et al.*, 2010, Tiwari *et al.*, 2011

2.4.2 Transmission

Whiteflies in the *Bemisia* and *Trialeurodes* genera were the major virus vectors. In the genus *Bemisia*, only *B. tabaci* has been shown to be a vector whereas in the *Trialeurodes* genus, *T. vaporariorum*, *T. abutilonea* and *T. ricini* transmit the virus. Nymphs and adults of *B. tabaci* insert their proboscises into the leaf, penetrating the phloem and withdrawing sap during feeding. It is during this process the plant viruses are acquired. After acquisition by whiteflies, begomoviruses are persistent and are retained for periods ranging from a few weeks to life (Duffus, 1987). Begomoviruses are the most numerous of the *B. tabaci*-transmitted viruses and cause crop yield losses of between 20% and 100% (Brown and Bird, 1992).

Giri and Mishra (1986) reported that BDMV will transmit by *B. tabaci* to members of cucurbitaceae and solanaceae families. 2-3 weeks old seedlings of cucurbits and solanaceous plants were inoculated with BDMV using *B. tabaci*. The adult whiteflies were given 12 h of each acquisition access period and inoculation access period in specially made PVC microcages. After inoculation the plants were sprayed with 0.05 per cent Decis 2.8 EC to kill the insects and kept in the glass house for 6-8 weeks for symptom development. He observed that, incubation period of the virus in the hosts was varied from 8-20 days depending on the species of plants.

The transmission of BDMV by whiteflies was also reported by Rajinimala *et al.* (2005). According to her minimum of five whiteflies were required to transmit the virus, however, hundred per cent transmission of BGYMV disease was obtained when 45 whiteflies were released per plant. Twelve hours of acquisition access period and inoculation access period were required for the *B. tabaci* to transmit the disease. The percentage of transmission increased with increase in both acquisition and inoculation period (Rajinimala *et al.*, 2005). Zacharia (2006) has also standardized artificial transmission of BDMV.

2.5 VARIETAL SCREENING

Arunachalam (2002) has screened bitter gourd germplasm under field condition against BDMV and reported that IC 68296, IC68335, IC 682638, lC 68275, IC 68250A, IC 85620, IC 6831 2, lC 68285, IC68272 as highly resistant. IC 68330, IC 68338, IC 45339, IC 68310, IC 85618, IC 85633, IC 50523, IC 68286, IC 68232 as resistant variety. IC 68306, IC 85603, IC 444, IC 44436A, IC 85608 , IIHR-89, MDU local, IC 32817 IC 85619/1[85606), IC 45341 ,IC 45351, IC 68230, IC 68295, IC 50520A, IC 68345 as moderately resistant. IC 45346, IC 85611, IC

43261, IC 68294, IC 4441, Co 1, IC 683428, IC 85614, IC 68343, IC 85610, IC 68322, VKV 13s, IC 85605, IC 85616, IC 85624, KMK2, IC 68345, IC 85629, IC 68237, Preethi, IC 44419, IC 683 as moderately susceptible and Priyanka, IC 68326, IC 44-1.14, KMKI, PBl, Priya, IC 50516, IC 50527, IC 6825, IC 68292, IC 44438, VKV 134, IC 65626 as susceptible.

Thangamani *et al.* (2011) has conducted varietal screening of bitter gourd against CMV under natural condition. According to her, Kerala Rakshuse (KR) X USL and Preethi X MC 30 were resistant. CO 1, Preethi, KR, MC 30, Priyanka X CO 1, MC 105 X MC 10 were moderately resistant. Green long, Uchha small long (USL), Uchha bolder (UB), MC 105, MC 10, GL X Preethi, Preethi X CO1 and Preethi X UB were moderately susceptible. Priyanka, CO 1 X MC 10, MC10 X MC 105 were susceptible. MC 10 X GL was highly susceptible.

2.6 MANAGEMENT OF BITTER GOURD VIRUSES BY ENHANCING HOST RESISTANCE

2.6.1 Systemic acquired resistance

Plants can be induced to develop enhanced resistance to pathogen infection by treatment with a variety of abiotic and biotic inducers. Biotic inducers include infection by necrotizing pathogens and plant-growth promoting rhizobacteria, and treatment with non pathogens or cell wall fragments. Abiotic inducers include chemicals which act at various points in the signaling pathways involved in disease resistance, as well as water stress, heat shock, and pH stress. In the field, expression of induced resistance is likely to be influenced by the environment, genotype, and crop nutrition (Walters *et al.*, 2005).

The phenomenon of induced resistance in plants against disease was first reported by Bernad (1909). Later the systemic resistance in plants was reported by Chester (1933). However, the term systemic acquired resistance (SAR) was officially proposed by Ross (1961), who reported the resistance in tobacco plants following local infection with tobacco mosaic virus. Resistance through defense mechanism, is based on the expression of latent genetic information present in plants and is biologically safe (Kuc, 1987; Schonbeck *et al.*, 1993; Schneider *et al.*, 1996 and Kuc, 2001).

SAR is an inducible defense mechanism that plays a central role in disease resistance. A multitude of factors are reported to induce resistance in plants: pathogens (fungi, bacteria, viruses) causing hypersensitive necrotic reaction (HR); avirulent and attenuated pathogenic strains; pests (insects, nematodes); elicitors of biotic origin; abiotic elicitors, i.e. chemical products, such as benzothiadiazole (BTH), β-aminobutyric acid (BABA), 2-6, Dichloro iso nicotinic acid (INA), salicylic acid, inorganic salts, etc. (Cohen *et al.*, 1994; Kessman *et al.*, 1994; Schneider *et al.*, 1996; Siegrist *et al.*, 1997; Benhamou and Picard, 1999 and Kuc, 2001).

SAR is dependent on the production of salicylic acid (SA) in response to infection (Gaffney *et al.*, 1993) and is associated with the accumulation of pathogenesis-related proteins (PRs) both in the inoculated and in distant leaves (Ryals *et al.*, 1996, Loon and Kammen, 1970). Because exogenous application of SA induces both SAR and PRs, the latter are commonly taken as markers of the induced state. SAR is non-specific with respect to both the inducing and the challenging pathogen. Thus, a primary infection of cucumber with the fungus *Colletotrichum lagenarium* or with tobacco necrosis virus leads to enhanced resistance against various foliar and root diseases caused by fungi, bacteria, and viruses (Kuc, 1982).

A span of time is necessary for signals to be translocated to non-inoculated tissues and for triggering development of defense potential in the tissues (Kuc, 1987; Schneider *et al.*, 1996; Benhamou and Picard, 1999 and Kuc, 2001). The rise in SA parallels PR-1 protein induction in tobacco mosaic virus (TMV)-resistant Xanthi-nc tobacco cultivar. When leaves of Xanthi-nc tobacco were excised 24 hr after TMV inoculation and exudates from the cut petioles were collected, the increase in endogenous SA in TMV-inoculated leaves paralleled SA levels in exudates. A computer model predicts that SA should move rapidly in phloem (Yalpani *et al.*, 1991). Characterization of the biochemical changes associated with the induced resistance revealed a correlation between the establishment of the resistance and the accumulation of salicylic acid in the plant (Sticher *et al.*, 1997).

Mptraux, (1990) suggest that salicylic acid could function as the endogenous signal in the transmission of SAR in cucumber. SAR is mediated by an endogenous signal that is produced in the infected leaf and translocated in the phloem to other plant parts where it activates resistance mechanisms. According to Yalpani *et al.* (1991) in induced cucumber and tobacco plants, salicylic acid played a critical role in the conventional systemic acquired resistance to control the diseases of cucurbits. Raskin (1992) considered salicyclic acid as the plant hormone and found it to be helpful in reducing the diseases caused by fungi, bacteria and as well as viruses.

A study was conducted by Rajinimala *et al.* (2009) on management of BDMV using biotic and abiotic defense inducers viz., *Pseudomonas chlororaphis, P. fluorescens*-A, *Bougainvillae spectabilis* leaf extract, Bion 200 ppm and insecticide monocrotophos. All the treatments, except *Pseudomonas* spp were applied four times starting from 15 DAS at 15 days interval and the *Pseudomonas* spp was applied as seed treatment. The BDMV was artificially inoculated at 15 days after

sowing. Observation on the per cent disease incidence (PDI) and the growth parameters were recorded at 15 days intervals. The results revealed that the *B. spectabilis* treated plots recorded the least disease incidence of 33.33%. Other treatments recorded 66.66 % where as control recorded 100 % incidence of BDMV at 75 DAS. Louis *et al.,* (2011) has studied the effect of resistance inducing substances viz., mosaic affected bitter gourd plant tissue, ash of mosaic affected bitter gourd plant tissue, plumbago and salicylic acid for the management of bitter gourd mosaic by prophylactic and curative application. It was reported that the lowest PDI and PDS was found in salicylic acid (1 ppm) treated plants.

Induced resistance through external application of SA to control CMV in squash was carried out by Naylor *et al.* (1998). The newly emerged seedlings of squash were watered with 2 mM SA or water for 5 days prior to inoculation with CMV at 50 or 5 µg/ml. Upper, non inoculated leaves were monitored for subsequent 10 days for the development of systemic symptom. Salicylic acid treatment was able to delay or prevent the onset of symptoms in plants inoculated with the lower inoculum concentration. Even in plants inoculated with the higher virus concentration, pre treatment with SA was still able to slow the progress of symptom development. Thus, in squash, SA can delay the onset of CMV induced disease even in plants inoculated with relatively high concentrations of purified virus.

The potential of Benzo-(1,2,3)-thiazole-7-carboxylic acid *S*-methyl ester (BTH) to trigger SAR in tomato *(Lycopersicon esculentum.* Mill cv. Vollendung) plants against yellow strain of cucumber mosaic virus (CMV-Y) was investigated. Application of BTH, as a drench, 7 days before inoculation with the virus, protected plants against the necrosis caused by CMV-Y. The resistance was evident as decreased disease incidence and severity in BTH-treated plants. Twenty-one days

after challenge inoculation with CMV-Y, the disease incidence in plants with SAR did not exceed 12.5% whereas, 91.7% of control plants were severely infected. The development of primary disease symptoms in BTH-treated plants was delayed for 7 days. The disease spread rapidly in control plants and by the end of the experiment almost all the control plants showed severe mosaic and leaf necrosis. Results of enzyme-linked immunosorbent assay (ELISA) indicated that BTH treatment affected virus replication in protected leaf tissues. Analysis of the newly developed leaves of BTH-treated plants for virus antigen revealed that symptomless plants failed to support the replication of CMV-Y and the concentration of the virus in these plants was similar to that in uninoculated control plants. A single application of BTH to the roots of young tomato plants protected them against one of the most serious strains of CMV that is known to cause severe necrosis and yield loss in different countries (Galliteli *et al.,* 1991).

