IDENTIFICATION AND MANAGEMENT OF CHILLI MOSAIC VIRUS

BY

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Thesis Submitted to Faculty of Postgraduate Studies
in partial fulfillment of the requirements
for the degree of

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(PLANT PATHOLOGY)

DIVISION OF PLANT PATHOLOGY
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CERTIFICATE – I

This is to certify that the thesis entitled **"Identification and Management of Chilli Mosaic Virus"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Plant Pathology)** to the faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agriculture Sciences and Technology of Jammu is a record of bonafide research, carried out by **Ms. Sonali Bhagat,** Registration No.**J-15-M-421,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of investigation have been duly acknowledged.

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We, the members of Advisory Committee of **Ms. Sonali Bhagat,** Registration No. **J-15-M-421,** a candidate for the degree of **Master of Science in Agriculture (Plant Pathology)** have gone through the manuscript of the thesis entitled **"Identification and Management of Chilli Mosaic Virus"** and recommend that it may be submitted by the student in partial fulfillment of the requirements for the degree.

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ABSTRACT

Title of the thesis	:	Identification and management of chilli mosaic virus
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ABSTRACT

The investigation regarding the identification and management of chilli mosaic virus was conducted in the year 2016-2017. Extensive survey were conducted in the various chilli growing areas of Jammu and Udhampur districts of Jammu and Kashmir. It was observed that in Jammu district maximum incidence of chilli mosaic was recorded in Arnia (31.42%) followed by Dasgal (29.71%), Bayaspur (24.00%), Ambran (21.14%) and Kalyana (17.14%) while in Udhampur district, maximum disease incidence was recorded in Chenani (32.57%) followed by Basht (30.28%), Kandwal (27.42%), Sudhmahadev (25.71%) and Gaurikund (19.42%). To confirm the presence of different viruses causing the disease, infected samples collected during survey were tested by serological means (DAS-ELISA). The results showed that *cucumber mosaic virus* (CMV) was found in Arnia, Bayaspur, Kalyana, Dasgal, Basht, Chenani, Gaurikund and Sudhmahadev, *potato virus Y* (PVY) was found in Bayaspur, Kalyana, Ambran, Basht, Chenani, Kandwal, Gaurikund and *pepper veinal mottle virus* (PVMV) was found in Arnia, Dasgal, Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev. Screening of fifteen germplasm collected from different sources against the disease showed that out of fifteen germplasm Pusa Sadabhar, Punjab Gucchedar and Punjab Lal were found resistant, CH-1, Arka Meghna and Anmol BSS-273 were found moderately resistant, Surajmukhi, G-4, Nishant, Crystal 906, K-Long 1 and Chandani were found moderately susceptible while Pusa Jwala, NP-46-A and local were found susceptible. Serological identification of chilli mosaic viruses in different germplam showed that Pusa Sadabhar, Punjab Gucchedar and Punjab Lal were not infected by any virus which proved the resistant nature of these germplasm while in other chilli germplasm there was presence of one or more virus. Different insecticides *viz.* imidacloprid, malathion, demeton-o-methyl, dimethoate, acetamiprid and a bio-insecticide (neem oil) were evaluated under field conditions and it was found that foliar application of imidacloprid showed lowest disease intensity of 9.01 per cent and was found most effective in controlling the spread of the disease under field conditions.

Key words: Chilli mosaic virus, identification, disease incidence, management.

Ranbir Singh

Signature of Major Advisor

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LIST OF ABBREVIATIONS USED IN THE MANUSCRIPT

viz.	*videlicet* (namely)
ha	Hectare
et al	*et alibi* (and others)
%	Per cent
@	at the rate
µl	Micro litre
$CD_{(p=0.05)}$	Critical difference at 5% level of significance
l	Litre
ml	Milli litre
g	Gram
kg	Kilo gram
DAS	Double Antibody Sandwich
DAT	Days After Transplanting
ELISA	Enzyme Linked Immuno-Sorbent Assay
FYM	Farm Yard Manure
CMV	Cucumber Mosaic Virus
PVY	Potato Virus Y
PVX	Potato Virus X
PVMV	Pepper Veinal Mottle Virus
TMV	Tobacco Mosaic Virus
TSWV	Tomato Spotted Wilt Virus
NSKE	Neem Seed Kernel Extract
NSO	Neem Seed Oil
PDI	Per cent Disease Incidence
RBD	Randomized Block Design

CHAPTER-1

INTRODUCTION

Chilli (*Capsicum annum* L.) is one of the most important cash crop among the spices and grown widely around the year. It belongs to the family *Solanaceae* and genus *Capsicum*. The fruits are consumed as fresh, dried, processed products as well as for spice and condiments. Chilli is consumed both green and ripe. Chilli, despite of its fiery hotness, is well known for its chemical, medicinal, and health benefiting properties. Red chilli is rich in caretonoid pigment. It is a good source of potassium and folic acid and contains more vitamin A and C than carrots and citrus fruits (Howard, 2000 and Marin *et al*., 2004). The pungency in chilli is due to presence of crystalline volatile alkaloid capsaicin while the bright red colour of the fruit at the ripe stage is due to the presence of crystalline volatile alkaloid capsanthin. India accounts for 25% of the world's total production of chilli which is a significant source of income making it the world's single largest producer and exporter. In India, chilli is cultivated on an area of 7.94 thousand hectare with an annual production of 1.30 million tonnes. In Jammu region of Jammu and Kashmir, 794 hectare area is under chilli cultivtion with an annual production of 1304 tonnes (Anonymous, 2013).

The sustainability of chilli based agriculture is threatened by a number of factors. The biotic stresses such as bacterial wilt, anthracnose, viruses and several insect pests have been reported to impair the crop productivity (Issac, 1992). Among the viral diseases chilli mosaic disease is one of the major disease. The disease was first time reported from India by Kulkarni (1924) and McRae (1924). The disease was reported to be caused by large number of viruses, out of which, only eight viruses were found in India, of which *pepper vein banding virus* (PVBV) and *cucumber mosaic virus* (CMV) are the most predominant ones, causing heavy loss in yield (Prasad Rao, 1976; Bidari, 1982 and Nagaraju and Reddy, 1983). While the other viruses such as *potato virus Y, pepper veinal mottle virus, tobacco etch virus, tobacco mosaic virus, tobacco ring spot and tomato spotted wilt virus* are also responsible for causing the disease (Satyaprakash *et al.*, 2002).

Chilli mosaic virus under field conditions spread mainly by different aphid vectors. The important vectors which are actively involved in transmission of chilli mosaic virus include *Aphis gossypii¸ Aphis craccivora, Myzus persicae* (Dubey and Joshi, 1974; Khatri and Sekhon, 1974; Singh and Singh, 1999). In chilli, *A. gossypii* is the most efficient vector in warmer regions, whereas, *M. persicae* is the predominant vector in the cold climates (Conti & Marte, 1983). Bidari and Reddy (1990) reported that 50 per cent of commercial chilli growing areas in Karnataka were infected by chilli mosaic and the isolates were identified as *potato virus Y, pepper vein-banding virus, pepper veinal mottle virus, tobacco etch virus, tobacco mosaic virus, cucumber mosaic virus, tobacco ring spot and tomato spotted wilt virus*. Further, Bidari and Reddy (1990) reported that incidence due to chilli mosaic disease in Karnataka varied from 11.8 to 94.8 per cent. Damiri (2014) reported that chilli mosaic disease affected the plant height and yield components of chilli at different days after transplanting in Indonesia. Serological detection of the causal virus of the disease is important to identify the disease at early stage for its management (Biswas *et al*., 2005 and Myti *et al*., 2014) and use of systemic insecticide is an effective management strategy in reducing the incidence of the disease under field conditions (Nandihalli and Thontadarya, 1986; Devi and Reddy, 1995).

In Jammu region, the disease occur at regular intervals in different chilli growing areas resulting in great loss to the crop. The information regarding the status and serological detection of the chilli mosaic virus is very scanty as no work has been done regarding the early detection of the disease by serological mean. So considering all the aspects the present study was undertaken with the following objectives :

1. Identification of chilli mosaic viruses by Enzyme Linked Immuno Sorbant Assay (ELISA).

2. Management of the disease.

CHAPTER-2

REVIEW OF LITERATURE

Chilli (*Capsicum annum* L.) is among the most frequently consumed spices and condiments throughout the world. One of the major constraints in the production of chilli, is infection by viruses which are responsible for causing great loss in production. Among these different viral disease, chilli mosaic is one of the important disease infecting chilli. The virus is commonly transmitted by aphids. The work done on various aspects of chilli mosaic disease in India as well as in other countries is reviewed as under :-

2.1 Occurrence :

Doolittle and Walker (1923) for the first time reported the occurrence of chilli mosaic virus from USA. In addition to this, the virus was reported from Trinidad (Dale, 1954) and Nigeria (Lana *et al*., 1975). McRae (1924) and Kulkarni (1924) reported chilli mosaic disease for the first time from Bombay province.

