Characterization and management of yellow mosaic disease of black gram (*Vigna mungo* (L.) Hepper)

By

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **'Characterization and management of yellow mosaic disease of black gram (*Vigna mungo* (L.) Hepper)'** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other university or society.

Vellanikkara
12/09/2018

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CERTIFICATE

Certified that this thesis entitled **'Characterization and management of yellow mosaic disease of black gram (*Vigna mungo* (L.) Hepper)'** is a record of research work done independently by **Ms. Divya Jayakumar V. J.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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ABBREVIATIONS

a.i	- Active ingredient
AAP	- Acquisition access period
ACMV	- *African cassava mosaic virus*
ACPV	- Advanced Centre of Plant Virology
AVPs	- Antiviral principles
BLAST	- Basic Local Alignment Search Tool
bp	- Base pair
cm	- Centimeter
CP	- Coat Protein
CR	- Common region
CRD	- Completely Randomized Design
CTAB	- Cetyl trimethyl ammonium bromide
cv.	- Cultivar
DAI	- Days after inoculation
DAS	- Days after sowing
DAS-ELISA	- Double Antibody Sandwich ELISA
DNA	- Deoxyribo Nucleic Acid
dNTP	- Deoxyribonucleotide triphosphate
DoYMV	- *Dolichos yellow mosaic virus*
ds	- Double stranded
EDTA	- Ethylene Diamine Tetra Acetic acid
ELISA	- Enzyme Linked Immuno Sorbent Assay

g	- Gram
GBNV	- *Groundnut bud necrosis virus*
ha	- Hectare
hrs	- Hours
HYMV	- *Horsegram yellow mosaic virus*
ICAR	- Indian Council of Agricultural Research
IAP	- Inoculation access period
IARI	- Indian Agricultural Research Institute
ICMV	- *Indian cassava mosaic virus*
IIPR	- Indian Institute of Pulses Research
KAU	- Kerala Agricultural University
kb	- Kilo base pairs
kg	- Kilo gram
l	- Litre
M	- Molar
MAb	- Monoclonal Antibody
MaGMV	- *Macroptilium golden mosaic virus*
mg	- Milligram
$MgCl_2$	- Magnesium Chloride
ml	- Millilitre
mM	- Millimolar
MYMIV	- *Mungbean yellow mosaic India virus*
MYMV	- *Mungbean yellow mosaic virus*

NaCl	- Sodium chloride
NASH	- Nucleic acid spot hybridization
NCBI	- National Centre for Biotechnology Information
ng	- Nanogram
nm	- Nanometer
NSKE	- Neem Seed Kernel Extract
OD	- Optical Density
ORF	- Open Reading Frame
PAL	- Phenyl alanine ammonia lyase
PCR	- Polymerase Chain Reaction
PO	- Peroxidase
ppm	- Parts per million
PPO	- Polyphenol oxidase
PTA	- Phosphotungstic acid
PVP	- Poly Vinyl Pyrollidone
q	- Quintal
RAPD	- Random Amplified Polymorphic DNA
RARS	- Regional Agricultural Research Station
RBD	- Randomized Block Design
RCA	- Rolling Circle Amplification
RNA	- Ribo Nucleic Acid
rpm	- Revolutions per minute
SDS	- Sodium dodecyl sulphate

SL	- Soluble concentrate
SLCMV	- *Sri Lankan cassava mosaic virus*
ss	- Single stranded
TAS-ELISA	- Triple Antibody Sandwich ELISA
TBE	- Tris Borate EDTA
TE	- Tris EDTA
ToLCV	- *Tomato leaf curl virus*
ToMoV	- *Tomato mottle virus*
TSWV	- *Tomato spotted wilt virus*
ULCV	- *Urdbean leaf crinkle virus*
UV	- Ultra violet
V	- Voltage
v/v	- Volume by volume
var.	- Variety
w/v	- Weight by volume
WASP	- Web Agri Stat Package
WG	- Wettable granule
YMD	- Yellow Mosaic Disease
YMVs	- Yellow Mosaic Viruses
µg	- Microgram
µl	- Microlitre

Introduction

1. INTRODUCTION

Pulses are unique crops which serve as an important source of protein for a large portion of global population. India is the largest producer (25% of global production), consumer (27% of world consumption) and importer (14%) of pulses in the world. Bengal gram, pigeon pea, cowpea, green gram, black gram and lentils are the major pulses grown and consumed in India. Besides serving as an important source of protein in human diet, pulses enrich soil and mitigate climate change through their unique nitrogen fixing properties. The present production of pulses in India is 24510 tonnes from an area of 28831.40 ha (India stat, 2018).

Black gram (*Vigna mungo* (L.) Hepper) also known as urd bean and mash is the third important pulse crop grown in India (Sharma *et al*., 2016) which contributes 70 per cent of the world's total black gram production. It is an erect, sub erect, densely hairy annual herb belonging to the family leguminosae. Black gram production is distributed mainly in tropical and subtropical countries. The traditional area for cultivation is confined to south Asia and adjacent regions. The global production of black gram is around 8.5 million tonnes from the major producing countries such as India, Myanmar and Thailand. India is the largest producer as well as consumer of black gram. It is cultivated in India in an area of about 4478.16 ha with an average productivity of 632 kg ha^{-1} (India stat, 2017). The major producing states are Madhya Pradesh, Maharashtra, Rajasthan, Andra Pradesh, Uttar Pradesh, Karnataka and Tamil Nadu.

Black gram is an excellent source of dietary protein (24-26%) and a good source of fodder. The crop is known as 'mini fertilizer factory' as it restores soil fertility by atmospheric nitrogen fixation (22 kg ha^{-1}). It is having medicinal property as it helps in mitigating elevated cholesterol levels (Menon and Kurup, 1976 and Indira and Kurup, 2013). Black gram contains essential nutrients like protein (25g/100g), potassium (983 mg/100g), calcium (138 mg/100g), iron (7.57 mg/100g),

niacin (1.447 mg/100g), thiamine (0.273 mg/100 g) and riboflavin (0.254 mg/100g) (USDA, 2016).

Black gram is popular in northern India as it is largely used to make dal but it is extensively used in south India also in culinary preparations like idli, dosa, vada, papad *etc.* The present area under pulse cultivation in Kerala is 3601 ha and black gram is 403 ha (GoK, 2016). Black gram is cultivated mainly in Palakkad and Malappuram disrticts of Kerala. The crop prefers dry weather condition with ideal temperature range of 25-35°C and is being cultivated in both seasons (*kharif* and *rabi*) in different parts of the country.

Black gram is affected by a number of diseases such as powdery mildew, anthracnose, leaf spot, rust, dry root rot, yellow mosaic and leaf crinkle. Among these, yellow mosaic disease (YMD) is the major constraint in black gram cultivation in India (Rathi, 2002; Gupta and Pathak, 2009 and Panigrahi *et al.*, 2016) and it causes severe losses in different leguminous species in India (Capoor and Varma, 1950; Nariani, 1960; Nene, 1973; Suteri, 1974; Muniyappa *et al.*, 1975; Singh, 1979 and Singh *et al.*, 2007). Most of the cultivated varieties are susceptible to yellow mosaic disease and causes 100 per cent yield losses under severe conditions. The first report of YMD affecting pulse crop in India was in lima bean (Capoor and Varma, 1948) followed by mung bean (Nariani, 1960). Yellow mosaic disease emerged as a serious problem on black gram in northern plains of India during 1970s (Murtaza *et al.*, 1983 and Gaffor *et al.*, 1992), during which it was difficult to identify the viruses specifically due to biologically indistinguishable symptoms. The disease caused an annual loss exceeding US $ 300 million in mung bean, urd bean and soybean (Varma *et al.*, 1992 and Varma and Malathi, 2003).

Yellow mosaic disease affected black gram exhibits alternate green and yellow patches on leaves. Leaf size is generally not affected but sometimes the green area are slightly raised in appearance and leaves show puckering and reduction in size. In some cases, leaves become papery white and thin.

Yellow mosaic disease of black gram is caused by the genus *Begomovirus* belonging to the family *Geminiviridae*, which is the second largest family of plant viruses. Geminiviruses are economically important group of diverse small plant viruses that have a circular, single stranded (ss) DNA genome of size ranging from 2.5-3.0 kilo bases and are encapsulated within twinned isometric particles of size 15-18 × 30 nm (Stanley, 1983). Depending on their vector, host range and genome characteristics, geminiviruses are grouped into nine different genera namely *Becurtovirus, Begomovirus, Curtovirus, Eragovirus, Mastrevirus, Topocuvirus* and *Turncurtovirus* and recently added *Capulavirus* and *Grablovirus* (Brown *et al.*, 2015 ; Adams *et al.*, 2016 and Varsani *et al.*, 2017). Geminiviruses belonging to the genus *Begomovirus* have either monopartite (DNA A) or bipartite (DNA A and DNA B) genome (Harrison and Robinson, 1999 and Rojas *et al.*, 2005).

Majority of begomoviruses consist of characteristic two circular ss DNA components, DNA A and DNA B (Varma and Malathi, 2003) each of about 2.6-2.8 kb genome size. DNA A encodes proteins for encapsidation and viral replication and DNA B for intra and intercellular movement function (Harrison *et al.*, 1977). Monopartite begomoviruses have one or more small circular satellite DNA molecules having ~1.3 kb size, designated as DNA β instead of DNA B (Briddon *et al.*, 2002). Coat protein gene is considered for identification of different strains of yellow mosaic viruses as it is well characterized and widely used for virus identification and classification (Mahajan *et al.*, 2011).

Four different species of begomoviruses, *viz.*, *Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic India virus* (MYMIV), *Dolichos yellow mosaic virus* (DoYMV) and *Horsegram yellow mosaic virus* (HYMV) cause yellow mosaic disease in different legumes and they are collectively called as yellow mosaic viruses (YMVs) (Qazi *et al.*, 2007). Among these MYMIV is predominant in northern, central and eastern regions of India (Usharani *et al.*, 2004). Whereas MYMV is

predominant in southern regions of India and it causes severe yellow mosaic disease in green gram and black gram (Karthikeyan *et al.*, 2004).

Yellow mosaic disease of pulses is extensively studied from different parts of the country. But no study has been conducted in Kerala on any aspects of the yellow mosaic disease in black gram. Hence, in an endeavor to generate information on the viruses causing YMD in black gram in Kerala, the present study was undertaken with the following objectives

- To identify and characterize the virus causing yellow mosaic disease in black gram
- To evolve a suitable strategy for the management of the disease

Review of Literature

2. REVIEW OF LITERATURE

Urd bean (*Vigna mungo* (L.) Hepper), the third important pulse crop of India is a rich source of dietary protein (23.9%) and used in daily diet of Indians. India contributes 70 per cent to the annual production of black gram in the world and is the major exporter of black gram. The average productivity of black gram in India has remained around 0.41 to 0.53 t ha^{-1} for the past two decades. Losses due to various diseases are always a major limiting factor for increasing productivity in black gram. Among these diseases, yellow mosaic disease caused by yellow mosaic virus is one of the major constraints in black gram production.

The availability of literature on yellow mosaic disease on black gram is limited. Hence, publications on yellow mosaic diseases of different pulses are also reviewed to support the present study.

2.1 OCCURRENCE OF YELLOW MOSAIC DISEASE

Yellow mosaic disease was first reported in cowpea from Lyllpur (now known as Faisalabad) now a part of Pakistan (Vasudeva, 1942). Later reported from different countries like Sri Lanka (Shivanathan, 1977), Bangladesh (Jalaluddin and Shaikh, 1981) and Thailand (Thongmeearkom *et al.*, 1981).

In India, occurrence of yellow mosaic disease was first reported in late 1940s in limabean from the College of Agriculture, Pune (Capoor and Varma, 1948). Later it was reported in dolichos (Capoor and Varma, 1950), mung bean (Nariani, 1960), horse gram (William *et al.*, 1968), black gram (Nair and Nene, 1973), cowpea (Nene, 1973) and soybean (Suteri, 1974).

2.2 ECONOMIC IMPORTANCE OF YELLOW MOSAIC DISEASE

Nene (1973) reported that YMD of black gram, soybean, mung bean and cowpea causes yield loss of 100 per cent under severe conditions and the annual loss

due to YMD was estimated to be $300 million in three leguminous pulses *viz.*, black gram, mung bean and soybean (Varma and Malathi, 2003).

Khattak *et al.* (2000) reported that incidence of yellow mosaic disease on susceptible mung bean plants at Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan during summer resulted in a reduction in seed size from 10.6 to 52.3 per cent and reduction in grain yield from 32.2 to 78.6 per cent over the healthy plants at the peak incidence of disease. It indicates that YMD may cause severe yield loss under heavy incidence of disease in the field.

2.3 SYMPTOMATOLOGY

During 1950s, Nariani observed typical bright yellow mosaic symptoms on mung bean (*Vigna radiata* (L.) Wilczek) affected by a virus disease at New Delhi and identified the virus as *Mungbean yellow mosaic virus* (MYMV) in 1960. He reported that the symptoms start on leaf lamina as irregular small specks or yellow spots, which later enlarge in size and spread all over the lamina and the entire lamina become completely yellow in colour. Plants produce fewer pods and flowers.

Nene (1973) reported that symptoms like yellow specks are observed even in the first trifoliate leaf at the seedling emergence stage in black gram plants. Some varieties of black gram plants show necrotic mottling on leaves (Nene, 1972; Singh *et al.*, 2002 and Qazi *et al.*, 2007). Not only leaves but seeds are also affected by YMD and are ill filled, reduced in size and weight, deformed and exhibited yellow discolouration (Nene, 1973 and Varma *et al.*, 1992).

Singh and De (2006) observed yellow mosaic symptoms on black gram plants as irregular green patches on leaf lamina of older leaves later spreading to younger leaves causing total yellowing of leaves in susceptible varieties.

Rouhibakhsh *et al.* (2008) reported that YMD infected black gram plants show alternate green and yellow patches on the leaves. Sometimes the green areas on

the leaves are slightly raised and shows puckering and reduction in size. In some cases, leaves become papery white and thin.

Naimuddin *et al.* (2011) also reported yellow mosaic symptoms such as yellow patches between veins of leaf lamina. Initially very few patches were observed and later almost more than 70 per cent of leaf area turned yellow on wild species of black gram (*Vigna mungo* var. *silvestris*) in the field.

2.4 ETIOLOGY OF YMD IN BLACK GRAM

Yellow mosaic disease of black gram is caused by Geminivirus, belonging to *Begomovirus* genus of family *Geminiviridae*, which is the second largest family of plant viruses. Geminiviruses are an economically important group of plant viruses which are transmitted either by leafhoppers or whiteflies (*Bemisia tabaci* Gennadius). Whitefly transmitted geminiviruses cause serious loss to many important food crops like bean, cucurbits, tomato, pepper and cassava in tropical and subtropical regions (Brown, 1991 and Brown and Bird, 1992). These geminiviruses are grouped into nine different genera based on their vector, host range and genetic characteristics as *Becurtovirus, Begomovirus, Curtovirus, Eragovirus, Mastrevirus, Topocuvirus, Turncurtovirus* and recently added *Capulavirus* and *Grablovirus* (Brown *et al.*, 2015 ; Adams *et al.*, 2016 and Varsani *et al.*, 2017). Majority (80%) of the geminivirus belong to whitefly transmitted *Begomovirus*. Begomoviruses are differentiated from other genera, particularly by the transmission by whiteflies (*Bemisia tabaci* Genn.) in a persistent manner (Malathi and John, 2008).

Four different species of whitefly transmitted begomoviruses *viz.*, *Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic India virus* (MYMIV), *Dolichos yellow mosaic virus* (DoYMV) and *Horsegram yellow mosaic virus* (HYMV) are known to cause YMD in different legumes and are commonly known as yellow mosaic viruses (Qazi *et al.*, 2007 and Ilyas *et al.*, 2010). Among these, MYMV (Morinaga *et al.*, 1993) and MYMIV (Mayo and Pringle 1998) are the major

viruses associated with YMD in black gram. DoYMV is very recently recognized and named as a distinct species of *Begomovirus* affecting the host Dolichos (*Lablab purpureus*) (Maruthi *et al.*, 2006) and HYMV was first reported from horse gram (Muniyappa *et al.*, 1987). Type species of MYMV from mung bean was isolated from Thailand (Thongmeearkom *et al.*, 1981) and MYMIV from black gram from New Delhi. MYMIV is predominant in northern, central and eastern regions of India (Usharani *et al.*, 2004), Pakistan, Nepal and Bangladesh (Varma *et al.*, 1992 and Jones, 2003) whereas MYMV is predominant in southern and western regions of India (Karthikeyan *et al.*, 2004), Pakistan (Ahmad, 1975) and Thailand (Thongmeearkom *et al.*, 1981).

2.4.1 Genome organization

Geminiviruses are characterized by 15-18 × 30 nm sized geminate isometric virions with single stranded circular DNA genome with a size ranging from 2.5-3.0 kilo bases (Stanley, 1983). Genome of begomoviruses may be monopartite or bipartite. The bipartite genome consists of two circular single stranded DNA components, DNA A and DNA B whereas, monopartite genome consist of DNA A component only, lacking DNA B (Harrison and Robinson, 1999; Varma and Malathi, 2003 and Rojas *et al.*, 2005). The DNA A of monopartite viruses are reported to be associated with a small circular ss DNA molecule of ~ 1.3 kb length, designated as satellite DNA molecule (DNA β) (Saunders *et al.*, 2000 ; Briddon *et al.*, 2003 ; Zhou *et al.*, 2003 and Briddon and Stanley, 2006). The *Begomovirus* is differentiated into 'old world' and 'new world' begomoviruses based on the coat protein and replication initiation protein sequences which corresponds to the geographical origin of the virus. Old world viruses include those from Asia, Africa, Mediterranean region and Australia and new world viruses belong to America (Howarth and Vandermark, 1989). Old world begomoviruses can be either monopartite or bipartite whereas, new world begomoviruses are exclusively bipartite with one recently described monopartite virus (Brown, 2001).

There is no sequence similarity between DNA A and DNA B components except for a short stretch of ~200 nt, which is referred to as the common region (CR). The common region has a highly conserved region of TAATATTAC sequence in both DNA components and is responsible for replication and transcription (Eagle *et al.*, 1994; Padidam *et al.*, 1996 and Harrison and Robinson, 2002). DNA A component consist of one gene (AV1) on viral sense strand and three genes (AC1, AC2, AC3) on complementary sense strand for new world bipartite begomoviruses (Harrison and Robinson, 1999) whereas an additional gene AV2 in the viral sense strand and AC4 on complementary sense strand for the old world bipartite begomoviruses (Hanley-Bowdoin *et al.*, 1999). AV1 gene codes for coat protein of the virus, AV2 for pre-coat protein, AC1 for replication initiator protein, AC2 for transcription activator protein, AC3 for replication enhancer protein and AC4 for symptom determinant protein. DNA B component codes for only two ORFs, BV1 gene on viral sense strand, which codes for nuclear shuttle protein and BC1 on complementary sense strand which codes for movement protein (Sanderfoot *et al.*, 1996 and Glick *et al.*, 2009).

β satellites associated with the monopartite begomoviruses are very essential for typical symptom development on the susceptible plants (Stenger *et al.*, 1991 ; Saunders *et al.*, 2000 ; Glick *et al.*, 2009 and Sivalingam and Varma, 2012).

2.4.2 Agro-inoculation and proving Koch's postulate for YMV

Agrobacterium mediated transfer of viral genome components provides a way for proving Koch's postulates in case of viral diseases. Biswas and Varma (2001) carried out agro-inoculation with dimeric constructs of cloned DNA A and DNA B components of MYMV-Bg to six different grain legumes *viz.*, black gram, cowpea, french bean, mung bean, pigeon pea and soybean through sprouted seed and seedling inoculation methods and accumulation of virus was confirmed through NASH (Nucleic acid spot hybridization). Out of the 14 varieties tested by agro-inoculation on sprouted seeds, three varieties of french bean and two varieties of

mung bean developed typical symptoms whereas, out of 16 varieties tested by agro-inoculation on seedlings, four varieties of french bean and one variety each of mung bean and black gram developed typical symptoms. Agro-inoculation thus proved the pathogenicity of the virus identified.

The tandem viral constructs of MYMV-[IN:Vam:05] and MYMIV-[II:ND:Bg 3:91] were agro-inoculated using *Agrobacterium tumefaciens* strain EHA105 onto 2-days-old sprout seeds of french bean cv. selection 9, cowpea cv. Pusa Komal, mung bean cv. Pusa Baisakhi, PS16 and black gram cv. T-9. Agro-inoculation of MYMV produced typical yellow mosaic symptoms in black gram (50.95%) and mung bean (66%) and atypical symptoms in french bean (100%) and cowpea (44%). These atypical symptoms were almost similar to symptoms caused by MYMIV infection, in black gram (49%) and mung bean (63%). The reassortment of viral components (DNA A and DNA B) of MYMV and MYMIV produced symptoms only in French bean (100%) (Haq *et al.*, 2011).

Maheshwari *et al.* (2014) agro-inoculated the viral constructs of MYMV, using *Agrobacterium tumefaciens* strain Ach 5 and C 58 onto seeds of black gram Co5. They found that Co5 variety is highly susceptible to agro-inoculation and *A. tumefaciens* strain (Ach 5) showed an average infectivity of 63 per cent whereas, *A. tumefaciens* strain (C 58) showed 16 per cent infectivity. Accumulation of viral DNA was confirmed through PCR analysis of the symptomatic plants.

The average infectivity of the cloned components of DNA A and DNA B of a non-sap transmissible isolate MYMIV-[IN:Ana:CpMBKA25:04] was tested by agro-inoculation on different legumes *viz.*, mung bean, black gram, french bean and cowpea using seed inoculation method and in tobacco using seedling inoculation and observed cent per cent typical symptoms in french bean, mung bean (cv. K.851) and flecking symptoms in mung bean cultivars, GM-2-12-24 and local Delhi. Whereas, plants remained symptom free in mung bean cultivars, GM-9907, GM-9908, GM-9922 and GM-02-01, cowpea and tobacco (John *et al.*, 2016).

2.5 TRANSMISSION

It has been reported that the yellow mosaic virus occurring in India is transmitted by insect vector, whitefly (*Bemisia tabaci*) and not by soil, seed or mechanical inoculation to plants belonging to leguminosae family (Ahmad and Harwood, 1973 and Nair and Nene, 1973) and also to plants belonging to other families like graminae and compositae (Nene *et al.*, 1971; Nene, 1973 and Rathi and Nene, 1974). Begomoviruses emerged as a major threat to different crops in different countries due to the incredible increase in population density of the vector, *Bemisia tabaci* in 1970's (Bird and Maramorosch, 1978) and establishment of B-biotype of *Bemisia tabaci* in USA and other countries (Brown and Bird, 1992). Begomoviruses, transmitted by whitefly (*Bemisia tabaci* Genn.) were formerly included in a subgroup III geminiviruses (Polston and Anderson, 1997 and Chakraborty *et al.*, 2008).

The latent period required for whitefly to perform as a good vector for the virus is less than four hours. A most efficient female and male adult can retain infectivity for 10 days and 3 days, respectively and female adults are three times more efficient than male adults (Rathi and Nene, 1974a). Nymphs of whiteflies can acquire virus from infected leaves (Nene, 1972) but viruses do not pass through eggs (Ahmad and Harwood, 1973).

The persistent and circulative transmission of MYMV by whiteflies was reported by Nair and Nene (1974) and also mentioned that there is no need of pre-acquisition and pre-inoculation starvation for increasing the efficiency of virus transmission.

Malathi and John (2008a) reported that a single whitefly can acquire virus from the source plant with a single bite and the transmission efficiency of virus increases with time on source plant during acquisition as well as on the healthy plants during inoculation.

Naimuddin *et al.* (2011) reported the transmission of yellow mosaic disease from a wild relative of urd bean (*Vigna mungo* var. *silvestris*) to black gram seedlings with a transmission efficiency of 46.7 per cent and the symptoms appeared 10 days after inoculation.

Kumar *et al.* (2014) studied and confirmed the efficacy of vector *Bemisia tabaci* in transmission of MYMV through artificial inoculation under controlled conditions in glass house on black gram plants (Barabanki local). It was observed that plants showed systemic infection on leaves, typical yellow mosaic symptoms like yellow specks were formed and covered more than 75 per cent of leaf area within 7 to 10 days after inoculation with viruliferous whiteflies and an average infectivity of 81 per cent was recorded.

Maheshwari *et al.* (2014) confirmed transmission of MYMV through whitefly on black gram (Co5) plants under glasshouse conditions. Five plants out of 15 plants inoculated showed typical yellow specks on leaves 12 -15 days after inoculation of viruliferous whiteflies and an average infectivity of 33 per cent was recorded at an acquisition access period and inoculation access period of 24 hrs.

Govindan *et al.* (2014) found that the efficiency of transmission of MYMV to mung bean plants by whiteflies was maximum upto 85 per cent when an acquisition access period (AAP) of 48 hrs and inoculation access period (IAP) of 24 hrs were given compared to 70 per cent transmission efficiency at 24 hrs of AAP and IAP.

Anokhe *et al.* (2018) studied the transmission of *Mungbean yellow mosaic virus* by whiteflies on mung bean, by investigating the effect of number of whiteflies, AAP and IAP on transmission efficiency and found that transmission efficiency increased with the number of whiteflies used. 36.6 and 66.6 per cent transmission was recorded with one and five whiteflies, respectively. At different AAP *viz.*, 60, 90 and 240 minutes, transmission of 35, 60 and 100 per cent were obtained respectively.

Transmission efficiency of 16, 38, 57 and 70 per cent were observed at 15, 30, 60 and 90 minutes of IAP, respectively.

2.6 HOST RANGE

Host range of *Mungbean yellow mosaic virus* varies according to geographical locations. The host range for MYMV isolates from Thailand is restricted to leguminosae family such as soybean, black gram, mung bean, french bean, jack bean and lima bean (Honda *et al.*, 1983). Whereas, isolates from India can infect species from other families like graminae (*Brachiaria ramosa*) and compositae (*Eclipta alba*) (Nene *et al.*, 1971).

Nariani (1960) reported certain hosts of MYMV such as *Vigna acontifolia*, *Vigna radiata*, *Vigna mungo*, *Glycine max*, *Phaseolus lathyroides* and *Dolichos biflorus*. Nene *et al.* (1971) reported the transmissibility of MYMV to *Cajanus cajan*, *Xanthium strumarium*, *Brachiaria ramosa* and *Eclipta alba*.

Rathi and Nene (1974) found *Cosmos bipirimatus* and *Phaseolus vulgaris* var. *monitor* as the hosts of MYMV.

Usharani *et al.* (2004) reported the transmission of YMD from soybean plants to black gram, cowpea, mung bean and soybean and expression of yellow and golden mosaic symptoms on these plants through inoculation of viruliferous whiteflies.

Naimuddin *et al.* (2014) reported the occurrence of yellow mosaic virus on a widely occurring weed, *Ageratum conyzoides* and PCR results confirmed the virus as MYMIV and later the virus was successfully transmitted by whiteflies from weed to cultivated hosts like mung bean and urd bean. *Ageratum conyzoides* might be acting as a collateral host for the virus during almost all seasons.

Bhanu *et al.* (2015) identified *Sonchus arvensis*, *Acalifa indica*, *Acalifa celiyata*, *Hemidesmus indicus*, *Rincozia minima*, *Corchorus olitorius*, *Croton*

sparsiflorus, *Tephrosia purpurea*, *Cida acuta* and *Aciranthus aspira* as collateral host for yellow mosaic disease.

Gupta (2016) observed and recorded 10 to 80 per cent incidence of whitefly transmitted MYMV on different plant species like *Alternanthera eassilus*, *Corchorus solitarus*, *Sidarhom bifolia* and *Paracalyx cubisus* other than crop plants in different fields in *kharif*, *rabi* and summer seasons.

Deepa *et al.* (2017) conducted a host range study of MYMV from green gram to different crop plants as well as weed species and revealed that six species, *Nicotiana benthamiana*, black gram (*Vigna mungo*), horse gram (*Macrotyloma uniflorum*), soybean (*Glycine max*), pigeon pea (*Cajanus cajan*) and cowpea (*Vigna ungiculata*) and weed species like *Croton bonplandianum*, *Euphorbia geniculata*, *Phyllanthus madraspatenses*, *Malvastrum coromandelium*, *Acalypha indica* and *Alternanthera sessile* showed successful transmission and acted as potential source of MYMV while *Parthenium hysterophorus* did not show any systemic symptoms. *Nicotiana benthamiana* and *Croton bonplandianum* showed maximum transmission.

Jayappa *et al.* (2017) carried out whitefly transmission of MYMV to different plant species belonging to leguminosae and solanaceae and found that MYMV could be transmitted only to leguminous crops such as black gram (*Vigna mungo* (L.) Hepper), pigeon pea (*Cajanus cajan* (L.) Mill), horse gram (*Macrotyloma uniforum* (Lam.) Verdc.), soybean (*Glycine max* (L.) Merr.) and french bean (*Phaseolus vulgaris*) with per cent transmission of 86.66, 13.33, 80, 40 and 60 per cent, respectively. This study revealed that transmission efficiency decreases as the age of the host seedlings increases. Similar observations on host plants were recorded by Karthikeyan *et al.* (2004) and Malathi *et al.* (2005).

2.7 DETECTION OF BEGOMOVIRUSES

Detection of disease causing agent is very important for the management of diseases. Though symptomatology gives a clue to the type of causal organism

involved, many times it can be confusing, particularly in case of viral diseases as the symptoms are very much similar to that of nutrient deficiency. Electron microscopy, serological tools and molecular tools are generally used for plant virus detection.

2.7.1 Electron microscopy

Thongmeearkom *et al.* (1981) observed loose aggregates of isometric virus-like particles of 15-20 nm diameter in mung bean phloem cells infected with yellow mosaic disease. They also reported that the nuclei of phloem cells are filled with virus-like particles and fibrillar and hypertrophied nucleoli were visible just two days before the symptom appearance. Honda *et al.* (1983) also observed 18 × 30 nm sized geminate particles of *Mungbean yellow mosaic virus* in the infected mung bean plants.

Muniyappa *et al.* (1987) reported the presence of geminate particles of 15 to 18 × 30 nm size resembling geminiviruses in yellow mosaic infected horse gram samples in leaf dip and purified virus preparations. They also suggested that uranyl acetate is more effective for staining the virus particles than the phosphotungstic acid (PTA).

Hajong and Naveenkumar (2016) conducted electron microscopic studies of the soybean plants affected with yellow mosaic disease and revealed the presence of geminate particles of size approximately 30 × 20 nm.

2.7.2 Serodiagnosis

Serological techniques are generally employed for the detection of different plant viruses. The coat protein region of begomoviruses are highly conserved. Hence, polyclonal antibody raised against one begomovirus can be used to detect other begomoviruses. But these polyclonal antibodies are inefficient for species level detection due to the highly conserved coat protein region. However monoclonal antibodies developed against specific epitopes on coat protein can be used for species level detection. Though there are a few reports on serological detection of

begomoviruses using monoclonal antibodies, there are no reports on serological detection of YMV. Low virus titre of YMV in the host plants makes it difficult to detect serologically.

Swanson *et al.* (1998) reported TAS-ELISA with monoclonal antibodies (MAb SCR 18) as the most sensitive method for the detection of ToLCV isolates in India than the DAS-ELISA with polyclonal antibodies by raising 25 MAbs against two Begomoviruses like ToLCV (26 indian isolates) and *Croton yellow vein mosaic virus* (13 isolates) and detection with TAS-ELISA.

Rivera-Vargas *et al.* (2001) used three different monoclonal antibodies like 3F7, 2G5 and 5C5 for the detection of *Bean golden yellow mosaic virus* (BGMV) in common bean (*Phaseolus vulgaris*) cultivars and *Macroptilium lathyroides*. They reported that, MAb 3F7 was most effective one and it detected the virus in susceptible cultivars of bean, *viz.*, Top crop and Quest but could not be detected in the line DOR 303, which showed typical virus symptoms. None of the MAb were able to detect virus in *Macroptilium lathyroides* and suggested that ELISA was not effective for the detection of virus in *Macroptilium lathyroides.*

Lima *et al.* (2012) detected a *Begomovirus*, which is serologically related to *Macroptilium golden mosaic virus* (MaGMV) in 318 leaf samples of *Macroptilium lathyroides* collected from field and four graft inoculated samples with golden mosaic by indirect ELISA technique with antiserum for MaGMV.

Paul (2014) serologically revealed the close relationship of ToLCV with *Afircan cassava mosaic virus* (ACMV) through DAC-ELISA using antisera of three different geminiviruses like *Indian cassava mosaic virus* (ICMV), *African cassava mosaic virus* (ACMV) and *Sri Lankan cassava mosaic virus* (SLCMV) and confirmed the presence of *Begomovirus* in all the samples under study.

2.7.3 Molecular diagnosis

Currently, molecular techniques like Polymerase Chain Reaction (PCR) are the most popular technique used for the detection of plant viruses. PCR is becoming an alternative way to the serological techniques due to its high accuracy and sensitivity in the detection of plant viruses even from the small amount of samples. In PCR technique, the genetic material of diseased samples is extracted, purified and amplified with viral nucleic acid specific primers. PCR is the widely used and effective tool for identification of begomoviruses as they form a double stranded DNA intermediate during replication. This ds DNA can act as template for the PCR.

2.7.3.1 *Isolation of genomic DNA*

Doyle and Doyle (1987) reported a rapid method for DNA isolation from small quantities of fresh soybean leaf samples which used an extraction buffer consisting of 1M Tris-HCl, 0.5M EDTA, 5M NaCl, 2 per cent CTAB and 0.2 per cent ß-mercaptoethanol at pH 8.0 to compensate for higher water content in fresh tissue. The DNA was purified using chloroform: isoamyl alcohol (24:1) and precipitated using 10 mM ammonium acetate. John *et al.* (2008) also followed same protocol for extraction of total DNA from the leaves infected with cowpea golden mosaic disease in cowpea. More recently, genomic DNA was extracted by Ramesh *et al.* (2016 and 2017) from soybean and *Vigna trifoliata* leaves showing typical yellow mosaic symptoms using the same procedure.

Rouhibhaksh *et al.* (2008) reported a Gem-CTAB method which is a relatively inexpensive, simple and easy protocol for the extraction of *Begomovirus* DNA from yellow mosaic virus infected legume hosts. This method differed from other methods in the ratio of the extraction buffer added to the leaf sample extracted (10:1), concentration of β-mercaptoethanol (5%) and NaCl (2M). Using this method, it was possible to extract PCR amplifiable DNA from mature leaves of legume hosts rich in polyphenols, tannins and polysaccharides. Satya *et al.* (2013) adopted the

same protocol for genomic DNA extraction from the yellow mosaic diseased samples of urd bean and mung bean with slight modification of using 2 per cent β-mercaptoethanol in extraction buffer. Later the same procedure was followed by Bhagyashree *et al.* (2017) for extraction of total genomic DNA from the yellow mosaic affected lima bean leaves.

Biswas *et al.* (2008) isolated the total DNA from yellow mosaic infected leaves of young mung bean, black gram and pigeon pea plants in an extraction buffer containing 100 mM Tris-Cl, pH 8.0, 100 mM NaCl, 10 mM EDTA and 0.05 per cent SDS. DNA was purified from protein with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) mixture and DNA was precipitated using 2.5 volume of absolute alcohol and $1/10^{th}$ volume of 3 M sodium acetate (pH 4.8).

Choudhary *et al.* (2008) reported a simple and easy protocol for the isolation of total DNA from the fresh as well as dried leaves of *Vigna acontifolia* and *Vigna trilobata.* The protocol was a modified CTAB method using an extraction buffer containing 3 per cent CTAB, 4 per cent β-mercaptoethanol, 2 M NaCl and 5 per cent PVP. DNA was purified with equal volume of mixture of chloroform: isoamyl alcohol (24:1) and precipitated with 1 M NaCl-TE and ice cold isopropanol. A good quality which is suitable for analysis with restriction enzyme digestion and random amplification of polymorphic DNA (RAPD) could be extracted using this method.

Gautam *et al.* (2014) carried out a modified CTAB method in order to extract total DNA from various wild *Vigna* species affected with yellow mosaic disease. CTAB extraction buffer containing CTAB (2%), 100 mM Tris (pH 8.0), 20 mM EDTA, 1.4 M NaCl and β-mercaptoethanol (2%) was used for extraction, chloroform: isoamyl alcohol (24:1) for purification and 0.1 volume of 3 M sodium acetate (pH 5.2) for precipitation of the DNA.

Manjunatha *et al.* (2015) followed CTAB method for extracting total nucleic acid from the red gram leaves showing typical yellow mosaic symptoms with a

CTAB extraction buffer containing CTAB 2 per cent (w/v), 1.4 M NaCl, 2-mercaptoethanol (0.2%) (v/v), 20 mM EDTA and 100 mM Tris–HCl (pH 8.0). DNA was purified with 0.75 volume chloroform: isoamy lalcohol (24:1) and precipitated with 0.6 volume cold isopropanol. Later the same protocol was followed by Deepa *et al.* (2017a) for genomic DNA isolation from green gram leaves infected with yellow mosaic virus.

The total genomic DNA was extracted from yellow mosaic diseased tender bean leaves by Reinzie *et al.* (2016) following CTAB method developed by Lodhi *et al.* (1994) with slight modifications. Ten times more concentration of EDTA (0.2 M), ß-mercaptoethanol (2%) and an extra 1 per cent PVP were included in the extraction buffer, the treatment with NaCl was omitted and single chloroform: isoamyl alcohol mixture (equal volume) treatment was used instead of double chloroform: octanol treatment for purification. The DNA was precipitated with isopropanol.

Obaiah *et al.* (2014) determined the presence of *Begomovirus* in symptomatic black gram leaves using a modified CTAB method for genomic DNA isolation which contained an extraction buffer of 100 mM Tris (pH 8.0), 1.4 M NaCl, 20 mM EDTA, CTAB (2%), PVP (1%) and ß-mercaptoethanol (0.1%) and a purification step with equal volume of chloroform : isoamyl alcohol mixture and precipitation step with 0.1 volume of 3 M sodium acetate (pH 4.8) and 0.6 volume ice cold isopropanol.

2.7.3.2 *Polymerase Chain Reaction (PCR)*

2.7.3.2.1 *Detection of begomovirus*

Deng *et al.* (1994) designed a universal degenerate primer pair (DengA/DengB) amplifying an approximately 500 bp region including portions of common region and 5' end of coat protein for detecting *Begomovirus* group. This primer pair is used by many workers for the detection of *Begomovirus*.

Maheshwari *et al.* (2014) used universal degenerate primers (Deng A/Deng B) for the detection of geminivirus in the yellow mosaic disease affected black gram, cowpea and green gram and reported amplicons of 539 bp size.

PCR amplification of genomic DNA extracted from the yellow mosaic diseased red gram samples was carried out by Manjunatha *et al.* (2015) using universal deng primers, which confirmed presence of *Begomovirus* in the sample with the amplification of 520 bp coat protein region of the virus. Red gram isolates showed maximum sequence similarity of 95-98 per cent with HYMV of different pulses reported from southern India, followed by MYMV (87%).

Deepa *et al.* (2017) detected the presence of *Begomovirus* in older as well as younger leaves of green gram showing yellow mosaic symptom by PCR amplification with begomovirus coat protein specific primers (AV494 (F)/ AC1048 (R)) and an amplicon of ~520 bp was obtained.

2.7.3.2.2 *Detection of different yellow mosaic virus species*

Amplification of full length DNA A as well as different regions of DNA A was employed for identification of yellow mosaic viruses. Several workers have designed species-specific primers for the detection of yellow mosaic viruses. Most of these primers are designed to amplify full length of a part of coat protein gene.

Naimuddin and Akram (2010) designed four sets of species-specific primer pairs (MYMIV CP-F/MYMIV CP-R, MYMV CP-F/MYMV CP-R, HYMV CP-F/ HYMV CP-R and DoYMV CP-F/DoYMV CP-R) specific to coat protein gene for the detection these viruses. They detected the presence of MYMIV and mixed infection of MYMIV with DoYMV in yellow mosaic infected cowpea samples by PCR amplification with these primers which yielded amplicons of ~1000 bp with MYMV and DoYMV specific primer.

Mishra *et al.* (2010) identified the presence of MYMIV in three pulses (mung bean, urd bean and pigeon pea) showing typical yellow mosaic symptoms by PCR

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using three sets of primers, AC2-F/AC2-R, AC3-F/AC3-R and AC4-F/AC4-R which yielded amplicons of sizes ~480 bp, ~450 bp and ~500 bp, respectively. They reported that weeds have no role in the epidemics of the yellow mosaic disease in pulses, as they did not get amplification in weeds, *Acalypha indica, Croton bonplantianum* and *Clerodendron* sp.

Ali and Ahmad (2010) designed six sets of primers specific to DoYMV (DAC1F/DAC1R, DAC2 F/DAC2R, DAC3F/DAC3R, DAC4F/DAC4R, DAV1F/DAV1R and DAV2F/DAV2R) and tried to amplify targeted DNA fragments of the yellow mosaic infected dolichos *(Lablab purpureus* L. Sweet) samples from the field. Five primer pairs, DAC1F/DAC1R, DAC2F/DAC2R, DAC3F/DAC3R, DAC4F/DAC4R and DAV2F/DAV2R gave positive PCR reaction with amplicons of size 1100, 500, 380, 390 and 400 bp, respectively.

The first report on identification and characterization of the virus species associated with yellow mosaic disease in wild species of *Vigna mungo* var. *silvestris* was by Naimuddin *et al.* (2011). They identified the virus by PCR using gene specific primers (AV1PF/AV1PR, AC1PF/AC1PR, AC2PF/AC2PR, AC3PF/AC3PR and AC4PF/AC4PR) and yielded expected amplicons of size ~1100 bp, ~480 bp, ~450 bp, ~390 bp and ~950 bp, corresponding to genes AV1, AC1, AC2, AC3 and AC4 of MYMIV, respectively in five field samples and in the symptomatic leaf sample from whitefly-inoculated plants. Analysis of coat protein gene sequences revealed that the virus species has a nucleotide sequence similarity of 97 per cent with MYMIV-Mung bean (EU523045), 80 per cent with MYMV-Soybean (AY271896), 81 per cent with HgYMV (NC_005635), 70 per cent with DoYMV (AY309241) and 68 per cent with *Cowpea golden mosaic virus* (CGMV).

