STUDIES ON TRANSMISSION, HOST RANGE AND MANAGEMENT OF ASH GOURD MOSAIC DISEASE

By

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(2009-11-123)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Studies on transmission, host range and management of ash gourd mosaic disease"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "Studies on transmission, host range and management of ash gourd mosaic disease" is a bonafide record of research work done independently by **Ms. Divya M.** (2009-11-123) under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Divay.M

To my loving family, friends and Department of Plant Pathology

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Introduction

1. INTRODUCTION

Vegetables are protective supplementary foods as they contain large quantities of minerals, vitamins and essential amino acids required for our daily diet.

Ash gourd, *Benincasa hispida* (Thunb.) is an important cucurbit vegetable that occupies a pivotal position among fruit vegetables, particularly South India. It is also known as Chinese preserving melon, wax gourd, white gourd, white pumpkin, hairy melon or winter melon. Ash gourd is grown on homesteads, rice fallows and even in riverbeds as well as on commercial scale for its valuable fruits. It is an annual, climbing herb producing large fruits which are fleshy, succulent and densely hairy when young, but thickly covered with white waxy coating on maturity. It is cultivated in an area of 2497 hectare with an annual production and productivity of 15326 tonnes and 6.13t/ha respectively (IIVR, 2010).

The fruits are used in culinary preparations, confectionaries (Petha) and also for various medicinal preparations. Ash gourd fruits are used in ayurvedic preparations and in naturopathy treatments. The famous ayurvedic preparation Kushmanda rasayanam used as a nerval tonic and health rejuvenator is prepared using fruits of ash gourd cultivar called Vaidyakumbalam or Neikumbalam. The diluted juice of ash gourd is beneficial in the treatment of peptic ulcer and obesity. It acts as a blood coagulant also. Shelled seeds of ash gourd are used for de-worming. The peels and seeds boiled in coconut oil promote hairgrowth and prevent dandruff and scalp dryness. Fruits contain on an average 96.7 per cent moisture, 1.9 per cent carbohydrates, 0.4 per cent protein, 0.1g fat, 0.06mg thiamine, 0.01mg riboflavin, 1mg vitamin C, 30mg calcium, 0.8mg iron, 10 calories of energy per 100g of edible portion (Gopalan *et al.*, 1994).

Inspite of the economic importance of this vegetable in our country, very little attempt has so far been made to improve this crop. Yield of ash gourd remain low due to poor genetic stocks, inadequate and improper management practices and incidence of many parasitic and non-parasitic diseases. Among parasitic diseases, viral diseases are of major concern for the farmers due to the heavy economic loss they cause (Hansen, 1989, Mink 1993). Cucurbit crops are subjected to severe losses due to several potyviruses and cucumber mosaic virus (Grumet *et al.*, 2000).

Of the viral diseases, mosaic is an important disease of ash gourd and it is caused by different viruses resulting in various types of symptoms. In India, ash gourd mosaic was first reported by Singh in 1970. Although various workers have studied different aspects of ash gourd mosaic, this disease has not received any attention in Kerala so far.

Since widely accepted therapeutic agents have not yet been developed for the control of viral diseases, the incidence of the disease increases year after year. Indiscriminate use of chemical protectants to control vector population cause adverse impact on ecological balance and health hazards.

In the recent past, mosaic has been found to be the major constraint for ash gourd cultivation in Kerala. Investigation was hence carried out on the following aspects of the mosaic.

- Symptomatology
- Transmission
- Biological indexing
- Host range
- Varietal screening under net house conditions
- Management of ash gourd mosaic disease under field conditions

Review of Literature

2. REVIEW OF LITERATURE

The present investigation, "Studies on transmission, host range and management of ash gourd mosaic disease" was carried out to study the occurrence, symptoms, etiology, host range and management of ash gourd mosaic on which no work has been conducted so far in Kerala. The relevant literature on various aspects related to the studies done so far on the disease in India and abroad are reviewed in this chapter under the following heads.

The Cucurbitaceae is a unique family of at least 9 genera and nearly 16 species cultivated as vegetables. In India, at least 15 types of cucurbits, *viz.*, ash gourd, bitter gourd, bottle gourd, cucumber, sponge gourd, long melon or snake cucumber, kundru, musk melon, pointed gourd, pumpkin, ridge gourd, snake gourd, squash, round gourd and water melon are grown. Ash gourd is an important warm-season cucurbit vegetable grown for its fruits. It is grown throughout the Old World tropics and less commonly in the New World (Peter and Pradeepkumar, 2008).

Cucurbits are known to be infected by a large number of viruses. These viruses are, cucumber mosaic (Doolittle, 1916; Jagger (1916), cucumber green mottle mosaic (Ainsworth, 1935), bottle gourd mosaic, (Vasudeva and Lal, 1943), pumpkin mosaic, pumpkin yellow vein mosaic, squash mosaic (Middleton, 1949; Linderberg *et al.*, 1956), muskmelon vein necrosis, cucumber vein yellowing and cucumber (wild) mosaic (Freitag, 1952), tobacco mosaic strain (bottle gourd mosaic), tobacco ringspot cantaloupe mosaic, cucumber ring spot, watermelon mosaic (Anderson, 1954), melon mosaic (Linderberg *et al.*, 1956), cucumber necrosis (McKeen, 1959), cucurbit latent (Webb and Bohn, 1961), squirting cucumber mosaic (Cohen and Nitzany, 1963), cucumber stunt mottle (Hollings, 1963), Benincasa mosaic, bitter gourd mosaic, kakri mosaic, tori mosaic (Mitra and Nariani, 1965), cucurbit mosaic, muskmelon necrotic ringspot (Kishi, 1966),

muskmelon mosaic (Bandhopadhyay and Mukhopadhyay, 1977), pumpkin enation mosaic and pumpkin mild mosaic (Ghosh and Mukhopadhyay, 1979).

Mosaic is a serious disease of cucurbits which cause severe reduction in crop yield. Cucumber green mottle mosaic virus and white mosaic caused by Cucumis virus strain 2 A (strain of green mottle mosaic virus) reduced the yield 10 to 15 per cent and 70 to 90 per cent respectively in green house (Ges, 1965). Cucumber green mottle mosaic virus caused a yield loss of 15 per cent in cucumber in Lea Valley, United Kingdom (Fletcher *et al.*, 1964). The infection of cucumber plants with cucumber mosaic virus at the cotyledonary stage reduced the yield by 80 per cent in summer and 96 per cent in winter (Kazda *et al.*, 1975)

2.1. OCCURRENCE

Bhargava & Bhargava (1976) reported a strain of watermelon mosaic virus from ash gourd. The incidence of mosaic diseases in ash gourd was estimated 5 to 10 per cent (Singh, 1976). Papaya ringspot virus - watermelon strain and papaya ringspot virus - papaya strain was reported by Akanda *et al.*, (1991) in white gourd.

Fukumoto *et al.*, (1993) isolated zucchini yellow mosaic virus from wax gourd and balsam pear. Chen *et al.*, (1995) carried out the characteristaion of a tospovirus like virus isolated from wax gourd which showed symptoms of light to darker green mottle on leaves, leaf crinkling and tip necrosis and reported that the virus shown serological relationship with watermelon silver mottle virus.

A yellow leaf disease has been observed in cantaloupe melons and wax gourd in Thailand and the virus was identified as tomato leaf curl virus by Samretwanich *et al.*, (2000). The biochemical alteration of cellular components (chlorophyll a and b, β-carotene, total nitrogen and protein, total phosphorus) of

ash gourd due to the infection of bottle gourd mosaic virus, watermelon mosaic virus 2 and papaya ringspot virus was determined by Muqit *et al.*, (2007).

Bhargava and Joshi (1960) detected watermelon mosaic virus from vegetable marrow in UP with mosaic symptoms which differed from other previously described viruses. Reddy and Nariani (1963) reported three types of symptoms in vegetable marrow mosaic *viz.*, mosaic, filiform and witches' broom and their incidence was found to be 46.5, 7.6 and 42.3 per cent respectively. Hariharasubramaniam and Badami (1964) reported the widespread occurrence of a mosaic virus disease on pumpkin causing severe blistering, distortion and reduction in size of leaves. He concluded that the causal virus resembled the bottle gourd mosaic virus and filiform type of vegetable marrow mosaic.

Janardhanan *et al.*, (1969) studied a mosaic disease of bottle gourd from Mysore state and reported that the causal virus resembled Cucumis virus 2 B in symptomatology and certain other characteristics, it was assumed to be a new virus or a new strain of *Lagenaria siceraria* virus. Pillai (1971) was the first to record a mosaic disease of snake gourd in Kerala. The causal virus was reported as a strain of CMV. Dubey *et al.*, (1974) identified the snake gourd mosaic virus as Cucumis virus 1 from New Delhi.

Sangar and Raj (1988) identified a cucumber mosaic cucumovirus in Madhya Pradesh differing from Cucumis virus 3 of summer squash which was characterised by mosaic mottling, leaf distortion and filiform upward rolling. The incidence of vegetable marrow mosaic virus was found to range between 80 to 85 per cent (Bansal *et al.*, 1990). Mantri *et al.*, (2005) gave first report of papaya ringspot virus-W in bottlegourd from India.

Ainsworth (1935) described three cucumber mosaic diseases as green-mottle mosaic (Cucumber virus 3), yellow mosaic (Cucumber virus 4) and yellow-mottle mosaic (Cucumber virus 1). A survey of widely growing area of

zucchini squash (*Cucurbita pepo*) in Chile showed that watermelon mosaic virus is of widespread occurrence (Auger *et al.*, 1974).

According to Hseu *et al.*, (1987), among zucchini yellow mosaic potyvirus (ZYMV), watermelon mosaic I potyvirus (WMIV), cucumber green mottle mosaic tobamovirus (CGMMV), watermelon mosaic II potyvirus and cucumber mosaic cucumovirus infecting cucumber, *Luffa* spp., bitter gourd, wax gourd, pumpkin and bottle gourd in Taiwan, ZYMV was the most prevalent followed by WMIV and CMV. He also reported that CGMMV was rare in cucumber, *Luffa* spp., wax gourd and pumpkin but predominant in bottle gourd.

Lecoq *et al.*, (1992) observed a yellowing disease of melon, cucumber and zucchini squash in France with the infected plants showing yellowing and thickening of the older leaves and they proposed it as cucurbit aphid borne yellows virus belonging to luteo virus group. Dahal *et al.*, (1997) reported on the occurrence of papaya ringspot potyvirus and cucurbit viruses in Nepal. Jawdah *et al.*, (1997) reported cucurbit aphid-borne yellows luteovirus in Lebanon and the disease was widely distributed in the major cucurbit growing areas year round alone or in mixed infections with mosaic-inducing viruses with the highest frequencies being recorded between May and October.

In a survey of viruses in vegetable crops in Western Samoa conducted by Pearson and Liyanage (1997), squash mosaic comovirus was recorded for the first time infecting *Citrullus lanatus, Cucurbita maxima* and *Trichosanthes cucumerina* var. *anguina*. Other new host records were papaya ringspot potyvirus infecting *C. maxima and Cucumis sativus*, zucchini yellow mosaic potyvirus infecting these two hosts and also *Momordica charantia* and *Capsicum annuum*, and cucumber mosaic cucumovirus infecting *Benincasa hispida* and *Psophocarpus tetragonolobus*.

Zouba *et al.*, (1997) conducted a survey of virus diseases of cucurbits (squash, watermelon, muskmelon, cucumber, pumpkin and bottle gourd) and revealed the presence of watermelon mosaic 2 potyvirus, zucchini yellow mosaic potyvirus, papaya ringspot virus (watermelon strain), cucumber mosaic cucumovirus, squash mosaic comovirus, tomato ringspot nepovirus, tobacco ringspot nepovirus and tomato spotted wilt tospovirus.

Gu *et al.*, (2002) detected zucchini yellow mosaic virus in watermelon, melon, pumpkin, luffa, marrows, bitter melon, cucumber and squash samples and not in two wax gourd samples in Northern China. A survey was done to determine the incidence and distribution of virus diseases of cucurbit crops in North Western Australia by Coutts and Jones (2005) and reported the occurrence of squash mosaic virus, cucumber mosaic virus, watermelon mosaic virus, papaya ringspot virus-cucurbit strain, zucchini yellow mosaic virus and luteovirus.

Papayiannis *et al.*, (2005) reported the prevalence of zucchini yellow mosaic virus, papaya ringspot virus type W, watermelon mosaic virus, cucurbit aphid-borne yellows virus, cucumber mosaic virus, squash mosaic virus, cucurbit yellow stunting disorder, beet pseudo-yellows virus and cucumber vein yellowing virus in cucumber, zucchini, melon and watermelon in Cyprus.

Kassem *et al.*, (2007) reported that 90 per cent of *Cucumis melo* and *Cucurbita pepo* in eastern Spain was infected by atleast one of the viruses *viz.*, beet pseudo-yellows virus, cucurbit aphid-borne yellows virus, cucumber mosaic virus, cucumber vein yellowing virus, cucurbit yellow stunting disorder virus, melon necrotic spot virus, papaya ringspot virus, watermelon mosaic virus and zucchini yellow mosaic virus.

Xiang *et al.*, (2008) observed the occurrence of cucurbit aphid-borne yellows virus on nine cucurbitaceous species including *Cucurbita moschata*, *Cucurbita pepo*, *Lagenaria siceraria*, *Cucumis sativus*, *Luffa cylindrica*, *Citrullus*

lanatus, *Cucumis melo*, *Benincasa hispida* and *Momordica charantia* in many regions of China.

2.2. SYMPTOMATOLOGY

Raj (1969) reported the symptomatology of a seed borne virus disease of ash gourd as yellowing, distortion and crinkling of the leaves, stunting of the plant and non-bearing of fruits.

The symptoms of an ash gourd mosaic disease was described by Singh, (1976) as irregular yellow areas first starting from the veins and tips of the young leaves followed by the yellow mosaic mottled area interspersed by dark green blisters on the leaf surface. The severely infected vines did not bear any mature fruit because of stunted growth. Xiang *et al.*, (2008) described a cucurbit aphid-borne yellows virus on nine cucurbitaceous field crops including *Benincasa hispida* causing yellowing, sometimes combined with severe mosaic and in some cases, fruit malformation.

Vasudeva and Lal (1943) reported that small circular spots appear on leaves of vegetable marrow which later become chlorotic and subsequently leaves developing vein clearing and marginal chlorosis along with blistering or puckering on the interveinal areas in some leaves. Vasudeva *et al.*, (1949) reported the virus disease of bottle gourd which caused general stunting and reduction in flower and fruit production. Young leaves exhibited dark green blisters on a crinkled pale green surface and was identified as Cucumis virus 2 C.

Bhargava and Joshi (1960) described the symptoms on *Cucurbita pepo* as coarse mosaic to diffused pattern without any clear demarcation of light and dark green areas and also vein banding pattern on the leaves. The severely infected leaves showed distortion, malformation, extensive reduction of lamina and usually

leaf apices reduced very much into thread like structure. The affected plants become weak with a fewer fruits of small size.

Reddy and Nariani (1963) reported that vegetable marrow infected with mosaic type virus showed typical mosaic pattern of light and dark green areas on leaf and slight reduction in leaf size. In filiform type, the symptoms were distortion of lamina, filiforming of the leaves, vein clearing in younger leaves and development of dark green blister on older leaves. Flowering was delayed, size of flowers was reduced and in severe cases there was no normal setting of fruits. Witches' broom type was seen in the later stage and characterised by a dense tuft of irregularly bent, stunted branches producing severely reduced and malformed leaves. The petioles and internodes were very severely reduced resulting in witches' broom like appearance. The mosaic type was identified as Cucumis virus 1 and filiform type as melon mosaic virus.

Mitra and Nariani (1965) reported a mosaic disease in Tori (*Luffa acutangula*) which is characterised by light and dark green mosaic mottling, downward curling of leaf margins and general stunting in the plant growth. The causal virus was identified as Cucumis virus 3. Shankar *et al.*, (1969) identified a mosaic disease of snake gourd characterised by a mosaic pattern of irregular dark green and yellow chlorotic patches on the lamina. The affected plants were stunted, produced fewer flowers and showed leaf crinkling. The causal virus was identified as Cucumis virus 1.

Verma *et al.*, (1970) investigated the virus disease of snake gourd, bottle gourd and pumpkin in UP. The leaves of the infected snake gourd were variously malformed, reduced in size and showed dark green mosaic mottling symptoms. In bottle gourd, leaves showed mosaic mottling and blistering symptoms. Leaves were smaller, deformed and plants were stunted. Mosaic mottling, blistering and deformity of leaves were the symptoms in Pumpkin. The viruses of snake gourd

and bottle gourd were identified as Cucumis virus 2 B and that of pumpkin as Cucumis virus 1.

Nagarajan and Ramakrishnan (1971b) reported the occurrence of watermelon mosaic virus on snake gourd. The plants were stunted and leaves were affected by prominent mosaic mottling with considerable reduction in leaf size. In advanced stages, the leaves were crowded together to give a bushy appearance. When young plants were infected, considerable malformation were seen in leaves. The outer edge of lamina became serrated. In severely infected plants, the leaves showed blistering and inward cupping of leaf margins. The symptoms appeared within 7 to 9 days of inoculation.

Pillai (1971) reported mosaic mottling, crinkling and reduction in leaf size, stunted growth and production of fewer flowers and fruits as the symptoms of snake gourd mosaic. Dubey *et al.*, (1974) identified the symptoms of Cucumis virus 1 infection of snake gourd mosaic as mosaic mottling accompanied by chlorosis, vein banding and blistering of leaf lamina, sparse production of branches, flowers and fruits.

Ghosh and Mukhopadhyay (1979) observed cucumber plants with typical symptoms of yellowish green foliage, pinpoint yellow chlorotic spots, leaf crumpling, mottling and stunting.

Mantri *et al.*, (2005) reported the symptoms of papaya ringspot virus-W infection of bottlegourd as mosaic mottling, interveinal chlorotic bands, leaf distortion, malformation of fruits and fruit size reduction.

The three cucumber mosaic diseases were described by Ainsworth (1935). The green mottle mosaic caused a dark green mottle with blistering and distortion of leaves, but the fruit was not usually marked. The yellow mosaic gave rise to a distinct type of leaf mottle, yellow to silver white in colour and the fruit may be

seriously marked. The yellow mottle mosaic was characterised by a diffuse yellow mottle of cucumber leaves and fruit.