In India, Prasada Rao and Yaraguntaiah (1979) Gahukar and Nariani (1982), Pandurange Gouwda and Reddy (1982), Anjaneyulu and Apparao (1967), Singh and Shukla (1990), Bidari and Reddy (1990) and Vijayabhanu (1991) have also reported that the reduced yield of chilli in Northern Telangana zone of Andhra Pradesh was due to chilli mosaic disease.

2.2 Disease incidence and yield losses :

Joshi and Dubey (1973); Hameed *et al*., (1995) and Ong *et al*., (1980) reported that yield losses due to chilli mosaic virus was 50-60 per cent in Pakistan and some other parts of the world due to *pepper veinal mottle virus* and *cucumber mosaic virus*. In Karnataka mosaic disease was the most predominant disease causing 12-95 per cent incidence and 65-75 per cent yield losses (Narasimhan and Alagianagalingam, 1986; Bidari and Reddy, 1990). In tropical areas 97 per cent yield loss due to CMV was reported (Anonymous, 1993).

Jagdeeshwar *et al.,* (2007) conducted a survey in commercial chilli growing areas of Northern Telangana zone of Andhra Pradesh during *kharif* season 2000-2003 and showed an average incidence of chilli mosaic as 18.47 per cent. Shah *et al.,* (2013) conducted a survey in Srinagar and Pulwama districts of Kashmir valley to assess the

incidence of chilli mosaic disease and found that the disease was prevalent in both the districts with the incidence of 8.15 per cent and 3.01 per cent, respectively. Myti *et al*., (2014) conducted a research on identification of chilli mosaic virus in Northern and Eastern part of Bangladesh and revealed that the disease incidence was recorded as 11.74 to 55.90 per cent.

2.3 Symptomatology :

The virus characteristically produces mosaic mottling, vein clearing, filiform leaves, leaf curling, stunting, reduced fruit size with warty dark green out growths (Doolittle, 1921; Doolittle and Walker, 1923 and 1925; Anjaneyulu and Apparao, 1967; Nitzany, 1975; Lockhart and Fischer, 1976; Prasada Rao, 1976; Shukla and Shri Ram, 1977; Gahukar and Nariani, 1982; Vijayabhanu, 1991; Jagadeeshwar, 2004 and Jagadeeshwar *et al*., 2005a). Jagdeeshwar *et al*., (2007) observed different symptoms of chilli mosaic under field conditions as upward curling, slight to severe mottling, vein clearing, vein banding, smalling of leaves, slight to severe stunting of infected plants, excessive flowering and pre-mature drooping of flowers.

Infection of PVMV causes various symptoms in chilli including irregular dark green spot on the leaf (mottle), vein banding and leaf malformation (Latifah *et al*., 2008, Siriwong *et al*., 1995, Tsai *et al*., 2008). Van Fanbing (1999) also reported that the viruses caused different symptoms on chilli as mosaic, ring spot, curling, yellowing etc. Thakur *et al.,* (2014) found that *Pepper veinal mottle virus* (PVMV) infecting hot pepper (*Capsicum annum* L.) under natural conditions in Himachal Pradesh, exhibited mottling, mild mosaic and stunted growth.

2.4 Causal viruses :

Prasada Rao and Yaraguntaiah (1979) reported that among the several viruses attacking chilli, *cucumber mosaic virus, tobacco mosaic, potato virus Y, tomato ring spot virus, pepper veinal mottle virus, tomato spotted wilt virus, pepper vein banding virus, tobacco etch virus* were found common and responsible for causing chilli mosaic disease under south Indian conditions.

Brunt *et al*., (1990) reported that most commonly recorded viruses of chilli as *cucumber mosaic virus* (CMV), *potato virus Y* (PVY), *pepper veinal mottle virus* (PVMV), *tomato spotted wilt virus* (TSWV) and *tobacco mosaic virus* (TMV). Hameed *et al*., (1995) reported that the main viral disease infecting chilli crop in Pakistan and

particularly in Sindh province was *leaf curl virus* and *pepper veinal mottle virus* , *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY) and *Potato virus X* (PVX). They also reported that PVMV and CMV have been found the most economically important viruses causing 40 per cent yield loss in Pakistan.

2.4.1 *Cucumber mosaic virus* :

Doolittle (1916) and Jagger (1916) first described *Cucumber mosaic virus* (CMV) and the virus was assigned to the Cucumovirus group as the type member. Domingo (1994) and Ding *et al*., (1995) revealed that CMV is tri-partite, single-stranded, positive sense RNA virus.

2.4.2 *Potato virus Y* :

Potato virus Y was reported in India by many workers (Jeyrajan and Ramakrishna 1969; Suryachandra and Narayana, 1987) and is transmitted by insect vector like *M. persicae* and *A. fabae* (Lana *et al.*, 1975; Thakur *et al.*, 1988; Gowada and Reddy,1989), *A. gossypii* (Fereres *et al.*,1993).

2.4.3 *Pepper veinal mottle virus* :

Pepper veinal mottle virus was reported from South India by Prasadarao and Yaraguntaiah (1979), from Bangalore by Nagaraju and Reddy (1980) and from Nigeria by Atiri and Dale (1985).

2.5 Transmission :

Dubey and Joshi (1974); Prasada Rao (1976); Singh and Shukla (1990) conducted studies on vector transmission of *Cucumber mosaic virus* in chilli and reported that *A. gossypii* and *M. persicae* effectively transmit the virus to healthy plants in a non-persistant manner. Ong *et al*., (1979) reported that PVMV is easily transmitted in the field by many aphid species in a non-persistant manner.

The transmission of chilli mosaic virus by different species of aphids such as *A. gossypii*, *A. craccivora* and *M. persicae* (Pandurange Gowda and Reddy, 1982; Gahukar and Nariani, 1982; Bidari and Reddy, 1990). Palukaitis and Garcia-Arenal (2003), reported that *cucumber mosaic virus* is naturally transmitted by 80 aphid species. Jagadeeshwar (2004) and Jagadeeshwar *et al*., (2005a and 2005b) conducted a survey in the Northern Telangana Zone of Andhra Pradesh and revealed that aphid vectors, *A.*

gossypii, M. persicae, A. craccivora and *Rhopalosiphum maidis* were most predominant vectors for chilli mosaic virus.

2.6 Serological detection :

Shah *et al*., (2001) conducted a survey of chilli crop in three provinces of Pakistan during 1996-98 to ascertain the prevalence and distribution of four major pepper viruses *viz. pepper veinal mottle virus*, *cucumber mosaic virus*, *tobacco mosaic* and *potato virus Y*. At each location 3-5 farmers fields were inspected for identification of virus through DAS-ELISA. *Pepper veinal mottle virus, cucumber mosaic virus* appeared as the most prevalent viruses in almost all the surveyed areas. Shah *et al*., (2011) conducted an experiment for detection of chilli mosaic virus in different genotypes/lines and found that CV-1, CV-2, CV-3, CV-7, CV-11, and CV-12 did not show any symptoms and were found ELISA negative while the genotypes Gola Peshawari and Rawala showed mild vein mottling symptoms and were found ELISA positive.

Iqbal *et al*., (2012) conducted a survey in Pakistan during 2006-2007 in order to monitor and determine the incidence of *cucumber mosaic virus* in chilli through DAC-ELISA and the result indicated that *cucumber mosaic virus* prevails throughout the Pakistan with a relative incidence of 44.7 per cent. Arogundade *et al*., (2012) recorded the presence of PVMV and CMV as a single infection as well as mixed infection in chilli at Ibadan (Nigeria). They reported that 36.79 per cent were positive to PVMV, 22.14 per cent tested positive to CMV, 10 per cent tested positive to both PVMV and CMV through serological test, while 31.07 per cent tested negative to the two antisera.

Baruah *et al*., (2016), detected the infection of *cucumber mosaic virus* (CMV) and *potato virus Y* (PVY) in chilli in Assam by DAS-ELISA and concluded that 55 per cent infection was due to CMV and 44.9 per cent infection was due to PVY. Rahman *et al*., (2016) tested different varieties like BARI Marich- 1, Chittagong, Comilla-1, Jamalpur, Gazipur, Chandpur, Pusa jawla, Comilla-2, Kustia, Bogra and Balujhuri against *cucumber mosaic virus* by DAS-ELISA and confirmed that all the germplasm were infected by *Cucumber mosaic virus*.

