"EFFECT OF BOTANICALS ON SEED BORNE LEAF CRINKLE VIRUS IN BLACK GRAM (*Vigna mungo* L)."

By

Mr. Tikhe Dnyanev Ashok

(Reg. No. 015/248)

A Thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI-413 722, DIST. AHMEDNAGAR
MAHARASHTRA STATE (INDIA)

In partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY

POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI - 413 722, DIST. AHMEDNAGAR
MAHARASHTRA STATE (INDIA).

2017

"EFFECT OF BOTANICALS ON SEED BORNE LEAF CRINKLE VIRUS IN BLACK GRAM (*Vigna mungo* L)."

By

Mr. Tikhe Dnyandev Ashok

(Reg. No. 015/248)

A Thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI-413 722, DIST. AHMEDNAGAR
MAHARASHTRA STATE (INDIA)

In partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (AGRICULTURE)

in

PLANT PATHOLOGY

Approved by

Dr. S. B. Latake
(Chairman and Research Guide)

Dr. B. M. Ilhe
(Committee Member)

Dr. G. P. Deshmukh
(Committee Member)

Dr. S. V. Kolse
(Committee Member)

Prof. L. B. Mhase
(Committee Member)

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY

POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI - 413 722, DIST. AHMEDNAGAR
MAHARASHRA STATE (INDIA).

2017

CANDIDATE'S DECLARATION

I hereby declare that this thesis or a part thereof has not been submitted by me or any other person to any other University or Institution for a Degree or Diploma.

Place : MPKV, Rahuri *(Tikhe D. A.)*

Dated : / / 2017

Dr. S. B. Latake
Cotton Pathologist,
Cotton Improvement Project
M.P.K.V., Rahuri (413 722)
Maharashtra State India

CERTIFICATE

This is to certify that the thesis entitled, **"EFFECT OF BOTANICALS ON SEED BORNE LEAF CRINKLE VIRUS IN BLACK GRAM (*Vigna mungo* L)."** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfillment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY**, is a record of *bona fide* research work carried out by **Mr. Tikhe Dnyandev Ashok** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation and sources of literature referred to have been duly acknowledged.

Place : MPKV., Rahuri (Dr. S. B. Latake)
Date : / /2017 Research Guide

Dr. J. V. Patil
Associate Dean
Post Graduate Institute
Mahatma Phule Krishi Vidyapeeth
Rahuri - 413 722, Dist: Ahmednagar
Maharashtra State (India)

CERTIFICATE

This is to certify that the thesis entitled, **"EFFECT OF BOTANICALS ON SEED BORNE LEAF CRINKE VIRUS IN BLACK GRAM (*Vigna mungo* L)."** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfillment of the requirement for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY,** is a record of *bona fide* research work carried out by **Mr. Tikhe Dnyandev Ashok,** under the guidance and supervision of **Dr. S. B. Latake,** Cotton Pathologist, Cotton Improvement Project, M.P.K.V., Rahuri and that no part of the thesis has been submitted for any other university for degree or diploma.

Place : MPKV., Rahuri (Dr. J. V. Patil)
Date : / /2017 Associate Dean

ACKNOWLEDGEMENT

I consider myself to be very fortunate for getting an opportunity to express my deepest gratitude and enormous indebtedness to my Chairman and Research Guide ***Dr. S. B. Latake****, Cotton Pathologist, Cotton Improvement Project, M.P.K.V., Rahuri for his keen interest, dynamic guidance constant inspiration, constructive criticism and encouragement throughout my research work. Without his insistence and persistence this manuscript would not have seen light of the day. I feel extremely honored for getting a golden opportunity to work under his perpetual motivation where I developed insight as how to carryout scientific research work due to his highly technical and critical methodical approach towards work.*

I wish to express my profound sense of gratitude to ***Dr. C. D. Deokar****, Head, Department of Plant Pathology and Agricultural Microbiology for providing excellent laboratory facilities and never ending inspiration during the investigation.*

I gratefully acknowledge my indebtedness and sincere thanks to the advisory committee member, ***Dr. B. M. Ilhe****, Mycologist, ARS, Niphad.* ***Dr. G. P. Deshmukh****, Assistant Professor (Plant Pathology) Oil Seed Research Station Jalgaon.* ***Dr. S. V. Kolse*** *Jr. Pathologist, AICRP on Fruits, M.P.K.V., Rahuri,* ***Prof. L. B. Mhase****, Scientist (Plant Breeding) Pulse Improvement Project, M.P.K.V., Rahuri. for precise advice, helpful comments, co-operation throughout this investigation, their timely guidance and critical evaluation of this manuscript.*

I take this golden opportunity to express my sincere thanks to ***Dr. J. V. Patil*** *Associate Dean PGI, M.P.K.V., Rahuri providing necessary facilities for research work and whole hearted co-operation.*

I also express my deepest thanks to ***Dr. B. G. Barhate****,* ***Dr. K, S. Raghuvanshi,*** ***Dr. A. M. Nawale,*** ***Dr. S. R. Zanjare*** *and* ***Shri. N. A. Musmade*** *for their excellent teaching and persistent help in my academic career.*

I am also thankful to Shri. Ranjale sir, Sau. Manisha Nangare,

madam Sau Harishchandre madam, Miss. Kamini Madam and other staff members of Department of Plant Pathology and Agricultural Microbiology, PGI, Rahuri for their helpful comments, suggestions and co-operation in completion of the present study.

I express my regards to ***Shri. P.A. Shinde****, University librarian, MPKV, Rahuri for their kind co-operation during the course of my post graduate study.*

I am thankful to my senior's Sanjay Bhos, Balaji (bhau) Chawan and Mahesh Shitole sir for their kind co-operation and suggestions during this investigation.

I am extremely thankful to all my batchmates Sharad Pawar (PSI), Vishal Suryawanshi, Shivkumar Sadaphule (Motabhai), Amajad Tamboli, Suhas Kaulage, Suhas Yadav, Abhimanyu Kharade, Mahaling Bhadekar, Arjun Rajarpallu, Dipankar Sarkar, Pallavi Rukhe (Rukhsana), Shital Khobragade (Guddi) and Balika Ghogare, Poonam Chawan for their excellent company and valuable help.

Really fortunate I am to have a juniors I record my deep sense of appreciation of their cheerful company co-operation during my investigation.

I can't express the depth of feelings to thanks for being my friends forever to, Vaijinath funde, Swapnil Londhe (PSI), Prashant Dighe, Hrishikesh Ulhare, Vijay Jawane, Soma Kolhe, Rahul Jawale, Deepak Aher, Shreepad Gawali and all chhawa group for their inspiring attitude and support and company of whom brought joy and happiness.

Words are not enough any vocabulary will be insufficient to express my gratitude, love and affection to my loving and caring father ***Shri. Ashokrao Nana Tikhe (Aaba),*** *mother* ***Mrs. Indubai Ashokrao Tikhe (Aai)****, their efforts, blessings and affection made me able to build up my academic career. I am thankful to my sister's* ***Mukta (Baidy) and Mira (Taidy)*** *, brother* ***Deepak*** *for their continuous inspiration and love helped me to build up my abilities and my strength to bring a long cherished dream into reality.*

Also I am great thankful to my maushi ***Mrs. Sindhu Bhimrao Chaudhari****, brother-in-law* ***Shri. Vithal Kantilal Satpute,*** *and other relatives for their everlasting love inexhaustible support, constant inspiration, moral support and encouragement during my life.*

I am grateful to all authors past and present whose literature has been cited.

I would like to express my sincere thanks to the Mahatma Phule Krishi Vidyapeeth, Rahuri. Dist-Ahmednagar for providing me an opportunity to undertake my post graduate studies in this "Institute of Excellence".

Lastly I thank one and all who have directly and indirectly helped me in course of this investigation and whose name could not appear in this acknowledgement.

Place: MPKV., Rahuri *(Tikhe D. A.)*

Date : / /2017

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF ABBREVIATIONS

ºC	:	Degree Centigrade (s)
C.D.	:	Critical Difference
cm.	:	Centimeter (s)
cv.	:	Cultivar
DAS	:	Days after sowing
et al.	:	And others (et alli)
Fig.	:	Figure
g	:	Gram (s)
mg	:	Milligram (s)
ha	:	Hectare (s)
hrs	:	Hours
i.e.	:	That is
kg	:	Kilogram (s)
min	:	Minute
ml	:	Millilitre (s)
nm	:	Nanometer
No.	:	Number
S.Em.	:	Standard Error of mean
spp.	:	Species
viz.	:	Videlicet (Namely)
w/v	:	Weight by volume
ULCV	:	Urdbean Leaf Crinkle Virus
etc.	:	And so forth (*et cetera*)
PDI	:	Per cent Disease Incidence
CRD	:	Completely Randomized Design

ABSTRACT

"EFFECT OF BOTANICALS ON SEED BORNE LEAF CRINKLE VIRUS IN BLACK GRAM (*Vigna mungo* L)."

By

Mr. Tikhe Dnyandev Ashok

A candidate for the degree

Of

MASTER OF SCIENCE (AGRICULTURE)

In

PLANT PATHOLOGY

Mahatma Phule Krishi Vidyapeeth

Rahuri - 413 722 (M.S.), India

2017

Research Guide	: **Dr. S. B. Latake**
Department	: **Plant Pathology**

The present investigation entitled "Effect of botanicals on seed borne Leaf Crinkle Virus in Black Gram (*Vigna mungo* L)." was undertaken with a view to find the per cent seed transmission of leaf crinkle virus through the infected black gram seeds to next generation, to evaluate the efficacy of botanicals in controlling the seed borne infection of ULCV in black gram and to find out the resistant reaction of promising

black gram genotypes against Urdbean Leaf Crinkle Virus (ULCV) under field conditions.

In symptomatology, first characteristic virus symptom appeared three to four weeks after sowing of the crop. The third trifoliate leaf developed symptoms by an increase in size and turning light green in colour. Approximately after a week the typical leaf crinkling became conspicuous. As the plants grew further, the extent of crinkling on the younger leaves was more pronounced. Considerable malformation of the inflorescence was observed in early infected plants. Some plants became bushy in appearance and remain green in the field even after the healthy plants attained senescence.

Seeds collected from the ULCV infected plants from various eight locations were sown under glass house conditions and the per cent transmission for each sample was worked out. The rate of virus transmission through the infected seeds ranged between 40.23 to 53.66 per cent indicating that seed play an important role in transmission of ULCV.

Eleven plant extracts were evaluated for their efficacy in controlling the seed borne infection of ULCV in black gram/ urdbean. Prior to sowing, seeds were dipped in the individual crude extracts of the plant species for 6, 12 and 18 hours and observations were recorded.