Middleton (1944) reported that cucumber mosaic virus caused filiform symptoms on the leaves in *Cucurbita pepo* var. *condensa*. The mosaic mottling, stunting and reduction in size of flowers and fruits were reported on vegetable marrow by Naqvi *et al.*, (1975). Severe leaf narrowing, distortion and dome shaped protuberances on the fruit was observed on vegetable marrow by Ragozzino and Stefanis (1977). Makkouk and Lesemann (1980) reported WMV-1 causing severe mottling, blistering and malformation in cucumber leaves from Lebanon.

Crosslin *et al.*, (1988) first reported zucchini yellow mosaic virus (ZYMV) on *Cucurbita pepo* var. *melo pepo* in Pacific North West. The infected plants showed mosaic and leaf deformation and produced mottled, irregularly shaped unmarketable fruits.

The papaya ringspot potyvirus watermelon strain and cucurbit viruses isolated from pawpaw and ten cucurbitaceous vegetables (ash gourd, zucchini, watermelon, cucumber, pumpkin, bottlegourd, snake gourd, sponge gourd, bitter gourd and choyote) were characterised by the presence of severe mosaic, leaf distortion, oily streaks or spots on papaya, leaf distortion, blisters and shoe stringing on zucchini and mosaic or yellow mosaic, blisters and leaf distortion on other cucurbits (Dahal *et al.*, 1997).

The symptoms on cucumber plants infected by viral diseases in Lithuania are characterised by vein clearing, mosaic, mottling and malformation of leaves, deformation and stunting of plants (Zitikaite, 2002). Ariyaratne *et al.*, (2005) identified a new mosaic disease of snake gourd in Sri Lanka with leaf distortion, reduction of internode length and fruit distortion as the prominent symptoms.

Gal (2007) reported that zucchini yellow mosaic virus causes stunting and major foliar deformation with dark green blisters and mosaics in cucurbit hosts, eventually developing a filamentous leaf phenotype.

2.3. STUDIES ON TRANSMISSION

2.3.1. Sap Transmission

Sidhu (1965) reported artificial transmission of vegetable marrow mosaic virus (Cucumis virus-1) to ash gourd. Shanker *et al.*, (1972) transmitted pumpkin mosaic virus (PMV) which resembled Cucumis virus-3 and watermelon mosaic virus to ash gourd using potassium phosphate buffer 0.066*M* (pH 7.6) and found t that 0.1 *M* citrate phosphate buffer was the most effective buffer for PMV stability and infectivity as it gave 40 per cent infection even after 24h of storage.

Sharma and Chohan (1973) reported that Cucumis virus I of ash gourd was sap transmissible using phosphate buffer (pH 7) but not transmissible through the seeds of ash gourd.

Umamaheswaran (1985) reported that pumpkin mosaic virus was sap transmissible and gave a maximum infection of 85 per cent. He also reported that percentage of transmission varied with extraction medium used.

Tripathi and Joshi (1985) reported a sap transmissible virus producing mosaic and leaf distortion symptoms of pumpkin. Sandhu and Kang (2007) identified cucumber mosaic virus and watermelon mosaic virus-1 causing mosaic syndrome of cucurbits in Punjab and reported that the viruses were transmissible through sap and seed.

Wakman *et al.*, (2002) reported a mosaic disease of pumpkin (*Cucurbita maxima*) in Sulawesi, Indonesia that was transmitted mechanically from crude sap

of different leaf samples to healthy pumpkin seedlings. The mosaic disease was associated with possibly a potyvirus such as watermelon mosaic virus rather than papaya ringspot virus or zucchini yellow mosaic virus. Ariyaratne *et al.*, (2005) identified a PRSV related virus in snake gourd in Sri Lanka that was mechanically transmitted to ash gourd. Gal (2007) reported that zucchini yellow mosaic virus was mechanically transmitted efficiently both in laboratory and naturally.

2.3.2. Insect Transmission

2.3.2.1. Aphid transmission

Singh (1970) studied the mosaic disease of *Benincasa* and its transmission by *Myzus persicae*. Tewari *et al.*, (2004) studied the efficiency of aphids as vectors of Benincasa mosaic virus and revealed that *Aphis gossypii* on *Lagenaria vulgaris* (*L. siceraria*) was the most efficient vector (66.6 per cent of infection), followed by *Myzus persicae* on *Raphanus sativus* (60.0 per cent), *Lipaphis pseudobrassicae* (*L. erysimi*) on *Brassica campestris* (53.3 per cent) and *Aphis caraecovora* on *Calotropis procera* (40.0 per cent). *A. gossypii* acquired the virus even without preliminary fasting, but the efficiency increased upto a period of 4 h of fasting (46.6 per cent infection).

Joshi (1962) reported a strain of watermelon mosaic virus infecting vegetable marrow which was transmitted by *Aphis gossypii* and *Myzus persicae*. Reddy and Nariani (1963) found that the mosaic disease of vegetable marrow caused by CMV was transmitted by *Aphis craccivora* which was the first report of that insect to be a vector of CMV. In addition, *Aphis gossypii*, *Aphis evonymi* and *Myzus persicae* were identified as vectors. The per cent transmission increased when inoculation feeding was given on cotyledons of healthy seedlings.

Hariharasubramaniam and Badami (1964) reported a virus disease of pumpkin which was found to be transmitted to cucurbits only by *Aphis laburni*.

Verma *et al.*, (1970) reported *Myzus persicae* and *Aphis gossypii* as vectors of snake gourd mosaic, bottle gourd mosaic and pumpkin mosaic.

Jaganathan and Ramakrishnan (1971) studied a mosaic disease of muskmelon and pumpkin and reported that *Myzus persicae* alone transmitted the muskmelon mosaic while *M. persicae* and *Aphis gossypii* transmitted the pumpkin mosaic. They found that a minimum five aphids are required to transmit the virus. The vector-virus relationships were studied in detail. The bitter gourd mosaic virus as described by Nagarajan and Ramakrishnan (1971a) was transmitted by five species of aphid vectors, *viz.*, *Myzus persicae*, *Aphis gossypii*, *Aphis malvae*, *Aphis nerii* and *Brevicoryne brassicae*.

Pillai (1971) reported the non-transmission of mosaic disease of snake gourd (CMV) by *Aphis craccivora* and *Myzus persicae*. Goel and Varma (1973) identified a new strain of CMV (Luffa strain) and the virus was found to be transmitted by *Myzus persicae*, *Aphis gossypii* and *Brevicoryne brassicae*. Dubey *et al.*, (1974) identified a snake gourd mosaic virus and designated as Cucumis virus 1 which was found to be transmitted by *Aphis gossypii, Myzus persicae* and not by *Aphis craccivora* and three other aphid species.

Sanger and Raj (1988) reported the transmission of cucumber mosaic cucumovirus from summer squash by *Myzus persicae*. Purushothaman (1994) reported the transmission of bitter gourd mosaic by the aphid vectors viz., *Aphis gossypii*, *Aphis malvae*, *Aphis craccivora* and *Myzus persicae* of which *Aphis gossypii* and *Aphis malvae* are found to be the most efficient ones. He also found that 10 viruliferous aphids were required for the successful transmission of the virus and the acquisition and inoculation threshold were 20 min.

Louis (2003) reported 62.5 per cent transmission of pumpkin mosaic virus through *Aphis gossypii*. Sandhu and Kang (2007) reported the aphid (*Aphis*

gossypii) transmission of cucumber mosaic virus and watermelon mosaic virus-1 of cucurbits in Punjab.

Aphid transmission of cucurbit viruses are reported from other countries also. Hoggan (1933) reported that single individual of green peach aphid (*Myzus persicae*) was able to transit CMV to tobacco, but the per cent infection increased with the number of aphids. The entire process of picking up the virus and transmitting it to the healthy plants required only 30 min. No incubation period was noticed and the viruliferous aphids found to loose their infectivity after feeding for two hours on healthy plants or after starvation for 18 to 27h.

Severin (1947) found that CMV was transmitted by *Aphis gossypii*, *Aphis rumicis* and *Myzus persicae*. Simons (1955) studied the host-vector-virus relationship of southern cucumber mosaic virus. It was transmitted by *Aphis gossypii, Myzus persicae* and *Aphis fabae* in the order of efficiency. The acquisition threshold of the first two vectors ranged from 5-10 seconds. According to Yamamoto (1986) high rate of watermelon mosaic virus infection of cucumber seedlings was due to transmission by *A. gossypii* from many kinds of cucurbit crops.

Yonaha *et al.*, (1988) reported that the two viruses isolated from *Trichosanthes rostrata* and other Cucurbitaceae in Okinawa were identified as Trichosanthes mottle potyvirus and watermelon mosaic I potyvirus which infected only cucurbits and were transmitted by *Myzus persicae* and *Aphis gossypii*.

Yellowing disease of cucurbits caused by luteovirus (cucurbit aphid-borne yellows virus) was found to be readily transmitted in a persistent manner by the aphids *Myzus persicae* and *Aphis gossypii* (Lecoq *et al.*, (1992). The zucchini yellow mosaic virus from *Cucurbita moschata* was transmitted by an aphid, *Myzus persicae* (Kim *et al.*, 1995).

Dukic *et al.*, (2002) studied the biological and serological characterisation of cucumber mosaic cucumovirus, zucchini yellow mosaic potyvirus and watermelon mosaic potyvirus 2 and reported that the viruses were transmissible by *Aphis gossypii* in a non-persistent manner, but possible role of seed in virus transmission was not confirmed. Wakman *et al.*, (2002) reported the aphid transmission of a watermelon mosaic virus of pumpkin (*Cucurbita maxima*) from Sulawesi, Indonesia.

2.3.2.2. Whitefly transmission

Giri and Misra (1986) reported white fly as the vector of leaf distortion virus disease of bitter gourd. Mathew *et al.*, (1991) noticed 40 per cent transmission of BGMV with a single viruliferous whitefly. They also observed that the rate of transmission increased with increase in number of insects and cent per cent transmission was obtained with ten viruliferous whiteflies per plant.

Muniyappa *et al.*, (2003) reported that pumpkin yellow vein mosaic virus (PYVMV) was transmitted readily in a persistent manner by the whitefly, *Bemisia tabaci* with a minimum acquisition and inoculation access periods of 30 min and 10 min respectively.

Zacharia (2006) reported cent per cent transmission of bitter gourd distortion mosaic virus by *Bemisia tabaci* with an incubation period of 14 days.

The virus diseases transmitted by whitefly are often referred to as rugaceous and cause mosaic and leaf distortion symptoms in infected plants and *Bemisia tabaci* is the most important and widespread vector that transmit most of the known whitefly transmitted viruses and they feed mainly on phloem tissues and the viruses are not usually transmitted by mechanical means (Walkey, 1985).

McCreight and Kishaba (1991) reported that the squash leaf curl gemini virus (SLCV) on squash is transmitted by the sweet potato whitefly, *Bemisia tabaci* and *Benincasa hispida*, *Cucurbita ficifoli*, *Lagenaria siceraria*, *Luffa acutangula*, *Luffa aegyptiaca* and *Luffa graveolens* were resistant to SCLV in green house and field tests.

2.4. STUDIES ON IDENTIFICATION OF VIRUS

2.4.1. Biological indexing

The virus (Cucumis virus-1) obtained from ash gourd produced pinhead size, yellowish-brown necrotic local lesions on *Chenopodium amaranticolor* (Sharma and Chohan, 1973). Ash gourd mosaic virus induced necrotic lesions on *Beta vulgaris* (Singh, 1976).

Vasudeva and Nariani (1952) reported that bottle gourd mosaic virus caused localised infection on inoculated leaves of tobacco, *Solanum nigrum* and *Solanum nodiflorum*. Ross (1953) reported *Physalis floridana* as a local lesion test plant for PVY. Cucumis virus-1 induced reddish local lesions, dark brown lesions and brown circular to oval lesions on *Vigna sinensis*, *Beta vulgaris* and *Spinacea oleracea* respectively (Nariani and Nyako, 1963).

Vegetable marrow mosaic virus produced systemic lesions on *Nicotiana tabacum*, *Nicotiana rustica* and *Nicotiana glutinosa* whereas reddish local lesions on *Chenopodium* species (Reddy and Nariani, 1963). Verma *et al.*, (1970) reported *Chenopodium amaranticolor* as a local lesion host of snake gourd mosaic virus. Bitter gourd mosaic virus produced local lesion symptom on *Chenopodium amaranticolor* whereas *Datura stramonium* and *Datura metel* were the symptomless carrier of the virus (Purushothaman, 1994).

Nisha (2007) reported the presence of chlorotic lesions in *Petunia hybrida* within 48h of inoculation whereas absence of lesions in cowpea by potyviruses of vanilla.

Samuel (1931) showed that CMV produces local lesions on tobacco. Hoggan (1933) reported that CMV produces local lesions on sugar beets. Roberts *et al.*, (1951) reported *Chenopodium hybridum* L. and some varieties of bean (*Phaseolus vulgaris*) as local lesion hosts for cucumber mosaic virus. Webster (1951) reported that Cucumber virus 1 from cucumber produced local lesions on black cowpea.

Sinclair and Walker (1955) reported that certain strains of CMV induced local lesion reaction in resistant varieties and systemic infection in susceptible varieties of *Vigna sinensis*. Velson (1960) identified a strain of melon mosaic virus on *Cucurbita moschata* which produced local lesions on *Chenopodium amaranticolor*. According to Smith (1972) *Chenopodium amaranticolor* was a good local lesion host of type strain of cucumber mosaic virus whereas the *Datura stramonium* gave pale spots followed by mosaic mottle with formation of chlorotic rings on leaves.

Zucchini yellow mosaic virus produced local lesions on inoculated leaves of *Chenopodium amaranticolor, Chenopodium quinoa and Gomphrena globosa. Nicotiana clevelandii* and cucurbits were infected systemically (Kim *et al.*, 1995).

Sousa *et al.*, (1996) identified a strain of cowpea aphid-borne mosaic virus (potyvirus) and reported that the virus caused systemic symptoms in *Vigna unguiculata*. Gal (2007) reported that zucchini yellow mosaic virus produced local lesions on *Chenopodium amaranticolor*, *Chenopodium quinoa* and *Gomphrena globosa*.

2.5. HOST RANGE

Cucumis virus-1 strain of ash gourd resulted in systemic infection on *Nicotiana glutinosa* L. and *Gomphrena globosa* L. and other cucurbitaceous hosts (Sharma and Chohan, 1973). Singh (1976) reported systemic infection of *Benincasa hispida*, *Cucumis melo*, *Lagenaria siceraria*, *Luffa cylindrica*, *Luffa acutangula*, *Cucurbita pepo*, *Nicotiana tabacum* var. White Burley, *Petunia hybrida* and *Gomphrena globosa* by an ash gourd mosaic virus. Cucumber, green melon, zucchini, watermelon and ash gourd plants were reported as hosts of watermelon mosaic virus of pumpkin (Wakman *et al.*, 2002).

Vasudeva and Pavgi (1945) reported transmission of melon mosaic virus from cucumber to number of solanaceous plants. Vasudeva *et al.*, (1949) reported a virus disease in bottle gourd caused by Cucumis virus 2C which differed from the early mentioned strain of cucumber green mottle mosaic virus by its ability to produce symptoms on watermelon and *Datura stramonium*. The virus was carried asymptommatically on bitter gourd and *Luffa acutangula*. Joshi (1962) detected a strain of watermelon mosaic virus infecting vegetable marrow, *Cyclanthera pedata*, squash and cucumber.

Reddy and Nariani (1963) reported that Cucumis virus 1 caused systemic infection in snake gourd. The filiform type (Cucumis virus 3) also caused mosaic on snake gourd but the host range was restricted to Cucurbitaceae. Vegetable marrow mosaic virus was successfully transmitted to *Cucumis sativus* L., *Cucumis melo* L. var. *utilissima*, *Cucumis anguria*, *Cucurbita moschata*, *Cucurbita maxima*, *Lagenaria siceraria*, *Trichosanthes anguina*, *Momordica charantia*, *Luffa acutangula*, *Luffa cylindrica*, *Citrullus vulgaris*, *Zinnia elegans* and *Gomphrena globosa* exhibiting mild diffused mottling on the younger leaves. *Brassica campestris* and *Hesoeris matronalis* were symptomless carriers.

Hariharasubramaniam and Badami (1964) reported that the host range of pumpkin mosaic was limited to Cucurbitaceae. According to Allam (1965) the host range of vegetable marrow mosaic was restricted to Cucurbitaceae.

Mitra and Nariani (1965) reported that the host range of tori mosaic (Cucumis virus 3) was restricted to Cucurbitaceae and confined to *Lagenaria siceraria*, *Cucurbita moschata*, *Cucurbita pepo*, *Momordica charantia*, *Trichosanthes anguina* and *Citrullus vulgaris*. Verma *et al.*, (1970) recorded a severe mosaic disease on snake gourd and the host range was restricted to members of Cucurbitaceae.

Jaganathan and Ramakrishnan (1971) showed that the host range of viruses from muskmelon and pumpkin were confined to Cucurbitaceae. Among different species of cucurbits tested, snake gourd and bitter gourd were found to be the hosts. The viruses were identified as strains of melon mosaic virus. Nagarajan and Ramakrishnan (1971b) reported the occurrence of watermelon mosaic virus on snake gourd, the host range being restricted to Cucurbitaceae.

Shanker *et al.*, (1972) reported that host range of pumpkin mosaic virus was restricted to Cucurbitaceae viz., *Trichosanthes anguina*, *Lagenaria siceraria*, *Momordica charantia*, *Citrullus vulgaris*, *Luffa acutangula*, *Cucurbita pepo* and *Cucumis melo*. Naqvi *et al.*, (1975) reported that the host range of vegetable marrow mosaic virus was restricted to cucurbitaceous and solanaceous families. Sastry (1982) reported PVY isolate of brinjal produced severe mosaic mottle in brinjal, mosaic mottle in chilli, *Nicotiana glutinosa* and *Datura metel* and necrotic lesions in *Physalis floridana* and *Chenopodium amaranticolor*.

Host range of bitter gourd mosaic virus include *Cucumis melo*, *Cucumis metuliferus*, *Luffa acutangula*, *Citrullus vulgaris*, *Trichosanthes anguina*, *Musa* sp. *cv. palayankodan*, *Antigonon leptopus*, *Capsicum annuum*, *Nicotiana glutinosa* and *Physalis minima* due to infection by BGMV. *Cucumis sativus*,

Benincasa hispida and *Lagenaria siceraria* were found to be non-hosts of BGMV (Purushothaman, 1994).