2.7 Screening :

Anand *et al*., (1961) screened different varieties of chilli against chilli mosaic virus and reported that varieties *viz.* Puri red, Puri orange, Kondiverum, G-2 and local showed resistant reaction against chilli mosaic virus. Chowfla and Sharma (1990)

screened different germplasm of bell pepper (*C. annum* L.) against mosaic caused by poty and cucumo virus in Himachal Pradesh and found that Punjab Lal, Gauhati black, were found resistant to both the groups of viruses. Pinaki and Acharyya (1999) screened twenty five genotypes of *Capsicum annum* L. against *cucumber mosaic virus* under both field and artificial conditions and found that Pusa sadabhar and Punjab lal were resistant while Utkal Ragini and HC-44 were moderately resistant.

Shah *et al*., (2011) screened 32 exotic and indigenous chilli germplasm against PVMV through symptomatology and DAS-ELISA under glass house conditions and found that all local cultivars *viz.* NARC-4, Red chilli, Red top, Sanum, Swat Local, Ghotki, BSS-269, Loungi, Sufi and Choo were susceptible to PVMV, while the lines CV-1, CV-2, CV-3, CV-7, CV-11 and CV- 12 were highly resistant. Hidayat *et al.,* (2012) screened 29 chilli accessions against PVMV under greenhouse conditions and reported that the genotypes IPB C1, IPB C10, and PBC 521 were highly resistant, IPB C8, IPB C14, IPB C17 and Keriting Sumatra were resistant, IPB C48, IPB C60, Tegar, Toro, and Taring were moderately susceptible, IPB C6, IPB C15, and Tanjung were susceptible, and IPB C13, IPB C20, IPB C21, IPB C24, IPB C33, IPB C55, IPB C73, IPB C81, IPB C99, Tornado, Andalas, Tegak, Beauty Bell, and Polaris were highly susceptible. Ashfaq *et al.,* (2014) screened 40 Chilli genotypes, against CMV by visual observations and enzyme-linked immune sorbent assay (DAS-ELISA). Nine genotypes *viz.* C-2, CV-2, CV-5, BSS-269, PGRI, M-2001, CM-2001, M-97 and CP-328 were remained free of infection and catagorised as highly resistant. Rahman *et al.,* (2016) reported that the incidence of CMV was 21.21 per cent in Kustia and 3.00 per cent in variety Comilla-2.

2.8 Management :

The control of aphids which are the major vectors of chilli mosaic disease had been reported by many workers (Schmutterer, 1987; Isman *et al*., 1990; Mordue and Blackwell, 1993 and Lowery and Isman, 1996).

Maskale and Lingappa (1991) used neem products (neem rich -1 and neem guard) as an insecticide apart from chemical and synthetic insecticides for the management of chilli mosaic disease. Lowery *et al*., (1993) reported that neem seed kernel extract (NSKE) and neem seed oil (NSO), have shown significant reduction in number of green peach aphid (*Myzus persicae*) on pepper. Devi and Reddy (1995) tested

different insecticides like malathion, methyl parathion, chloropyriphos, monocrotophos, dimethoate against aphid vectors transmitting chilli mosaic disease and found that most of them were effective in reducing aphid population.

Basavarajappa and Patil (1999) reported that chilli mosaic caused by several viruses can be managed by using plant extracts as well as by use of insecticides. Jagadeeshwar (2004) found that seed treatment, nursery protection, seedling dip and field application of imidacloprid greately reduced chilli mosaic by increasing yield of dry chilli by 2421 kg/ha.

Baruah *et al*., (2016) conducted a research on the integrated management of chilli mosaic disease in Assam and revealed that all the treated chemicals had significantly reduced disease incidences as compared to untreated control while out of the various treatment used, seed treatment with imidacloprid @ 0.25 ml/l + nursery net + foliar spray with imidacloprid @ 2ml/l at 15, 30, 45 & 60 DAT was proved to be the most effective in reduction of disease incidence.

CHAPTER-3

MATERIALS AND METHODS

The field experiments were carried out in the research farm, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha during the year 2016-2017 to ascertain the incidence, intensity and performance of different insecticides while serological detection of chilli mosaic virus was done under laboratory conditions. The various materials and methods adopted for the field as well as laboratory experiments are given below:

3.1 Survey :

The survey of chilli mosaic virus was undertaken in parts of two district *viz*. Jammu and Udhampur. In Jammu, the survey was conducted in the villages of Arnia, Bayaspur, Kalyana, Ambran and Dasgal while in Udhampur, the survey was conducted in Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev during the year 2016-2017 (Table 1). The per cent disease incidence was calculated by the formula:

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Table 1: Locations selected for survey :

District	**Location**
Jammu	Arnia
	Bayaspur
	Kalyana
	Ambran
	Dasgal
Udhampur	Basht
	Chenani
	Kandwal
	Gaurikund
	Sudhmahadev

3.2 Serological detection of chilli mosaic virus :

Samples of chilli mosaic virus showing the symptoms like vein banding, curling of leaves and yellow and green patches on leaves (Plate I, II and III) were collected from different locations of Jammu and Udhampur. The serological detection was carried out under laboratory conditions in Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu. The samples were subjected to DAS-ELISA (Double Antibody Sandwich-Enzyme Linked Immuno Sorbant Assay) as per instruction of manufacture. The samples were exposed against antisera of *cucumber mosaic virus* (CMV), *potato virus Y* (PVY) and *pepper venial mottle virus* (PVMV) procured from Agdia.

3.2.1 Buffers used :

- **Carbonate coating buffer (1X)**

  For the preparation of carbonate coating buffer, 1 volume of 10X carbonate coating buffer concentrate was diluted with 9 volumes of distilled water.

- **Phosphate Buffered Saline with Tween 20 (wash buffer) (1X)**

  For the preparation of PBST buffer, PBST buffer powder was dissolved in distilled water as under :

  Buffer – 5 g

  Distilled water - 500 ml

  The solution was stirred for 15-30 minutes or until the powder was dissolved.

- **Enzyme conjugate buffer (ECI) (1X)**

  For the preparation of 1X ECI enzyme conjugate diluents, ECI buffer powder was dissolved in disitilled water as under :

Buffer powder	Distilled water
0.5 g	15 ml
1 g	30 ml
5 g	150 ml

  Stir the solution for 15-30 minutes after the addition of the buffer powder in the distilled water.

Plate I: Vein Banding due to chilli mosaic disease

Plate II: Curling of chilli leaves

Plate III: Yellow and green patches on chilli leaves

- **General extraction buffer**

  For the preparation of the 1X general extract buffer, general extract buffer powder was added in distilled water as under :

  Buffer – 16.5 g

  Distilled water – 500 ml

  Tween 20 – 10 ml

- **p-nitrophenyl phosphate (PNP) substrate buffer**

  For the preparation of PNP substrate buffer, 1 volume of 5X PNP buffer concentrate was diluted with 4 volumes of distilled water. Like for preparing 10 ml of working PNP substrate buffer, 2 ml of 5X PNP buffer concentrate was mixed with 8 ml of distilled water.

3.2.2 Sample preparation :

Leaves showing symptoms of chilli mosaic virus were collected and rinsed with water and then grinded in sample extraction buffer at a 1:10 ratio with the help of pestle and mortar. The content was then filtered with filter paper in order to get the extract.

3.2.3 Procedure for Double Antibody Sandwich-Enzyme Linked Immuno Sorbant Assay (DAS-ELISA) :

For the serological detection of chilli mosaic virus, DAS-ELISA was performed by the procedure as described by Clark and Adams (1977). The procedure was :

- The ELISA plate (96 wells) was coated with 100 µl coating buffer in each well.
- After coating the plate, the plate was covered tightly and was incubated at 37˚C for 4 hours.
- After incubation washing of plate was done. The plate was washed for 3-4 times with PBST buffer.
- The infected plant extract, healthy samples and buffer were added in each plate at the rate of 100 µl and the plate was then covered tightly.
- The plate was again incubated at 6˚C for 16 hours followed by washing with PBST buffer for 3-4 times.
- 100 µl of enzyme (secondary antibody) was added in each well of the plate.
- The plate was covered tightly and was incubated at 37˚C for 4 hours followed by 3-4 times washing with PBST.