Abstract contd.... **Mr. D. A. Tikhe**

Seed treatment with kanher (*Nerium indicum*) i.e. dipping of urdbean seeds in the plant extract gave maximum control of seed borne infection of ULCV at 6, 12 and 18 hrs (53.34, 60.22 and 66.11 per cent, respectively). The incubation period of the virus was also prolonged with this treatment. It was followed by seed treatment with extract of babool (*Acacia nilotica*) and lantana (*Lantana camera*).

Spray treatment alone with plant extracts was not much effective in controlling ULCV. The best treatment was of kanher which recorded lowest (34.72 per cent) infected plants with 41.95 per cent disease control. It was followed by treatment of babool plant extract.

Seed treatment with kanher leaf extract followed by spraying of the same gave significantly superior control of ULCV (75.00 per cent). It was closely followed by treatment of babool (73.70 per cent).

Twenty four genotypes were screened under field conditions for their reaction to ULCV. Out of these, three genotypes *viz*; PDKV black gold, Phule U-0609-43 and TBU-4 exhibited resistant reaction to the disease.

1. INTRODUCTION

Grain legumes are the most important nutritional component of Indian diet for vegetarian and also for poor among the population. Black gram (*Vigna mungo* L. Hepper), commonly known as urdbean is one of the thirteen different grain legumes grown in India. It belongs to the family "Leguminosae" and is being called by different names such as urd, mash, urid and urad etc. In India, urdbean has been cultivated since ancient times. India is a primary centre of origin of the crop whie central Asia is secondary. According to Zukovskij (1962) urdbean originated from its wild progenitor *Phaseolus sublobatus* in India.

Urdbean is consumed in various forms, (as dal, whole or split, husked and unhusked or parched), whole plant is used as fodder for cattle and is also a green manure crop. The crop plants possess deep root system which binds soil particles and thus, prevents soil erosion. Many delicious food items can be prepared from urdbean eg. dosa, idli, curry, papad, bari (spiced balls), pudding (halwa) and imrati (a delicious sweet).

In India, urdbean is mainly cultivated as *kharif* crop. The major states cultivating urdbean are Madhya Pradesh, Uttar Pradesh, Uttaranchal, Punjab, Maharashtra, West Bengal, Andhra Pradesh, Tamil Nadu and Karnataka. In India, the crop is cultivated over 3.06 million hectare area with total production of 1.70 million tonnes and productivity

of 555 kg/ha in India (Anonymous, 2013-14). In Maharashtra, the crop is cultivated over 2.80 lakh hectare area with total production of 0.59 lakh tonnes and productivity of 211 kg/ha (Anonymous, 2015-16).

The crop is highly prone to a number of pathogens *viz.* fungi, bacteria, viruses and nematodes etc. which are responsible for its low production. Important diseases affecting its productivity are powdery mildew (*Erysiphe polygoni*), anthracnose *(Colletotrichum lindemuthianum*), leaf spot (*Cercospora canescens*), rust (*Uromyces appendiculatus*), dry root rot (*Rhizoctonia bataticola*), bacterial blight (*Xanthomonas campestris* pv. *phaseoli*), leaf crinkle (urdbean leaf crinkle virus), yellow mosaic (mungbean yellow mosaic virus), black gram mottle virus and bean common mosaic virus. Among the viruses infecting the urdbean crop, urdbean leaf crinkle virus (ULCV) is much prevalent and in recent years has become a potential threat to the cultivation of urdbean crop in many states of India, as most of the high yielding varieties are susceptible to this virus (Gautam *et al.*, 2016).

Urdbean leaf crinkle disease was first reported from Delhi and Uttar Pradesh in the year 1966 by Williams *et al.*(1968). Later in 1967, the disease appeared in tarai region of UP (Kolte and Nene, 1970). These workers, for the first time named the disease as urdbean leaf crinkle disease, proved the infectious nature of the pathogen and designated it as urdbean leaf crinkle virus (ULCV). There is no information

available on the occurrence of the disease in any other country of the world except India (Williams *et al.* 1968), Sri Lanka (Shivanathan, 1980) and Pakistan (Bashir *et al.* 1991).

Though, the disease is restricted in its occurrence, it is economically important. The loss in seed yield in ULCV affected black gram crop ranges from 35 to 81 per cent which is dependent upon type of genotype, location and infection time (Gautam *et al. 2016*). The yield losses estimated may go up to 76-100 % depending' upon the stage at which plants become infected (Brar and Rataul, 1989 and Bashir *et al.*, 1991). Urdbean leaf crinkle virus is both seed as well as sap transmissible (Dubey and Sharma (1985) and Brar and Rataul (1986). Of the various methods, transmission of virus through seed is considerably ecological significance for virus perpetuation, perennation and dissemination, as well as economic consequence for the plant grower. The rate of transmission through seed is from 2.7 to 46 per cent (Ahmad *et al.* 1997). Virus perpetuation by infected seeds is seemingly the perfect survival strategy since it serves as a protective link between crop growing seasons and is a sole source of primary inoculum in the field.

As present the main strategy employed to combat the plant viruses is application of chemical to control the insect vector population transferring the virus. However, the chemical application has certain limitation and drawbacks. This includes phytotoxic effect on plants, development of resistance in insect towards the insecticides and

environmental pollution, etc. Considering all these, the present investigation was undertaken with a view to evaluate plant extracts for their efficacy in controlling the seed borne ULCV with following objectives

(i) To estimate frequency of transmission of leaf crinkle virus from one generation to other through seed.

(ii) To find out effect of extracts of botanicals on seed germination and transmission of leaf crinkle virus of black gram.

2. REVIEV OF LITERATURE

The literature pertaining to the various aspects of urdbean leaf crinkle virus is being reviewed in this chapter.

2.1 Occurrence

Leaf crinkle, a disease of urdbean caused by Urdbean Leaf Crinkle Virus (ULCV) was first reported by Williams *et al.* (1968) from Delhi. Since, then it has been reported from various urdbean growing states of India, *viz.*, Uttar Pradesh (Nene, 1968; Kolte and Nene, 1970; Beniwal and Chaubey, 1979), Punjab (Khatri *et al.* 1971; Brar and Rataul, 1986), Tamil Nadu (Narayanasamy and Jaganathan, 1975), Haryana (Singh, 1980; Kadian, 1983), Himachal Pradesh (Gupta, 1974; Dubey and Sharma, 1985), Gujarat (Mishra *et al.* 1994; Patel *et al.* 1999), Maharashtra (Mahajan and Joi, 1999), and North Eastern hill Region of India (Sahay *et al.* 1999). Apart from India, this disease has also been reported from Sri Lanka (Shivanathan, 1980) and Pakistan (Bashir *et al.* 1991).

2.2 Symptoms and losses caused by urdbean leaf crinkle virus

2.2.1 Symptomatology

Williams *et al.* (1968) and Nene (1968) observed that the urdbean leaf crinkle virus infected plants remained stunted, developed rugosity, showed crinkling on affected leaves and produced few pods under field conditions.

Khatri *et al.* (1971) reported that the disease is characterized by leaf crinkling, reduction in leaf size and witches broom, etc.

Kolte and Nene (1970, 1972) and Kolte (1971) described in detail the symptoms of urdbean leaf crinkle virus both under natural and artificial conditions. They stated that initial symptoms of the disease appeared three to four weeks after sowing on the third trifoliate leaf and characterized by an increase in the size and a lighter green colour before typical crinkling became conspicuous a week later. The affected trifoliates showed enlargement of the leaflet followed by crinkled surfaces of the laminae. After the appearance of initial symptoms the affected trifoliates particularly 4th, 5th and 6th curved downward. In case of severely affected plants, two free stipules at the base of the affected trifoliate became thicker and broader than the normal ones. In affected plants flowering was delayed by 8 to 10 days.

Kolte and Nene (1979) reported abnormal pollen grains to be present in the anthers of affected buds which contained 10 per cent sterile pollen. This resulted in reduction in pod formation to 41.77 per cent in diseased plants as against 84.96 per cent in healthy plants.

Brar and Rataul (1986) described the most characteristic symptoms of the disease as wavy appearance on the third trifoliate followed by typical crinkling, shortening of petiole of central leaflet and thickening of leaf veins. The leaf

area of the diseased trifoliate was more than that of healthy ones.

Srivastava and Singh (2010) reported that the ULCV disease was characterized by severe crinkling, puckering and rugosity of the leaves associated with significant reduction in yield.

Gautam *et al*. (2016) reported that ULCV resulted in extreme crinkling, puckering and rugosity of leaves inflicting heavy yield losses annually. The loss in seed yield in ULCV affected urdbean crop ranged from 35-81 per cent.

2.2.2 Yield Losses

Kolte (1971); Nene (1973) and Kolte and Nene (1979) reported about 62 to 100 per cent yield losses in field grown urdbean plants due to urdbean leaf crinkle virus.

Beniwal and Chaubey (1979) reported that ULCV infection significantly and adversely affected number of pods per plant and number of seeds per pod in urdbean cv. Pant Urd 30 and Pant Urd 26. Maximum yield reduction of 70.7 and 83.8 per cent was noticed in Pant U 30 and Pant U 26, respectively. The virus infection significantly affect the texture of urdbean seeds by increasing the percentage of shrivelled, oversized and brown coloured seeds.

Kadian (1982) reported that losses from leaf crinkle virus were 2.12 to 93.98 per cent in the *Vigna mungo* cv. Varsha and 2.82 to 95.17 per cent in *V. mungo* variety T 9.

There was a significant decrease in yield in terms of pods per plant, seeds per pod and 1000 grain weight.

Brar and Rataul (1989) recorded yield losses due to ULCV in urdbean up to 84 per cent in severely infected and 55.2 per cent in partially infected urdbean plants.

Mishra *et al.* (1994) reported a reduction of 0.6, 1.3 and 16.6 per cent in terms of pod size, number of seeds per pod and 1000 grain weight, respectively of ULCV infected urdbean plants.

Patel *et al.* (1999) reported 28.9 per cent reduction in yield of urdbean plants as a result of ULCV infection.

2.3 Transmission of Urdbean Leaf Crinkle Virus through seed

Like many other viruses, urdbean leaf crinkle virus spreads mainly through seed, sap and insect vectors.

Kolte (1971) and Kolte and Nene (1972) reported 18.39 per cent transmission of ULCV through seeds in urdbean cultivar T- 9.

Gupta (1974) reported seed transmission of ULCV in addition to sap and graft transmission.

Narayanaswamy and Jaganathan (1975) observed that susceptibility of the plants and percentage of seed transmission of the ULCV reduced as the age of plants increased. Higher percentage of infection in young infected plants induced a higher rate of seed transmission.