Pumpkin mosaic virus showed systemic infection on *Cucurbita maxima*, *Trichosanthes anguina*, *Citrullus vulgaris*, *Momordica charantia* and *Benincasa hispida* (wild ash gourd) of family Cucurbitaceae, *Capsicum annuum* and *Datura metel* of Solanaceae, *Vigna unguiculata* and *Glycine max* of Fabaceae. *Lagenaria siceraria* and *Luffa acutangula* were found to be symptomless carriers of the virus (Louis, 2003).

Zacharia (2006) reported that bitter gourd distortion mosaic virus was not transmissible to ash gourd, bottle gourd, ivy gourd, cucumber, pumpkin, snake gourd and watermelon.

Cucumber mosaic virus causing mosaic disease of vegetable marrow also attack *Lycopersicon esculentum*, *Nicotiana tabacum* var. White Burley, *Nicotiana glutinosa* and *Datura stramonium* (Doolittle, 1920).

Ainsworth (1935) reported that green mottle mosaic (Cucumber virus 3) and yellow mosaic (Cucumber virus 4) were not transmissible to solanaceous plants whereas yellow mottle mosaic (Cucumber virus 1) was transmissible to solanaceous plants.

Velson (1960) identified a strain of melon mosaic virus on *Cucurbita moschata* which had a limited host range causing systemic infection in cucurbitaceous plants. Inouque *et al.*, (1967) studied a mosaic disease in Japan on cucumber and identified as a cucumber green-mottle mosaic virus and found that many cucurbit hosts were systemically affected.

Sousa *et al.*, (1996) reported that a strain of cowpea aphid-borne mosaic virus (potyvirus) caused systemic symptoms in *Calapagonium* sp., *Canavalia*

brasiliensis, *Canavalia ensiformis*, *Cassia iccidentalis*, *Centrosema brasilianum*, *Centrosema pascuorum*, *Clitoria ternatea*, *Glycine max*, *Macroptilium lathyroides*, *Nicotiana benthamiana*, *Phaseolus membranescens*, *Sesamum orientale* and *Vigna unguiculata*.

Chen *et al*., (2004) reported that watermelon silver mottle virus infected *Benincasa hispida*, *Cucumis melo*, *Cucumis sativus*, *Cucumis melo conomon* group, *Cucurbita pepo*, *Luffa aegyptica*, *Lagenaria siceraria*, *Solanum nigrum* and *Amaranthus viridis*.

The cucumber green mottle mosaic virus of snake gourd did not infect plants of Leguminosae, Caricaceae, Compositae and Solanaceae families. PRSV related virus of snake gourd infected cucurbitaceous host plants like *Trichosanthes cucumerina*, *Cucurbita maxima*, *Benincasa hispida* and *Momordica charantia* (Ariyaratne *et al*., 2005).

2.6. VARIETAL SCREENING

Bhargava and Bhargava (1976) studied resistance of ash gourd varieties and reported that *Benincasa hispida* var Petha Local was resistant to five strains of watermelon mosaic virus.

Khan *et al*., (2000) conducted an experiment to find out the impact of trichome density on the infestation of *Aphis gossypii* Glover and the incidence of aphid transmitted viral diseases in ash gourd (*Benincasa hispida* Thunb.) using four genotypes *viz*., Local Sylhet, Local Round, High Female and CQ-10-90. He reported that the Local Sylhet genotype was found to have the highest trichome density compared with those of Local Round, High Female and CQ-10-90 and was least infested by *Aphis gossypii*. He also reported that the percent disease incidence was also found to be lowest on the Local Sylhet genotype.

2.7. MANAGEMENT OF ASH GOURD MOSAIC

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

Mathew (1998) adopted an integrated approach for the management of mosaic disease in bitter gourd which include high seed rate, rouging of infected plants, basal application of thimet 10g/plant, weekly sprays of 5 per cent neem oil-soap suspension and fortnightly sprays of 0.03 per cent dimethoate.

2.7.1. Botanicals

Cherian and Menon (1944) observed that cold extracts of neem seed kernel was efficient as an insecticide against *Aphis gossypii* and the toxicity was found to increase by addition of soap. Asari and Nair (1972) reported the effectiveness of neem seed suspension against brinjal aphid. Narayanaswamy and Ramiah (1983) observed that leaf extracts of *Cocos nucifera* was effective against tomato spotted wilt virus. Neem oil (0.5 %) inhibited aphid transmission of CMV by 40, 33.3, 23.3 and 30 per cent when sprayed with the oil before acquisition, during acquisition, between acquisition and inoculation and at the time of inoculation respectively (Srivastava *et al.*, 1986).

The effect of antiviral principles from the extracts of *Mirabilis jalapa*, *Prosopis chilensis*, *Datura metel*, *Cocos nucifera*, *Sorghum vulgare* against virus disease in blackgram was studied by Manjuvani (1987) and reported that extract of *M. jalapa* reduced the per cent infected plants followed by leaf extracts of *P.*

chilensis, *D. metel*, *C. nucifera* and *S. vulgare* which was 37.5, 43.7, 50 and 50 per cent infection respectively.

Kannan and Doraiswamy (1993) conducted field experiments to investigate the efficacy of plant extracts of *Prosopis chilensis*, *Vitex negundo*, *Azadirachta indica* and *Madhuca longifolia* against cowpea aphid-borne mosaic virus (blackeye cowpea mosaic potyvirus) on cowpeas. All the extracts reduced the incidence of disease and increased the yield. However, incidence of the disease was most reduced by application of one per cent emulsion of *A. indica* and this resulted in a significant increase in yield.

Reghunath and Gokulapalan (1994) reported the effectiveness of neem oil against cowpea aphids and thereby reducing cowpea mosaic virus incidence. Samuel and Mariappan (1996) reported reduction in survival of aphids and transmission of mosaic in chillies with neem seed oil.

Umamaheswaran (1996) studied on the management of cowpea aphid-borne mosaic virus using products from 25 plants including *Cocos nucifera* and found that it had 81.33 per cent inhibition over control. Manickam and Rajappan (1998) reported the effect of antiviral principles from dry leaves of coconut against tomato spotted wilt virus.

Kumar (1999) studied the effect of neem oil (3 per cent) on CMV infecting snake gourd and reported that neem oil had 52.53 per cent reduction over control. Rajakumar and Byadgi (2002) studied the efficacy of viricides (2 per cent Virex-H and Action-100) and leaf extracts of neem and bougainvillea in controlling the aphid vectors of tomato mosaic virus causing tomato mosaic diseases in Dharwad.

Sunkad *et al.*, (2002) reported that groundnuts sprayed 20 and 30 days after sowing, with sorghum and coconut leaf extract reduced peanut bud necrosis

disease (groundnut bud necrosis virus) incidence and significantly increased yields. The greatest reduction in disease incidence was observed in plots sprayed with sorghum leaf extract (47.3 per cent), followed by coconut leaf extract (44.5 per cent).

Vanitha and Suresh (2002) conducted a study to investigate the effect of botanical insecticides in controlling tomato spotted wilt virus in tomato cv. KM-1 transmitted by *Thrips tabaci* and *Frankliniella* sp. The botanicals sprayed were *Adathoda* sp. leaf extract at 10 per cent, coconut leaf extract at 10 per cent, neem oil at 3 per cent, neem seed kernel extract at 15 per cent and sorghum leaf extract at 10 per cent. He reported that adathoda leaf extract was effective in controlling the disease with maximum yields.

The aqueous extracts from different parts (root, leaf, stem, flower and seed samples) of *Boerhaavia diffusa* was found to be significantly active against tomato yellow leaf curl virus, papaya ringspot virus, papaya green mottle mosaic virus, watermelon mosaic virus, bottle gourd mosaic virus in muskmelon, ridge gourd and bottle gourd, cucumber mosaic virus in cucumber and muskmelon and watermelon mosaic virus in watermelon (Aswathi *et al.*, 2003). Louis (2003) studied the inhibitory effect of one per cent distilled water extract of *Plumbago rosea* against pumpkin mosaic virus and reported that weekly spray of the extract decreased disease severity and increased yield.

Kulkarni *et al.*, (2003) conducted an experiment to study the management of groundnut bud necrosis virus disease (BND) on variety JL-24 using coconut and sorghum leaf extracts and neem seed kernel extract 20 and 35 DAT in Karnataka. He reported that coconut and sorghum leaf extracts sprayed 20 and 35 days after planting significantly reduced the incidence of BND and increased pod yield by 60 to 100 per cent.

Pandey *et al.*, (2003) studied the antiviral properties of extracts (5 and 10 per cent) of selected medicinal plants (*Catharanthus roseus*, *Rauvolfia serpentina*, *Bacopa monnieri*, *Eclipta alba and Phyllanthus niruri*) against Benincasa mosaic and reported that the viral inhibition of *C. roseus* and *B. monnieri* decreased with increasing application duration. *C. roseus* and *R. serpentina* extracts at 5 per cent concentration were more effective than at 10 per cent. Pre-inoculation was better compared to post inoculation in terms of effectiveness against the virus. For *E. alba* and *P. niruri* extracts, a time gap was required between application and viral inoculation to obtain maximum inhibition. For *R. serpentina* time gap increase did not affect viral inhibition.

Singh *et al.*, (2005) dealt with inhibitory activity of bark extracts of 15 different angiospermic plants against the infectivity of bottle gourd mosaic virus using *Terminalia arjuna*, *Psidium guajava*, *Ocium sanctum*, *Ficus elastica*, *Ficus religiosa*, *Terminalia tomentosa*, *Artocarpus integrifolia*, *Calotropis procera*, *Vinca rosea* and *Jatropha curcas* and found that the bark extract obtained from *Terminalia arjuna*, has maximum inhibitory activity for bottlegourd mosaic virus.

Bhyan *et al.*, (2007) reported that karamja (*Pongamia pinnata*) leaf extract performed best among the extracts of neem (*Azadiracta indica*) fruits, garlic (*Allium sativum*) bulbs and mahagoni (*Swietenia macrophylla*) seeds against TYLCV and yield related parameter of tomato. Use of neem oil 2.5 or 5 per cent with garlic (20g/plant) was found effective in controlling epilachna beetle, jassids, aphids and mites in bitter gourd (KAU, 2007).

Kumar and Aswathi (2007) reported that the symptom severity and the time of appearance of bottle gourd mosaic disease in bottle gourd plants was delayed, if the aqueous root extract of *Boerhaavia diffusa* was sprayed weekly on to the leaves of bottle gourd plants since seedling stage. The incidence of bottle gourd mosaic disease was gradually reduced as the number of sprays with *B. diffusa* was increased. The maximum protection was observed in plants which

received six sprays followed by five and four sprays. Three and less sprays were not found much effective. Plants treated with *B. diffusa* performed superior over control regarding all the growth parameters.

2.7.2. Biological Control

Kumar (1999) studied the effect of *Pseudomonas fluorescens* as soil drenching and soil drenching + foliar spray (2 per cent), on CMV infected snake gourd and reported that *P. fluorescens* (soil drenching) had 8.9 per cent reduction and *P. fluorescens* (soil drenching + foliar application) had 23.39 per cent reduction over control. Venkatesan (2000) reported that among AVPs, chemical oils and biocontrol agents for the management of blackgram yellow mosaic, application of *Pseudomonas fluorescens* (2 per cent) recorded the lowest percentage disease incidence (39.14 per cent).

Saravanan (2006) reported that seed treatment, soil drenching and foliar application of *Pseudomonas fluorescens* controlled yellow mosaic disease of black gram and enhanced yield.

Raupach *et al.*, (1996) tested the capacity of plant growth promoting rhizobacteria (PGPR) strains 89B-27 (*Pseudomonas fluorescens*) and 90-166 (*Serratia marcescens*) to protect *Cucumis sativus* L. cv. Straight 8 from cucumber mosaic cucumovirus infection. He reported that seed treatment with both PGPR strains significantly and consistently reduced the disease incidence.

Zhender *et al.*, (2001) reported the effect of plant growth promoting rhizobacteria against cucumber mosaic virus in cucumber and tomato. Ipper *et al.*, (2005), El-Badry *et al.*, (2006) and Megahed (2008) showed the control of CMV in cucumbers and tomato by rhizosphere bacteria by an ISR mechanism.

Kandan *et al.*, (2005) investigated the biocontrol efficacy of strains of *Pseudomonas fluorescens* against tomato spotted wilt virus (TSWV) in tomato both alone and in mixtures. He reported that the *P. fluorescens* strains applied to seed, soil and foliage or as a seedling dip significantly reduced TSWV with a concomitant increase in growth promotion and yield compared to control plants in both glass house and field.

Galal (2006) screened nine *Streptomyces* strains to protect Cucumis sativus from cucumber mosaic virus (CMV). Foliage treatment with the filtrate of five *Streptomyces* strains showed more activities when applied before virus inoculation and he also reported *that S. violaceusniger* filtrate recorded the highest percentage of viral inhibition.

Assarany and Galal (2008) investigated on the induction of resistance of cucumber plants (*Cucumis sativus*) against cucumber mosaic virus (CMV) by either ethyl alcohol extracts or cultural filtrates of twenty fungal isolates from *Aspergillus clavatus*, *Aspergillus fumigatus*, *Aspergillus glaucus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus versicolor*, *Penicillium expansum*, *Penicillium funiculosum*, *Penicillium griseofulvum*, *Penicillium janczewskii*, *Penicillium janthinellum*, *Penicillium nigicans*, *Penicillium notatum*, *Penicillium rubrum*, *Penicillium chrysogenum*, *Botrytis squamosa*, *Botrytis byssoidea*, *Fusarium solani* and *Fusarium oxysporum* and he reported that the most potent fungal extract is *F. oxysporum*.

El-Dougdoug *et al.*, (2010) described the antiviral activity from the heat stable culture filtrate of *Pseudomonas fluorescens* against a satellite cucumber mosaic virus.

2.7.3. Chemical Methods

Out of 247 viral diseases of plants, 164 are stated to be transmitted by nearly 200 species of aphids. In view of their short life cycle and high reproductive rate, aphids can multiply in large numbers and cause severe yield loss in economically important crop plants. Chemical insecticides have been used regularly for the management of aphid pests. Resurgence has been reported in several species of aphid pests as a consequence of indiscriminate application of chemical pesticides.

Rathore and Agnihotri (1985) reported 39 per cent reduction in incidence of yellow mosaic by spraying with dimethoate (0.02 per cent) at 15 days interval. Devi and Reddy (1995) studied the effects of insecticides (quinalphos, dimethoate) on the transmission of pepper vein banding virus (PVBV) and cucumber mosaic virus (CMV) on *Capsicum annuum* by *Myzus persicae* and reported that spraying of insecticides on plants before inoculating with viruses were effective in inhibiting the transmission of PVBV and CMV. Transmission percentage decreased with increase in aphid mortality and concentration of the insecticides. Kumar (1999) reported 19 per cent reduction over control of CMV infection in snake gourd by 0.1 per cent spray of dimethoate.

The efficacy of a new insecticide acetamiprid 20 SP along with imidacloprid 70WS was investigated against severity and incidence of Okra yellow vein mosaic virus (YVMV), whitefly (*Bemisia tabaci*) and yield by Gowdar *et al.*, (2007) in Karnataka. The results reveal that acetamiprid and imidacloprid gave a significant reduction of YVMV incidence and mean whitefly population compared to the control with acetamiprid being the best treatment.

Combined treatment of nylon net covering tomato nursery beds for 25 days to protect seedlings and four spray application of imidacloprid (0.005 per cent) at 15 days interval after transplanting was found to reduce leaf curl

incidence (23 per cent at 90 DAT) and increase yields in tomato cv. Megha (Reddy *et al.*, 2010).

Cosmi *et al.*, (1999) recommended that the control of tobacco viruses in tobacco crop (cultivars Bright, Burley and Kentucky) should rely on a mixture of insecticide treatments against aphid vectors, removal of weed hosts bordering the field, good nutrition of the crop and the use of tolerant or resistant cultivars.

Successful control of watermelon viral diseases include regular and planned insecticide treatments to reduce aphid population, herbicide treatments to eliminate alternative virus hosts and planting less susceptible cultivars (Bulajic *et al.*, 2008).

Karim *et* al., (2008) conducted an experiment to study the effect of insecticides against yellow leaf curl virus in tomato in Bangladesh. He reported that among the treatments Gaucho (Imidacloprid) 70WP @ 5g/kg seed performed best in reducing the disease incidence as well as disease severity in all the plant growth stages and increased the yield.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation "Studies on transmission, host range and management of ash gourd mosaic disease" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2009 to 2010 with a view to understand the symptomatology, mode of transmission, host range, varietal resistance and to chalk out a suitable management practice. The details of the materials used and the techniques adopted for the investigation are described below.

3.1. SURVEY

A purposive sampling survey was conducted for collection of mosaic samples from five different locations of Thrissur district. The details of survey are presented in the Table 1.

Various types of mosaic symptoms appeared on the crop and the disease incidence were recorded. Per cent disease incidence was calculated using the formula;

$$\text{Per cent disease incidence (PDI)} = \frac{\text{No. of plants infected}}{\text{Total no. of plants observed}} \times 100$$

Table 1. Details of survey on ash gourd mosaic at different locations

Location	Period of survey	Variety	Stage of the crop
Farmer's field, Puthanchira	October to December 2009	Local	Flowering and fruiting
Central Nursery, KAU-Plot I and II	February to March 2010	KAU Local	Flowering and fruiting
Department of Olericulture, COH, Vellanikkara	December 2009	RCAG-28, PAG-72, BH-21, RCAG-15, KAG-1, IVAG-90	Flowering and fruiting
Agricultural Research Station, KAU, Mannuthy	January 2010	KAU Local	Flowering and fruiting
Farmer's field, Pananchery	August to September 2010	Local	Flowering and fruiting

3.2. STUDY ON SYMPTOMATOLOGY

Symptomatology of major types of ash gourd mosaic was studied under natural condition during survey. Plants were artificially inoculated through sap transmission for studying symptoms under artificial conditions.

3.2.1. Maintenance of Virus Cultures

The culture of different ash gourd mosaic viruses was maintained on ash gourd plants by periodical sap transmission. These plants were used as source plants for transmission, biological indexing, host range and varietal screening studies.

Ash gourd variety, KAU Local at two leaf stage was used for all studies. Mosaic with marginal yellowing symptom was the predominant type of ash gourd mosaic noticed in the survey. Hence marginal yellowing type ash gourd mosaic was used for various studies unless otherwise mentioned.

3.3. TRANSMISSION STUDIES

The mode of transmission of ash gourd mosaic was studied by sap transmission and vector transmission.