- p-nitrophenyl phosphate solution was prepared as substrate and was added in each well.
- The plate was then incubated at room temperature in the dark for 2 hours.
- After the incubation the readings were measured at 405 nm.
- The positive samples were determined by visual observation. Yellow colour was developed in the positive samples (virus infected samples).

3.3 Location of the field experiment for screening and management studies :

The field experiment was conducted at the research field Division of Plant Pathology, Chatha, SKUAST Jammu which is located at 32.69˚ N latitude, 74.65˚ E longitude and at an altitude of 336 m above the mean sea level during the *Rabi* season of the year 2016-17.

3.3.1 Nursery raising :

The soil was turned into fine tilth first and then 3.5 kg of FYM was mixed with it. Raised and levelled seed bed was made and then chilli germplasm collected from different sources were sown in lines on the raised bed. The seed bed was watered and mulched with dry straw. After 35 days, seedlings were ready for transplanting. Healthy seedlings were then transplanted to the main experimental field. The seedlings were grown on sandy loam soil.

3.3.2 Layout of the experimental plot :

Land preparation, design, manure and fertilization :

Before transplanting the field was ploughed two times to make it into a fine tilth. All the weeds and stubbles were removed from the plot. Chilli seedlings were transplanted in a Randomized Block Design (RBD) 1x1.5 m plot size having spacing of 45 cm between the row and 30 cm between plants in three replications. Well decomposed farmyard manure (FYM) @ 25 tonnes/ha was thoroughly mixed with the soil at the time of field preparation and supplemented with inorganic N:P:K fertilizers at the rate of 200:100:80 kg/ha. Along 1/4N, other fertilizers were applied as basal application and the remaining N was top dressed in three split doses at 30 days interval after transplanting.

3.3.3 Screening of chilli germplasm/varities/cultivar :

Fifteen chilli germplasm/varities/cultivar *viz*. Pusa Sadabhar, Surajmukhi, G-4, CH-1, Pusa Jwala, Arka Meghna, Punjab Gucchedar, Punjab Lal, NP-46-A, Nishant, Crystal

906, Anmol BSS 273, K-Long 1, Chandani, and local variety were screened for determining resistance against chilli mosaic virus diseases under natural conditions (Table 2).

Table 2: Germplasm/varieties/cultivar used in the study with source :

Germplasm/varieties/cultivar	**Source**
Pusa Sadabhar	Department of Agriculture, Talab Tillo Jammu
Surajmukhi	Department of Agriculture, Talab Tillo Jammu
G-4	Department of Agriculture, Talab Tillo Jammu
CH-1	Local market, Jammu
Pusa Jwala	Department of Agriculture, Talab Tillo Jammu
Arka Meghna	Local market, Jammu
Punjab Gucchedar	Department of Agriculture, Talab Tillo Jammu
Punjab Lal	Department of Agriculture, Talab Tillo Jammu
NP-46-A	Department of Agriculture, Talab Tillo Jammu
Nishant	Local market, Jammu
Crystal 906	Local market, Jammu
Anmol BSS 273	Department of Agriculture, Talab Tillo Jammu
K- Long 1	Department of Agriculture, Talab Tillo Jammu
Chandani	Local market, Jammu
(Local)	Local market, Jammu

3.3.4 Disease Scoring :

Per cent disease incidence was recorded by using the following formula:

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Observations of disease incidence were recorded at 15 days interval starting from 40 days after transplanting by using the scale given by Shah *et al*., (2011). The scale of grading varietal response is mentioned in Table 3.

Table 3: Scale for grading varietal response of chilli germplasm/varieties/cultivar against chilli mosaic virus :

Per cent Disease Incidence	**Grade**	**Reaction group**
Resistant	0-10%	R
Moderately Resistant	>10-30%	MR
Moderately Susceptible	>30-50%	MS
Susceptible	>50%	S

(Modified scale by Shah *et al*., 2011)

3.4 Management :

The field experiment was conducted at research farm Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu (Plate IV). Three sprays were given for the management of the disease. First spray was given at the appearance of the disease symptoms followed by two sprays at 15 days interval. In control plots only water was sprayed. The schedule of insecticides used are presented in Table 4.

Plate IV: Field trial of chilli crop laid out at Research Farm, SKUAST-J

Table 4: Schedule of insecticides used in the study is given as under :

S.No.	Chemical Name	Schedule and dosage
1.	Malathion	Foliar spray @ 1.5ml/l of water (3 sprays at 15 days interval).
2.	Dimethoate	Foliar spray @ 1.5ml/l of water (3 sprays at 15 days interval).
3.	Demeton-O-Methyl	Foliar spray @ 1.5ml/l of water (3 sprays at 15 days interval).
4.	Imidacloprid	Seed treatment @ 3g/kg of seed. Seedling dip @ 0.3ml/l of water. Foliar spray @ 0.5ml/l of water (3 sprays at 15 days interval).
5.	Acetamiprid	Foliar spray @ 0.2g/l of water (3 sprays at 15 days interval).
6.	Neem oil	Foliar spray @ 3ml/l of water (3 sprays at 15 days interval).
7.	Control	Water only.

Severity of chilli mosaic disease was determined on disease scoring scale suggested by Biswas *et al.*, 2005.

Grade	Symptoms
0	Mosaic symptoms absent
1	1-5% of leaves showed mosaic symptoms
2	5-20% of leaves showed mosaic symptoms
3	20-30% of leaves showed mosaic symptoms
4	30-50% of leaves showed mosaic symptoms
5	50-70% of leaves showed mosaic symptoms
6	>70% of leaves showed mosaic symptoms

The per cent disease index in treated and untreated plots was calculated by using standard formula (Mckinney, 1923).

$$\text{Percentage of Disease Index} = \frac{\text{Sum of all numerical rating}}{\text{Maximum disease Grade} \times \text{Total number of plants observed}} \times 100$$

3.4.1 Statistical analysis :

The experimental data was analyzed by using standard methods to test the significance (Gomez and Gomez, 1984).

CHAPTER-4

EXPERIMENTAL RESULTS

Chilli (*Capsicum annum* L.) is one of the world's most popular vegetable consumed as fresh or processed and used mainly as a spice and condiment. Viral diseases are one of the most severe constraints in the production of the crop. Among viral diseases, chilli mosaic disease is the most important one. An investigation was carried out on various aspects *viz.* survey, incidence, symptomatology, screening, serological detection and management of the disease under field and laboratory conditions. The results observed during the investigation have been discussed as under :

4.1 Survey :

Survey was carried out in two district of Jammu division including Jammu and Udhampur. In Jammu district, the incidence of the disease was recorded from Arnia, Bayaspur, Kalyana, Ambran and Dasgal while from Udhampur district, the survey was conducted in Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev. The disease was recorded on the basis of symptomatology and then confirmed in laboratory by using serological detection method. The results are presented in Table 5.

In Jammu district, maximum disease incidence was recorded at Arnia (31.42%) followed by Dasgal (29.71%), Bayaspur (24.00%), Ambran (21.14%), Kalyana (17.14%) while the mean percentage of disease incidence was 24.68 per cent. In Udhampur district, maximum incidence of chilli mosaic virus disease was recorded in Chenani (32.57%) followed by Basht (30.28%), Kandwal (27.42%), Sudhmahadev (25.71%) and Gaurikund (19.42%) and the mean percentage of disease incidence was 27.08 per cent.

Table 5: Incidence of chilli mosaic disease in different locations of Jammu and Udhampur district :

District			
Jammu		**Udhampur**	
Location	Disease incidence (%)	Location	Disease incidence (%)
Arnia	31.42	Basht	30.28
Bayaspur	24.00	Chenani	32.57
Kalyana	17.14	Kandwal	27.42
Ambran	21.14	Gaurikund	19.42
Dasgal	29.71	Sudhmahadev	25.71
Range	17.14-31.42	Range	19.42-32.57
Mean	24.68	Mean	27.08
Overall Range	17.14-32.57		
Overall Mean	25.88		

4.2 Serological detection :

Samples collected from different locations of Jammu and Udhampur district were brought under laboratory conditions for confirmation of the pathogens by DAS-ELISA. Three antibodies *viz. cucumber mosaic virus* (CMV), *potato virus Y* (PVY) and *pepper veinal mottle virus* (PVMV) procured from Agdia were used to test the presence or absence of respective causal viruses.