The *Begomovirus* species present in the yellow mosaic diseased mung bean plants in Bangladesh was first characterized through PCR with MYMV coat protein gene specific primers (MYMV cpFwd and MYMV cpRev) which yielded amplification of ~750 bp fragment of the CP gene. The isolates showed maximum

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sequence similarity with the MYMIV-Bangladesh strain (AF314145.1) in the database searches by NCBI-BLAST program (Islam *et al.*, 2012).

Obaiah *et al.* (2014) detected *Mungbean yellow mosaic India virus* from infected black gram plants from Andhra Pradesh by PCR using coat protein gene specific primers (RHA F and AC-abut) which amplified approximately 900 bp CP gene region of viral DNA A fragment. They standardized the genomic DNA concentration required for PCR and reported the minimum concentration of genomic DNA as 2.32 ng/µl or 10^{-3} dilutions upto which the virus can be detected from the infected samples. Using the same primer set Reddy *et al.* (2015) detected the *Mungbean yellow mosaic India virus* (MYMIV) in the yellow mosaic affected black gram samples from Andhra Pradesh which also resulted in 900 bp gene product but no amplification was obtained with degenerate primers tested for MYMV.

Maheshwari *et al.* (2014) amplified coat protein gene of YMV using YMV-CP gene specific primers (YFP1/YRP1, YFP2/YRP2) from the DNA isolated from YMV infected black gram, cowpea and green gram samples from different locations of Tamil Nadu. An expected amplification of YMV CP gene of size 704 bp and 648 bp was observed with YFP1/YRP1 and YFP2/YRP2 primers respectively. Sequence analysis of these amplicons revealed highest similarity with MYMV-Tamil Nadu isolates but the YMV infected sample of horse gram was found to be similar with HYMV-Tamil Nadu isolate.

Akram *et al.* (2015) carried out PCR in yellow mosaic disease affected dolichos plant samples, using primer pairs to amplify ~900 bp fragment covering coat protein region of DNA-A of four viruses (MYMIV, MYMV, HgYMV and DoYMV) and degenerate primers (DgIMHBC1-F/ DgIMHBC1-R) to amplify BC 1 gene of DNA B component. They obtained expected size of amplicon only with primers (DoYMVCP-F/DoYMVCP-R) specific to DoYMV DNA-A, but not with other primers. They obtained full length genome of DoYMV and confirmed the presence of

DNA B component with rolling circle amplification and restriction analysis which revealed DNA fragments of ~2.7 kb.

Hajong and Naveenkumar (2016) detected the presence of *Mungbean yellow mosaic virus* which causes yellow mosaic disease of soybean in the Vidharba region of Maharashtra. The viral DNA fragment was amplified to an amplicon size of 1000 bp with the coat protein gene specific primer (NABF and NABR).

Ramesh *et al.* (2016) characterized the viruses causing yellow mosaic disease in soybean in India. They collected yellow mosaic affected soybean samples from different regions of India and amplified coat protein region of two *Begomovirus* species (MYMV and MYMIV) using specific primers, RUGEMF1/ RUGEMR1, MYMV F/MYMV R and MYMIVF/MYMIVR, which amplified 447, 391 and 391bp from CP region of DNA A, respectively. They reported that samples from northern and central India showed infection due to MYMIV and samples obtained from southern and western India were infected with MYMV.

Bhagyashree *et al.* (2017) characterized the *Begomovirus* causing lima bean yellow mosaic disease in Karnataka by sequencing the CP and MP genes amplified through PCR with HYMV specific primers (HYMV CP-F/ HYMV CP-R and HYMV MP-F/ HYMV MP-R) which resulted in PCR products of ~1000 bp and ~900 bp corresponding to coat protein and movement protein genes of the virus respectively. The GKVK virus isolate showed 98 per cent sequence homology with the HYMV strain and this report was contrary to the earlier reports that lima bean is infected by *Mungbean yellow mosaic India virus* (MYMIV) (Shahid *et al.*, 2012).

2.7.3.3 *Rollong circle amplification (RCA)*

More recently, rolling circle amplification is becoming the most versatile tool for the amplification of ss DNA viruses from ultra low concentration of the viruses. Different from PCR, RCA is an isothermal nucleic acid amplification technique (Haible *et al.*, 2006 and Ali *et al.*, 2014). RCA is better, easier and cheaper method

than the polymerase chain reaction. RCA exponentially amplifies ss or ds circular DNA templates using φ29 DNA polymerase (Lizardi *et al.* 1998).

Satya *et al.* (2013) amplified an expected size of 2.7 kb DNA A component of MYMV from the yellow mosaic disease affected mung bean and urd bean samples through RCA where amplification with PCR failed because of extremely low virus concentration. The RCA products were subjected to restriction digestion with different endonucleases like *Hind*III, *Bam*HI, *Pst*I and *Xba*I and the restriction products from *Hind*III and *Bam*HI were purified and cloned into pUC18 vector and sequenced.

Akram *et al.* (2015) detected the presence of 2.7 kb DNA B component of DoYMV from yellow mosaic disease affected dolichos leaf samples (Do1 and Do2) through restriction digestion of the RCA products with *Drd*1, *Eco*RV and *Hin*dIII endonucleases. The DNA fragment from one dolichos sample successfully cloned into pJET/1.2 blunt vector and was sequenced.

Ramesh *et al.* (2017a) detected the presence of 2.7 kb DNA A component *of Mungbean yellow mosaic India virus* from the yellow mosaic diseased soybean leaves through RCA of viral genome and selected endonucleases like *Bam*HI, *Eco*RI, *Hin*dIII and *Pst*I were used for the restriction digestion analysis of the RCA derived DNA and obtained 2.7 kb fragments, which were cloned and sequenced.

2. 8 MANAGEMENT OF YELLOW MOSAIC DISEASE

Lot of efforts were under taken by researchers all over the world for developing strategies for the management of MYMV. There are a number of reports on cultural control, vector control by chemicals, breeding of resistant cultivars, resistant transgenic plants and application of antiviral principles for the management of the MYMV in different crop species.

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2.8.1 Cultural control

Cultural control methods are always safe and eco-friendly approach for the control of viruses by reducing its incidence by altering sowing time and spacing in combination with other methods.

Pramanik and Ali (2001) investigated the effects of plant to plant spacing and sowing dates in winter mung bean and observed that late sown mung bean at 25th September showed reduced disease incidence, increased growth and yield than early sown mung bean at 15th September. In case of plant to plant spacing, yellow mosaic was lower in plots with 30 × 5 cm spacing with disease incidence and severity of 63.93 per cent and 3.7 per cent, respectively than in 25 × 5 cm spacing with 68.91 per cent and 4.02 per cent disease incidence and severity, respectively. They also reported that single or combined application of boron (2 kg/ha) and molybdenum (1.5 kg/ha) reduced the disease severity to 4.48 and 3.96 per cent, respectively compared to 5.79 per cent in control.

Islam and Faruq (2009) tested various types of mulch sheets, different insect traps and reflective tapes for the management of yellow mosaic disease in mung bean. Among the mulches, yellow polythene mulch was more effective and showed only 8.0 per cent disease incidence and 6.91 per cent severity and in case of traps, yellow pot and cloth traps were more effective and showed 8-9 per cent disease incidence and 7-8 per cent severity compared to 24% incidence and 9.69% severity in control plots at 50 DAS. Similar findings were obtained later by Hossain *et al.* (2010) using yellow trap and reflective tape for the management of yellow mosaic disease in mung bean.

Swathi and Gaur (2017) reported the effect of combination of maize, sorghum and bajra as border crops in soybean fields with seed treatments and foliar spray of insecticides at 45-50 DAS. The maize combination with soybean recorded 1.02 mean whitefly population, 14.63 per cent YMV incidence and highest grain yield of 19.42

q/ha compared to 1.42 whitefly population, 21.54 per cent incidence and 17.75 q/ha yield in the plots without barrier crop. The same results were already observed by Raghupathi and Sabitha (1994) that maize and bajra planted as border crops separately in soybean field reduced disease incidence as 9.88 and 9.81 per cent, respectively compared to control plots (17.4% and 16.8%) without barriers and a whitefly population of 2.36 and 2.46 per plant, respectively compared to 8.62 in control plots. But as a contrary to this, Salam *et al.* (2009) reported that maize as border crop with mung bean against MYMV was not effective and recorded a high whitefly population of 6.20/plant and a high disease incidence of 44.51 per cent.

2.8.2 Resistant cultivars

Extensive use of pesticides is very harmful to ecological balance. So breeding for resistance to MYMV disease has been known to virologists as a key scheme for the control of MYMV. Breeding for resistant cultivars have started since 1970s. The use of virus resistant genotypes has become an imperative measure for an effective disease control but little success is achieved in this aspect. The highly evolving nature of begomoviruses helps them breaking down the resistance of varieties in a short period of time.

Shukla and Pandya (1985) reported that two recessive genes are involved in controlling the resistance mechanism in mung bean, whereas susceptibility was controlled by single recessive gene and hence susceptibility was dominant over resistance.

Iqbal *et al.* (2011) screened 100 genotypes or lines of mung bean germplasm against MYMV under field conditions on the basis of an arbitrary scoring scale and categorized into six different classes. None of the genotypes was highly resistant against MYMV whereas four genotypes 014043, 014133, 014249 and 014250 were resistant to the disease with 1-10 per cent MYMV infection and 8 genotypes were moderately resistant while 30 were susceptible and 43 genotypes were highly

susceptible. Similarly, nine lines showed resistance in field conditions against MYMV (Awasthi and Shyam, 2008).

500 soybean germplasm lines were collected from all over the world and screened for disease reaction at YMV hotspot consecutively for 3 years (2007-2009) and found that 96 genotypes showed stable disease reaction over the years comprising 48 each of resistant and susceptible genotypes. Among the resistant ones there were 21 genotypes like UPSM534, DS9712, DS9814, PK1169 *etc.* which exhibited complete resistance with '0' score (Kumar *et al.*, 2014a).

Equbal *et al.* (2015) conducted field screenings for four consecutive seasons during summer and *kharif*, 2013 and 2014 identifying resistance or tolerance in urd bean germplasm against MYMV and found that out of 100 test entries, only 9 genotypes (IPU 10-23, KUG 586, Mash-338, NDU 12-300, PU 09-35, UH 07-06, Uttara, VBG 10-008 and VBN 6) were highly resistant and 4 genotypes (Kopergaon, RUG-44, VBG 09-005 and NDU 11-201) showed resistance consistently in both seasons over two years. Majority of them were susceptible and highly susceptible to MYMV in any one of the season.

An attempt was made by Akhtar *et al.* (2016) during two crop seasons at three locations to identify resistant sources of green gram and black gram against YMD and out of 12 genotypes of green gram tested only two genotypes *viz.*, Megha and ML 1477 showed high resistance whereas out of 8 genotypes of black gram, BS 23-13 recorded high resistance during all the seasons.

Gopi *et al.* (2016) carried out an experiment to identify genetic sources of resistance to YMV in black gram and out of 49 germplasm lines, two lines PU-31 and PU-35 were found resistant with 1-2 disease rating scale whereas 6 genotypes, PU-13, PU-10, T-9, T-91, NRILBG-20 and PU-30 were moderately resistant.

Reang *et al.* (2017) undertook a field screening during *kharif* season for two years with 8 varieties of mung bean to ascertain its resistance against YMD but no

lines showed complete resistance during both years. Least incidence was recorded in Sukumar (9.12%) with a yield of 1.80 kg/plot which was on par with Megha having 9.39 per cent disease incidence and 1.71 kg/plot yield.

In order to find out a suitable resistant mung bean cultivar against MYMV an experiment with 9 cultivars were carried out during *kharif* and found that IPM-2-3 cultivar from Indian Institute of Pulses Research (ICAR - IIPR), Kanpur was the most resistant cultivar with lowest disease incidence of 6.13 per cent and highest yield of 701 kg/ha followed by PDM-139 (Meti *et al.*, 2017).

2.8.3 Chemical control

Vector management is one of the most widely practiced methods for the management of virus diseases. Since MYMV is transmitted by whiteflies, chemicals can be sprayed for the control of the whitefly population which in turn can reduce the disease incidence. Several rounds of spraying are needed to manage the vector.

It was reported that three sprays of anthio (0.2%) (Chenulu *et al.*, 1979), three sprays of monocrotophos (0.25 kg a.i/ha) from 15 DAS at 10 days interval and a combination of aureofungin (0.003%) and phosphamidon 0.25 kg a.i/ha (Ahmed and Gane, 1982) were effective for the management of whitefly population.

Ghosh *et al.* (2009) noted that two sprays of dimethoate (2ml/l) or imidacloprid (confidor @ 0.25 ml/l) or thiomethoxam (actara @ 0.25 ml/l) or azadirachtin (econeem @ 1.5 ml/l) or monocrotophos (1.5 ml/l) or chlorpyriphos (2.5 ml/l) at 38 and 53 DAS were effective for the reduction of whitefly population and spread of mung bean yellow mosaic disease during pre and post *kharif* seasons. They also found that imidacloprid and thiomethoxam have a potentiality to replace monocrotophos which is facing restrictions for use in agriculture.

Gupta and Pathak (2009) conducted an experiment to test efficacy of neem products and insecticides during three consecutive *kharif* seasons on black gram plants against whitefly population and YMV and found that an admixture of NSKE

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(in cow's urine) (3%) + dimethoate 0.03 per cent showed a reduced whitefly population of 8.6 and 2.3 per cent YMV infected plants compared to untreated control with whitefly population of 20.5 and 5 per cent YMV infected plants. NSKE (in cow's urine) (3%) + methyl demeton (0.03%), neem oil (0.5%) + dimethoate (0.03%) and cow's butter milk (4%) also showed effectiveness against yellow mosaic disease.

Lal and Jat (2015) reported that the combination of seed treatment with imidacloprid (5 ml/kg) and 0.04 per cent spray of triazophos drastically reduced the YMV percentage to 2.2 and yield increased to 18.03 q/ha compared to 5.5 per cent YMV and 11.16 q/ha yield in control on mung bean in *kharif* season 2010-11 but in 2011-12 the combination was not that much effective but the spray of NSKE (5%) showed long time effect against whitefly compared to control.

Akram *et al.* (2016) investigated on seed treatment, spraying and combination of both with different insecticides on mung bean for reducing YMD and increasing the grain yield and observed that none of them were effective in reducing YMD incidence significantly but some treatments like foliar spray of the insecticide Nurelle D 505 (50% chlorpyriphos and 5% cypermethrin) @ 0.1 per cent at 15 and 45 DAS, seed treatment with imidacloprid 17.8 SL @ 5 ml/kg seeds and seed treatment with *Trichoderma* @ 6g/kg were effective in increasing grain yield and a combination of seed treatment with imidacloprid and 2 sprays with Nurelle showed a highest average grain yield of 710.3 kg/ha compared to 437.7 kg/ha in control.

Sharma *et al.* (2016) found that thiomethoxam 25 WG significantly reduced mung bean yellow mosaic disease incidence (7.43%) and increased yield (13.13 q/ha) than other insecticides and was on par with imidacloprid compared to 29.78 per cent incidence and 4.57 q/ha yield in control. Contact insecticides *viz.*, malathion and chlorpyriphos showed least effectiveness against mung bean yellow mosaic disease incidence. The same results were observed by earlier workers also, that imidacloprid minimized intensity of YMD on urd bean crop (Zhang *et al.*, 2004) and reduced

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whitefly population on mung bean (Kumar *et al.*, 2006). Gopalaswamy *et al.* (2012) reported that YMV on black gram can be controlled with various neonicotinoides through the control of whitefly.

Combination of seed treatment with imidacloprid @ 5 ml/kg seed followed by two sprays of imidacloprid (0.5 ml/1) showed highest yield in mung bean, 1017.50 kg/ha followed by two sprays of imidacloprid @ 0.5 ml/l alone, seed treatment with imidacloprid followed by two sprays of neemazal (3ml/l) and seed treatment with imidacloprid followed by 2 sprays of thiomethoxam. While imidacloprid seed treatment followed by triazophos (sprays) and seed treatment with imidacloprid alone showed reduced yield than other combinations (Jayappa *et al.* 2017). This result was supportive to the findings of Lal and Jat (2015) who reported seed treatment with imidacloprid alone was not effective against YMV infection which showed highest infection. But this result differed from the findings of Khan *et al.* (2012) who reported that imidacloprid alone is most effective to control mung bean yellow mosaic disease.

2.8.4 Antiviral principles (AVPs)

The labour intensive nature of cultural methods, frequent breakdown of resistance and the restricted usage of insecticides and pesticides due to cost and adverse environmental hazards make the management of YMD difficult. AVPs offer an ecofriendly approach for the management of viral diseases. AVPs are naturally occuring plant products with antiviral properties and have been reported to reduce the incidence of a wide range of viruses in different crop species under field conditions. They have been identified from several plants, isolated and characterized and found effective against various viruses. This can be very well utilized for the management of yellow mosaic disease.

Antiviral principles from coconut, sorghum and finger millet are effective against *Tomato spotted wilt virus* (TSWV) (Narayanaswamy and Ramiah, 1983c) and

leaf extracts of *Mirabilis jalapa, Cocos nucifera* and *Sorghum vulgare* reduced the transmission of *Rice tungro virus* and increased the incubation period of the virus (Srinivasulu and Jeyarajan, 1986).

Verma and Singh (1994) reported the virus inhibitory activity of leaf extract of *Clerodendrum aculeatum* as spray together with soil amendment as dry leaf powder or leaf extract against MYMV infection in mung bean.

Surendran *et al.* (1999) observed the antiviral activity of pre-inoculation sprays using 10 per cent leaf extract or oil formulation of *Azadirachta indica* against brinjal mosaic virus under field conditions through whitefly population control.

Leaf extracts from ten different plant species were reported to be effective against *Pumpkin yellow vein mosaic virus* infection in pumpkin in varying degrees. Among these, *Bougainvillea spectabilis* showed maximum reduction (93.3%) followed by *Boerhaavia diffusa*, *Caoton honplandianum*, *Azadirachta indica*, *Prosopis chilensis*, *Oscimum basilium* and *Mirabilis jalapa* (68.3%). Animal products like buttermilk also reduced virus infection by 80 per cent over the control (Jayashree *et al.*, 1999). The goat milk, buffalo milk and cow milk were reported to be effective in suppressing urd bean yellow mosaic virus under glasshouse condition (Singh *et al.*, 1985).

Venkatesan *et al.* (2010) reported that *Pseudomonas fluorescens* treated black gram showed only 39 per cent MYMV incidence and an increased grain yield was obtained compared to control and 10 per cent foliar spray of *Mirabilis jalapa* and *Datura metel* leaf extract at 15 days intervals following sowing significantly reduced the MYMV incidence to 40.56 per cent and 62.53 per cent, respectively and increased activity of peroxidase (PO), polyphenol oxidase (PPO), phenyl alanine ammonia lyase (PAL) and phenolic content was also observed.

Murphy *et al.* (2000) explained the efficacy of biocontrol agent *Pseudomonas fluorescens* against *Cucumber mosaic virus* and *Tomato mottle virus* under field

conditions by inducing systemic resistance. Kandan *et al.* (2002) reported that *Pseudomonas fluorescens* induced resistance activates multiple potential defense mechanism that induces increased activity of PO, PPO, PAL and phenol which provides resistance to TSWV in tomato.

Karethikeyan *et al.* (2009) reported that among different non host plant extracts, 10 per cent *Mirabilis jalapa*, *Bougainvillea spectabilis* and *Prosopis chilensis* leaf extracts were most effective in reducing the *Urdbean leaf crinkle virus* (ULCV) irrespective of time of application on urd bean plants and also found that these AVPs induce defense mechanism in plants by accumulating defense related enzymes.

It was recorded that leaf extract of *Clerodendrum aculeatum* (5% spray) followed by root extract of *Boerhaavia diffusa* and leaf extract of *Azadirachta indica* were effective against mung bean yellow mosaic disease and resulted a reduction in disease incidence to 66.70, 60.27 and 42.43 per cent in mung bean and 63.65, 58.20 and 42.92 per cent in urd bean, respectively (Singh and Awasthi, 2009). Further, plant extracts induced a significant increase in the plant growth like plant height, nodules, pod and seed yield compared with control.

An experiment was conducted for checking the effectiveness of coconut and sorghum leaf extracts against *Groundnut bud necrosis virus* in cowpea plants and found that application of leaf extracts 48 hrs after virus inoculation showed less number of local lesion formation than virus inoculated 24 hrs after spraying of leaf extracts (Manjunatha *et al.*, 2010).

Six sprays of *Clerodendrum aculeatum* showed 53.76 and 48.22 per cent reduction in mung bean yellow mosaic disease control in mung bean and urd bean, respectively (Singh *et al.*, 2011). Whereas, six sprays of *Boerhaavia diffusa* showed 42.48 and 40.55 per cent disease control and six sprays of *Azadirachta indica* showed

33.07 and 28.55 per cent disease control in mung bean and urd bean, respectively under field conditions.

An improved management strategy was obtained for viral disease control in watermelon through coupling of seed treatment and foliar application of different AVPs from different plants at fortnightly intervals. Coupling of seed treatment and foliar spray (4-6 days after germination) of root extracts of *Boerhaavia diffusa* showed 37.68 per cent disease incidence over the control (82.35%) whereas *Clerodendrum aculeatum* (leaf), *Azadirachta indica* (leaf) and *Terminalia arjuna* (bark) extract treated plants showed 44.77, 39.46 and 53.70 per cent disease incidence, respectively. Almost doubling of the vine length, no. of fruits, fruit diameter and fruit yield was also observed compared to control (Sharma *et al.*, 2017).

Materials and Methods

3. MATERIALS AND METHODS

The experiments pertaining to the present investigation on "Characterization and management of yellow mosaic disease in black gram (*Vigna mungo* (L.) Hepper)'' was conducted during 2016-2018. The laboratory experiments were carried out at the molecular laboratory, Plant Pathology division, RARS, KAU, Pattambi, Palakkad. The field experiment was also laid out in the field of RARS. The materials used and methods followed during the present investigation is described in detail in this chapter.

3.1 SURVEY

A purposive sampling survey was carried out during the *rabi* and summer seasons at different pulse growing areas of Palakkad and Malappuram districts of Kerala. Black gram leaves showing different types of yellow mosaic symptoms were collected freshly in polythene bags and stored in deep freezer at -20°C for molecular analysis. YMV infected green gram, cowpea and red gram samples were also collected from nearby survey fields. First survey was conducted during March 2017 in two fields at Othaloor and Koodallur of Palakkad district. Second survey was carried out during December 2017, *rabi* season in two black gram fields at Vadavannur as well as Karippode of the same district. Later samples were collected during March 2018 from Sankaramangalam of Palakkad district and from Pulamanthole, Amarambalam and Chokkad of Malappuram district. Simultaneously samples were also collected from the research fields of RARS, Pattambi, Palakkad. Disease incidence (%), different weed hosts observed in the fields surveyed and different symptoms observed in the fields on black gram plants were recorded during the survey. The disease incidence (%) was assessed by recording the number of plants infected out of total plants examined by the following formula.

$$\text{Disease Incidence (DI)} = \left\{ \frac{\text{Number of plants infected}}{\text{Total number of plants examined}} \right\} \times 100$$

3.1.1 Symptomatology of the disease

Different symptoms observed on the infected plants in the survey fields were recorded.

3.2 MAINTENANCE OF VECTOR POPULATION AND ESTABLISHMENT OF THE VIRUS CULTURE

Virus culture was established in black gram plants using whiteflies since yellow mosaic viruses are reported to be transmitted by whiteflies. Healthy whiteflies maintained on brinjal plants were used for transmission of the virus.

3.2.1 Raising healthy brinjal seedlings

Healthy brinjal seeds were sown in protrays filled with potting mixture. Ten days old seedlings were transplanted into plastic pots of size 27 cm diameter and 22 cm depth filled with potting mixture and the pots were kept inside insect proof cages (51×51×90 cm) designed with insect proof net on three sides, glass top and front door and a fibre bottom (Plate 3.1a). Such insect proof cages were used for maintenance of healthy whitefly culture. Brinjal plants were replaced periodically with healthy seedlings.

3.2.2 Maintenance of vector population

Egg masses of whiteflies were collected from the field and the adults emerged were allowed to feed on healthy brinjal plants kept inside the insect proof cages (Plate 3.1b). The second generation whiteflies released after 30 days were transferred to new healthy brinjal seedlings. Subsequent generations were used for transmission studies. Every month, whiteflies were transferred to fresh brinjal seedlings to maintain the culture.

3.2.3 Source of virus inoculum

Infected black gram plants collected during survey from Vadavannur and Sankaramangalam of Palakkad district, served as the initial virus inocula (Plate 3.1c). The virus culture was established in the net house by vector transmission to healthy black gram plants and these plants served as virus inoculum for host range studies (Plate 3.1d).

3.2.4 Maintenance of YMV culture on black gram plants

Healthy whiteflies were collected from the insect proof rearing cage using an aspirator made up of a glass tube (16 cm length and 1cm diameter) connected to a plastic tube of 50 cm length (Plate 3.2a) and released onto the virus inoculum kept inside the acquisition cage (Plate 3.2b) for acquiring virus for 48 hrs. After acquisition access period, the whiteflies were collected from the inoculum source and released on to 10 days old healthy black gram seedlings raised in polythene bags (12×12 inch size) and kept inside the inoculation cage (Plate 3.2c) for 48 hrs. Then the inoculated black gram seedlings were kept inside insect proof cages for expression of yellow mosaic symptoms.

3.3 DETECTION OF THE ETIOLOGICAL AGENT

Electron microscopic studies were conducted to check the presence of virus particles. This was followed by molecular detection by Polymerase Chain Reaction (PCR). The molecular works were conducted at the molecular biology laboratory, Plant Pathology division, RARS, Pattambi.

3.3.1 Electron microscopy (EM)

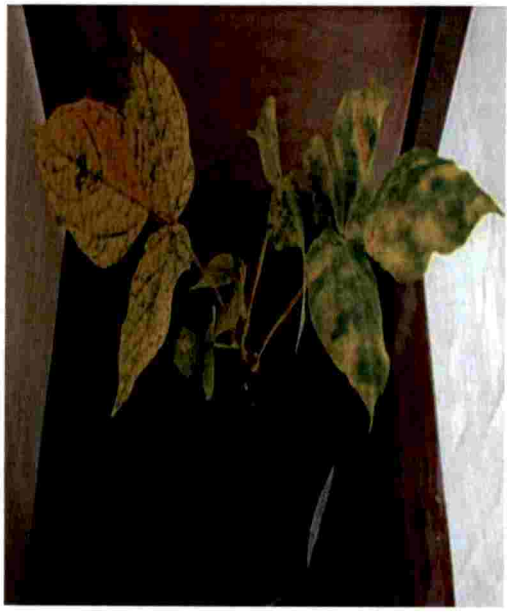
Electron microscopic studies were conducted to study the morphological characters of the virus particles. Yellow mosaic virus infected black gram leaves were collected from two different fields, one from Sankaramangalam survey field and other from the Pattambi research field and send to Advanced Centre of Plant

Plate 3.1 Maintenance of virus culture

a. Cage for rearing whiteflies

b. Maintenance of healthy whiteflies on brinjal plants

c. Yellow mosaic virus inoculum



d. Maintenance of YMV on black gram (Co6) seedlings under greenhouse condition

Virology (ACPV), Division of Plant Pathology, Indian Council of Agricultural Research - Indian Agricultural Research Institute (ICAR - IARI), New Delhi. Leaf dip preparations of the sample adsorbed on carbon coated copper grids were negatively stained using 2 per cent aqueous uranyl acetate. The grids were further examined under the electron microscope (JEOL 100 CX-11) at ACPV, IARI).

3.3.2 Molecular detection

3.3.2.1 *Isolation of total genomic DNA*

Total nucleic acid was extracted from the leaves of infected black gram plants collected during survey and stored in deep freezer following modified CTAB method (Murray and Thompson, 1980) and Gem CTAB DNA extraction protocol (Rouhibaksh *et al.*, 2008) with slight modifications. The composition of DNA extraction buffer used in two different methods is mentioned in Table 3.1.

Table 3.1 Composition of DNA extraction buffer

Sl No.	Reagents	Concentration	
		Modified CTAB method	**Gem CTAB method**
1	Tris base (pH 8.0)	100 mM	100 mM
2	NaCl	1.4 M	2 M
3	EDTA (pH 8.0)	20 mM	10 mM
4	CTAB	2% (W/V)	2% (W/V)
5	β-Mercaptoethanol	0.1%	5%
6	Polyvinylpyrrolidone	1%	1%

Other reagents used

a. Chloroform:Isoamyl alcohol (24:1 v/v)
b. Chilled isopropanol (0.8 V)
c. 70 per cent ethyl alcohol (wash buffer)
d. Sterile distilled water (DNA pellet storage)

The reagents used in DNA extraction buffer, Tris base, NaCl and EDTA stock solutions were prepared in distilled water and autoclaved. The stock solution of CTAB was prepared in sterile distilled water.

Protocol

1. 100 mg (0.1g) of the fresh leaf tissue or leaf tissue stored at -20°C was ground in liquid nitrogen in autoclaved pre-chilled mortar and pestle. Ground samples were transferred into autoclaved 2 ml micro centrifuge tubes.
2. 1 ml of pre-warmed (65°C) extraction buffer was added into ground samples, mixed well by inverting the tubes.
3. The tubes were incubated at 65°C for 1 hr in a hot water bath. The contents were mixed 3-4 times by gentle inversion.
4. After incubation, contents in the tubes were centrifuged at 10000 rpm for 10 minutes at 4°C.
5. The supernatant was transferred into autoclaved, 1.5 ml microcentrifuge tubes and simultaneously added equal volume of freshly prepared Chloroform:Isoamyl alcohol (24:1 v/v). Tubes were incubated at room temperature for 30 minutes with occasional mixing by inversion of the tubes.
6. Tubes were centrifuged at 10000 rpm for 10 minutes at 4°C after incubation.
7. Supernatant was transferred into fresh 1.5 ml microcentrifuge tubes and 0.8 volume of ice cold isopropanol was added into the tubes, mixed well and incubated overnight at 4°C.
8. The total nucleic acid was precipitated by centrifugation at 12000 rpm for 15 minutes at 4°C.
9. Gently poured off the supernatant and saved the pellet in the tube. The pellets were washed twice with 70 per cent ethyl alcohol and centrifuged at 10000 rpm for 10 minutes at 4°C.
10. Alcohol was poured off and pellets were air dried at room temperature.
11. Pellets were dissolved in 50µl sterile distilled water and stored at -20°C.

3.3.2.1.1 *Agarose gel electrophoresis*

Agarose gel electrophoresis was carried out in GeNei™ electrophoresis unit to check the quality of genomic DNA. The details of different chemicals used for Agarose gel electrophoresis are given in Table 3.2

Table 3.2 Reagents used for agarose gel electrophoresis

Sl. No.	Reagents	Concentration
1	Low EEO agarose for molecular biology	0.8% for genomic DNA 1.5% for PCR product
2	TBE buffer	10X
	a. Tris base – 108 g	
	a. Boric acid – 55 g	
	b. 20 mM EDTA – 100 ml	
	c. Sterile distilled water – 1L	
3	Ethidium bromide	0.5 µg / ml
4	Orange loading dye	6X
5	Molecular marker	
	a. 100 bp ladder	50 µg (0.1 µg/ µL)
	b. 1 kb ladder	50 µg (0.1 µg/ µL)

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Protocol

1. Gel casting tray was cleaned and placed on a horizontal surface and comb was placed in properly.
2. Prepared 0.8 per cent (0.8 g in 100 ml) agarose by dissolving 0.96g agarose in 120 ml 1X TBE buffer in a 500 ml conical flask by heating in a microwave oven till the agarose was fully dissolved and becomes a clear solution.
3. Solution was allowed to cool to 40-45°C and 2µl ethidium bromide was added into the conical flask and mixed well.
4. Prepared gel solution was poured into gel casting tray and allowed to solidify for 40-45 minutes.
5. After the gel got solidified, comb was removed gently without disturbing the wells and the gel casting tray was kept inside the electrophoresis unit containing 300 ml 1X TBE gel running buffer.
6. DNA samples were prepared for loading by mixing 1µl DNA loading dye and 2 µl sterile distilled water to 3 µl of DNA sample and the total 6 µl volume was loaded into each well carefully. In one lane, 1 kb molecular ladder was loaded.
7. The samples were resolved by performing electrophoresis at a constant voltage of 70 V for 2-3 hours.

3.3.2.1.2 *Documentation of gel*

Total DNA as well as amplicons were viewed under UV light and documented using mediccare GELSTAN 4x Advanced documentation system at Division of Plant Breeding, RARS, Pattambi, Palakkad.

3.3.2.1.3 *Quantification and purity analysis of genomic DNA*

Quantification and purity analysis of the isolated DNA samples were carried out using Bio-Photometer plus. The nucleic acid shows maximum absorption of light at a wavelength of 260 nm. Hence this wavelength is used for quantification of DNA.

Protein shows maximum absorption at 280 nm. OD260/OD280 ratio indicates the purity of DNA. The ratio of 1.8 to 2 indicates that the preparation of DNA is pure whereas the value below 1.8 indicates protein contamination and above 2.0 shows RNA contamination in the DNA sample.

Procedure for setting the device

1. Device was switched on and it was immediately ready for the measurement.

2. Using the cursor keys selected the desired method of measurement (ss DNA) from the device display list (ds DNA, ss DNA, RNA *etc.*) and pressed enter.

3. The cuvette (reusable) was filled with a blank solution (sterile distilled water as a reference) and inserted into the cuvette shaft by sliding blue cover back on the shaft and pressed the 'blank' key on the screen to set the blank value at zero. The instrument was then ready for sample analysis.

4. The cuvette was filled with minimum of 100µl sample solution (2µl DNA sample + 98µl sterile distilled water) and inserted into the cuvette shaft and pressed 'Sample' key on the screen.

5. The quantity of the DNA (ng/µl) and absorption ratios displayed on the screen were recorded for documentation.

3.3.2.2 *Polymerase chain reaction*

Polymerase Chain Reaction was used to detect the virus associated with yellow mosaic disease in black gram. Already reported primers were used for the PCR assay and the details of the primers are mentioned in Table 3.3. Deng primer, a universal degenerate primer for detection of begomovirus and three sets of species specific primers amplifying the coat protein gene region of yellow mosaic viruses were used. The details and composition of reagents in PCR reaction mixture is mentioned in Table 3.4

Table 3.3 Details of oligonucleotides (Primers) used in the study

Primer name	Oligonucleotides	No. of nucleotides	Expected size of amplicon	Reference
Deng A	5'- TAATATTACCKGWKGVCCSC -3'	20	550 bp	Deng *et al.* (1994)
Deng B	5'- TGGACYTTRCAWGGBCCTTCACA -3'	23		
MYMIV -CP F	5'- GTA TTT GCA KCA WGT TCA AGA -3'	21	1000 bp	Naimuddin and Akram (2010)
MYMIV -CP R	5'- AGG DGT CAT TAG CTT AGC -3'	18		
HYMV-CP-F	5'- ATG CTT GCA ATT AAG TAC TTG CA -3'	23	1050 bp	Naimuddin and Akram (2010)
HYMV-CP-R	5'- TAG GCG TCA TTA GCA TAG GCA -3'	21		
MYMV-CP-F	5' ATG GG(T/G) TCC GTT GTA TGC TTG -3'	21	1000 bp	Naimuddin and Akram (2010)
MYMV-CP-R	5'- GGC GTC ATT AGC ATA GGC AAT -3'	21		

Degeneracy codes, K=G/T, R=A/G, S=C/G, W=A/T, Y=C/T, B=C/G/T and V=A/C/G

Table 3.4 Composition of PCR reaction mixture

No	Reagents	Concentration of the stock	Volume added (µl)	Final concentration
1	PCR buffer (Mg^{2+} free)	10X	2.5 µl	1X
2	$MgCl_2$	25 mM	2.5 µl	2.5 mM
3	dNTP mix	10 mM	1 µl	0.4 mM
4	Taq DNA polymerase	3U/µl	1 µl	3 Units
5	Forward primer	100 pmol/ µl	1 µl	10 pmol
6	Reverse primer	100 pmol/ µl	1 µl	10 pmol
7	DNA	95 ng/ µl	1 µl	95 ng
8	Sterile distilled water		15 µl	
	Total		**25 µl**	

Protocol

1. Autoclaved PCR tubes of 0.2 ml volume were taken, labelled and kept on ice crystals in ice box.
2. Master mix was prepared adding all reagents except the template DNA. 24 µl of the master mix was pipetted into PCR tubes.
3. 1 µl of the DNA templates were separately added into the PCR tubes and the contents of the tubes were given a short spin.
4. The tubes were kept in the wells of the thermocycler (eppendorf Mastercycler gradient). Then the PCR was run with the YMV species specific programmes. The details of the different PCR programmes adopted are mentioned below.
5. After completion of the PCR programme, the tubes were taken out and kept at 4°C for further analysis of the PCR product.

PCR conditions used for different primers

- Universal degenerate primer (Deng primer)

1.	Initial denaturation	- 92°C for 2 minutes	
2.	Denaturation	- 92°C for 1 minutes	35 cycles
3.	Annealing	- 56.8°C for 1 minutes	
4.	Extension	- 72°C for 30 seconds	
5.	Final extension	- 72°C for 10 minutes	

- YMV species specific primers (MYMV, MYMIV & HYMV)

1.	Initial denaturation	- 94°C for 3 minutes	
2.	Denaturation	- 94°C for 30 seconds	35 cycles
3.	Annealing	- 55.3°C for 1 minutes	
4.	Extension	- 72°C for 1 minutes	
5.	Final extension	- 72°C for 10 minutes	

3.3.2.2.1 *Analysis of the PCR products by agarose gel electrophoresis*

10 µl of each amplicons were resolved in 1.5% agarose gel prepared in 1X TBE buffer containing ethidium bromide. Electrophoresis was performed at 70V for 2 hrs using 1X TBE buffer as electrophoresis buffer as described under section 3.3.2.1.1. Viral DNA was viewed under UV light in the gel documentation unit and documented.

3.4 MOLECULAR CHARACTERIZATION OF THE ETIOLOGICAL AGENT

3.4.1 Sequencing of the amplicons

Amplicons obtained for MYMV and HYMV coat protein gene specific primers were sequenced. Three representative MYMV isolates and two HYMV isolates were sequenced. The sequencing of the PCR products was done using automated sequencing facility at Agrigenome Labs Pvt. Ltd., Kakkanad, Kochi, Kerala, India.

3.4.2 *In silico* analysis

The nucleotide sequence as well as predicted amino acid sequence of the coat protein gene were analysed *in silico.*

3.4.2.1 *Analysis of nucleotide sequence*

The sequence data obtained using the forward primer was assembled with the reverse complimentary sequence of the sequence data obtained using the reverse primer, using reverse compliment bioinformatics tool. The assembled sequences were compared with viral DNA sequences available at NCBI (http://ncbi.nlm.gov./blast) Genbank sequence data base, using BLAST (Basic Local Alignment and Search Tool) available at NCBI. Comparisons were made with other YMV DNA sequences obtained from NCBI Genbank sequence data base. The sequences were aligned using MUSLE algoritham in MEGA 7.0.26 software. Dendrograms were constructed from the aligned sequences using the neighbor joining method with bootstrapping (1000 replicates) and complete deletion. Phylogenetic tree was made using 26 reported YMV sequences available in the NCBI database belonging to four major viruses affecting pulses, *viz.*, MYMV, MYMIV, HYMV and DoYMV. The Genbank accession numbers of sequences used for comparison are listed in Table 3.5.

3.4.2.2 *Analysis of predicted coat protein*

As the amplified region includes sequence coding for coat protein, the amino acid sequence was predicted using ExPASy translation tool available online. The predicted amino acid sequences were then compared with that of other yellow mosaic viruses using CLUSTALW algoritham in BioEdit version 7.0. Yellow mosaic viruses used for comparison are given in Table 3.6.