3.3.1. Sap Transmission

Sap transmission of the virus was studied using different buffers, *viz.*, potassium phosphate (0.1 *M*, pH 7.2), 0.1 per cent sodium sulphite + 1 per cent K_2HPO_4, sodium phosphate (0.1 *M*, pH 7.2), citrate phosphate (0.1 *M*, pH 7) and sterile distilled water (Appendix I). Mosaic infected young ash gourd leaves were selected, washed with tap water, dried with blotting sheet and weighed separately. For preparation of standard extract, buffer volume equal to the weight of the leaves was added into a mortar and leaves were ground with the pestle. After thorough grinding, the whole leaf pulp was filtered through double layer of muslin cloth to get filtered standard extract of the leaves. A pinch of carborundum was added to the extracted sap. Cotton pad soaked in standard extract was rubbed on the leaves of test plants supporting the leaves from the centre with a piece of cardboard, in one direction only that was from the petiole to the apex of the leaf. After five minutes of inoculation, test plants were washed with distilled water to remove excessive inoculum and extraneous particles. For this purpose a wash bottle was used. Eleven plants were inoculated with each buffer. Uninoculated plants served as control. Plants were kept in insect proof net house and observed daily for the development of symptoms.

3.3.2. Vector Transmission

Vector transmissions were conducted using aphid, *Aphis gossypii* Glover and whitefly, *Bemisia tabaci* Genn.

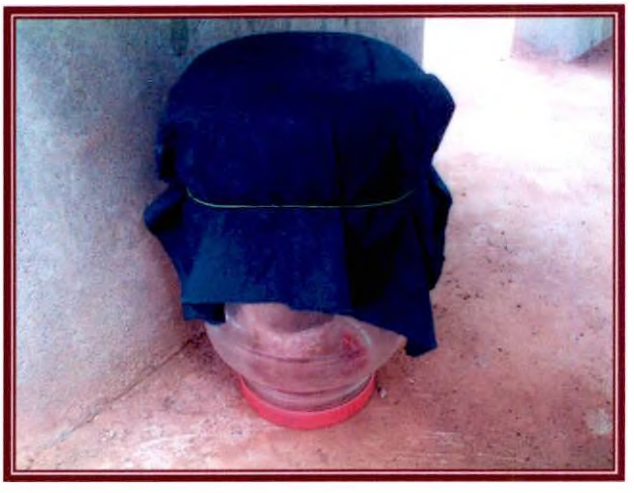
3.3.2.1. Rearing of insects

Pure culture of aphids and whiteflies were reared on healthy bhindi and brinjal plants respectively in insect proof cages for transmission studies. Old plants were changed periodically with healthy young plants for the proper maintenance of the insect cultures.

3.3.2.2. Aphid transmission

Aphid transmission was conducted using *Aphis gossypii* Glover (Plate 1). Aphids were collected from the culture maintained on healthy bhindi plants by giving slight disturbance. After ensuring the withdrawal of their stylets from plant tissue, the moving aphids were picked up with the moistened camel brush and put into a Petri plate. After collecting adequate number of aphids, the Petri plate was covered with black paper to provide dark condition to avoid movement of the aphids and they were subjected to pre-acquisition fasting for a period of one hour. These aphids were then released on mosaic infected young ash gourd leaves for acquisition feeding. After an acquisition access period (AAP) of 30 min., viruliferous aphids were released on healthy ash gourd plants @ 10/plant and covered with plastic cages for inoculation feeding. Windows were provided on top and sides of the plastic cage which were covered with black muslin cloth (Plate 1). After 24 h of inoculation access period (IAP), the aphids were killed by spraying quinalphos (0.05 per cent) and inoculated plants were kept in the insect proof net house for the development of symptoms. Plants without inoculation served as control.

Aphis gossypii



Inoculation feeding of aphids

Plate 1. Aphid transmission studies

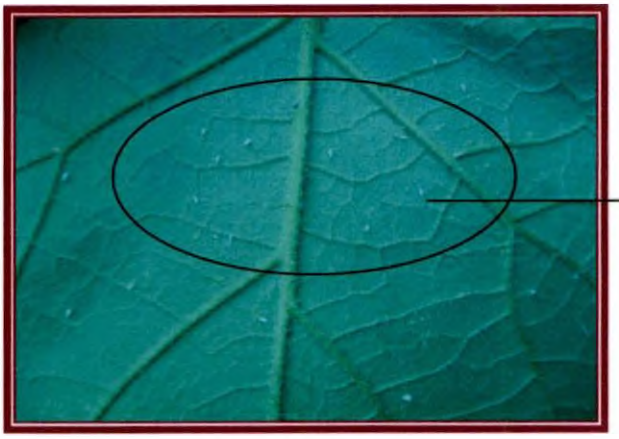
3.3.2.3. Whitefly transmission

Whitefly transmission was done using *Bemisia tabaci* Genn. (Plate2). Whiteflies were caught using an aspirator (Plate 2) which consists of a test tube (10 cm length and 2.5 cm diameter) covered with a stopper with two holes through which two glass tubes (12 cm length and 0.5 cm diameter) were fixed. One glass tube was bent at the outer end to which a rubber tube (15 cm length and 0.5 cm diameter) was attached through which air was sucked in and the other end of which was covered with a muslin cloth for avoiding the entry of whiteflies into the tube. The other glass tube was a straight one and was used to catch whiteflies. Whiteflies were collected from the lower surface of brinjal leaves and then released on infected plant kept in a plastic cage. After an acquisition access period of 12 h, they were released @ 10/plant on healthy ash gourd seedlings in plastic cages for inoculation feeding. Windows were provided on top and sides of the plastic cage which were covered with muslin cloth (Plate 2). Ten plants were used for the transmission studies. After 24 h of inoculation access period, insects were killed by spraying 0.05 per cent quinalphos. The inoculated plants were kept in insect proof net house for the symptom development. Plants without inoculation served as control.

3.4. STUDIES ON IDENTIFICATION OF VIRUS

3.4.1. Biological Indexing

The type of viruses associated with the different mosaic symptoms were ascertained by using indicator plants *viz.*, *Petunia hybrida* and *Vigna unguiculata* following sap transmission. Citrate phosphate buffer (0.1*M* pH 7) was used for all sap transmission studies since it gave maximum infection. Five varieties of *Vigna unguiculata viz.* Lola, Krishnamony, Kanakamony, CoVu7 and V118 were used at two leaf stage of the plant. The symptoms were recorded.



Bemisia tabaci

Aspirator

Inoculation feeding of whiteflies

Plate 2. Whitefly transmission studies

3.4.2. Electron Microscopy

Electron microscopic studies were conducted at Indian Institute of Horticultural Research, Bangalore.

3.5. HOST RANGE

The host range of ash gourd mosaic was studied using members of Cucurbitaceae, Solanaceae and Fabaceae following sap transmission. Ash gourd plants were also inoculated for comparison. Respective host plants without inoculation served as control. The plants were observed daily for the development of symptoms. The details of the plants used for host range studies are furnished below (Table 2).

3.5. VARIETAL SCREENING

Available genotypes of ash gourd in Department of Olericulture, College of Horticulture, Vellanikkara and farmers' varieties collected from different locations were evaluated for resistance to ash gourd mosaic under insect proof net house condition following sap transmission (Table 3). Ten plants of each genotype were inoculated and another ten plants were kept as control without inoculation. The plants were observed daily for the development of symptoms. Disease incidence was observed and the genotypes were categorized using disease reaction scale described by Aghora *et al.*, (2010) as given below.

Per cent disease incidence	**Disease reaction**
0-5	Resistant
5.1-15	Moderately resistant
15.1-50	Moderately susceptible
>50.1	Susceptible

Table 2. List of plants used for host range studies

Family	Common name	Scientific name
Cucurbitaceae	Pumpkin	*Cucurbita moschata*
	Cucumber	*Cucumis melo var. conomon*
	Bottle gourd	*Lagenaria siceraria*
	Watermelon	*Citrullus lanatus*
	Ridge gourd	*Luffa acutangula*
	Bitter gourd	*Momordica charantia*
	Snake gourd	*Trichosanthes anguina*
	Ivy gourd/little gourd	*Coccinia indica*
Solanaceae	Chilli	*Capsicum annuum*
	Tomato	*Lycopersicon esculentum*
	Brinjal	*Solanum melongena*
Fabaceae	Cowpea	*Vigna unguiculata*
	Cluster bean	*Cyamopsis tetragonoloba*

Table 3. List of genotypes screened against ash gourd mosaic

Sl.No.	Genotypes	Place of collection
1	BH-205	Department of Olericulture
2	BH-206	Department of Olericulture
3	BH-210	Department of Olericulture
4	BH-216	Department of Olericulture
5	BH-219	Department of Olericulture
6	Indu	Department of Olericulture
7	BHF-1 (Jeevas)	Puthanchira
8	BHF-2	Puthanchira
9	BHF-3	Nenmeni
10	BHF-4	Vellanikkara
11	BHF-5	Puthanchira
12	BHF-6	Puthanchira
13	BHF-7	Puthanchira
14	BHF-8	Cherumkuzhy
15	BHF-9	Vaikom

3.7. STUDIES ON MANAGEMENT OF ASH GOURD MOSAIC DISEASE

A field experiment was conducted to evaluate the effect of botanicals, chemicals and biocontrol agents on the management of ash gourd mosaic disease. A field experiment was laid out at College of Horticulture, Vellanikkara (Plate 3). The layout of the field experiment is given in Figure 1. The weather data during the period of field experiment is mentioned in Appendix II.

Experimental details were as follows:

Season	- November 2010 to March 2011
Variety	- KAU Local
Design	- RBD
Replication	- 3
Treatments	- 7
Number of plants/channel	- 6
Spacing	- 4.5 m between channels; 50 cm between plants in the channel
Plot size	- 4.5 X 3 m

Preparation of land

Land was prepared thoroughly and channels were taken at a spacing of 4.5 m. Well dried farmyard manure and fertilizers were applied as per the Package of Practices Recommendations: Crops (KAU, 2007). Plants were allowed to spread on wooden twigs.

The first foliar spray of the treatments was given 15 days after sowing. Other three sprays were given at 20 days interval after the first spray. Treatments adopted for the field experiments are given in Table 4. The method of preparation of different extracts is given in Appendix III.

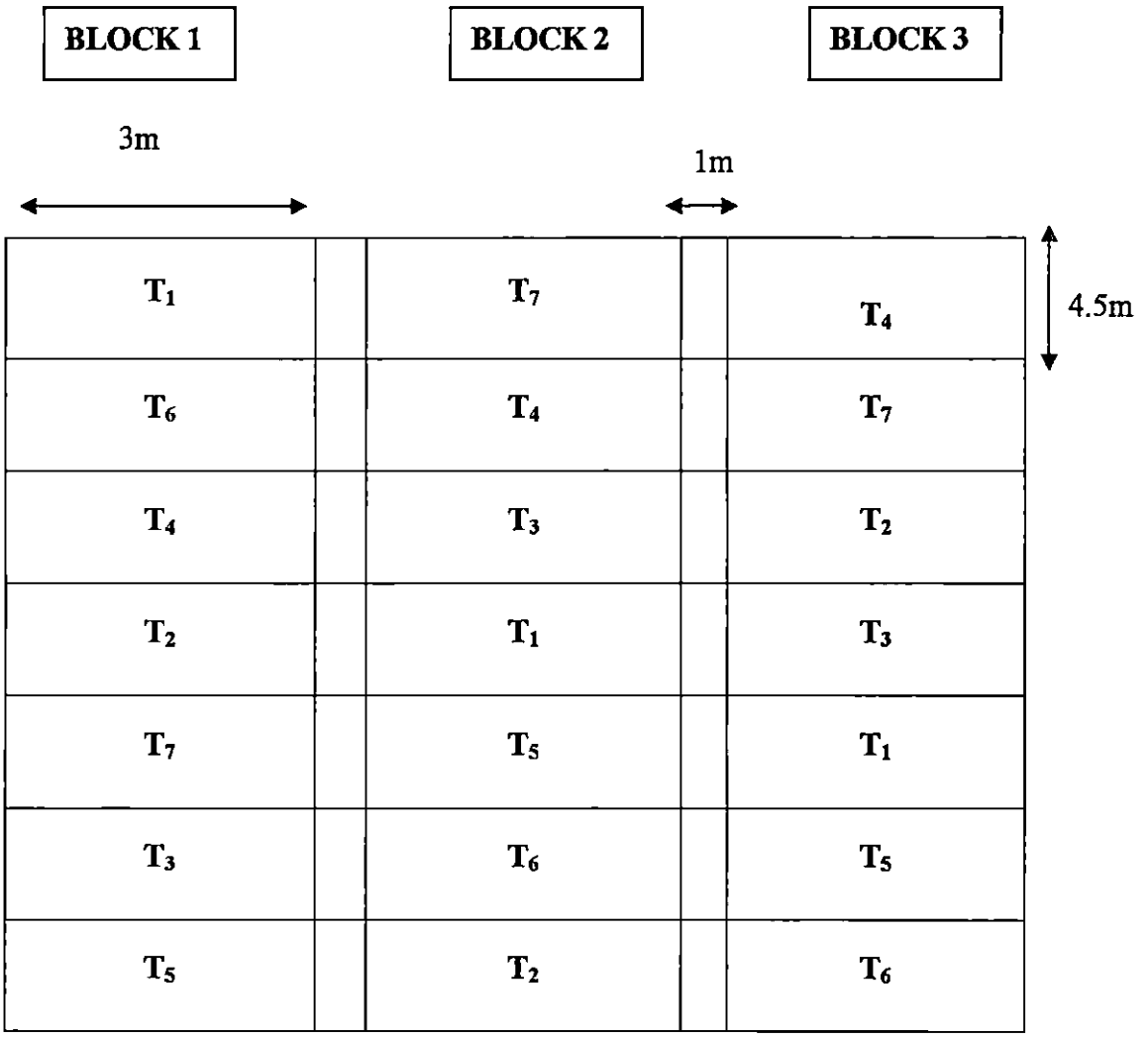


Figure 1. Layout of the field experiment

Plate 3. Field general view

3.7.1. Symptoms

The types of mosaic symptoms appeared on the plants were recorded.

Table 4. Treatment details of field experiment

Treatments	Treatment details	Concentration	Method of application
T_1	Neem oil garlic emulsion	2 per cent	Foliar spraying
T_2	Coconut leaf extract	10 per cent	Foliar spraying
T_3	*Pseudomonas fluorescens* (talc based formulation)	2 per cent	Foliar spraying
T_4	Action-100	0.2 per cent	Foliar spraying
T_5	Action-100 + Quinalphos	0.2 per cent + 0.05 per cent	Foliar spraying
T_6	Quinalphos	0.05 per cent	Foliar spraying
T_7	Control		

3.7.2. Disease Incidence and Severity

Number of plants infected in each treatment were recorded and the per cent disease incidence (PDI) was calculated as indicated in 3.1 (Materials and Methods).

Disease severity was recorded using 0-5 scale suggested by Deo *et al.*, (2000) as given below.

Grade	**Per cent leaves infected**
0	No symptom
1	< 25 per cent
2	25-50 per cent
3	51-75 per cent
4	76-90 per cent
5	>91 per cent

Per cent disease severity (PDS) was calculated using the formula given below;

$$PDS = \frac{\text{Sum of all numerical ratings}}{\text{Total number of plants observed X maximum disease grade}} \times 100$$

Based on per cent disease incidence and severity, coefficient of infection (CI) was calculated as described by PDVR (1997).

$$CI = \frac{PDI \times PDS}{100}$$

Incidence and severity of the mosaic disease were recorded ten days after each spray.

3.7.3. Biometric Characters of the Plant

Biometric characters of the plant like number of branches and fruits and length of main branch and lateral branches and fruit yield were recorded separately.

3.8. Statistical Analysis

Data was analysed following analysis of variance for randomized block design (Gomez and Gomez, 1984). Multiple comparison among treatment means where the F test was significant was done with Duncan's Multiple Range Test using MSTAT package. The data was transformed if necessary and statistically analysed.

Results

4. RESULTS

The results of the investigation "Studies on transmission, host range and management of ash gourd mosaic disease" are presented in this chapter under the following heads.

4.1. SURVEY

The various mosaic symptoms observed on the leaves of ash gourd were classified into different types *viz.*, marginal yellowing (MY) type, yellow-green patch (YG) type, severe puckering (PK) type, filiform (FF) type and light green and dark green patch (LG-DG) type. Symptoms observed on fruits were yellow - green mottling, small dome shaped protuberances on the surface, cracking and fruit size reduction. The various types of mosaic observed during survey from different locations are given in Plate 4 (a to f). Mosaic infected ash gourd leaves were collected from the surveyed locations and used for further studies.

The mean per cent incidence of various mosaic symptoms recorded during sampling survey revealed that MY type was the prominent one with 31.13 per cent incidence followed by YG type (10.65 per cent), PK type (3.55 per cent), FF type (2.72 per cent) and LG-DG type (1.56 per cent) and are presented in Table 5 and illustrated in Figure 2.

4.2. STUDY ON SYMPTOMATOLOGY

Symptomatology of four major types of mosaic in ash gourd that was noticed during survey was studied under natural and artificial conditions.

Marginal yellowing

Light green-dark green patch

Puckering

Filiform

Yellow-green mottling and cracking on fruit

Plate 4a.Ash gourd mosaic symptoms observed at different locations during survey - Puthanchira

Marginal yellowing

Yellow -green patch

Mosaic on fruit

Plate 4b. Ash gourd mosaic symptoms observed at different locations during survey – Plot I, Central Nursery, KAU.