Chilli samples collected from Arnia, kalyana, Bayaspur, Dasgal, Chenani, Basht, Gaurikund and sudhmahadev were found infected with *cucumber mosaic virus* while samples collected from Ambran and Kandwal were found negative against *cucumber mosaic virus*. The samples collected from each location were loaded into nine wells of the ELISA plate which were coated with specific antibodies while three wells each of ELISA plate were used for the healthy tissue and buffer respectively. The absorbance value in Arnia (0.3604-1.1957), Bayaspur (0.2481-0.9036), Kalyana (0.1312-0.3403), Ambran

(0.0291-0.0515), Dasgal (0.1689-0.6163), Chenani (0.4074-1.0280), Basht (0.0991-0.3016), Kandwal (0.0433-0.0546), Gaurikund (0.1586-0.5016) and Sudhmahadev (0.2347-0.9349) was recorded. However the wells which were charged with healthy tissue and buffer, the absorbance value was 0.0284 and 0.0352 respectively at 405 nm (Table 6 and Plate V).

Table 6: Serological detection of *cucumber mosaic virus* (CMV) from different locations of Jammu and Udhampur district :

District	**Location**	**No. of wells charged**	**OD values of CMV at 405 nm**	**Presence (+) or absence (-) of virus**	**Categorization of viruses on the basis of absorbance**
Jammu	Arnia	9	0.3604-1.1957	+	+++
	Bayaspur	9	0.2481-0.9036	+	+++
	Kalyana	9	0.1312-0.3403	+	+
	Ambran	9	0.0291-0.0515	-	-
	Dasgal	9	0.1689-0.6163	+	++
Udhampur	Chenani	9	0.4074-1.0280	+	+++
	Basht	9	0.0991-0.3016	+	+
	Kandwal	9	0.0433-0.0546	-	-
	Gaurikund	9	0.1586-0.5016	+	++
	Sudhmahadev	9	0.2347-0.9349	+	+++
Healthy tissue		3	0.0284	-	-
Buffer		3	0.0352	-	-

***Categorization of viruses on the basis of absorbance :**

0-10 times = '+', 11-20 times = '++', 21-30 times = '+++'

For identification of *potato virus Y* the samples collected from Bayaspur, Ambran, Kalyana, Chenani, Basht, Kandwal and Gaurikund showed positive results while samples collected from Arnia, Dasgal and Sudhmahadev showed negative results. The absorbance value at 405 nm was 0.0495-0.0712 in Arnia, 0.3105-0.7797 in Bayaspur, 0.2512-0.4684 in Kalyana, 0.1436-0.3853 in Ambran, 0.0515-0.0687 in Dasgal, 0.4261-1.9803 in Chenani, 0.3494-1.0049 in Basht, 0.1692-0.4227 in Kandwal, 0.0837-0.2264 in Gaurikund and 0.0563-0.0736 in Sudhmahadev while in healthy samples and buffer it was 0.0370 and 0.0394 respectively (Table 7 and Plate VI).

Table 7: Serological detection of *potato virus Y* (PVY) from different locations of Jammu and Udhampur district :

District	Location	No. of wells charged	OD values of PVY at 405 nm	Presence (+) or absence (-) of virus	Categorization of viruses on the basis of absorbance
Jammu	Arnia	9	0.0495-0.0712	-	-
	Bayaspur	9	0.3105-0.7797	+	++
	Kalyana	9	0.2512-0.4684	+	+
	Ambran	9	0.1436-0.3853	+	+
	Dasgal	9	0.0515-0.0687	-	-
Udhampur	Chenani	9	0.4261-1.9803	+	+++
	Basht	9	0.3494-1.0049	+	++
	Kandwal	9	0.1692-0.4227	+	+
	Gaurikund	9	0.0837-0.2264	+	+
	Sudhmahadev	9	0.0563-0.0736	-	-
Healthy tissue		3	0.0370	-	-
Buffer		3	0.0394	-	-

***Categorization of viruses on the basis of absorbance :**

0-10 times = '+', 11-20 times = '++', 21-30 times = '+++'

The samples collected from Arnia, Dasgal, Chenani, Basht, Kandwal, Gaurikund and Sudhmahadev were positive for *pepper veinal mottle virus* having optical density (OD) value of 0.3546-2.5953, 0.1882-0.4118, 0.3982-1.6477, 0.2868-1.5426, 0.1536-0.7946, 0.0962-0.3726 and 0.3056-1.1957 respectively at 405 nm while the samples collected from Bayaspur, Kalyana and Ambran were negative against the virus having OD value of 0.0386-0.0573, 0.0532-0.0610, 0.0441-0.0623 respectively. However in healthy tissue and buffer charged wells the OD value was 0.0412 and 0.0323 respectively at 405 nm (Table 8 and Plate VII).

Table 8: Serological detection of *pepper veinal mottle virus* (PVMV) from different locations of Jammu and Udhampur district :

District	Location	No. of wells charged	OD values of PVMV at 405 nm	Presence (+) or absence (-) of virus	Categorization of viruses on the basis of absorbance
Jammu	Arnia	9	0.3546-2.5953	+	+++
	Bayaspur	9	0.0386-0.0573	-	-
	Kalyana	9	0.0532-0.0610	-	-
	Ambran	9	0.0441-0.0623	-	-
	Dasgal	9	0.1882-0.4118	+	+
Udhampur	Chenani	9	0.3982-1.6477	+	+++
	Basht	9	0.2868-1.5426	+	+++
	Kandwal	9	0.1536-0.7946	+	++
	Gaurikund	9	0.0962-0.3726	+	+
	Sudhmahadev	9	0.3056-1.1957	+	++
Healthy tissue		3	0.0412	-	-
Buffer		3	0.0323	-	-

***Categorization of viruses on the basis of absorbance :**

0-10 times = '+', 11-20 times = '++', 21-30 times = '+++'

The positive samples produced yellow colour where as the negatively reacted samples, healthy samples and buffer showed no colour.

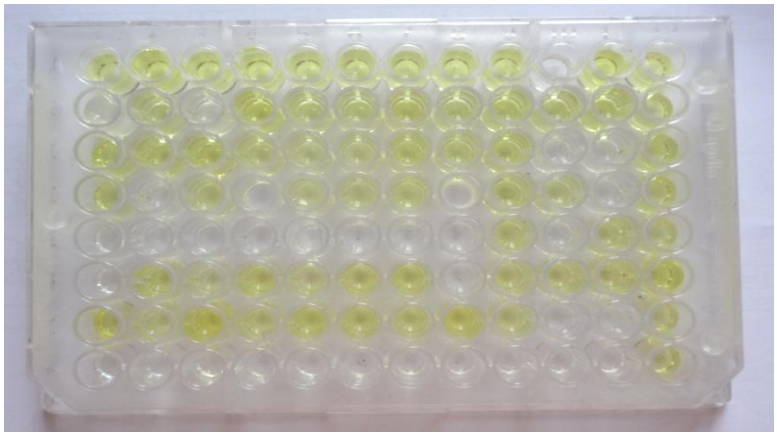

Plate V: Serological detection of cucumber mosaic virus (CMV) by DAS-ELISA

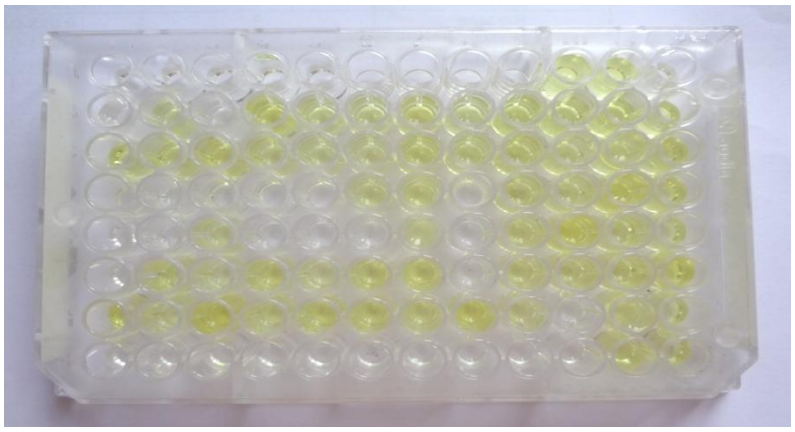

Plate VI: Serological detection of potato virus Y (PVY) by DAS-ELISA

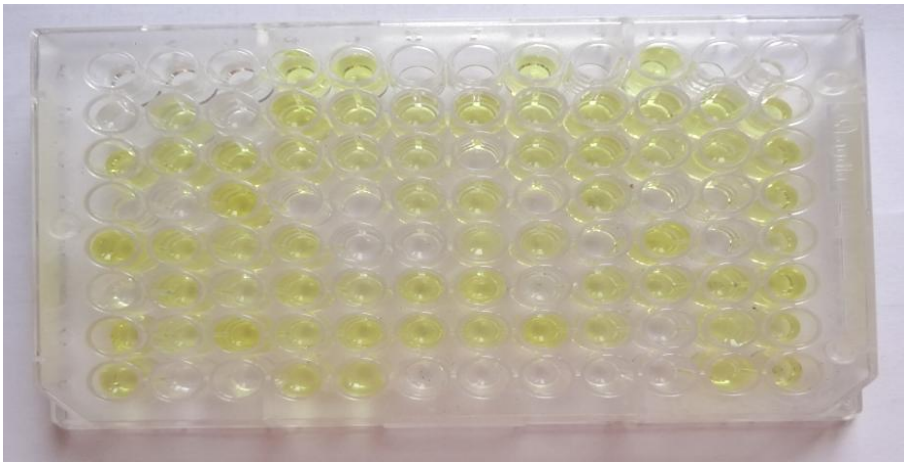

Plate VII: Serological detection of pepper veinal mottle virus (PVMV) by DAS-ELISA