Table 3.5 List of YMV species or isolates infecting different legume hosts used for *in silico* analysis of nucleotide sequence

No.	Reference sequences	Abbreviation	Host	Accession No.
1	*Mungbean yellow mosaic virus* segment DNA A, complete sequence	MYMV[Tmil_Nadu-Bg]	Black gram	DQ400848.1
2	*Mungbean yellow mosaic virus* pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	MYMV[Nagpur-Sb]	Soybean	DQ389146.1
3	*Mungbean yellow mosaic virus* isolate Tirupati segment DNA-A, complete sequence	MYMV[Tirupati-Bg]	Black gram	KP455992.1
4	*Mungbean yellow mosaic virus*-Vigna segment A, complete sequence	MYMV[Madurai-Gg]	Green gram	AJ132575.1
5	*Mungbean yellow mosaic virus*-Soybean[Madurai] segment DNA A, complete sequence, strain Madurai	MYMV[Madurai-Sb]	Soybean	AJ421642.1
6	*Mungbean yellow mosaic virus* isolate Namakkal segment DNA-A, complete sequence	MYMV[Namakkal-Mb]	Mothbean	DQ865201.1
7	*Mungbean yellow mosaic virus* isolate Haryana segment DNA-A, complete sequence	MYMV[Haryana-Gg]	Green gram	AY271896.1
8	*Mungbean yellow mosaic virus*-[NAV] pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	MYMV[Navasari-Sb]	Soybean	DQ389144.1
9	*Mungbean yellow mosaic India virus* segment DNA A, complete sequence	MYMIV[New_Delhi-Cp]	Cowpea	DQ389153.1
10	*Mungbean yellow mosaic India virus* segment DNA A, complete sequence	MYMIV[Ludhiana-Bg]	Black gram	DQ400847.1
11	*Mungbean yellow mosaic India virus* genomic DNA, segment DNA-A, complete sequence, isolate: Jabalpur	MYMIV[Jabalpur-Bg]	Black gram	LC271790.1
12	*Mungbean yellow mosaic India virus* isolate Vizianagaram pre-coat protein (AV2) gene, partial cds; and coat protein (AV1) gene, complete cds	MYMIV[Vizianagaram-Bg]	Black gram	JN181006.1
13	*Mungbean yellow mosaic India virus* isolate, pre-coat protein (AV2) gene, partial cds; and coat protein (AV1) gene	MYMIV[Hyderabad-Bg]	Black gram	KC243784.1

No.	Reference sequences	Abbreviation	Host	Accession No.
14	*Mungbean yellow mosaic India virus* - [Akola] segment DNA-A, complete genome	MYMIV[Akola-Gg]	Green gram	AY271893.1
15	*Mungbean yellow mosaic India virus* isolate India:Varanasi:French bean:2011 segment DNA-A, complete sequence	MYMIV[Varanasi-Fb]	French bean	KC019304.1
16	*Horsegram yellow mosaic virus* isolate Chittoor segment DNA-A, partial sequence	HYMV[Chittoor-Hg]	Horse gram	KR053204.1
17	*Horsegram yellow mosaic virus* isolate India:Banglore:French bean:2011 segment DNA-A, complete sequence	HYMV[Bangalore-Fb]	French bean	KC019306.1
18	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate lima bean	HYMV[Bangalore-Lb]	Lima bean	AM932429.1
19	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate horse gram	HYMV[Bangalore-Hg]	Horsegram	AM932427.1
20	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate french bean	HYMV[Bangalore-Fb]	French bean	AM932425.1
21	*Dolichos yellow mosaic virus* isolate Dolichos-Tirupati-Andhrapradesh segment DNA-A, partial sequence	DoYMV[Tirupati-Dol]	*Dolichos lablab*	KT282129.1
22	*Dolichos yellow mosaic virus* isolate CBE2 segment DNA-A, complete sequence	DoYMV [Coimbatore-Dol]	*Dolichos lablab*	KP784662.1
23	*Dolichos yellow mosaic virus* isolate DYMV-CpKn coat protein (AV1) gene, complete cds	DoYMV[Kanpur-Cp]	Cowpea	GU591170.1
24	*Dolichos yellow mosaic virus* DNA-A segment, complete sequence, isolate Bangalore-2	DoYMV[Bangalore-Dol]	*Dolichos lablab*	AM157413.1
25	*Dolichos yellow mosaic virus* segment DNA-A, complete sequence	DoYMV [New Delhi-Dol]	*Dolichos lablab*	AY309241.1
26	*Dolichos yellow mosaic virus* complete DNA-A segment	DoYMV[Mysore-Dol]	*Dolichos lablab*	AJ968370.1

Table 3.6 List of YMV isolates used for *in silico* analysis of amino acid sequence

Sl No.	Reference amino acid sequence	Abbreviation	Host	Accession No.
1	*Mungbean yellow mosaic virus* isolate - Vamban, Tamil Nadu	MYMV-TN	Black gram	ABD67444.1
2	*Horsegram yellow mosaic virus* isolate - Bangalore	HYMV	Horse gram	CAP69631.1
3	*Dolichos yellow mosaic virus* isolate - Kanpur	DoYMV	Cowpea	ADD97723.1
4	*Mungbean yellow mosaic India virus* isolate – New Delhi	MYMIV	Cowpea	ABD60107.1

3.5 WHITEFLY TRANSMISSION AND HOST RANGE STUDIES OF YELLOW MOSAIC VIRUS OF BLACK GRAM

The yellow mosaic virus is transmitted by an insect vector, whitefly (*Bemisia tabaci* Genn.). So the insect transmission studies were conducted using pure culture of whiteflies raised in insect proof cages at the net house of Plant Pathology division, RARS, Pattambi during September 2017 to July, 2018. The accessories used for transmission is detailed below.

3.5.1 Accessories used for transmission studies

3.5.1.1 *Aspirator*

An aspirator made up of a glass tube (16 cm length and 1cm diameter) connected to a plastic tube of 50 cm length (Plate 3.2a) was used for collecting the healthy whitefly colonies from the brinjal plants which was further used for transmission studies.

3.5.1.2 *Cages for acquisition of YMV from source plants*

The cylindrical shaped plastic bottles of 20 L water carrying capacity with 26 cm diameter and 32 cm depth were collected and cut at the both ends with a soldering rod and one end was covered with white coloured muslin cloth and a hole was cut out

on the cylindrical side of the bottle 15 cm from the bottom of the bottle for releasing the whiteflies and hole was closed with cotton plug to avoid escaping of the whiteflies from the plant (Plate 3.2b).

3.5.1.3 *Cages for inoculation of YMV on test seedlings*

Inoculation cages were prepared using plastic bottles of 6.5 cm diameter and 19 cm length for test seedlings of pulse crops and 6.5 cm diameter and 10 cm length for seedlings of different weeds. The bottles were cut at both ends, one end was covered with a piece of white muslin cloth and a small hole was cut out on the cylindrical side of the bottle and closed with a cotton plug (Plate 3.2c).

3.5.2 Raising of test plants for host range studies

The yellow mosaic virus culture maintained on black gram plants were transmitted through whiteflies onto different pulse crops *viz.*, cowpea (Kanakamani), green gram (Co-8), horse gram (CRSG-19) and pigeon pea (Co-7) and to three different weed hosts which were commonly observed in the black gram fields surveyed *viz.*, *Ageratum conyzoides*, *Synedrella nodiflora* and *Cleome viscosa.* The seeds of pulse crops were collected from the Pulses Division, Regional Agricultural Research Station, Pattambi. Weed seeds were collected from the different fields of RARS, Pattambi and stored. Host range studies were conducted during February-July 2018 inside the net house of Pathology division, Regional Agricultural Research Station, Pattambi. The seeds of each pulse were sown in separate polybags (15×15 inch) (Plate 3.3 a-e) and weed seeds were sown in small polybags (10×5) filled with potting mixture and kept inside the net house (Plate 3.4 a-c).

3.5.3 Acquisition and inoculation of YMV on test plants

Healthy whiteflies were collected using an aspirator from the brinjal plants maintained in insect proof cages (Plate 3.5a) and released on to the virus culture maintained on black gram plants inside acquisition cage to acquire virus for 48 hrs, (Plate 3.5b). These viruliferous whiteflies were then inoculated on to ten numbers of

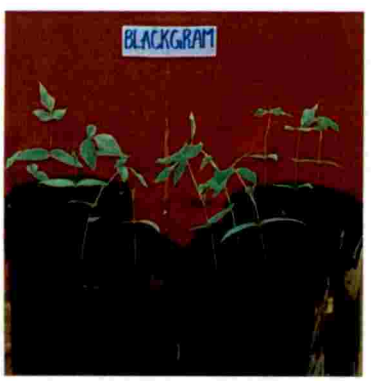
Plate 3.2 Accessories for transmission studies

a. Aspirator

b. Acquisition access cage

c. Inoculation micro-cages

Plate 3.3 Seedlings of different pulses (Ten days old)



a. Black gram

b. Cowpea

c. Green gram

d. Horse gram

e. Pigeon pea

Plate 3.4 Seedlings of different weeds (15 days old)

a. Cleome viscosa

b. Synedrella nodiflora

c. Ageratum conyzoides

Plate 3.5 Acquisition of virus from the inoculum

a. Collection of healthy whiteflies from brinjal plants

b. Acquisition of virus by whiteflies

10 days old test seedlings of each pulse crop and ten numbers of 15 days old test seedlings of different weeds kept inside the inoculation cage for 48 hrs (Plate 3.6 a & b). After inoculation access period the cages were removed and whiteflies were killed by spraying 0.03% imidacloprid on the test plants. Later the seedlings were kept inside the insect proof glasshouse and observed for the development of the symptoms (Plate 3.7a & b).

3.6 MANAGEMENT OF YELLOW MOSAIC DISEASE

3.6.1 Pot culture studies

Pot culture study was conducted during summer 2017 at Plant Pathology division, RARS, Pattambi for testing the effectiveness of different concentrated plant extracts, animal product, biocontrol agent and commercially available herbal extracts with antiviral property.

3.6.1.1 *Experiment details*

Completely randomized block design was used in the experiment with three replications for each ten treatments. Four healthy black gram seeds (Co6) were sown in each of the three 12"×12" sized polybags filled with potting mixture. Each polybag with four seedlings was considered as a single replication of each treatment.

3.6.1.2 *Treatments*

Treatments were applied as four foliar sprays at 15 days intervals starting from 15 DAS *i.e.*, 15, 30, 45 and 60 DAS. Concentrated plant extracts (T1-T4) of 10 per cent was prepared for application. The representative plant parts (leaf and root) were collected freshly, cleaned and ground in clean, pre-chilled mortar and pestle with required quantity of pre-chilled sterile distilled water and the crude extract was strained through a clean muslin cloth. The filtrate was collected in a separate clean beaker, kept on ice crystals before application and made up to the required volume using sterile distilled water while spraying. The animal product, buttermilk (T5) was applied as 10 per cent spray. Biocontrol agent, *Pseudomonas fluorescens* and

Plate 3.6 Inoculation of viruliferous whiteflies on to different seedlings

a. Pulse seedlings

b. Weed seedlings

Plate 3.7 Incubation of different seedlings in insect proof cage for symptom development

a. Pulse seedlings

b. Weed seedlings

commercial antiviral product, perfekt were applied as seed treatment as well as foliar spray. For seed treatment, *Pseudomonas fluorescens* (10g kg seed^{-1}) was sprinkled on moistened black gram seeds and mixed thoroughly just before sowing. In case of perfekt, 0.5 ml of the formulation was added to 1 L of water and seeds were dipped in it for 20-30 minutes, remaining solution was poured out, seeds were shade dried and sown immediately. A commercial antiviral product, virochek and an insecticide containing azadirachtin (1%) were applied as foliar spray. Sterile distilled water was applied in the control plots. The details of treatments applied are given below.

Treatments

T1 : Leaf extract of *Mirabilis jalapa* (10%)

T2 : Leaf extract of *Bougainvillea spectabilis* (10%)

T3 : Root extract of *Boerhaavia diffusa* (10%)

T4 : Leaf extract of *Cocos nucifera* (10%)

T5 : Buttermilk (10%)

T6 : *Pseudomonas fluorescens* - Seed treatment (10g kg seed^{-1}) and foliar spray (10g l^{-1})

T7 : Perfekt - seed treatment (1ml l^{-1}) and foliar spray (0.5ml l^{-1})

T8 : Virochek - foliar spray (2 ml l^{-1})

T9 : Neem based insecticide containing azadirachtin 10000ppm @ 2ml l^{-1}

T10 : Untreated Control (sterile distilled water)

3.6.1.3 *Observation*

Observations on different types of symptoms, disease incidence (%), vulnerability index or per cent disease severity and whitefly count were taken at 5 days after each treatment at 15 days intervals. Disease incidence was recorded by counting the number of plants infected out of total four plants in each pot. Vulnerability index was calculated by following the formulae given by Bos (1982) based on the 0-5 arbitrary scale for MYMV disease scoring given by Bashir (2005).

The formulae and scale are given below. Whitefly count was taken by visual observations on underside of the leaves of the plant.

$$\text{Disease Incidence (\%)} = \left\{ \frac{\text{Number of plants infected}}{\text{Total number of plants examined}} \right\} \times 100$$

MYMV disease scoring 0-5 arbitrary scale :-

0 = No virus symptoms seen

1= Occasional mild symptoms (1-10% infection)

2 = Moderate infections (11-20% infection)

3 = 21-30% infection

4 = Severe and wide spread symptoms (30-50% infection)

5 = Severe with likely loss in yield (More than 50% infection)

$$\text{Vulnerability Index (VI)} = \frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5)}{N_t (n_c - 1)} \times 100$$

Where, n_1 = Number of plants in disease category 1 etc.

n_t = Total number of plants

n_c = Total number of categories

3.6.2 Field study

In order to find out the effectiveness of different concentrated plant extracts, animal product, biocontrol agent and commercially available herbal extracts with antiviral property a field experiment was conducted during January-March, 2018 at Regional Agricultural Research Station (RARS), Pattambi, Palakkad. The experiment included ten treatments with three replications.

3.6.2.1 *Experiment details*

Design	: RBD
No. of treatments	: 10
No. of replications	: 3
Plot size	: 3 x 2 m^2
Variety	: Co6
No. of plants / treatment	: 160

3.6.2.2 *Preparation of land*

Land for sowing was prepared by ploughing and pulverizing the soil. Field was laid out as 30 plots of 6 m^2 size and channels of 60 cm width running between the plots for watering the field. A plant to plant spacing of 20 cm and row to row spacing of 30 cm was maintained in each plot.

3.6.2.3 *Treatments*

The details of treatments are given under section 3.6.1.2.

3.6.2.4 *Observations*

Observations on different types of symptoms, Disease Incidence (%), Vulnerability Index (VI) or per cent disease severity and whitefly count were taken 5 days after each treatment at 15 days intervals. Disease Incidence (%) was recorded by counting the number of plants infected out of total number of plants in each plot. The Vulnerability Index or per cent disease severity (PDS) was calculated as detailed in section 3.6.1.3. Observations on 15 randomly selected plants were taken for calculating vulnerability index. The whitefly count from the field was taken by visual counting of the number of whiteflies on underside of the leaves on each plant out of 5 randomly selected plants from each plot. The total yield from the each plot (g/m^2) was also recorded, after harvesting.

3.6.3 Statistical analysis

The data recorded from the pot culture and field experiment was analysed by analysis of variance for completely randomized design and randomised block design respectively using 'WASP statistical software'. The observations expressed in percentage were transformed appropriately for analysis.

Results

4. RESULTS

A study was conducted on identification, characterization and management of virus causing yellow mosaic disease (YMD) of black gram in Kerala. YMD infected samples of black gram were collected from different fields of Palakkad and Malappuram districts of Kerala. The virus involved was identified by PCR using virus specific primers. Vector transmission and host range were studied. For the management of the disease, various antiviral principles, botanicals and biocontrol agents were evaluated. The findings of different experiments conducted are described in detail below.

4.1 SURVEY

Purposive sampling surveys were conducted in black gram growing areas of Palakkad and Malappuram districts during the period, March 2017- April 2018. The surveys were conducted in six locations of Palakkad district *viz*., Othaloor, Koodallur, Vadavannur, Karippode, Sankaramangalam and Pattambi covering eight fields (Plate 4.1a). In Malappuram district, three locations *viz*., Pulamanthole, Amarambalam and chokkad covering five fields were also surveyed (Plate 4.1b). The data on various parameters like age of the crop, variety, disease incidence (%), different symptoms and different weeds observed in the field were recorded and are presented in Table 4.1 and Table 4.2.

4.1.1 Incidence of yellow mosaic disease in black gram

The incidence of yellow mosaic disease incidence in black gram varied from location to location. The disease incidence was highest in Palakkad district with an average incidence of 50.00 per cent whereas in Malappuram district, the average disease incidence was 31 per cent. The disease incidence showed an increasing pattern with the age of the crop. In Palakkad district, YMD incidence varied from 12 to 100 per cent (Table 4.1). Incidence was highest in Vadavannur - I and lowest in Othaloor. The disease incidence in Malappuram district varied from 20 to 60 per cent

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Plate 4.1a General view of the survey fields - Palakkad district

Vadavannur - Field I

Vadavannur - Field II

Karippode

Sankaramangalam

Pattambi - Field I

Plate 4.1b General view of the survey fields - Malappuram district

Pulamanthole

Amarambalam

Chokkad

(Table 4.2). Incidence was highest in the Amarambalam -I and lowest in Pulamanthole.

Local varieties, VBN 3, VBN 4, VBN 6, VBN 8 and Co6 were the varieties cultivated in the surveyed locations. Highest incidence of yellow mosaic disease (100%) was observed in a local variety. Lowest incidence of 12 per cent was noticed in variety, VBN 6. Yellow mosaic disease incidence upto 80 per cent was noticed in variety, VBN 4. Though the variety, VBN 8 was released as a yellow mosaic disease resistant in the year 2016, the disease incidence was observed in that also.

Table 4.1 Incidence of yellow mosaic disease of black gram in Palakkad district

Field No.	Location	Variety	Area	Stage of the crop (DAS)	Disease incidence (%)	Common weeds
1	Othaloor	VBN 6	1 acre	30	12	*Ageratum conyzoides* *Parthenium* sp. *Malvastrum coromandelianum* *Cleome viscosa*
2	Koodallur	VBN 6	2 acre	30	50	*Heliotropium annum* *Scoparia dulcis*
3	Vadavannur I	Local	30 cent	45	100	*Euphorbia hirta* *Synedrella nodiflora*
4	Vadavannur II	VBN 4	26 cent	45	60	*Amaranthus spinosus* *Cleome viscosa*
5	Karippode	VBN 4	1 acre	55	80	*Cleome viscosa*
6	Sankaramangalam	Local	0.5 acre	35	26	*Synedrella nodiflora* *Heliotropium annum* *Cleome viscosa*
7	Pattambi I	VBN 3	16 cent	45	44	No weeds
8	Pattambi II	Co 6	8 cent	60	28	*S. nodiflora* *Cleome viscosa* *Ageratum conyzoides*

Table 4.2 Incidence of yellow mosaic disease of black gram in Malappuram district

Sl. No.	Location	Variety	Area	Stage of the crop (DAS)	Disease incidence (%)	Common weeds
1	Pulamanthole	VBN 8	22 cent	50	20	*Cleome viscosa* *Synedrella nodiflora*
2	Amarambalam I	VBN 6	1 acre	55	60	*S. nodiflora*
3	Amarambalam II	Local	0.5 acre	30	20	*S. nodiflora*
4	Chokkad I	Local	60 cent	40	25	*S. nodiflora*
5	Chokkad II	Local	60 cent	40	30	*S. nodiflora*

4.1.2 Symptamatology

During the survey, various types of symptoms of YMD were observed in black gram (Table 4.3 & 4.4. and Plate 4.2a & b). The predominant symptom observed was yellow mosaic. Typical yellow mosaic was observed in all fields except Othaloor, Koodallur, Pulamanthole and Pattambi-II. Only a mild form of yellow mosaic was observed in these plots. The symptom appeared as yellow specks initially and later develops to yellow mosaic. Yellow mosaic with whitish discolouration was noticed in Vadavannur - II, Pattambi – I, Amarambalam– I and Amarambalam – II. Papery white leaves were observed in Vadavannur – II. Yellow mosaic with necrosis was also observed in Vadavannur – I and Amarambalam – II. Complete yellowing with interveinal bronzing was recorded in Vadavannur – II and Amarambalm – II. Reduction in size of younger leaves was also noticed in Vadavannur – II and Amarambalam –I. Distorted and elongated younger leaves were observed in Chokkad-II. The size of leaf lamina was reduced. Vein banding was observed in Amarambalam-I. Crinkling of leaves was also observed in Vadavannur-II, Karippode, Vadavannur-I, Koodallur and Othaloor. Stunting of the infected plants was also noticed in Sankaramangalam, Pattambi-II and Vadavannur-I. Stunting was more prominent only in local varieties, towards the later stages of the crop. Reduction in number and size of pods and size of seeds were also noticed.

Vamban (VBN) varieties exhibited wide variation in symptoms such as yellow mosaic pattern on leaves, reduction in size of younger leaves, total yellowing of leaves with white discolouration, crinkling and cupping of the leaves with yellowing, papery white leaves on maturity, vein banding and complete yellowing along with interveinal bronzing. Six different type of symptoms were observed on VBN 4 at Vadavannur-II followed by five symptoms in VBN 6 at Amarambalam-I. Generally stunting was not observed in Vamban varieties. In the resistant variety, VBN 8, only mild form of mosaic was observed.

Various weeds observed in and around the survey fields were *Ageratum conyzoides*, *Parthenium* sp., *Malvastrum coromandelianum*, *Cleome viscosa*, *Heliotropium annum*, *Scoparia dulcis*, *Euphorbia hirta*, *Synedrella nodiflora* and *Amaranthus spinosus* (Table 4.1 & 4.2). Among these, yellowing type symptoms were observed in *Ageratum conyzoides*, *Synedrella nodiflora* and *Cleome viscosa*. Hence these weeds were included in host range studies.

Table 4.3 Different symptoms observed in survey fields of Palakkad district

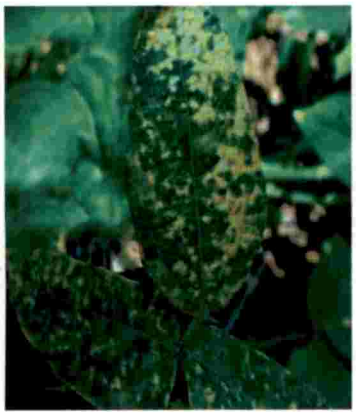
Sl. No.	Location	Variety	Symptoms observed
1	Othaloor	VBN 6	Yellow mosaic pattern on green leaves White discolouration along the veins of the leaves Curling and crinkling of the leaves Mild chlorosis along the veins
2	Koodallur	VBN 6	Yellow mosaic starting as small yellow specks Crinkling and chlorosis of leaves Total yellowing of the leaf Interveinal chlorosis with necrotic spots
3	Vadavannur I	Local	Intermingled green and yellow mosaic pattern Crinkling, puckering and yellowing of younger leaves Yellow mosaic with necrosis of leaves Interveinal chlorosis of younger leaves

Sl. No.	Location	Variety	Symptoms observed
4	Vadavannur II	VBN 4	Yellow specks on green leaves Reduced size of younger leaves Total yellow leaves with whitish discolouration Typical yellow mosaic pattern and crinkling and cupping of younger leaves Papery white leaves Complete yellowing along with interveinal bronzing
5	Karippode	VBN 4	Typical yellow mosaic pattern with crinkling Yellow mosaic with necrosis
6	Sankaramangalam	Local	Typical yellow mosaic pattern on leaves Total yellowing of leaf
7	Pattambi I	VBN 3	Yellow mosaic pattern with white discolouration Reduced size of younger leaves
8	Pattambi II	Co 6	Mild yellow mosaic symptom White discolouration on leaves Puckering of leaves

Table 4.4 Different symptoms observed in survey fields of Malappuram district

Sl. No.	Location	Variety	Symptoms observed
1	Pulamanthole	VBN 8	Yellow mosaic patches Total yellowing of the leaves starting as vein clearing
2	Amarambalam I	VBN 6	Typical yellow mosaic Reduced leaf size Yellow mosaic with whitish discolouration Yellow mosaic intermingled with white patches Vein banding
3	Amarambalam II	Local	Yellow mosaic with necrosis of the leaves Complete yellowing along with interveinal bronzing Typical yellow mosaic Yellow mosaic with whitish discolouration
4	Chokkad I	Local	Yellow mosaic with whitish discolouration Yellow specks on elongated younger leaves cupping and marginal yellowing of the leaves
5	Chokkad II	Local	Yellow mosaic with green thick veins Typical mosaic with distorted and elongated leaves

Plate 4.2a. Different symptoms observed in survey fields - Palakkad



Typical yellow mosaic

Total yellowing with white discolouration

Papery white leaves

Reduced leaf size

Cupping of leaves

Puckering

Interveinal chlorosis

Complete yellowing with interveinal bronzing

Stunting of plant

Healthy Infected Healthy Infected

Reduction in size of the seed

Reduction in size of the pod

Plate 4.2b. Different symptoms observed in survey fields - Malappuram

Yellow mosaic

White discolouration

Distorted long leaf

Reduction in leaf size

Vein banding

4.2 ESTABLISHMENT OF VIRUS CULTURE

The yellow mosaic disease is reported to be transmitted by the insect vector whitefly (*Bemisia tabaci* Genn.). So insect transmission was carried out to study the infectious nature of yellow mosaic disease in black gram.

Whiteflies were used for transmission of the virus from infected black gram plants to healthy black gram seedlings and 80 per cent transmission rate of virus was recorded on 10 days old healthy black gram seedlings with 48 hrs of AAP and IAP (Table 4.16)

The symptoms appeared on black gram plants 15-18 days after inoculation. Initially symptoms started to appear as small chlorotic specks on the newly emerging leaves of black gram plants 15 days after inoculation (DAI) with viruliferous whiteflies on 10 days old black gram seedlings (Plate 4.3a). Later yellow spots started to appear on matured leaves of the black gram plant 24 DAI (Plate 4.3b) which indicating the systemic spread of the virus within the affected plant. By 35 DAI typical yellow mosaic symptoms were developed on all the leaves (Plate 4.3c).

4.3 DETECTION OF THE VIRUS CAUSING YELLOW MOSAIC DISEASE

4.3.1 Electron microscopy

Electron microscopic examination of two black gram samples infected with yellow mosaic disease collected from Sankaramangalam and Pattambi fields in Palakkad district, revealed the presence of geminate particles of size 15-18 × 30 nm which confirmed the presence of geminivirus in the samples (Plate 4.4).

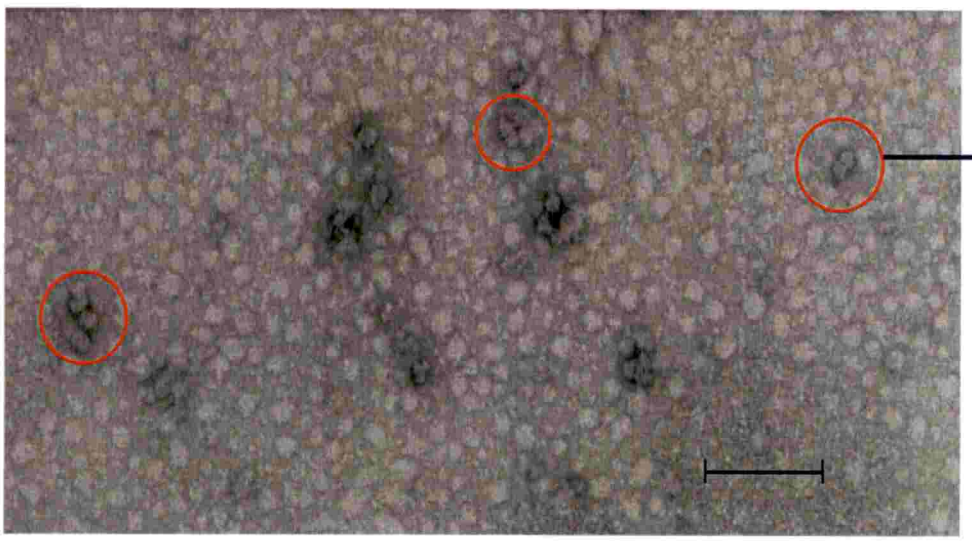
4.3.2 Molecular detection

All the samples were screened for the presence of *Begomovirus*, the whitefly transmitted geminivirus by amplification with universal degenerate primer for *Begomovirus* group (Deng primer). The virus species was identified with species specific primers for YMV.

Plate 4.3 Symptom development in black gram plants through whitefly transmission

a. 15 DAI b. 24 DAI c. 35 DAI

Plate 4.4 Electron micrograph showing geminate particles in infected black gram samples



Location : Sankaramangalam. The magnification bar represents 200nm.

4.3.2.1 *Total nucleic acid isolation from yellow mosaic infected black gram samples*

The total nucleic acid was isolated from the leaves collected during survey from apparently healthy as well as infected black gram plants following two different protocols. Good quality PCR amplifiable viral DNA was obtained by following the Gem-CTAB protocol of Rouhibaksh *et al.* (2008) with slight modifications as explained in materials and methods. The other protocol (modified CTAB method) yielded poor quality DNA which was not amplifiable. Hence the Gem-CTAB method, which was found suitable for isolation of DNA from the samples having high phenolic content, was used throughout the research programme (Plate 4.5).

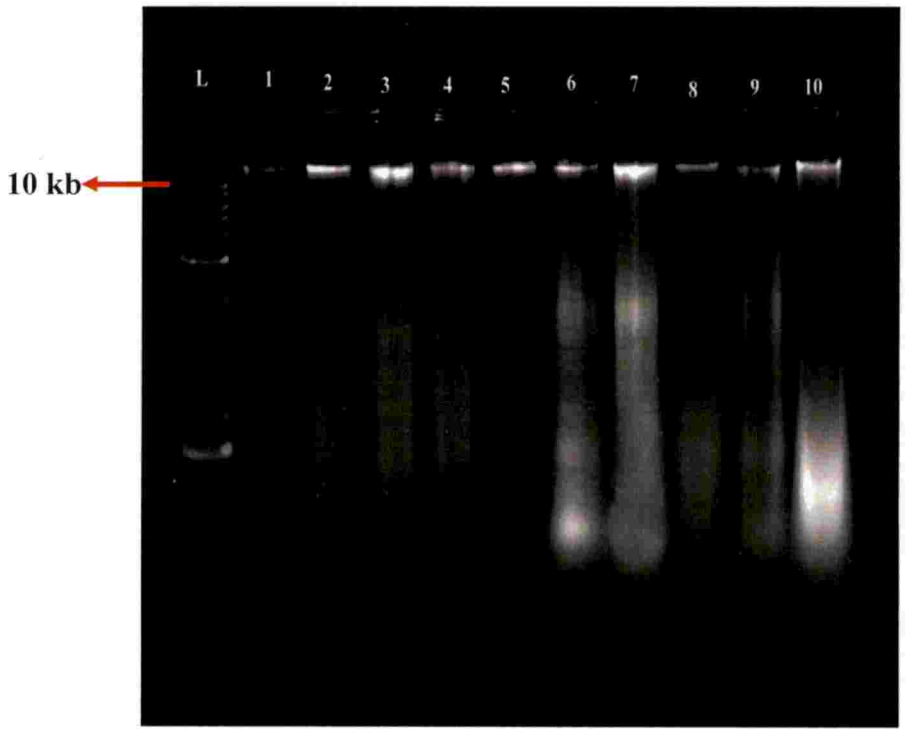
4.3.2.2 *Quantification and purity analysis of total DNA*

The quantity and purity of the total DNA isolated from the samples were estimated using Bio-Photometer plus. The concentration of the isolated total DNA from different samples ranged from 140 – 1775 ng/µl (Table 4.5 and 4.6). The OD260/OD280 ratios for different samples were in the range of 1.71 to 2.22 (Table 4.5 and Table 4.6) which indicated that the total DNA extracted is pure without much protein and RNA contaminations.

4.3.2.3 *PCR*

As the electron microscopic studies and whitefly transmission indicated the presence of *Begomovirus*, the whitefly transmitted geminivirus in the samples, PCR was performed with a universal degenerate primer specific for *Begomovirus* (Deng primer). The results of PCR with deng primer confirmed presence of *Begomovirus* in the samples. The samples were further screened for the presence of various species of yellow mosaic viruses, the begomoviruses reported from black gram, *viz.*, MYMV, MYMIV and HYMV using primers specific to the coat protein region of these viruses.

Plate 4.5 Total DNA extracted from black gram samples



Lane L – 1 kb ladder, Lane 1 – Othaloor, Lane 2 – Koodallur, Lane 3 – Vadavannur I, Lane 4 – Karippode, Lane 5 – Sangaramangalam, Lane 6 – Pattambi I, Lane 7 – Pulamanthole, Lane 8 – Amarambalam, Lane 9 – Chokkad, Lane 10 – Healthy black gram sample

Table 4.5 Quantity and purity of the total DNA extracted from infected samples collected from Palakkad district

No.	Location	Isolate name	Quantity of DNA (ng/µl)	260 nm	280 nm	OD 260/ OD 280
1	Othaloor	BgOL – 1	140	0.109	0.067	1.74
2	Koodallur	BgKL – 2	485	0.198	0.125	1.81
3	Vadavannur I	BgV I – 1	675	0.489	0.361	1.91
		BgV I – 2	215	0.093	0.049	2.01
		BgV I – 3	755	0.169	0.038	1.77
4	Vadavannur II	BgV II -1	740	0.317	0.174	1.95
		BgV II – 2	1185	0.494	0.258	1.99
		BgV II – 3	390	0.168	0.097	1.85
		BgV II – 4	1020	0.435	0.239	1.93
		BgV II – 5	640	0.266	0.137	2.02
		BgV II – 6	385	0.154	0.076	2.03
5	Karippode	BgK – 1	475	0.200	0.103	2.05
		BgK – 2	685	0.300	0.162	2.02
6	Sankaramangalam	BgS – 1	575	0.254	0.135	2.08
		BgS – 2	295	0.214	0.154	2.03
		BgS – 3	120	0.064	0.038	1.85
7	Pattambi I	BgP I – 1	395	0.166	0.091	1.92
		BgP I – 2	590	0.318	0.209	1.85
8	Pattambi II	BgP II – 1	885	0.561	0.400	1.83
		BgP II – 2	1300	0.805	0.562	1.88

Table 4.6 Quantity and purity of the total DNA extracted from infected samples collected from Malappuram district

No.	Location	Isolate name	Quantity of DNA (ng/µl)	260 nm	280 nm	OD 260/ OD 280
1	Pulamanthole	BgPM - 1	1775	0.860	0.459	1.87
		BgPM - 2	400	0.217	0.112	1.93
		BgPM - 3	1435	0.776	0.368	2.10
		BgPM - 4	1530	0.627	0.336	1.86
2	Amarambalam I	BgA I - 1	1335	0.723	0.391	1.85
		BgA I - 3	855	0.461	0.222	2.07
		BgA I - 5	120	0.064	0.038	1.85
		BgA I - 6	270	0.102	0.048	2.125
3	Amarambalam II	BgA II - 1	1345	0.727	0.367	1.98
		BgA II - 4	1105	0.598	0.286	2.09
4	Chokkad I	BgC I - 1	405	0.219	0.098	2.22
		BgC I - 2	135	0.296	0.156	1.89
		BgC I - 3	1255	0.679	0.346	1.96
5	Chokkad II	BgC II - 1	1140	0.615	0.300	2.05
		BgC II - 2	1340	0.724	0.370	1.95

4.3.2.3.1 *Standardization of annealing temperature of primers*

The annealing temperatures for four sets of primers were standardized by checking positive amplification at different temperature ranges selected based on the melting point of the primer. Annealing temperature in the range of 51 - 59°C was tested for amplification by deng, MYMV, MYMIV and HYMV primers. Deng primer amplified the viral DNA at 55.3°C and 56.8°C with a band size ranging between 500 to 550 bp (Plate 4.6a). Since the amplification at 56.8°C was better than that at

55.3°C, it was selected for further amplifications. MYMV and HYMV primers showed amplification at 55.3°C with a band size of 1000 bp (Plate 4.6b) and 1050 bp (Plate 4.6c) respectively and faint amplification was obtained at 53.6°C and 54.4°C for HYMV specific primer. Whereas, MYMIV primer did not amplify viral DNA at any temperature range tested.

4.3.2.3.2 *Standardization of DNA concentration*

The total DNA isolated was diluted to different concentrations *viz.*, 15, 35, 55, 75, 95, 115, 135, 150, 175, 200 and 250 ng/µl and tested for PCR amplification. DNA concentration between 35-155 ng/µl showed amplification of viral DNA in the case of MYMV specific primer. HYMV specific primer amplified viral DNA at DNA concentrations ranging between 55-155 ng/µl. But the best amplicons of expected size was observed at a DNA concentration, 95 ng/µl for both the primers. The size of the amplicons were 1000 bp and 1050 bp for MYMV and HYMV primer respectively (Plate 4.7a & 4.7b), whereas MYMIV primer did not amplify viral DNA at any DNA concentration tested.

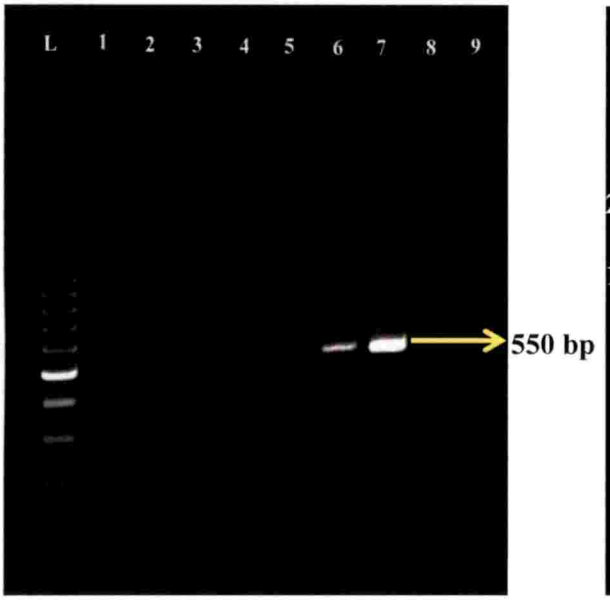
4.3.2.3.3 *Identification of the virus*

4.3.2.3.3.1 Confirmation of the presence of *Begomovirus*

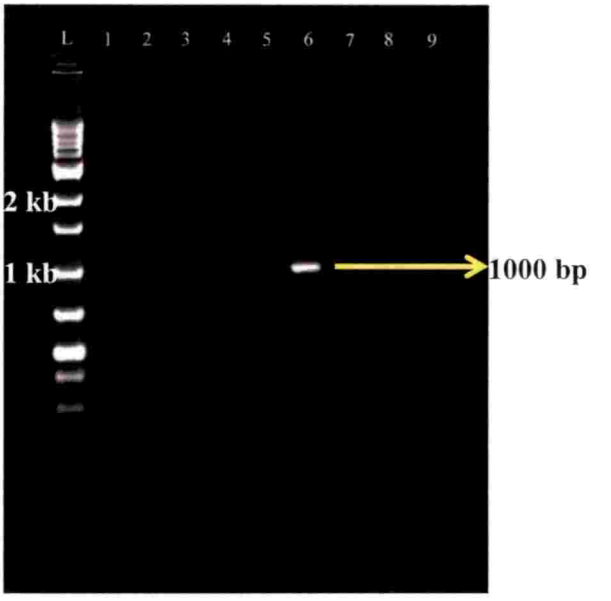
All samples collected were tested for the presence of *Begomovirus* using Deng A/Deng B primer, a universal degenerate primer specific to *Begomovirus*. *Begomovirus* was detected in 31out of 48 samples collected from Palakkad district and 12 out of 20 samples from Malappuram district (Table 4.7).

Positive amplification was obtained in samples collected from six fields out of the eight fields surveyed in Palakkad district (Plate 4.8a). Samples collected from Othalloor and Koodallur where typical yellow mosaic symptoms were not observed did not show any amplification. Mild chlorosis observed in these fields may be due to some other viruses or nutrient deficiency.