Shakoor *et al.* (2011), used salicylic acid against seed-borne fungi of bitter gourd at the concentrations of 20, 30, 40 mg/10 ml of water. The seed sample was naturally infected with six seed-borne fungi such as *Aterneria alternata, Myrothecium roridum, Fusarium solani, Rhizopus* spp. *Aspergillus niger and A. flavus.* Application of salicylic acid controlled all the pathogens at dose of 30 and 40 mg/10ml. Only *A. alternata, M. oridum and A. flavus* could survive after treatment with salicylic acid at 30 mg/10 ml.

2.6.2 Induced systemic resistance

The term "induced systemic resistance" (ISR) was introduced to designate the resistance induced in plants by inoculation of roots with non-pathogenic rhizobacteria. This novel type of induced resistance was first described in Arabidopsis plants, inoculated with the root-colonizing non pathogenic bacteria *Pseudomonas fluorescens*. Leaves of tomato plants exhibited resistance against the

bacterial leaf pathogen *Pseudomonas syringae pv. tomato* (Pieterse *et al.*, 1998). Treatment of plants with selected strains of plant growth-promoting rhizobacteria (PGPR) can induce systemic resistance in carnation, cucumber, radish, tobacco and arabidopsis as evidenced by an enhanced defensive capacity upon challenge inoculation with a pathogen (Karthikeyan, 2005).

Pathogens induce SAR and non-pathogenic rhizobacteria inducing ISR and have different signal-transduction pathway SAR depends on the accumulation of SA and activation of PR-genes, but ISR depends on perception of ethylene and jasmonic acid. SAR associated SA production induces pathogenesis-related proteins (PRs), but no accumulation of PRs in ISR. Rhizobacteria activates a signal-transduction pathway different from the one leading to SAR and requires perception of ethylene and jasmonic acid rather than salicylic acid, even though both pathways result in a phenotypically similar enhanced defensive capacity expressed upon challenge inoculation. Both ethylene and JA are produced by and act as hormones in plants. Recognition of the inducing bacteria by the roots may result in a change in ethylene and JA production or metabolism in the plant (Loon *et al.*, 1998).

To protect cucumber plants, PGPR strains have been applied by seed treatment, cotyledon injection, or as a soil drench. The bacteria could invade the plant vascular system and be carried to the aerial parts of the plants and induced resistance against the pathogens (Kluepfel, 1993).

The *P. fluorescens* strain Pf1 was assessed for its efficiency in induction of defense genes against leaf blight fungus in resistant and susceptible varieties of onion under green house conditions. The bulbs of onion were dipped in water containing talc formulation (20 gm /1) for 2 hr, and then planted in pots at the rate of four bulbs per pot. Twenty five days after sowing, foliar application with 10 ml of bacterial

suspension was done and after one day, the plants were challenge inoculated with a conidial suspension of *A. palandui.* Leaf blight disease index of *P. fluorescens* treated plants was 19.9 and 27.1%, respectively in the resistant and susceptible cultivars whereas that of control plants was 27.6 % and 37.3 % respectively (Karthikeyan, 2005).

Materials and Methods

3. MATERIALS AND METHODS

The present study on the "Management of bitter gourd mosaic by enhancing host resistance" was carried out during 2014-2015 in the Department of Plant Pathology, College of Horticulture, Vellanikkara. The management was mainly concentrated on major mosaic causing viruses of bitter gourd *viz*., cucumber mosaic virus (CMV), potyvirus and bitter gourd distortion mosaic virus (BDMV). The details of materials used and techniques adopted for the study are described below.

3.1 SEPARATION OF VIRUS CULTURES

As mixed infection of bitter gourd plants by different viruses occurs in field, it is difficult to identify the disease and screen the resistance of varieties. Hence the viruses were separated using different systemic indicator host plants.

3.1.1. Separation of CMV from mixed infection

The ornamental crop *Cosmos sulphureus,* was used as a systemic indicator host for separation of CMV from mixed infected field sample. Young leaves of bitter gourd plants having CMV symptom were collected from field, washed with tap water, dried with blotting paper and weighed for the preparation of standard extract. Ten ml of 0.1 M potassium phosphate buffer and ten gm of the infected leaves were added into a chilled mortar and ground with pestle. After thorough grinding, the homogenized leaf sap was filtered through double layered muslin cloth to get filtered extract. A pinch of carborandum powder (600 mesh) was added to the extracted sap and it was swabbed using a cotton pad soaked in the standard extract, on the leaves of seven days old *C. sulphureus*. Swabbing was done only in one direction that was from petiole to apex of the leaf by supporting it from below using a cardboard. After

five minutes of inoculation, the leaves were washed with sterile distilled water to remove excessive inoculum and extraneous particles. The plants inoculated with the buffer without the infected sap was served as control. The inoculated plants were kept in the insect proof net house and observed daily for the development of symptoms.

3.1.2. Separation of potyvirus from mixed infection

For separation of potyvirus, *Carica papaya* was used as systemic indicator host. Seven days old papaya seedlings were used for mechanical inoculation of the virus inoculum. Young leaves of bitter gourd plants having potyvirus symptom were collected from field and the standard extract was prepared and inoculated on the leaves of seven days old papaya seedlings as mentioned in 3.1.1.

3.1.3. Separation of BDMV from mixed infection

BDMV belongs to begomovirus group which is transmitted only through whiteflies. So the separation of the virus was carried out using *B. tabaci* (whitefly) as a vector.

3.1.3.1. Rearing of whiteflies

The whiteflies were collected using test tubes from brinjal field of Department of Olericulture, College of Horticulture, Vellannikkara. Whitefly culture was maintained on healthy brinjal seedlings raised in polythene cover and kept in insect proof cages. Plants were periodically replaced with healthy young plants for the proper build up of whitefly population.

3.1.3.2. Separation of BDMV

Whitefly transmission was adopted for the separation of BDMV from the infected field sample. Infected twig of bitter gourd plants exhibiting characteristic symptoms of BDMV like mosaic, puckering, leaf malformation and profuse hairy growth were collected from the field. Healthy whiteflies collected from the whitefly culture were given an acquisition access period of 24 h on infected twigs in an insect proof cage. After 24 h, infected twigs were replaced by healthy bitter gourd seedlings. Viruliferous whiteflies were given an inoculation access period of 24 h on healthy bitter gourd seedlings at the rate of 10 whiteflies per plant and later the whiteflies were killed by spraying quinalphos.

3.2 MAINTENANCE OF VIRUS CULTURE

The pure cultures of CMV and potyvirus obtained in *C. sulphureus* and *C. papaya* respectively were subsequently transferred to healthy bitter gourd seedlings by mechanical inoculation. Periodical sap transmission was carried out on bitter gourd seedlings for the maintenance of CMV and potyvirus. The pure cultures of BDMV obtained in bitter gourd were maintained by periodical whitefly transmission on healthy bitter gourd seedlings.

3.2.1 DAC ELISA for confirmation of virus

DAC ELISA was performed as per the standard procedure to confirm the presence of the virus in the indicator plants and back inoculated bitter gourd seedlings. This was done at the virus indexing laboratory of Banana Research Station, Kannara using antiserum of banana CMV and banana potyvirus (bract mosaic).

3.3 SYMPTOMATOLOGY

Symptomatology of major types of bitter gourd mosaic viruses *viz.*, CMV, potyvirus and BDMV was studied under natural and artificial condition. Symptoms under natural condition were observed and recorded from field grown bitter gourd plants and symptoms under artificial condition were recorded from bitter gourd plants inoculated mechanically (CMV and potyvirus) and using whiteflies (BDMV).

3.4 STANDARDIZATION OF BUFFER FOR SAP TRANSMISSION

The mechanical transmission of sap transmissible viruses *viz.*, CMV and potyvirus was standardized with different buffers at three pH levels *viz.*, 7.0, 7.2 and 7.4. The compositions of buffers used are given in Appendix I.

Table 2. Buffers used for sap transmission of CMV and potyvirus of bitter gourd.

Sl. No.	Buffer (0.1 M)	pH
1	Potassium phosphate buffer	7.0, 7.2 and 7.4
2	Sodium phosphate buffer	
3	Citrate buffer	
4	Acetate buffer	
5	Boric acid buffer	

The standard extract was prepared by crushing young leaves of bitter gourd leaves with pure cultures of CMV and potyvirus with each buffer at different pH. Buffer volume equal to the weight of the leaves was added into a chilled mortar and the leaf sample was ground with pestle to get standard extract. After thorough grinding, the homogenized leaf pulp was filtered through double layered muslin cloth to get filtered standard extract. A pinch of carborandum powder (600 mesh) was

added to the extracted sap. Cotton pad soaked in standard extract was rubbed on the primary leaves of 10 days old seedlings of bitter gourd variety Preethi, in one direction from the petiole to the apex of the leaf by supporting it from below with a cardboard. After five minutes of inoculation, test plants were washed with distilled water to remove excessive inoculum and extraneous particles. Ten numbers of plants were inoculated for all buffers at each pH. The plants inoculated with buffer without the infected sap was served as the control. The inoculated plants were kept in insect proof net house and observed daily for development of symptoms.

3.5 VARIETAL EVALUATION FOR HOST RESISTANCE

The genotypes of bitter gourd available in NBPGR, Thrissur, Kerala Agricultural University released variety, commercial hybrids and farmers varieties were collected (Table 3) for the evaluation of resistance to bitter gourd mosaic viruses. Resistance to CMV and potyvirus was evaluated by sap transmission and to BDMV was evaluated by whitefly transmission. Five plants of each accession were inoculated with CMV, potyvirus and BDMV individually and were kept under insect proof net house. The plants were observed daily for the development of symptoms.

The disease incidence and severity was recorded after 14 and 21 days of virus inoculation for CMV and poty and after 10 days for BDMV.

Table 3. Bitter gourd accessions evaluated for resistance to mosaic viruses.

Sl. No.	Accessions	Source
1	Preethi	Department of Olericulture, College of Horticulture, Vellanikkara.
2	Biliagala	Karnataka
3	White long	
4	Green long	
5	TCR2	NBPGR, Thrissur.
6	TCR39	
7	TCR53	
8	TCR76	
9	TCR103	
10	TCR149	
11	TCR179	
12	TCR196	
13	TCR202	
14	TCR285	
15	TCR364	
16	TCR380	
17	TCR416	
18	TCR463	
19	TCR471	
20	TCR492	
21	TCR493	
22	TCR494	

The disease severity was recorded using standard scoring scale of the virus after 14 and 21 days of inoculation for CMV and potyvirus and after 10 days of

inoculation for BDMV. The disease severity of CMV and potyvirus was scored using the 0-5 scale (Song *et al.*, 2013) with some modification (Table 4) and that of BDMV was scored using the 0-5 scale of Arunachalam (2002) with some modification (Table 5).