***Yellow coloured well shows the presence of virus**
*** Colourless well shows the absence of virus**

Based on the above experiment, it was concluded that *cucumber mosaic virus* (CMV) was prevalent in Arnia, Bayaspur, Kalyana, Dasgal, Basht, Chenani, Gaurikund and Sudhmahadev , *potato virus Y* (PVY) was predominant in Bayaspur, Kalyana, Ambran, Basht, Chenani, Kandwal, Gaurikund and *pepper veinal mottle virus* (PVMV) was prevalent in Arnia, Dasgal, Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev (Table 9).

Table 9: Serological confirmation of chilli mosaic viruses in different locations of Jammu and Udampur district :

Virus	Location
CMV	Arnia, Bayaspur, Kalyana, Dasgal, Basht, Chenani, Gaurikund and Sudhmahadev
PVY	Bayaspur, Kalyana, Ambran, Basht, Chenani, Kandwal, Gaurikund
PVMV	Arnia, Dasgal, Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev

4.3 Screening :

Fifteen germplasm of chilli *viz*. Pusa Sadabhar, Surajmukhi, G-4, CH-1, Pusa Jwala, Arka Meghna, Punjab Gucchedar, Punjab Lal, NP-46-A, Nishant, Crystal 906, Anmol BSS-273, K-Long 1, Chandani and Local obtained from different sources were screened against the chilli mosaic virus under field conditions. Three germplasm *viz*. Pusa Sadabhar, Punjab Gucchedar and Punjab Lal were found resistant against the disease and Pusa Jwala, NP-46-A and Local were found susceptible having the mean disease incidence of 59.52 per cent, 56.24 per cent and 58.72 per cent respectively. While Surajmukhi, G-4, Nishant, Crystal 906, K-Long 1 and Chandani were found moderately susceptible having mean disease incidence of 47.61 percent, 37.85 per cent, 42.06 per cent, 39.48 per cent, 43.64 per cent and 46.86 per cent respectively. However CH-1, Arka Meghna and Anmol BSS-273 were found moderately resistant with disease incidence of 23.80 per cent, 28.56 per cent and 29.36 per cent respectively. The results are presented in Table 10 and Table 11.

At 40 DAT, maximum disease incidence was recorded in NP-46-A (50.00%) followed by Pusa Jwala (47.61%), Local (45.23%), Surajmukhi (35.71%), Chandani (33.33%), K-Long 1 (30.95%), Nishant (26.19%), G-4 (23.08%), Crystal 906 (23.08%), Anmol BSS-273 (21.42%), Arka Meghna (19.04%), CH-1 (11.90%), Pusa Sadabhar (0.00%), Punjab Gucchedar (0.00%) and Punjab Lal (0.00%).

At 55 DAT, maximum disease incidence was recorded in Local (59.52%) followed by NP-46-A (57.14%), Pusa Jwala (52.38%), Surajmukhi (52.38%), Chandani (42.85%), K-Long 1 (45.23%), Nishant (40.47%), G-4 (40.47%), Crystal 906 (30.95%), Anmol BSS-273 (28.57%), Arka Meghna (26.19%), CH-1 (21.42%), Pusa Sadabhar (0.00%), Punjab Gucchedar (0.00%) and Punjab Lal (0.00%).

At 70 DAT, Pusa Jwala showed mosaic disease incidence of 78.57 per cent followed by Local (71.42%), Chandani (64.42%), Crystal 906 (64.42%), NP-46-A (61.90%), Nishant (59.52%), Surajmukhi (54.76%), K-Long 1 (54.76%), G-4 (50.00%), Arka Meghna (40.47%), CH-1 (38.09%), Anmol BSS-273 (38.09%), Pusa Sadabhar (0.00%), Punjab Gucchedar (0.00%) and Punjab Lal.

Table 10: Screening of chilli germplasm against chilli mosaic virus under field conditions :

S.No	Germplasm	Disease Incidence (%)			Mean (%)	Grade
		40 DAT	55 DAT	70 DAT		
1	Pusa Sadabhar	0	0	0	0	R
2	Surajmukhi	35.71	52.38	54.76	47.61	MS
3	G-4	23.08	40.47	50.00	37.85	MS
4	CH-1	11.90	21.42	38.09	23.80	MR
5	Pusa Jwala	47.61	52.38	78.57	59.52	S
6	Arka Meghna	19.04	26.19	40.47	28.56	MR
7	Punjab Gucchedar	0	0	0	0	R
8	Punjab Lal	0	0	0	0	R
9	NP-46-A	50.00	57.14	61.90	56.24	S
10	Nishant	26.19	40.47	59.52	42.06	MS

11	Crystal 906	23.08	30.95	64.42	39.48	MS
12	Anmol BSS-273	21.42	28.57	38.09	29.36	MR
13	K-Long 1	30.95	45.23	54.76	43.64	MS
14	Chandani	33.33	42.85	64.42	46.86	MS
15	(Local)	45.23	59.52	71.42	58.72	S

Table 11: Disease reaction of different germplasm against chilli mosaic virus under field conditions :

Reaction	Disease Incidence (%)	No. of entries	Germplasm
Resistant	0	3	Pusa Sadabhar, Punjab Gucchedar and Punjab Lal
Moderately Resistant	>10-30	3	CH-1, Arka Meghna and Anmol BSS-273
Moderately Susceptible	>30-50	6	Surajmukhi, G-4, Nishant, Crystal 906, K-Long1 and Chandani
Susceptible	>50	3	Pusa Jwala, NP-46-A and local

All the germplasm screened were tested serologically by DAS-ELISA for the confirmation of causal virus. The samples collected during screening were loaded into three wells of the ELISA plate which were coated with specific antibody while three wells each of ELISA plate were loaded with buffer, healthy tissue and positive control. The result showed negative result in case of Pusa Sadabhar, Punjab Gucchedar and Punjab Lal which confirmed that there was no virus present in these three germplasm (Table 12).

Table 12: Serological detection of chilli mosaic virus in different germplasm:

S.NO.	Germplasm	Number of well charged	ELISA readings of CMV at 405 nm		ELISA readings of PVY at 405 nm		ELISA readings of PVMV at 405 nm	
1	Pusa Sadabhar	3	0.0288-0.0347	-	0.0418-0.0463	-	0.0439-0.0565	-
2	Surajmukhi	3	0.2387-0.4978	+	0.0299-0.0395	-	0.2540-0.5267	+
3	G4	3	0.1026-0.6240	+	0.0849-0.1124	+	0.0463-0.0592	-
4	CH-1	3	0.2869-0.5435	+	0.2587-0.3230	+	0.3347-0.5553	+
5	Pusa Jwala	3	0.5218-2.5953	+	0.0407-0.0528	-	0.1076-0.3792	+
6	Arka Meghna	3	0.3073-0.5016	+	0.2019-0.3105	+	0.0475-0.0603	-
7	Punjab Gucchedar	3	0.0392-0.0411	-	0.0425-0.0498	-	0.0426-0.0558	-
8	Punjab Lal	3	0.0405-0.0456	-	0.0469-0.0537	-	0.0407-0.0610	-
9	NP-46-A	3	0.4564-1.0280	+	0.2133-03363	+	0.3027-0.9514	+
10	Nishant	3	0.2073-1.2230	+	0.0349-0.0542	-	0.2741-0.8175	+
11	Crystal 906	3	0.0385-0.0442	-	0.3542-0.4261	+	0.1520-0.2481	+
12	Anmol BSS-273	3	0.0423-0.0446	-	0.2603-0.3542	+	0.1931-0.2744	+
13	K-Long 1	3	0.2335-0.7303	+	0.3462-0.4335	+	0.0379-0.0443	-
14	Chandani	3	0.0265-0.0394	-	0.1858-0.3428	+	0.2117-0.3632	+
15	(Local)	3	0.2997-1.0115	+	0.3471-0.9267	+	0.3835-0.4768	+
Healthy tissue		3	0.0232	-	0.0284	-	0.0308	-
Buffer		3	0.0406	-	0.0337	-	0.0363	-

All other germplasm showed the presence of one or more virus. It was also observed during the screening that *cucumber mosaic virus* (CMV) detected in Surajmukhi, G-4, CH-1, Pusa Jwala, Arka Meghna, NP-46-A, Nishant, Anmol BSS-273 and Local germplasm, while G-4, CH-1, Arka Meghna NP-46-A, Nishant, K-Long 1 and Local showed positive results against *potato virus Y* (PVY). However, *pepper veinal mottle virus* (PVMV) was found in *viz*. Surajmukhi, CH-1, Pusa Jwala, NP-46-A, Nishant, Crystal 906, Anmol BSS-273, Chandani and Local (Table 13).