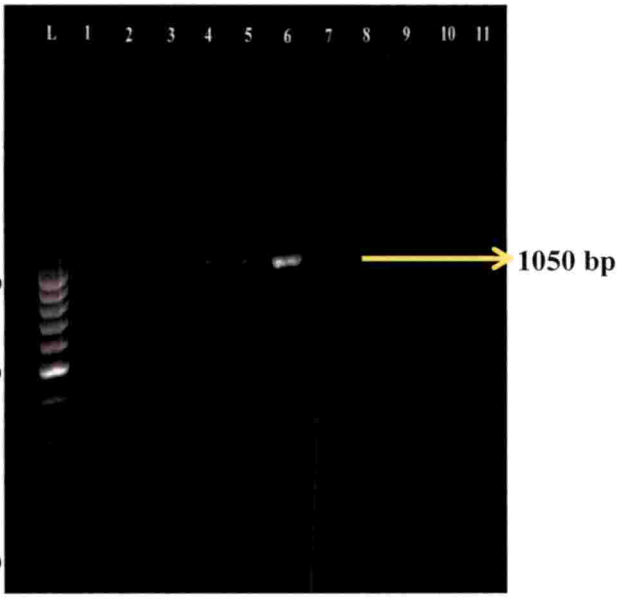
Plate 4.6 Standardization of annealing temperature



a. *Begomovirus* specific primer



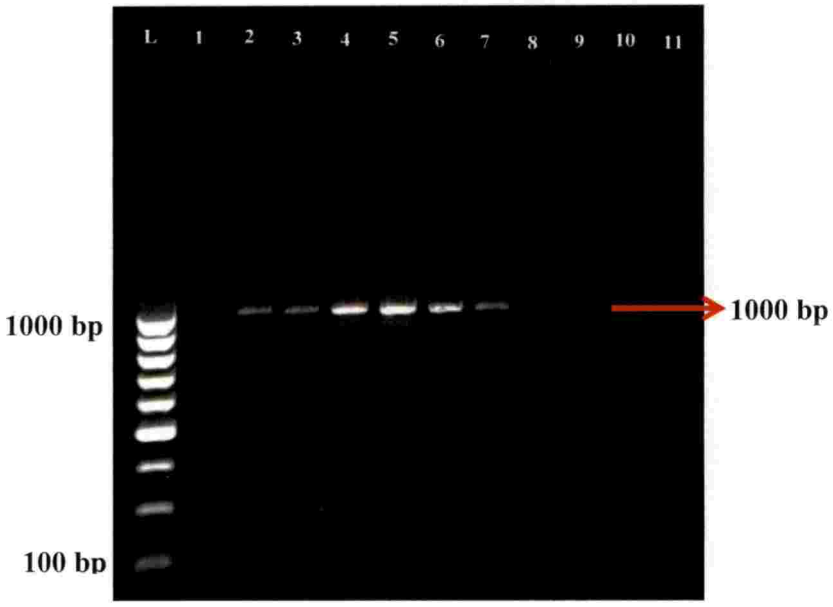
b. MYMV primer



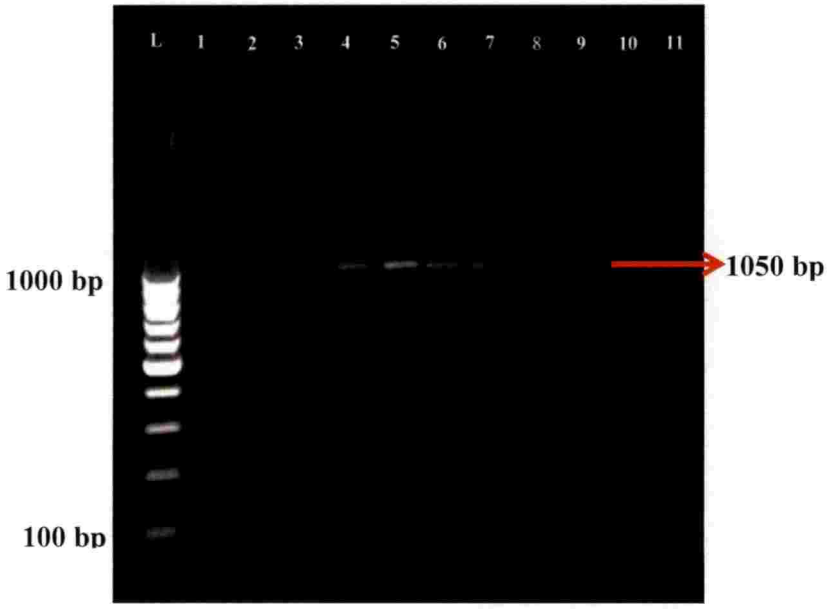
c. HYMV primer

Lane L (a & c) – 100 bp ladder, Lane L (b) – 1 kb ladder, Lane 1 – 51.0°C, Lane 2 – 52.1°C, Lane 3 – 53.0°C, Lane 4 – 53.6°C, Lane 5 – 54.4°C, Lane 6 – 55.3°C, Lane 7 – 56.8°C, Lane 8 – 57.5°C, Lane 9 – 58.3°C

Plate 4.7 Standardization of DNA concentration for YMV species specific primers



a. MYMV primer



b. HYMV primer

Lane L – 100 bp ladder, Lane 1 – 15 ng/ul, Lane 2 – 35 ng/ul, Lane 3 – 55ng/ul, Lane 4 – 75 ng/ul, Lane 5 – 95 ng/ul, Lane 6 – 115 ng/ul, Lane 7 – 135 ng/ul, Lane 8 – 155 ng/ul , Lane 9 – 175 ng/ul, Lane 10 – 200 ng/ul, Lane 11 – 250 ng/ul

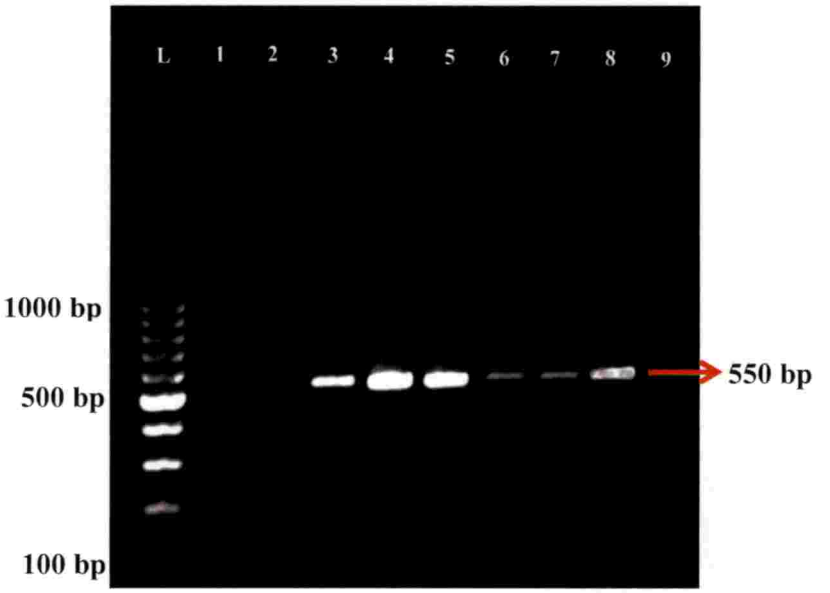
Table 4.7 Confirmation of *Begomovirus* in yellow mosaic infected black gram samples with deng primer

District	Location	Isolate name	Total no. of samples screened	No. of samples positive
Palakkad	Othaloor	BgOL	4	0
	Koodallur	BgKL	5	0
	Vadavannur I	BgV I	11	10
	Vadavannur II	BgV II	7	5
	Karippode	BgK	4	3
	Sankaramangalam	BgS	3	2
	Pattambi I	BgP I	7	5
	Pattambi II	BgP II	7	5
	Total		**48**	**31**
Malappuram	Pulamanthole	BgPM	4	0
	Amarambalam I	BgA I	6	5
	Amarambalam II	BgA II	4	3
	Chokkad I	BgC I	4	2
	Chokkad II	BgC II	2	2
	Total		**20**	**12**

Amplicons of expected size was observed in four fields out of the five fields surveyed in Malappuram district (Plate 4.8b). None of the samples collected from Pulamanthole gave amplification. The YMD resistant variety, VBN 8 was cultivated in the field. Though a mild form of mosaic symptoms were observed in the field, the presence of the virus could not be detected. This indicates the resistance of the variety to YMV and the symptoms observed may be due to nutritional disorder.

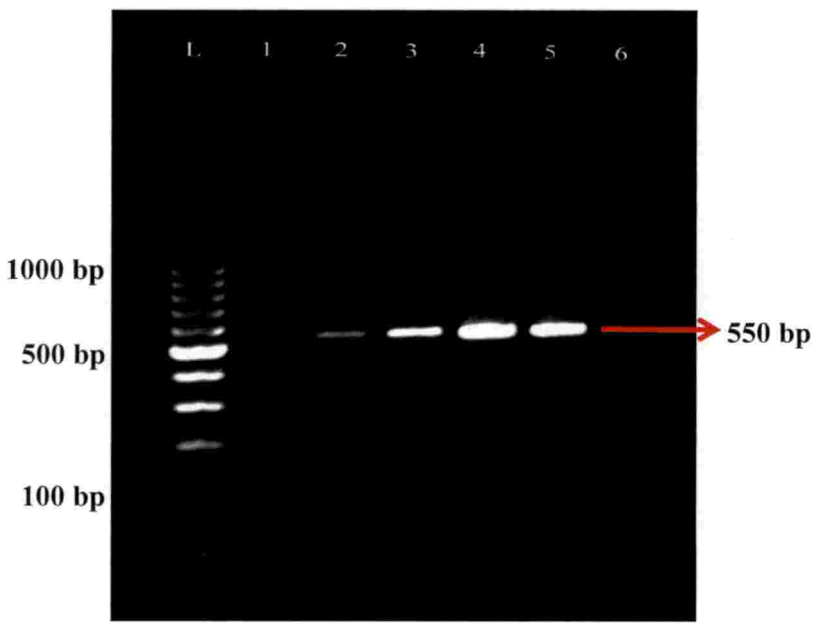
100

Plate 4.8 Detection of *Begomovirus* in different fields



a. Palakkad district

Lane L – 100 bp ladder, Lane 1 – BgOL, Lane 2 – BgKL, Lane 3 – BgV I, Lane 4 – BgV II, Lane 5 – BgK , Lane 6 – BgS, Lane 7 – BgP I, Lane 8 – BgP II, Lane 9 – Healthy black gram sample



b. Malappuram district

Lane L - 100 bp ladder, Lane 1 - BgPM, Lane 2 - BgA I, Lane 3 - BgA II, Lane 4 - BgC I, Lane 5 - BgC II, Lane 6 - Healthy black gram sample

4.3.2.3.3.2 Identification of the virus species

For identification of the virus species, the samples collected were subjected to PCR amplification using virus specific primers MYMV-CPF/MYMV-CPR, HYMV-CPF/HYMV-CPR and MYMIV-CPF/MYMIV-CPR amplifying a portion of DNA A including the coat protein region of MYMV, HYMV and MYMIV respectively.

PCR with MYMV specific primer yielded amplicons of expected size (~1000 bp) in samples collected from five fields out of eight fields surveyed in Palakkad district (Plate 4.9a). MYMV could be detected in all the samples which gave positive amplification for deng primer except the sample from second field of Pattambi. PCR analysis of samples collected from Malappuram district using MYMV specific primer revealed the presence of MYMV in all the fields which gave positive amplification for *Begomovirus* specific primer (Plate 4.9b). MYMV was present in all the samples positive for *Begomovirus* except one sample from second field of Chokkad. This indicates that the major virus causing yellow mosaic of black gram in Kerala is MYMV. But MYMV could not be detected in any of the samples from Othaloor, Koodallur and Pulamanthole which gave negative results for *Begomovirus* specific primer.

PCR amplification using HYMV primer yielded positive amplification in samples collected from four fields in Palakkad district and two fields in Malappuram district (Plate 4.10a and 4.10b). HYMV was detected in second field of Pattambi, where MYMV was not present. The symptom observed in second field of Pattambi was mild mosaic. Mixed infection of MYMV and HYMV was noticed in fields of Vadavannur I and II, Pattambi I, Amarambalam II and Chokkad II. HYMV and MYMV were present in same samples revealing the co-occurrence of both viruses.

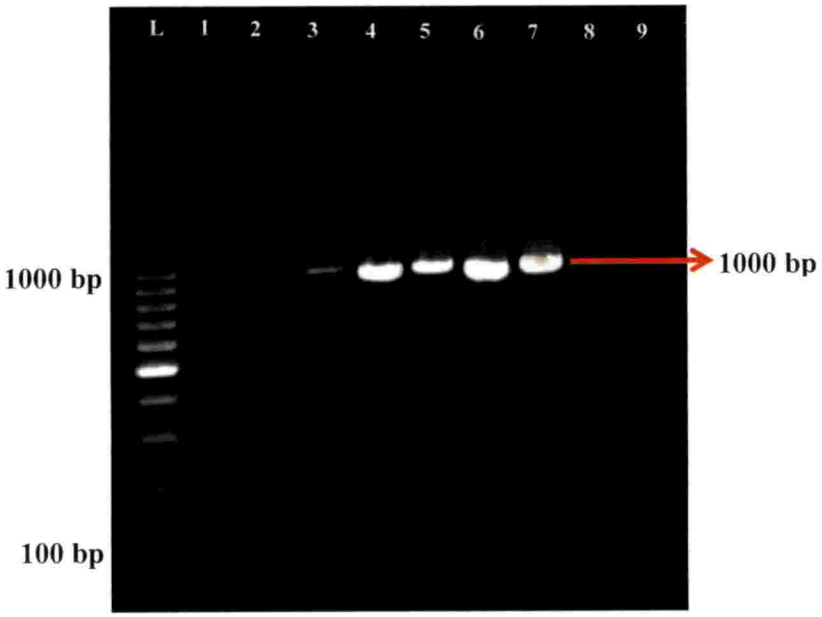
None of the samples collected gave positive amplification for MYMIV specific primer (Plate 4.11). To confirm these results, a MYMIV positive sample (sample from IARI) was included in the study. Amplification of expected size (~1000

bp) was observed in the positive sample confirming the absence of MYMIV in the yellow mosaic samples collected. The PCR result recorded in each sample having different symptoms are mentioned in Table 4.8a and 4.8b.

PCR analysis revealed the presence of *Begomovirus* in all samples showing typical yellow mosaic symptoms. MYMV could be detected in various samples showing symptoms such as typical yellow mosaic, yellow mosaic coupled with cupping and crinkling of leaves, yellow specks on leaves, yellow specks with elongation and distortion of younger leaves, reduction in size of younger leaves, complete yellowing of leaves with whitish discolouration and papery white leaves. The varieties in which MYMV was detected include local varieties, VBN 3, VBN 4, VBN 6 and Co6. Though light yellow mosaic patches were observed on VBN 8, yellow mosaic virus could not be detected in any of the samples collected from that field.

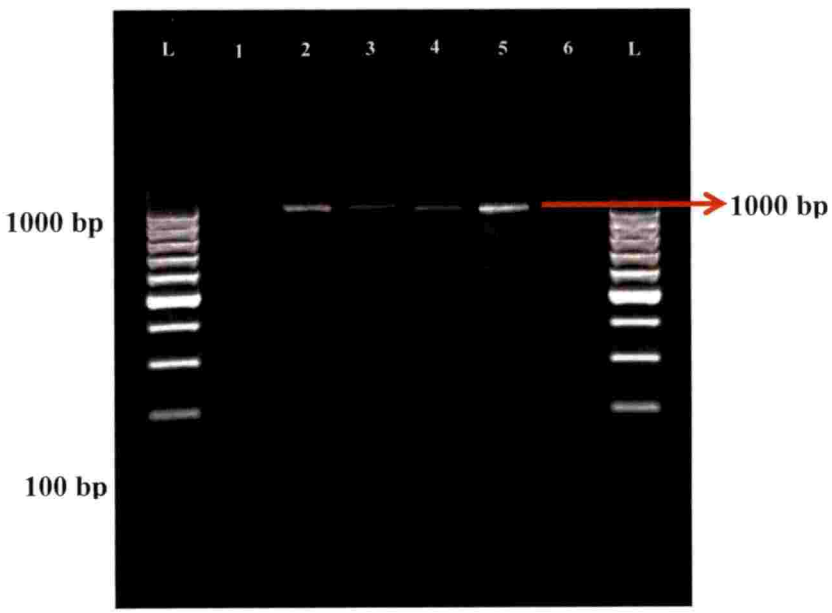
Maximum variation in symptoms was observed in second field of Vadavannur. Samples showing six types of symptoms were collected from the field and all of these showed the presence of MYMV and HYMV. Mixed infection was noticed in the first field of Vadavannur also. First field in Pattambi, second field in Amarambalam and second field in Chokkad also showed mixed infection. Second field in Pattambi, where only mild yellow mosaic was observed showed amplification for HYMV alone. Whereas, one sample from Chokkad, which was positive for HYMV alone showed typical yellow mosaic with distorted and elongated leaves. But a clear cut distinction between the symptoms caused by MYMV and HYMV was not observed in the present study. Similar kind of symptoms, *viz*., yellowing of leaves with whitish discolouration showed the presence of MYMV alone in the second field of Chokkad, whereas both viruses could be detected from samples showing similar symptoms in second field of Vadavannur and second field of Amarambalam.

Plate 4.9 Detection of MYMV in different fields



a. Palakkad district

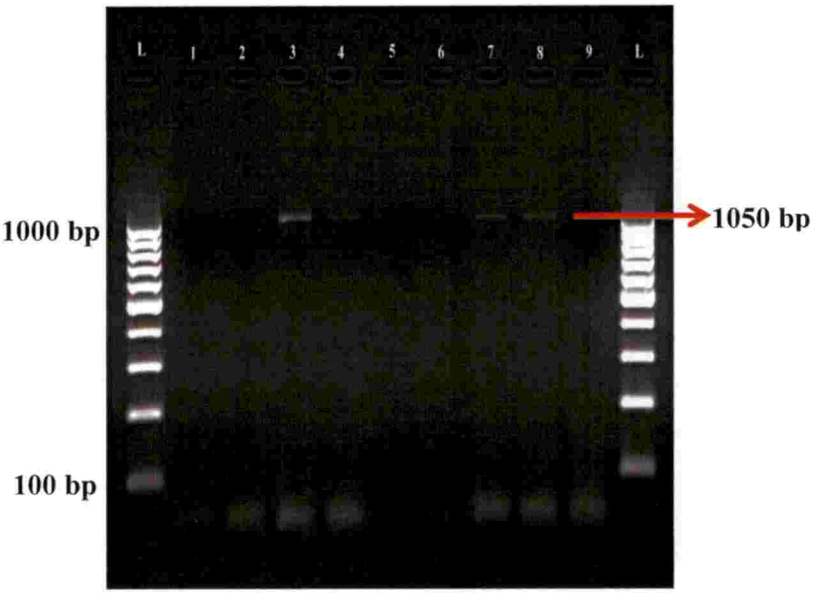
Lane L – 100 bp ladder, Lane 1 – BgOL, Lane 2 – BgKL, Lane 3 – BgV I, Lane 4 – BgV II, Lane 5 – BgK , Lane 6 – BgS, Lane 7 – BgP I, Lane 8 – BgP II, Lane 9 – Healthy black gram sample



b. Malappuram district

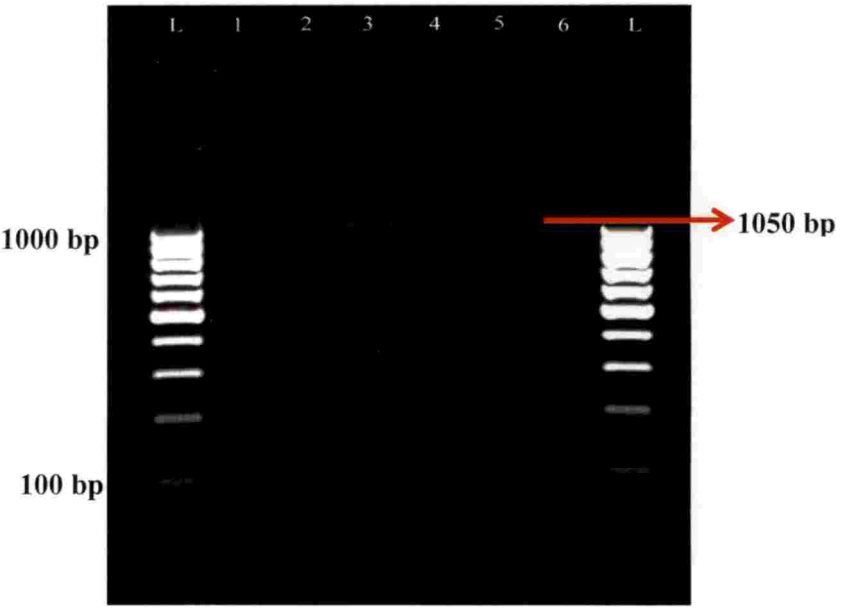
Lane L - 100 bp ladder, Lane 1 - BgPM, Lane 2 - BgA I, Lane 3 - BgA II, Lane 4 - BgC I, Lane 5 - BgC II, Lane 6 - Healthy black gram sample

Plate 4.10 Detection of HYMV in different fields



a. Palakkad district

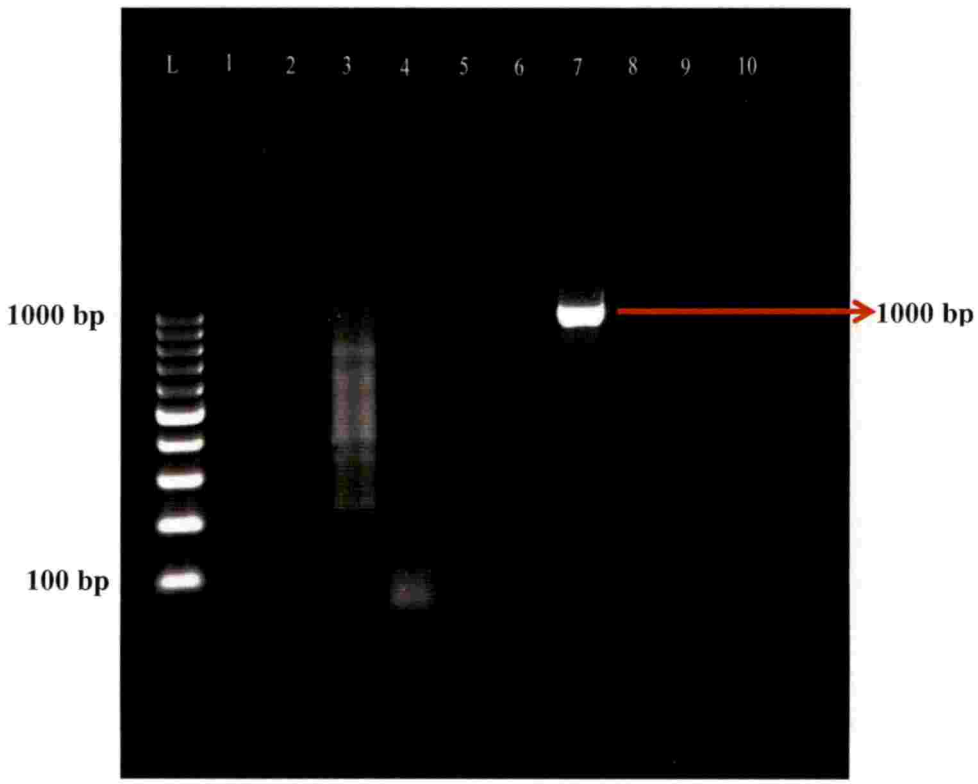
Lane L – 100 bp ladder, Lane 1 – BgOL, Lane 2 – BgKL, Lane 3 – BgV I, Lane 4 – BgV II, Lane 5 – BgK , Lane 6 – BgS, Lane 7 – BgP I, Lane 8 – BgP II, Lane 9 – Healthy black gram sample



b. Malappuram district

Lane L - 100 bp ladder, Lane 1 - BgPM, Lane 2 - BgA I, Lane 3 - BgA II, Lane 4 - BgC I, Lane 5 - BgC II, Lane 6 - Healthy black gram sample

Plate 4.11 Detection of MYMIV in different fields



Lane L – 100 bp ladder, Lane 1 – BgOL, Lane 2 – BgKL, Lane 3 – BgV , Lane 4 – BgK, Lane 5 – BgS, Lane 6 – BgP, Lane 7 – Positive sample, Lane 8 – BgPM, Lane 9 – BgA, Lane 10- BgC

Table 4.8a Detection of yellow mosaic virus species infecting black gram in Palakkad district

No.	Location	Isolate name	Symptom	PCR result			
				Deng	MYMV	HYMV	MYMIV
1	Othaloor	BgOL - 1	Mild yellow mosaic pattern	-	-	-	-
		BgOL - 2	White discolouration along the veins	-	-	-	-
		BgOL - 3	Curling and crinkling	-	-	-	-
		BgOL - 4	Mild chlorosis along the veins	-	-	-	-
2	Koodallur	BgKL - 1	Yellow mosaic starting as small yellow specks	-	-	-	-
		BgKL – 2	Crinkling and chlorosis	-	-	-	-
		BgKL – 3	Total yellowing of the leaf	-	-	-	-
		BgKL – 4	Interveinal chlorosis with necrotic spots	-	-	-	-
3	Vadavannur I	BgV I - 1	Intermingled green yellow mosaic pattern	+	+	+	-
		BgVI - 2	Crinkling, puckering and yellowing of younger leaves	+	+	+	-
		BgV I - 3	Interveinal chlorosis	+	+	+	-
		BgVI - 4	Yellow mosaic with necrosis of leaves	-	-	-	-

Table 4.8a Detection of yellow mosaic virus species infecting black gram in Palakkad district

No.	Location	Isolate name	Symptom	PCR result			
				Deng	MYMV	HYMV	MYMIV
4	Vadavannur II	BgV II - 1	Yellow specks	+	+	+	-
		BgV II - 2	Total yellow leaves with whitish discolouration	+	+	+	-
		BgV II - 3	Reduced size of younger leaves	+	+	+	-
		BgV II - 4	Papery white leaves	+	+	+	-
		BgV II - 5	Yellow green specks with crinkling and cupping of younger leaves	+	+	+	-
		BgV II - 6	Complete yellowing along with interveinal bronzing	+	+	-	-
5	Karippode	BgK - 1	Typical yellow mosaic with crinkling	+	+	-	-
		BgK - 2	Yellow mosaic with necrosis	-	-	-	-
		BgK - 3	Typical mosaic pattern with reduced leaf size	+	+	-	-
6	Sankaramangalam	BgS - 1	Typical yellow mosaic pattern with reduced leaf size	+	+	-	-
		BgS - 2	Total yellowing of the leaf	-	-	-	-
7	Pattambi I	BgP I - 1	Typical yellow mosaic pattern with white colouration	+	+	+	-
		BgP I - 2	Reduced size of younger leaves	+	+	+	-
8	Pattambi II	BgP II - 7	Mild yellow mosaic pattern	+	-	+	-

Table 4.8b Detection of yellow mosaic virus species infecting black gram in Malappuram district

No.	Location	Isolate name	Symptom	PCR result			
				Deng	MYMV	HYMV	MYMIV
1	Pulamanthole	BgPM -1	Mild yellow green patches	-	-	-	-
		BgPM -2	Total yellowing of the leaves starting as vein clearing	-	-	-	
2	Amarambalam I	BgA I -1	Typical yellow mosaic	+	+	-	-
		BgA I -2	Typical YM with white patches	+	+	-	-
		BgA I - 3	Vein banding	+	+	-	-
		BgA I -4	White discoloration on green leaves	+	+	-	
		BgA I -5	Reduced leaf size				
3	Amarambalam II	BgA II -1	Typical yellow mosaic on new young leaves	+	+	+	-
		BgA II -2	Yellow mosaic with necrosis	-	-	-	-
		BgA II -3	Yellow mosaic with whitish discolouration	+	+	+	-
		BgA II -4	Complete yellowing along with interveinal bronzing	+	+	-	-
4	Chokkad I	BgC I -1	Green yellow specks on elongated younger leaves	+	+	-	-
		BgC I -2	Cupping & marginal yellowing of leaves	-	-	-	-
		BgC II -3	Yellow mosaic with whitish discolouration	+	+	-	-
5	Chokkad II	BgC II -1	Typical YM with green thick vein	+	+	-	-
		BgC II -2	Typical YM, shapeless, distorted & elongated leaves	+	-	+	-

Some symptoms which were not reported earlier were observed in the present study and the presence of YMV was confirmed in such symptoms. Samples showing complete yellowing of leaves along with interveinal bronzing collected from Vadavannur II and Amarambalam II showed the presence of MYMV and HYMV. Vein banding observed in Amarambalam - I is also a new symptom not reported earlier. MYMV was detected in the sample showing vein banding. However some of the samples collected did not yield any amplification for YMV. The symptoms observed in these samples include a general yellowing of leaves, yellow mosaic with necrosis of leaves, cupping and marginal yellowing of leaves, crinkling and curling of leaves without yellow mosaic pattern and white discolouration or chlorosis along veins of the leaves.

4.4 PARTIAL CHARACTERIZATION OF THE ETIOLOGICAL AGENT

4.4.1 Sequencing of the amplicons

Amplicons of three representative isolates of MYMV and two representative isolates of HYMV were sequenced with automated sequencing machine at Agrigenome labs, Kakkanad. The details of the five representative black gram samples subjected for sequencing are mentioned in Table 4.9. The nucleotide sequence obtained and lengths of each isolates are mentioned in Table 4.10a – 4.14a.

Table 4.9 Representative isolates selected for sequencing

Sl No.	Isolate	Location	Primer	Abbreviation
1	BgV II - 6	Vadavannur	MYMV	BgVM
2	BgS - 1	Sankaramangalam	MYMV	BgSM
3	BgA I - 1	Amarambalam	MYMV	BgAM
4	BgP I - 1	Pattambi	HYMV	BgP1H
5	BgC II - 1	Chokkad	HYMV	BgC2H

4.4.2 *In silico* analysis

4.4.2.1 *Homology analysis of nucleotide sequence*

Homology analysis of MYMV isolates from present study with other reported *Begomovirus* sequences in NCBI database revealed significant nucleotide identity with different DNA A segments and coat protein region of different yellow mosaic virus species in different legume hosts.

Sequence of BgVM isolate showed 99 per cent identity with *Mungbean yellow mosaic virus* [DQ400848.1] of black gram from Tamil Nadu with maximum query coverage (100%) and score of 1086 which was followed by *Mungbean yellow mosaic virus* isolate from Namakkal [DQ865201.1] and *Mungbean yellow mosaic virus*-Soybean strain from Madurai [AJ421642.1]. *Mungbean yellow mosaic virus isolate* Tirupati [KP455992.1] exhibited 100 per cent query coverage and 98 per cent identity with BgVM isolate. *Horsegram yellow mosaic virus* isolate of horse gram [AM932427.1] showed query coverage of 99 per cent and 99 per cent identity but E value was not zero. MYMIV isolates showed below 80 per cent identity to the sequence. The details are mentioned in Table 4.10(b) and Plate (4.12).

The BgSM isolate also shared 99 per cent identity with *Mungbean yellow mosaic virus* [DQ400848.1] and *Mungbean yellow mosaic virus* isolate from Tirupati [KP455992.1] with a maximum query coverage (100%). This was followed by *Mungbean yellow mosaic virus*-Soybean strain from Madurai [AJ421642.1] and *Mungbean yellow mosaic virus* isolate from Namakkal [DQ865201.1] with which BgSM shared 98 per cent sequence identity at maximum query coverage (100%). The isolate shared only 86 per cent identity with *Horsegram yellow mosaic virus* isolate of horse gram [AM932427.1]. As in the case of BgVM isolate, BgSM also showed below 80 per cent identity with MYMIV isolates. The details are mentioned in Table 4.11(b) and Plate (4.13).

Table 4.10 (a) Nucleotide sequence of a portion of DNA A of MYMV isolate BgVM including the coat protein gene

Sequence (5'- 3')	Size (bp)
5'-AGCTTTACGCATAATGTTCAATACAAACTTTATTAATTTGAAATCGAATCATAAAAATAGATCCGA ATTTTCAATGTTGCGTACACAGGATTTGATGCATGAGTACATGCCATATACAATAACAATGCATTCTC AGTATGGTTTTCATACTTCGCTGCCTCTTGGTGGTTGTAAACAACATAATTATTAACACGATAAAATT TACTAACTATGGCTTGCTCCTTGCTCGCATATTGGCCACCAGTAACTGTGGCTTGAAACTTACGCACG ACCTGATAACGATCACGAAGATCGTTCTTCACAGTTGCAGTACTGGGTTCATTGTCATACATGTTGAA CACTTGACCAAAGTCTTGTGGCGTACCAAATGGACGTCTGTCACGCACTAAACTTGAACATCACAGT ATTAGTGTGATTCTTTGTCTTGATGTTCTCATCCATCCAGATTTTACCTGTAACCCAGATAGACTTAAC ACAAAATCGTTTCCCAACTCTATGAGTGATGCCCATGCCTCGTGTCACATCAGTGACACATATTACCT TGCCGACATGCGAAATATCATGTTTCGCCTCAAAGGACTGGACCTTACACGGTCCTTCACAACCACGT GGGACATCAGGAGAGCGGTATAACCGATAGTACCTCGGTTTCCTCCACATGGGTCGATTGGTCCACC TCCTCCTTTTCGCGCTGGCAGGGACACTTGCCGCAGTGGCAGGTAAGGAAAGCGGGGTGTCGAAGGT CAGCCTCCTCCGCACATTCGATATCGGGGTAGAGAAGGCGGTATCGTAATTCCGCTTTGGCATGTTTC CGTATACGTAAAACCTGTATTAAATCTGTTAAATACTCAAAACCAAGAGAATTAGAAGGAT-3'	873

Table 4.10(b) Sequence homology observed for isolate BgVM in BLASTn analysis

Sl No.	Description	Maximum score	Total score	Query coverage	E value	Identity (%)	Accession
1	*Mungbean yellow mosaic virus* segment DNA A, complete sequence	1086	1861	100%	0.00	99	DQ400848.1
2	*Mungbean yellow mosaic virus* isolate Namakkal segment DNA-A, complete sequence	1059	1823	100%	0.0	98	DQ865201.1
3	*Mungbean yellow mosaic virus* - soybean [Madurai] segment DNA-A, complete sequence	1059	1828	100%	0.0	98	AJ421642.1
4	*Mungbean yellow mosaic virus* isolate Tirupati segment DNA-A, complete sequence	1048	1811	100%	0.0	98	KP455992.1
6	*Mungbean yellow mosaic virus - Vigna* [Maharashtra] segment DNA-A, complete sequence	1042	1800	100%	0.0	97	AF314530.1
7	*Mungbean yellow mosaic virus* isolate Haryana segment DNA-A, complete sequence	937	1623	100%	0.0	94	AY271896.1
8	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate horse gram	632	1119	99%	7e-177	99	AM932427.1
9	*Horsegram yellow mosaic virus* isolate India:Banglore:French bean:2011 segment DNA-A, complete sequence	621	1102	99%	1e-173	85	KC019306.1
10	*Mungbean yellow mosaic India virus* isolate Pigeon pea segment DNA-A, complete sequence	422	683	98%	2e-113	79	KX363947.1

Plate 4.12 Homology analysis of MYMV isolate BgVM

BLASTn graphical output

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mungbean yellow mosaic virus segment DNA A, complete sequence	1555	1555	100%	0.0	99%	DQ400848.1
Mungbean yellow mosaic virus clone CA2 segment DNA-A, complete sequence	1544	1544	100%	0.0	99%	KC911718.1
Mungbean yellow mosaic virus isolate Namakkal segment DNA-A, complete sequence	1522	1522	100%	0.0	98%	DQ865201.1
Mungbean yellow mosaic virus-Soybean[Madurai] segment DNA A, complete sequence, strain Madurai	1522	1522	100%	0.0	98%	AJ421642.1
Mungbean yellow mosaic virus clone CA1 segment DNA-A, complete sequence	1517	1517	100%	0.0	98%	KC911721.1
Mungbean yellow mosaic virus-Vigna segment A, complete sequence	1517	1517	100%	0.0	98%	AJ132575.1
Mungbean yellow mosaic virus-Vigna[Maharashtra] A component, complete sequence	1511	1511	100%	0.0	98%	AF314530.1
Mungbean yellow mosaic virus clone YA1 segment DNA-A, complete sequence	1506	1506	100%	0.0	98%	KC911722.1
Mungbean yellow mosaic virus clone PA1 segment DNA-A, complete sequence	1506	1506	100%	0.0	98%	KC911717.1
Mungbean yellow mosaic India virus pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1500	1500	100%	0.0	98%	DQ389150.1
Mungbean yellow mosaic virus-[NAV] pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1495	1495	100%	0.0	98%	DQ389144.1
Mungbean yellow mosaic virus isolate Tirupati segment DNA-A, complete sequence	1489	1489	99%	0.0	98%	KP455992.1
Mungbean yellow mosaic virus isolate Green AV1-like gene, complete sequence	1454	1454	96%	0.0	98%	KY824800.1
Mungbean yellow mosaic virus isolate VN10 segment DNA-A, complete sequence	1445	1445	100%	0.0	97%	JX244175.1
Mungbean yellow mosaic virus isolate VN7 segment DNA-A, complete sequence	1445	1445	100%	0.0	97%	JX244174.1
Mungbean yellow mosaic virus isolate VN15 segment DNA-A, complete sequence	1439	1439	100%	0.0	96%	JX244176.1
Mungbean yellow mosaic virus isolate VN5 segment DNA-A, complete sequence	1439	1439	100%	0.0	96%	JX244173.1

BLASTn text output

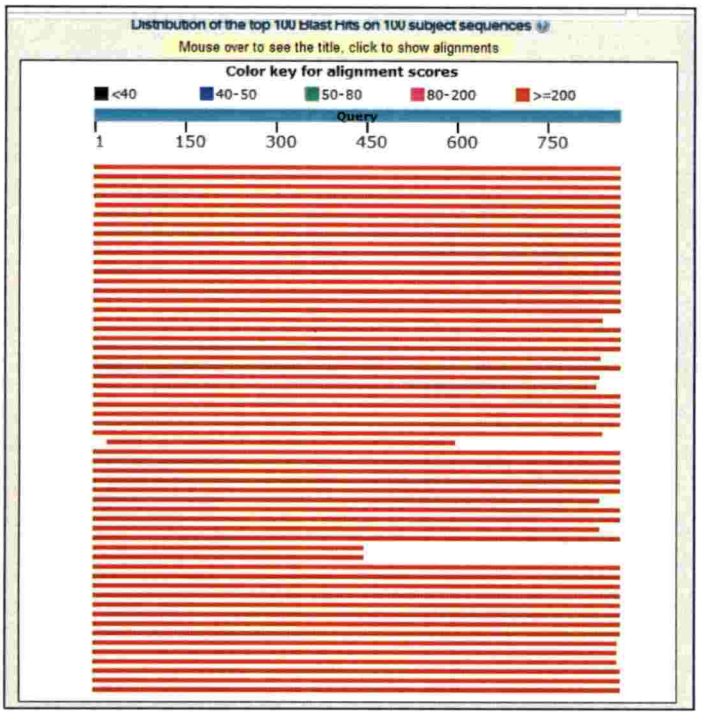
Table 4.11 (a) Nucleotide sequence of a portion of DNA A of MYMV isolate BgSM including the coat protein gene

Sequence (5'- 3')	Size (bp)
5'-GCATAATGTTCAATACAAACTTTATTAATTTGAAATCGAATCATAAAAATAGATCCGAATT TTCAATGTTGCGTACACAGGATTTGATGCATGAGTACATGCCATATACAATAACAATGCATTC TCAGTATGGTTTTCATACTTCGCTGCTTCTTGGTGGTTGTAAACAACATAATTGTTAACACGAT AAAATTTACTAACTATGGCTTGCTCCTTGCTCGCATACTGGCCACCAGTAACTGTGGCTTGAA ACTTACGCACGACCTGATAACGATCACGAAGATCGTTCTTCACAGTAGCAGTACTGGGTTCAT TGTCATACATGTTGAACACTTGACCAAAGTCTTGTGGCGTGCCAAATGGACGTCTGTCACGCA CTAACTTGAACATCACAGTATTAGTGTGATTCTTTGTCTTGATGTTCTCATCCATCCAGATTTT ACCTGTAACCCAGATAGACTTAACACAAAATCGTTTCCCAACTCTATGAGTGATGCCCATGCC TCGTGTCACATCAGTGACACATATTACCTTGCCGACATGCGAAATATCATGTTTCGCCTCAAA GGACTGGACCTTACACGGTCCCTCACAACCACGTGGGACATCAGGAGAGCGGTATAACCGAT AGTACTTCGGTTTCCTCCACATGGGTCGGTTGGTCCACCTCCTCCTTTTCGCGCTGGCAGGGA CACTTCCCGCAGTGGCAGGTAAGGAAAGCGGGGTGTCGAAGGTCAGCCTCCTCCGCACATTC GATATCGGGGTAGAGAAGGCGGTATCTAATTCCGCTTTGGCATGTTTCCGTATACGTAAAACC TGTATTAACTCTGTTAAATACTCAAAACCAAGAGAATTAGAAGAATAA-3'	866

Table 4.11(b) Sequence homology observed for isolate BgSM in BLASTn analysis

Sl No.	Description	Maximum score	Total score	Query coverage	E value	Identity (%)	Accession
1	*Mungbean yellow mosaic virus* segment DNA A, complete sequence	1223	2136	100%	0.0	99	DQ400848.1
2	*Mungbean yellow mosaic virus* isolate Tirupati segment DNA-A, complete sequence	1212	2136	100%	0.0	99	KP455992.1
3	*Mungbean yellow mosaic virus* - soybean [Madurai] segment DNA-A, complete sequence	1201	2109	100%	0.0	98	AJ421642.1
4	Mungbean yellow mosaic virus isolate Namakkal segment DNA-A, complete sequence	1190	2081	100%	0.0	98	DQ865201.1
5	*Mungbean yellow mosaic virus* isolate VN5 segment DNA-A, complete sequence	1151	2009	100%	0.0	97	JX244173.1
6	*Mungbean yellow mosaic virus* isolate Haryana segment DNA-A, complete sequence	1046	1834	100%	0.0	94	AY271896.1
7	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate horse gram	730	1305	100%	0.0	86	AM932427.1
8	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate lima bean	713	1278	100%	0.0	85	AM932429.1
9	*Mungbean yellow mosaic India virus* isolate Bengal, segment A, complete viral segment	481	925	94%	3e-131	80	HF922628.1
10	*Mungbean yellow mosaic India virus* segment DNA A, complete sequence, clone MI15	455	768	96%	2e-123	79	AM950268.1

Plate 4.13 Homology analysis of MYMV isolate BgSM



BLASTn graphical output

Sequences producing significant alignments:

Select: All None Selected:0

Alignments

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mungbean yellow mosaic virus clone CA2 segment DNA-A, complete sequence	1539	1539	100%	0.0	99%	KC911718.1
Mungbean yellow mosaic virus isolate Tirupati segment DNA-A, complete sequence	1533	1533	100%	0.0	99%	KP455992.1
Mungbean yellow mosaic virus clone VA1 segment DNA-A, complete sequence	1533	1533	100%	0.0	99%	KC911722.1
Mungbean yellow mosaic virus segment DNA A, complete sequence	1533	1533	100%	0.0	99%	DQ400846.1
Mungbean yellow mosaic virus-Soybean[Madurai] segment DNA A, complete sequence, strain Madurai	1515	1515	99%	0.0	98%	AJ421642.1
Mungbean yellow mosaic virus clone CA1 segment DNA-A, complete sequence	1511	1511	100%	0.0	98%	KC911721.1
Mungbean yellow mosaic virus isolate Namakkal segment DNA-A, complete sequence	1506	1506	100%	0.0	98%	DQ865201.1
Mungbean yellow mosaic virus-Vigna[Maharashtra] A component, complete sequence	1495	1495	100%	0.0	98%	AF314530.1
Mungbean yellow mosaic virus-Vigna segment A, complete sequence	1495	1495	100%	0.0	98%	AJ132575.1
Mungbean yellow mosaic virus clone PA1 segment DNA-A, complete sequence	1483	1483	100%	0.0	98%	KC911717.1
Mungbean yellow mosaic virus-IN[AV1 pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1483	1483	100%	0.0	98%	DQ389144.1
Mungbean yellow mosaic India virus pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1478	1478	100%	0.0	97%	DQ389150.1
Mungbean yellow mosaic virus isolate VN10 segment DNA-A, complete sequence	1456	1456	100%	0.0	97%	JX244175.1
Mungbean yellow mosaic virus isolate VN7 segment DNA-A, complete sequence	1456	1456	100%	0.0	97%	JX244174.1
Mungbean yellow mosaic virus isolate VN15 segment DNA-A, complete sequence	1450	1450	100%	0.0	97%	JX244176.1
Mungbean yellow mosaic virus isolate VN5 segment DNA-A, complete sequence	1450	1450	100%	0.0	97%	JX244173.1
Mungbean yellow mosaic virus isolate Black pre-coat protein-like gene, partial sequence, and AV1-like gene, complete sequence	1448	1448	96%	0.0	98%	KY824799.1

BLASTn text output

The BgAM isolate also showed maximum 99 per cent identity with *Mungbean yellow mosaic virus* [DQ400848.1] of black gram from Tamil Nadu with a maximum query coverage of 100 per cent. This was followed by *Mungbean yellow mosaic virus* isolate from Namakkal [DQ865201.1] and *Mungbean yellow mosaic virus* isolate from Tirupati [KP4992.1] with 98 per cent identity and 100 per cent query coverage. The sequence showed only 86 per cent identity with the *Horse gram yellow mosaic virus* isolate [AM932427.1]. The identity with MYMIV isolates were less than 80 per cent (Table 4.12b and Plate 4.14).