Table 4. Disease severity scale for CMV and potyvirus (Song *et al.*, 2013)

Description of symptoms	Score
No symptom	0
Very mild mosaic specks	1
Mosaic symptom with 1-10 % reduction of leaf area of top 25 % leaves	2
Mosaic symptom with 10-25 % reduction of leaf area of top 25 % leaves	3
Mosaic symptom with 25-50 % reduction of leaf area of top 25 % leaves	4
Mosaic symptom with more than 50 % reduction of leaf area of top 25 % leaves	5

Table 5. Disease severity scale for BDMV (Arunachalam *et al.*, 2002)

Description of symptom	Score
No symptom	0
Minute chlorotic specks/patches on leaf	1
Wide area of mosaic symptom on whole leaf without distortion of top 25 % leaves	2
Distortion and reduction of about 25 % of the normal leaf area of top 25 % leaves	3
Distortion and reduction of about 25 -75 % of the normal leaf area of top 25 % leaves	4
Distortion and reduction of more than 75 % of the normal leaf area of top 25 % leaves	5

The per cent disease incidence (PDI) and per cent disease severity (PDS) was worked out as given below.

Per cent disease incidence = Number of plants infected X100
Total number of plants observed

Per cent disease severity = Sum of all numerical ratings X 100
Total number of plants observed X
Maximum disease grade

Using PDI and PDS the coefficient of infection (CI) was calculated as given below.

Coefficient of infection = PDI x PDS
100

Based on CI, the genotypes were categorized into six groups, as given by Ravishankar, 2000.

CI	Disease reaction
0- 5	Highly resistant (HR)
5.1- 10	Resistant (R)
10.1- 20	Moderately resistant (MR)
20.1- 40	Moderately susceptible (MS)
40.1- 70	Susceptible (S)
70.1 – 100	Highly susceptible (HS)

3.6 EFFECT OF DEFENSE INDUCERS ON GERMINATION OF BITTER GOURD SEEDS

Fifteen seeds of bitter gourd variety Preethi was soaked overnight in different defense inducers viz., 25 ppm salicylic acid, 0.1 % barium chloride and 2 % *Pseudomonas fluorescence* along with control in water. The overnight soaked seeds were sown in poly bags filled with 1:1:1 potting mixture. The germination of each

treatment was recorded on eighth day of sowing and per cent germination was worked out.

3.7 MANAGEMENT OF BITTER GOURD MOSAIC BY ENHANCING HOST RESISTANCE

Field experiment for the "Management of bitter gourd mosaic by enhancing host resistance" was conducted at COH, Vellanikkara during February 2015 to May 2015. The experimental details were as follows:

Season - February 2015 to May 2015
Varieties – White long (moderately resistant) and Preethi (susceptible)
Design – Randomized block design
Replications – Three
Treatments – Eight
Number of plants/ replication – Five
Spacing – 0.75 X 1.5 m

Land preparation

The land was ploughed and levelled. Ten days old seedlings of bitter gourd was planted in the channels at 0.75 m apart, and at 5 cm depth. Manures and fertilizers were applied as per Package of Practice, KAU 2011. The quantity of manures and fertilizers used were 20 t/ha FYM, 35:25:25 kg N, P_2O_5 and K_2O as basal and 35 kg N as top dressing at 15 and 30 days after planting. General view of experiment plot given in plate1.

Plate 1. General view of experimental field

Table 6. Treatment details of field experiment

Treatments	Treatment details	Method of application
T_1	Control, moderately resistant variety – White long	--
T_2	Salicylic acid, 25ppm	Seed treatment and four foliar sprays, at 15 days interval starting from 20 days of sowing.
T_3	Barium chloride, 0.1%	
T_4	*Pseudomonas fluorescens,* 2%	
T_5	Control, susceptible variety – Preethi	--
T_6	Salicylic acid, 25ppm	Seed treatment and four foliar sprays, at 15 days interval starting from 20 days of sowing.
T_7	Barium chloride, 0.1%	
T_8	*P. fluorescens,* 2%	

Observation

Incidence of disease was recorded and severity was scored using 0-5 scale after five, ten and fifteen days of each spray. The fruits were harvested at regular intervals and yield was recorded.

3.8 STATISTICAL ANALYSIS

Data was analyzed statistically using the statistical package MSTAT Freed (1986).

Results

4. RESULTS

The results of the study "Management of bitter gourd mosaic by enhancing host resistance" are presented in this chapter under the following heads.

4.1 SEPARATION OF VIRUS CULTURES

4.1.1. Separation of CMV from mixed infection

The ornamental crop summer cosmos (*Cosmos sulphureus),* was used as a systemic indicator host for separation of CMV from the mixed infected bitter gourd field samples. The virus infected leaf samples were collected and were crushed using 0.1M potassium phosphate buffer to prepare the standard extract. It was mechanically inoculated on young leaves of seven days old cosmos seedlings. Inoculated plants were maintained under insect proof net house condition for development of symptoms.

Initial symptoms were developed on newly emerging leaves of the virus inoculated plants after 21 days of inoculation. Mosaic accompanied with yellowing was the first symptom and this was followed by vein clearing and mild curling. Leaf distortion accompanied with shoestring appearance was found as prominent symptoms in later stages of infection. Necrosis of the infected leaves was also observed in severely infected plants. Leaf size of the infected plants was drastically reduced as compared to the healthy plants (Plate 2a). On the basis of the observed symptoms it was concluded that the summer cosmos plants act as a systemic indicator host for CMV.

4.1.2 Separation of potyvirus from mixed infection

Carica papaya plants were used as a systemic indicator host for separation of potyvirus from field infected bitter gourd samples. Virus infected samples were

collected from bitter gourd plants and were crushed with 0.1M potassium phosphate buffer to prepare the standard extract which was mechanically inoculated on young leaves of seven days old papaya seedlings. Inoculated plants were maintained under insect proof net house condition for development of symptoms.

Initial symptoms were observed after 15 days of inoculation on newly emerging leaves. Mosaic, down ward puckering of leaves and stunted growth of plants were the symptoms observed (Plate 2b).

4.1.3 Separation of BDMV from mixed infection

Whitefly transmission was carried out as mentioned in 3.1.3 with 24 h acqusition access period and 24 h of inoculation access period for separation of BDMV from the infected field samples to healthy bitter gourd seedlings. Cent per cent transmission was observed in the inoculated bitter gourd seedlings. Initial symptom was observed after 10 days of inoculation. The symptoms produced were mosaic, puckering of leaves, reduced leaf size, profuse hairy growth on twigs and stunted growth of plant.

4.2 MAINTENANCE OF VIRUS CULTURE

The pure culture of CMV and potyvirus obtained from *C. sulphureus* and *C. papaya* were mechanically inoculated to healthy seedlings of susceptible bitter gourd variety Preethi.

The CMV inoculated bitter gourd seedlings developed symptom after 10 days of inoculation and the symptoms observed were vein clearing, puckering, deformation and reduced leaf size. Leathery leaf and marginal leaf rolling was also observed in someplants.

Vein clearing

Shoestring

a. Symptoms of CMV on *Cosmos sulphureus*

Puckering

Mosaic

b. Symptoms of potyvirus on *Carica papaya*

Plate 2. Symptom of CMV on *Cosmos sulphureus and* potyvirus on *Carica papaya*

the potyvirus inoculated bitter gourd seedlings produced symptom after 13 days of inoculation and the symptoms observed were vein clearing, puckering, deformation, and reduced leaf size.

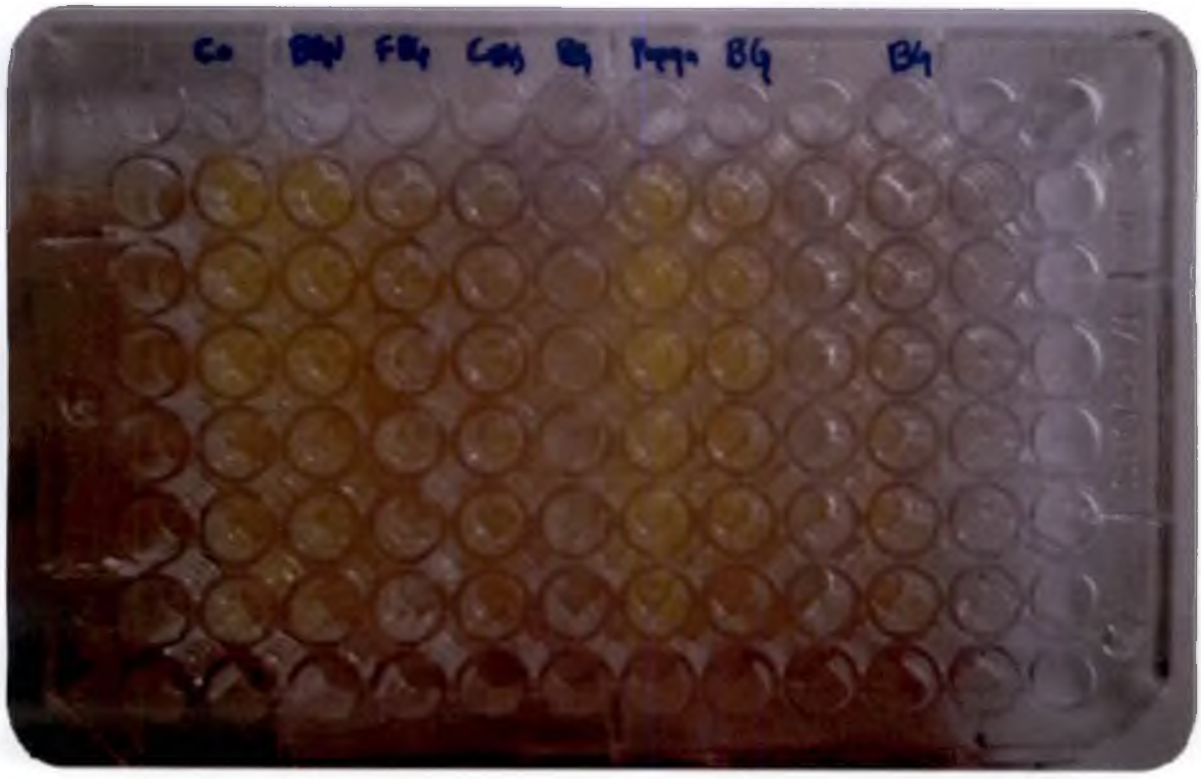
The pure cultures of CMV and potyvirus were maintained in bitter gourd by periodical sap transmission and that of BDMV was maintained in bitter gourd by periodical whitefly transmission.

4.2.1 DAC ELISA for confirmation of virus

DAC ELISA was performed to confirm the presence of viruses using infected sap of the indicator plants and the back inoculated bitter gourd seedlings using banana CMV and banana potyvirus (bract mosaic) antiserum. The absorbance value of infected cosmos and bitter gourd samples were 0.381 and 0.374 where as that of healthy were 0.163 and 0.153 respectively. Similarly absorbance value of infected papaya and bitter gourd samples were 0.608 and 0.328 where as that of healthy were 0.272 and 0.162 respectively. Plate 3.

Table 7. DAC ELISA reaction

Sl. No.	Virus and host plant	Mean absorbance value at 405 nm (infected / healthy)
1	CMV from cosmos	0.381/0.163
2	CMV from bitter gourd	0.374/0.153
3	Potyvirus from papaya	0.608/0.272
4	Potyvirus - bitter gourd	0.328/0.162



C – *Cosmos sulphurus*
BG – Bitter gourd
P – *Carica papaya*

Plate 3. DAC ELISA reaction

4.2 SYMPTOMATOLOGY

Symtomatology of major types of bitter gourd mosaic viruses was studied under natural condition from field grown bitter gourd plants and under artificial condition from bitter gourd plants inoculated through sap (CMV and potyvirus) and whitefly (BDMV).