Marginal yellowing

Yellow -green patch

Puckering

Plate 4c. Ash gourd mosaic symptoms observed at different locations during survey - Plot II , Central Nursery, KAU

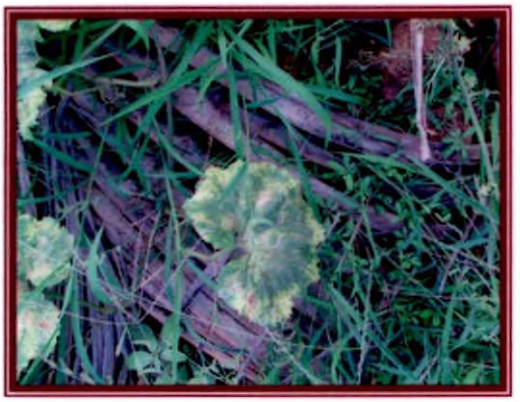
Marginal yellowing

Yellow -green patch

Puckering

Filiform

Plate 4d. Ash gourd mosaic symptoms observed at different locations during survey - Department of Olericulture



Marginal yellowing

Yellow -green patch

Filiform

Plate 4e. Ash gourd mosaic symptoms observed at different locations during survey - ARS, KAU

Marginal yellowing

Yellow-green patch

Puckering

Filiform

Mottling and dome-shaped protuberances on fruit

Plate 4f. Ash gourd mosaic symptoms observed at different locations during survey - Pananchery

Table 5. Per cent incidence of different types of ash gourd mosaic observed during survey

Location	Per cent disease incidence				
	MY	YG	PK	FF	LG-DG
Farmer's field, Puthanchira	51.04	-	6.25	2.08	6.25
Central Nursery, KAU-Plot I	19.70	7.58	-	-	-
Department of Olericulture, COH, Vellanikkara	26.98	30.16	7.94	6.35	-
Agricultural Research Station, KAU, Mannuthy	26.80	4.87	-	2.43	-
Mean	**31.13**	**10.65**	**3.55**	**2.72**	**1.56**

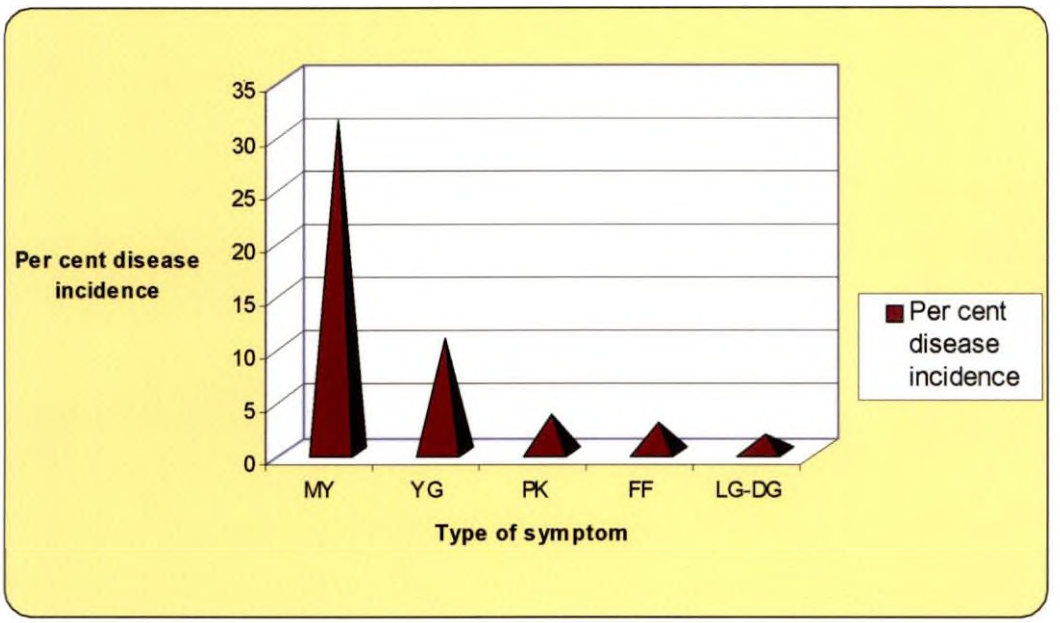


Figure 2. Mean per cent incidence of different types of ash gourd mosaic observed during survey

4.2.1. Symptoms Observed Under Natural Conditions

Symptoms were observed on leaves and fruits. Four major types of mosaic symptoms were observed on leaves of ash gourd plants infected under natural conditions. The various symptoms are described as follows (Plate 5);

4.2.1.1. Symptoms on Leaves

Marginal yellowing type

This type of mosaic symptom was characterised by the presence of prominent yellowing along the entire leaf margin. There was no distortion or size reduction of leaves.

Yellow-green patch type

This type of mosaic symptom was characterised by the presence of yellow and green patches on the leaves with slight reduction in size. The formation of yellow and green patch resulted in mottled appearance.

Puckering type

The leaves of the plant with this type of mosaic symptom were characterised by severe puckering (blister). There was reduction in size and deformation of leaf.

Filiform type

The leaves with this type of mosaic disease presented a filiform appearance. There was reduction in size and deformation of leaf.

Marginal yellowing

Yellow-green patch

Puckering

Filiform

Yellow-green mottling and cracking on fruit

Mottling and dome-shaped protuberances on fruit

Plate 5. Mosaic symptoms of ash gourd observed under natural condition

4.2.1.2. Symptoms on fruits

On fruits the symptoms appeared as yellow - green mottling. Surface of some of the fruits had an uneven appearance with small dome shaped protuberances. Some fruits exhibited fruit cracking and reduced size.

4.2.2. Symptoms Observed Under Artificial Conditions

The symptomatology of four major types of mosaic was studied under artificial conditions. The symptoms under artificial conditions varied slightly from that under natural conditions and is described as follows;

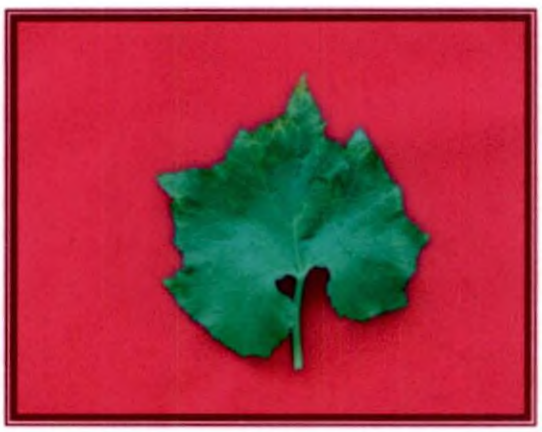
Marginal yellowing type

In mechanically inoculated plants, symptom initiated as light yellowing from tip of the leaf lobe after 23 days of inoculation. Then yellowing spread inward to base of the leaf through vein and veinlets. Thickening of the vein and yellowing of the leaves were appeared in later stages. In this type, yellowing was prominent along veins and veinlets compared to natural condition where yellowing was prominent along margin only. There was slight reduction in leaf size and shape. The severity of the symptom was more as compared to natural conditions (Plate 6).

Yellow-green patch type

In mechanically inoculated plants, symptom initiated as yellowish patches on leaves. Yellow and green patches spread and entire leaf turned yellow in later stages. The severity of the symptom was less as compared to natural conditions. There was no change in size or shape of leaf (Plate 7).

Stage 1



Stage 2

Stage 3

Stage 4

Plate 6. Stages of development of marginal yellowing type ash gourd mosaic under artificial condition

Stage 1

Stage 2

Stage 3

Plate 7. Stages of development of yellow-green patch type ash gourd mosaic under artificial condition

Puckering type

In mechanically inoculated plants, symptom initiated as yellowing along vein near to margin with slight blister. In the later stages, the leaves became more yellowish with prominent blisters. Reduction of leaf size and deformation was also observed. In this type, the severity of symptom was less compared to natural conditions (Plate 8).

Filiform type

In mechanically inoculated plants, symptom initiated as dark green colouration (banding) along veins. In later stages, vein banding spread to entire leaf and finally exhibited a filiform appearance. There was reduction in leaf size and change in shape. The severity of symptom was less compared to natural conditions (Plate 9).

4.3. TRANSMISSION STUDIES

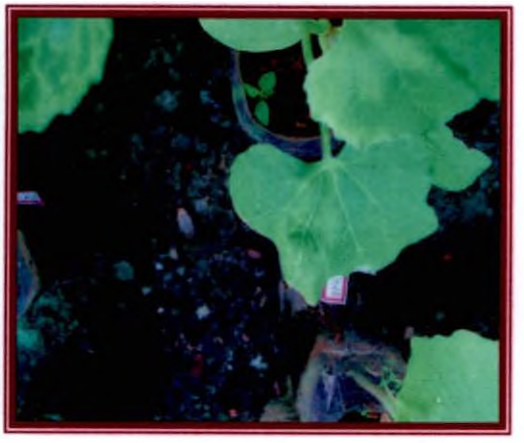
The transmission studies of ash gourd mosaic were carried out by sap and vector transmission. The details of the transmission studies are described below.

4.3.1. Sap Transmission

Symptoms were appeared in healthy ash gourd plants by inoculation with infected sap prepared in different buffers, which showed that marginal yellowing type mosaic was sap transmissible (Plate 10). Among different buffers used, citrate phosphate buffer (0.1*M*, pH 7) recorded highest disease incidence (73 per cent) and sodium phosphate buffer (0.1*M*, pH 7.2) recorded lowest incidence (27 per cent). Citrate phosphate buffer showed the minimum incubation period (IP) and sterile distilled water showed the maximum incubation period and is

Stage 1

Stage 2



Stage 3

Stage 4

Plate 8. Stages of development of puckering type ash gourd mosaic under artificial condition

Stage 1

Stage 2

Stage 3

Plate 9. Stages of development of filiform type ash gourd mosaic under artificial condition

presented in Table 6. Citrate phosphate buffer (0.1*M*, pH 7) was selected for further sap inoculation studies.

Table 6. Comparison of buffers for sap transmission of ash gourd mosaic

Buffer	No. of plants		Per cent disease incidence	Incubation period (days)
	Inoculated	Infected		
Potassium phosphate (0.1*M*, pH 7)	11	4	36	29-34
0.1% Sodium sulphite + 1% K_2HPO_4	11	4	36	34-41
Sodium phosphate (0.1*M*, pH 7.2)	11	3	27	28-29
Citrate phosphate (0.1*M*, pH 7)	11	8	73	23-28
Sterile distilled water	11	4	36	40-41

4.3.2. Insect Transmission

Transmission studies using aphids and whiteflies were conducted to find out vector of ash gourd mosaic.

4.3.2.1. Aphid transmission

In the transmission studies using aphid, *Aphis gossypii*, the inoculated plants produced symptoms which showed that *A. gossypii* was able to transmit the

virus (Plate 10). A mosaic incidence of 59.5 per cent was obtained through *Aphis gossypii* with an incubation period of nine to ten days and the results are presented in Table 7.

Table 7. Transmission of ash gourd mosaic through *Aphis gossypii*

Experiment No.	No.of plants		Per cent disease incidence	Incubation period (days)
	Inoculated	Infected		
I	13	9	69	9
II	10	5	50	10
Mean			**59.5**	

4.3.2.2. Whitefly transmission

In the transmission studies using whitefly, *Bemisia tabaci*, the inoculated plants did not produce symptoms which showed that *B. tabaci* is not a vector of this disease.

4.4. STUDIES ON IDENTIFICATION OF VIRUS

4.4.1. Biological Indexing

Systemic infection was produced on *Petunia hybrida* by inoculation with filiform type whereas no symptom was produced by marginal yellowing type. Brown necrotic spots were produced on inoculation with yellow-green patch and puckering type mosaic, in *Petunia hybrida*. The necrotic spot produced on inoculation with puckering type was small in size compared to that produced by yellow-green patch type mosaic (Plate 11).

Mosaic symptom produced on sap inoculation

Mosaic symptom produced on aphid transmission

Plate 10. Mosaic symptoms of ash gourd by sap and aphid transmission

Dark brown necrotic spot in *Petunia hybrida* on inoculation with YG patch type mosaic

Dark brown necrotic spot on Petunia on inoculation with PK type mosaic

Systemic infection on *Petunia hybrida* on inoculation with FF type mosaic

Plate 11. Symptoms produced on *Petunia hybrida* on inoculation with different types of ash gourd mosaic

Systemic mosaic infection was produced in Kanakamony, Krishnamony and CoVu 7 varieties of cowpea on inoculation with filiform type mosaic. Chlorotic spots were produced on inoculation with yellow-green patch and puckering type mosaic in Lola. The size of chlorotic spot produced on inoculation with puckering type was small as compared to that produced by yellow-green patch type. No symptom was produced on inoculation with marginal yellowing type (Plate 12).

Based on the symptoms produced on *Vigna unguiculata*, it was ascertained that the virus causing yellow-green patch type mosaic belonged to Cucumber mosaic virus group and the virus causing filiform type of mosaic belonged to Potato virus-Y group. Marginal yellowing and puckering type of mosaic which were not able to ascertain using biological indexing and was identified by electron microscopy.

4.4.2. Electron Microscopy

The electron microscopic study of marginal yellowing and puckering type of mosaic revealed that both types of mosaic were caused by members of potyvirus group with flexuous rod shaped particles.

4.5. HOST RANGE

Systemic infection was observed in three members of Cucurbitaceae *viz.*, *Trichosanthes anguina* (snake gourd), *Lagenaria siceraria* (bottle gourd) and *Coccinia indica* (ivy gourd), two members of Solanaceae *viz.*, *Lycopersicon esculentum* (tomato) and *Capsicum annuum* (chilli) and one member of Fabaceae, *Cyamopsis tetragonoloba* (cluster bean). Marginal yellowing symptom was not observed in any of the infected plants and symptoms produced were varied with the crop. In snake gourd, vein clearing appeared on entire leaf lamina with moderate puckering. In bottle gourd, vein clearing appeared on certain areas of the

Chlorotic spot in cowpea on inoculation with YG type mosaic

Small chlorotic spot in cowpea on inoculation with PK type mosaic

Systemic infection on cowpea on inoculation with FF type mosaic

Plate 12. Symptoms produced on *Vigna unguiculata* on inoculation with different types of ash gourd mosaic

Snake gourd

Bottle gourd

Ivy gourd

Tomato

Chilli

Cluster bean

Plate13. Symptoms of ash gourd mosaic infection in different host plants

leaf and resulted in leaf malformation. In ivy gourd, vein banding and yellowing of interveinal areas resulted in typical mosaic appearance. In tomato, vein clearing was the major mosaic symptom. In chilli and cluster bean, vein thickening, downward curling and reduction of leaf size were the symptoms (Plate 13). Among these, maximum incubation period (39 days) was exhibited by ivy gourd and minimum incubation period (19 days) by bottle gourd and cluster bean (Table 8) and are illustrated in Figure 3.

4.6. VARIETAL SCREENING

A total of fifteen genotypes collected from Department of Olericulture, College of Horticulture, Vellanikkara and farmers were screened for resistance against ash gourd mosaic disease.

Out of the fifteen genotypes screened, genotype BHF-1 (Jeevas) showed no mosaic incidence. The per cent disease incidence was lowest for BH-205 (10 per cent) and highest for BH-216, BH-219 and BHF-5 (70 per cent). BH-219 exhibited the lowest incubation period (IP) (8 days) and BHF-3, the highest IP (39 days).

Based on the disease reaction scale described in 3.6, BHF-1 (Jeevas) was recorded as the resistant variety, BH-205 as moderately resistant, BH-206, BH-210, Indu, BHF-2, BHF-3, BHF-4, BHF-6, BHF-7, BHF-8, BHF-9 as moderately susceptible and BH-216, BH-219, BHF-5 as susceptible genotypes. (Table 9).

The per cent disease incidence and incubation period (IP) and disease reaction of different genotypes are presented in Table 9 and Figure 4. The symptoms expression of different genotypes are shown in Plate 14.

Table 8. Symptoms of ash gourd mosaic infection in different host plants

Host plant	Symptom	Incubation period (days)
Cucurbitaceae		
Trichosanthes anguina	VC, PK	21
Lagenaria siceraria	VC, MF	19
Coccinia indica	VB, MO	39
Cucurbita moschata	NS	-
Cucumis melo	NS	-
Citrullus vulgaris	NS	-
Luffa acutangula	NS	-
Solanaceae		
Lycopersicon esculentum	VC, MO	23
Capsicum annuum	DC, MF, SR	23
Solanum melongena	NS	-
Fabaceae		
Cyamopsis tetragonoloba	DC, MF, SR	19

VC- Vein clearing, PK- Puckering, MF- Malformation, VB- Vein banding, MO- Mosaic, DC- Downward curling, SR-Size reduction, NS- No symptom

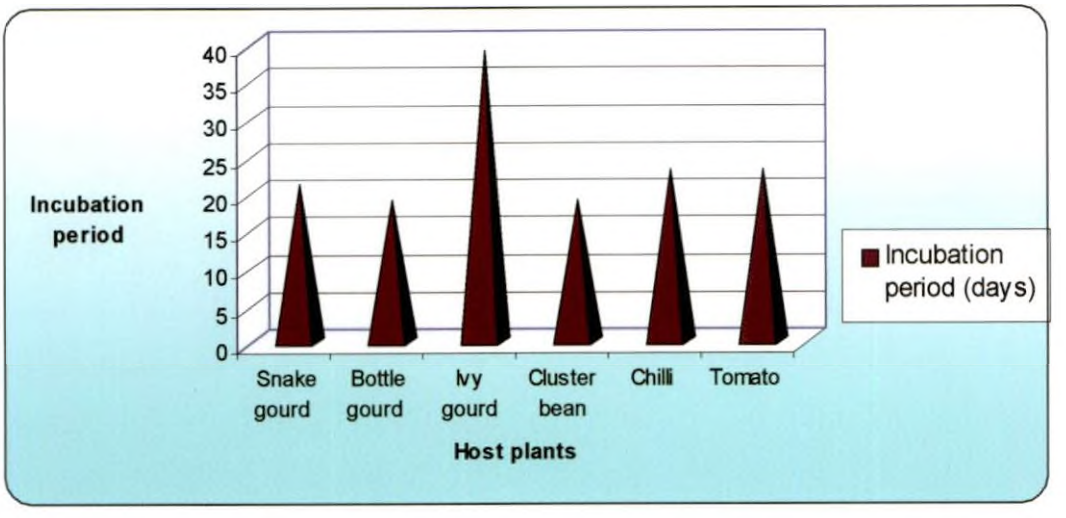


Figure 3. Incubation period of different host plants to ash gourd mosaic virus

Table 9. Evaluation of ash gourd genotypes for resistance against mosaic disease

Genotypes	Per cent disease incidence	Incubation period (days)	Disease reaction
BH-205	10	15	MR
BH-206	20	15	MS
BH-210	20	9	MS
BH-216	70	10	S
BH-219	70	8	S
Indu	30	17	MS
BHF-1 (Jeevas)	-	-	R
BHF-2	20	22	MS
BHF-3	30	39	MS
BHF-4	30	10	MS
BHF-5	70	20	S
BHF-6	20	12	MS
BHF-7	30	22	MS
BHF-8	30	20	MS
BHF-9	20	17	MS