Homology analysis of HYMV isolates from present study with other reported *Begomovirus* sequences in NCBI database also revealed significant nucleotide identity with different MYMV isolates itself (Table 4.13b and Plate 4.15). Sequence of BgP1H isolate showed maximum identity (99%) with MYMV cloneVA1 of black gram from Pudukottai, Tamil Nadu [KC911722.1] with maximum query coverage (100%) which was followed by MYMV isolate of black gram from Tirupati [KP455992.1] with 99 per cent identity and query coverage. Whereas, MYMV isolate from Namakkal [DQ865201.1] showed 98 per cent identity with 100 per cent query coverage. The BgC2H isolate also showed more than 90 per cent identity with MYMV isolates itself. Isolate shared 99 per cent identity and 100 per cent query coverage with MYMV cloneVA1 of black gram from Pudukottai, Tamil Nadu [KC911722.1] and MYMV isolate of black gram from Tirupati [KP455992.1]. A 98 per cent identity and 100 per cent query coverage with MYMV isolate from Namakkal [DQ865201.1] (Table 4.14b and Plate 4.16). Different HYMV isolates shared only 86-87 per cent identity with these two isolates.

This indicates that the isolates BgP1H and BgC2H which were originally identified as HYMV by PCR amplification using species specific primer was MYMV itself or is a recombinant virus evolved as a result of co-existence of MYMV and HYMV.

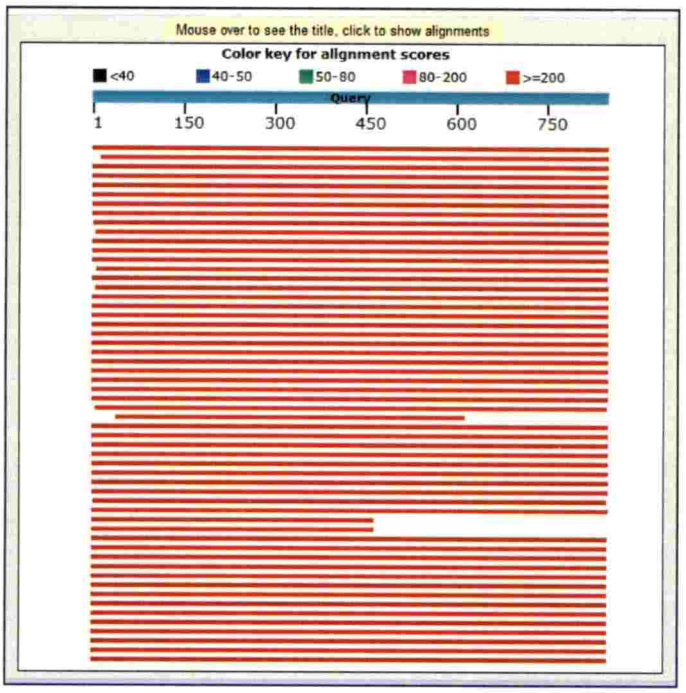
Table 4.12 (a) Nucleotide sequence of a portion of DNA A of MYMV isolate BgAM including the coat protein gene

Sequence (5'- 3')	Size (bp)
5'-AGGATGTAAGCTTGACGCATAATGTTCAATACAAACTTTATTAATTTGAAATCGAATCAT AAAAATAGATCCGAATTTTCAATGTTGCATACACAGGATTGGATGCATGAGTACATGCCATA TACAATAACAATGCATTCTCAGTATGGTATTCATACTTCGCTGCTTCTTGGTGGTTGTAAACA ACATAATTGTTAACACGATAAAATTTACTAACTATGGCTTGCTCCTTGCTCGCATACTGGCCA CCAGTAACTGTGGCTTGAAACTTACGCACGACCTGATAACGATCACGAAGATCGTTCTTCAC AGTAGCAGTACTGGGTTCATTGTCATACATGTTGAACACTTGACCAAAGTCTTGTGGCGTGC CAAATGGACGTCTGTCACGCACTAACTTGAACATCACAGTATTAGTGTGATTCTTTGTCTTGA TATTCTCATCCATCCAGATTTTACCTGTAACCCAGATAGACTTAACACAAAATCGTTTCCCAA CTCTATGAGTGATGCCCATGCCTCGTGTCACATCAGTGACACATATGACCTTGCCGACATGC GAAATATCATGTTTCGCCTCAAAGGACTGGACCTTACACGGTCCTTCACAACCACGTGGGAC ATCAGGAGAGCGGTATAACCGATAATACCTCGGTTTCCTCCACATGGGTCGGTTGGTCCACC TCCTCCTTTTCGCGCTGGCAGGGACACTTCCCGCACTGGCAGGTAAGGATAGCGGGGTGTCG AAGGTCAGCCTCCTCCGCACATTCGATATCGGGGTAGAGAAGGCGGTATCGTAATTCCGCTT TGGCATGTTTCCGTATACGTAAAACCTGTATTAACT-3'	844

Table 4.12(b) Sequence homology observed for isolate BgAM in BLASTn analysis

Sl No.	Description	Maximum score	Total score	Query coverage	E value	Identity (%)	Accession
1	*Mungbean yellow mosaic virus* segment DNA A, complete sequence	1086	1861	100%	0.0	99	DQ400848.1
2	*Mungbean yellow mosaic virus* isolate Namakkal segment DNA-A, complete sequence	1059	1823	100%	0.0	98	DQ865201.1
3	*Mungbean yellow mosaic virus* isolate Tirupati segment DNA-A, complete sequence	1048	1811	100%	0.0	98	KP455992.1
4	*Mungbean yellow mosaic virus - Vigna* [Maharashtra] segment DNA-A, complete sequence	1042	1800	100%	0.0	97	AF314530.1
5	*Mungbean yellow mosaic virus* isolate Haryana segment DNA-A, complete sequence	937	1623	100%	0.0	94	AY271896.1
6	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate horse gram	632	1119	99%	7e-177	86	AM932427.1
7	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate lima bean	616	1091	99%	7e-172	85	AM932429.1
8	*Mungbean yellow mosaic India virus*, segment DNA-A, complete sequence, clone A1E	449	748	100%	7e-122	80	JQ398669.1
9	*Mungbean yellow mosaic India virus* isolate Bengal, segment A, complete viral segment	424	718	98%	4e-114	79	HF922628.1
10	*Mungbean yellow mosaic India virus* isolate Vizianagaram pre-coat protein (AV2) gene, partial cds; and coat protein (AV1) gene, complete cds	396	657	98%	9e-106	79	JN181006.1

Plate 4.14 Homology analysis of MYMV isolate BgAM



BLASTn graphical output

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mungbean yellow mosaic virus clone VA1 segment DNA-A, complete sequence	1531	1531	100%	0.0	99%	KC911722.1
Mungbean yellow mosaic virus isolate Tirupati segment DNA-A, complete sequence	1485	1485	98%	0.0	99%	KP455992.1
Mungbean yellow mosaic virus isolate Namakkal segment DNA-A, complete sequence	1471	1471	100%	0.0	98%	DQ865201.1
Mungbean yellow mosaic virus-Soybean[Madurai] segment DNA A, complete sequence, strain Madurai	1471	1471	100%	0.0	98%	AJ421642.1
Mungbean yellow mosaic virus segment DNA A, complete sequence	1467	1467	99%	0.0	98%	DQ400848.1
Mungbean yellow mosaic virus-Vigna[Maharashtra] A component, complete sequence	1465	1465	100%	0.0	98%	AF314530.1
Mungbean yellow mosaic virus-Vigna segment A, complete sequence	1459	1459	100%	0.0	98%	AJ132575.1
Mungbean yellow mosaic virus isolate Green AV1-like gene, complete sequence	1450	1450	99%	0.0	98%	KY824800.1
Mungbean yellow mosaic virus isolate Black pre-coat protein-like gene, partial sequence; and AV1-like gene, complete sequence	1450	1450	99%	0.0	98%	KY824799.1
Mungbean yellow mosaic virus clone CA2 segment DNA-A, complete sequence	1445	1445	99%	0.0	98%	KC911718.1
Mungbean yellow mosaic virus clone PA1 segment DNA-A, complete sequence	1443	1443	100%	0.0	98%	KC911717.1
Mungbean yellow mosaic virus isolate Macroptilium AV1-like gene, complete sequence	1441	1441	99%	0.0	97%	KY824802.1
Mungbean yellow mosaic virus clone CA1 segment DNA-A, complete sequence	1437	1437	100%	0.0	97%	KC911721.1
Mungbean yellow mosaic virus-[NAM] pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1435	1435	99%	0.0	98%	DQ389144.1
Mungbean yellow mosaic virus isolate Croton AV1-like gene, complete sequence	1434	1434	99%	0.0	98%	KY824804.1
Mungbean yellow mosaic India virus pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1434	1434	99%	0.0	97%	DQ389150.1

BLASTn text output

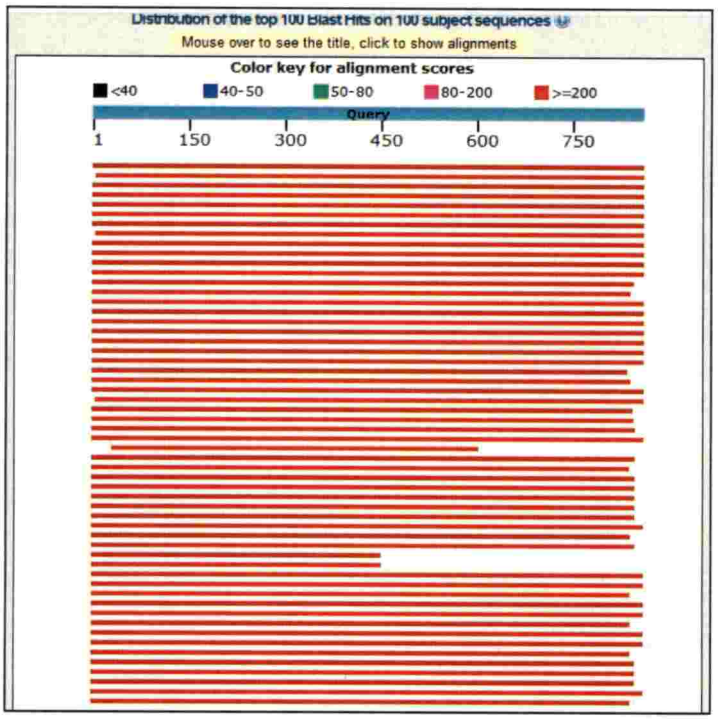
Table 4.13 (a) Nucleotide sequence of a portion of DNA A of HYMV isolate BgP1H including the coat protein gene

Sequence (5'- 3')	Size (bp)
5'-AGCTTGACGCATAATGTTCAATACAAACTTTATTAATTTGAAATCGAATCATAAAAATAGATC CGAATTTTCAATGTTGCGTACACAGGATTTGATGCATGAGTACATGCCATATACAATAACAATGC ATTCTCAGTATGGTTTTCATACTTCGCTGCTTCTTGGTGGTTGTAAACAACATAATTGTTAACACG ATAAAATTTACTAACTATGGCTTGCTCCTTGCTCGCATACTGGCCACCAGTAACTGTGGCTTGAA ACTTACGCACGACCTGATAACGATCACGAAGATCGTTCTTCACAGTAGCAGTACTGGGTTCATTG TCATACATGTTGAACACTTGACCAAAGTCTTGTGGCGTGCCAAATGGACGTCTGTCACGCACTAA CTTGAACATCACAGTATTAGTGTGATTCTTTGTCTTGATATTCTCATCCATCCAGATTTTACCTGT AACCCAGATAGACTTAACACAAAATCGTTTTCCAACTCTATGAGTGATGCCCATGCCTCGGGTCA CATCAGTGACACATATGACCTTGCCGACATGCGAAATATCATGTTTCGCCTCAAAGGACTGGACC TTACACGGTCCTTCACAACCACGTGGGACATCAGGAGAGCGGTATAACCGATAATACCTCGGTTT CCTCCACATGGGTCGGTTGGTCCACCTCCTCCTTTTCGCGCTGGCAGGGACACTTCCCGCAGTGG CAGGTAAGGATAGCGGGGTGTCGAAGGTCAGCCTCCTCCGCACATTCGATATCGGGGTAGAGAA GGCGGTATCGTAATTCCGCTTTGGCATGTTTCCGTATACGTAAAACCTGTATTAACTCTGTTAAAT ACTCGACACCAAGAGA-3'	861

Table 4.13(b) Sequence homology observed for isolate BgP1H in BLASTn analysis

Sl No.	Description	Maximum score	Total score	Query coverage	E value	Identity (%)	Accession
1	*Mungbean yellow mosaic virus* clone VA1 segment DNA-A, complete sequence, Pudukottai	1563	1563	100%	0.0	99	KC911722.1
2	*Mungbean yellow mosaic virus* isolate Tirupati segment DNA-A, complete sequence	1526	1526	99%	0.0	99	KP455992.1
3	*Mungbean yellow mosaic virus* isolate Namakkal segment DNA-A, complete sequence	1513	1513	100%	0.0	99	DQ865201.1
4	*Mungbean yellow mosaic virus* segment DNA A, complete sequence	1502	1502	100%	0.0	98	DQ400848.1
5	*Mungbean yellow mosaic virus*-Soybean[Madurai] segment DNA A, complete sequence, strain Madurai	1502	1502	100%	0.0	98	AJ421642.1
6	*Mungbean yellow mosaic virus* isolate Haryana segment DNA-A, complete sequence	1308	1308	100%	0.0	94	AY271896.1
7	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate horse gram	961	961	98%	0.0	87	AM932427.1
8	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate lima bean	928	928	98%	0.0	86	AM932429.1
9	*Mungbean yellow mosaic India virus*, segment DNA-A, complete sequence, clone A1E	822	822	98%	0.0	84	JQ398669.1
10	*Mungbean yellow mosaic India virus* isolate Bengal, segment A, complete viral segment	710	710	97%	0.0	82	HF922628.1

Plate 4.15 Homology analysis of HYMV isolate BgP1H



BLASTn graphical output

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mungbean yellow mosaic virus clone VA1 segment DNA-A, complete sequence	1563	1563	100%	0.0	99%	KC911722.1
Mungbean yellow mosaic virus isolate Tirupati segment DNA-A, complete sequence	1526	1526	99%	0.0	99%	KP455992.1
Mungbean yellow mosaic virus isolate Namakkal segment DNA-A, complete sequence	1513	1513	100%	0.0	98%	DQ886201.1
Mungbean yellow mosaic virus segment DNA A, complete sequence	1502	1502	100%	0.0	98%	DQ400848.1
Mungbean yellow mosaic virus-SorbeanIIMadurai segment DNA A, complete sequence, strain Madurai	1502	1502	100%	0.0	98%	AJ421642.1
Mungbean yellow mosaic virus-VignaIIMaharashtra1 A component, complete sequence	1496	1496	100%	0.0	98%	AF314530.1
Mungbean yellow mosaic virus-Vigna segment A, complete sequence	1491	1491	100%	0.0	98%	AJ132575.1
Mungbean yellow mosaic virus clone CA2 segment DNA-A, complete sequence	1485	1485	99%	0.0	98%	KC911718.1
Mungbean yellow mosaic virus-IN[A/I pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1474	1474	100%	0.0	98%	DQ389144.1
Mungbean yellow mosaic virus clone CA1 segment DNA-A, complete sequence	1469	1469	100%	0.0	97%	KC911721.1
Mungbean yellow mosaic virus clone PA1 segment DNA-A, complete sequence	1469	1469	100%	0.0	97%	KC911717.1
Mungbean yellow mosaic India virus pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1469	1469	100%	0.0	97%	DQ389150.1
Mungbean yellow mosaic virus isolate Black pre-coat protein-like gene, partial sequence, and AV1-like gene, complete sequence	1458	1458	98%	0.0	98%	KY824799.1
Mungbean yellow mosaic virus isolate Green AV1-like gene, complete sequence	1454	1454	97%	0.0	98%	KY824800.1
Mungbean yellow mosaic virus isolate VN10 segment DNA-A, complete sequence	1447	1447	100%	0.0	97%	JX244175.1
Mungbean yellow mosaic virus isolate VN7 segment DNA-A, complete sequence	1447	1447	100%	0.0	97%	JX244174.1
Mungbean yellow mosaic virus isolate VN15 segment DNA-A, complete sequence	1441	1441	100%	0.0	97%	JX244176.1

BLASTn text output

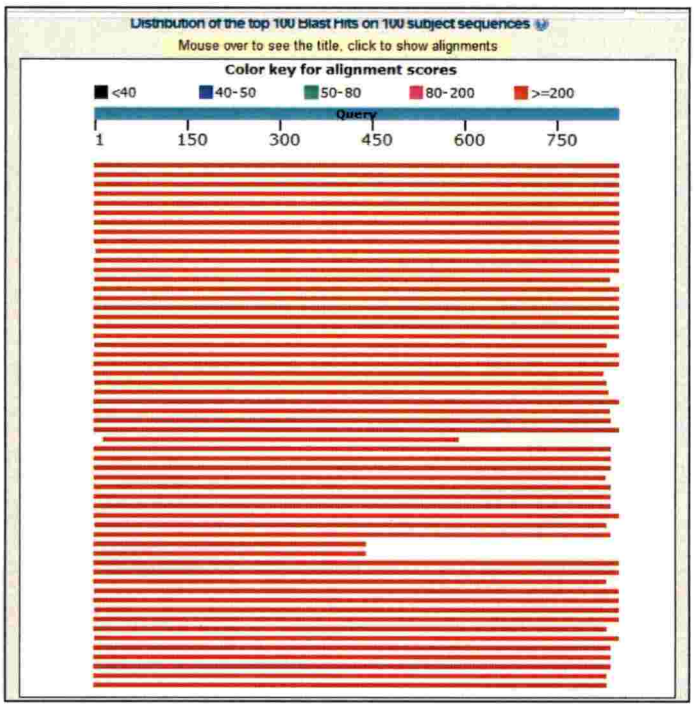
Table 4.14 (a) Nucleotide sequence of a portion of DNA A of HYMV isolate BgC2H including the coat protein gene

Sequence (5'- 3')	Size (bp)
5'-GTTCAATACAAACTTTATTAATTTGAAATCGAATCATAAAAATAGATCCGAATTTTCA ATGTTGCATACACAGGATTTGATGCATGAAGTACATGCCCATATACAATAACAATGAAT TCTCAGTATGGTTTTCATACTTCGCTGCTTCTTGGTGGTTGTAAACAACATAATTGTTAAC ACGATAAAATTTACTAACTATGGCTTGCTCCTTGCTCGCATACTGGCCACCAGTAACTGT GGCTTGAAACTTACGCACGACCTGATAACGATCACGAAGATCGTTCTTCACAGTAGCAGT ACTGGGTTCATTGTCATACATGTTGAACACTTGACCAAAGTCTTGTGGCGTGCCAAATGG ACGTCTGTCACGCACTAACTTGAACATCACAGTATTAGTGTGATTCTTTGTCTTGATATTC TCATCCATCCAGATTTTACCTGTAACCCAGATAGACTTAACACAAAAATCGTTTCCCAAC TCTATGAGTGATGCCCATGCCTCGTGTCACATCAGTGACACATATGACCTTGCCGACATG CGAAATATCATGTTTCGCCTCAAAGGACTGGACCTTACACGGTCCTTCACAACCACGTGG GACATCAGGAGAGCGGTATAACCGATAATACCTCGGTTTCCTCCACATGGGTCGGTTGGT CCACCTCCTCCTTTTCGCGCTGGCAGGGACACTTCCCGCAGTGGCAGGTAAGGATAGCGG GGTGTCGAAGGTCAGCCTCCTCCGCACATTCGATATCGGGGTAGAGAAGGCGGTATCGT AATTCCGCTTTGGCATGTTTCCGTATACGTAAAACCTGTATTAACTCTGTTAAATACTCGA CACCAAGAGA-3'	849

Table 4.14(b) Sequence homology observed for isolate BgC2H in BLASTn analysis

Sl No.	Description	Maximum score	Total score	Query coverage	E value	Identity (%)	Accession
1	*Mungbean yellow mosaic virus* clone VA1 segment DNA-A, complete sequence, Pudukottai	1535	1535	100%	0.0	99	KC911722.1
2	*Mungbean yellow mosaic virus* isolate Tirupati segment DNA-A, complete sequence	1504	1504	100%	0.0	99	KP455992.1
3	*Mungbean yellow mosaic virus* segment DNA A, complete sequence	1465	1465	100%	0.0	98	DQ400848.1
4	*Mungbean yellow mosaic virus*-Soybean[Madurai] segment DNA A, complete sequence, strain Madurai	1465	1465	100%	0.0	98	AJ421642.1
5	*Mungbean yellow mosaic virus* isolate Namakkal segment DNA-A, complete sequence	1459	1459	100%	0.0	99	DQ865201.1
6	*Mungbean yellow mosaic virus* isolate Haryana segment DNA-A, complete sequence	1282	1282	100%	0.0	94	AY271896.1
7	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate horse gram	924	924	98%	0.0	87	AM932427.1
8	*Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate lima bean	891	891	98%	0.0	86	AM932429.1
9	*Mungbean yellow mosaic India virus* isolate Vizianagaram pre-coat protein (AV2) gene, partial cds; and coat protein (AV1) gene, complete cds	651	651	97%	0.0	81	JN181006.1
10	*Mungbean yellow mosaic India virus* isolate Bengal, segment A, complete viral segment	673	673	97%	0.0	82	HF922628.1

Plate 4.16 Homology analysis of HYMV isolate BgC2H



BLASTn graphical output

Sequences producing significant alignments:

Select: All None Selected 0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mungbean yellow mosaic virus clone VA1 segment DNA-A, complete sequence	1531	1531	100%	0.0	99%	KC911722.1
Mungbean yellow mosaic virus isolate Tirupati segment DNA-A, complete sequence	1504	1504	100%	0.0	99%	KP455992.1
Mungbean yellow mosaic virus segment DNA A, complete sequence	1465	1465	100%	0.0	98%	DQ400848.1
Mungbean yellow mosaic virus-Soybean[Madurai] segment DNA A, complete sequence, strain Madurai	1465	1465	100%	0.0	98%	AJ421642.1
Mungbean yellow mosaic virus isolate Namakkal segment DNA-A, complete sequence	1459	1459	100%	0.0	98%	DQ865201.1
Mungbean yellow mosaic virus clone CA2 segment DNA-A, complete sequence	1454	1454	100%	0.0	98%	KC911718.1
Mungbean yellow mosaic virus-Vigna[Maharashtra] A component, complete sequence	1448	1448	100%	0.0	98%	AF314530.1
Mungbean yellow mosaic virus-Vigna segment A, complete sequence	1448	1448	100%	0.0	98%	AJ132575.1
Mungbean yellow mosaic virus-INAV[pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1437	1437	100%	0.0	97%	DQ389144.1
Mungbean yellow mosaic India virus pre-coat protein (AV2) and coat protein (AV1) genes, complete cds	1432	1432	99%	0.0	97%	DQ389150.1
Mungbean yellow mosaic virus clone CA1 segment DNA-A, complete sequence	1426	1426	100%	0.0	97%	KC911721.1
Mungbean yellow mosaic virus clone PA1 segment DNA-A, complete sequence	1426	1426	100%	0.0	97%	KC911717.1
Mungbean yellow mosaic virus isolate Black pre-coat protein-like gene, partial sequence, and AV1-like gene, complete sequence	1424	1424	97%	0.0	98%	KY824799.1
Mungbean yellow mosaic virus isolate VN10 segment DNA-A, complete sequence	1421	1421	100%	0.0	97%	JX244175.1
Mungbean yellow mosaic virus isolate VN7 segment DNA-A, complete sequence	1421	1421	100%	0.0	97%	JX244174.1
Mungbean yellow mosaic virus isolate VN15 segment DNA-A, complete sequence	1415	1415	100%	0.0	97%	JX244176.1
Mungbean yellow mosaic virus isolate VN5 segment DNA-A, complete sequence	1415	1415	100%	0.0	97%	JX244173.1

BLASTn text output

As the homology analysis indicates them to be MYMV, these isolates are referred to as MYMV isolates hereafter.

4.4.2.2 *Phylogenetic analysis*

The portion of DNA A including the sequences coding for coat protein gene amplified from the isolates collected were aligned with the corresponding region in DNA A of other yellow mosaic viruses. Phylogenetic tree was constructed for understanding the relationship of isolates from present study with four yellow mosaic viruses, *viz*., MYMV, MYMIV, HYMV and DoYMV reported from India.

The dendrogram (Fig. 4.1) revealed four major clusters of MYMV, HYMV, MYMIV and DoYMV. Each cluster consisted of strains of the corresponding virus species which are reported from different parts of India. The MYMV isolates in the present study, BgVM, BgSM and BgAM clustered with the reported MYMV strains. The isolates from Palakkad district, BgVM and BgSM branched together. The black gram isolate from the Malappuram district formed a separate branch and was phylogenetically more closely related to the MYMV strain from Tirupati [KP455992.1]. The isolates BgP1H and BgC2H, which were originally amplified using HYMV primer, formed a separate cluster. Though these two isolates were identified as MYMV isolate by homology analysis, phylogenetic analysis indicates that they are phylogenetically different from other MYMV isolates. HYMV isolates reported from other parts of India formed a separate cluster and it was phylogenetically more related to MYMV. The third major cluster included isolates of MYMIV infecting different legume hosts in different regions of India. In that also two separate clusters are formed, one includes MYMIV isolates from southern parts of India and second includes MYMIV isolates from north India. DoYMV isolates formed a fourth major cluster separately and DoYMV cluster also exhibited a clustering based on geographical location.

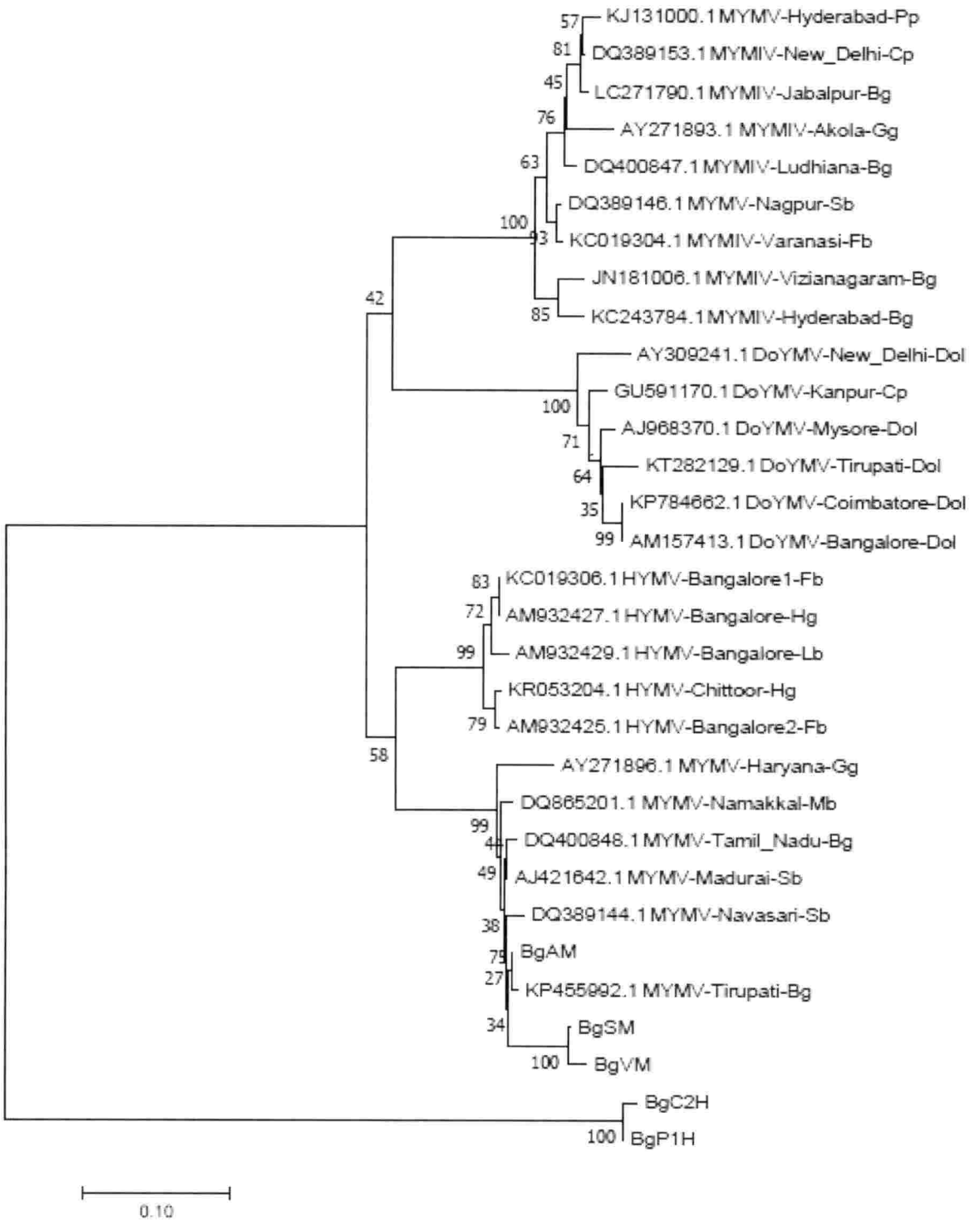


Fig. 4.1 Phylogenetic tree showing relationship among different yellow mosaic viruses. Dendrogram was constructed by alignment of MYMV black gram isolates from Kerala with other YMV isolates using MEGA 7.0.26 software

4.4.2.3 *Prediction of amino acid sequence of coat protein*

The amplified region of MYMV isolates from black gram contained a single open reading frame (ORF) of 774 nucleotide and 257 amino acids. This encodes for coat protein. The amino acid sequence was predicted using ExPASy translate tool and compared with that of other YMVs (Table 4.15 and Fig. 4.2). MYMV black gram isolates, BgVM, BgSM, BgAM, BgP1H and BgC2H shared 97-99 per cent amino acid identity among themselves. These isolates shared 97-99 per cent amino acid sequence identity with MYMV isolate from Tamil Nadu [ABD67444.1], 90-92 per cent amino acid sequence identity with HYMV [CAP69631.1], 85-86 per cent identity with MYMIV [ABD60107.1] and 79-80 per cent identity with DoYMV [ADD97723.1]. DoYMV exhibited least identity with other YMVs.

4.4 HOST RANGE STUDIES

Host range studies were carried out to find out different host plants of MYMV, by whitefly transmission. Transmission was tested on four different pulse crops and three different weed plants (Table 4.16).

Whiteflies were given 48 hrs of AAP on virus inoculum and 48 hrs of IAP on each of the ten days old pulse seedlings and fifteen days old weed plant seedlings. A minimum of five whiteflies were released on each plant. The studies revealed that virus was not transmissible to other pulses except horse gram. In horse gram, the symptoms appeared 20-22 days after inoculation (DAI). Five horse gram seedlings out of ten seedlings inoculated showed infection resulting in 50 per cent transmission. The symptoms started as a mild interveinal chlorosis (Plate 4.17a). Prominent interveinal chlorosis was observed 30 DAI (Plate 4.17b). This was observed in young leaves as well indicating systemic spread of virus within the plant. Typical yellow mosaic symptoms were observed 45 DAI (Plate 4.17c). The virus could be detected in these symptomatic plants by PCR confirming the role of horse gram as a host of the virus.

Table 4.15 Pairwise identity (%) matrix of predicted amino acid sequences of coat protein of MYMV black gram isolates with other YMVs

Isolate	BgSM	BgVM	BgAM	BgP1H	BgC2H	MYMV-TN	HYMV	MYMIV	DoYMV
BgSM	**	99.2	99.8	99.6	98.0	99.6	91.8	85.9	79.7
BgVM		**	98.8	99.6	98.0	98.8	91.8	85.9	80.1
BgAM			**	99.2	97.6	98.4	92.2	86.3	80.1
BgP1H				**	98.4	99.2	92.2	86.3	80.1
BgC2H					**	97.6	90.6	84.8	78.5
MYMV-TN						**	91.4	86.3	79.3
HYMV							**	85.2	82.1
MYMIV								**	80.5
DoYMV									**

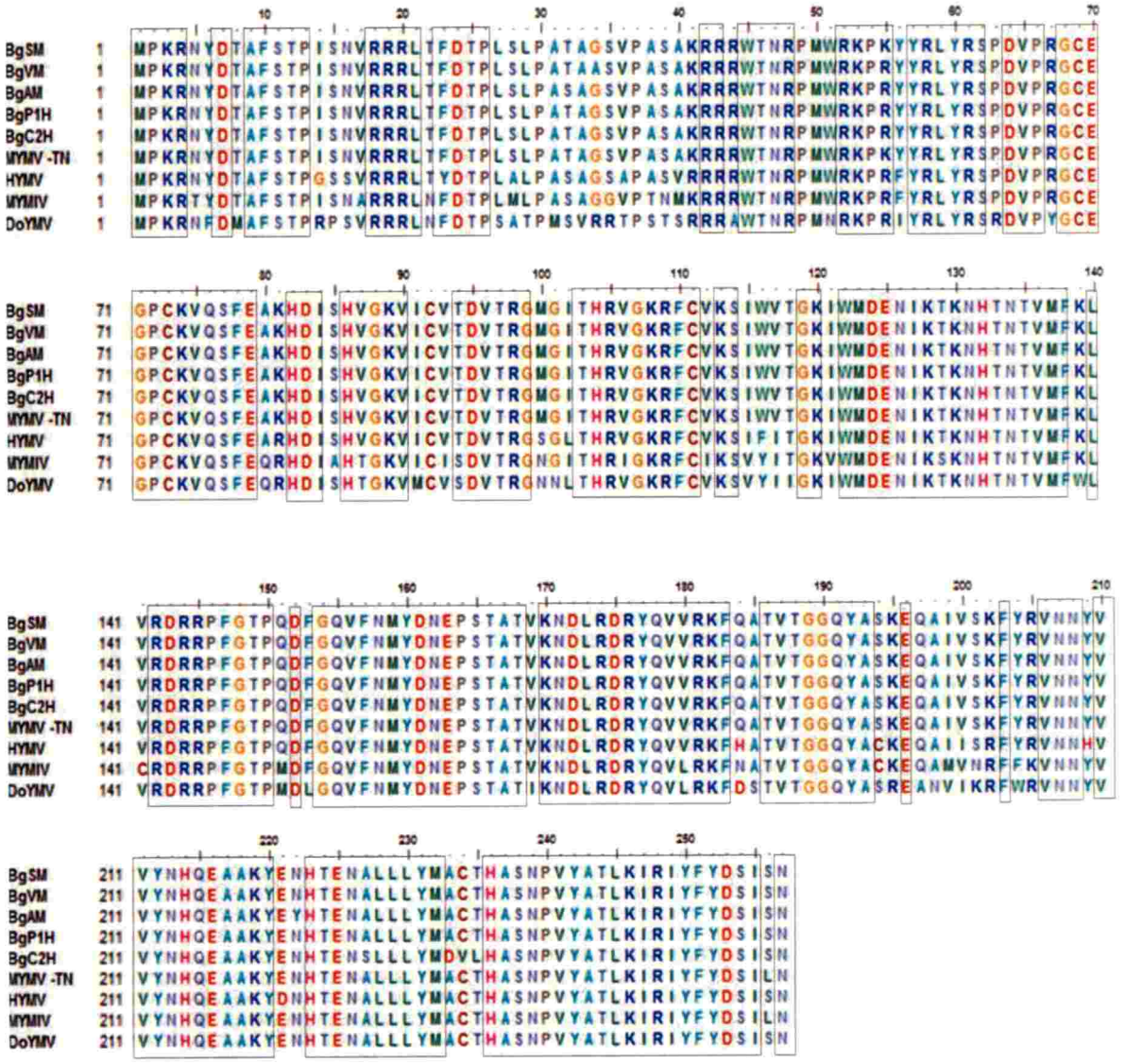


Fig. 4.2 Predicted amino acid sequence of MYMV isolates in comparison with that of other yellow mosaic viruses. Conserved regions are given in box. Accession numbers of other YMVs used: MYMV – TN – ABD67444.1, HYMV - CAP69631.1, MYMIV - ABD60107.1, DoYMV - ADD97723.1

Table 4.16 Transmission of MYMV by insect vector, whitefly (*Bemisia tabaci*) to different pulse crops and weeds

Inoculated plant species	Common name	Family	Variety used	No. of plants inoculated	No. of plants infected	Per cent infection (%)	No. of days for symptom development	Conformation of infectivity by PCR
Vigna mungo (L.) Hepper	Black gram	Leguminosae	Co 6	10	8	80	15-18	+
Vigna unguiculata	Cowpea	Leguminosae	Kanakamani	10	0	-	-	-
Vigna radiata	Green gram	Leguminosae	Co 8	10	0	-	-	-
Macrotyloma uniflorum (Lam.) Verdec.	Horse gram	Leguminosae	CRSG 19	10	5	50	20-25	+
Cajanus cajan (L) Mill sp.	Pigeon pea	Leguminosae	Co 7	10	0	-	-	-
Weeds								
Synedrella nodiflora	Nodeweed, Pig grass	Asteraceae		10	4	40	20-22	-
Cleome viscosa	Asian spider flower	Capparaceae		10	0	-	-	-
Ageratum conyzoides	Goatweed, Whiteweed,	Asteraceae		10	0	-	-	-

Among weed plants tested, symptoms were observed on *Synedrella nodiflora* only. Symptoms appeared in four seedlings out of 10 seedlings giving 40 per cent transmission rate. Symptoms appeared as yellow discolouration 20 DAI (Plate 4.18a). Later crinkling and puckering was observed 45 DAI (Plate 4.18b). However virus could not be detected in these symptomatic plants in PCR. So the symptom noticed may be the damage caused due to feeding by whiteflies or nutritional disorder.

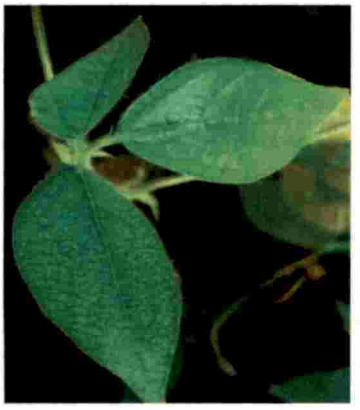
4.5 MANAGEMENT OF YELLOW MOSAIC DISEASE IN BLACK GRAM

In order to find out the effectiveness of different concentrated plant extracts, animal product, biocontrol agent, insecticide and commercially available herbal extracts against yellow mosaic disease in black gram, a pot cultue as well as a field experiment was conducted and observations on disease incidence (%), disease severity, whitefly population and yield were recorded.

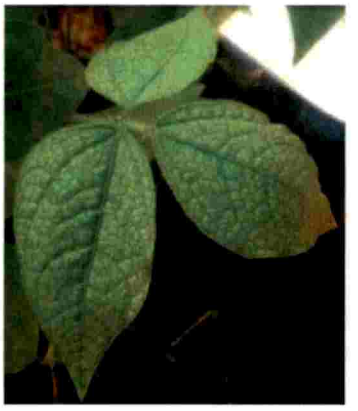
4.5.1 Pot culture studies

The effectiveness of concentrated plant eaxtracts, buttermilk, biocontrol agent and commercially available products were tested against yellow mosaic in black gram in pot culture under natural incidence. The experiment was conducted during summer 2017 in CRD design with three replications (Plate 4.19a and 4.19b). 10 per cent aqueous extracts of leaves of *Mirabilis jalapa*, *Bougainvillea spectabilis* and *Cocos nucifera* and roots of *Boerrhavia diffusa*, 10 per cent buttermilk, a commercial formulation of herbal extracts, virochek and a neem based pesticide, azadirachtin 10000 ppm @ 2 ml l^{-1} were sprayed at 15 days interval starting from 15 DAS. Biocontrol agent, *Pseudomonas fluorescence* and a commercial formulation, perfekt was applied as seed treatment @ 10g/kg seed and 1 ml l^{-1} respectively followed by foliar spray at 15 days interval @ 10 g l^{-1} and 0.5 ml l^{-1} respectively. Observations like disease incidence (%) and per cent disease severity were recorded at five days after each spray.