Beniwal and Bharathan (1980, 1983a) reported 0-15 per cent seed transmission of urdbean leaf crinkle virus in different germplasm and varieties of urdbean. Plant age at the time of infection affected seed transmission as higher percentage of transmission occurred in plants infected at early growth stage than those infected later in the season.

Beniwal *et al.* (1984) detected ULCV in all the floral and seed parts. The virus was found distributed in all the five parts of flower i.e. epicalyx, calyx, corolla, androecium and gynoecium. Similarly, it was detected in all the three parts of the seed i.e. seed coat, cotyledon and primary axis, thereby indicating the internal seed borne nature of virus.

Dubey and Sharma (1985) confirmed that ULCV was seed transmitted up to 17.6 per cent in naturally infected plants. The virus survived in cotyledons and embryos of infected seeds and did not affect germination. They also found that highest transmission of 68 per cent occurred in seeds from 10 days inoculated plants. No transmission was obtained through seeds collected from 50 days old inoculated plants.

Brar and Rataul (1986) found ULCV to be seed transmissible to the extent of 77.64 and 45 per cent in severely diseased and partially diseased plants, respectively.

Morales (1987) reported that bean common mosaic virus (BCMV) was transmitted in a high but variable proportion of the seed produced by mosaic affected plants,

depending on the bean cultivar and growth stage at which plants become infected.

Kadian (1994) reported that the ULCV in urdbean was seed transmitted (21 per cent) and transmission rate increased when infected seeds were continuously reused. The transmission rate was higher in seeds collected from mechanically sap inoculated one week old plants and decreased with the plant age. No transmission occurred from seeds of the plants inoculated seven weeks after sowing.

Mishra *et al.* (1994) reported seed transmission of ULCV and the percentage varied from 2.0 to 31.25.

Many other workers had also reported ULCV transmited through seed in urdbean crop *viz*; 1.16-16 per cent (Mahajan and Joi, 1999), 10-30 per cent (Patel *et al.* 1999), 1-83 per cent (Pushpalatha *et al.* 1999) and up to 33 per cent (Negi and Vishunavat, 2003).

Reddy *et al.* (2005) conducted studies of seed transmission of ULCV. They reported that seed lots showing 2-3.6 seed borne infection recorded 45.2 - 86.5 per cent disease incidence at the post flowering stage.

Lanoiselet (2008) reported that seed transmission rate for wheat streak mosaic virus ranged from 0 to 0.22 per cent, with a mean seed transmission rate of 0.06 per cent.

Hanssen (2010) reported that Pepino Mosaic Virus in tomato could be transmitted to the next generation via

contaminated seed and provided a statistically sound estimation of 0.026 per cent as the seed transmission rate in tomato.

2.4 Use of botanicals for control of plant viruses

The interest in antiviral agents inhibiting plant infection originated with the study of mosaic disease of pokeweed (*Phytolacca decandra*) (Allard, 1918). It is worthy of attention, however that in 1925, Duggar and Armstrong reported that the crude extract of pokeweed (*Phytolacca decandra* L.) markedly inhibited the infectivity of tobacco mosaic virus. Doolittle and Walker (1925) also reported that cucumber mosaic cucumovirus infected pokeweed leaf juice could produce no infection upon inoculation into naturally susceptible cucumber plants. Kuntz and Walker (1947) reported that mixing of extracts of leaves of spinach, garden beet, sugar beet and chard in equal parts with the inocula of TMV and Cabbage mosaic virus, completely or almost completely inhibited their activity.

Leaf extracts of pepper, geranium and jimson weed inhibited the development of local lesions induced by tobacco mosaic virus (TMV) on Pinto bean leaves (Apablaza and Bernier, 1972).

Tomlinson *et al.* (1974) reported that the extracts of leaves of *Prunus americana* and their partially purified preparations caused marked inhibition of the infection of on *Chenopodium quinoa* by Cucumber mosaic virus (CMV).

Verma and Mukherjee (1975) found that brinjal leaf extract induced local and systemic resistance in *Nicotina glutinosa* against TMV and in *Nicotina tabacum* var. NP31 against Tobacco ring spot virus (TRSV) when applied 24 hours before virus inoculation.

Singh and Varma (1981) screened leaf extracts of twenty plant species known to possess medicinal properties for TMV inhibition. They found that leaf extract of *Datura metel* L. inhibited 100 per cent TMV on *Chenopodium amaranticolor*.

Similarly, aqueous leaf extract of *Chenopodium ambrosoides* was shown to prevent infection of tobacco mosaic and sunnhemp rosette virus when applied to the leaves (Verma and Baranwal, 1983).

Chowdhary and Saba (1985) tested 15 crude plant extracts against urdbean leaf crinkle virus and found that under *in vitro condition*, ginger extract gave the highest percentage of inhibition after 1 hr. incubation and turmeric after 2 hr.

Murthy and Nagarajan (1986) found that twig extract of *Pithecolobium dulce* was most promising in checking the mosaic incidence in field planted tobacco crop.

Lin and Qui (1987) reported that among extracts from 90 plant species in 37 families, extracts of *Chenopodium serotinum* and *Hosta plantaginea* shown to have some effects in reducing the severity of mosaic disease of tomato caused by

tobacco mosaic tobamovirus (TMV-T). Disease was controlled more than 60% in tomato seedlings sprayed with the extract of *Eupatorium serotinum* 2-3 times before and 1- 2 times after inoculation of the virus.

Zaidi *et al.* (1988) studied the inhibition of spinach mosaic virus by extracts of some medicinal plants. Extracts of all medicinal plants inhibited the spinach mosaic virus to varying degree. Highest inhibition of SMV was achieved in the extracts of leaves of *Ocimum sactum* L.

Kubo *et al.* (1990) found an inhibitor of plant virus infection in the extracts of *Mirabilis jalapa* L. The inhibitor protein MAP showed highly potent activity against mechanical transmission of Tobacco mosaic virus (TMV), Cucumber green mottle mosaic, Potato Y potyvirus, Turnip mosaic potyvirus and Cucumber mosaic cucumovirus.

Baranwal and Verma (1992) observed that pre-inoculation treatment with *Celosia cristata* leaf extract prevented lesion production by Sunnhemp rosette virus, Tobacco mosaic virus and Potato virus X in several local lesion hosts but not in *Chenopodium amaranticolor*.

Louis and Balakrishnan (1996) reported that extracts from five medicinal plants showed more pumpkin mosaic virus inhibitory activity when applied before virus inoculation than after inoculation.

Nagaraju *et al.* (1997) evaluated two neem based products, neemark (5 per cent) and neem seed kernel extract

(4 per cent) and leaf extracts from seven plant species (*Azadiracta indica, Pongamia glabra, Ocimum sanctum, Vinca rosea, Phyllanthus niruri, Tagetes errecta* and *Spinacea oleracea*) against Pepper vein banding virus on bell pepper under glasshouse conditions. *S. oleracea* was found to be inhibitory recording only 25 per cent transmission against 86.6 per cent in inoculated check.

Malik (1998) found neem oil to be the best inhibitor of seed borne inoculum of ULCV as it checked the symptom development.

Jayashree *et al.* (1999) tested ten plant extracts for their efficacy in controlling pumpkin yellow vein mosaic virus. They found that among these ten plant extracts, *Bougainvillea spectabilis* showed maximum inhibition of virus transmission followed by *Boerhavia diffusa*. In plant derivatives, neem oil and Thuja inhibited the virus effectively.

Patel *et al.* (1999) reported that among 29 tested botanicals, treatment mixture containing leaf extract of *Maadhuca indica* inhibited TMV infection completely (100 per cent) on *Nicotiana glutinosa* whereas, leaf extract of *Glycyrrhiza glabra*, *Cassia angustifolia*, *Emblica officinalis*, *Sesbania sesbana* were found to possess potent inhibitors of TMV infection and the percentage of inhibition being reported to be 97.6, 96.5, 93.5 and 92.1, respectively. Leaf extracts of *Ocimum sanctum, Annona squamosa, Aegla marmelos, Lepidium sativum, Adhatoda vasica* and *Eucalyptus*

teretocornis were also found to contain relatively strong TMV inhibitors.

Tewari *et al.* (2001) reported different fern species to have virus inhibitory properties. They tested thirteen species of ferns for their inhibitory effects against Cucumber green mottle mosaic virus (CGMMV) on *Cucurbita pepo* cv. Caserta. The extracts of *Adiantum caudatum*, *Dryoteris filixmas* and *Polypodium parasiticum* were found to exhibit highest inhibitory effect (more than 90 per cent).

Bharathimatha *et al.* (2003) reported the existence of antiviral principles in the seed extracts of *Harpullia cupanioides* against Tomato spotted wilt virus (TSWV), Rice tungro virus (RTV) and Cowpea aphid borne mosaic virus (CABMV).

Thirumalaisamy *et al.* (2003) reported that extract from *Zingiber officinale*, *Piper longum* and *Prosopis julijlora* possessed the most potent anti ULCV properties.

Othman & Shoman (2004) studied the antiphytoviral activity of the crude extracts of *Plectranthus tenuiflorus* against different plant viruses *viz*; Tobacco necrosis virus (TNV), Tobacco mosaic virus (TMV) and Tomato spotted wilt virus (TSWV). They observed that when the crude extract was applied onto *Proteus vulgaris*, *Datura stramonium* and *Chenopodium amaranticolor* as pre-inoculation spray, it reduced the infectivity of above viruses by 90.68, 85.68 and 77.7 per cent respectively. However, when the extract was

mixed with the virus inoculum, it inhibited the local lesion development by 100 per cent after one hour of mixing for TNV, and three hours for both TMV and TSWV.

Ashfaq *et al.* (2006) evaluated water extracts (1:1) of nine plant species of diverse botanical families and four chemicals for their effectiveness against Urdbean leaf crinkle virus (ULCV) in the field during spring and summer 2006. Extracts from five plant species (*Aloe barbadensis*, *Calotropis procera*, *Bougainvellea spectabilis*, *Allium cepa* and *Allium sativum*) reduced the incidence to 20-30 per cent as against 80 per cent in control, and delayed symptom expression by 10-14 days.

Deepthi *et al.* (2007) evaluated the effect of leaf extracts and acetone precipitated proteins of medicinal plants on seed borne Tobacco mosaic virus (TMV) and Tomato mosaic virus (ToMV) infection. They found that the aqueous leaf extracts of guava, Phyllanthus and Thuja were effective in reducing the infection by ToMV.