R - Resistant

MR - Moderately resistant

MS - Moderately susceptible

S - Susceptible

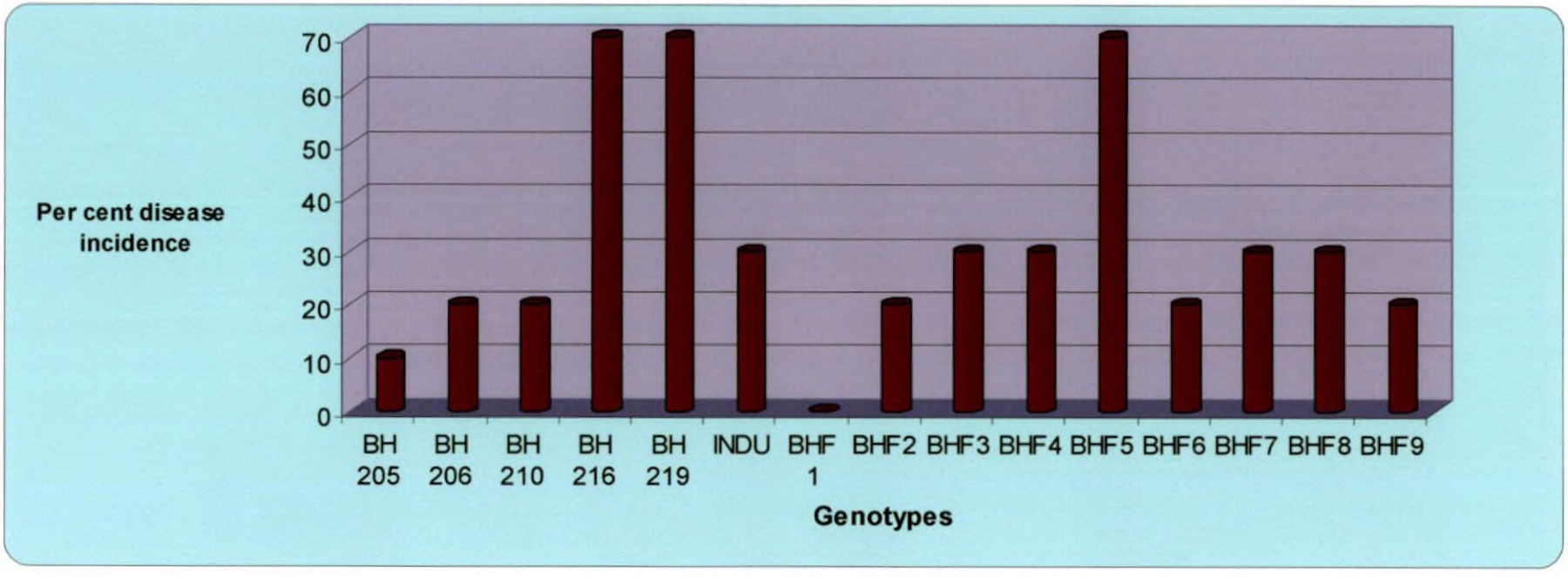


Figure 4. Per cent mosaic incidence of different genotypes on inoculation with ash gourd mosaic

BH-205

BH-206

BH-210

BH-216

BH-219

Indu

Plate 14. Symptoms produced in different genotypes on inoculation with ash gourd mosaic

BHF -2

BHF -3

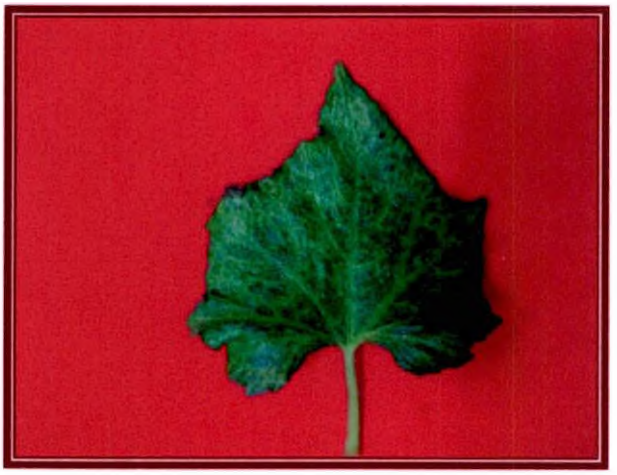
BHF -4

BHF -5

BHF -6

BHF -7

Plate 14. Symptoms produced in different genotypes on inoculation with ash gourd mosaic



BHF- 8

BHF -9

BHF- 1- Jeevas-
Resistant

Plate 14. Symptoms produced in different genotypes on inoculation with ash gourd mosaic

4.7. STUDIES ON MANAGEMENT OF ASH GOURD MOSAIC DISEASE

4.7.1. Symptoms

Five types of symptom were observed in the field experiment. The symptoms included marginal yellowing type, filiform type, puckering type, vein clearing type and general mosaic. Plants were found to be infected by any of the five symptoms (Plate 15).

4.7.2. Per Cent Disease Incidence

From the results in Table 10, it was observed that all the treatments were superior to control in checking the mosaic incidence.

During first observation the per cent disease incidence was minimum in T_5 (16.67). The other treatments T_1, T_2, T_3 and T_6 were on par with T_5. The highest per cent disease incidence was in the control (76.67). The reduction in disease incidence over the control was maximum in T_5 (60 %) followed by T_6 (58.90 %) and minimum in T_4 (21.1 %).

During the second observation also, the per cent disease incidence was minimum in T_5 (47.20) and was on par with T_1, T_2, T_3 and T_6. The per cent disease incidence was maximum in control (94.33). The reduction in disease incidence over the control was maximum in T_5 (47.23 %) followed by T_6 (46.63 %) and minimum in T_4 (22.2 %).

It was found that during the third observation the per cent disease incidence was minimum in T_6 (58.90) and T_5 and T_3 were on par with T_6. The highest per cent disease incidence was recorded in control (100.00). The reduction in disease incidence over the control was maximum in T_6 (41.1 %) followed by T_3 (35.53 %) and minimum in T_4 (16.7 %).

Marginal yellowing

Puckering

Filiform

General mosaic

Vein clearing

Plate 15. Different types of ash gourd mosaic symptoms in field experiment

Table 10. Effect of different treatments on per cent disease incidence of ash gourd mosaic (10 days after spray)

Treatments	Treatment details	1st spray		2nd spray		3rd spray		4th spray	
		PDI	% reduction over control	PDI	% reduction over control	PDI	% reduction over control	PDI	% reduction over control
T_1	Neem oil-garlic emulsion (2 %)	22.20^{bc} (4.11)	54.67	58.90^{b}	35.53	71.13^{c}	28.87	71.13^{c}	28.87
T_2	Coconut leaf extract (10 %)	42.23^{abc} (6.36)	34.43	62.23^{b}	32.20	75.57^{bc}	24.43	87.77^{ab}	12.23
T_3	*Pseudomonas fluorescens* (2 %)	35.57^{abc} (5.87)	41.10	53.33^{b}	41.10	64.47^{cd}	35.53	76.67^{bc}	23.33
T_4	Action-100 (0.2 %)	55.57^{ab} (7.47)	21.10	72.23^{ab}	22.20	83.30^{b}	16.70	88.87^{ab}	11.13
T_5	Action-100 (0.2 %) + Quinalphos (0.05 %)	16.67^{c} (2.84)	60	47.20^{b}	47.23	69.47^{cd}	30.53	80.53^{bc}	19.47
T_6	Quinalphos (0.05 %)	17.77^{bc} (3.68)	58.90	47.80^{b}	46.63	58.90^{d}	41.10	71.13^{c}	28.87
T_7	Control	76.67^{a} (8.78)		94.43^{a}		100.00^{a}		100.00^{a}	
	CD	3.11		28.25		10.22		13.09	

Figures in parentheses are square root transformed values

Figures followed by same letters do not differ significantly according to DMRT

In the fourth observation it was found that the per cent disease incidence was minimum in T_6 and T_1 (71.13). T_5 and T_3 were on par with T_6 and T_1. The highest per cent disease incidence was recorded in control (100.00). The reduction in disease incidence over the control was maximum in T_6 and T_1 (28.87%), followed by T_3 (23.33 %) and minimum in T_4 (11.13 %).

4.7.3. Per Cent Disease Severity

Effect of different treatments on per cent disease severity is presented in Table 11.

From the Table, it was observed that there was no significant difference between the treatments during the first and second observation.

During the third observation, the per cent disease severity was found to be the minimum in T_6 (20.00). All the treatments were on par with T_6 except control (T_7), which had the highest per cent disease severity (50.00). The reduction in disease severity over the control, was maximum in T_6 (30 %) followed by T_5 (27.78 %) and minimum in T_4 (16.66 %).

In the fourth observation also the same trend was followed as in the third observation. The per cent disease severity was minimum in T_6 (25.56). All the treatments were on par with T_6 except control (T_7) which had the highest per cent disease severity (51.11). The reduction in disease severity over the control was maximum in T_6 (25.55 %) followed by T_1 (23.34 %) and minimum in T_4 (15.56 %).

4.7.4. Coefficient of Infection

The effect of different treatments on the coefficient of infection is presented in Table 12. During the first observation the coefficient of infection was

minimum in T_5 (1.67). All the other treatments were on par with T_5 except the control which had the highest coefficient of infection (28.96). The reduction in coefficient of infection over the control, was maximum in T_5 (27.29 %) followed by T_6 (27.18 %) and minimum in T_4 (19.69 %).

During the second observation also the coefficient of infection was minimum for T_5 (7.68) and all the treatments except control were on par with T_5. Control recorded the highest coefficient of infection (44.63). The reduction in coefficient of infection over the control was maximum in T_5 (36.95 %) followed by T_6 (35.33 %) and minimum in T_4 (24.07 %).

During the third observation, the coefficient of infection was minimum for T_6 (12.26) and all the other treatments were on par with T_6 except the control which had the highest coefficient of infection (50.00). The reduction in coefficient of infection over the control, was maximum in T_6 (37.74 %) followed by T_5 (34.53 %) and minimum in T_4 (22.23 %).

In the fourth observation also the minimum coefficient of infection was recorded for T_6 (18.38). All the other treatments were on par with T_6 except the control which showed the highest coefficient of infection (51.11) (Plate 16). The reduction in coefficient of infection over the control was maximum in T_6 (32.73%) followed by T_1 (31.85 %) and minimum in T_4 (19.82 %).

The effect of different treatments on disease incidence, disease severity and coefficient of infection ten days after the fourth (final) spray are illustrated in Figure 5. Comparison on the effect of different treatments after fourth spray revealed that all treatments were superior to control in checking disease incidence, disease severity and coefficient of infection among which quinalphos 0.05 per cent (T_6) was the best one. All treatments were on par with T_6 in checking coefficient of infection. All treatments except T_2 and T_4 were on par with T_6 in

Table 11. Effect of different treatments on per cent disease severity of ash gourd mosaic (10 days after spray)

Treatments	Treatment details	1st spray*	2nd spray*	3rd spray		4th spray	
		PDS	PDS	PDS	% reduction over control	PDS	% reduction over control
T_1	Neem oil-garlic emulsion (2 %)	11.11 (2.94)	18.89 (4.26)	25.55[b]	24.45	27.78[b]	23.34
T_2	Coconut leaf extract (10 %)	15.56 (3.72)	18.89 (4.30)	23.33[b]	26.67	28.89[b]	22.23
T_3	*Pseudomonas fluorescens* (2 %)	11.11 (3.27)	17.78 (4.22)	24.45[b]	25.55	30.00[b]	21.11
T_4	Action-100 (0.2 %)	16.67 (4.09)	28.89 (5.41)	33.34[ab]	16.66	35.56[ab]	15.56
T_5	Action-100 (0.2 %) + Quinalphos (0.05 %)	3.33 (1.55)	13.33 (3.60)	22.22[b]	27.78	28.89[b]	22.22
T_6	Quinalphos (0.05 %)	6.67 (2.39)	15.55 (3.79)	20.00[b]	30	25.56[b]	25.55
T_7	Control	36.67 (5.96)	46.67 (6.79)	50.00[a]		51.11[a]	
	CD	NS	NS	17.53		16.85	

*Not significant

Figures in parentheses are square root transformed values

Table 12. Effect of different treatments on coefficient of infection of ash gourd mosaic (10 days after spray)

Treatments	Treatment details	1st spray		2nd spray		3rd spray		4th spray	
		CI	% reduction over control	CI	% reduction over control	CI	% reduction over control	CI	% reduction over control
T_1	Neem oil-garlic emulsion (2 %)	3.70^{b} (1.85)	25.27	11.63^{b} (3.33)	33.00	17.63^{b}	32.37	19.27^{b}	31.85
T_2	Coconut leaf extract (10 %)	8.74^{b} (2.63)	20.22	12.00^{b} (3.44)	32.62	17.04^{b}	32.96	25.10^{b}	26.01
T_3	*Pseudomonas fluorescens* (2 %)	4.63^{b} (2.13)	24.33	10.29^{b} (3.17)	34.33	15.71^{b}	34.29	23.52^{b}	27.60
T_4	Action-100 (0.2 %)	9.26^{ab} (3.08)	19.69	20.56^{ab} (4.59)	24.07	27.78^{b}	22.23	31.29^{b}	19.82
T_5	Action-100 (0.2 %) + Quinalphos (0.05 %)	1.67^{b} (1.25)	27.29	7.68^{b} (2.62)	36.95	15.47^{b}	34.53	23.24^{b}	27.88
T_6	Quinalphos (0.05 %)	1.78^{b} (1.42)	27.18	9.30^{b} (2.85)	35.33	12.26^{b}	37.74	18.38^{b}	32.73
T_7	Control	28.96^{a} (5.26)		44.63^{a} (6.62)		50.00^{a}		51.11^{a}	
	CD	3.67		2.19		13.65		14.48	

Figures in parentheses are square root transformed values
Figures followed by same letters do not differ significantly according to DMRT

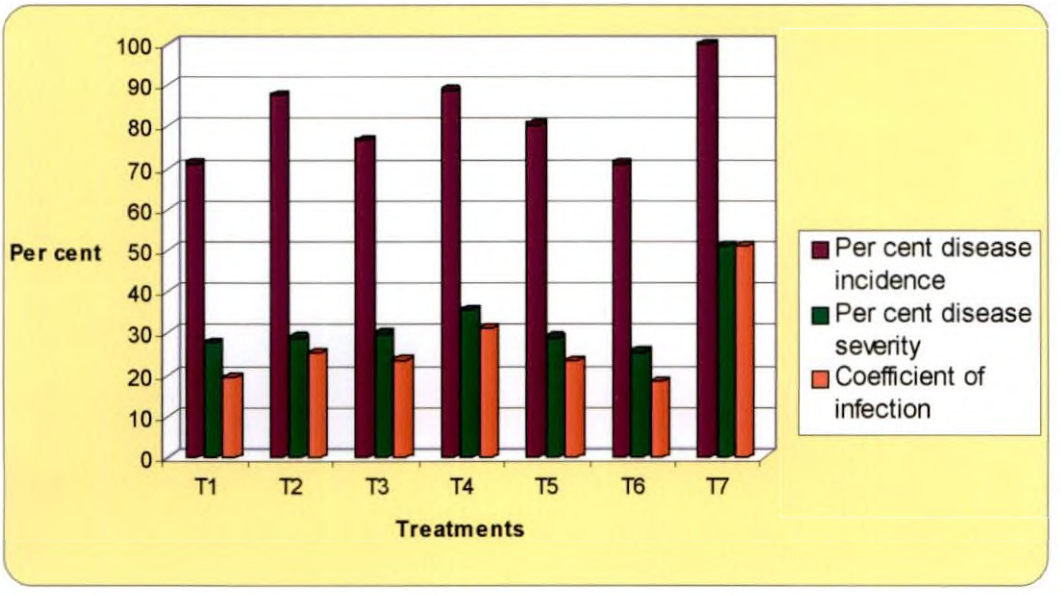


Figure 5. Effect of different treatments on per cent disease incidence, per cent disease severity and coefficient of infection of ash gourd mosaic

bringing down the disease incidence. All treatments except T_4 were on par with T_6 in reducing the disease severity.

4.7.5. Effect of Treatments on Biometric Characters of the Plant

The biometric characters of the plant like number of branches per plant, length of main and lateral branches per plant and number of fruits per plot were recorded and are presented in Table 13 and illustrated in Figure 6 and 7.

4.7.5.1. Number of branches

The number of branches per plant were found to be the highest for T_6 (4.17) and was on par with T_5. The number of branches per plant was recorded lowest for the control (2.11).

4.7.5.2. Length of main branch

The length of main branch (in centimeters) was found to be maximum for T_6 (243.14). All the other treatments were on par with T_6 except control which had the minimum length of main branch (135.58).

4.7.5.3. Length of lateral branches

The length of lateral branches (in centimeters) was maximum for T_6 (105.42) and was on par with T_1, T_3 and T_5. The minimum length was recorded for the control (67.22).

4.7.5.4. Number of fruits

The number of fruits per plot was recorded highest for T_6 (2.33) and was on par with T_2, T_3 and T_4. The number of fruits was recorded lowest for T_7 and T_1 (1.00).

4.7.5.5. Yield of ash gourd

Effect of different treatments on yield of ash gourd was recorded during the season and is presented in Table 13. The yield (in kilograms) per plant was found to be highest for T_6 (2.08). All the other treatments were on par with T_6 except control which recorded the lowest yield (0.60). The yield per plot was also found to be highest for T_6 (4.85) and was followed by T_2 and T_4. The yield per plot was lowest for T_7 (0.60).