Table 13: Serological identification of chilli mosaic viruses in different Germplasm/ varieties/cultivar :

Virus	Germplasm/varieties/cultivar
CMV	Surajmukhi, G-4, CH-1, Pusa Jwala, Arka Meghna, NP-46-A, Nishant, Anmol BSS-273 and local
PVY	G-4, CH-1, Arka meghna NP-46-A, Nishant, K-Long 1 and local
PVMV	Surajmukhi, CH-1, Pusa Jwala, NP-46-A, Nishant, Crystal 906, Anmol BSS-273, Chandani and local

4.4 Management :

Different insecticides (imidacloprid, malathion, demeton-o-methyl, dimethoate, acetamiprid, and neem oil) were evaluated against chilli mosaic disease in variety Pusa Jwala under field conditions and the results of the study are presented in Table 14.

At 40 DAT, the minimum disease index was recorded in foliar application of imidacloprid treatment (6.06%) followed by dimethoate (6.16%), demeton-o-methyl (6.78%), acetamiprid (7.07%), seedling treatment with imidacloprid (7.40%), seed treatment with imidacloprid (8.54%), malathion (8.63%), neem oil (10.49%) and control (18.00%).

While at 55 DAT, foliar application of imidacloprid was found most effective with minimum disease index of 8.63 per cent followed by seed treatment with imidacloprid (9.01%), dimethoate (10.49%), demeton-o-methyl (11.10%), seedling treatment with imidacloprid (11.10%), acetamiprid (11.35%), malathion (12.95%), neem oil (14.19%) and then control (20.18%). However at 70 DAT, maximum percentage of disease index was recorded in control (23.45%) followed by neem oil (17.89%), malathion (17.27%), seed

treatment with imidacloprid (16.66%), demeton-o-methyl (15.42%), acetamiprid (14.19%), seedling treatment with imidacloprid (14.19%), dimethoate (13.57%).

Table 14: Evaluation of different insecticides against chilli mosaic virus disease under field conditions on variety Pusa Jwala :

Treatment	Dose	Per cent Disease Index			Mean
		40 DAT	55 DAT	70 DAT	
Imidacloprid (ST)	3g/kg of seed	8.54	9.01	16.66	11.40
Imidacloprid (Sd T)	0.3ml/l of water	7.40	11.10	14.19	10.89
Imidacloprid (FS)	0.5ml/l of water	6.06	8.63	12.34	9.01
Malathion (FS)	1.5ml/l of water	8.63	12.95	17.27	12.95
Demeton-o-methyl (FS)	1.5ml/l of water	6.78	11.10	15.42	11.10
Dimethoate (FS)	1.5ml/l of water	6.16	10.49	13.57	10.07
Acetamiprid (FS)	1.5ml/l of water	7.07	11.35	14.19	10.87
Neem oil (FS)	0.2g/l of water	10.49	14.19	17.89	14.19
Control		18.00	20.18	23.45	20.54
C.D ($p \leq 0.05$))		3.153	4.087	2.711	
SE(m)±		1.043	1.351	0.911	

*ST= Seed Treatment

*Sd T= Seedling Treatment

*FS= Foliar spray

*DAT= Days After Transplanting

At 40 DAT, there was no significant variation among seed treatment with imidacloprid, seedling treatment with imidacloprid, foliar application with imidacloprid, malathion, demeton-o-methyl, dimethoate and acetamiprid. At 55 DAT, there was no

significant variation among foliar application of imidacloprid, seed treatment of imidacloprid, seedling treatment of imidacloprid, demeton-o-methyl, dimethoate, acetamiprid, while at 70 DAT, there was no significant variation among foliar application of imidacloprid, seedling treatment with imidacloprid, dimethoate and acetamiprid.

CHAPTER-5

DISCUSSION

Chilli (*Capsicum annum* L.) is an important commercial crop widely cultivated all over the world. Commercial chilli production sustains losses from infection by many bacterial, fungal and viral pathogens. Viral diseases are considered to be the major limiting factor in chilli production. Among the viral diseases, chilli mosaic disease has caused a serious loss in chilli production in Jammu division. Therefore an investigation was carried out on various aspects to know the status, detection and management of the disease. The data recorded during the course of investigation is discussed as under :

Survey was conducted in various locations of Jammu (Arnia, Bayaspur, Kalyana, Ambran and Dasgal) and Udhampur (Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev) district to record the incidence of chilli mosaic disease. The chilli mosaic disease was prevalent in all the locations surveyed. In Jammu district, maximum disease incidence was recorded from Arnia (31.42%) and minimum from Kalyana (17.14%), whereas, in Udhampur district maximum disease incidence was recorded from Chenani (32.57%) and minimum from Gaurikund (19.42%). The overall incidence of chilli mosaic in Jammu district was 24.68 per cent while in Udhampur district, it was 27.08 per cent. This difference in incidence in two district may be due to source of virus inoculum, population of aphid responsible for transmission of virus and environmental conditions. Bidari and Reddy (1990) reported 12 to 95 per cent incidence of chilli mosaic disease in Karnataka. Shah *et al.,* (2013) also recorded 8.15 per cent and 3.01 per cent disease incidence of chilli disease from Srinagar and Pulwama district of Kashmir valley while 11.74-55.90 per cent disease incidence of chilli mosaic in northern and eastern part of Bangladesh was also reported by Myti *et.al*, (2014).

Identification of chilli mosaic virus under field conditions was done on the basis of visual symptoms and confirmation of virus was done under laboratory conditions through DAS-ELISA. It was observed that *cucumber mosaic virus* (CMV) was found in Arnia, Bayaspur, Kalyana, Dasgal, Basht, Chenani, Gaurikund and Sudhmahadev , *potato virus Y* (PVY) was found in Bayaspur, Kalyana, Ambran, Basht, Chenani, Kandwal, Gaurikund and *pepper veinal mottle virus* (PVMV) was found in Arnia, Dasgal, Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev. The difference in the distribution of viruses in different locations may be due to the presence of source of inoculum of that particular virus in the

particular location. Similar results were shown by Shah *et al.*, (2001) who reported through DAS-ELISA that *pepper veinal mottle virus* and *cucumber mosaic virus* were prevalent in major provinces of Pakistan. Myti *et al*., (2014) also reported that DAC-ELISA was the most reliable test for the detection of the plant viruses. They used three antisera of *cucumber mosaic virus* (CMV), *potato virus Y* (PVY) and *pepper veinal mottle virus* (PVMV) to detect the virus separately. Other workers who used the similar technique for the detection of chilli mosaic virus were Iqbal *et al*., (2012); Barauh *et al*., (2016) and Rahman *et al.,* (2016).

Identification of resistant germplasm is one of the important aspect in management of viral disease. Different chilli germplasm were screened against chilli mosaic virus under field conditions. It was found that out of 15 germplasm, Pusa Sadabhar, Punjab Gucchedar and Punjab Lal were categorized as resistant, CH-1, Arka Meghna and Anmol BSS-273 as moderately resistant, Pusa Jwala, NP-46-A and Local as susceptible and Surajmukhi, G-4, Nishant, Crystal 906, K-Long1 and Chandani as moderately susceptible. It was observed that disease incidence of chilli mosaic ranged from 0.00-78.57 per cent at 70 days after transplanting. Pinaki and Acharyya, (1999) reported that Pusa Sadabhar and Punjab Lal were resistant against *cucumber mosaic virus*. The screening of different germplasm of chilli against chilli mosaic disease has been also reported by Anand *et al*., (1961); Arora *et al.,* (1996); Shah *et al*., (2011); Hidayat *et al.,* (2012) and Ashfaq *et al.,* (2014).