The symptom of CMV on naturally infected bitter gourd plants were confined mostly to leaves. The young leaves in the secondary branches of the plant showed symptoms of the disease. It consist of scattered small irregular yellow patches. Some leaves showed vein clearing symptom. Severe infection caused reduction in leaf size. Leathery appearance and down ward rolling of leaf margins was also a prominent symptoms of the virus. Fruits produced were normal without any symptom (Plate 4).

In artificially inoculated plants the symptom was observed after 10 days of inoculation as vein clearing. Puckering, filiform leaves, reduced leaf size, leathery leaf and downward rolling of leaf margins were observed subsequently (Plate 5).

The symptoms of potyvirus were similar in natural and artificial conditions. The symptoms observed in infected plants were vein clearing, filiform leaves, reduced leaf size and yellowing. The flowering was reduced but the fruits produced were normal without any symptom (Plate 6 and Plate 7).

The symptoms of BDMV infection was appeared in all stages of plant growth. It was first appeared in the newly formed leaves and rapidly spreads to other leaves on the same vine. The symptom was initially appeared as small chlorotic specks on the outer margins of leaves, which spread rapidly to the whole leaf. Later the infected leaves got distorted and reduced in size. In severe case, enhanced hairy growth was

observed on vines and leaves. Shortening of internodes and clustering of leaves were also observed. The early infection of BDMV led to stunted growth, clustering of leaves and sterility of plants. However infection occurring after flowering resulted in small sized and deformed fruits (Plate 8 and Plate 9).

4.4 STANDARDIZATION OF BUFFER FOR SAP TRANSMISSION

The standardization of buffer for sap transmission of both CMV and potyvirus of bitter gourd was carried out using susceptible variety Preethi with five different buffers at three pH, viz., 7.0, 7.2 and 7.4. The disease incidence was observed after 14 days and the results are presented in Table 8 and Table 9.

Table 8. Standardization of buffer for sap transmission of CMV

Sl. No.	Buffer (Molarity - 0.1 M)	Mean per cent disease incidence		
		pH 7.0	pH 7.2	pH 7.4
1	Potassium phosphate buffer	90	60	80
2	Sodium phosphate buffer	60	60	50
3	Citrate buffer	70	50	70
4	Acetate buffer	50	20	40
5	Boric acid buffer	10	30	20

Of the different buffers tested for the sap transmission of CMV, potassium phosphate buffer at pH 7.0 showed the maximum disease incidence (90 %) followed by pH 7.4 with 80 % disease incidence. The minimum incidence of 10 % was recorded in Boric acid buffer at pH 7.

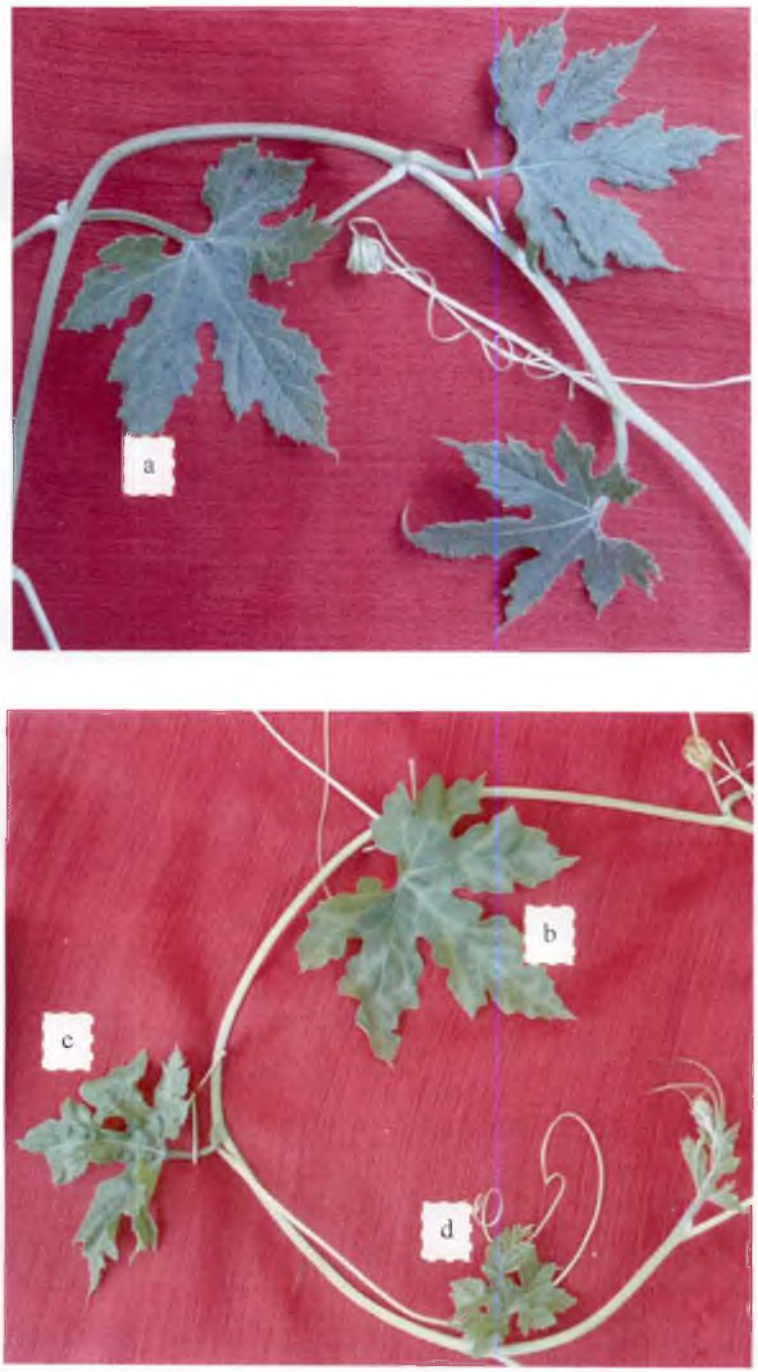
Mosaic

Yellowing

Leathery leaf and marginal leaf rolling

Leathery leaf with mosaic

Plate 4. Symptoms of CMV under natural condition



a. Leathery leaf b. Vein clearing
c. Puckering d. Reduced leaf size

Plate 5. Symptoms of CMV under artificial condition

Mosaic

Malformed leaf

Rugosity

Reduced leaf size

Plate 6. Symptoms of potyvirus under natural condition

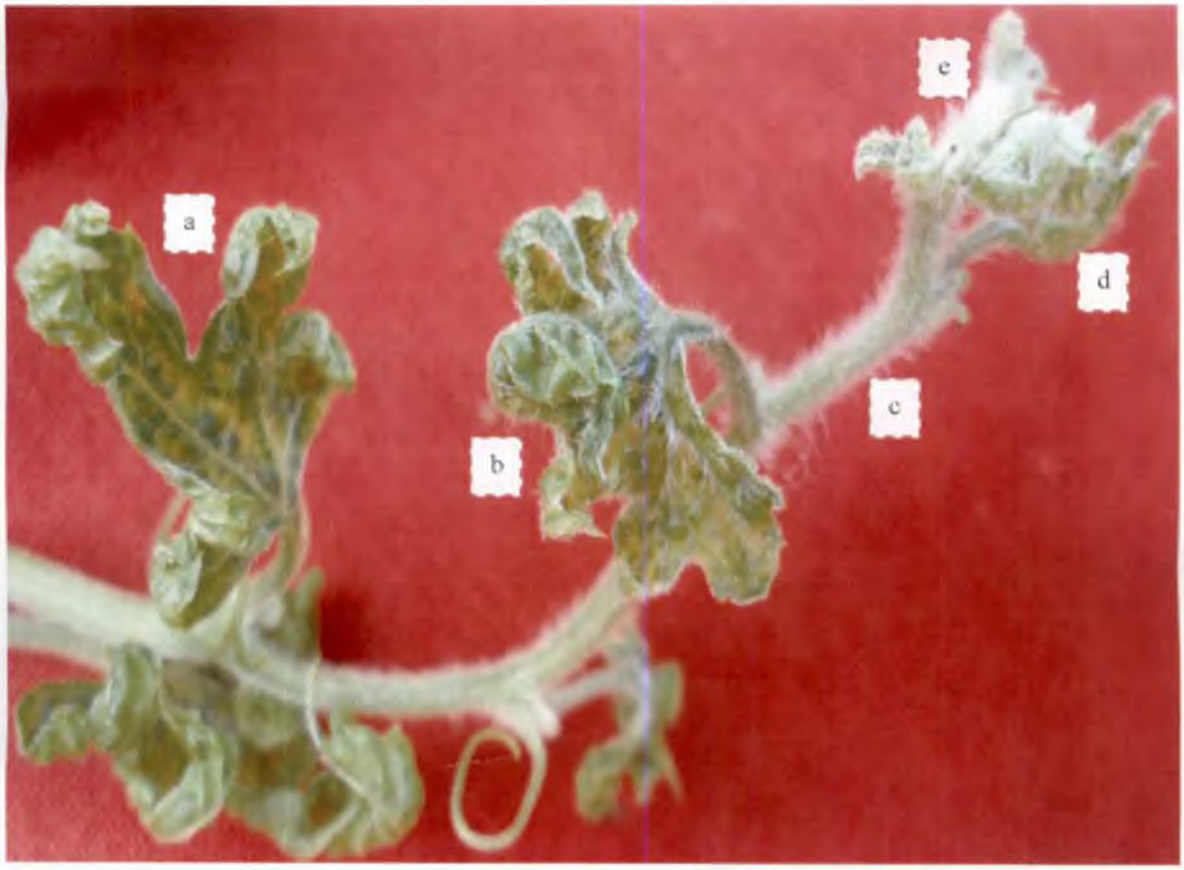
Vein clearing

Puckering

Malformed leaf

Reduced leaf size

Plate 7. Symptoms of potyvirus under artificial condition



a. Mosaic and puckering b. Cupping c. Hairy growth

d. Reduced leaf size e. Clustering of internodes

Plate 8. Symptoms of BDMV under natural condition

Puckering

Cupping

Reduced leaf size

Hairy growth

Plate 9. Symptoms of BDMV under artificial condition

Table 9. Standardization of buffer for sap transmission of potyvirus

Sl. No.	Buffer (Molarity - 0.1 M)	Mean per cent disease incidence		
		pH 7.0	pH 7.2	pH 7.4
1	Potassium phosphate buffer	80	60	70
2	Sodium phosphate buffer	30	70	70
3	Citrate buffer	70	70	60
4	Acetate buffer	50	40	40
5	Boric acid buffer	10	40	10

Of the different buffers tested for potyvirus sap transmission, potassium phosphate buffer pH 7 showed the maximum disease incidence (80 %) followed by pH 7.4, sodium phosphate buffer pH 7.2 and 7.4 and citrate buffer pH 7.0 and 7.2. The minimum disease incidence was recorded in Boric acid buffer at pH 7.0 and 7.4.