Plate 4.17 Symptom development in host range studies (Horse gram)

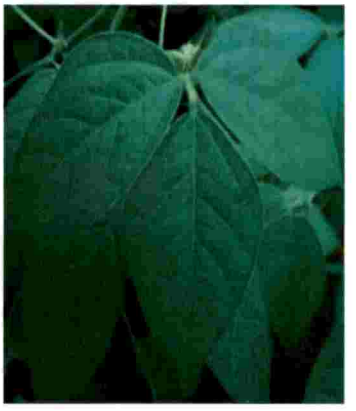


a. 23 DAI



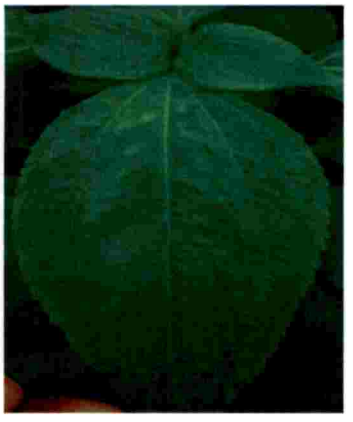
b. 30 DAI

c. 45 DAI

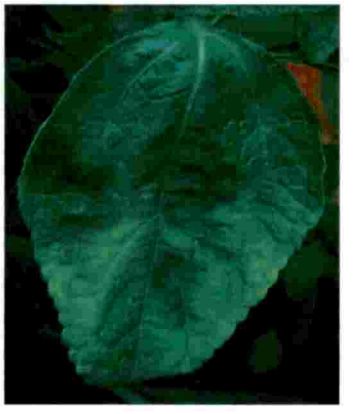


Uninoculated horse gram leaf

Plate 4.18 Symptom development in host range studies (*Synedrella nodiflora*)



a. 20 DAI



b. 45 DAI

Uninoculated leaf

Plate 4.19a General view of pot culture

Plate 4.19b Different symptoms observed on black gram plants (Variety : Co6) in pot culture

Yellow mosaic

Whitish discolouration

Chlorotic spots

Puckering

Crinkling and puckering

Interveinal chlorosis

4.5.1.1 *Effect of treatments on YMD incidence*

The disease appeared in untreated control plots 20 days after sowing and at 30 DAS, the disease incidence was 66.67 per cent which steadily increased and reached 100 per cent at 60 DAS in control (Table 4.17). At 30 DAS, T3, root extract of *Boerhaavia diffusa* exhibited complete inhibition of virus and none of the plants were infected. T9, Azadirachtin and T5, buttermilk exhibited maximum effectiveness at 30 DAS. Only 8.33 per cent disease incidence was observed in these treatments. This was on par with T1 (*Mirabilis jalapa*), T2 (*Bougainvillea spectabilis*) and T6 (*Pseudomonas fluorescens*). 25 per cent disease incidence was recorded on these treatments 30 DAS. T2 (*Bougainvillea spectabilis*), T3 (*Boerhaavia diffusa*), T5 (Buttermilk), T6 (*Pseudomonas fluorescens*) and T9 (Azadirachtin) retained its effectiveness till the end of the crop and the disease incidence in these treatments were significantly lower than that in control plots at 45 DAS and 60 DAS. The disease incidence in T1 (*Mirabilis jalapa*), increased drastically at 45 DAS (83.33%) and 91 per cent incidence was observed at 60 DAS. Commercial antiviral products, perfekt and virochek and T4 (*Cocos nucifera*) did not show any effect on mosaic incidence. The increase in disease incidence over days were lowest in T2 (*Bougainvillea spectabilis*), and T6 (*Pseudomonas fluorescens*), whereas a sudden increase in disease incidence was noticed in T3 (*Boerhaavia diffusa*), T5 (Buttermilk) and T9 (Azadirachtin) 45 DAS (Fig. 4.3).

Table 4.17 Effect of different treatments on disease incidence (Pot culture)

Tr. No.	Treatments	Disease incidence (%)		
		30 DAS	45 DAS	60 DAS
T1	Leaf extract of *Mirabilis jalapa* (10%)	25.00^{bc} (30.00)	83.33^{ab} (79.04)	91.67^{ab} (79.04)
T2	Leaf extract of *Bougainvillea spectabilis* (10%)	25.00^{bcd} (25.48)	25.0^{d} (25.48)	33.33^{d} (35.00)
T3	Root extract of *Boerhaavia diffusa* (10%)	0.00^{d} (1.43)	41.67^{bcd} (40.00)	41.67^{cd} (40.00)
T4	Leaf extract of *Cocos nucifera* (10%)	50^{ab} (45.00)	50.00^{abcd} (45.00)	66.67^{bcd} (59.52)
T5	Buttermilk (10%)	8.33^{cd} (10.95)	33.33^{cd} (30.48)	50.00^{cd} (45.00)
T6	*Pseudomonas fluorescens* - Seed treatment (10g kg seed^{-1}) and foliar spray (10g l^{-1})	25.00^{bc} (30.00)	33.33^{cd} (35.00)	33.33^{d} (35.00)
T7	Perfekt - seed treatment (1ml l^{-1}) and foliar spray (0.5ml l^{-1})	50.00^{ab} (45.00)	75.00^{abc} (64.52)	75.00^{abc} (64.52)
T8	Virochek - foliar spray (2 ml l^{-1})	50.00^{ab} (45.00)	75.00^{abc} (64.52)	91.67^{ab} (79.04)
T9	Neem based insecticide containing azadirachtin 10000ppm @ 2ml l^{-1}	8.33^{cd} (10.95)	41.67^{cd} (35.48)	50.00^{cd} (45.00)
T10	Untreated Control (sterile distilled water)	66.67^{a} (59.52)	91.67^{a} (79.04)	100.00^{a} (88.57)
	CD(0.05)	26.06	36.26	26.06

*Figures in parenthesis are arc sin transformed values

4.5.1.2 *Effect of treatments on disease severity*

Percentage leaf area showing mosaic symptoms were recorded and per cent disease severity (vulnerability index) was calculated by the formula given by Bos (1982) based on the 0-5 MYMV disease score given by Bashir (2005) (Table 4.18).

T3 *Boerhaavia diffusa*, T2 *Bougainvillea spectabilis*, T5 buttermilk , T9 neem based insecticide and T6 *Pseudomonas fluorescens* were found to be effective in reducing disease severity as in the case of disease incidence. T1, *Mirabilis jalapa* which showed effectiveness at 30 DAS (16.67%), lost its effectiveness by 45 DAS

(70.00%). The disease severity increased at slower pace in T2, *Bougainvillea spectabilis* and T6, *Pseudomonas fluorescens* (Fig. 4.4). At 60 DAS, the most effective treatments were T2, *Bougainvillea spectabilis* (21.67%), T3, *Boerhaavia diffusa* (28.33%) and T6, *Pseudomonas fluorescens* (26.67%). Treatments T7, perfekt T8, virochek and T4, *Cocos nucifera* were ineffective throughout the crop period.

Table 4.18 Effect of different treatments on disease severity or vulnerability index (Pot culture)

Tr. No.	Treatments	Per cent disease severity		
		30 DAS	45 DAS	60 DAS
T1	Leaf extract of *Mirabilis jalapa* (10%)	16.67^{bcde} (23.16)	70.00^{ab} (57.10)	80.00^{ab} (64.63)
T2	Leaf extract of *Bougainvillea spectabilis* (10%)	15.00^{cde} (18.71)	16.67^{d} (20.16)	21.67^{d} (23.64)
T3	Root extract of *Boerhaavia diffusa* (10%)	0.00^{e} (1.43)	18.33^{d} (24.31)	28.33^{cd} (31.74)
T4	Leaf extract of *Cocos nucifera* (10%)	50.00^{ab} (45.29)	46.67^{abcd} (43.08)	80.00^{ab} (63.55)
T5	Buttermilk (10%)	6.67^{de} (9.81)	28.33^{cd} (27.00)	63.33^{bc} (52.88)
T6	*Pseudomonas fluorescens* - Seed treatment (10g kg seed^{-1}) and foliar spray (10g l^{-1})	15.00^{bcde} (22.59)	26.67^{bcd} (30.94)	26.67^{cd} (30.94)
T7	Perfekt - seed treatment (1ml l^{-1}) and foliar spray (0.5ml l^{-1})	45.00^{abc} (42.13)	63.33^{abc} (54.71)	86.67^{ab} (73.12)
T8	Virochek - foliar spray (2 ml l^{-1})	35.00^{abcd} (31.44)	63.33^{abc} (53.10)	81.67^{ab} (66.01)
T9	Neem based insecticide containing azadirachtin 10000ppm @ 2ml l^{-1}	3.33^{de} (7.71)	33.33^{bcd} (30.48)	68.33^{b} (57.91)
T10	Untreated Control (sterile distilled water)	58.33^{a} (50.38)	76.67^{a} (63.59)	96.67^{a} (82.90)
	CD(0.05)	25.74	28.786	22.31

*Figures in parenthesis are arc sin transformed values.

4.5.2 Field experiment

A field experiment was conducted to evaluate the effect of different concentrated plant extracts, animal products, bio control agent, commercially

Fig. 4.3 Effect of different treatments on yellow mosaic disease incidence in pot culture

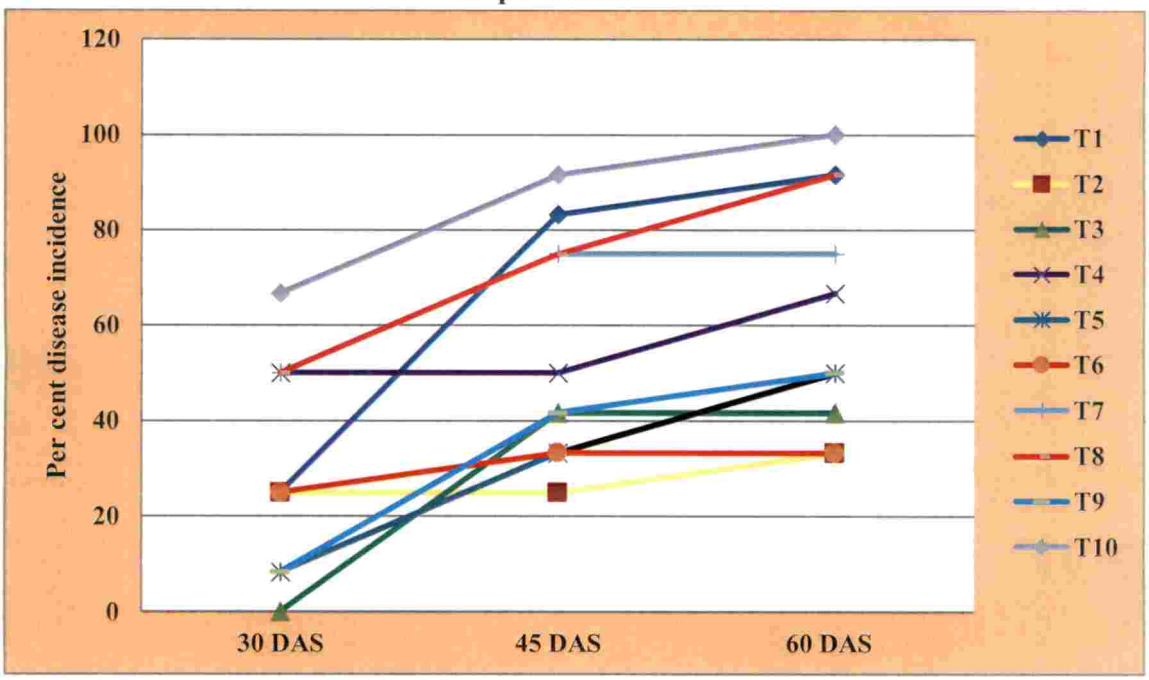
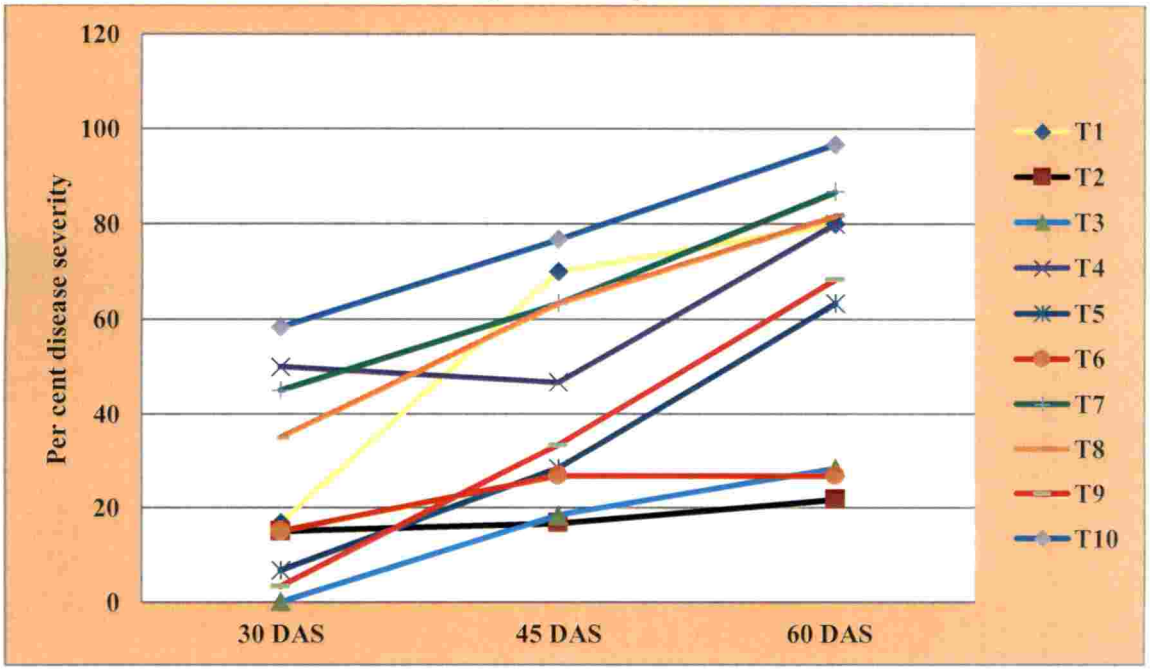


Fig. 4.4 Effect of different treatments on yellow mosaic disease severity or vulnerability index in pot culture



available antiviral principles and neem based insecticide, which were tested in pot culture studies on yellow mosaic disease in black gram plants (Plate 4.20). Observations on disease incidence (%), per cent disease severity, whitefly count and yield were recorded at five days after each spray.

4.5.2.1 *Effect of treatments on disease incidence*

The disease appeared in the field 30 days after sowing. The disease incidence recorded after each spray is given in Table 4.19. The lowest disease incidence was observed in the treatment T6, *Pseudomonas fluorescens* (1.47%) compared to incidence in control plot (6.78%) at 30DAS. But as the incidence was less, a significant difference was not observed between the treatments.

At 45 DAS also, disease incidence was lowest in T6, *Pseudomonas fluorescens* applied plots (8.23%). This was significantly lower than that in untreated control plots (17.14%) and statistically on par with T2, *Bougainvillea spectabilis* (9.04%), T3, *Boerhaavia diffusa* (8.96%), T7 Perfekt (10.63%) and T8 Virochek (9.53%). T1 *Mirabilis jalapa* (12.15%) and T4 *Cocos nucifera* (13.31%) were not effective in reducing disease incidence and were on par with untreated control (17.14%).

The effectiveness of *Pseudomonas fluorescens* was evident even 60 days after sowing. The lowest disease incidence was recorded in T6 *Pseudomonas fluorescens* (16.0%) at 60DAS. T3 *Boerhaavia diffusa* (18.85%) and T9 neem based pesticide, Azadirachtin 10000 ppm (22.56%) were on par with T6 *Pseudomonas fluorescens* (16.01%). T5 (buttermilk) was also effective in reducing the disease incidence at 60 DAS, though not on par with T6 (*Pseudomonas fluorescens*). The effectiveness of Perfekt (T7) (25.69%) and Virochek (T8) (24.99%) reduced with age of the plant and the disease incidence was on par with that of the untreated control. The effect of nine different treatments on disease incidence as percentage reduction over the control is represented in Fig. 4.5.

Plate 4.20 General view of experimental field

Pseudomonas fluorescens treated plot

Boerhaavia diffusa treated plot

Untreated control plot

Table 4.19 Effect of different treatments on disease incidence

Tr. No.	Treatments details	Disease incidence (%)		
		30 DAS	45 DAS	60 DAS
T1	Leaf extract of *Mirabilis jalapa* (10%)	3.29 (1.90)	12.15^{abc} (3.46)	33.96^{a} (5.80)
T2	Leaf extract of *Bougainvillea spectabilis* (10%)	1.81 (1.49)	9.04^{bc} (2.99)	21.98^{bcd} (4.68)
T3	Root extract of *Boerhaavia diffusa* (10%)	1.61 (1.35)	8.96^{bc} (2.98)	18.85^{cd} (4.30)
T4	Leaf extract of *Cocos nucifera* (10%)	1.5 (1.36)	13.31^{ab} (3.59)	29.24^{ab} (5.27)
T5	Buttermilk (10%)	1.32 (1.28)	10.88^{b} (3.29)	23.04^{bc} (4.78)
T6	*Pseudomonas flourescens* - Seed treatment (10g kg seed^{-1}) and foliar spray (10g l^{-1})	1.47 (1.21)	8.23^{c} (2.82)	16.01^{d} (3.90)
T7	Perfekt - seed treatment (1ml l^{-1}) and foliar spray (0.5ml l^{-1})	1.82 (1.41)	10.63^{bc} (3.24)	25.69^{abc} (5.04)
T8	Virochek - foliar spray (2 ml l^{-1})	1.82 (1.40)	9.53^{bc} (3.08)	24.99^{abc} (4.98)
T9	Neem based insecticide containing azadirachtin 10000ppm @ 2ml l^{-1}	2.35 (1.55)	9.59^{bc} (3.09)	22.56^{bcd} (4.74)
T10	Untreated Control (sterile distilled water)	6.78 (2.67)	17.14^{a} (4.14)	35.73^{a} (5.79)
	CD(0.05)	NS	0.749	0.801

*The figures in the paranthesis are square root transformed values.

4.5.2.2 *Effect of treatments on per cent disease severity*

Per cent disease severity (PDS) or vulnerability Index (VI) was calculated using the formula given by Bos (1982) from the percentage leaf area affected applying the scoring pattern given by Bashir (2005).

The per cent disease severity recorded in various treatments is given in Table 4.20. Lowest disease severity was recorded in *Pseudomonas* treated plants (T6) throughout the period of experiment. At 30 DAS, T2 (*Bougainvllea spectabilis*), T3 (*Boerhaavia diffusa*), T5 (Buttermilk), T8 (Virochek) and T9 (Azadirachtin) were

effective in reducing disease severity and were statistically on par with T6, *Pseudomonas fluorescens* (3.56%) with per cent disease severity of 5.10, 2.67, 3.68, 4.89 and 7.09 per cent respectively.

Table 4.20 Effect of different treatments on disease severity or vulnerability index

Tr. No.	Treatments	Disease severity (%)		
		30 DAS	45 DAS	60 DAS
T1	Leaf extract of *Mirabilis jalapa* (10%)	10.43^{ab} (3.24)	26.67^{ab} (31.01)	43.11^{ab} (41.04)
T2	Leaf extract of *Bougainvillae spectabilis* (10%)	5.10^{bcd} (2.32)	20.00^{bc} (25.54)	33.33^{de} (35.25)
T3	Root extract of *Boerhaavia diffusa* (10%)	2.67^{cd} (1.76)	18.67^{bc} (25.11)	31.11^{e} (33.86)
T4	Leaf extract of *Cocos nucifera* (10%)	8.14^{abc} (2.91)	24.44^{ab} (29.49)	42.22^{abc} (40.52)
T5	Buttermilk (10%)	3.68^{cd} (1.95)	25.33^{ab} (30.11)	40.00^{bcd} (39.21)
T6	*Pseudomonas flourescens* - Seed treatment (10g kg seed^{-1}) and foliar spray (10g l^{-1})	3.56^{d} (1.58)	12.00^{c} (20.12)	19.11^{f} (25.91)
T7	Perfekt - seed treatment (1ml l^{-1}) and foliar spray (0.5ml l^{-1})	8.68^{abc} (3.00)	24.00^{ab} (29.31)	46.22^{ab} (42.82)
T8	Virochek - foliar spray (2 ml l^{-1})	4.89^{bcd} (2.10)	22.22^{b} (28.05)	34.22^{cde} (35.73)
T9	Neem based insecticide containing azadirachtin 10000ppm @ 2ml l^{-1}	7.09^{bcd} (2.73)	22.22^{b} (28.09)	41.33^{bcd} (39.99)
T10	Untreated Control (sterile distilled water)	16.00^{a} (4.06)	34.67^{a} (36.05)	50.67^{a} (45.38)
	CD (0.05)	1.294	7.520	4.916

*The figures in the paranthesis are transformed values (30 DAS – square root transformation. 45 and 60 DAS – arcsin transformation).

The effectiveness of T2, *Bougainvillea spectabilis* (20%) and T3, *Boerhaavia diffusa* (18.67%) in reducing disease severity continued upto 45 DAS and they were on par with T6, *Pseudomonas fluorescens* (12.00%). T8, Virochek (22.22%) and T9, neem based pesticide, Azadirachtin 10000 ppm (22.22%) were also significantly superior over untreated control (34.67%). T5, buttermilk lost its effectiveness by 45

DAS, though it was found to be effective in pot culture studies, indicating that buttermilk losses its efficacy in field evaluation.

At 60 DAS, the second lowest disease severity was recorded in T3, *Boerhaavia diffusa* (31.11%) which was on par with that of T2, *Bougainvillea spectabilis* (33.33%) and T8, Virochek (34.22%). T9, neem based pesticide, Azadirachtin 10000 ppm (41.33%) was also effective in reducing disease severity at 60 DAS. None of the treatments were as effective as *Pseudomonas*. T8, virochek which was not effective in the pot culture study was found to be effective in field study. This indicates that it is effective at low level of incidence of the virus and losses its effectiveness if the disease incidence is higher as in pot culture where 100 per cent incidence was observed in control plants. The effect of nine different treatments on disease severity is represented in Figure 4.6.

4.5.1.3 *Effect of treatments on whitefly population*

The whitefly count on different treatments from each plot was recorded five days after each spray. After the first spray countable whitefly population was not observed in the field. The whitefly population was observed after second spray onwards. The data recorded are given in Table 4.21.

The lowest number of whiteflies was observed on T9, Neem based insecticide containing azadirachtin (0.77) was on par with that in T6, *Pseudomonas fluorescens* (0.81). While the number of whiteflies remained same in T9 even at 60 DAS, *Pseudomonas fluorescens* applied plots showed a drastic decline in the number of whiteflies (0.56) at 60 DAS showing the effectiveness of *Pseudomonas* for long period (Fig. 4.7). T2 (*Bougainvillea spectabilis*) and T3 (*Boerhaavia diffusa*) were not effective in reducing whitefly population though they were as effective as T6, *Pseudomonas fluorescens* in reducing disease incidence and severity. This indicates that their action is not based on vector management.

Fig. 4.5 Effect of different treatments on reduction in disease incidence over the control under field condition

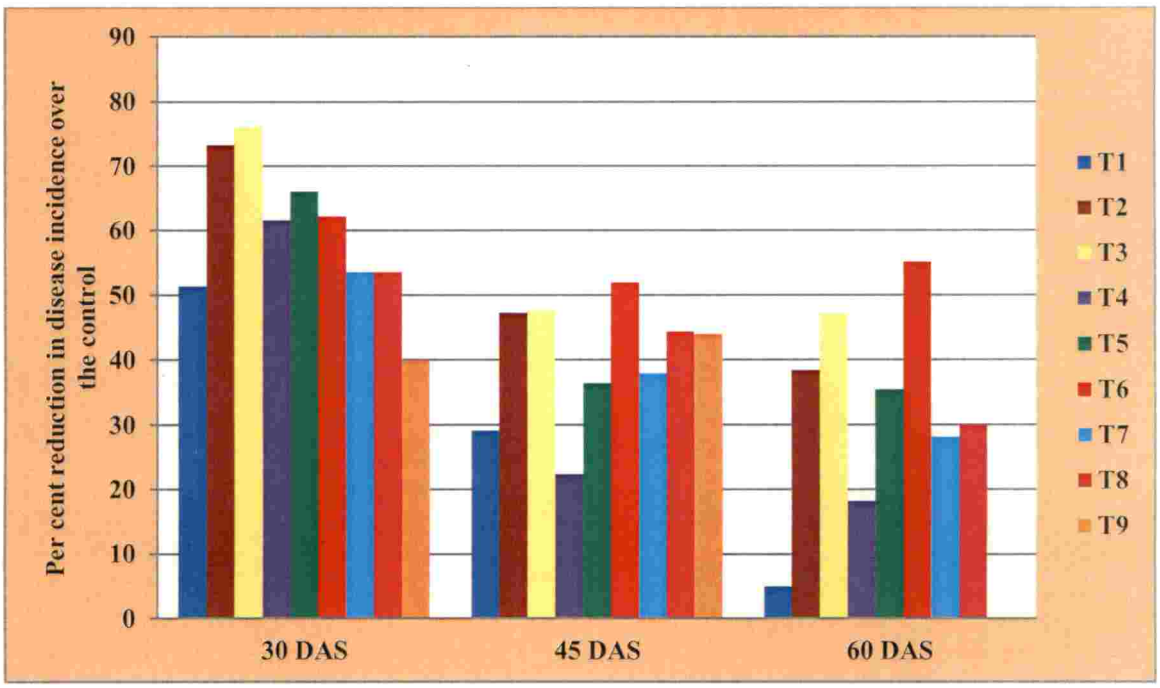


Fig. 4.6 Effect of different treatments on disease severity or vulnerability index under field condition

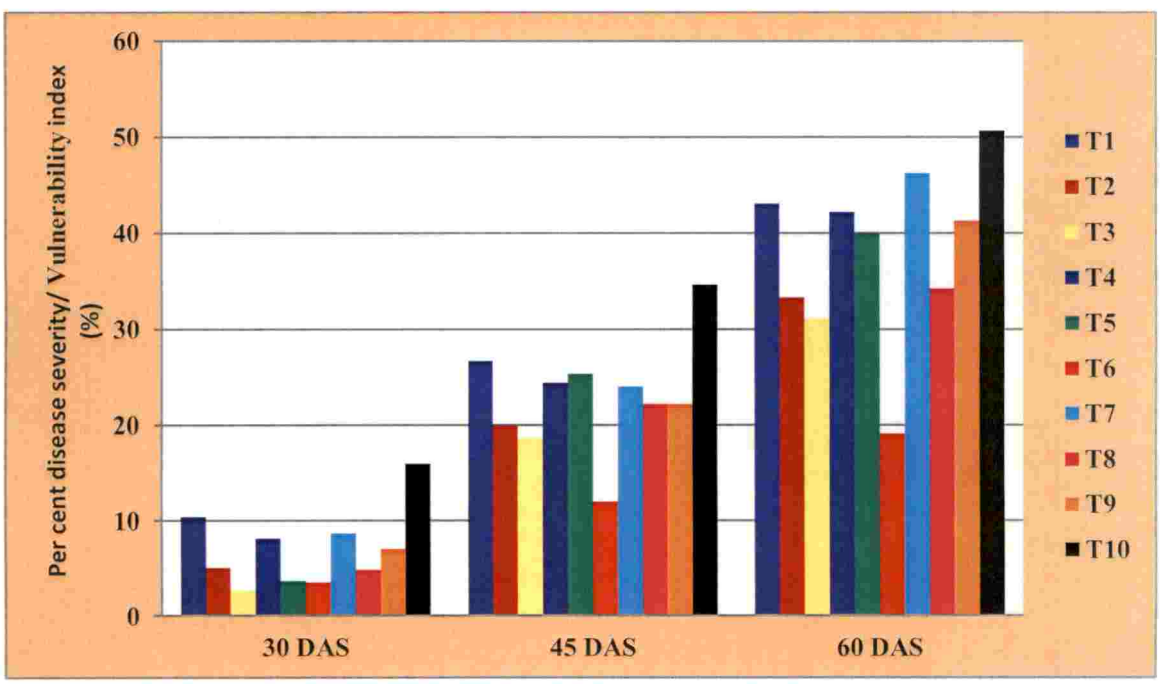


Table 4.21 Effect of different treatments on whitefly count

Tr. No.	Treatments	Mean whitefly count		
		30 DAS	45 DAS	60 DAS
T1	Leaf extract of *Mirabilis jalapa* (10%)	0.87^{bcd} (0.92)	1.67^{a} (1.27)	1.67^{a} (1.29)
T2	Leaf extract of *Bougainvillea spectabilis* (10%)	1.47^{abc} (1.20)	1.60^{ab} (1.25)	1.33^{a} (1.15)
T3	Root extract of *Boerhaavia diffusa* (10%)	1.27^{abc} (1.11)	0.93^{bcd} (0.96)	1.33^{a} (1.13)
T4	Leaf extract of *Cocos nucifera* (10%)	1.20^{abc} (1.09)	1.20^{abc} (1.09)	1.53^{a} (1.24)
T5	Buttermilk (10%)	0.87^{cd} (0.90)	1.13^{abcd} (1.06)	1.40^{a} (1.18)
T6	*Pseudomonas fluorescens* - Seed treatment (10g kg seed^{-1}) and foliar spray (10g l^{-1})	0.67^{d} (0.81)	0.73^{cd} (0.83)	0.33^{b} (0.56)
T7	Perfekt - seed treatment (1ml l^{-1}) and foliar spray (0.5ml l^{-1})	0.93^{bcd} (0.96)	1.13^{abcd} (1.06)	1.33^{a} (1.15)
T8	Virochek - foliar spray (2 ml l^{-1})	0.93^{bcd} (0.95)	1.00^{abcd} (0.98)	1.27^{a} (1.12)
T9	Neem based insecticide containing azadirachtin 10000ppm @ 2ml l^{-1}	0.60^{d} (0.77)	0.60^{d} (0.77)	0.53^{a} (0.72)
T10	Untreated Control (sterile distilled water)	1.40^{ab} (1.18)	1.40^{ab} (1.18)	1.87^{a} (1.35)
	CD (0.05)	0.262	0.310	0.266

*The figures in the paranthesis are square root transformed values.

4.5.1.4 *Effect of treatments on yield*

The yield from black gram field was recorded at the time of harvesting and given in Table 4.22. The analysis of yield data from the field revealed that highest yield was recorded in T6, *Pseudomonas fluorescens* (489.16 Kg/ha), this was on par with that of T2, *Bougainvillea spectabilis* (395.56 Kg/ha) and T3, *Boerhaavia diffusa* (393.78 Kg/ha) and the lowest yield was recorded in untreated control (282.58Kg/ha) (Fig. 4.8).

Table 4.22 Effect of different treatments on yield

Tr No.	Treatments	Mean yield	
		Yield (g/6m^2)	Yield (kg/ha)
T1	Leaf extract of *Mirabilis jalapa* (10%)	184.27	307.11^{bc}
T2	Leaf extract of *Bougainvillae spectabilis* (10%)	237.33	395.56^{ab}
T3	Root extract of *Boerhaavia diffusa* (10%)	236.27	393.78^{ab}
T4	Leaf extract of *Cocos nucifera* (10%)	211.89	353.16^{bc}
T5	Buttermilk (10%)	231.15	385.24^{b}
T6	*Pseudomonas flourescens* - Seed treatment (10g kg seed^{-1}) and foliar spray (10g l^{-1})	293.49	489.16^{a}
T7	Perfekt - seed treatment (1ml l^{-1}) and foliar spray (0.5ml l^{-1})	186.45	310.76^{bc}
T8	Virochek - foliar spray (2 ml l^{-1})	229.12	381.87^{b}
T9	Neem based insecticide containing azadirachtin 10000ppm @ 2ml l^{-1}	234.56	390.93^{b}
T10	Untreated Control (sterile distilled water)	169.55	282.58^{c}
	CD (0.05)		96.348

Fig. 4.7 Effect of different treatments on whitefly population in field

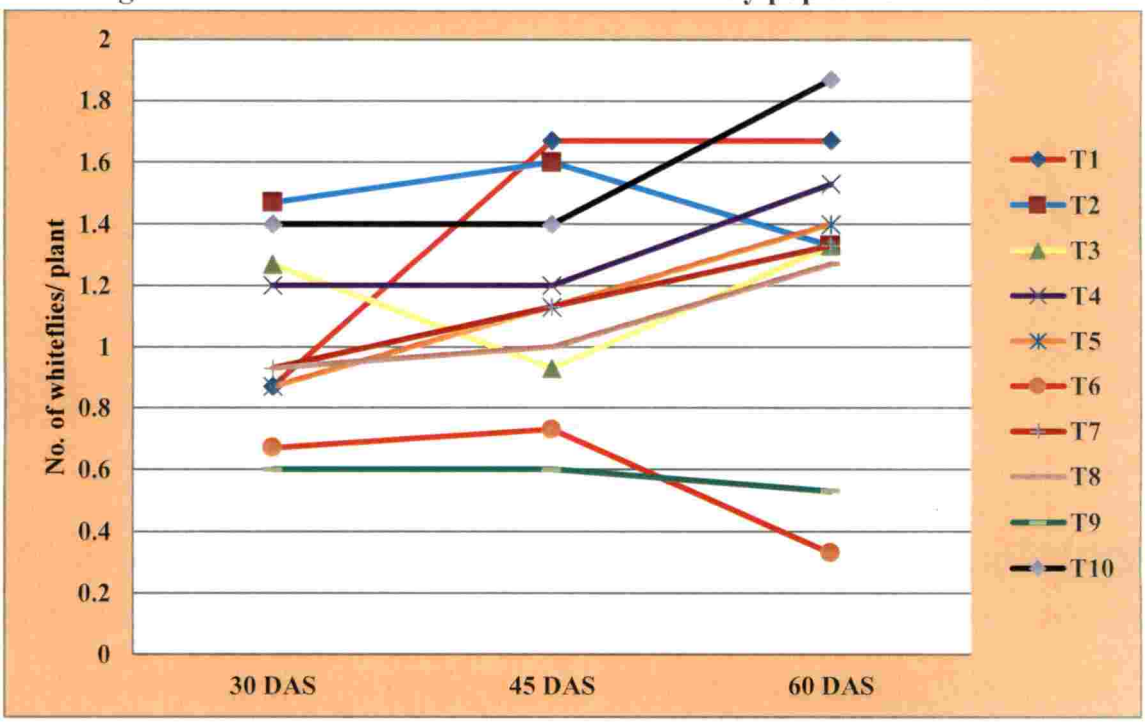
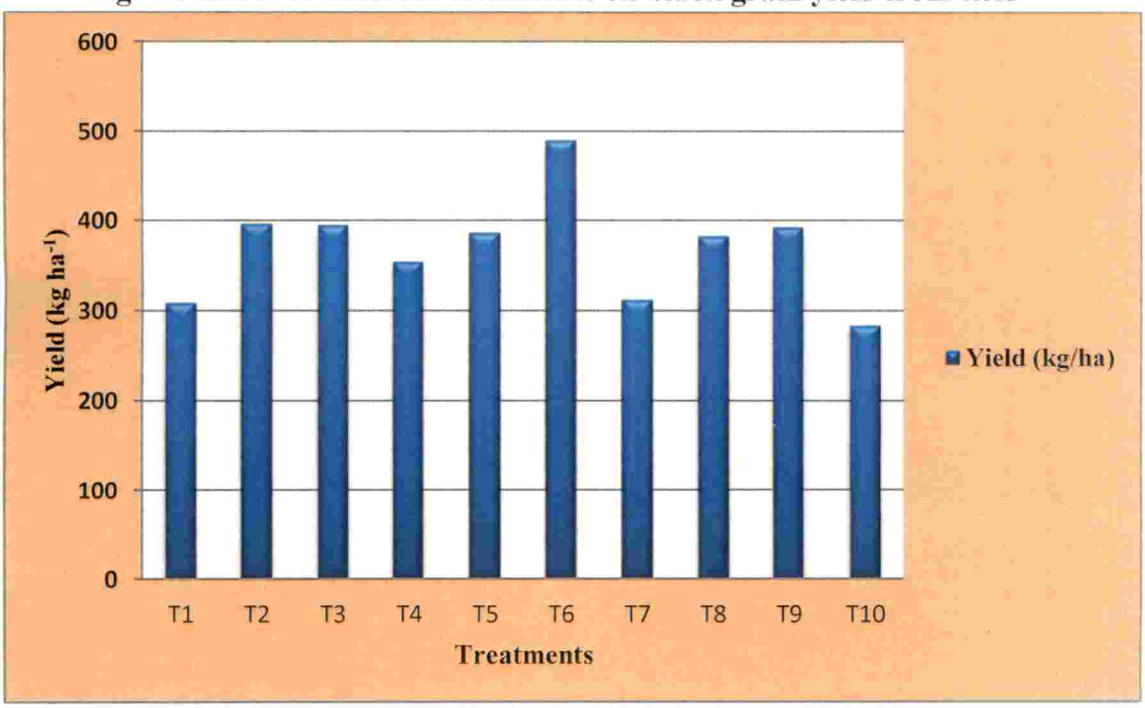


Fig. 4.8 Effect of different treatments on black gram yield from field



Discussion

5. DISCUSSION

Yellow mosaic disease (YMD) affects almost all legume crops and causes a major threat to pulse production in India. YMD is the major constraint in the black gram production in India. It was first reported in urd bean by Nair and Nene in 1973. Later it was recorded from various parts of the country. Various studies were done on yellow mosaic disease affecting various pulse crops in India. But no studies were undertaken on the yellow mosaic disease affecting black gram in Kerala.

The present study was undertaken on identification, characterization and management of yellow mosaic disease of black gram in Kerala. A survey was conducted in pulse growing areas of Palakkad and Malappuram districts, symptomatology was studied and the virus involved was identified by PCR. Coat protein region of the viral genome was amplified, sequenced and compared with that of the other yellow mosaic viruses deposited in NCBI database. The virus culture was established through whitefly transmission and host range was studied. Different botanicals, biocontrol agent, animal product and commercially available antiviral products were evaluated for the management of the disease. The results obtained are briefly discussed in this chapter.

A purposive sampling survey was carried out in black gram growing areas of Palakkad and Malappuram districts during March 2017 - April 2018 at six different locations *viz*., Othaloor, Koodallur, Vadavannur, Karippode, Sankaramangalam and Pattambi of Palakkad district covering eight fields and at three different locations *viz*., Pulamanthole, Amarambalam and Chokkad of Malappuram district covering five fields. The survey revealed that the disease incidence vary from location to location. The highest as well as the lowest incidence was observed in Palakkad district. 100 per cent incidence was observed in Vadavannur during December 2017, where as the lowest disease incidence was observed in Othaloor during March 2017. The average YMD incidence was highest in Palakkad district with an average incidence of 50.00

per cent whereas in Malappuram district, the average incidence was 31 per cent. Disease incidence increased with the age of the crop. The same trend was reported by other scientists also in urd bean (Biswas *et al.*, 2009) and in mungbean in Uttarpradesh (Nene, 1972), Haryana (Singh, 1979), south Karnataka (Manjunatha *et al.*, 2013), southern Gujarat (Pawar and Mahatma, 2013) and north eastern Karnataka (Meti *et al.*, 2017) with disease incidence varying between 65.5 to 72, 5-100, 70-100, 31-100, 45-70 and 5.66-33.33 per cent respectively.

Different symptoms observed in the survey fields were intermingled yellow and green mosaic patches on the leaves, slight puckering of the leaves with yellow mosaic symptom, green and yellow specks on the leaves with cupping of the leaves, yellow mosaic pattern with necrotic spots on the mature leaves of infected plants, total yellowing of the leaves with irregular whitish discolouration turning to papery white leaves on maturity, deformation of leaves with reduced size and narrow elongated leaves. Stunting of infected plants and reduction in number of pods, size of pods and size of seeds were also observed in the survey fields. Similar symptoms were observed by Nene (1972), Singh and De (2006), Rouhibakhsh *et al.* (2008), Naimuddin *et al.* (2011) and Satya *et al.* (2013) on black gram plants. However the yellow discolouration of the YMD infected seeds which was reported by Nene (1973) and Varma *et al.* (1992) on black gram seeds was not observed in this study.

Vein banding and complete yellowing of the leaves with interveinal bronzing were the two new symptoms observed in the present study which were not reported earlier. PCR confirmed the presence of virus in these samples. Further transmission studies are required to know whether the symptom is caused by virus alone or some other factors are also involved.

Varieties grown in the survey fields were local varieties, VBN 3, VBN 4, VBN 6, VBN 8 and Co-6. Cent per cent incidence was observed on a local variety in first field of Vadavannur. VBN 3, VBN 4, VBN 6 and VBN 8 are varieties released from National Pulses Research Centre, Vamban, Tamil Nadu in the year 2000, 2003,

2011 and 2016 respectively. High level of incidence ranging from 44 to 80 per cent was observed on varieties VBN 3 and VBN 4. VBN 6 and VBN 8 were released as yellow mosaic resistant varieties. 60 per cent disease incidence was recorded on VBN 6 at Amarambalam II. The virus could be detected in these samples by PCR. This confirms the breakdown of resistance in this variety which was earlier reported by Equbal *et al.* (2015). The resistance against yellow mosaic is easily broken down due to the highly evolving nature of begomoviruses. Though mild yellow mosaic symptoms were observed in VBN 8, the presence of virus could not be detected in PCR, confirming the resistance of the variety to yellow mosaic disease.

Two representative samples were sent for electron microscopic studies at Advanced Centre for Plant Virology, IARI, New Delhi. Electron micrograph revealed the presence of geminate particles in the samples. Since this indicates the presence of virus belonging to the genus *Begomovirus*, whitefly transmitted geminivirus, which is the most widely occurring geminivirus and reported to cause considerable yield loss in black gram (Varma and Malathi, 2003), white fly transmission of the virus was attempted by releasing viruliferous whiteflies on to healthy black gram seedlings. Symptoms were observed in the black gram plants 15-18 days after inoculation. This confirmed the presence of a *Begomovirus* in the samples collected.

PCR offers a sensitive method for detection of begomoviruses, which is present in a very low concentration in the host. The quality of DNA extracted from the infected samples is very important for the accurate detection of virus. The high level of phenolic compounds and polysaccharides present in the black gram plants and low level of viral DNA in yellow mosaic infected samples makes the isolation of total nucleic acid for PCR amplification difficult (Swanson *et al.*, 1992). The total genomic DNA was extracted from the black gram samples collected from various locations following two different isolation protocols, modified CTAB method (Murray and Thompson, 1980) and Gem CTAB method (Rouhibakhsh *et al.*, 2008). But good quality DNA suitable for PCR amplification was obtained with Gem CTAB

method only. The method is reported to be suitable for the extraction of good quality DNA from mature leguminous leaves highly rich in polyphenols, tannins and polysaccharides (Rouhibakhsh *et al.*, 2008). The procedure was followed with slight modifications like increasing the speed of centrifugation for precipitation of DNA from 10,000 rpm to 12,000 rpm, increasing the time of incubation in chloroform: isoamyl alcohol for removal of proteins and increasing number of alcohol washes. The quality of DNA extracted using the modified CTAB method was poor. OD260/OD280 values of the DNA were much below 1.8 indicating protein contamination in the samples. Whereas, OD260/OD280 values of DNA isolated using Gem CTAB method was in the range of 1.71 to 2.2 which indicates good quality DNA without much RNA and protein contamination. The DNA extracted using modified CTAB method did not yield amplification in PCR. Haq *et al.* (2011) isolated total DNA from the mature black gram leaves infected with yellow mosaic disease using the same protocol. Satya *et al.* (2013) also used the Gem CTAB method for isolating good quality DNA from yellow mosaic infected mung bean samples. The same procedure was followed by Bhagyashree *et al.* (2017) for isolation of DNA from the yellow mosaic infected lima bean leaf samples. But as a contrary to the findings of the present study, modified CTAB method was successfully adopted by Obaiah *et al.* (2014) and Gautam *et al.* (2014) and isolated PCR amplifiable total plant DNA from yellow mosaic infected black gram samples and wild *Vigna* species respectively.