Prasad *et al.* (2007) evaluated the efficacy of certain plant extracts in reducing Bean common mosaic potyvirus strain blackeye cowpea mosaic (BCMV-BlCM) disease in cowpea. The disease incidence was reduced to 7 per cent when 0.75 per cent (w/v) of *Boerhavia diffusa* leaf extract was used as seed treatment under greenhouse conditions as compared to 80 per cent in control. Under field conditions *Boerhavia diffusa* reduced the disease incidence up to 40 per cent at

0.75 per cent (w/v) concentration of extract. In spray treatment, *Boerhavia diffusa* and *Bougainvellea spectabilis* reduced the disease incidence up to 13 and 12 per cent under greenhouse conditions, whereas *Boerhavia diffusa* and *Canthium inerme* reduced the disease incidence up to 31 and 32 per cent under field conditions.

Bhyan *et al.* (2007) tested four plant extracts *viz*; neem (*Azadiracta indica*) fruits, garlic (*Allium sativum*) bulbs, karamaja (*Pongamia pinnata*) leaves and mehogoni (*Swietenia macrophylla*) seed as phytopesticide against Okra mosaic virus. All the phytopesticides were found to perform better than the control (no phytopesticidal management), but karamja extract treated plants had minimal rate of incidence of the virus with maximum plant height, flower production, fruit formation and that yield.

Mahdy *et al.* (2007) reported delaying the sowing date and spraying faba bean seedlings with six aqueous botanical extracts at fortnightly under field conditions was achieved as a simple strategy for Bean yellow mosaic potyvirus (BYMV) control. They also found that root and leaf extracts of both *Mirabilis jalapa* and *Prunus americana* gave the highest inhibition rate at all concentrations followed by leaf extracts of *Dianthus caryophyllus*, *Canthium inerme*.

Lavanya *et al.* (2009) assessed the efficacy of anti-viral principles from botanicals for the suppression of Sunflower necrosis virus (SFNV). The AVP extracts from

Bougainvillea spectabilis and *Prosopis chilinesis* were found to be effective in reducing the viral infection both in cowpea and sunflower plants.

Singh and Awasti (2009) conducted an experiment to test the efficacy of a few botanical extracts on the yellow mosaic of mung bean. They recorded maximum reduction in disease incidence i.e. 66.70 and 63.65 per cent in mungbean and urdbean, respectively by eight sprays of *Clerodendrum aculeatum*. Whereas, eight sprays of *Boerhavia diffusa* root extract could reduce the disease incidence by 60.27 and 58.20 per cent followed by *Azadirachta indica* leaf extract by 42.43 and 42.92 percent in mungbean and urdbean, respectively.

Karthikeyan *et al.* (2009) evaluated antiviral principles from *Mirabilis jalapa* and *Bougainvillea spectabilis* for the induction of systemic resistance in blackgram (*V. mungo*) against Urdbean leaf crinkle virus. They found that pre inoculation spraying of blackgram plants with leaf extracts of *Mirabilis jalapa* or *Bougainvillea spectabilis* reduced the leaf crinkle disease to about 90 per cent.

Ali *et al.* (2010) evaluated plant extracts against *Bemisia tabaci* and cotton leaf curl virus disease under field conditions. Treatment with *Azadirachta indica* extract and salicylic acid were found to be most effective followed by *Eucalyptus globules* and *Allium sativum* extracts.

Reddy *et al.* (2010) found that the botanicals *viz*; Glyricidia leaf extract, Clerodendron leaf extract and sorghum

leaf extract were effective in reducing Tomato leaf curl virus incidence in tomato.

Srivastava *et al.* (2010) investigated the effect of six medicinal plant extracts on the inhibition of three strains of Watermelon mosaic virus. It was observed that medicinal plant extracts were inhibitory for all the three strains wherein maximum reduction in disease incidence was noted in case of leaf extracts of *Rauvolfia serpentina* for all the three strains up to 75 days.

Awasthi *et al.* (2011) found the antiviral agents from *Boerhavia diffusa* root extract and *Clerodendrum aculeatum* leaf extract were significantly effective in inducing systemic resistance against Papaya ring spot virus in papaya. They observed maximum reduction in disease incidence (72.25 and 74 per cent) when *Boerhavia diffusa* root extract was applied as seed treatment + nursery treatment + field treatment.

Binyamin *et al.* (2011) reported that the neem extract gave better results for reducing vector population as well as disease incidence in ULCV.

Singh *et al.* (2011) observed maximum reduction in the incidence of mungbean yellow mosaic virus disease in mungbean and urdbean when crop was given six sprays of each extract of *Azadirachta indica* and *Cleodendrum* aculeatm separately. In case of *Cleodendrum aculeatum* treatments reduction in disease incidence in mungbean and urdbean was

53.76 and 48.22 per cent, respectively, whereas, *Boerhavia diffusa* root extract could reduce the incidence by 42.48 and 40.55 per cent in mungbean and urdbean followed by 33.07and 28.55 per cent by *Azadirachta indica*.

2.5 Screening of urdbean genotypes against ULCV

Kolte (1971) tested 13 cultivars of urdbean against ULCV and found all of them to be susceptible.

Narayanasamy and Jaganathan (1973) observed black gram varieties Karaikal, Mattikalai, Palladam, Parvathipuram, BR 16 and BR 68 to be highly resistant against ULCV without showing any symptoms, while varieties Kahandur, PHM 8, P 33, P 49, P 58 and P 223 showed only less than 5 per cent infection of ULCV.

Kadian (1980) tested 338 varieties of urdbean for resistant against ULCV but only two varieties DLU-90 and DLU-487 were found resistant. Varieties HPU 19, 33, 55, 56, 72, 75, 91, 109, 167, 200, 232, 240, 246, 252, 264, 269 and 277 were found moderately resistant to ULCV infection.

Out of 280 varieties and germplasms screened by Sharma and Dubey (1984), urdbean cultivars HPU 27, 102, 164 and 315 were found to be highly resistant to ULCV.

Annapan *et al*. (1988) observed that Co-S was moderately resistant to ULCV besides powdery mildew (*Erysiphe polygony*), tip blight and pod borer.

Joshi (1988) found 10 germplasm accessions *viz*. shU 9504, shU 9505, shU 9511, shU 9513, shU 9515, shU 9516, shU 9519, shU 9520, shU 9522 and shU 9528 to be resistant against ULCV infection both under field as well as glass house conditions.

Iqbal *et al*. (1991) screened 19 genotypes/ cultivars of mash selected from local races against urdbean leaf crinkle virus for two consecutive years (1988-1989) under natural infection conditions. Four genotypes *viz*; S 210, MM 5-60, S 250 and Mash Sialkot were found resistant.

Narendra Urd 1 was reported to be resistant against mungbean yellow mosaic virus (MYMV) and urdbean leaf crinkle virus (Singh and Singh, 1994).

Prasad *et al*. (1998) reported that out of 25 varieties of urdbean screened for ULCV, maximum disease was observed in T 9-150 (39.5%) and no disease was recorded in case of NDU-94-6. Also the difference in the disease incidence between the varieties was found to be significant.

Malik (1998) observed minimum infection of ULCV (0.67%) in Pant Urd 35, while the infection was maximum (5.35%) in Pant Urd 30.

According to Patel *et al*. (1999) germplasm lines of urdbean i.e. OU-90-47, OU-90-60, OU-90-69, OU-90-741, OU-90-72 were found moderately resistant, while OU-90-54, OU-90-66, OU-90-61 as resistant and OU-90-44 highly resistant against ULCV infection.

Ashfaq *et al.* (2007) reported that 11 out of 87 urdbean genotypes were highly resistant during spring 2005 i.e., 2cm-703, 90cm-015, 90cm-056, 93cm-006, 94cm-109, 99cm-001,SKG-Local, IAM382-1, IAM382-9, IAM382-15 and IAM 133 against leaf crinkle virus.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection of Urdbean leaf crinkle virus (ULCV) infected seed samples

The ULCV infected seed samples of urdbean were collected from various eight locations as detailed in Table 1. The plants showing typical symptoms of ULCV in the field were tagged before harvesting. These plants were harvested separately and the seed were collected for further studies.

Table: 1 Collection of ULCV infected seed sample from various locations

Sr. no.	Location	Variety
1.	Kokangaon, Tal. Karjat, Dist. Ahmednagar	Local
2.	Shilegaon, Tal. Karmala, Dist. Solapur	TPU-4
3.	Pulses Improvement project, MPKV, Rahuri	TAU-1
4.	Pulses Improvement project, MPKV, Rahuri	TPU-4
5.	Oilseeds Research Station, Jalgaon	TAU-1
6.	Karanji, Tal. Pathardi, Dist. Ahmednagar	Local
7.	Gondegaon, Tal. Shrirampur, Dist. Ahmednagar	Local
8.	Jawala, Tal. Shegaon, Dist. Buldhana	PKV udid 15

3.1.2 Miscellaneous materials

All the miscellaneous materials required for virological study used during present investigations *viz.*, earthen pots, tags, beakers, pestle and mortar, distilled water, hand sprayer and muslin cloth, etc. were obtained from Department of Plant Pathology and Agril. Microbiology, MPKV, Rahuri.

3.2 Methods

3.2.1 Estimation of frequency of transmission of ULCV through seed

The seeds collected from ULCV infected plants from various locations were sown under glasshouse conditions in sterilized earthen pots filled with sterilized soil. Five seeds per pot were sown and observations on germination of seed, incubation period of the virus and appearance of virus symptom were recorded. Three pots were maintained for each location/seed sample. The transmission percentage was worked out for each of the seed sample by following formula-

$$\text{Per cent transmission} = \frac{\text{No. of plants showing virus symptoms}}{\text{No. of germinated plants}} \times 100$$

3.2.2 Evaluation of efficacy of botanicals for control of seed borne infection of ULCV

Eleven different botanicals were evaluated under *in vitro* conditions for their efficacy to control seed borne infection of ULCV in urdbean. The ULCV infected plants of variety TPU-4 from the experimental field of Pulses Improvement Project, M.P.K.V., Rahuri were tagged before harvesting. The seeds from such tagged i.e. virus infected plants were harvested separately and these seeds were used for testing the efficacy of botanicals under glasshouse conditions.

3.2.2.1 Preparation of plant extracts

Crude extracts of plant species were prepared by using either leaves or bulbs or rhizome. The various plant species and their plant part used during present study are listed in Table 2 (Plate 1)

The fresh plant parts were firstly surface sterilized with 0.1% $HgCl_2$ solution and then washed thoroughly with distilled water. The respective plant parts (leaves, rhizome, bulbs, clove etc.) were macerated in pestle and mortar in equal volume of distilled water (1:1 w/v). These macerated material was first filtered through double fold muslin cloth and then through Whatman filter paper no. 1 (Plate 2). The supernatant was collected and used for further studies.