Serological detection of different causal viruses was also done in different germplasm tested through DAS-ELISA and the results showed that Pusa Sadabhar, Punjab Gucchedar and Punjab Lal were not infected by any of the virus which proved the resistant nature of these germplasm. While in other chilli germplasm one or more virus was present. It was found that *cucumber mosaic virus* (CMV) was present in germplasm *viz*. Surajmukhi, G-4, CH-1, Pusa Jwala, Arka Meghna, NP-46-A, Nishant, Anmol BSS-273 and Local. Germplasm G-4, CH-1, Arka Meghna NP-46-A, Nishant, K-Long 1 and Local showed positive results against *potato virus Y* (PVY). While *pepper veinal mottle virus* (PVMV) showed positive results in germplasm *viz*. Surajmukhi, CH-1, Pusa Jwala, NP-46-A, Nishant, Crystal 906, Anmol BSS-273, Chandani and Local. On the basis of symptomatology all the chilli samples showed some disease incidence in the field but ELISA test confirmed the presence or absence of virus in the infected samples. Although symptomatology, is the first step to diagnose the disease yet it is not a reliable criterion because symptoms development is influenced by many factors such as environmental conditions, insect sucking, nutritional deficiency, type of infections, virus strains etc. Shah

et al., (2011) reported that Peshawari, Rawala, CV-5, CV-6, CV-10, Ghotki, BSS-269 and ELPASO lines showed positive result against chilli mosaic virus through ELISA. Rahman *et al.,* (2016) reported that different varities of chilli like BARI Marich-1, Chittagong, Comilla-1, Jamalpur, Gazipur, Chandpur, Pusa Jwala, Comilla-2, Kustia, Bogra and Balujhuri showed positive results against *cucumber mosaic virus* through ELISA.

Different chemicals *viz*. imidacloprid, malathion, demeton-o-methyl, dimethoate, acetamiprid, and neem oil were evaluated under field conditions against chilli mosaic disease. The results revealed that foliar spray of imidacloprid at 70 DAT was found most effective treatment in maintaining the disease intensity of 12.34 per cent. The other chemicals *viz*. malathion, demeton-o-methyl, dimethoate, acetamiprid, and neem oil were also effective in reducing the disease intensity as compared to untreated plots. Similar results were found by Santharam *et al*., (2003) who reported that imidacloprid had shown excellent response as seed dresser, root dip and foliar spray for the management of mosaic disease in chilli. The secondary spread of chilli mosaic disease under field conditions totally depends upon the presence of vectors and source plants. If the vectors and the source plants are abundant, the chance of disease spread will be much more. Under this situation to minimize the spread of the disease, application of proper insecticides at right time is most essential. Basavarajappa and Patil (1999) found that systemic insecticides were found most effective in reducing mosaic disease of chilli. Baruah *et al*., (2016) also revealed that seed treatment with imidacloprid @ 0.25 ml/l + nursery net + foliar spray with imidacloprid @ 2ml/l was proved to be the most effective in reduction of disease incidence. For ecofriendly management of aphids which are the major vector of chilli mosaic under field conditions, application of neem seed and neem seed kernel extract were also found effective to check the further spread of the disease (Rembold *et al*., 1987; Isman *et al*., 1990).

During the course of study it was observed that chilli mosaic disease is one of the major disease of chilli and a major constraint in its production. During the field survey it was observed that the disease was prevalent at all the locations. Early detection of the virus through serological means is very important as this will help the farmer to adopt effective control measures at early stages like removal of virus infected plant, removal of weeds and application of systemic insecticides which will control the insect vector and check further spread of the disease.

CHAPTER-6

SUMMARY AND CONCLUSION

Chilli (*Capsicum annum* L.) is an important vegetable crop grown worldwide and has a tremendous export potential due to its demand in the international market and its non-perishable nature on drying. Many bacterial, viral and fungal diseases cause economic losses to chilli cultivation. Among the various viral diseases chilli mosaic disease is the major limiting factor responsible for low productivity of the crop. So considering the importance of the crop, study was conducted in Jammu and Udhampur district with the objectives to indentify the virus responsible for disease and its management. The findings of the study are summarized as under:

- Survey was conducted in two district of Jammu region *viz*. Jammu and Udhampur to ascertain the incidence of chilli mosaic. In Jammu, maximum disease incidence of 31.42 per cent was recorded in Arnia and minimum of 17.14 per cent was recorded in Kalyana. The mean percentage of disease incidence recorded in Jammu was 24.68 per cent. In Udhampur, maximum disease incidence of 32.57 per cent was recorded in Chenani and minimum 19.42 per cent was recorded in Gaurikund with mean percentage of 27.08 per cent.
- Infected samples collected during the survey were tested by serological means (DAS- ELISA) under laboratary conditions and the result showed that *Cucumber mosaic virus* (CMV) was found in Arnia, Bayaspur, Kalyana, Dasgal, Basht, Chenani, Gaurikund and Sudhmahadev, *potato virus Y* (PVY) was found in Bayaspur, Kalyana, Ambran, Basht, Chenani, Kandwal and Gaurikund while *pepper veinal mottle virus* was found in Arnia, Dasgal, Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev.
- Screening of different germplasm of chilli against chilli mosaic disease showed that out of the fifteen germplasm Pusa Sadabhar, Punjab Gucchedar and Punjab Lal were found resistant while CH-1, Arka Meghna and Anmol BSS-273 were found moderately resistant. However Pusa Jwala, NP-46-A and Local showed susceptible reaction and Surajmukhi, G-4, Nishant, Crystal 906, K-Long 1 and Chandani showed moderately susceptible reaction against the disease.
- Detection of chilli mosaic virus in different chilli germplasm through DAS-ELISA showed that no infection was found in Pusa Sadabhar, Punjab Gucchedar and Punjab

Lal, which proved the resistant nature of these three germplasm. While in other germplasm there was presence of one or more virus. Surajmukhi, G-4, CH-1, Pusa Jwala, Arka Meghna, NP-46-A, Nishant, Anmol BSS-273 and Local showed positive results against *cucumber mosaic virus* (CMV), while G-4, CH-1, Arka Meghna, NP-46-A, Nishant, K-Long 1 and Local showed positive results, against *potato virus Y* (PVY). While *pepper veinal mottle virus* (PVMV) was found in Surajmukhi, CH-1, Pusa Jwala, NP-46-A, Nishant, Crystal 906, Anmol BSS-273, Chandani and Local.

- Effect of various chemicals *viz*. imidacloprid, malathion, demeton-o-methyl, dimethoate, acetamiprid and botanical insecticides (neem oil) was studied against chilli mosaic disease under field conditions in the susceptible variety Pusa Jwala. The results revealed that foliar application of imidacloprid was found most effective with minimum mean disease intensity of 9.01 per cent followed by dimethoate, acetamiprid, seedling treatment of imidacloprid, demeton-o-methyl, seed treatment with imidacloprid, malathion and neem oil.

CONCLUSION

- In Jammu district, maximum incidence of chilli mosaic was recorded in Arnia (31.42%) and minimum was recorded in kalyana (17.14%). While in Udhampur district, maximum disease incidence was recorded in Chenani (32.57%) and minimum was recorded in Gaurikund (19.42%).
- *Cucumber mosaic virus* (CMV) was detected serologically in Arnia, Bayaspur, Kalyana, Dasgal, Basht, Chenani, Gaurikund and Sudhmahadev , while *potato virus Y* (PVY) was detected in Bayaspur, Kalyana, Ambran, Basht, Chenani, Kandwal, Gaurikund and *pepper veinal mottle virus* (PVMV) was detected in Arnia, Dasgal, Basht, Chenani, Kandwal, Gaurikund and Sudhmahadev areas of Jammu and Udhampur district.
- Pusa Sadabhar, Punjab Gucchedar and Punjab lal were found resistant during screening of different germplasm and showed negative results in DAS-ELISA, where as Pusa Jwala, NP-46-A and Local were found susceptible and showed positive results during serological detection.
- Foliar application of imidacloprid was found most effective to check the spread of chilli mosaic virus under field conditions.

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CERTIFICATE- IV

Certified that all necessary corrections as suggested by the external examiner and the advisory committee have been duly incorporated in the thesis entitled **"Identification and Management of Chilli Mosaic Virus"** submitted by **Ms. Sonali Bhagat,** Registration No. **J-15-M-421.**

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