Polymerase chain reaction was carried out to identify the virus present in the samples. Total nucleic acid extracted from the symptomatic black gram samples from different locations were subjected to PCR amplification using virus specific primers. Initial screening of the samples was done using a universal degenerate primer (Deng primer) specific to *Begomovirus* group (Deng *et al.* 1994) for confirming the presence of *Begomovirus* in the samples collected. Out of the 13 fields surveyed, positive amplification for universal begomovirus primer was obtained in 10 fields confirming

the presence of *Begomovirus* in majority of the samples tested. Deng primer was used for the initial detection of *Begomovirus* in yellow mosaic infected leaf samples of various leguminous crops by several workers (Islam *et al.*, 2012, Maheshwari *et al.*, 2014 and Reddy *et al.*, 2015).

For the detection of the virus species present in the diseased samples, the samples were subjected to PCR amplification using virus specific primers targeting the coat protein region of yellow mosaic viruses, the group of begomoviruses affecting legumes. MYMV, MYMIV and HYMV are the yellow mosaic viruses reported from black gram. Species specific primers designed by Naimuddin and Akram (2010) to amplify a region of DNA A including the coat protein gene of these viruses were used in the present study to identify the virus.

Standardization of PCR conditions are very essential for the successful detection of viral DNA. The annealing temperature and DNA concentration were optimized for each primer. Universal degenerate primer showed amplification at 56.8°C with an amplicon of 550 bp size. Species specific primers for MYMV and HYMV showed amplification at 55.3°C and yielded amplicons of 1000 bp and 1050 bp size respectively. Amplification of viral DNA was best at 95 ng/µl concentration for both MYMV and HYMV primers. MYMV was detected in nine fields out of the 13 fields surveyed. HYMV was detected in six out of 13 fields surveyed. Mixed infections of MYMV and HYMV were observed in five fields out of these six fields. These mixed infections can lead to emergence of new recombinant strains of the virus. Mixed infections in the field are reported to be one of the major factors in development of new strains of the virus through recombination and reassortment of DNA A and B components (Padidam *et al.*, 1999). MYMIV was not detected in any sample. This is in accordance with the earlier findings that MYMIV is prevalent in northern, central and eastern India and MYMV in south India (Usharani *et al.*, 2004 and Karthikeyan *et al.*, 2004). Obaiah *et al.* (2014) detected MYMIV from the yellow mosaic infected black gram samples in Andhra Pradesh by PCR using coat protein

gene specific primers, RHA F and AC-abut. Naimuddin *et al.* (2011) detected MYMIV in the wild relative of *Vigna* species with primers specific to AV1, AC1, AC2 and AC3 gene sequences.

Even though mixed infection was detected, MYMV was the predominant virus which was observed in most of the fields surveyed. The presence of MYMV species was detected in yellow mosaic infected black gram samples from Tamil Nadu through PCR with full length DNA A (AV-abut and AC-abut) and DNA B (BV-abut and BC-abut) abutting primers (Haq *et al.* 2011). Maheshwari *et al.* (2014) identified two different YMV species *viz.*, MYMV and HYMV from Tamil Nadu in different pulses like black gram, cowpea, horse gram and green gram using YMV coat protein gene specific primers. They observed mixed infection of MYMV and HYMV in green gram. Mixed infections of more than one virus are reported in pulses by many workers. Biswas *et al.* (2009) reported mixed infections of MYMIV, *Urdbean leaf crinkle virus* and ground nut bud necrosis virus in black gram.

The PCR amplified products of five representative isolates were sequenced and homology analyzed. The results showed that three MYMV isolates, BgVM, BgSM and BgAM are more similar to MYMV isolates reported from other parts of the country. The three isolates of MYMV, BgVM, BgSM and BgAM showed maximum nucleotide identity (94-99%) with different *Mungbean yellow mosaic virus* isolates reported from different regions of India. All three isolates showed maximum identity of 99 per cent with *Mungbean yellow mosaic virus* segment DNA A, complete sequence [DQ400848.1] reported from black gram from Tamil Nadu. BgSM isolate showed 99 per cent identity with *Mungbean yellow mosaic virus* isolate Tirupati segment DNA-A, complete sequence [KP455992.1] as well. Whereas, BgVM and BgAM showed 98 per cent identity with this isolate. These isolates showed 94 per cent identity with the *Mungbean yellow mosaic virus* isolate Haryana segment DNA-A, complete sequence [AY271896.1] which indicates that the isolates are more similar to the isolates from neighbouring states than that from distant places.

The isolate BgVM showed 99 per cent identity with *Horsegram yellow mosaic virus* segment DNA-A complete sequence, isolate horse gram [AM932427.1] whereas BgSM and BgAM showed only 86 per cent identity with HYMV horse gram isolate. The sample BgVM had given positive amplification for HYMV as well using the virus specific primer. The coexistence of both these viruses in the sample may be the reason for higher identity of BgVM isolate with the reported HYMV isolate. All the three isolates showed less than 80 per cent identity with the MYMIV isolates available in NCBI databases.

Interestingly, isolates BgP1H and BgC2H, which were detected as HYMV by PCR amplification shared maximum nucleotide homology with MYMV isolates. Both the isolates shared 99 per cent sequence homology with MYMV isolates from Pudukottai and Tirupati. Hence these isolates may be MYMV itself or a recombinant virus evolved as a result of mixed infection. Full genome sequencing of DNA A of these isolates is required to confirm its identity.

Fauquet *et al.* (2008) advocated whole genome sequence comparison for identifying new species. But coat protein genes are used by several workers for species detection due to the highly conserved nature of CP gene (Brown *et al.* 2001). ICTV has accepted coat protein region as a desirable marker for identification of virus where whole genome sequence is not available (Rybicki, 1994). Naimuddin and Akram (2010) detected the presence of MYMIV and DoYMV in cowpea using the same set of virus specific primers amplifying the coat protein region. Maheshwari *et al.* (2014) amplified the coat protein region of YMV infecting black gram, green gram, cowpea and horse gram. They identified the virus affecting black gram, green gram and cowpea as a variant of MYMV and YMV affecting horse gram as HYMV based on the nucleotide identity in the coat protein region. They observed a considerable variability in the 5' end of CP gene and reported coat protein gene sequencing as a rapid and reliable method for *Begomovirus* detection and identification. Prema and Rangaswamy (2018) identified yellow mosaic virus

infecting mungbean at Hebbal area in Bangalore to be a variant of MYMV and not MYMIV based on the homology of the coat protein gene sequence with various isolates.

Naimuddin and Akram (2010) reported the presence of MYMIV and DoYMV in cowpea. Maheshwari *et al.* (2014) detected the presence of MYMV and HYMV in green gram samples collected from Vamban and Coimbatore regions of Tamil Nadu. Yang *et al.* (2008) reported mixed infection of two different begomoviruses in *Malvastrum coromandelianum.* Present study also revealed the occurrence of mixed infection of MYMV and HYMV in black gram in Kerala. This indicated that mixed infection of *Begomovirus* are very common in nature. These mixed infections can lead to development of new strain or species through recombination and reassortment of viral DNA components. Presence of *Begomovirus* with bipartite and monopartite genomes in a single host and presence of recombinant begomoviruses are reported earlier (Ravi *et al.*, 2006).

Malathi *et al.* (2005) and John *et al.* (2008) detected MYMIV with a variant DNA B component affecting cowpea in north and western India. Javaria *et al.* (2007) detected MYMIV from cowpea and suggested that the presence of a variant DNA B component might be the reason for widening the host range of MYMIV. These studies suggest that mixed infection plays a major role in the evolution of begomoviruses.

Phylogenetic tree constructed based on the alignment of coat protein gene sequences of the yellow mosaic viruses infecting black gram plants collected from the survey locations with that of 26 different isolates of yellow mosaic viruses available in NCBI database revealed four major clusters of MYMV, HYMV, MYMIV and DoYMV. The dendrogram revealed a clear demarcation between the species, and each species formed a separate cluster. HYMV and MYMV were more related to each other. MYMIV and DoYMV isolates clustered into two major clusters. Within the major cluster, the isolates branched according to the geographic location. The

isolates BgVM, BgSM and BgAM clustered with the MYMV isolates which indicated that the yellow mosaic virus infecting black gram in Kerala is MYMV. The isolates from Palakkad are closer to each other while the isolate from the Malappuram branched with the MYMV black gram isolate from Tirupati [KP455992.1]. However, the isolates BgP1H and BgC2H, which were originally amplified with HYMV primer formed a separate branch indicating that they are phylogenetically different from the other isolates. This might have happened due to the recombination between viruses. Full genome sequencing should be carried out to know whether the isolates show recombination. The sequence of coat protein region can only give an indication. MYMIV and DoYMV isolates branched into entirely separate clusters and hence it is evident that the YMV infecting black gram in Kerala are more related to MYMV followed by HYMV phylogenetically rather than the MYMIV and DoYMV strains.

This is in accordance with earlier findings where the dendrogram consisting of four major clusters were formed by multiple sequence alignment of nucleotides and deduced aminoacid sequences of coat protein gene of different legumes infecting begomoviruses and with that of MYMIV-[CpKn] and DoYMV-[CpKn] cowpea isolates (Naimuddin and Akram, 2010). The first cluster of MYMIV strains included isolate MYMIV-[CpKn] and the fourth cluster of DoYMV strains included isolate DoYMV-[CpKn] and both isolates were distantly related.

A geographic relationship existing between the isolates of YMV was earlier reported by several workers. Haq *et al.* (2011) found out that in a phylogenetic dendrogram based on the alignment of complete DNA A sequences of MYMV isolates from Tamil Nadu (Pudukottai district) with other known *Begomovirus* sequences, the isolate segregate with the MYMV isolates from southern India and one isolate from western India.

Obaiah *et al.*, (2011) constructed a phylogenetic tree based on the alignment of DNA A sequence of yellow mosaic virus isolates collected from black gram from

Andhra Pradesh with 22 geminivirus sequences from NCBI database. It formed two major clusters of MYMIV and MYMV in which the isolates from Andhra Pradesh was a part of the sub cluster of the MYMIV.

Maheshwari *et al.* (2014) reported that the YMV isolated from black gram, cowpea and green gram clearly grouped with the MYMV-TN [DQ400848.1] in phylogenetic tree. In addition, YMV from the horse gram and green gram grouped with the HYMV strains from Tamil Nadu. Phylogenetic tree formed by the alignment of coat protein gene sequences of the YMV infecting horse gram with other begomoviruses affecting legumes revealed three major clusters, MYMIV, HYMV and MYMV. The horse gram isolate formed unique cluster with HYMV group that cause yellow mosaic disease symptoms in horse gram [AJ627904.1] and french bean [GU323321.1].

Prema and Rangaswamy (2018) reported two clustered phylogram formed by the phylogenetic analysis of coat protein gene sequence of the YMV affecting mung bean [MYMV-Hebbal, Bangalore isolate] with known *Begomovirus* sequences obtained from GenBank database and found that the MYMV-Hebbal, Bangalore isolate falls under the cluster II containing MYMV strains and the isolate clustered with MYMV-Nammakal, MYMV-Madurai, MYMV-Maharashtra and MYMV-Tamil Nadu isolates infecting moth bean, soybean and mung bean respectively.

Analysis of the predicted amino acid sequence of the coat protein gene revealed that coat protein is highly conserved within the same species. The MYMV isolates showed 97-99 per cent amino acid sequence identity among themselves. Among the MYMV isolates, the lowest identity (97%) was exhibited by BgC2H, this may be due to some recombination which might have occurred due to mixed infection. MYMV was more closely related to HYMV than other YMVs and shared 90-92 per cent amino acid sequence identity. Phylogenetic analysis of nucleotide also reveals this relationship and MYMV cluster was closer to HYMV cluster. MYMIV coat protein shared 85-86 per cent amino acid sequence identity with MYMV

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isolates. Coat protein of DoYMV differed from other yellow mosaic viruses and shared only 78-82 per cent identity. Naimuddin and Akram (2010) also reported 72-73 per cent similarity among DoYMV and MYMV based on amino acid sequence identity of coat protein.

Since yellow mosaic viruses are insect transmitted and a lot of successful works were done on transmission of MYMV to different crops, the insect vector whitefly (*Bemisia tabaci* Genn.) was used for the transmission of MYMV from infected black gram plants to healthy ten days old black gram seedlings. The vector was given an acquisition access period of 48 hrs and an inoculation access period of 48 hrs. 80 per cent transmission was obtained. The time taken for the appearance of symptoms was 15-18 days after inoculation. The presence of MYMV was confirmed with PCR using coat protein region specific primer. Similar results were recorded by Ahmad and Harwood (1973) in MYMV infected black gram plants. Salalrajan (1988) found that minimum 15-20 numbers of whiteflies are required for transmission of MYMV in black gram. The result was confirming the findings of Maheshwari *et al.* (2014). But they observed 33 per cent transmission rate with 24 hrs of AAP and IAP. Govindan *et al.* (2014) found that the transmission efficiency of the whiteflies increased upto 85 per cent by increasing the acquisition access period of the vector from 24 hrs to 48 hrs on MYMV infected mung bean plants. Jayappa *et al.* (2017a) reported that as the age of the mung bean seedlings increases, the transmission rate of the MYMV decreases and the maximum transmission of 93 per cent obtained upon inoculation to 10 days old seedlings. The reports of Jayappa *et al.* (2017) confirmed the results of Malathi *et al.* (2005). In the present study also, 80 per cent transmission was attained on 10 days old seedlings.

In order to understand the hosts of MYMV that can act as a reservoir for the virus, a host range study was conducted. Four different pulse crops (10 days old) coming under leguminosae family *viz.*, green gram, cowpea, horse gram and pigeon pea and three different weed species (15 days old), *viz.*, *Synedrella nodiflora*, *Cleome*

viscosa and *Ageratum conyzoides* belonging to different families and which were predominantly observed in the survey fields were inoculated with viruliferous whiteflies giving 48 hrs of acquisition access period and inoculation access period and observed for symptom development. The symptoms developed only on horse gram and *Synedrella nodiflora* 20 days after inoculation. Systemic symptoms developed on horse gram plants 30 days after inoculation and the presence of virus in these plants was confirmed by PCR. However symptomatic plants of *Synedrella nodiflora* did not yield PCR amplification for YMV specific primers. The chlorosis and puckering noticed on the plants may be due to feeding injury of whiteflies or some nutritional deficiency. This indicates that a certain level of host specificity occurs in this virus. The co-existence of MYMV and HYMV might have contributed to its transmission to horse gram plants. However various workers have reported transmission of MYMV to other legume hosts. Naimuddin *et al.* (2014) reported the occurrence of MYMIV and not MYMV in weed, *Ageratum conyzoides* using PCR and it was successfully transmitted by whiteflies to hosts like mung bean and urd bean. Whereas, Deepa *et al.* (2017) reported that the MYMV infecting mung bean can be successfully transmitted to black gram, horse gram, cowpea and pigeon pea through whiteflies. But Jayappa *et al.*, (2017) reported transmission of MYMV from mung bean to black gram, horse gram, cowpea and pigeon pea only and not to cowpea. However Prema (2013) reported that the HYMV infecting horse gram plants could not be transmitted to black gram, green gram, pigeon pea, cowpea and cluster bean, which is similar to the results of present study.

Aqueous extracts of plants in which antiviral principles were reported, botanicals, biocontrol agents and other organic products were tested for its effectiveness against yellow mosaic disease under natural incidence as a pot experiment as well as in field conditions. Among the treatments applied, *Pseudomonas fluorescens*, leaf extract of *Bougainvillea spectabilis* (10%) and root extract of *Boerhaavia diffusa* (10%) were found to be very effective for the

management of yellow mosaic in black gram and increasing the grain yield. Azadirachtin 10000 ppm and a commercial product virochek was also found to be effective in reducing disease severity. Buttermilk, though found to be effective in pot culture experiment, lost its effectiveness upon large scale application in field. Under field condition, buttermilk was effective in reducing disease incidence but not disease severity. On contrary virochek, a commercial product, which was found to be ineffective in pot experiment, was found to be effective in field. The yellow mosaic incidence in untreated control plots in pot culture experiments was 100 per cent at 60 DAS, whereas it was only 28 per cent in field. Hence it can be inferred that virochek losses its effectiveness at higher levels of mosaic incidence. The whitefly count was significantly lower than that in control plots only in the case of azadirachtin and *Pseudomonas fluorescens* treated plots. Hence it is clear that the action of leaf extract of *Bougainvillea spectabilis* and root extract of *Boerhaavia diffusa* is not due to its effect on vector, but due to its action on virus directly. This might be due to the antiviral principles present in it which have been reported to reduce virus accumulation by several mechanisms such as induced systemic resistance and not by vector management. The slight increase in grain yield observed in *Pseudomonas* treated plants may be due to the PGP effect of the *Pseudomonas*.

Studies conducted by Venkatesan *et al.,* in 2010 also revealed the effectiveness of *Pseudomonas fluorescens* in reducing the incidence of yellow mosaic disease in black gram to 30 per cent compared to 80 per cent in control and an increased grain yield of 733 kg ha^{-1} was recorded in *Pseudomonas fluorescens* treated plots compared to 493 kg ha^{-1} in control plots and in increasing the level of defense related enzymes *viz.*, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in treated plants compared to control plants. However *Mirabilis jalapa* which was found to be effective in the study by Venkatesan *et al.,* was not found to be effective in the present study. In the same way Kandan *et al.* (2002) revealed the

effectiveness of *Pseudomonas fluorescens* against *Tomato spotted wilt virus* (TSWV) and Paul (2014) against *Tomato leaf curl virus* (ToLCV) with highest yield.

In the present study, along with *Pseudomonas fluorescens,* 10 per cent root extract of *Boerhaavia diffusa* was found to be effective in reducing disease incidence as well as severity. The effectiveness of *Boerhaavia diffusa* in reducing the incidence of yellow mosaic in black gram was earlier reported by Singh and Awasthi (2009). Singh *et al.* (2011) also observed that 10 per cent root extracts of *Boerhaavia diffusa* showed yellow mosaic disease incidence of 45.90 per cent compared to 73.50 per cent incidence in control achieving a 40.55 per cent reduction of disease in black gram plants treated with *Boerhaavia diffusa* and recorded a higher grain yield of 3.10 g plant^{-1} compared to 2.10 g plant^{-1} from control plot. Similar results were recorded by Singh *et al.* (2004) and Verma *et al.* (1985) on yellow mosaic disease of green gram and black gram by application of root extracts of *Boerhaavia diffusa*. Recently, Sharma *et al.* (2017) found that application of 10 per cent root extract of *Boerhaavia diffusa* as seed treatment as well as foliar spray was effective against various viral diseases caused by gemini, cucumo and poty viruses in watermelon and a disease incidence of 75 per cent, 50 per cent, 30 per cent was recorded in seed treatment, foliar spray and seed treatment + foliar spray, respectively compared to 82 per cent incidence in control. Fruit yield also followed the same trend. This effect of *Boerhaavia diffusa* may be due to the presence of a glycoprotein in its roots which have been reported earlier (Verma *et al.*, 1979) and these glycoprotein act as resistance inducer in plants which acts systemically and inhibits virus infection by interfering with virus replication in the host (Awasthi and Verma, 2006). It was also found that the inhibitors in *Boerhaavia diffusa* induces production of some translocatable virus inhibitory substances in host plants, when the extracts were applied on host plants before virus inoculation (Verma *et al.*, 1985).

In the present investigation 10 per cent leaf extract of *Bougainvillea spectabilis* was also found to be effective against yellow mosaic disease of black

gram. In accordance with the results, Jayashree *et al.* (1999) recorded a 93 per cent reduction of *Pumpkin yellow vein mosaic virus* over the control through the application of 10 per cent leaf extract of *Bougainvillea spectabilis* under glasshouse condition. Findings of Karthikeyan *et al.* (2009) also supports that 10 per cent leaf extract of *Bougainvillea spectabilis* effectively reduced the *Urdbean leaf leaf crinckle virus* in black gram by 90 per cent over the control and the effect of this AVP was described by the increased activity of defense related enzymes like peroxidase and polyphenol oxidase in treated plants. The action of defense related peroxidase enzymes are polymerization reaction on cell wall which include suberization, lignifications and cross linking of structural cell wall proteins (Fry, 1986) so it indirectly blocks the entry of the virus particles into host plant. The active principle present in *Bougainvillea* sp., a glycoprotein named BAP was isolated and the mechanism of action was studied by Narwal and co-workers in 2001. It was found to induce the synthesis of a new virus inhibitory substance or enhance the synthesis of an already existing one which can alter the cellular metabolism of the host giving protection against viruses.

Other than these three treatments, an organic insecticide containing 10000 ppm azadirachtin was found to be effective, though not as effective as *Pseudomonas fluorescens*. Findings of Surendran *et al.* (1999) that spraying of 10 per cent oil formulation of *Azadirachta indica* is effective against brinjal mosaic virus under field condition was in congruence with the present study. It was found that oil formulation of *Azadirachta indica* was more effective than neem seed kernel extract (NSKE) against *Pumpkin yellow vein mosaic virus* (PYVMV) which showed 78 per cent and 66 per cent reduction over the control respectively under glasshouse condition (Jayashree *et al.* 1999). Later the findings of Gupta and Patak (2009) was also in concordance with the findings of present study that oil formulation (1%) of neem could reduce the yellow mosaic disease in black gram through effectively reducing the whitefly count in the field and it increased the grain yield also. So the effect of the

oil formulations of *Azadirachta indica* may be due to its effect on vector control instead of its direct effect on virus, which was already reported by many scientists that it possesses repellent, antifeedent and insecticidal action against virus vector (Mariappan and Saxena, 1983) which make the treated plots unattractive and non feedable to the vectors so indirectly giving minor effect against disease.

Among the commercially available antiviral products tested, virochek was found to be effective to some extent against the disease but perfekt was found to be ineffective. The literatures on the application of virochek against any viral disease was not available. So it may be the first attempt on checking the effectiveness of virochek. Whereas in case of perfekt, reports against yellow mosaic disease was not available but 2 per cent spray of perfekt was reported to be effective against ToLCV (Paul, 2014).

An animal product, buttermilk (10%) was found to be effective against the yellow mosaic disease of black gram only during initial stages of infection, which lost its effect in due course of time in the present study. But it was found to be effective against PYVMV with 80 per cent reduction in disease over the control in a study conducted under glasshouse condition (Jayashree *et al.*, 1999). In the present study also, it was found to be effective in the pot experiment.

Ten per cent leaf extract of *Cocos nucifera* was not found to be effective in the present study whereas, Manjunatha *et al.* (2010) found that coconut leaf extract is effective against GBNV (*Groundnut bud necrosis virus*) in cowpea under glass house condition and an increased activity of PAL and PPO was also estimated in treated plants.

Leaf extract of *Mirabilis jalapa* was also not effective against yellow mosaic disease of black gram. But as a contrary to this finding, Venkatesan *et al.* (2010) found that leaf extract of *Mirabilis jalapa* could effectively control the YMD in black gram. It was also found to be effective against *Bean common mosaic virus* in bean

plants under field as well as glasshouse conditions (Elsharkawy and El-Sawy, 2015). However, in the present study, a sudden increase in severity and incidence of yellow mosaic was observed in *Mirabilis jalapa* treated plants 45 DAS in experiment conducted in pot and in the field it was ineffective from the beginning itself.

In case of whitefly count, azadirachtin and *Pseudomonas fluorescens* were effective against whitefly. If we compare both the treatments, *Pseudomonas fluorescens* gave a slow and steady action on decreasing whitefly population as it showed a drastic decline in whitefly count during later stages of the crop,. This effect of *Pseudomonas fluorescens* may be due to its induced systemic resistance which acts through activation of various compounds and accumulation of defense related enzymes. This may be causing some histological changes on the host plants which make them non feedable to the whiteflies. A study conducted by Shefali *et al.* (2014) revealed that a combined treatment of chitosan with *Pseudomonas* sp. can effectively reduce the whitefly count on tomato plants through its ISR activity and indirectly reduce tomato leaf curl disease also. Pangnakorn and Chuenchooklin (2016) also found out that *Pseudomonas fluorescens* effectively reduce the whitefly population than yellow sticky traps. In *Pseudomonas* treated plots, a mean whitefly count of 10.00 was recorded compared to 40.83 in the control plots. There are only few studies of *Pseudomonas* on insect pest control.

So, from the management study it can be inferred that the prophylactic application of *Pseudomonas fluorescens* as seed treatment as well as foliar spray at fortnightly intervals starting from 15 days after sowing or 10 per cent aqueous extracts of roots of *Boerhaavia diffusa* or leaves of *Bougainvillea spectabilis* at fortnightly intervals starting from 15 days after sowing can effectively control the yellow mosaic disease of black gram. The increased yield obtained in these plots along with the reduced disease incidence confirms the results.

The present study reveals that a PGPR, having induced systemic action can act as an effective management tool for the yellow mosaic disease of black gram and

the antiviral principles which are present in different plants will be a boon for the protection of plants against various plant viral diseases, if suitable strategies are developed for prolonging the effect of these inhibitors and using them at commercial level. More than that these are advantageous over the chemicals as they are easily biodegradable, nonphytotoxic and have no residual effect. The study indicates that an effective management strategy against viral diseases should aim at induction of systemic resistance in plants and should not be based on vector management alone.

Summary

6. SUMMARY

Urd bean (*Vigna mungo* (L.) Hepper) is the third important pulse crop in India, a rich source of dietary protein (23.9%) and used in daily diet of Indians. The area under black gram cultivation is increasing over years. Among various diseases affecting black gram, yellow mosaic disease is the major constraint in black gram production. Yellow mosaic disease of pulses is extensively studied from different parts of the country but no study has been conducted in Kerala on any aspect of the yellow mosaic disease (YMD) in black gram. Hence, in an endeavor to get the information on the viruses causing YMD in pulses in Kerala and developing a suitable management strategy, the present study was undertaken.

Purposive sampling surveys were conducted in black gram growing areas of Palakkad and Malappuram districts during March 2017-April 2018. Survey was conducted in six different locations of Palakkad district and three different locations of Malappuram district. The per cent disease incidence varied from location to location. In Palakkad district, disease incidence varied from 12-100 per cent whereas it varied from 20-60 per cent in Malappuram district.

The major symptoms observed in the survey fields were intermingled yellow and green patches on the leaves, slight puckering of the leaves with yellow mosaic symptoms and cupping of the leaves. In some cases, leaves became totally yellowish in colour with irregular whitish discolouration and such leaves appeared papery white upon maturity. Reduction in leaf size with distortion of the shape of the leaves was observed in many fields. Stunting of infected plants and reduction in no. of pods, size of pods and size of seeds were also observed in survey fields. Vein banding and complete yellowing of the leaves with interveinal bronzing are two new symptoms observed in the present study which was not reported earlier.

Electron microscopic studies of the samples collected revealed the presence of geminate particles of 15-18 x 30nm size. The presence of geminivirus was further

confirmed by PCR. As the geminiviruses reported from pulses belong to the genus, *Begomovirus* transmitted by whiteflies, whitefly transmission of the virus present in the samples was attempted and found successful. The virus culture was established in glass house through transmission of yellow mosaic from the samples collected to healthy black gram plants by whiteflies.

Total genomic DNA from the yellow mosaic infected black gram samples were extracted following the Gem CTAB protocol suggested by Rouhibakhsh *et al.* (2008) with slight modifications like increasing the speed of precipitation, incubation period in chloroform: isoamyl alcohol and number of alcohol washes. This was found to be the best method for good quality DNA extraction from black gram plants which are rich in phenols and tannins. The quantity and purity of the isolated DNA was estimated using UV spectrophotometer. The concentration of the isolated total DNA ranged from 120 to 1775 ng/µl. The purity of DNA extracted expressed as the OD260/OD280 value varied from 1.71 to 2.2. Since the range of OD260/OD280 value was almost within 1.8 to 2.00, the genomic DNA extracted was pure without much protein and RNA contamination.

The total nucleic acid extracted from the infected black gram samples were subjected to PCR amplification with a universal degenerate primer specific to *Begomovirus* group. The annealing temperature for the primer was standardized as 56.8°C. Out of the total 58 samples collected from 13 different fields, 43 were positive confirming the presence of *Begomovirus* in these samples. The begomoviruses reported from black gram belong to three different virus species *viz.*, *Mungbean yellow mosaic virus* (MYMV), *Mungbean yellow mosaic India virus* (MYMIV) and *Horsegram yellow mosaic virus* (HYMV). For the detection of the virus species infecting black gram plants, PCR was carried out with three sets of primers specific to coat protein region of MYMV, MYMIV and HYMV. Annealing temperature and the optimum DNA concentration required for amplification were standardized. Amplicons of expected band size were obtained at an annealing

temperature of 55.3°C and the optimum template concentration was standardized to be 95 ng/µl.

MYMV could be detected in five fields in Palakkad district out of the eight fields surveyed and four fields in Malappuram district out of the five fields surveyed. This reveals that the major virus causing yellow mosaic in black gram in Kerala is MYMV. HYMV was detected in samples collected from four fields in Palakkad district and two fields in Malappuram district. Mixed infection of HYMV and MYMV was noticed in three fields in Palakkad district and two fields in Malappuram district. Such mixed infections can lead to recombination and reassortment of the viral DNA resulting in the emergence of new strains or even species. One field from Palakkad district showed the presence of HYMV alone.

The amplicons obtained in PCR was sequenced for further confirmation of the virus species. Five representative isolates were sequenced, three amplified using MYMV specific primer and two amplified with primer specific to HYMV. *In silico* analysis revealed the homology of the isolates with MYMV isolates reported from other parts of the country, particularly those from the southern region. The isolates exhibited more than 90 per cent identity to MYMV strains and 86 per cent to HYMV strains. Interestingly the coat protein gene sequence of the two isolates amplified with HYMV primer also exhibited more than 90 per cent similarity to MYMV strains and only 86 per cent similarity with HYMV, suggesting the possibility of recombinants occurring in the field as an outcome of the mixed infections. The complete sequencing of DNA A is required for the further confirmation of the result.

Dendrogram was constructed to understand the phylogenetic relationship of isolates reported in the present study with that reported from other regions of India and with other begomoviruses affecting pulses, such as MYMIV and *Dolichos yellow mosaic virus*. The isolates reported in the present study are closely related to each other and they show closer relation to isolates from Tamil Nadu and one isolate from

Tirupati. Isolates from the present study are more distantly related to MYMIV isolates which is reported from north India alone.

Analysis of the predicted amino acid sequence of the cp gene of the isolates from the present study revealed that they share 97-99 per cent amino acid sequence identity with MYMV Tamil Nadu isolate, 90 to 92 per cent identity with HYMV, 85 to 86 per cent identity with MYMIVand 78-82 per cent identity with DoYMV. These results indicate that the virus involved in YMD in black gram plants in Kerala is MYMV.

Host range studies were carried out to find out the possible hosts that can act as a reservoir of the virus. Whitefly transmission of the virus was attempted to four predominant pulses of Kerala, green gram, cowpea, horse gram and pigeon pea and three predominant weeds found in the survey fields *viz.*, *Ageratum conyzoides, Synedrella nodiflora* and *Cleome viscosa.* An acquisition access period of 48 hrs and inoculation access period of 48 hrs were given to the whiteflies. Interveinal chlorosis was observed in horse gram 20-25 days after inoculation with a transmission rate of 50 per cent. Systemic symptoms developed on horse gram plants 30 days after inoculation and the presence of virus was confirmed by PCR. Chlorosis and puckering were observed in *Synedrella nodiflora* 20-22 days after inoculation. However, the presence of YMV in symptomatic plants of *Synedrella nodiflora* could not be confirmed by PCR. Symptoms were not produced in other test plants. The virus could not be transmitted to green gram, pigeon pea and cowpea indicating the possibility of occurrence of species specific strains within the virus species.

Botanicals, biocontrol agents, commercially available antiviral products and animal product were evaluated for its effectiveness against natural incidence of yellow mosaic disease in black gram plants in pots as well as under field condition. The results reveal that *Pseudomonas fluorescens* as seed treatment at the rate of 10g/kg seed and foliar sprays at the rate of 10g/l at fortnightly intervals starting from 15 days after sowing and foliar sprays of 10 per cent aqueous extract of leaves of

Bougainvillea spectabilis and roots of *Boerhaavia diffusa* at fortnightly intervals starting from 15 days after sowing are effective in reducing the yellow mosaic disease and thereby increasing the grain yield in black gram. Azadirachtin 10,000 ppm and a commercial formulation, virocheck was also found to be effective for the management of the virus, though not as effective as *Pseudomonas fluorescens*. Whitefly population was found to be significantly lower than the untreated control in plants treated with azadiractin and *Pseudomonas fluorescens*. Application of *Bougainvillea spectabilis* and *Boerhaavia diffusa* did not reduce whitefly population indicating that the effect of these treatments might due to its antiviral property rather than vector control.

References

REFERENCES

Adams, M. J., Lefkowitz, E. J., King, A. M., Harrach, B., Harrison, R. L., Knowles, N. J., Kropinski, A. M., Krupovic, M., Kuhn, J. H., Mushegian, A. R., Nibert, M., Sabanadzovic, S., Sanfacon, H., Siddell, S. G., Simmonds, P., Varsani, A., Zerbini, F. M., Gorbalenya, A. E., and Davison, A. J. 2016. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses. *Arch. Virol.* 161: 2921–2949.

Ahmad, M. 1975. Screening of mungbean (*Vigna radiata*) and urd bean (*V. mungo*) for germplasms resistance to yellow mosaic virus. *J. Agric. Res.* 13: 349-354.

Ahmad, M. and Harwood, R. E. 1973. Studies on whitefly transmitted yellow mosaic diseae of cowpea (*Vigna ungiculata*). *Plant Div. Rep.* 62: 224-226.

Ahmed, S. R. and Gane, R. B. 1982. Chemotherapeutic approach to check yellow mosaic virus of mung (*Phaseolus aureus* L.). *Hindusthan Antibiot. Bull.* 24 (3): 197 – 199.

Akhtar, J., Lal, H. C., Singh, P. K., Karmakar, S., Gautam, N. K., and Kumar, A. 2016. Identification of resistant sources of Vigna spp. against yellow mosaic disease. *Virol. Mycol.* 5(1): 1- 4.

Akram, M., Akram, N. M., and Naimuddin 2016. Management of mungbean yellow mosaic disease and effect on grain yield. *Indian J. Plant Prot.* 44(1) :127-131.

Akram, M., Naimuddin, Agnihotri, A. K., Gupta, S., and Singh, N. P. 2015. Characterization of full genome of *Dolichos yellow mosaic virus* based on sequence comparison, genetic recombination and phylogenetic relationship. *Ann. Appl. Biol.* 167: 354–363.

Ali, M., Li, F., Zhiqing, Z., Kaixiang, Z., Dong-Ku, K., James A., Chris, L. X., and Weian, Z. 2014. Rolling circle amplification: a versatile tool for chemical biology, materials science and medicine. *Chem. Society Rev.* 43 (10): 3324.

Ali, S. and Ahmad, R. 2010. Polymerase Chain Reaction based detection of *Dolichos yellow mosaic virus* infecting *dolichos. Trends Biosci.* 3(2): 133-134.

Anokhe, A., Mandal, B., and Subramanian, S. 2018. Characterization of *Mung bean yellow mosaic virus* transmission by Asia I and Asia II-1 genetic group of *Bemisia tabaci* Gennadius. *J. Entomol. Zool. Stud.* 6(1): 487-491.

Awasthi, L. P. and Shyam, S. 2008. Screening of mungbean germplasm for field resistance to mungbean yellow mosaic virus. *Int. J. Plant Sci. Res.* 35:1-4.

Awasthi, L. P. and Verma, H. N. 2006. *Boerhaavia diffusa*-A wild herb with potent biological and antimicrobial properties. *Asian Agri. History*, 10 (1): 55-68.

Bashir, M. 2005. Studies on viral diseases of major pulse crops and identification of resistant sources. Technical Annual Report (April, 2004 to June, 2005) of ALP Project. Crop Sciences Institute, Nat Agri. Res Centre, Islamabad. pp. 1-169.

Bhagyashree, M., Basavaraj, S., Prameela, H. A., Jyoti, G., Manjunath, S., Hurakadli, and Rangaswamy, K. T. 2017. Coat protein and movement protein based characterization of lima bean yellow mosaic virus disease in Karnataka. *Legume Res.* 40 (2): 358-363.

Bhanu, S. H., Jayalakshmidevi, R. S., Reddy, B. V. B., and Prasanthi, L. 2015. Host range studies for yellow mosaic virus (YMV) infecting pulses. *Int. J. Trop. Agric.* 33(2): 1173-1185.

Bird, J. and Maramorosch, K. 1978. Viruses and virus diseases associated with whiteflies. *Adv. Virus Res.* 22: 55-110.

Biswas, K. K. and Varma, A. 2001. Agroinoculation: a method of screening germplasm resistance to mungbean yellow mosaic geminivirus. *Indian Phytopath.* 54 (2): 240-245.

Biswas, K. K., Malathi, V. G., and Varma, A. 2008. Diagnosis of Symptomless Yellow mosaic begomovirus Infection in Pigeonpea by Using Cloned *Mungbean yellow mosaic India virus* as Probe. *J. Plant Biochem. Biotechnol.* 17(1): 09-14.

Biswas, K. K., Tarafdar, A., Kumar, A., Dikshit, H. K., and Malathi, V. G. 2009. .Multiple infection in urd bean (*Vigna mungo*) in natural condition by begomovirus, tospovirus and urdbean leaf crinkle virus complex. *Indian Phytopath.* 62 (1) : 75-82.

Bos, L. 1982. Crop losses caused by viruses. *Crop Prot.* 1(3): 263-282.

Briddon, R. W., Bull, S. E., Amin, I., Idris, A. M., Mansoor, S., Bedford, I. D., Dhawan, P., Rishi, N., Siwatch, S. S., Abdel-Salam, A. M., Brown, J. K., Zafar, Y., and Markham, P. G. 2003. Diversity of DNA b a satellite molecule associated with some monopartite begomoviruses. *Virol.* 312:106–121.

Briddon, R. W., Bull, S. E., Mansoor, S., Amin, I., and Markham, P.G., 2002. Universal primers for the PCR-mediated amplification of DNA beta: a molecule associated with some monopartite begomoviruses. *Mol. Biotechnol.* 20 : 315–318.

Briddon, R. W. and Stanley, J. 2006. Subviral agents associated with plant single stranded DNA viruses. *Virol.* 344 : 198-210.

Brown, J. K. 1991. An update on the whitefly transmitted geminiviruses in the Americas and the Caribbean basin. *FAO plant prot. Bull.* 39:5-23.

Brown, J. K. 2001. Molecular markers for the identification and global tracking of whitefly vector-begomovirus complexes. *Virus Res.* 71: 233-260.

Brown, J. K. and Bird, J. 1992. Whitefly transmitted geminiviruses and associated disorders in the Americas and the Caribbean basin. *Plant dis.* 76: 220-225.

Brown, J. K., Torres-Jerez, I., Idris, A. M., Banks, G. K., and Wyatt, S. D. 2001. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. *Arch. Virol.* 146: 1581-1598.

Brown, J. K., Zerbini, F. M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva, J. C., Fiallo-Olive, E., Briddon, R. W., Hernandez-Zepeda, C., Idris, A., Malathi, V. G., Martin, D. P., Rivera-Bustamante, R., Ueda, S., and Varsani,

A. 2015. Revision of Begomovirus taxonomy based on pairwise sequence comparisons. *Arch. Virol.* 160:1593–1619.

Capoor, S. P. and Varma, P. M. 1948. Yellow mosaic of *Phaseolus lunatus*. *Curr. Sci.* 17 : 152–153.

Capoor, S. P. and Varma, P. M. 1950. A new virus disease of *Dolichos lablab*. *Curr. Sci.* 19:248–289.

Chakraborty, S., Vanitharani, R., Chattopadhyay, B., and Fauquet, C. M. 2008. Supervirulent pseudorecombination and asymmetric synergism between genomic components of two distinct species of begomovirus associated with severe tomato leaf curl disease in India. *J. gen. virol.* 89: 818-828.

Chenulu, V. V., Venkteswarulu, V., and Rangaraju, R. 1979. Studies on yellow mosaic disease on mungbean. *Indian Phytopathol.* 32 : 230 – 235.

Choudhary, K., Mathur, N., Choudhary, O. P., and Pillai, U. 2008. Protocol for isolation of genomic DNA from dry and fresh leaves of *Vigna* Species suitable for rapid and restriction digestion. *Adv. Biological Res.* 2 (5-6): 83-89.

Deepa, H., Govindappa, M. R., Kulakarni, S., Kenganal, M., and Biradar, S. A. 2017. Biological Evidence on Host Range of Yellow Mosaic Disease of Greengram [*Vigna radiata* (L.) Wilczek]. *Int. J. Curr. Microbiol. App. Sci.* 6(10): 678-684.

Deepa, H., Govindappa, M. R., Kulkarni, S. A., Kenganal, M., and Biradar, S. A. 2017a. Molecular detection of yellow mosaic disease of greengram through polymerase chain reaction. *Bull. Env. Pharmacol. Life Sci.* 6(1): 507-509.