Table 2 : Plant species evaluated against seed borne ULCV infection

Sr. no	Plant species	Plant part used
1.	Kanher (*Nerium indicum*)	Leaves
2.	Gajargawat (*Parthenium hysterophorus*)	Leaves
3.	Babool (*Acacia nilotica*)	Leaves
4.	Tobacco (*Nicotina tabacum*)	Leaves
5.	Tulsi (*Ocimum sanctum*)	Leaves
6.	Onion (*Allium cepa*)	Bulb
7.	Lantana (*Lantana camera*)	Leaves
8.	Garlic (*Allium sativum*)	Clove
9.	Pudina (*Mentha arvensis*)	Leaves
10.	Neem (*Azadirachta indica*)	Leaves
11.	Ginger (*Zingiber officinale*)	Rhizome

3.2.2.2 Efficacy of botanicals as seed treatment

The seeds of urdbean var. TPU-4 collected from ULCV infected plants were first surface sterilized with 0.1 % $HgCl_2$ for 30 second followed by three subsequent washing with sterilized distilled water. The sterilized seeds were dipped separately in the respective plant extract for 6, 12 and 18 hrs. at room temperature. Such treated seeds were sown separately in earthen pots. The seeds dipped in distilled water served as control. Ten plants for each treatment were maintained. The observations on germination of seed, incubation period of the virus and appearance of virus

symptoms on the plants were recorded. The per cent infected plants were workout on the basis of germination and number of plants showing virus symptoms.

3.2.2.3 Efficacy of botanicals as spray treatment

To evaluate the efficacy of botanical only as a spray treatment the infected seeds were first sown in earthen pots. After germination of seedlings, two sprays of plant extracts were given. First spray at eight days and second fifteen days after germination of seedling. Ten plants for each treatment were maintained. The observations on germination of seed, incubation period of the virus and appearance of virus symptom were recorded. The per cent infected plants were workout on the basis of germination and number of plants showing virus symptoms.

3.2.2.4 Efficacy of botanicals as seed and spray treatment

The virus infected seeds were treated with individual plant extract by dipping the seed for eighteen hrs. The seeds were then sown in earthen pots and eight days after germination of seedlings, plants were sprayed with the respective plant extract. Ten plants for each treatment were maintained. Second spray was repeated after eight days. The plants were observed for appearance of symptoms and incubation of the virus was recorded. The per cent infected plants were workout on the basis of germination and number of plants showing virus symptoms.

3.2.3 Screening of urdbean genotypes against ULCV

Twenty four genotypes of urdbean obtained from Pulses Improvement Project, M.P.K.V., Rahuri were screened under natural field condition for their reaction to ULCV. Each genotype was sown in a row of five meter length at 45×10 cm spacing. Two rows per replication were maintained and each genotype was sown in two replications. Periodic observations on appearance of symptoms and number of infected plants were recorded. The per cent disease incidence was worked out by following formula given by Mayee and Datar (1985)

$$PDI = \frac{\text{No. of plants showing disease symptoms}}{\text{Total no. of plant observed}} \times 100$$

The plants were grouped as per the following disease rating scale (Ashfaq *et al.*, 2007)

Sr. No.	Per cent infection		Infection category
1.	0-10% infection	:	Resistant (R)
2.	11-20% infection	:	Moderately resistant (MR)
3.	21-30% infection	:	Moderately susceptible (MS)
4.	31-40% infection	:	Susceptible (S)
5.	31-40% infection	:	Highly susceptible (HS)

4. EXPERIMENTAL RESULTS

The results obtained during the present investigation on, “Effects of Botanicals on Seed Borne Leaf Crinkle Virus in Black Gram (*Vigna mungo* L).” are presented in this chapter.

4.1 Symptomatology of ULCV

Seeds were collected from leaf crinkled infected urdbean plants from various eight locations as detailed in Table 1 in materials and methods. First characteristic virus symptom in the field appeared three to four weeks after sowing of the crop. The third trifoliate leaf developed symptoms by an increase in size and turning light green in colour. Approximately after a week the typical leaf crinkling became conspicuous. As the plats grew further, the extent of crinkling on the younger leaves was more pronounced. Considerable malformation of the inflorescence was observed in early infected plants. Some plants became bushy in appearance and remain green in the field even after the healthy plants attained senescence (Plate 3).

4.2 Transmission of ULCV from infected seeds to next generation

Seeds collected from the ULCV infected plants were sown under glass house conditions and the per cent transmission for each sample was worked out (Plate 4).

From the data presented in Table 3 it is clear that ULCV was transmitted from one generation to another through

the infected seeds. However, the virus did not affect the germination of seeds and it was between 83-97 per cent for the samples under study. Maximum i.e. 53.66 per cent transmission of the virus was observed in the seed sample collected from Karjat Tahsil followed by sample from Pathardi Tahsil were local variety was cultivated. Minimum (40.23 per cent) virus transmission was recorded in variety PKV udid 15.

Table 3: Transmission of ULCV from infected seed to next generation

Sr. no.	Location	Variety	Germination (%)	Transmission (%)
1.	Kokangaon, Tal. Karjat, Dist. Ahmednagar	Local	85.45	53.66
2.	Shilegaon, Tal. Karmala, Dist. Solapur	TPU-4	90.75	43.57
3.	Pulses Improvement project, MPKV, Rahuri	TAU-1	95.65	41.33
4.	Pulses Improvement project, MPKV, Rahuri	TPU-4	97.21	44.24
5.	Oilseed Research Station, Jalgaon	TAU-1	94.28	42.78
6.	Karanji, Tal. Pathardi, Dist. Ahmednagar	Local	83.33	53.45
7.	Gondegaon, Tal. Shrirampur, Dist. Ahmednagar	Local	87.25	52.94
8.	Jawala, Tal. Shegaon, Dist. Buldhana	PKV Udid 15	95.32	40.23

4.3 Control of seed borne infection of ULCV with seed treatment of plant extracts

Eleven plant extracts were evaluated for their efficacy in controlling the seed borne infection of ULCV in urdbean. Prior to sowing, seeds were dipped in the individual crude extracts of the plant species for 6, 12 and 18 hours and observations were recorded (Plate 5).

4.3.1 Seed treatment for 6 hrs.

The results obtained on seed treatment with plant extracts for controlling transmission of ULCV are presented in Table 4. All the plants extract were found effective in controlling the virus infection over the untreated control treatment. Minimum (25.19 per cent) virus infected plants were recorded in kanher plant extract treatment followed by treatment of babool plant extract (26.85 per cent) which gave 53.34 and 50.19 per cent disease control, respectively. Moreover, these two treatments were found at par with each other. Maximum (53.70 per cent) infected plants were recorded in the untreated control. Germination percentage was however not affected by the treatment under study. Incubation period of the virus ranged between 14-26 days in the treatments, lowest in the untreated control and highest in the kanher treatment.

Table 4: Effect of plant extracts as seed treatment on ULCV transmission (6 hrs. dipping)

Sr. no	Plant extract	Germination %	Virus infected plants %	% Disease control	Incubation period
1.	Kanher (*Nerium indicum*)	93.33	25.19 (30.12)	53.34 (46.91)	26
2.	Gajargawat (*Parthenium hysterophorus*)	83.33	39.81 (39.12)	25.84 (30.55)	16
3.	Babool (*Acacia nilotica*)	86.67	26.85 (31.21)	50.19 (45.11)	25
4.	Tobacco (*Nicotiana tabacum*)	83.33	36.11 (36.94)	32.50 (34.76)	19
5.	Tulsi (*Ocimum sanctum*)	80.00	29.17 (32.69)	45.84 (42.61)	22
6.	Onion (*Allium cepa*)	86.67	38.43 (38.31)	28.62 (34.34)	16
7.	Lantana (*Lantana camera*)	83.33	27.78 (31.81)	48.34 (44.05)	24
8.	Garlic (*Allium sativum*)	86.67	30.56 (33.56)	42.78 (40.85)	22
9.	Pudina (*Mentha arvensis*)	83.33	31.94 (34.42)	40.28 (39.40)	21
10.	Neem (*Azadirachta indica*)	80.00	33.33 (35.26)	37.50 (37.76)	22
11.	Ginger (*Zingiber officinale*)	86.67	34.26 (35.83)	36.67 (37.27)	20
12.	Control	86.67	53.70 (47.12)	- -	14
	S.Em. ±		2.06	3.66	
	C.D. at 5 %		6.05	10.75	

Table 5: Effect of plant extracts as seed treatment on ULCV transmission (12 hrs. dipping)

Sr. no	Plant extract	Germination %	Virus infected plants %	% Disease control	Incubation period
1.	Kanher (*Nerium indicum*)	93.33	21.48 (27.61)	60.22 (50.90)	26
2.	Gajargawat (*Parthenium hysterophorus*)	80.00	29.17 (32.69)	46.25 (42.85)	19
3.	Babool (*Acacia nilotica*)	90.00	22.22 (28.13)	58.89 (50.12)	24
4.	Tobacco (*Nicotiana tabacum*)	83.33	28.24 (32.10)	47.87 (43.78)	20
5.	Tulsi (*Ocimum sanctum*)	86.67	23.61 (29.07)	56.64 (48.82)	23
6.	Onion (*Allium cepa*)	86.67	30.56 (33.56)	43.89 (41.49)	18
7.	Lantana (*Lantana camera*)	86.67	23.15 (28.76)	57.27 (49.18)	24
8.	Garlic (*Allium sativum*)	83.33	24.07 (29.38)	55.42 (48.11)	20
9.	Pudina (*Mentha arvensis*)	93.33	24.81 (29.88)	53.70 (47.12)	21
10.	Neem (*Azadirachta indica*)	86.67	26.85 (31.21)	50.74 (45.42)	21
11.	Ginger (*Zingiber officinale*)	83.33	27.78 (31.81)	48.75 (44.28)	20
12.	Control	80.00	54.23 (47.43)	- -	15
	S.Em. ±		2.26	3.60	
	C.D. at 5 %		6.64	10.56	

4.3.2 Seed treatment for 12 hrs.

From the data presented in Table 5 it is revealed that all the plant extract treatments were superior over the untreated control in reducing the transmission of ULCV. Minimum incubation period (15 days) for the virus with maximum 54.23 per cent infected plants were recorded in the untreated control. Whereas, the treatment with kanher plant extract gave highest (60.22 per cent) control of virus infection with lowest i.e. 21.48 per cent infected plant (Plate 6). It was at par with treatment of babool plant extract which gave 58.89 per cent disease control. The virus symptoms in these two treatments also appeared late as the incubation period recorded was 26 and 24 days, respectively. The next better treatments were of tulsi, lantana and garlic extract. The germination percentage was in the range of 80-93 per cent in the all the treatment including control indicating that plant extract did not have much effect on germination of urdbean seedlings.