4.5 VARIETAL EVALUATION FOR HOST RESISTANCE

Available genotypes of bitter gourd in NBPGR, Thrissur, KAU released variety, farmers varieties and commercial hybrids were evaluated for resistance to bitter gourd mosaic under insect proof net house condition. Sap transmission was followed for CMV and potyvirus and whitely transmission was followed for BDMV. Five plants of each accession was inoculated with viruses separately and was kept under insect proof net house. The inoculated plants were observed daily for the development of symptoms. Disease incidence and severity was recorded and the genotypes were categorized based on coefficient of infection (CI). The results of

evaluation of 22 bitter gourd accessions against CMV, potyvirus and BDMV were given in table 10, 11 and 12 respectively.

Of the 22 accessions, three accessions were highly resistant (HR), three accessions were resistant (R), seven accessions were moderately resistant (MR), six accessions were moderately susceptible (MS) and three accessions were susceptible (S) to CMV.

From the table 10, it is clear that the bitter gourd accessions TCR 53, TCR 285 and TCR 39 were highly resistant; TCR 2, TCR 494 and TCR 103 were resistant; TCR 179, TCR 416, Biliagala, TCR 202, White long, TCR 76 and TCR 492 were moderately resistant; TCR 364, TCR 471, TCR 149, TCR 463, TCR 196 and TCR 380 were moderately susceptible and TCR 493, Green long and Preethi were susceptible to CMV.

Of the 22 accessions, one accession was highly resistant, two accessions were resistant, thirteen accessions were moderately resistant, one accession was moderately susceptible and five accessions were susceptible to potyvirus (Table 11).

Biliagala was highly resistant accession to potyvirus. Resistant accessions were TCR 493 and TCR 380; moderately resistant accessions where TCR 2, TCR 149, TCR 492, TCR 494, White long, TCR 463, TCR 53, TCR 285, TCR 39, Green long, TCR 416, TCR 471 and TCR 196; moderately susceptible accession was TCR 76 and susceptible accessions were TCR 364, TCR 103, TCR 179, TCR 202 and Preethi (Table 11).

Table 10. Evaluation of bitter gourd accessions against CMV

Sl. No.	Accessions	PDI		PDS		CI		Disease reaction
		14 DAI	21 DAI	14 DAI	21 DAI	14 DAI	21 DAI	
1	TCR 53	0	0	0	0	0	0	HR
2	TCR 285	0	0	0	0	0	0	HR
3	TCR 39	0	0	0	0	0	0	HR
4	TCR 2	0	40	0	16	0	6.40	R
5	TCR 494	20	40	08	20	1.60	8.00	R
6	TCR 103	20	40	08	20	1.60	8.00	R
7	TCR 179	40	40	20	32	8.00	12.80	MR
8	TCR 416	20	40	08	32	1.60	12.80	MR
9	Biliagala	20	60	08	28	1.60	16.80	MR
10	TCR 202	40	60	16	28	6.40	16.80	MR
11	White long	20	60	08	32	1.60	19.20	MR
12	TCR 76	20	60	08	32	1.60	19.20	MR
13	TCR 492	20	60	08	32	1.60	19.20	MR
14	TCR 364	60	60	28	36	16.80	21.60	MS
15	TCR 471	60	60	24	36	14.40	21.60	MS
16	TCR 149	40	60	20	36	8.00	21.60	MS
17	TCR 463	40	80	16	40	6.40	32.00	MS
18	TCR 196	40	80	16	40	6.40	32.00	MS
19	TCR 380	20	80	08	40	1.60	32.00	MS
20	TCR 493	60	80	24	48	14.40	38.40	S
21	Green long	80	80	36	56	28.80	44.80	S
22	Preethi	80	100	32	68	25.60	68.00	S

Table 11. Evaluation of bitter gourd accessions against potyvirus

Sl. No.	Accessions	PDI		PDS		CI		Disease reaction
		14 DAI	21 DAI	14 DAI	21 DAI	14 DAI	21 DAI	
1	Biliagala	0	0	0	00	0	0	HR
2	TCR 493	0	20	0	28	0	5.60	R
3	TCR 380	0	40	0	16	0	6.40	R
4	TCR 2	0	60	0	20	0	12.00	MR
5	TCR 149	20	60	12	24	2.40	14.40	MR
6	TCR 492	0	60	0	24	0	14.40	MR
7	TCR 494	0	60	0	24	0	14.40	MR
8	White long	0	60	0	24	0	14.40	MR
9	TCR 463	0	80	0	20	0	16.00	MR
10	TCR 53	0	80	0	21	0	16.80	MR
11	TCR 285	0	0	60	0	28.00	16.80	MR
12	TCR 39	0	60	0	28	0	16.80	MR
13	Green long	0	60	0	28	0	16.80	MR
14	TCR 416	00	60	0	32	0	19.20	MR
15	TCR 471	40	60	16	32	6.40	19.20	MR
16	TCR 196	0	60	0	32	0	19.20	MR
17	TCR 76	20	80	8	40	1.60	32.00	MS
18	TCR 364	40	100	16	44	6.40	44.00	S
19	TCR 103	20	100	12	48	2.40	48.00	S
20	TCR 179	40	100	16	52	6.60	52.00	S
21	TCR 202	40	100	20	52	8.00	52.00	S
22	Preethi	80	100	28	64	22.40	64.00	S

Of the 22 accessions, eleven accessions were highly resistant; three accessions were resistant; three accessions were moderately resistant; four accessions were moderately susceptible and one accession was highly susceptible to BDMV.

Table 12. Evaluation of bitter gourd accessions against BDMV

Sl. No	Accessions	PDI	PDS	CI	Disease reaction
1	TCR 380	0	0	0	HR
2	Biliagala	20	4	0.80	HR
3	TCR 285	10	12	1.20	HR
4	TCR 493	20	8	1.60	HR
5	TCR 416	20	8	1.60	HR
6	TCR 202	20	8	1.60	HR
7	TCR 39	20	8	1.60	HR
8	TCR 149	20	12	2.40	HR
9	TCR 494	20	12	2.40	HR
10	TCR 492	20	16	3.20	HR
11	Green long	20	20	4.00	HR
12	TCR 179	40	16	6.40	R
13	TCR 2	60	12	7.20	R
14	TCR 463	40	20	8.00	R
15	TCR 196	40	36	14.40	MR
16	TCR 471	40	40	16.00	MR
17	White long	40	45	18.00	MR
18	TCR 364	60	44	26.40	MS
19	TCR 53	60	48	28.80	MS
20	TCR 76	60	52	31.20	MS
21	TCR 103	60	60	36.00	MS
22	Preethi	100	92	92.00	HS

The highly resistant accessions were TCR 380, Biliagala, TCR 285, TCR 493, TCR 416, TCR 202, TCR 39, TCR 149, TCR 494, TCR 492 and Green long; resistant accessions were TCR 179, TCR 2 and TCR 463; moderately resistant accessions were TCR 196, TCR 471 and White long; moderately susceptible accessions were TCR 364, TCR 53, TCR 76 and TCR 103 and highly susceptible accession was Preethi (Table 12).

4.6 EFFECT OF DEFENSE INDUCERS ON GERMINATION OF BITTER GOURD SEEDS

The seeds of bitter gourd variety Preethi was soaked overnight in selected defense inducers and sown in poly bags. Seed germination was observed on eighth day of sowing. The per cent seed germination was in the range of 93.33 % to 53.33 % the maximum germination of 93.33 % was recorded in *Pseudomonas fluorescens* and the minimum germination of 53.33 % was recorded in salicylic acid. The germination per cent of seeds soaked in water (control) was 80. The result showed that germination of bitter gourd was reduced by salicylic acid and barium chloride and the per cent reduction over control was 33 and 8 respectively.

4.7 MANAGEMENT OF BITTER GOURD MOSAIC BY ENHANCING HOST RESISTANCE

A field experiment was conducted to evaluate selected defense inducers for management of bitter gourd mosaic using moderately resistant variety White long and susceptible variety Preethi. The treatments were applied five times during the entire crop period as seed treatment and four foliar sprays at 20, 35, 50 and 65 days after sowing. The incidence of mosaic was observed at five days interval after third treatment application and the data were presented in Table 13. The disease severity was scored at five days intervals after fourth and fifth treatment application and the data were presented in table 14 and 15.

The mosaic symptoms were appeared at 40 days of sowing and after third treatment application. The per cent disease incidence (PDI) was recorded at 5, 10 and 15 days of the spray and the results are given in table 13.

Table 13. Mosaic incidence of bitter gourd after third treatment application

Groups	Treatment	PDI			Days for symptom appearance
		5 day	10 day	15 day	
Moderately resistant (White long)	T_1 - Control	53.30	100	100	40
	T_2 - Salicylic acid 25 ppm	0	0	46.60	51
	T_3 - Barium chloride 0.1 %	0	53.30	100	47
	T_4 -1 *P. fluorescens* 2 %	0	46.60	100	47
Susceptible (Preethi)	T_5 - Control	53.30	100	100	40
	T_6 - Salicylic acid 25 ppm	0	0	46.60	51
	T_7- Barium chloride 0.1 %	0	46.60	100	47
	T_8- *P. fluorescens* 2 %	0	46.60	100	47

After five days, the disease was observed only in control (T1 and T5) and the PDI was 53.30 %. After 10 days, the PDI was increased to 100 % in control and among the treatments the maximum PDI of 53.30 % was observed in barium chloride (T3). After 15 days, mosaic was noticed in all the treatments and the PDI was varied from 46.60 % in salicylic acid (T2 and T6) to 100 % in all the remaining treatments. The above data clearly showed that, salicylic acid was the most effective treatment to check the disease incidence.

The incubation period of the treatments was varied from 40 days in control (T1 and T5) to 51 days in salicylic acid (T2 and T6). The data clearly showed that untreated plants produced symptom after 40 days of sowing where as the treated plants exhibited symptoms at a later stage and it was maximum in salicylic acid.

At five days of fourth treatment application, there was significant difference between treatments in both moderately resistant and susceptible groups. Salicylic acid

recorded the lowest disease severity in moderately resistant group (12.33 %) and in susceptible group (12 %). In moderately resistant group salicylic acid was on par with barium chloride and *P. fluorescens.* In susceptible group *P. fluorescens* recorded significantly higher disease severity than salicylic acid.

When compared the per cent reduction in disease severity over control, 41 % reduction was recorded in salicylic acid and 30 % reduction was recorded in other treatments of moderately resistant group. In susceptible group, salicylic acid recorded 66 % reduction and it was followed by barium chloride (40 %) *P. fluorescens* (18 %).

At ten days of fourth treatment application, treatments of both groups were significantly different in their disease severity. In moderately resistant group the lowest disease severity of 10.33 % was recorded in salicylic acid and other treatments were on par with salicylic acid. The highest disease severity of 21.33 % was recorded in control and it was significantly different from other treatments. In susceptible group the lowest disease severity of 10. 66 % was recorded in salicylic acid and it was on par with barium chloride. *P. fluorescens* recorded the highest disease severity of 34.66 % and this was on par with control.