Table 13. Effect of different treatments on biometric characters of ash gourd

Treatments	Treatment details	No.of branches/plant	Length of main branch/plant (cm)	Length of lateral branches/plant (cm)	No.of fruits/plot	Yield/plant (kg)	Yield/plot (kg)
T_1	Neem oil-garlic emulsion (2 %)	3.80^{ab}	231.50^{a}	101.68^{a}	1.00^{b}	1.57^{ab}	1.57^{bc}
T_2	Coconut leaf extract (10 %)	3.35^{ab}	219.33^{a}	80.97^{b}	2.00^{ab}	1.55^{ab}	3.10^{ab}
T_3	*Pseudomonas fluorescens* (2 %)	3.72^{ab}	228.33^{a}	103.39^{a}	1.67^{ab}	1.55^{ab}	2.59^{abc}
T_4	Action-100 (0.2 %)	3.28^{b}	211.94^{a}	76.78^{b}	2.00^{ab}	1.40^{ab}	2.80^{ab}
T_5	Action-100 (0.2 %) + Quinalphos (0.05 %)	3.83^{a}	234.58^{a}	104.92^{a}	1.33^{b}	1.33^{ab}	1.77^{bc}
T_6	Quinalphos (0.05 %)	4.17^{a}	243.14^{a}	105.42^{a}	2.33^{a}	2.08^{a}	4.85^{a}
T_7	Control	2.11^{c}	135.58^{b}	67.22^{c}	1.00^{b}	0.60^{b}	0.60^{c}
	CD	3.05	64.82	6.66	0.24	0.38	1.89

Figures followed by same letters do not differ significantly according to DMRT

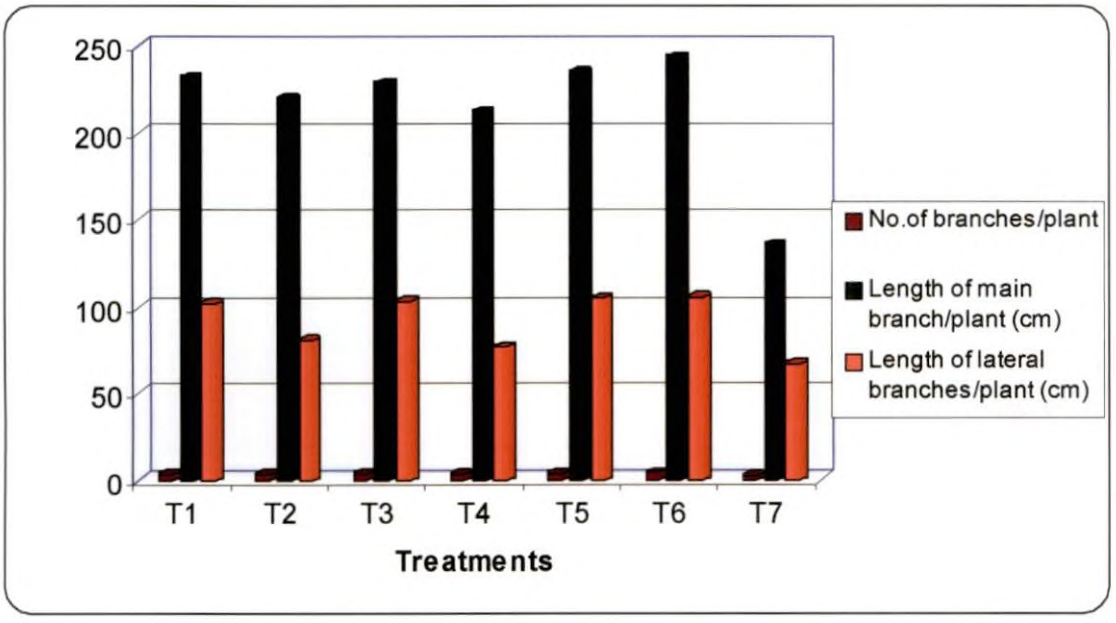


Figure 6. Effect of different treatments on biometric characters of ash gourd

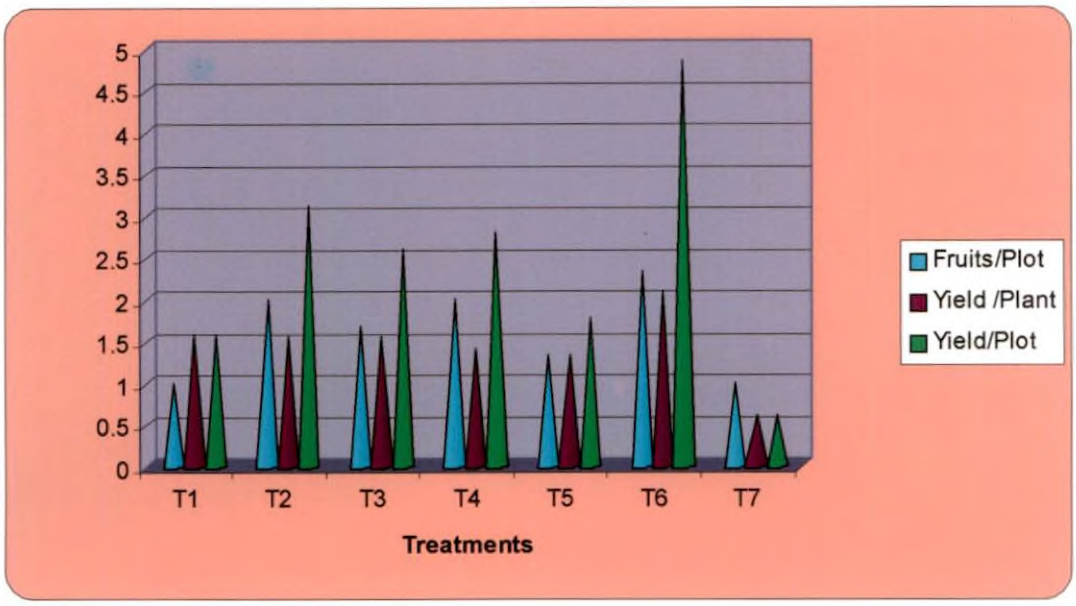


Figure 7. Effect of different treatments on fruits/plot, yield/plant and yield/plot of ash gourd

T_6 - Quinalphos 0.05 %

T_7 - Control

Plate 16. Field view of treatments


Discussion

5. DISCUSSION

Ash gourd, *Benincasa hispida* (Thunb.) is an important tropical cucurbit vegetable that is grown throughout India especially South India. In recent years, mosaic incited by virus is spreading fastly and becoming a prominent disease in Kerala compared to various other fungal diseases of ash gourd. Singh (1976) conducted studies on mosaic disease of ash gourd in Punjab. This study has provided some useful information on symptomatology, etiology and host range of ash gourd mosaic. Information regarding the varietal screening and management of this disease are lacking. Hence, the present study is a serious attempt to enter into certain aspects of the disease with an emphasis on symptomatology, transmission, identification of the virus, host range, varietal screening and the disease management.

A purposive sampling survey was conducted in different locations of Thrissur district for collection of mosaic samples and to study the incidence of various mosaic symptoms. From the sampling surveys, mainly four different types of mosaic symptom were observed *viz.*, marginal yellowing, yellow-green patch, filiform and severe puckering. Considering the per cent incidence of the four types of symptom observed during survey, it was found that marginal yellowing type mosaic was the prominent one with an average disease incidence of 31.13 per cent. Singh (1976) reported 5 to 10 per cent incidence of mosaic disease in ash gourd and mosaic mottling was reported to be the major symptom. The occurrence of different type of viruses was reported by Bhargava and Bhargava (1976), Akanda *et al.*, (1991) and Muqit *et al.*, (2007). The earlier reports revealed the presence of different viruses in ash gourd. In the present investigation also, different types of mosaic were observed among which marginal yellowing type mosaic was found to be the most prominent.

Symptoms are the observable effects that a virus causes on the growth, development and metabolism of an infected host plant. Study on symptomatology was undertaken in natural and artificial conditions. In the case of marginal yellowing type symptom, the yellowing of leaf margin was prominent under natural conditions. But under artificial conditions, yellowing of veins and veinlets from the leaf margin were prominent and the symptom was severe under artificial condition compared to the natural conditions. The marginal yellowing symptom observed in the present study was in accordance with the marginal chlorosis symptom described by Vasudeva and Lal (1943) for vegetable marrow mosaic virus. The symptom initiation of marginal yellowing type mosaic under artificial condition was from the tip of the leaf lobe and then spreading inward. Singh (1976) reported that symptoms of mosaic disease of ash gourd was started from the veins and tips of the young leaves followed by the yellow mosaic mottled area interspersed by dark green blisters on the leaf surface. In the present study also symptom initiation was from leaf tip.

Under natural condition, yellow-green patch type mosaic was characterised by the presence of yellow and green patches (mottling appearance) on the leaves with slight reduction in size. Under artificial condition, similar symptom was appeared with reduced severity. Raj (1969) reported on a mosaic disease of ash gourd which causes mosaic mottling, yellowing, distortion and crinkling of the leaves. Verma *et al.*, (1970) reported mosaic mottling symptom in snake gourd and bottle gourd along with blistering, malformation and leaf size reduction. In the yellow-green patch type, mosaic mottling without blistering and malformation was the symptom.

Puckering type mosaic was characterised by severe puckering, reduction of size and deformation of leaf under natural condition. Similar symptoms were recorded under artificial condition also with low disease severity. Puckering type symptoms were reported earlier by different workers. Mosaic disease of ten cucurbitaceous vegetables including ash gourd were characterised by severe

mosaic, yellow mosaic, blisters and leaf distortion and viruses were identified as Papaya ringspot potyvirus watermelon strain and cucurbit viruses (Dahal *et al.*, 1997).

In filiform type of mosaic, leaves showed a filiform appearance and there was reduction in size and deformation of leaf. Under artificial condition, similar symptom was observed with low severity. Reddy and Nariani (1963) also reported filiform type mosaic in *Cucurbita pepo* characterised by distortion of lamina, filiformy of leaves, vein clearing in younger leaves and development of dark green blisters on older leaves and was identified as melon mosaic virus.

In field experiments, general yellowing symptom was also noticed. Xiang *et al.*, (2008) described a cucurbit aphid-borne yellows virus on wax gourd causing yellowing, sometimes combined with severe mosaic and in some cases, fruit malformation.

In the present study, under natural conditions, some of the fruits exhibited mosaic patches. Surface of some of the fruits had an uneven appearance with dome shaped protuberances. Some fruits exhibited fruit cracking. Though fruit cracking has not been reported earlier, Xiang *et al.*, (2008) described a cucurbit aphid-borne yellows virus in wax gourd (*Benincasa hispida*) causing fruit malformation.

Transmission is an important experimental tool to establish the etiology of viral diseases. With this view, an attempt was made to understand the mode of transmission of the ash gourd mosaic through sap and vector.

In the present investigation, transmission studies conducted using the infected sap prepared in different buffers, produced symptoms on the inoculated plants. The virus was sap transmissible and among the different buffers used, citrate phosphate buffer (0.1*M*, pH 7) recorded highest disease incidence with

minimum incubation period. Sidhu (1965) reported artificial transmission of vegetable marrow mosaic virus (Cucumis virus-1) on ash gourd. Shanker *et al.*, (1972) transmitted pumpkin mosaic virus and watermelon mosaic virus to ash gourd and found out that 0.1 *M* citrate phosphate buffer was the most effective buffer for PMV stability and infectivity as it produced 40 per cent infection even after 24h of storage. Sharma and Chohan (1973) reported the sap transmissibility of Cucumis virus- 1 infecting ash gourd using phosphate buffer (pH 7).

As the transmission through sap showed positive indication, the next investigation was to find out the role of insects as vectors. The present study using *Aphis gossypii* showed that it was able to transmit the virus in a non-persistent manner giving a mosaic incidence of 59.5 per cent with an incubation period of nine to ten days. The transmission of ash gourd mosaic by *Myzus persicae* was reported by Singh (1970). The present investigation using *Aphis gossypii* is in accordance with Tewari *et al.*, (2004) who reported *A. gossypii* as the most efficient vector of Benincasa mosaic virus among *Myzus persicae*, *Lipaphis pseudobrassicae* (*L. erysimi*) and *Aphis caraecovora.*

The transmission of cucumber mosaic virus, watermelon mosaic potyvirus 2, zucchini yellow mosaic potyvirus, cucurbit aphid-borne yellow luteovirus in cucurbits by aphids viz., *Aphis gossypii* and *Myzus persicae* was reported by several workers (Lecoq *et al.*, 1992; Dukic *et al.*, 2002). Sandhu and Kang (2007) also reported that cucumber mosaic virus and watermelon mosaic virus- 1 causing mosaic syndrome of cucurbits were transmissible by *A. gossypii.*

Aphids play an important role in the fast spread of the disease under field conditions. According to Yamamoto (1986) high rate of WMV infection of cucumber seedlings was due to transmission by *A. gossypii* from many kinds of cucurbit crops.

The transmission study using whitefly revealed the inefficiency of whiteflies as vectors of the ash gourd mosaic virus. Walkey (1985) reported that whiteflies feed mainly on phloem tissues and the viruses transmissible by whitefly are not sap transmissible to the same host. In the present study also ash gourd mosaic showed sap transmission but no whitefly transmission.

Biological indexing was done to ascertain the type of virus associated with the mosaic disease using *Petunia hybrida* and *Vigna unguiculata*. All types except marginal yellowing type produced symptoms on *P. hybrida*. Dark brown necrotic spot was produced on inoculation with yellow-green patch and severe puckering type whereas systemic mosaic infection was produced on inoculation with filiform type. Singh (1976) reported systemic infection on *P. hybrida* by an ash gourd mosaic virus which exhibited mosaic mottling and blistering of ash gourd leaves. But in the present study, even though systemic infection was produced in Petunia, symptoms produced in ash gourd was of filiform type. Chlorotic local lesions were reported in *P. hybrida* by potyviruses of vanilla (Nisha, 2007). In the present investigation, dark brown necrotic spots were observed in *P. hybrida* on inoculation with yellow-green patch and severe puckering types of ash gourd mosaic.

All types except marginal yellowing type produced symptoms on cowpea. Chlorotic spots were produced on inoculation with yellow-green patch and puckering type mosaic. Sinclair and Walker (1955) reported that certain strains of CMV induced local lesion in resistant varieties of *Vigna sinensis*. According to Nariani and Nyako (1963) Cucumis virus-1 induced reddish local lesions on *Vigna sinensis*. Based on these reports and symptomatology of the virus in ash gourd, the yellow-green type mosaic was tentatively identified to be caused by a Cucumber mosaic virus. Systemic mosaic symptom was prôduced by filiform type mosaic in cowpea. Sousa *et al.*, (1996) reported a strain of aphid-borne mosaic potyvirus that caused systemic symptoms in *V. unguiculata*. In accordance

with this report and symptomatology of the virus in ash gourd, the virus of filiform type was tentatively identified as Potato virus-Y.

Since marginal yellowing and puckering types of mosaic could not be identified by biological indexing, they were subjected to electron microscopic study. It revealed that marginal yellowing and puckering type mosaic were caused by virus that belongs to the potyvirus group.

Collateral hosts play an important role in the perpetuation of the pathogen and the vectors. The knowledge on this aspect is very useful for the successful management of virus diseases. It was observed that snake gourd, bottle gourd, ivy gourd, tomato, chilli and cluster bean showed systemic infection. Symptoms varied with the crop and marginal yellowing symptom was not produced in any of the crops.

Sharma and Chohan (1973) observed systemic infection on cucurbitaceous hosts by Cucumis virus-1 strain of ash gourd. Singh (1976) reported a mosaic disease of ash gourd which caused systemic infection on *Cucumis melo*, *Lagenaria siceraria*, *Luffa cylindrica*, *Cucurbita pepo*. In the present study, infection was not observed in *C. melo* and *Luffa acutangula*.

Reddy and Nariani (1963) reported Cucumis virus-3 showing filiform type symptom, on *Citrullus vulgaris*, *Cucurbita moschata*, *Cucumis melo*, *Momordica charantia* and *Luffa acutangula*. Mitra and Nariani (1965) reported *Cucurbita moschata*, *Momordica charantia* and *Citrullus vulgaris* as the hosts of Cucumis virus–3. Louis (2003) reported that PMV produced systemic infection in *C. vulgaris*, *M. charantia*, *Benincasa hispida* (wild), *Capsicum annuum* and *Vigna unguiculata* and no infection on *L. acutangula*, *C. melo*, *Solanum melongena*, *Lycopersicon esculentum* and *Cyamopsis tetragonoloba*. Ariyaratne *et al.*, (2005) reported that a PRSV related virus from snake gourd did not infect plants of

Solanaceae and Leguminosae whereas it found infected cucurbitaceous crops including *Benincasa hispida* and *Trichosanthes cucumerina*.

Solanaceous crops as host of cucumber mosaic virus was reported by Doolittle (1920), Ainsworth (1935) and Naqvi *et al.*, (1975). Vasudeva and Pavgi (1945) identified a melon mosaic virus infecting a number of solanaceous crops. Sastry (1982) reported PVY infection on solanaceous crops.

Sousa *et al.*, (1996) reported a strain of cowpea aphid-borne mosaic virus (potyvirus) that caused systemic symptoms in *Vigna unguiculata*.

Based on available literature pumpkin, cucumber, watermelon, ridge gourd, bitter gourd, brinjal and cowpea were found to be infected by potyviruses. But in the present investigation these crops were not infected. But the electron microscopic studies revealed the PVY nature of ash gourd mosaic. Hence the virus under present investigation may be different from the earlier reported strains of potyvirus.

The virus causing marginal yellowing symptom in ash gourd did not show similar symptom in any of the test crops. So presence of the virus infected host crops in the surroundings may spread the mosaic disease to ash gourd.

Appropriate method for disease management is the use of resistant varieties supplemented with cultural, chemical and biological methods.

In the present investigation, 15 genotypes obtained from Department of Olericulture, College of Horticulture and farmers were screened under net house conditions and found that one genotype, BHF-1 (Jeevas) was resistant to the mosaic with no mosaic incidence and BH-205 was moderately resistant with ten per cent of incidence. Bhargava and Bhargava (1976) reported the resistance of

ash gourd variety *Benincasa hispida* var. Petha Local to five strains of watermelon mosaic virus.

Effective viricides are not available for the control of plant viral diseases. Hence disease management aims to prevent or to reduce the incidence and severity of infection. Botanicals and biocontrol agents are reported to have antiviral effect. Hence in the present investigation, an attempt was made to find out the effect of botanicals, biocontrol agents and insecticides against ash gourd mosaic.

From the field experiment, it was found that after the fourth spray (final spray), all the treatments showed effectiveness in reducing disease incidence, severity and coefficient of infection compared to the control (Figure 8). Maximum reduction in disease incidence was obtained in T_6 and T_1 (28.87 %) and minimum in T_4 (11.13 %). The reduction in disease severity was also found to be maximum in T_6 (25.56 %) followed by T_1 (23.34 %) and minimum in T_4 (15.56 %). The coefficient of infection also showed maximum reduction in T_6 (32.74 %) followed by T_1 (31.85 %) and minimum in T_4 (19.82 %). The inhibitory effect of quinalphos and neem oil-garlic emulsion was may be due to reduction in vector transmission of the virus.

Devi and Reddy (1995) reported the effect of quinalphos in reducing the transmission of pepper vein banding virus (PVBV) and cucumber mosaic virus on *Capsicum annuum*. The use of insecticides for the control of cucurbit virus diseases had been reported by Kumar (1999) and Bulajic *et al.*, (2008).

The application of neem-based products for control of virus diseases in cucurbits had been reported by Srivastava *et al.*, 1986 and Kumar, 1999. The effectiveness of leaf extracts from dry leaves of coconut (*Cocos nucifera*) in reducing tomato spotted wilt virus was also reported Narayanaswamy and Ramiah (1983) and Manickam and Rajappan (1998).