4.3.3 Seed treatment for 18 hrs.

Similar trend of results as that of 6 and 12 hrs. seed treatment was also observed in the seed treatment for 18 hrs. (Table 6). Though all the plant extract treatment recorded significantly lower number of virus infected plants over the untreated control, minimum i.e. 17.78 per cent virus infected plant were recorded in the treatment of kanher plant extract followed by treatment of babool plant extract which recorded 18.15 per cent infected plants. Per cent disease control in

these two treatments was 66.11 and 65.55 per cent respectively. Incubation period of the virus was also highest in these treatments. The minimum incubation period (15 days) with maximum virus infected plants (52.38 per cent) was recorded in untreated control.

Table 6: Effect of plant extracts as seed treatment on ULCV transmission (18 hrs. deeping)

Sr. no	Plant extract	Germination %	Virus infected plants %	% Disease control	Incubation period
1.	Kanher (*Nerium indicum*)	93.33	17.78 (24.94)	66.11 (54.40)	27
2.	Gajargawat (*Parthenium hysterophorus*)	80.00	33.33 (35.26)	36.46 (37.14)	19
3.	Babool (*Acacia nilotica*)	93.33	18.15 (25.21)	65.55 (54.06)	24
4.	Tobacco (*Nicotiana tabacum*)	86.67	30.56 (33.56)	41.67 (40.20)	21
5.	Tulsi (*Ocimum sanctum*)	86.67	19.44 (26.17)	62.04 (51.96)	21
6.	Onion (*Allium cepa*)	83.33	32.41 (34.70)	38.31 (38.24)	20
7.	Lantana (*Lantana camera*)	86.67	18.98 (25.83)	63.89 (53.06)	24
8.	Garlic (*Allium sativum*)	93.33	21.48 (27.61)	58.89 (50.12)	21
9.	Pudina (*Mentha arvensis*)	90.00	22.41 (28.25)	57.04 (49.04)	20
10.	Neem (*Azadirachta indica*)	90.00	22.22 (28.13)	57.41 (49.26)	20
11.	Ginger (*Zingiber officinale*)	86.67	23.15 (28.76)	55.55 (48.19)	19
12.	Control	76.67	52.38 (46.36)	- -	15
	S.Em. ±		1.91	3.03	
	C.D. at 5%		5.60	8.89	

Table 7: Effect of spraying of plant extracts on seed borne ULCV transmission

Sr. no	Plant extract	Germination %	Virus infected plants %	% Disease control	Incubation period
1.	Kanher (*Nerium indicum*)	86.67	34.72 (36.10)	41.95 (40.37)	24
2.	Gajargawat (*Parthenium hysterophorus*)	86.67	50.00 (45.00)	16.30 (23.81)	18
3.	Babool (*Acacia nilotica*)	83.33	35.65 (36.66)	40.46 (39.50)	22
4.	Tobacco (*Nicotiana tabacum*)	86.67	42.13 (40.47)	29.63 (32.98)	19
5.	Tulsi (*Ocimum sanctum*)	86.67	34.72 (36.10)	42.22 (40.53)	22
6.	Onion (*Allium cepa*)	83.33	48.15 (43.94)	19.63 (26.30)	17
7.	Lantana (*Lantana camera*)	90.00	37.04 (37.49)	37.78 (37.93)	24
8.	Garlic (*Allium sativum*)	80.00	41.67 (40.20)	30.84 (33.73)	22
9.	Pudina (*Mentha arvensis*)	86.67	42.59 (40.74)	28.52 (32.28)	20
10.	Neem (*Azadirachta indica*)	83.33	43.98 (41.54)	26.30 (30.85)	22
11.	Ginger (*Zingiber officinale*)	86.67	46.30 (42.88)	22.59 (28.38)	20
12.	Control	83.33	60.19 (50.88)	- -	14
	S.Em. ±		1.90	3.89	
	C.D. at 5%		5.58	11.35	

4.4 Evaluation of plant extracts as spray treatment

The plant extracts under study were also evaluated against transmission of virus as spray treatment alone. The results are presented in Table 7. Spray treatment with all plant extracts gave promising control of virus infection over untreated control. The per cent infected plants in the

treatments ranged between 50 to 34.72 as against 60.19 per cent in untreated control. The best treatment was of kanher which recorded lowest (34.72) per cent infected plants with 41.95 per cent disease control. It was followed by treatment of babool plant extract.

4.5 Evaluation of plants extracts as seed and spray treatments

The virus infected seeds were first treated with individual plant extracts by dipping the seeds in it for 18 hrs. After germination of seedlings, the plants were sprayed twice with respective plant extract. The results obtained are summarized in Table 8.

All the treatment were found significantly superior over the untreated control in minimizing the transmission of virus through seed as the per cent infected plants ranged between 14.44 to 22.22, only. In the untreated control the virus infected plants were 56.55 per cent. Per cent disease control obtained with the plant extract treatments was 60.37 to 75.00. Highest (75.00 per cent) disease control was obtained with kanher plant extract followed by treatment of babool (73.70 per cent). It was followed by treatment of Lantana and Tulsi. Incubation period of the virus was maximum (26 days) in treatment of kanher, whereas minimum was in untreated control.

Table 8: Effect of seed dipping and spraying of plant extracts on seed borne ULCV transmission

Sr. no	Plant extract	Germination %	Virus infected plants %	% Disease control	Incubation period
1.	Kanher (*Nerium indicum*)	93.33	14.44 (22.34)	75.00 (60.00)	26
2.	Gajargawat (*Parthenium hysterophorus*)	80.00	29.17 (32.69)	48.75 (44.28)	16
3.	Babool (*Acacia nilotica*)	90.00	14.81 (22.64)	73.70 (59.15)	26
4.	Tobacco (*Nicotiana tabacum*)	86.67	23.15 (28.76)	58.75 (50.04)	19
5.	Tulsi (*Ocimum sanctum*)	93.33	18.15 (25.21)	68.52 (55.87)	22
6.	Onion (*Allium cepa*)	80.00	25.00 (30.00)	55.42 (48.11)	16
7.	Lantana (*Lantana camera*)	86.67	15.28 (23.01)	73.33 (58.91)	23
8.	Garlic (*Allium sativum*)	83.33	19.91 (26.50)	63.93 (53.09)	22
9.	Pudina (*Mentha arvensis*)	86.67	19.44 (26.17)	65.00 (53.73)	21
10.	Neem (*Azadirachta indica*)	83.33	20.37 (26.83)	63.52 (52.84)	22
11.	Ginger (*Zingiber officinale*)	90.00	22.22 (28.13)	60.37 (50.98)	20
12.	Control	76.67	56.55 (48.76)	- -	15
	S.Em. ±		2.51	3.65	
	C.D. at 5%		7.36	10.70	

4.6 Screening of urdbean genotypes against ULCV

Twenty four promising urdbean genotypes received from Pulses Improvement Project, M.P.K.V., Rahuri were screened under natural field condition against ULCV (Plate 7). The data obtained on per cent disease incidence and reactions

of the individual entry to the disease are presented in Table 9. Out of the twenty four genotypes three genotypes *viz*; PDKV black gold, Phule U-0609-43 and TBU-4 exhibited resistant reaction to the disease. Whereas, thirteen genotypes were moderately resistant and six moderately susceptible to ULCV under field conditions. Only one genotype i.e. PKV udid 15 was found susceptible to the disease.

Table 9: Screening of urdbean genotypes against ULCV under natural condition

Sr. no.	Name of variety	% Disease incidence	Disease reaction
1.	AKU-15	12.40	MR
2.	PDKV Black Gold	8.98	R
3.	BDU-1	14.56	MR
4.	Phule U-601-23	18.20	MR
5.	Phule U-401-3	16.49	MR
6.	Phule U-0609-43	9.83	R
7.	Phule U-0611-8	21.36	MS
8.	Phule U-0612-42	28.10	MS
9.	AKU-10-2	21.36	MS
10.	AKU-11-15	26.40	MS
11.	AKU-13-16	22.87	MS
12.	TBU-2	12.34	MR
13.	TBU-4	9.64	R
14.	Phule U-608-28	16.34	MR
15.	Phule U-819-10	18.39	MR
16.	Phule U-819-18	20.27	MS
17.	Phule U-820-10	19.24	MR
18.	Phule U-807-16	14.94	MR
19.	Phule U-813-03	12.37	MR
20.	JLU-807-20	15.36	MR
21.	JLU-807-1	14.29	MR
22.	JLU-807-14	18.76	MR
23.	JLU-807-12	14.51	MR
24.	PKV udid 15	34.48	S

5. DISCUSSION

Blackgram or urdbean is one of the important major pulse crops in India and Maharashtra. Amongst various diseases infecting the crop, Urdbean Leaf Crinkle Virus (ULCV) is the most important one. An early crop infection with the virus can reduce grain yield up to 100 per cent (Kolte and Nene 1979). The disease has been reported in all states wherever urdbean is grown. Under natural field conditions leaf crinkle virus spreads mainly through seed and insect vectors. Seed transmission plays an significant role in spread of virus from one generation to another. Since the leaf crinkle virus is internally seed borne in urdbean (Beniwal and chaubey, 1984), as like many of seed transmitted viruses it is very difficult to control. In the absence of resistant cultivars, the available control measures are not adequate to manage the disease.

The present investigation was, therefore, carried out with a view to know the per cent seed transmission of ULCV from infected seed to next generation, effect of botanicals on ULCV transmission and disease control and screening of urdbean genotypes against Leaf Crinkle Virus. The findings of the present investigation are discussed in this chapter under suitable headings.

5.1 Symptomatology

The symptoms of leaf crinkle virus on urdbean plants under natural conditions were observed 20 to 30 days

after sowing. The common symptoms were *viz.*, crinkling, puckering and rugosity. After the initiation of the symptoms generally after a week the typical leaf crinkling became conspicuous. As the plants grew further, the extent of crinkling on the younger leaves was more pronounced. Considerable malformation of the inflorescence was observed in early infected plants. Some plants became bushy in appearance and remain green in the field even after the healthy plants attained senescence. Earlier many workers have described in detail the ULCV symptoms on urdbean. William *et al.* (1968) and Nene (1968) observed the common symptoms as crinkling of leaves, rugosity, stunting of plants and production of few pods. Kolte and Nene (1970, 1972) and Kolte (1971), Brar and Rataul (1986), Srivastava and Singh (2010) and Gautam *et al.* (2016) had also observed similar symptoms of the virus which are in confirmatory with the symptoms recorded during present investigation.

5.2 Transmission of ULCV from infected seeds to next generation

In the transmission studies it was found that the virus was transmitted through the seeds and rate of transmission was up to 53 per cent. Seed transmission nature of the virus has been confirmed by many workers *viz*; Beniwal *et al.* (1980, 1983a), Dubey and Sharma (1985), Brar and Rataul (1986), Mishra *et al.* (1994) and Reddy *et al.* (2005). Beniwal *et al.* (1980, 1983a) reported 0 to 15 per cent seed transmission of ULCV while Dubey and sharma (1980)

reported that ULCV was seed transmitted up to 17.6 per cent in naturally infected plants.