The percent reduction in disease severity was maximum in salicylic acid in both moderately resistant group (51 %) and susceptible group (75 %) and it was minimum in *P. fluorescens* in moderately resistant group (37 %) and susceptible group (19 %).

Table 14. Disease severity of bitter gourd after fourth treatment application

Group	Treatment	PDS					
		5 day		10 day		15 day	
		PDS	% reduction in disease severity	PDS	% reduction in disease severity	PDS	% reduction in disease severity
Moderately resistant (White long)	T_1 - Control	21.00[b] (4.63)	-	21.33[b] (4.66)	-	38.33[c] (6.23)	-
	T_2 - Salicylic acid 25 ppm	12.33[a] (3.58)	41	10.33[a] (3.28)	51	30.00[b] (5.52)	22
	T_3- Barium chloride 0.1 %	14.66[a] (3.89)	30	12.66[a] (3.61)	41	32.66[b] (5.75)	15
	T_4–*P. fluorescens* 2 %	14.66[a] (3.83)	30	13.33[a] (3.70)	37	22.66[a] (4.80)	40
CD (0.05)		0.81		0.68		0.48	
Susceptible (Preethi)	T_5 - Control	36.00[b] (6.02)	-	42.66[b] (6.51)	-	65.33[b] (8.11)	-
	T_6 - Salicylic acid 25 ppm	12.00[a] (3.50)	66	10.66[a] (3.32)	75	29.33[a] (5.44)	55
	T_7- Barium chloride0.1 %	21.33[ab] (4.64)	40	19.33[a] (4.44)	30	37.33[a] (6.05)	43
	T_8–*P. fluorescens* 2 %	29.33[b] (5.38)	18	34.66[b] (5.92)	19	41.33[a] (6.45)	37
CD (0.05)		1.45		1.19		1.42	

At 15 days of fourth treatment application, treatments of both groups were significantly different in their disease severity. In moderately resistant group *P. fluorescens* recorded the lowest disease severity of 22.66 % and it was followed by salicylic acid (30 %) and barium chloride (32.66 %) which were on par. The highest disease severity of 38.33 % was recorded in control. In the susceptible group, the lowest disease severity was recorded in salicylic acid (29.33 %) and it was on par with both barium chloride and *P. fluorescens*. Per cent reduction in disease severity was maximum for *P.fluorescens* in moderately resistant group and for salicylic acid in susceptible group.

When compared the disease severity of 5, 10 and 15 days of each treatment, a reduction in disease severity was observed in 10 days over 5 days. It was noticed in all treatments of moderately resistant group except control. But in susceptible group similar observation was noticed only in salicylic acid and barium chloride and disease severity of *P. fluorescens* was increased gradually as in control.

At 5, 10, 15 and 30 days of fifth treatment application, disease severity of treatments was not significantly different. Even though, at five days, the lowest severity was recorded in salicylic acid in both moderately resistant (40 %) and susceptible (42.66 %). Per cent reduction in disease severity was also more in salicylic acid compared to other treatments. At 10 and 15 days also, similar trend was observed with lowest disease severity and highest per cent reduction in salicylic acid (Table 15).

Table 15. Disease severity of bitter gourd after fifth treatment application

Groups	Treatment	5 days		10 days		15 days		30 days	
		PDS	% disease reduction	PDS	% disease reduction	PDS	% disease reduction	PDS	% disease reduction
Moderately resistant (White long)	T_1 - Control	53.33^{bc} (7.33)	-	60.00^{c} (7.77)	-	62.33^{c} (7.92)	-	63.00^{c} (7.96)	-
	T_2 - Salicylic acid 25ppm	40.00 ^{a} (6.34)	25	30.66^{a} (5.52)	48	29.00^{a} (5.38)	53	24.00^{a} (4.91)	62
	T_3 - Barium chloride 0.1 %	46.66^{ab} (6.85)	12	45.33^{b} (6.76)	24	45.66^{b} (6.79)	27	41.33^{b} (6.46)	34
	T_4 – *P. fluorescencs* 2 %	57.33 ^{c} (7.60)	-7	50.66^{bc} (7.15)	15	50.00^{b} (7.10)	20	42.66^{b} (6.56)	32
CD (0.05)		NS		NS		NS		0.71	
Susceptibile (Preethi)	T_5 - Control	70.66 ^{c} (8.43)	-	73.00^{c} (8.57)	-	75.33^{c} (8.70)	-	76.33^{c} (8.76)	-
	T_6 – Salicylic acid 25 ppm	42.66 ^{a} (6.56)	40	38.66^{a} (6.25)	47	39.00^{a} (6.28)	48	36.00^{a} (6.03)	53
	T_7 - Barium chloride0.1 %	50.66 ^{b} (7.14)	28	52.00^{b} (7.24)	28	52.00^{b} (7.24)	31	48.33^{b} (6.98)	37
	T_8 – *P. fluorescencs* 2 %	49.33 ^{b} (7.05)	30	53.33 ^{b} (7.33)	26	55.00 ^{b} (7.44)	27	52.33 ^{b} (7.26)	31
CD (0.05)		NS		NS		NS		0.38	

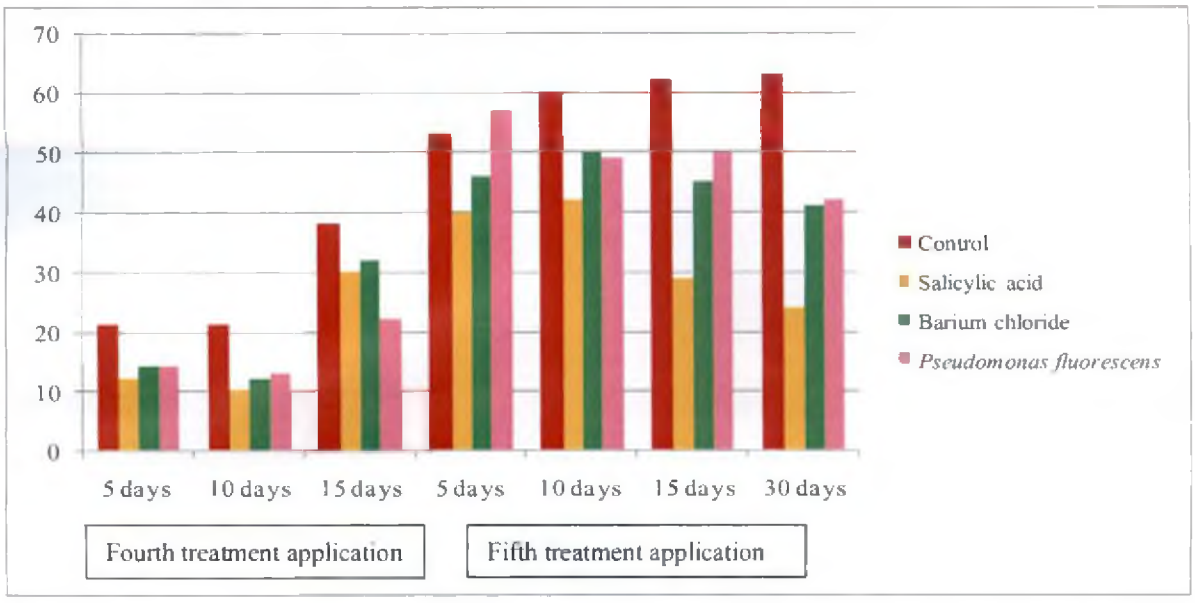


Fig. 1. Per cent disease severity – moderately resistant variety (White long)

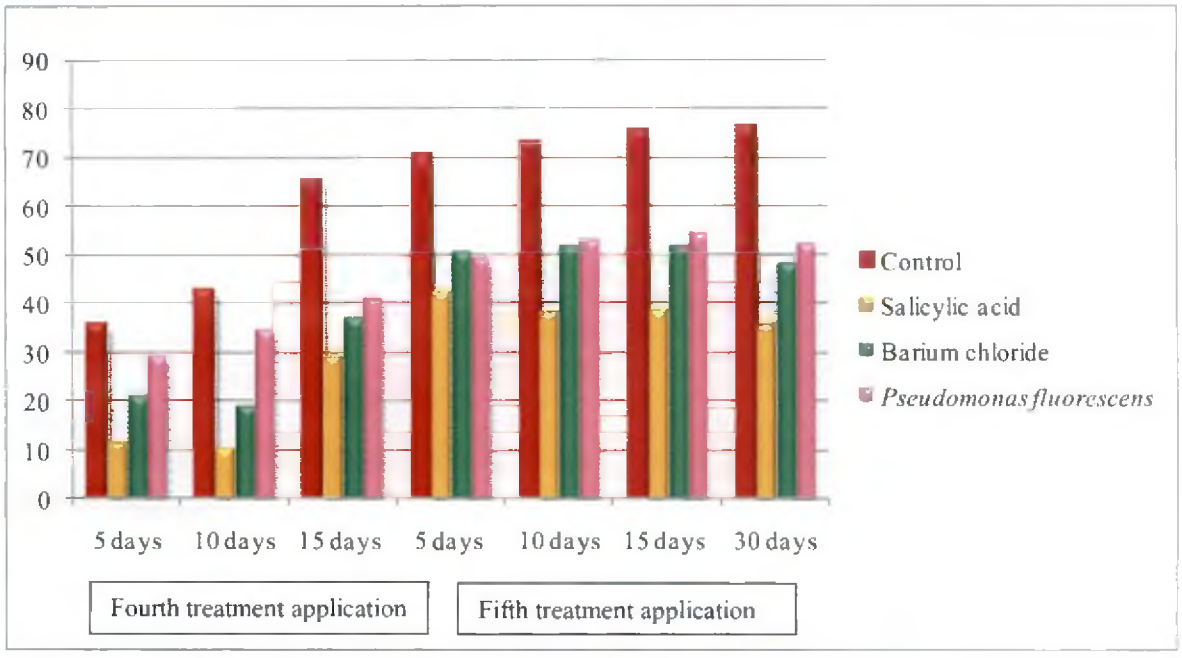


Fig. 2. Per cent disease severity – susceptible variety (Preethi)

At 30 days, there was significant difference between treatments in both moderately resistant and susceptible group. In moderately resistant group, disease severity of salicylic acid was significantly lower (24%) than other treatments. This was followed by barium chloride (41.33 %) and *P. fluoresces* (42.66 %) which were on par and was less than control (63 %). The per cent reduction in disease severity was also maximum (62 %) for salicylic acid. In susceptible group the lowest disease severity (36 %) was recorded in salicylic acid and it was followed by barium chloride 48.33 % and *Pseudomonas fluorescens* 52.33 % which were on par and were less than control (76.33 %). The per cent reduction was maximum (53 %) for salicylic acid than other treatments.