Deng, D., McGrath, P. F., Robinson, D. J., and Harrison, B. D. 1994. Detection and differentiation of whitefly transmitted geminiviruses in plants and vector insects by the polymerase chain reaction with degenerate primers. *Ann. Appl. Biol.* 125:327–336.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bull.* 19: 11-15.

Eagle, P. A., Orazco, B. M., and Hanley-Bowdoin, L. 1994. A DNA sequence required for geminivirus replication also mediates transcriptional regulation. *Plant Cell*, 6: 1157-1170.

Elsharkawy, M. M. and El-Sawy, M. M. 2015. Control of *Bean common mosaic virus* by plant extracts in bean plants. *Int. J. Pest Manag.* 61(1): 54-59.

Equbal, M. J., Sahni, S., Singh, D., and Kumar, B. 2015. Exploitation of urd bean germplasms for *Mungbean yellow mosaic virus* (MYMV) management and improved seed yield. *Int. J. Agric. Sci.* 7(6): 536-539.

Fauquet, C. M. and Stanley, J. 2003. Geminivirus classification and nomenclature; progress and problems. *Ann. Appl. Biol.* 142: 165-189.

Fauquet, C. M., Briddon, R. W., Brown, J. K., Moriones, E., Stanley, J., Zerbini, M., and Zhou, X. 2008. Geminivirus strain demarcation and nomenclature. *Arch. Virol.* 153 : 783–821

Fry, S. C. 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Ann. Rev. Plant Physiol.* 37:165 – 183.

Gaffor, A., Zubir, M., and Iqbal, S. M. 1992. Evaluation of selected germplasm of blackgram. *Pak. J. Botany*, 24: 112-118.

Gautam, N. K., Akram, M., Akhtar, J., Khan, Z., Dwivedi, N. K., Latha, M., and Ram, B. 2014. Responses of wild *Vigna* species/sub-species to yellow mosaic disease viruses, detected by a PCR-based method. *Phytopathologia Mediterranea,* 53(3): 428–437.

Ghosh, D., Laha, S. K., and Biswas, N. K. 2009. Effect of different pesticides on incidence of mungbean yellow mosaic virus incidence. *Int. J. Plant Prot.* 2 (1) : 67-70.

Glick, E., Levy, Y., and Gafni, Y. 2009. The viral etiology of tomato yellow leaf curl disease – a review. *Plant Prot. Sci.* 45: 81–97.

GoK [Government of Kerala] 2016. Agricultural statistics 2014-2015. Department of economics and statistics, Thiruvananthapuram, Kerala, 30 p.

Gopalaswamy, S. V. S., Ramana, M. V., and Krishna, R. 2012. Management of YMV of black gram by chemical control of *Bemisis tabaci* Gennadius. *Ann. Pl. Prot. Sci.* 20: 358-360.

Gopi, P., Satyanarayana, Krishna, R., and Rao, S. 2016. Evaluation of black gram germplasm for resistance against YMV. *J. Plant Pathol. Microbiol.* 7(7):1- 4.

Govindan, K., Nagarajan, P., and Angappan, K. 2014. Molecular studies on transmission *Mungbean yellow mosaic virus* (MYMV) by *Bemisia tabaci* Genn. In mungbean. *Afr. J. Agric. Res.* 9(38): 2874-2879.

Gupta, K. N. 2016. Surveillance of *Mungbean yellow mosaic virus* (MYMV) in weed plants of Jabalpur (MP), India. *Bioinfolet* 13(1A): 59.

Gupta, M. P. and Pathak, R. K. 2009. Bioefficacy of neem products and insecticides against the incidence of whitefly, yellow mosaic virus and pod borer in black gram. *Nat. Product Radiance.* 8(2): 133-136.

Haible, D., Kober, S., and Jeske, H. 2006. Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *J. Virol. Methods*, 135: 9–16.

Hajong, M. and Naveenkumar, K. L. 2016. Detection and diagnosis of *Mungbean Yellow Mosaic Virus* (MYMV) infecting soybean (*Glycine max* L.) in Maharashtra, India. *Adv. Life Sci.* 5(7):2779-2782.

Hanley-Bowdoin, L., Settloge, S. B., Orozco, B. M., Nagar, S., and Robertson, D. 1999. Geminiviruses: Models of plant DNA replication, transcription and cell cycle regulation. *Crit. Rev. Plant Sci.* 18: 71-106.

Haq, Q. M. I., Rouhibakhsh, A., Ali, A., and Malathi, V. G. 2011. Infectivity analysis of a black gram isolate of *Mungbean yellow mosaic virus* and genetic assortment with MYMIV in selective hosts. *Virus Genes*, 42:429–439.

Harrison, B. D and Robinson, D. J. 2002. Greens hoots of geminivirology. *Physiol. Mol. Plant pathol.* 60: 215-218.

Harrison, B. D. and Robinson, D. J., 1999. Natural genomic and antigenic variation in whitefly transmitted geminiviruses (Begomoviruses). *Ann. Rev. Phytopathol.* 37:369-398.

Harrison, B. D., Barker, H., Bock, K. R., Guthrie, E. J., Meredith, G., and Atkinson, M., 1977. Plant viruses with circular single-stranded DNA. *Nature*, 270 : 760-762.

Honda, Y., Iwaki, M., Saito, Y., Thongmeearkom, P., Kittisak, K., and Deema, N. 1983. Mechanical transmission purification and some properties of whitefly *bemisia tabaci* borne *Mung bean yellow mosaic virus* in Thailand. *Plant Dis.* 67(7): 801-804.

Hossain, S. M. A., Islam, S., Akhter, K., Akhtar, N., and Muqit, A. 2010. Effect of plant extracts, insecticides and cultural practices on growth characters and disease severity of mungbean yellow mosaic. *Int. J. Sustain. Crop Prod.* 5(2):16-20.

Howarth, A. J. and Vandermark, G. J. 1989. Phylogeny of geminiviruses. *J. Gen. Virol.* 70: 2717-2727.

India stat, 2018. Area and production of pulses in India. *www.indiastat.com.*

India stat, 2017. Area and production of black gram in India. *www.indiastat.com.*

Indira, M. and Kurup, P. A. 2013. Black Gram: A Hypolipidemic Pulse. *Nat. Product Radiance.* 2 (5): 16-20.

Iqbal, U., Iqbal, S. M., Afzal, R., Jamal, A., Farooq, M. A., and Zahid, A. 2011. Screening of mungbean germplasm against mungbean yellow mosaic virus (MYMV) under field conditions. *Pak. J. Phytopathol.* 23(1):48-51.

Islam, M. N., Sony, S. K., and Borna, R. S. 2012. Molecular characterization of mungbean yellow mosaic disease and coat protein gene in mungbean varieties of Bangladesh. *Plant Tissue Cult. Biotechnol.* 22(1): 73-81.

Islam, M. T. and Faruq, A. N. 2009. Effect of some cultural practices for the management of mungbean yellowmosaic disease. *J. Sher-e-Bangla Agric. Univ.* 3(1): 40-45.

Ilyas, M., Qazi, J., Mansoor, S., and Briddon, R. W. 2010. Genetic diversity and phylogeography of begomoviruses infecting legumes in Pakistan. *J. Gen. Virol.* 91: 2091-2101.

Jalaluddin, M and Shaikh, M. A. Q. 1981. Evaluation of mung bean (*Vigna radiata* L.) germplasm for resistance to mungbean yellow mosaic virus. *SABRAOJ.* 13: 40-45.

Javaria, Q., Muhammad, I., Mansoor, S., and Briddon, R. W. 2007. Legume yellow mosaic viruses: genetically isolated begomoviruses. *Mol. Plant Pathol.* 8: 343–348.

Jayappa, H. K., Ramappa, and Devamani, B. D. 2017. Management of *Mungbean yellow mosaic virus* (MYMV) in mungbean (*Vigna radiata* L.) *J. Entomol. Zool. Stud.* 5(5): 596-601.

Jayappa, H. K., Ramappa, and Devamani, B. D. 2017a. Host range of *Mungbean yellow mosaic virus* (MYMV) and influence of age of seedlings on the transmission of MYMV in mungbean. *Res. J. Agric. Sci.* 8(5):1235-1237.

Jayashree, K., Pun, K. B., and Doraiswamy, S. 1999. Effect of plant extracts and derivatives, butter milk and virus inhibitory chemicals on *Pumpkin yellow vein mosaic virus* transmission. *Indian Phytopathol.* 52 (4) : 357-361.

John, P., Mishra, A., and Malathi, V. G. 2016. Infectivity of cowpea isolate of by Agro-inoculation. *J. Mycol. Plant Pathol.* 46(4) : 381-386.

John, P., Sivalingam, P. N., Haq, Q. M. I., Kumar, N., Mishra, A., Briddon, R. W., and Malathi, V. G. 2008. Cowpea golden mosaic disease in Gujarat is caused by a *Mungbean yellow mosaic India virus* isolate with a DNA B variant. *Arch. Virol.* 153:1359–1365.

Jones, D. R. 2003. Plant viruses transmitted by whiteflies. *Eur. J. Plant Pathol.* 109:195-219.

Kandan, A., Radjacommare, R., Nandakumar, R., Raguchander, T., Ramiah, M., and Samiyappan, R. 2002. Induction of phenylpropanoid metabolism by Pseudomonas fluorescens against tomato spotted wilt virus in tomato. *Folia Microbiol.* 47:121–129.

Karthikeyan, A. S., Vanitharani, R., Balaji, V., Anuradha, S., Thillaichidambaram, P., Shivaprasad, P. V., Parameswari, C., Balamani, V., Saminathan, M., and Veluthambi, K. 2004. Analysis of an isolate of *Mungbean yellow mosaic virus* (MYMV) with a highly variable DNA B component. *Arch. Virol.* 149 : 1643-1652.

Karthikeyan, G., Doraisamy, S., Rabindran, R., and Ganapathy, T. 2009. Evaluation of antiviral principles for the induction of systemic resistance in black gram (*Vigna mungo*) against *Urdbean Leaf Crinkle Virus*. *Arch. Phytopathol. Plant Prot.* 42(12): 1172–1186.

Khan, M. A., Rashid, A., Mateen, A., Sajid, M., Rasheed, F., Anjum, M. A., Anjum, A., Muhammad, Fayyaz, M., and Farooq, M. 2012. Incidence of *Mungbean yellow mosaic virus* (MYMV), its epidemiology and management through mycotal, imidacloprid and tracer. *Agric. Biol. J. N. Am.* 3(11): 476-480.

Khattak, G. S. S., Haq, M. A., Rana, S. A., Abass, G., and Irfaq, M. 2000. Effect of *Mungbean yellow mosaic virus* (MYMV) on yield and yield components of mungbean (*Vigna radiata* (L.) Wilczek). *Kasetstart J.* (*Nat. Sci.*) 34: 12-16.

Kumar, M., Bag, N. K., Gautam, T. V., Prasad, S., Pandey, M., Dutta, and Roy, A. 2014. Evaluation of an Indian collection of black gram germplasm and identification of resistance sources to *Mungbean yellow mosaic virus*. *Crop Prot.* 61: 92-101.

Kumar, B., Talukdar, A., Verma, K., Girmilla, V., Bala, I., Lal, S. K., Singh, K. P., and Sapra, R. L. 2014a. Screening of soybean [*Glycine max* (L.) Merr.]

genotypes for yellow mosaic virus (YMV) disease resistance and their molecular characterization using RGA and SSRs markers. *Austr. J. Crop Sci.* 8(1): 27-34.

Kumar, R., Shamshad, A., and Rizvi, S. M. A. 2006. Efficacy of insecticides and neem oil against *Bemisia tabacii* Genn. and yellow mosaic virus in moongbean. *Ann. Plant Prot. Sci.* 14:431-434.

Lal, R. and Jat, B. L. 2015. Bioefficacy of insecticides and biorationals against the incidence of whitefly, *Bemisia tabaci* (Genn.) and yellow mosaic virus on mungbean. *Afr. J. Agric. Res.* 10(10) : 1050-1056.

Lima, A. D., Do Nascimento, A. K. Q., Amorim, V. B. O., Radaelli, P., and Filho, J. T. 2012. Serological detection and molecular characterization of a begomovirus isolate obtained from *Macroptilium lathyroides. José Revista Caatinga, Mossoró*, 25(2): 14-20.

Lizardi, P. M., Zhu, H., Bray-Ward, Z., Thomas, P., and Ward, D. C. 1998. Mutation detection and single-molecule counting using isothermal rolling-circle amplification. *Nature Genet.* 19 : 225-232.

Lodhi, M. A., Ye, G. N., Weeden, N. F., and Reisch, B. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Pl. Mol. Biol. Rep.* 12: 16-13.

Mahajan, N., Parameswari, C., and Veluthambi, K. 2011. Severe stunting in Black gram caused by the *Mungbean yellow mosaic virus* (MYMV) KA27 DNA B component is ameliorated by co-infection or post-infection with the KA22 DNA B: MYMV nuclear shuttle protein is the symptom determinant. *Virus Res.* 157 : 25–34.

Maheshwari, R., Gatikrushna Panigrahi, G., and Angappan, K. 2014. Molecular characterization of distinct YMV (Yellow mosaic virus) isolates affecting pulses in India with the aid of coat protein gene as a marker for identification. *Mol. Biol. Rep.* 41: 2635–2644.

Malathi, V. G. and John, P. 2008. Mungbean yellow mosaic viruses. In: Mahy, B. W. J. and Van Regenmortel, M. H. V. (eds), *Encyclopedia of Virology*, Vol.8, Elsevier, Amsterdam, pp. 364–371.

Malathi, V. G. and John, P. 2008a. Geminiviruses infecting legumes. In: Govind, P., Rao, P., Lava, K. P., and Holguin-Pena, R. J., (eds), *Characterization, Diagnosis & Management of Plant Viruses*. Stadium Press, LLC, Houston, TX, pp. 97-123.

Malathi, V. G., Surendranath, B., Naghma, A., and Roy, A. 2005. Adaptation to new hosts shown by the cloned components of *Mungbean yellow mosaic India virus* causing cowpea golden mosaic in northern India. *Can. J. Plant Pathol.* 27:439-447.

Manjunatha, B., Jayaram, N., Muniyappa, V. and Prameela, H. A. 2013. Status of yellow mosaic virus and whitefly *Bemesia tabaci* biotypes on mungbean in Southern Karnataka. *Leg. Res.* 36 (1): 62 - 66.

Manjunatha, L., Patil, M. S., Thimmegowda, P. R., Mahantesha, S. R. V., and Basamma. 2010. Effect of antiviral principle on groundnut bud necrosis virus. *J. Plant. Dis.* 5(1): 12 -15.

Manjunatha., Haveri, N. N., Reddy, B. A., Archana, S., and Hurakadli, M. S. 2015. Molecular detection and characterization of virus causing yellow mosaic disease of redgram (*Cajanus cajan* L. Millsp) in Karnataka. *Int. J. Pure App. Biosci.* 3 (4): 258-264.

Mariappan, V. and Saxena, Re. 1983. Effect of custard apple oil and neem oil on survival of *Nephotettix virescens* (Homoptera:Cicadellidae) on rice tungro virus transmission. *J. Econ. Entomol.* 76 : 573-576.

Maruthi, M. N., Manjunatha, B., Rekha, A. R., Govindappa, M. R., Colvin, J., and Muniyappa, V. 2006. *Dolichos yellow mosaic virus* belongs to a distinct lineage of Old World begomoviruses; its biological and molecular properties. *Ann. Appl. Biol.* 149: 187-195.

Mayo, M. A. and Pringle, C. R. 1998. Virus taxonomy - 1997. *J. General Virol.* 79: 649–657.

Menon, P. V. and Kurup, P. A. 1976. Dietary fibre and cholesterol metabolism: Effect of fibre rich polysaccharide from black gram (*Phaseolus mungo*) on cholesterol metabolism in rats fed normal and atherogenic diet. *Biomedicine*, 24 (4): 248–253.

Meti, M., Kenganal, M., Sunkad, G., Aswathanarayana, D. S., and Shanwad, U. K. 2017. Spatial variability of *Mungbean yellow mosaic virus* (MYMV) in North Eastern Karnataka. *Int. J. Plant Prot.* 10 (2) : 420-428.

Meti, M., Kenganal, M., Aswathanarayana, D. S., Sunkad, G., and Shanwad, U. K. 2017. Response of host resistance in mungbean cultivars against yellow mosaic disease caused by *Mungbean Yellow Mosaic Virus* (MYMV). *Int. J. Curr. Microbiol. App. Sci. 6*(10): 1559-1565.

Mishra, M., Sachan, M., Akram, M., and Naimuddin. 2010. Detection of *Mungbean yellow mosaic India virus* in *Kharif* Pulses and some weeds. *Trends Biosci.* 3(2): 117-119.

Morinaga, T., Ikegami, M., and Miura, K. I. 1993. The nucleotide sequence and genome structure of mungbean yellow mosaic geminivirus. *Microbiol. Immunol.* 37:471-476.

Muniyappa, V., Rajeshwari, R., Bharathan, N., Reddy, D. V. R., and Nolt, B. L.1987. Isolation and characterization of a geminivirus causing yellow mosaic disease of horsegram *(Macrotyloma uniflorum* [Lam.] Verde.) in India. *J. Phytopathol.* 119: 81-87.

Muniyappa, V., Reddy, H. R., and Shivshankar, C. 1975. Yellow mosaic disease of *Dolichos biflorus* Linn. *Curr. Sci.* 4: 176.

Murphy, J. F., Sehnder, G. W., Schuster, D. J., Sikora, E. J., Polston, J. E., and Kloepper, J. W. 2000. Plant growth promoting rhizobacterial mediated protection in tomato against tomato mottle virus. *Plant Dis.* 84:779–784.

Murray, M. G. and Thompson W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res*. 8: 4321-4326.

Murtaza, M. A., Bhatti, M. A., and Gayyam, H. A. 1983. Susceptibility of black gram lines to white fly and yellow mosaic. *Pak . J. Entomol*. 5: 49-56.

Naimuddin, K. and Akram, M. 2010. Detection of mixed infection of begomoviruses in cowpea and their molecular characterization based on CP gene sequences. *J. food legumes,* 23 (3&4): 191-195.

Naimuddin, K., Akram, M., Gupta, S., and Agnihotri, A. K. 2014. Ageratum conyzoides harbours *Mungbean yellow mosaic India virus*. *Pl. Pathol. J.* 13(1) : 59-64.

Naimuddin, K., Akram, M., and Sanjeev, G. 2011. Identification of *Mungbean yellow mosaic India virus* infecting *Vigna mungo* var. *silvestris* L. *Phytopathol. Mediterr*. 50: 94−100.

Nair, N. G. and Nene, Y. L. 1973. Studies on yellow mosaic of urd bean (*Phaseolus mungo* L.) caused by *Mungbean yellow mosaic virus* and Virus-vector relationships. *Indian J. Farm Sci.* 1: 62-70.

Nair, N. G. and Nene, Y. L., 1974. Studies on the yellow mosaic of urd bean caused by *Mungbean yellow mosaic virus* and factors influencing transmission and symptom expression. *Indian J. Farm Sci*. 2: 42-47.

Narayanasamy, P. and Ramiah, M. 1983c. Characterization of antiviral principles from sorghum leaves. In: *Proc. Null Sem. Management of Diseases of Oilseed crops,* Tamil Nadu Agric Univ., Madurai, India. pp.15-17.

Nariani, T. K. 1960. Yellow mosaic of mung (*Phaseolus aureus* L.). *Indian Phytopathol.* 13:24-29.

Narwal, S., Balasubrahmanyam, A., Sadhna, P., Kapoor, H.C., and Lodha, M. L. 2001. A systemic resistance inducing antiviral protein with N-glycosidase activity from *Bougainvillea xbuttiana* leaves. *Indian J. exp. biol.* 39: 600-603.

Nene, Y. L. 1972. A survey of viral diseases of pulse crops in Uttar Pradesh. *Res. Bull. No.* 4, Uttar Pradesh Agri. Univ., Pantnagar, 191p.

Nene, Y. L. 1973. Viral diseases of some warm weather pulse crops in India. *Plant Dis. Rep.* 57: 463- 467.

Nene, Y. L., Naresh, J. S., and Nair, N. G. 1971. Additional hosts of *Mungbean yellow mosaic virus*. *Indian Phytopathol.* 24: 415-417.

Obaiah, S. 2011. Molecular detection and characterization of yellow mosaic virus infecting black gram in Andhra Pradesh. M. Sc. (Ag) thesis, Acharya N.G. Ranga Agricultural University, Hyderabad, 99p.

Obaiah, S., Reddy, B. V. B., Reddy, N. P. E., and Sivaprasad, Y. 2014. Molecular detection of yellow mosaic virus infecting black gram (*Vigna mungo* (L.) Hepper) in Andhra pradesh. *Int. J. Plant Anim. Environ. Sci.* 4(1): 16-18.

Padidam, M., Beachy, R. N., and Faquet, C. M. 1996. The role of AV2 ("Precoat") and coat protein in viral replication and movement in tomato leaf curl geminivirus. *Virol.* 224: 390-404.

Padidam, M., Sawyer, S., and Fauquet, C. M. 1999. Possible emergence of new geminiviruses by frequent recombination. *Virol.* 265: 218–225.

Pangnakorn, U. and Chuenchooklin, S. 2016. Acute and chronic effect of biopesticide on infestation of whitefly *Bemisia tabaci* (Gennadius) on the cilantro cultivation. *Int. J. Agric. Biosystems Eng.* 10(2) : 118-122.

Panigrahi, K. K., Das, T. R., Baisakh, B., Mohanty, A., and Pradhan, J. 2016. Validation of CYR-1 marker linked with yellow mosaic virus resistance in black gram (*Vigna mungo* (L) *Hepper*). *Indian J. Genet.* 76(1) : 104-106.

Paul, A. 2014. Characterization and management of *Tomato leaf curl virus* in Kerala. M.Sc. (Ag) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala. 171p.

Pawar, D. M. and Mahatma, L. 2013. Occurrence and symptomatology of *Mungbean yellowmosaic virus* (MYMV) in mungbean [*Vigna radiata* (L.) Wilczek] in south Gujarat. *Asian J. Bio Sci.* 8 (2): 237-240.

Polston, J. E. and Anderson, P. L.1997. The emergence of whitefly transmitted geminiviruses in tomato in the Western Hemisphere. *Plant dis.* 81: 1358-1369.

Pramanik, B. K. and Ali, M. A. 2001. Cultural and nutritional management of yellow mosaic in winter mungbean. *Pakist. J. biological Sci.* 4(1): 59-62.

Prema, G. U. 2013. Molecular characterization of horsegram yelloe mosaic virus and its management. Ph. D (Ag) thesis, University of Agricultural Sciences, GKVK, Bangalore, 271p.

Prema, G. U. and Rangaswamy, K. T. 2018. Molecular detection and characterization of coat protein gene of *Mungbean yellow mosaic virus* (MYMV) from Karnataka. *Int. J. Agric. Sci.* 10(3): 5118-5122.

Qazi, J., Iyas, M., Mansoor, S., and Briddon, R. W. 2007. Legume yellow mosaic viruses : genetically isolated begomoviruses. *Molecular Plant Pathol.* 8 : 343-348.

Raghupathi, N. and Sabitha, D. 1994. Effect of barrier crops, mulching and yellow sticky traps in the management of yellow mosaic virus disease of soybean. In: Sivaprakasam, K. and Seetharaman, K. (eds), *Crop Diseases - Innovative Techniques and Management*. Kalyani Publishers, New Delhi, pp. 419-420.

Ramesh, S. V., Chouhan, B. S., and Ramteke, R. 2017. Molecular detection of *Begomovirus* (family: *Geminiviridae*) infecting *Glycine max* (L.) Merr. and associated weed *Vigna trilobata. J. Crop Weed*, 13(2): 64-67.

Ramesh, S. V., Chouhan, B. S., Gupta, G. K., Husain, S. M., and Chand, S. 2017a. Genomic sequence characterization of *Begomovirus* infecting soybean and molecular evolutionary genomics of Legume yellow mosaic viruses (LYMVs). *Plant Omics J.* 10(02):88-96.

Ramesh, S. V., Chouhan, B. S., Gupta, G. K., Ramteke, R., Chand, S., and Husain, S. M. 2016. Molecular diversity analysis of coat protein gene encoded by legume begomoviruses and PCR assay to detect yellow mosaic viruses infecting soybean in India. *Br. Biotechnol. J.* 12(3): 1-10.

Rathi, Y. P. S. 2002. Epidemiology, yield losses and management of major diseases of Kharif pulses in India, Plant Pathology and Asian congress of Mycology and Plant Pathology, University of Mysore, pp. 123-146.

Rathi, Y. P. S. and Nene, Y. L. 1974. The additional hosts of *Mungbean yellow mosaic virus*. *Indian phytopathol.* 27: 429-430.

Rathi, Y. P. S. and Nene, Y. L. 1974a. Some aspects of the relationship between *Mungbean yellow mosaic virus* and its vector *Bemisia tabaci*. *Indian Phytopathol.* 27: 459-462.

Ravi, K. S., Anuja, M., Meena, K., Mahatma, L., and Usha, Z. 2006. *Begomovirus* complexes in crops plants. *Indian J. Virol.* 17: 116.

Reang, D., Singh, P., Thapa, A., Rai, B., Sarkar, N., and Nath, P. S. 2017. Evaluation of Mungbean (*Vigna radiata* L.) cultivars for resistance to yellow mosaic Virus. *Int. J. Curr. Microbiol. App. Sci.* *6*(4): 88-98.

Reddy, B. V. B., Obaiah, S., Prasanthi, L., Sivaprasad, Y., Sujitha, A., and Krishna, T. G. 2015. Mungbean yellow mosaic India virus is associated with yellow mosaic disease of black gram (*Vigna mungo* L.) in Andhra Pradesh, India. *Arch. Phytopathol. Plant Prot.* 48(4): 345–353.

Rienzie, K. D. R. C., Wickramaarachchi, W. A. R. T., De Costa, D. M., and Wijesooriya, W. M. G. U. 2016. Molecular detection and characterisation of begomovirus causing bean yellowing disease in Sri Lanka. *J. Natn. Sci. Foundation Sri Lanka.* 44 (3): 249-255.

Rivera-Vargas, L. L., Bracero-Acosta, V., Beaver, J. S., Purcifull, D. E., Polston, J. E., and Hiebert, E. 2001.Detecting *Bean golden yellow mosaic virus* in bean

breeding lines and in the common legume weed *Macroptilium lathyroides* in Puerto Rico. *J. Agric. Univ. P.R.* 85(3-4):165-176.

Rojas, M. R., Hagen, C., Lucas, W. J., and Gilbertson, R. L. 2005. Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. *Ann. Rev. Phytopathol.* 43: 361–394.

Rouhibakhsh, A., Priya, J., Periasamy, M., Haq, Q. M. I., and Malathi, V. G. 2008. An improved DNA isolation method and PCR protocol for efficient detection of multicomponents of begomovirus in legume. *J. Virol. Methods*, 147: 37–42.

Rybicki, E. P. 1994. A phylogenetic and evolutionary justification for three genera of *Geminiviridae. Arch. Virol.* 139: 49-77.

Salalrajan, F. 1988. Studies on yellow mosaic virus disease of urd bean *(Vigna mungo* (L.) Hepper). M.Sc. (Ag) thesis, Tamil Nadu Agricultural University, Coimbatore. 75p.

Salam, S. A., Patil, M. S., and Byadgi, A. S. 2009. IDM of mung bean yellow mosaic disease. *Ann. Plant. Prot. Sci.* 17(1): 157-160.

Sanderfoot, A. A., Ingham, D. J., and Lazarowitz, S. G. 1996. A viral movement protein as a nuclear shuttle. *Plant Physiol.* 110: 23-33.

Satya, K., Malathi, V. G., Velazhahan, R., Rabindran, R., Jayamani, P., and Alice, D. 2013. Characterization of betasatellite associated with the yellow mosaic disease of grain legumes in southern India. *Acta Virologica*, 57: 405 – 414.

Saunders, K., Bedford, I. D., Briddon, R. W., Markham, P. G., Wong, S. M., and Stanley, J. 2000. A unique virus complex causes Ageratum yellow vein disease. *Proc. Natl. Acad. Sci. USA,* 97:6890–6895.

Shahid, M. S., Ikegami, M., and Natsuaki, K. T. 2012. First report of *Mungbean yellow mosaic India virus* on Lima bean affected by yellow mosaic disease in Nepal. *Austr. Plant Dis. Notes*, 7**:** 85-89.

Sharma, N. K., Singh, S., and Awasthi, L. P. 2017. Prevention and control of viral diseases in watermelon through botanical biopesticides. *Virol. Res. Rev.* 1(3): 1-8.

Sharma, S. R., Singh, V. K., and Tomar, A. K. 2016. Management of yellow mosaic disease of mung bean (*Vigna radiata*). *Indian Phytopathol.* 69(4s): 391-393.

Shefali, M., Jagadeesh, K. S., Krishnaraj, P. U., Byadagi, A. S., and Vastrad, A. S. 2014. Synergistic effect of chitosan and *Pseudomonas* sp. on the control of *Tomato leaf curl virus* in tomato. *Int. J. Pl. Prot.* 42 (4) : 437-447.

Shivanathan. 1977. Tropical Agriculture Research Series No. 10, Tropical Agriculture Research Center, Japan, pp. 65-68.

Shukla, G. P. and Pandya, B. P. 1985. Resistance to yellow mosaic in greengram. *SABRAO J.* 17:165-171.

Singh, A. K., Srivastava, S. K., and Singh, A. K. 1985. Effect of milk on the infectivity of urdbean mosaic virus. *Indian J. Mycol. Plant Pathol.* 15: 311-312.

Singh, D. K., Karjee, S., Malik, P. S., Islam, N., and Mukherjee, S. K. 2007. DNA replication and pathogenecity of MYMIV. In: Mendez-Vilas, A. (Ed.), *Communicating Current Research and Educational Topics and Trends In Applied Microbiology,* Series no-1, vol. 1 pp. 155-162.

Singh, R. A. and De, R. K. 2006. Major diseases and their management. In: Ali, M. and Kumar, S. (eds), *Advances in Mungbean and Urdbean.* IIPR, Kanpur, pp. 283-334.

Singh, R. A., Rajib K. D. E., Gurha, S. N., and Ghosh, A. 2002. Yellow mosaic of mung bean and urd bean. *IPM Syst. Agric.* 8: 395-408.

Singh, R. N. 1979. Natural infection of bean (*Phaseolus vulgaris*) by *Mungbean yellow mosaic virus. Indian J. Mycol. Plant Pathol.* 9 : 124-126.

Singh, S. and Awasthi, L. P. 2009. Plant products for the management of yellow mosaic disease of mungbean and urdbean. *J. Plant Prot. Sci.* 1(1) : 87-91.

Singh, S. K., Awasthi, L. P., Singh, S., and Sharma, N. K. 2011. Protection of mungbean and urdbean crops against vector borne *Mungbean yellow mosaic virus* through botanicals. *Curr. Bot.* 2(2):08-11.

Singh, S., Awasthi, L. P., and Verma, H. N. 2004. Prevention and control of yellow mosaic disease of mungbean by application of aqueous root extract of *Boerhaavia diffusa*. *Indian Phytopathol.* 57 (3): 303-307.

Sivalingam P. N. and Varma, A. 2012. Role of betasatellite in the pathogenesis of a bipartite begomovirus affecting tomato in India. *Arch. Virol.* 157(6):1081-1092.

Srinivasulu, B. and Jeyarajan, R. 1986. Effect of leaf extracts *on* the infection of *Rice tungro virus* in rice. *Indian J. Virol.* 2: 176-180.

Stanley, J. 1983. Infectivity of the cloned Gemini virus genome requires sequences from both DNA. *Nature* 305 : 643-645.

Stenger, D. C., Revington, G. N., Stevenson, M. C., and Bisaro, D. M. 1991. Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling circle replication of a plant viral DNA. *Proc. Natl. Acad. Sci. USA*. 88: 8029-8033.

Surendran, M., Shunmugam, V., Rajagopalan, B., and Ramanlan, N. 1999. Efficacy of botanicals on Brinjal mosaic virus. *Plant Dis. Res*. 14 (1): 63-66.

Suteri, B. D. 1974. Occurrence of soybean yellow mosaic virus in Uttar Pradesh. *Curr. Sci.* 43 : 689-690.

Swanson, M. M., Valand, G. B., Muniyappa, V., and Harrison, B. D. 1998. Serological detection and antigenic variation of two whitefly-transmitted geminiviruses: tobacco leaf curl and croton yellow vein mosaic viruses. *Ann. Appl. Biol.* 132: 427–435.

Swanson, M. M., Varma, A., Muniyappa, V., and Harrison, B. D. 1992. Comparative epitope profiles of the particle proteins of whitefly-transmitted geminiviruses from nine crop legumes in India. *Ann. Appl. Biol*. 120 : 425-433.

Swathi, M. and Gaur, N. 2017. Effect of border crops and insecticides on management of whitefly, *Bemisia tabaci* (Gennadius) transmitted yellow mosaic virus in soybean. *Int. J. Curr. Microbiol. Appl. Sci. 6*(5): 613-617.

Thongmeearkom, P., Honda, Y., Saito, Y., and Syamananda, R. 1981. Nuclear ultrastructural changes and aggregates of virus like particles in mungbean cells affected by mungbean yellow mosaic disease. *Phytopathol.* 71 (1):41-44.

USDA [United States Department of Agriculture] 2016. Mungo beans, mature seeds, raw. *National Nutrient Database for Standard Reference Release* , 28p.

Usharani, K. S., Surendranath, B., Haq, Q. M. R., and Malathi, V. G. 2004. Yellow mosaic virus infecting soybean in northern India is distinct from the species infecting soybean in southern and western India. *Curr. Sci.* 86(6): 845-850.

Varma, A., and Malathi, V. G. 2003. Emerging geminivirus problems : a serious threat to crop production. *Ann. Appl. Biol.* 142 : 145–164.

Varma, A., Dhar, A. K., and Mandal, B. 1992. MYMV transmission and control in India. In: Green, S. K., and Kim, D. (eds) Mungbean Yellow Mosaic Disease. Asian Vegetable Research and Development Centre, Taipei, pp. 8–27.

Varsani, A., Roumagnae, P., Fuchs, M., Navas-Castillo, J., Moriones, E., Idris, A., Briddon, R. W., Rivera-Bustamante, R., Zerbini, F. M., and Martin, D. P. 2017. *Capulavirus* and *Grablovirus*: two new genera in the family geminiviridae[online].Available:https://www.researchgate.net/publication/284807252 [27 Jan 2017].

Vasudeva, R. S. 1942. A mosaic disease of cowpea in India. *Indian J. Sci.* 12: 281–283.

Venkatesan, S., Radjacommare, R., Nakkeeran, S., and Chandrasekaran, A. 2010. Effect of biocontrol agent, plant extracts and safe chemicals in suppression of *Mungbean Yellow Mosaic Virus* (MYMV) in black gram (*Vigna mungo*). *Arch. Phytopathol. Plant Prot.* 43(1) : 59–72.

Verma, A. and Singh, R. B. 1994. *Clerodendrum aculeatum* a possible prophylactic agent against natural viral infection in mungbean. *Ann. Plant Prot. Sci.* 2: 60-63.

Verma, H. N., Awasthi, L. P., and Saxena. K. C. 1979. Isolation of the virus inhibitor from root extract of *Boerhaavia diffusa* inducing systemic resistance in plant. *Can. J. Bot.* 57: 1214-1218.

Verma, H. N., Rastogi, P., Prasad, V., and Srivastava, S. 1985. A possible control of natural virus infection of *Vigna radiata* and *V. mungo* by plant extracts. *Indian J. Plant Pathol.* 3(1):21-24

William, F. J., Grewal, J. S., and Amin, K. S. 1968. Serious and new disease of pulse crops in India in 1966. *Plant Dis. Rep.* 52 : 300–304.

Yang, C., Liu, J. Z., Gui, G., Xie, L., and Wu, Z. 2008. Mixed infection of two Begomoviruses in *Malvastrum coromandelianum* in Fujian, China. *J. Phytopathol.* 156 : 553-555.

Zhang, Q. B., Lei, H. D., Li, H. Y., Liu, H. Q., Yao, T. S., Tian, W. H., and Qian, K. M. 2004. Damage by citrus spiny white fly and its control. *S. China Fruits,* 33: 15-18.

Zhou, X., Xie, Y., Tao, X., Zhang, Z., Li, Z., and Fauquet, C. M. 2003. Characterization of DNA b associated with begomoviruses in China and evidence for co-evolution with their cognate viral DNA A. *J. Gen. Virol.* 84:237–247.

Appendices

APPENDIX – I

Compostion of various stock solutions and extraction buffer used for DNA isolation

1. Different stock solutions used for DNA isolation

a. 1 M Tris HCl (100 ml)

Tris base – 12.1 g in 100 ml distilled water (pH 8.0 by HCl)

b. 5 M NaCl

NaCl – 29.22 g in 100 ml distilled water

c. 200 mM EDTA

EDTA – 7.48 g in 100 ml distilled water (pH 8.0 by NaOH)

d. 20 per cent CTAB

CTAB – 20 g in 100 ml sterile distilled water

2. CTAB extraction buffer (100 ml)

a. 100 mM Tris HCl

1 M Tris HCl – 10 ml

b. 2 M NaCl

5 M NaCl – 40 ml

c. 10 mM EDTA

200 EDTA – 5 ml

d. 2 per cent CTAB

20 per cent CTAB – 10 ml

3. Chloroform – isoamyl alcohol (Freshly prepared)

To chloroform (24 ml), isoamyl alcohol (1 ml) was added and mixed thoroughly.

4. Chilled isopropanol

Isopropanol was stored in refrigerator at 0° C and was used for isolation.

APPENDIX - II

Composition of buffers and dyes used for agarose gel electrophoresis

1. **TBE buffer (10 X)**

   Tris base – 108 g

   Boric acid – 55 g

   20 mM EDTA (pH 8.0) – 100 ml

Made up final volume upto 1 L with distilled water and autoclaved.

2. **Orange DNA loading dye**

   Bromophenol blue – 0.25 per cent

   Xylene cyanol - 0.25 per cent

   Glycerol in water - 30 per cent

3. **Ethidium bromide**

   The dye was prepared as a stock solution of Ethidium bromide (stock 10 mg/ml; working concentration 0.5 μg/ml) and was stored at room temperature in a dark bottle.

Characterization and management of yellow mosaic disease of black gram (*Vigna mungo* (L.) Hepper)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Black gram (*Vigna mungo* (L.) Hepper) is one of the important pulse crop in India and an excellent source of good quality protein. Yellow mosaic disease (YMD), caused by a whitefly transmitted geminivirus is the major constraint for black gram cultivation. Yellow mosaic disease of pulses is extensively studied from different parts of the country. But no study has been conducted in Kerala. Hence, the present study was undertaken to characterize the virus causing yellow mosaic disease in black gram in Kerala and to evolve suitable strategies for its management.

Purposive sampling surveys were conducted in black gram growing areas of Palakkad and Malappuram districts, covering 13 fields at nine locations to study the incidence and symptomatology of yellow mosaic disease. The disease incidence in the fields varied from 12 to 100 per cent.

Common symptoms observed in the field were typical yellow mosaic, puckering and cupping of leaves, distortion of leaf lamina and drastic reduction in size of younger leaves. Irregular whitish discolouration of leaves which turned papery white on maturity was also observed in some fields. Complete yellowing of leaves along with brown discoloration between the veins and vein banding symptoms which were not reported by earlier workers were also observed in the field.

Electron microscopic studies revealed the presence of geminate particles of 15-18 x 30 nm size in infected black gram samples suggesting the association of a geminivirus with the disease. Whitefly transmission of the virus to healthy black gram plants was attempted and 80 per cent transmission was achieved.

PCR amplification using virus specific primers revealed the presence of *Mungbean yellow mosaic virus* (MYMV) and *Horsegram yellow mosaic virus* (HYMV) in the samples. MYMV was detected in infected samples from nine fields out of the 13 fields surveyed. HYMV was detected in six fields, which include five fields in which MYMV was also detected revealing the occurrence of mixed infection

in the field. Five representative isolates were sequenced at Agrigenome Labs, Kakkanad, Ernakulam. *In silico* analysis of these sequences revealed that the coat protein region of the isolates showed more than 90 per cent homology with MYMV isolates. This confirmed the presence of MYMV as the major virus in yellow mosaic disease of black gram in Kerala. Phylogenetic analysis revealed that the isolates from the present study are more closely related to MYMV isolates from southern parts of India and distantly related to *Mungbean yellow mosaic India virus* (MYMIV) isolates, which were reported from northern parts of India.

Host range studies conducted in insect proof cages under glass house condition showed that the virus could be transmitted through whiteflies only to horse gram. Symptoms were observed in horse gram and *Synedrella nodiflora*, a predominant weed found in the field 20 -25 days after inoculation. But the presence of virus was confirmed by PCR only in symptomatic horse gram and not in *Synedrella nodiflora*.

A field experiment was conducted during *Rabi* 2017-18 at RARS, Pattambi and evaluation of effectiveness of botanicals, biocontrol agents and other organic products revealed that application of *Pseudomonas fluorescens* as seed treatment @ 10g/kg seed and foliar sprays @ 10g/l at fortnightly intervals starting from 15 days after sowing or foliar sprays of 10 per cent aqueous extract of leaves of *Bougainvillea spectabilis* or roots of *Boerhaavia diffusa* at fortnightly intervals starting from 15 days after sowing are effective in reducing the yellow mosaic disease.

The present study reveals that MYMV is the virus associated with the YMD of black gram in Kerala and it can be effectively managed by prophylactic application of *Pseudomonas fluorescens*, 10 per cent leaf extract of *Bougainvillea spectabilis* or 10% root extract of *Boerhaavia diffusa.* This is the first report on identification of MYMV associated with yellow mosaic disease of black gram in Kerala.