During the present studies seeds were collected from leaf crinkle virus infected plants and transmission percentage from these seeds was worked out. Earlier Brar and Rataul (1986) reported 77.64 and 45.00 per cent ULCV seed transmission in severely diseased and partially diseased plant respectively, Moreover Reddy *et al.* (2005) reported that seed lots showing 2-3.6 seed borne infection recorded 45.2-86.5 per cent disease incidence. In our studies more or less similar results were obtained wherein the transmission rate ranged between 40.23-53.66 per cent.

The virus did not affect the germination of seeds and in general germination was above 83 per cent. Earlier also, Dubey and Sharma (1985) reported that virus survives in cotyledons and embryos of infected seeds and did not affect germination.

5.3 Control of seed borne infection of ULCV with seed treatment of plant extracts

Eleven different botanicals were evaluated as seed and spray treatment alone and seed plus spray treatment for their ability to control the seed borne infection of ULCV in urdbean. In the seed treatment, the seeds were dipped in the crude plant extracts for 6, 12 and 18 hrs. separately. All the treatments gave promising control of virus over the untreated control. Among the various plant spp. evaluated, extract of

kanher (*Nerium indicum*) gave maximum control of virus infection at all the three time intervals. It was followed by the treatment of babool (*Acacia nilotica*). The incubation period of the virus was also maximum in these two treatments. In general it was observed that virus symptoms appeared late than the untreated control in all the treatments under study indicating that seed treatments with plant extracts delayed the virus infection.

Spraying of plant extracts alone for controlling the ULCV was not that much effective as of seed treatment. The per cent disease control obtained with the best treatment i.e. Kanher was 41.95 per cent as against 66.11 per cent recorded in 18 hrs. seed treatment. However seed + spray treatment with the plant extracts gave maximum control of virus infection. The per cent disease control ranged between 48.85 to 75.00 in this experiment. The similar trend of results was also observed in seed +spray treatment of plant extracts where maximum disease control was shown by treatment of kanher leaf extract.

The use of botanicals for control of plant diseases is one of the eco-friendly approaches for maintaining the ecological balance and avoiding ill effects of chemical application. The potential of various plant spp. in controlling the plant viruses of economic significance has been investigated by many workers. Singh and Varma (1981) found that leaf extracts of *Datura metal* L. inhibited Tobacco mosaic virus on *Chenopodium amranticolor*. Chowdhary and Saba

(1985) obtained highest inhibition of ULCV with ginger leaf extract. Zaidy *et al.*, (1988) achieved highest inhibition of spinach mosaic virus by *Ocimum sanctum*. Nagaraju *et al.* (1997) recorded only 25 per cent transmission of pepper vein banding virus in bell pepper when treated with leaf extract of *Spinacea oleracea*. Bhyan *et al.* (2007) found that okra plants treated with Karamja extract had minimal rate of incidence of mosaic virus than the untreated control. Karthikeyan *et al.*(2009) reported that pre inoculation spraying of black gram plants with leaf extracts of *Mirabilis jalapa* or *Bougainvillea spectabilis* reduced the leaf crinkle disease to about 90 per cent.

The efficacy of plant extracts in controlling plant viruses has been also reported by many other workers *viz*; Lavanya *et al.* (2009), Singh and Awasti (2009) and Singh *et al.* (2011).

5.4 Screening of Urdbean genotypes against Urdbean Leaf Crinkle Virus (ULCV)

In all 24 Urdbean genotypes were screened against leaf crinkle virus under natural field conditions. Three genotypes *viz*; PDKV black gold, Phule U-0609-43 and TBU-4 showed resistant reaction towards the disease. Thirteen genotypes were found to be moderately resistant and six exhibited moderately susceptible reaction. Only one genotype was found susceptible to the disease.

Screening of genotypes for identifying resistance sources is a continuous process, since resistance in the cultivated varieties or hybrids is liable to be breakdown. The reasons for breakdown of resistance many be at the level of either host or pathogen or vector (in case of viral diseases). Earlier also many workers *viz*; Kolte (1971), Kadian (1980), Sharma and Dubey (1984), Joshi (1988), Prasad *et al.* (1998) and Patel *et al.* (1999) screened a number of urdbean genotypes for ULCV resistance and reported that a few of then showed resistant reaction to the disease.

6. SUMMARY AND CONCLUSIONS

Black gram or urdbean (*Vigna mungo* L.) is the well-known and important major pulse crop in India and Maharashtra. Several viruses have been reported to infect the urdbean and causes enormous losses in the yield.

The present investigation entitled "Effect of botanicals on seed borne Leaf Crinkle Virus in Black Gram (*Vigna mungo* L)." was undertaken with a view to find the per cent seed transmission of leaf crinkle virus through infected seeds to next generation, to evaluate the efficacy of botanicals in controlling the seed borne infection of ULCV and to find out resistant source of urdbean against Urdbean Leaf Crinkle Virus (ULCV) under natural field conditions. The overall finding of the investigations are summarized as below,

1. Commonly observed symptoms of urdbean leaf crinkle virus on urdbean crop under field condition were extreme crinkling, curling, puckering and rugosity of leaves. Stunting of plants and malformation of floral organ were also observed.
2. Seeds collected from the ULCV infected plants from various eight locations were sown under glass house conditions and the per cent transmission for each sample was worked out. The rate of virus transmission through the infected seeds was between 41 to 54 per cent.

3. Eleven plant extracts were evaluated for their efficacy in controlling the seed borne infection of ULCV in urdbean. Prior to sowing, seeds were dipped in the individual crude extracts of the plant species for 6, 12 and 18 hours and observations were recorded.

4. Seed treatment with kanher (*Nerium indicum*) i.e. dipping of urdbean seeds in the plant extract gave maximum control of seed borne infection of ULCV at 6, 12 and 18 hrs. (53.34, 60.22 and 66.11 per cent respectively). It was followed by seed treatment with extract of babool (*Acacia nilotica*) and Lantana (*Lantana camera*).

5. Only spray treatment with plant extracts was not much effective in controlling ULCV. The best treatment was of kanher which recorded lowest (34.72 per cent) infected plants with 41.95 per cent disease control. It was followed by treatment of babool plant extract.

6. Seed treatment with kanher leaf extract followed by spraying of the same gave significantly superior control of ULCV (75.00 per cent). It was closely followed by treatment of babool (73.70 per cent).

7. In general it was observed that seed treatment with the plant extracts had no effect on germination. The germination per cent ranged between 80 to 93 in all treatments under study including the untreated control.

8. The incubation period of the virus was prolonged due to seed treatment with various plant extracts. Maximum incubation period (26 days) was recorded in the treatment of kanher leaf extract followed by babool extract. In the untreated control the virus symptoms appeared early and the incubation period of virus was also only 14-15 days.
9. Twenty four urdbean genotypes were screened under natural field conditions for their reaction to ULCV. Out of these, three genotypes *viz*; PDKV black gold, Phule U-0609-43 and TBU-4 exhibited resistant reaction to the disease. Whereas, thirteen genotypes were moderately resistant and six moderately susceptible to ULCV under field conditions. Only one genotype i.e. PKV udid 15 was found susceptible to the disease.

7. Literatures cited

Ahmad, Z., M. Bashir and T. Mtsueda, 1997. Evaluation of legume germplasm for seed borne viruses in Harmonizing Agricultural Productivity and Conservation of Biodiversity, Breeding and Ecology Proc. 8th SABRAO J. Cong. Annu. Meeting Korean Breeding Soc. Seoul. Korea, **pp**: 117–120.

Ali, S., Khan, M. A., Sahi, S. T. and Hassan, M. U. 2010. Evaluation of plant extracts and salicylic acid against *Bemisia tabaci* and cotton leaf curl virus disease. *Pak. J. Phytopath,* **22**: 98–100.

Allard, H. A. 1918. The mosaic diseases of *Phytolacca decandra*. *Phytopath.* **8**: 51–54.

Annapan, R.S., Ayyemperumal, A., Rangasamy, S.R., Rajagopal, R., Shanmugasundara, P. and Soundarapandian, O. 1988. Co 5, a new black gram variety for dry farming. Madras Agric. J. **75**: 391-395.

Anonymous, 2014. Project Coordinator's report, AICRP on Chickpea, IIPR Kanpur.

Anonymous, 2016. District wise General Statistical information of Agriculture dept. (MS).

Apablaza, G. E. and Bernier C. C. 1972. Inhibition of tobacco mosaic virus infection by plant extracts. *Canadian J. Bot.* **50**: 1473–1478.

Ashfaq, M., Khan, M. A. and Mukhtar, T. 2006. Antiviral activity of plant extracts and chemicals against urdbean leaf crinkle virus (ULCV). *Pak. J. Phytopath*, **18**: 148–155.

Ashfaq, M., Khan, M. A., Mughal, S. M., Javed, N., Mukhtar, T. and Bashir, M. 2007. Evaluation of urdbean germplasm for resistance against urdbean leaf crinkle virus. *Pak. J. Bot.*, **39**(6):210-2111.

Awasthi, L. P., Singh, S., Sharma, N. K. and Singh, S. K. 2011. Induction of systemic resistance through antiviral agents of plant origin against Papaya ring spot virus disease in papaya (*Carica papaya* L.). *Int. J. Sustainable Agric.,* **3**: 54–57.

Bashir, M., Mughal, S.M. and Malik, B.A. 1991. Assessment of field losses due to leaf crinkle virus in urdbean. *Pak. J. Bot.*, **23**: 140-142.

Baranwal, V. K. and Verma, H. N. 1992. Localized resistance against virus infection by leaf extract of *Celosia cristata*. *Pl. Path.* **41**: 633–638.

Beniwal, S.P.S. and Chaubey, S.N. 1979. Urdbean leaf crinkle virus. Effect on yield contributing factors, total yield and seed characters of urdbean. *Seed Res.* **7**(2): 175-181.

Beniwal, S.P.S. and Bharathan, N. 1980. Beetle transmission of urdbean leaf crinkle virus. *Indian Phytopath.* **33**: 600-601.

Beniwal, S.P.S.; Chaubey, S.N. and Matheswaran, C. 1983a. Some factors affecting transmission of urdbean leaf crinkle virus through seeds in urdbean (Vigna mungo L. Hepper). *Seed Res.* **11** (1): 95-99.

Beniwal, S.P.S.; Chaubey, S.N. and Bharathan, N. 1984. Detection of urdbean leaf crinkle virus in urdbean seeds. *Seed Res.* **12** (1): 1 0 1-1 04 .