When compared the disease severity of 5, 10, 15 and 30 days of each treatment a gradual reduction was observed in all treatments except control of moderately resistant group. But in susceptible group, similar observation was noticed only in salicylic acid and barium chloride and disease severity of *P. fluorescens* was increased gradually as in control. The effect of best treatment and control given in plate 10.

Harvest was started after 60 days of sowing. Immature green coloured fruits were harvested and treatment wise yield was recorded and presented in table 16.

The treatments were varied significantly in yield. In the moderately resistant group, the maximum yield was recorded in *P. fluorescens* (5.04 t/ha) which was significantly superior than all other treatments. It was followed by control (3.42 t/ha) and salicylic acid (3.08 t/ha) which were on par. Barium chloride recorded significantly lower yield (2.08 t/ha) than all other treatments. In the susceptible group, the maximum yield was recorded in *P.fluorescens* (6.76 t/ha) which was significantly superior than all other treatments. It was followed by control (4.91 t/ha).

The yield of salicylic acid (3.89 t/ha) and barium chloride (3.45 t/ha) were on par and were significantly lower than control.

Salicylic acid (25 ppm) treated plants

Control plants

Plate 10. Effect of treatments on disease severity

Table 16. Fruit yield of bitter gourd field experiment

Group	Treatment	Yield (kg/ plot -5.6 sqm)	Yield (t/ha)
Moderately resistant (White long)	T_1 - Control	1.71 (41.39)	3.42
	T_2-Salicylic acid 25 ppm	1.54 (39.22)	3.08
	T_3 - Barium chloride 0.1 %	1.04 (32.27)	2.08
	T_4- *P. fluorescens* 2 %	2.51 (50.10)	5.04
CD (0.05)		3.76	
Susceptible (Preethi)	T_5 - Control	2.45 (49.54)	4.91
	T_6-Salicylic acid 25 ppm	1.94 (44.12)	3.89
	T_7-Barium chloride 0.1 %	1.71 (41.38)	3.45
	T_8- *P. fluorescens* 2 %	3.38 (58.12)	6.76
CD (0.05)		3.30	

Discussion

5. DISCUSSION

Bitter gourd, is one of the important vegetable crops in India. It is popular, due to its medicinal property apart from the high nutritional value. Diseases and pests were the major constraints in the cultivation of bitter gourd. The major disease for its low production is mosaic caused by different viruses like cucumber mosaic virus (CMV), potyvirus and bitter gourd distortion mosaic virus (BDMV). Hence in the present project, attempts were made to study the symptomatology and transmission of different bitter gourd viruses, varietal reaction and management of bitter gourd mosaic by defense inducers.

The major symptoms of CMV infection in bitter gourd were appearance of mosaic patches in leaves, yellowing and reduction of leaf size. A new symptom of CMV was also observed in bitter gourd field in which leaves were leathery in texture and downward rolling of leaves along the margins. Even though mosaic and yellowing symptom was reported earlier by Nagarajan and Ramakrishnan (1971) the leatheryness and marginal rolling of leaves were not reported and hence it was the first report.

The symptoms of potyvirus infection observed in bitter gourd were vein clearing, blistering and filiform leaves. Similar symptoms of potyvirus infection were reported earlier (CMI, 1984).

Mosaic, bilstering, reduced leaf size, distorted leaf, hairy growth on leaves and twig, stunted growth of plants were the symptoms of BDMV infection. The plants infected in early stage were not flowered. The incidence of BDMV was reported by first time in Kerala by Mathew *et al.* (1991) and the symptoms recorded were similar

to those observed in the present study. It was also reported from other state like Uttar Pradesh (Khan *et al.*, 2002) and Tamilnadu (Rajinimala *et al.*, 2005).

As mixed infection of CMV, potyvirus and BDMV occurs in field grown bitter gourd plants, separation of these viruses by the use of indicator hosts was tried to get pure cultures of different viruses. Cosmos an ornamental crop, was reported as a systemic host of CMV by Parvin *et al.,* (2007). According to him CMV from cosmos is transmissible to different host plants including pumpkin, a cucurbitaceous crop. Based on the study and as no other viruses were reported from cosmos, it was selected as a systemic host for separatation of CMV from bitter gourd. Standard sap transmission was followed for inoculation of infected bitter gourd sap to cosmos and back inoculation of healthy bitter gourd seedlings. The time taken for symptom development was 21 days and 11 days respectively in cosmos and back inoculated bitter gourd seedlings. The pure culture of CMV was maintained by transmitting the virus periodically to new bitter gourd seedlings. The presence of CMV in cosmos and back inoculated bitter gourd seedlings was confirmed through ELISA using banana CMV antiserum.

CMI (1984) has reported that papaya ring spot virus (PRSV), a potyvirus infecting papaya induces variable symptoms like vein clearing, mottling, filimorphism and malformed leaves in cucurbits. Chin and Ahamed 2007, has reported that the potyvirus, PRSV -P infect wild bitter gourd (*Momordica charantia* grown as weed plants. Mechanical transmission from the weed to cultivated bitter gourd plants produced vein clearing symptoms in two weeks after inoculation. Based on above references and as other sap transmissible viruses are not reported from papaya an attempt was made to transmit potyvirus from bitter gourd field sample to seven days old papaya seedling and back to bitter gourd seedlings by sap transmission. The potyvirus of bitter gourd got transmitted to papaya seedlings and

from papaya to bitter gourd seedlings. The time taken for symptom development was 15 days and 13 days respectively in papaya and back inoculated bitter gourd seedlings. The pure cultures of potyvirus were maintained by transmitting the virus periodically to new bitter gourd seedlings. Presence of potyvirus in papaya and back inoculated bitter gourd seedlings were confirmed through ELISA using banana potyvirus (bract mosaic) antiserum. The presence of inclusion bodies was checked in the infected papaya leaves by observing the thin section under microscope. Inclusion bodies of different shapes were observed which also confirmed the presence of potyvirus in infected papaya leaf. Shukla *et al.*, (1991) has reported that all members of the potyviridae family form cylindrical inclusion bodies in infected cells, and it is formed by virus-encoded protein which can be considered as the most important criterion for assigning viruses to the potyvirus group.

Selection of most suitable buffer for mechanical transmission of CMV and potyvirus was necessary for conducting screening of bitter gourd accessions against these viruses. Hence a study was conducted for standardization of sap transmission of CMV and potyvirus of bitter gourd using five different buffers at three pH levels. The buffers used were potassium phosphate, sodium phosphate, acete, citrate and Boric acid buffer of 0.1 M concentration at selected pH 7, 7.2 and 7.4. Among these buffers, 0.1 M potassium phosphate buffer (PPB) at pH 7 produced the maximum CMV transmission of 90 % after 14 days of inoculation. Therefore 0.1 M PPB, pH 7 was selected as the buffer for sap transmission and screening studies of CMV. Chandrakar (2013) used 0.1M potassium phosphate buffer (pH 7.5) supplemented with 0.1% sodium sulphite in a ratio of 1:10 (w/v) for the transmission of CMV to cucumber cultivar Pune Khira and it developed symptom by 10 to 14 days. In the present study, potassium phosphate buffer at lower pH (7.0) produced maximum transmission than at higher pH (7.4) of the same buffer.

As in the case of CMV, 0.1 M PPB at pH 7 also produced the maximum potyvirus transmission of 80 % after 14 days of inoculation. According to Nametha *et al.* (1985), 0.02 M potassium phosphate buffer, pH 7.0, containing 1% celite was effective in transmitting ZYMV a potyvirus, from field-grown squash to yellow squash (*Cucurbita pepo* L.) and cantaloupe (*Cucumis melo* L.) and mosaic symptom was developed after 14 days of inoculation. Verma *et al.* (2006) used 0.01 M potassium phosphate buffer (pH 7.3) to transmit ZYMV from bottle gourd plants to other cucurbits, members of chenapodeaceae and solanaceae families.

Based on the results of the present study and available literature, it is confirmed that 0.1 M PPB, pH 7.0 is an efficient buffer for the mechanical transmission of CMV and potyvirus of bitter gourd.

Cent per cent transmission of BDMV to healthy bitter gourd seedlings was obtained using whitefly (*Bemisia tabaci*) with 24 h of each acquisition access and inoculation access period and when used at the rate of ten numbers per plant. The symptoms were developed after 10 days of inoculation and the symptoms produced were mosaic, reduced leaf size and shortened internodes. Transmission of BDMV using *B. tabaci* was carried out by different workers. Giri and Mishra (1986) has reported that *B. tabaci* transmits BDMV with 12 h of acquisition access and inoculation access period and the time for symptom appearance was 6-8 weeks when 2-3 weeks old cucurbit seedlings were used. Rajinimala *et al.* (2005) has reported that 12 hours of acquisition access and inoculation access period were required for *B. tabaci* to transmit BDMV to bitter gourd seedlings. The percentage of transmission was increased with increased periods of acquisition and inoculation access period. As typical BDMV symptoms were produced in artificially inoculated bitter gourd seedlings and the procedure adopted for whitefly transmission was based on reports

of earlier workers, the presence of BDMV was confirmed in the artificially inoculated bitter gourd plants.

Artificial screening of 22 selected bitter gourd accessions against CMV, potyvirus and BDMV was the next aspect of the study. Of the 22 accessions, two were commercial hybrids, one was a released variety of Kerala Agriculture University, one was farmers variety from Karnataka and 18 were accessions of NBPGR, Thrissur. Artificial screening by sap transmission was carried out for CMV and potyvirus and by white fly transmission for BDMV. Number of plants screened for each accession per virus was five. After 21 days of inoculation, the accessions were grouped based on coefficient of infection into six disease reaction groups.

Screening of bitter gourd accessions by artificial inoculation was not reported earlier. But natural screening was conducted in the field and the results were reported by different workers. According to Thangamani *et al.,* (2011), bitter gourd accessions, Green long and Preethi was moderately susceptible for CMV. In the present study both Green long and Preethi were found susceptible to CMV. According to Arunachalam (2002), Preethi was moderately susceptible to BDMV but in the present study Preethi was highly susceptible to BDMV. As plants were inoculated individually, resistance of Preethi and Green long was less in the present study than that reported earlier.

Management of bitter gourd mosaic in the field was the next aspect of the study. For this, a field experiment was conducted using a moderately resistant variety White long and a susceptible variety Preethi. Defence inducers *viz.,* salicylic acid 25 ppm, barium chloride 0.1% and *Pseudomonas fluorescence* 2% was applied as different treatments for mosaic management. Five applications of the treatments were given which included seed treatment and four foliar sprays (Table 6).

Incubation period, disease incidence and severity were observed to evaluate the defense inducers in management of bitter gourd mosaic. The incubation period was 40 days in control (T1 and T5) where as 47 days in T3, T4, T7 and T8 and 51 days in T2 and T6. This clearly indicates that salicylic acid (T2 and T6) is the most effective treatment for delaying expression of mosaic symptoms. The delayed incidence of mosaic by application of defense inducers was reported earlier by different workers. Naylor *et al.,* (1998) has reported that watering of squash seedlings with 2nM of salicylic acid before artificial inoculation with CMV, delayed onset of symptom. According to Galliteli *et al.* (1991), defense inducer BTH, prevented CMV replication and delayed symptom expression in tomato. Rajanimala *et al.* (2009) has evaluated defense inducers on expression of BDMV symptom and reported that leaf extract of *Bougainvaillae spectabilis* was the most effective one in delaying expression of BDMV symptom.