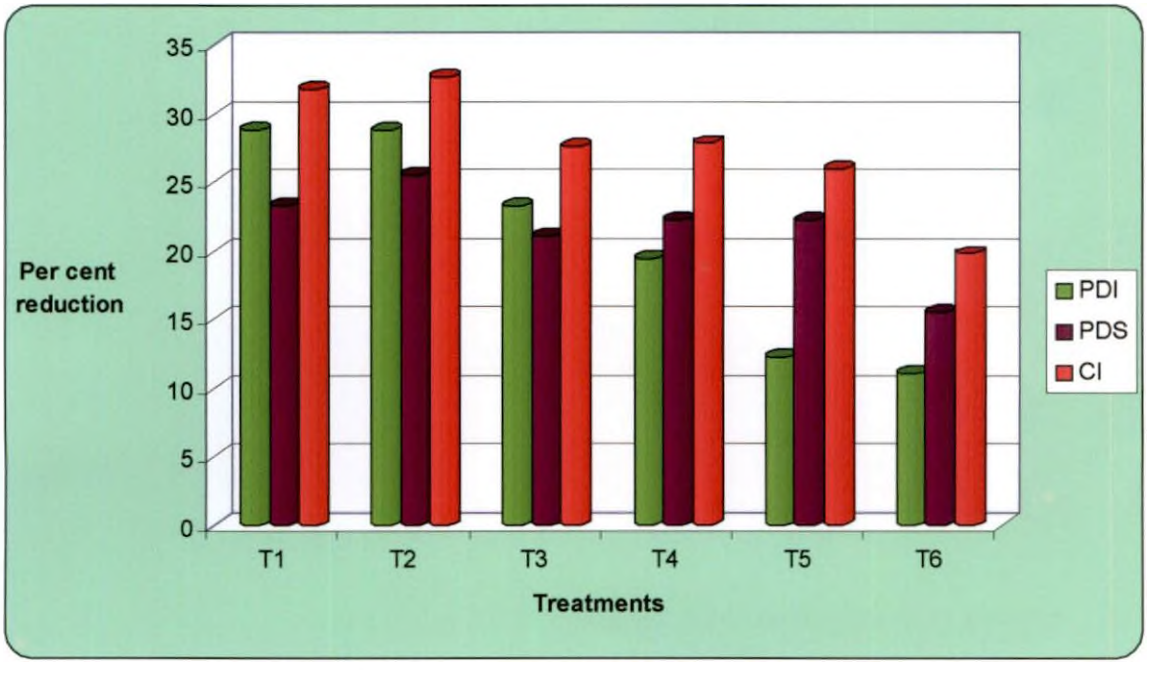


Figure 8. Per cent reduction of disease incidence, disease severity and coefficient of infection of ash gourd mosaic by different treatments over control

Rajakumar and Byadgi (2002) reported on the control of tomato mosaic diseases by the application of 2 per cent Action-100. Pandey *et al.*, (2003) reported on the antiviral properties of extracts (5 and 10 per cent) of selected medicinal plants (*Catharanthus roseus, Rauvolfia serpentina, Bacopa monnieri, Eclipta alba and Phyllanthus niruri*) against Benincasa mosaic.

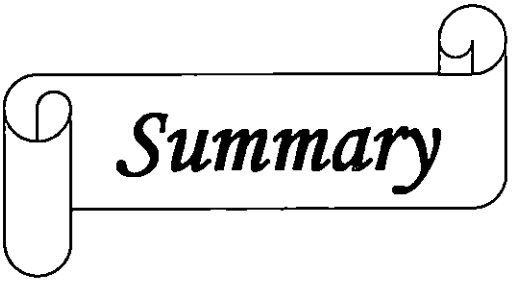
The effect of plant growth promoting Rhizobacteria, *Pseudomonas fluorescens* against cucurbit viruses has been reported by Raupach *et al.*, (1996) Kumar (1999) and Zhender *et al.*, (2001).

The biometric characters of the plants (number of branches, number of fruits, length of main and lateral branches) was also found to be better for all the treatments except the control with T_6 being the best one. The yield (per plant and per plot) of the plants was also found to be the highest for T_6 and there was 87.6 per cent increase in per plot yield over control.

Increase in yield of plants by application of botanicals have been reported by several workers (Kannan and Doraiswamy, 1993 in cowpea; Sunkad *et al.*, 2002 in groundnut; Vanitha and Suresh, 2002 and Bhyan *et al.*, 2007 in tomato). Yield enhancement by the application of *P. fluorescens* was reported by Kandan *et al.*, (2005) in tomato. The increase in yield by the application of insecticides has been reported by Karim *et al.*, (2008) and Reddy *et al.*, (2010) in tomato.

Maximum effect of treatments over control in reducing ash gourd mosaic was 32.73 per cent after four sprays of quinalphos (0.05 %). For reducing the mosaic disease further, cultivation of resistant/moderately resistant ash gourd varieties and application of quinalphos is recommended. Since ecofriendly treatments *viz.*, neem-oil garlic emulsion (2 %), coconut leaf extract (10 %), *Pseudomonas fluorescens* (2 %), Action-100 (0.2 %) and Action-100 (0.2 %) plus

quinalphos (0.05 %) is on par with quinalphos (0.05 %), there is scope for utilising it for controlling ash gourd mosaic disease in future.


Summary

6. SUMMARY

Ash gourd, *Benincasa hispida* (Thunb.) is an important tropical cucurbit vegetable that occupies a pivotal position among fruit vegetables particularly in South India. It is also known as Chinese preserving melon, wax gourd, white gourd, white pumpkin, hairy melon or winter melon. Mosaic disease of ash gourd has been reported by various workers from different parts of India. No work has been conducted so far on mosaic disease of ash gourd in Kerala.

Hence the present investigation, " Studies on transmission, host range and management of ash gourd mosaic disease" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2009 to 2010 with a view to understand the symptomatology, mode of transmission, host range, varietal resistance and to chalk out a suitable management practice.

For collection of mosaic samples and to study the incidence of various mosaic symptoms in ash gourd, a purposive sampling survey was conducted in major ash gourd growing regions of Thrissur district. Based on the per cent incidence of various mosaic symptoms, marginal yellowing type of mosaic was recorded as the prominent one with 31.13 per cent incidence followed by yellow and green patch type (10.65 per cent), puckering type (3.55 per cent), filiform type (2.72 per cent) and light and dark green patch type (1.56 per cent).

Symptomatology of four major types of mosaic in ash gourd viz., marginal yellowing, yellow-green patch, severe puckering and filiform that was noticed during survey was studied by observing the development of symptoms on naturally infected as well as artificially inoculated (sap inoculation) plants. Under natural conditions, marginal yellowing type was characterised by the presence of prominent yellowing along the leaf margin. Yellow-green type was characterised by the presence of yellow and green patches on entire leaf. Puckering type was

characterised by severe puckering (blister), size reduction and deformation of leaf. Filiform type was characterised by filiform (thread-like) appearance of leaf with size reduction and deformation. On fruits, the symptoms appeared as yellow and green mottling, uneven surface with dome shaped protuberances, cracking and size reduction.

The symptoms under artificial conditions varied slightly from natural conditions. In marginal yellowing type, yellowing along veins and veinlets was prominent and the disease severity was more under artificial conditions. In all other types viz., yellow-green patch, puckering and filiform, similar symptom with reduced severity was observed under artificial conditions.

The transmission of the ash gourd mosaic was carried out by sap and vector (aphid and whitefly). The sap transmission studies were conducted using different buffers and among them citrate phosphate buffer (0.1 *M*, pH 7) gave the maximum transmission (73 per cent) with 23-28 days of incubation. Aphid transmission using *Aphis gossypii* gave 59.5 per cent transmission with an incubation period of nine to ten days. The transmission study using whitefly (*Bemisia tabaci*) did not produce any symptom on inoculated plants which showed that whitefly is not a vector of this disease.

The type of viruses associated with different mosaic symptoms were ascertained using indicator plants like *Petunia hybrida* and *Vigna unguiculata*. Dark necrotic spot was produced on inoculation with yellow-green patch and severe puckering type whereas systemic mosaic infection was produced with filiform type in *P. hybrida*. In *V. unguiculata*, systemic mosaic infection was produced on inoculation with filiform type and chlorotic spots were produced with yellow-green patch type and severe puckering type. Symptoms were not produced on inoculation with marginal yellowing type in *P. hybrida* and *V. unguiculata*. Based on the symptoms produced on *V. unguiculata*, it was ascertained that the virus causing yellow-green patch type mosaic belong to

Cucumber mosaic virus group and the virus causing filiform type of mosaic belong to Potato virus-Y (PVY) group. Electron microscopic study revealed that marginal yellowing and puckering type of mosaic was caused by potyvirus.

The host range of ash gourd mosaic was evaluated using the members of Cucurbitaceae (pumpkin, cucumber, bottle gourd, watermelon, ridge gourd, bitter gourd, snake gourd and coccinia), Solanaceae (chilli, tomato and brinjal) and Fabaceae (cowpea and cluster bean). Systemic infection was observed in three members of Cucurbitaceae viz., snake gourd, bottle gourd and ivy gourd, two members of Solanaceae viz., tomato and chilli and one member of Fabaceae, cluster bean. Symptoms produced were varied with the crop and marginal yellowing symptom was not appeared in any of the host plants.

A total of fifteen genotypes including the genotypes from Department of Olericulture, College of Horticulture, Vellanikkara and farmers were screened for resistance against ash gourd mosaic disease. The sources of resistance were found out based on per cent disease incidence. Accordingly, BHF-1 (Jeevas) was recorded as the resistant, BH-205 as moderately resistant, BH-206, BH-210, BHF-2, BHF-6, BHF-9, Indu, BHF-3, BHF-4, BHF-7, BHF-9 as moderately susceptible and BH-216, BH-219, BHF-5 as susceptible genotypes.

An attempt was made to find out the effect of botanicals, biocontrol agents and insecticides against ash gourd mosaic. From the field experiment, it was found that after the fourth (final) spray, all the treatments showed effectiveness in reducing disease incidence, severity and coefficient of infection compared to the control. Considering the overall performance of various treatments, quinalphos 0.05 per cent (T_6) was the best treatment for reducing disease incidence, diseases severity and coefficient of infection and enhancing the biometric characters and yield of the plants. Ecofriendly treatments *viz.*, neem-oil garlic emulsion (2 %), coconut leaf extract (10 %), *Pseudomonas fluorescens* (2 %), Action-100 (0.2 %)

and Action-100 (0.2 %) plus quinalphos (0.05 %) was on par with quinalphos (0.05 %) in reducing coefficient of infection.

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Appendices

APPENDIX I

Different Buffers Used in Sap Transmission

1. Citrate phosphate buffer 0.1*M* (pH 7)

Stock solution

A: 0.1*M* solution of citric acid monohydrate (21.014g in 1000ml)

B: 0.3*M* solution of dibasic sodium phosphate (42.59g in 1000ml)

6.5ml of A + 43.6ml of B, diluted to total of 100ml.

2. One per cent dipotassium hydrogen orthophosphate (A) + 0.1 per cent sodium sulphite (B)

1g K_2HPO_4 and 0.1g sodium sulphite was dissolved in 100ml of ice-cold distilled water.

3. Sodium phosphate buffer 0.1*M* (pH 7.2)

Stock solution

A: 0.2*M* solution of monobasic sodium phosphate (27.8g in 1000ml)

B: 0.2*M* solution of dibasic sodium phosphate (42.59g in 1000ml)

28mlof A + 72ml of B, diluted to 200ml.

4. Potassium phosphate buffer 0.1*M* (pH 7.2)

Stock solution

A: 0.1*M* K_2HPO_4 (1.742g in 100ml)

B: 0.1*M* KH_2PO_4 (0.68g in 50ml)

71.7ml of A + 28.3ml of B, diluted to 100ml.

APPENDIX II

Weekly weather data during October 2010 to March 2011

Month	Days	Standard week	Maximum temp. (°C)	Minimum temp. (°C)	Humidity (morn) (%)	Humidity (eve) (%)	Rainfall (cm)
Oct 2010	1 TO 7	40	30.6	22.7	95	78	41.4
	8 TO 14	41	29.5	23.3	74	70	5.1
	15 TO 21	42	28.3	21.9	95	78	18.6
	22 TO 28	43	29.3	27.3	94	76	13.4
Nov 2010	29 TO 4	44	30.6	22.2	95	71	22
	5 TO 11	45	30.4	22.3	96	73	17
	12 TO 18	46	31.3	22.5	92	67	8.5
	19 TO 25	47	30.8	22.5	91	71	8.7
Dec 2010	26 TO 2	48	28.1	22.8	83	71	1.2
	3 RO 9	49	31	21.3	89	59	0.3
	10 TO 16	50	31.4	21.5	91	59	0.4
	17 TO 23	51	30.9	22.8	77	55	2.6
	24 TO 30	52	30.7	21.8	76	51	0
Jan 2011	1 TO 7	1	31.9	22.3	84	51	0
	8 TO 14	2	33.2	22.3	89	45	0
	15 TO 21	3	32.9	20.9	73	36	0
	22 TO 28	4	32.2	22.9	67	38	0
	29 TO 4	5	33.4	22.9	61	29	0
Feb 2011	5 TO 11	6	34.2	21	68	27	0
	12 TO 18	7	33.9	21.2	75	35	0
	19 TO 25	8	33.5	22.7	90	52	0
Mar 2011	26 TO 4	9	33.7	23	73	36	0
	5-11mar	10	35.57	23.56	90.86	40.29	0
	11-18mar	11	35.46	23.67	84	32.71	1.43
	19-25mar	12	33.83	24.17	88.29	54.14	0
	26-31mar	13	34.23	24.95	88.33	58.5	0

APPENDIX III

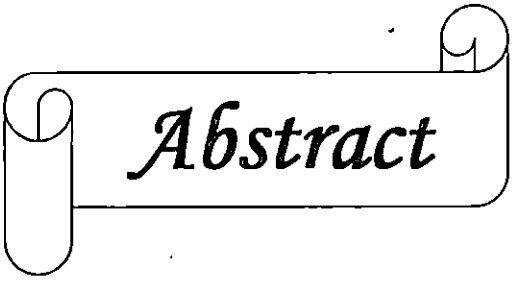
Preparation of Different Extracts

1. Neem Oil – Garlic Emulsion (2 per cent)

For the preparation of 10 litres of 2 per cent neem oil + garlic emulsion, 200ml neem oil, 200g garlic and 50g ordinary bar soap were required. Bar soap was sliced and dissolved in 500ml lukewarm water. 200g of garlic was ground and the extract was taken in 300ml of water. 500ml soap solution was poured in 200ml neem oil slowly and stirred vigorously to get a good emulsion. Garlic extract was mixed in the neem oil + soap emulsion. 1 litre of this stock solution was diluted by adding 9 litres of water to get 10 litres of 2 per cent neem oil - garlic emulsion.

2. Coconut Leaf Extract (10 per cent)

Fresh coconut leaves were chopped to small pieces and dried. The dried pieces were ground. 200g of the powder was weighed and one litre of water was added and kept in a water bath for one hour at 60^0C. After 1h, extract was strained and to this one litre of water was added to get 10 per cent coconut leaf extract.


Abstract

STUDIES ON TRANSMISSION, HOST RANGE AND MANAGEMENT OF ASH GOURD MOSAIC DISEASE

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The present investigation, "Studies on transmission, host range and management of ash gourd mosaic disease" was undertaken in the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2009-2011 with an aim to study the symptomatology of the mosaic disease, mode of transmission, host range of the virus, the resistance of available genotypes to mosaic under net house conditions and to evolve a suitable management practice under field conditions.

The sampling survey for the collection of mosaic samples conducted in different locations of Thrissur district revealed the incidence of five types of mosaic symptoms viz., marginal yellowing, yellow-green patch, severe puckering, filiform type and light and dark green patch type on ash gourd leaves. The marginal yellowing was found to be the prominent type of symptom compared to the other four types of mosaic. Under natural condition, yellowing of leaf margin was the major symptom of marginal yellowing type mosaic. But under artificial condition, yellowing of veins and veinlets of the leaf starting from the margin was the prominent symptom. In sap transmission studies, citrate phosphate buffer (0.1 *M*, pH 7) gave maximum disease incidence (73 per cent) with 23-28 days of incubation. In vector transmission studies, *Aphis gossypii* gave 59.5 per cent disease incidence and *Bemisia tabaci*, was unable to transmit the virus.

Biological indexing was done on *Petunia hybrida* and *Vigna unguiculata* to identify different viruses infecting ash gourd. Dark necrotic spot was produced in *P. hybrida* on inoculation with yellow-green patch type and severe puckering type mosaic whereas systemic infection was produced on inoculation with filiform type. Chlorotic spots were produced in *V. unguiculata* on inoculation with yellow-green patch type and puckering type mosaic whereas systemic infection was produced on inoculation with filiform type. Symptoms were not produced on inoculation with marginal yellowing type in *P. hybrida* and *V. unguiculata*. Based

on the symptoms produced on *V. unguiculata*, it was ascertained that the virus causing yellow-green patch type mosaic belong to Cucumber mosaic virus group and the virus causing filiform type of mosaic belong to potyvirus group. The electron microscopic study of the marginal yellowing type and puckering type revealed that they also belong to potyvirus group.

Host range studies of the ash gourd mosaic revealed systemic infection in snake gourd, bottle gourd, ivy gourd, tomato, chilli and cluster bean. Screening of 15 ash gourd genotypes against mosaic disease, revealed that one genotype, Jeevas was resistant to the mosaic with no disease incidence and one genotype BH-205 was moderately resistant (10 per cent incidence). The genotypes BH-206, BH-210, Indu, BHF-2, BHF-3, BHF-4, BHF-6, BHF-7, BHF-8 and BHF-9 were moderately susceptible (20-50 per cent incidence) and BH-216, BH-219 and BHF-5 were susceptible (70 per cent incidence) to mosaic.

Field experiment conducted to evaluate the effect of botanicals, biocontrol agent and chemicals on ash gourd mosaic revealed that all treatments reduced disease incidence, severity and coefficient of infection and increased yield and among them quinalphos (0.05%) was the best.

From the above study, it was concluded that marginal yellowing, yellow-green patch, puckering and filiformy were the major types of ash gourd mosaic and among them, mosaic with marginal yellowing symptom was the prominent one. The ash gourd mosaic was transmissible through sap and aphid. The virus causing marginal yellowing type mosaic belonged to potyvirus group. Snake gourd, bottle gourd, coccinia, tomato, chilli and cluster bean were found to be collateral hosts of the virus. Jeevas, a local genotype was identified as a resistant variety to ash gourd mosaic. The results of field experiment revealed that quinalphos (0.05 per cent) showed maximum effect in reducing mosaic infection.