The variation in incubation period of treatments also contributed to the variation in per cent disease incidence (PDI) of treatments. Even though the PDI was 100 % in all the treatments after 15 days of third treatment application, it was varied at 5 and 10 days. The PDI at 5 and 10 days of third treatment application was in the range of 0- 53.30 % and 0 – 100 % respectively. The effect of defense inducers *viz., B. spectabilis, P. chlorosaphis* and *P. fluorescence* on incidence of BDMV was reported by Rajanimala *et al.* (2009). According to them, the PDI after 60 days was 33.33 to 66.66% in treatments and cent per cent in control.

The per cent disease severity (PDS) was recorded after 4^{th} and 5^{th} treatment application. Disease severity was observed after 5, 10 and 15 days of each spray. Among the two varieties, susceptible variety Preethi recorded high PDS than moderately resistant variety White long. All treatments reduced severity of bitter gourd mosaic when compared with control. After fourth treatment application

salicylic acid recorded the lowest disease severity at five and ten days in both moderately resistant and susceptible group. But at fifteen days, the disease severity of *P. fluorescens* was on par with salicylic acid in both groups.

Reduction in disease severity of bitter gourd mosaic by application of defense inducers was reported by Louis *et al.* (2011). According to them, the maximum reduction in disease severity after ten days of treatment application was recorded in salicylic acid. The result of the present study is in conformity with the report.

An interesting observation noticed in the PDS was its reduction at 10 days and its increase at 15 days of treatment application. It indicates that a time interval of 5-10 days is required for the development of induced resistance in host plant by salicylic acid and barium chloride. The increase in PDS at 15 days of treatment application shows that resistance induced in the plant was active only up to 14 days of spray. This observation was more prominent in fourth application of treatment than in fifthapplication.

The need for a time span, after application of defense inducers was reported earlier by different workers. Mptraux *et al.* (1990) has reported that salicylic acid could function as an endogenous signal for development of systemic acquired resistance in cucumber and a span of time is necessary for signals to be translocated to non inoculated tissues and development of SAR. Similar observation was also reported by different workers (Kuc, 1987; Kuc, 2001; Schneider *et al.,* 1996; Benhamou and Picard 1999).

The susceptible variety Preethi recorded higher yield in all treatments compared to moderately resistant variety white long. Among the treatment *P. fluorescens* recorded higher yield than salicylic acid and barium chloride. As *P. fluorescens* is a

plant growth promoting rhizobacteria it enables to overcome the deleterious effect of virus infection in plants. The defense inducers salicylic acid and barium chloride, even though able to reduce the severity of bitter gourd mosaic was unable to enhance the growth of the crop. Growth retarding effect of salicylic acid and barium chloride also may be contributed to the low yield.

Bitter gourd plants of the experiment were grown in channels at a spacing of 0.75 X 1.5 m and were trailed on net tied on one side of the channel. Thick vegetative growth and lack of sufficient sunlight was a reason for the poor yield of experimental crop. Apart from this, high incidence of fruitfly attack was also observed, even with all precautions like covering of fruits and installation of pheromone traps.

Summary

6. SUMMARY

Bitter gourd (*Momordica charantia*) is a tropical and subtropical crop of the family cucurbitaceae and it is one of the important vegetable crops in Kerala. Bitter gourd is extensively cultivated in India, China and South East Asia. It is regarded as one of the world's major vegetable crop and has great economic importance. One of the major constraints in the production of this crop is virus diseases. Yield loss up to 100% has been reported by early infection of bitter gourd distortion mosaic virus (BDMV).

The present investigation "Management of bitter gourd mosaic virus by enhancing host resistance" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2013 to 2015 with a view to understand the symptomatology, mode of transmission, varietal reaction and to evaluate the defense inducers for management of bitter gourd mosaic.

As bitter gourd mosaic samples collected from field contains different viruses *viz.*, CMV, potyvirus and BDMV, separation and production of pure cultures of these viruses was carried out first. The systemic indicator host used for separation of CMV and potyviruses were *Cosmos sulphureus* and *Carica papaya* respectively. The infected bitter gourd samples extracted in suitable buffer was mechanically inoculated to indicator hosts for getting the virus pure culture and this was back inoculated to healthy bitter gourd seedlings and maintained in insect proof net house. The symptoms produced in cosmos plants were mosaic, vein clearing, reduced leaf size and shoestring appearance after 21 days of inoculation and in papaya symptoms observed were puckering, yellowing and reduced plant height after 15 days of inoculation.

The standardization of buffer for sap transmission of CMV and potyvirus was carried out using five different buffers each at three pH levels. The best buffer selected was potassium phosphate at pH 7.0 which showed 90% transmission of CMV and 80 % transmission of potyvirus after 14 days of inoculation.

The observation on PDI and PDS was recorded at 14 and 21 days after inoculation. Among 22 accessions screened, three accessions viz., TCR 285, TCR 39 and TCR 53 were highly resistant to CMV; one accession Biliagala was highly resistant to potyvirus and 11 accessions viz., TCR 285, TCR 39, TCR 493, TCR 416, TCR 492, TCR 494, TCR 380, TCR 202 and TCR 149, Green long and Biliagala were highly resistant to BDMV. The variety preethi was susceptible to all the virus and hybrid white long was moderately resistant to CMV, potyvirus and BDMV. So these two varieties were selected for the experiment "Management of bitter gourd mosaic".

Field experiment was conducted in the College of Horticulture, Vellanikara. The treatment selected were salicylic acid (25ppm), barium chloride (0.1%) and *Pseudomonas fluorescens* (2%) . the selected varieties were Preethi and white long. The treatments were applied as seed treatment and four foliar sprays at 15 days interval. The observation was taken on the disease incidence and on disease severity at 5, 10 and 15 days after each spray. Incidence of disease was delayed for 11 days in salicylic acid treated plants when compared to control. It will indicate that defense inducers delay the appearance of disease.

Even though a gradual increase was observed in the disease severity of control and treatments of both moderately resistant and susceptible varieties, the disease severity of treatments was always less than that of control. At 15 days of fourth treatment application, treatments of moderately resistant and susceptible groups were

significantly different in their disease severity. In moderately resistant group *P. fluorescens* recorded the lowest disease severity of 22.66 % and it was followed by salicylic acid (30 %) and barium chloride (32.66 %) which was on par. The highest disease severity of 38.33 % was recorded in control. In the susceptible group, the lowest disease severity was recorded in salicylic acid (29.33 %) and it was on par with both barium chloride and *P. fluorescens*. Per cent reduction in disease severity was maximum for *P.fluorescens* in moderately resistant group and for salicylic acid in susceptible group.

When compared the disease severity of 5, 10 and 15 days of each treatment, a reduction in disease severity was observed in 10 days over 5 days. It was noticed in all treatments of moderately resistant group except control. But in susceptible group similar observation was noticed only in salicylic acid and barium chloride.

The yield data of treatments were varied significantly. The maximum yield was recorded in *P. fluorescens* in the moderately resistant group (5.04 t/ha) and susceptible group (6.76 t/ha). So this study proves the effect of salicylic acid in decreasing disease with a reduction in yield but the effect of P. *fluorescens* in enhancing yield. So combined application of these two defense inducers may be useful to decrease the disease and enhancing yield.

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Appendix

APPENDIX 1

Composition of different buffers used in mechanical transmission

1. **0.1 M Potassium phosphate buffer**

   A. 0.1 M potassium dihydrogen phosphate

   B. 0.1 M Dipotassium hydrogen phosphate

2. **0.1 M sodium phosphate buffer**

   A. 0.1 M sodium phosphate monobasic anhydrous

   B. 0.1 M sodium phosphate dibasic dehydrate

3. **0.1 M citrate buffer**

   A. 0.1 M citric acid

   B. 0.2 M sodium phosphate

4. **0.1 M boric acid buffer**

   A. 0.1 M boric acid

   B. 0.05 M borax

5. **0.1 M acetate buffer**

   A. 0.1M acetic acid

   B. 0.1M sodium acetate (tri-hydrate)

MANAGEMENT OF BITTER GOURD MOSAIC BY ENHANCING HOST RESISTANCE

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ABSTRACT OF THE THESIS
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Abstract

Bitter gourd (*Momordica charantia* L.) is one of the important vegetable crops that occupy a pivotal position among fruit vegetables, particularly in south India. The fruits of this crop which have high commercial value and are being used for culinary preparations and various medicinal preparations. In spite of the economic importance of this vegetable, the research work carried out on protection of crop from viral disease is quite scanty. In many case, cent per cent mosaic incidence was recorded in the crop resulting in substantial economic loss. So the present study was focused on screening of bitter gourd accessions and management of bitter gourd mosaic by enhancing host resistance using defense inducers.

The three different viruses causing mosaic in bitter gourd are cucumber mosaic virus (CMV), potyvirus and bitter gourd distortion mosaic virus (BDMV). As these viruses causes mixed infection in field, the separation of individual viruses was carried out using systemic indicator host plants. For separation of CMV and potyvirus, systemic indicator host plants used were cosmos and papaya respectively. BDMV was separated by white fly transmission. The pure cultures of viruses were maintained on the susceptible bitter gourd variety Preethi.

The symptoms developed by different viruses were recorded under natural and artificial conditions were recorded CMV produced mosaic specks, yellow-green mosaic patches, leathery leaves and downward rolling of leaf margin. Symptoms of potyvirus infection were vein clearing, puckering, malformed leaf with reduced leaf size and rugosity. BDMV infection produced mosaic, puckering, leaf distortion, hairy growth on leaves and vines with reduction in leaf size and internodal length.

For the screening of bitter gourd accessions against CMV and potyvirus, potassium phosphate buffer pH 7.0 was found to be the most suitable buffer. Among 22 accessions screened, three accessions viz., TCR 285, TCR 39 and TCR 53 were highly resistant to CMV; one accession Biliagala was highly resistant to potyvirus and 11 accessions viz.,TCR 285, TCR 39, TCR 493 ,TCR 416, TCR 492, TCR 494,TCR 380, TCR 202 and TCR 149, Green long and Biliagala were highly resistant to BDMV.

The field experiment was undertaken with the objective of management of bitter gourd mosaic by using defense inducers. The three different defense inducers *viz.,* salicylic acid 25 ppm, barium chloride 0.1% and *Pseudomonas fluorescens* 2 % were evaluated on the moderately resistant cultivar white long and susceptible variety Preethi. The mosaic symptom was recorded after 51 days of sowing in salicylic acid treated plants and after 40 days of sowing in control. A time gap of 5-10 days after spray of defense inducer was required for development of resistance in plants. The lowest disease severity was observed in cultivar White long treated with salicylic acid. The highest yield was recorded in Preethi treated with *Pseudomonas fluorescens*.