Bharathimatha, C., Doraiswamy, S., Rabindran, R., Renukadevi, P. and Velazhahan, R. 2003. Existence of antiviral principles (AVPs) in seed extracts of *Harpullia cupanioides* (Roxb.) against tomato spotted wilt virus (TSWV), rice tungro virus (RTV) and cowpea aphid borne mosaic virus (CABMV). *Acta Phytopathologica et Entomologica Hungarica* **38**: 109–114.

Bhyan, S. B., Alam, M. M. and Ali, M. S. 2007. Effect of plant extracts on okra mosaic virus incidence and yield related parameters of okra. *Asian J. Agric. Res.* **1**: 112–118.

Binyamin, R., Khan M. A., Ahmad, N. and Ali. S., 2011. Relationship of epidemiological factors with urdbean leaf crinkle virus disease and its

management using plant extracts. *Int. J. Agric. Biol*, **13**: 411–414

Brar, J.S. and Rataul, H.S. 1986. Some field characteristics of leaf crinkle virus of urdbean, *Vigna mungo* (L.) Hepper. *Indian J. Virol.*, **2**(10): 49-56.

Brar, J.S. and Rataul, H.S. 1989. Incidence and extent of losses due to leaf crinkle virus in urdbean (*Vigna mungo* L.) Hepper. J. Res. Punjab Agric. Univ. 26(1): 65-70.

Chowdhury, A. K. and Saba, N. K. 1985. Inhibition of urdbean leaf crinkle virus by different plant extracts. *Indian Phytopath.* **38**: 566–568.

Deepthi, N., Madhusundhan, K. N., Udayshankar, A. C., Kumar, B. H., Prakash, H. S. and Shetty, H. S. 2007. Effect of plant extracts and acetone precipitated proteins from six medicinal plants against tobamovirus infection. *Int. J. Virol.* **3**: 80–87.

Doolittle, S. P. and Walker, M. N. 1925. Further studies on the overwintering and dissemination of cucurbit mosaic. *J. Agric. Res.* **31**: 1–58.

Dubey, G.S. and Sharma, I. 1985. Seed transmission of urdbean leaf crinkle Virus. *Seed Sci. & Technol.* **13**(1): 149-153.

Duggar, B. M. and Armstrong, J. K. 1925. The effect of treating the virus of tobacco mosaic with the juices of various plants. *Annals of Missouri Botanical Garden.***12**: 359–366.

Gautam, N. M., Kumar, K. and Prasad, M. 2016. Leaf crinkle disease in urdbean (*Vigna mungo* L. Hepper): An overview on causal agent, vector and host. *Protoplasma.* **253**: 729-746.

Gupta, V.K. 1974. Leaf crinkle, a viral disease of *Phaseolus mungo* L. in Himachal Pradesh. *Indian J. Exp. Biol.* **12**: 477-478.

Hanssen, I. M., Mumford, R., Blystad, D., Cortez, I., Hasiow-Jaroszewska, B., Hristova, D., Pagan, I., Pereira, A., Peters, J., Pospieszny, H., Ravnikar, M., Stijger, I., Tomassoli, L., Varveri, C., Vlugt, R. and Nielsen, S. L. 2010. Seed transmission of Pepino mosaic virus in tomato. *Eur. J. Pl. Path.* **126**: 145-152.

Iqbal, S.M., Ghafoor, A., Zubair, M. and Malik, B.A. 1991. Reaction of urdbean cultivars against leaf crinkle virus disease. *J. Agric. Res.* **29** (3): 411-415.

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 Phytopath.* **52**: 357–361.

Joshi, C. 1998. Studies on some aspects of urdbean leaf crinkle virus and its eco-friendly management. M. Sc. (Ag.) Thesis, O. B. Pant University of Agriculture and Technology, Pantnagar. 83p.

Kadian, D.P. 1980. Studies on leaf crinkle disease of urdbean (*Vigna mungo* (L.) Hepper)/ mungbean (*Vigna radiate* (L.) Wilczek) and its control. Ph. D. Thesis, Haryana Agricultural University, Hisar. 117p.o aphid species. *Indian Phytopath*. **28**: 80-82.

Kadian, D.P. 1982. Yield loss in mungbean and urdbean due to leaf crinkle disease. *Indian Phytopath*. **35**: 641-643.

Kadian, D.P. 1983. Studies on weed plants as host range of urdbean leaf crinkle virus. H.A.U. *J. Res.* **13**(4): 602-603.

Kadian, D.P. 1994. Mechanical and seed transmission of urdbean leaf crinkle virus (ULCV) in Haryana. *Crop Res.* **8**(3): 565-569.

Karthikeyan, G., Doraisamy, S., Rabindran, R. and Ganapathy, T. 2009. Evaluation of antiviral principles for the induction of systemic resistance in blackgram (*Vigna mungo*) against urdbean leaf crinkle virus. *Archives of Phytopathology and Plant Protection* **42**: 1172–1186.

Khatri, H.L., Bhatia, D.S. and Chohan, J.S. 1971. Brief account of the work done on diseases of kharif pulse crop at Department of Plant Pathology, PAU, Ludhiana during 1970-1971.

Kolte, S.J. and Nene, Y.L. 1970. Know the leaf crinkle disease of urd. *Indian Farmers' Digest*, **3**(11): 6-7.

Kolte, S.J. 1971. Studies on the leaf crinkle disease of urd bean (*Phaseolus mungo* L.). Ph.D. Thesis, G. B. Pant University of Agriculture and Technology, Pantnagar. 66p.

Kolte, S.J. and Nene, Y.L. 1972. Studies on symptoms and mode of transmission of leaf crinkle virus of urdbean (*Phaseolus mungo*). *Indian Phytopath*. **25**: 401-404.

Kolte, S.J. and Nene, Y.L. 1979. Noteworthy symptoms on host and influence of growth stages on host susceptibility. *Tropical Grain Legume Bulletin*, **15**: 5-8.

Kubo, S., Ikeda, T., Imaizumi, S., Takanami, Y. and Mikami, Y. 1990. A potent plant virus inhibitor found in *Mirabilis jalapa* L. *Annals of the Phytopatho. Soc. Japan* **56**: 481–487.

Kuntz, J. E. and Walker, J. C. 1947. Virus inhibition by extracts of spinach. *Phytopath.* **37**: 561–579.

Lanoiseslet, V. M., Hind-Lanoiseslet T. L. and Murrye, G. M. 2008. Studies on seed transmission of Wheat streak mosaic virus. *Australasian Pl. Patho.* **37**: 584-588.

Lavanya, N., Saravanakumar, D., Rajendran, L., Ramiah, M., Raguchander, T. and Samiyappan, R. 2009. Management of sunflower necrosis virus through anti– viral substances. *Archives of Phytopatho. and Pl. Protect.* **42**: 265–276.

Lin, C. J. and Qui, W. F. 1987. Control effects of some plant extracts on tomato mosaic disease caused by TMV–T. *Acta Phytophylactica Sinica* **14**: 217–220.

Louis, V. and Balakrishnan, S. 1996. Effect of application of selected medicinal plant extracts on the incidence of pumpkin mosaic virus. *Indian Phytopath.* **49**: 373–377.

Mahajan, P.D. and Joi, M.B. 1999. Survey and seed transmission of leaf crinkle virus of mung and urdbean. *Seed Res*. **27**(1): 131-133.

Mahdy, A. M. M., Fawzy, R. N., Hafez, M. A., Mohamed, H. A. N. and Shahwan, E. S. M. 2007. Inducing systemic resistance against bean yellow mosaic potyvirus using botanical extracts. *Egyptian J. Virology* **4**: 223–241.

Mayee, C.D. and Datar, V.V. 1985. Phytopathometry. Tech. Bull. 1, Marathwada Agril. Univ. Parbhani. pp.146.

Mohamed, E. F. 2011. Inhibition of Broad bean mosaic virus (BBMV) using extracts of Nigella (*Nigella sativa* L.) and Zizyphus (*Zizyphus spina*–Christi Mill.) plants. *J. American Sci.* **7**: 727–734.

Morales, F. J. 1987. Seed transmission characteristics of selected bean common mosaic virus strains in differential bean cultivars. *The American Phytopathological society Plant Disease*:51-53

Malik, S. 1998. Urdbean leaf crinkle virus: transmission, histopathology and management. Ph.D. Thesis, G. B. Pant University of Agriculture and Technology, Pantnagar. 74p.

Mishra, A., Gohel, V. R. and Patel, G. J. 1994. extent of seed transmission of urdbean leaf crinkle virus in Gujarat. *G.A.U. Res. J.* **19**(2): 130-132.

Murthy, N. S. and Nagarajan, K. 1986. Role of plant extracts in the control of TMV infection in nursery and field grown tobacco. *Indian Phytopatho.* **39**: 98–100.

Nagaraju, N., Reddy, H. R. and Ravi, K. S. 1997. Effect of exogenously applied plant products on pepper vein banding virus transmission, multiplication and symptom production in bell pepper (*Capsicum annuum* L.). *Indian J. Virol.* **13**: 161–163.

Narayanasamy, P. and Jagan athan, T. 1973b. Sources of resistance to black grarn virus disease. *Madras Agric. J.* **60**(9-12): 1836-1838.

Narayanasamy, P. and Jaganathan, T. 1975. Seed transmission of blackgram leaf crinkle virus. *Phytopath. Z.* **82**: 107-110.

Negi, H. and Vishunavat, K. 2003. Role of seedborne inoculum of urdbean leaf crinkle virus (ULCV) in disease development. Paper presented in National symposium on "Current technologies for plant disease management and future strategies" held at Department of Plant Pathology, Rajasthan Agriculture University, Agricultural Research Station, Durgapur, Jaipur, from October 8-10, 2003.

Nene, Y.L. 1968. A survey of viral diseases of pulse crops in Uttar Pradesh. First Annual Report, U.P. *Agric. Univ*. pp l-25.

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

Othman, B. A. and Shoman, S. A. 2004. Antiphytoviral activity of the Plectranthus tenuiflorus on some important viruses. *International Journal of Agriculture and Biology* **6**: 844–849.

Patel, A.B., Mishra, A. and Valand, G.B. 1999. Characterization of leaf crinkle virus disease of urdbean (*Vigna mungo* L.). *Indian J. Virol.* **15**(2): 101-105.

Pushpalatha, K.C., Prakash, H.S., Albrechtsen, S.E., Shetty, H.S. and mathur, S.B. 1999. Transmission of urdbean leaf crinkle virus through urdbean seeds. *Seed Res.* **27**(1): 112-115.

Prasad, M.S., Sarma, B.K., Kumar, S. and Prasad, M.S.L. 1998. Transmission test and varietal screening for urdbean leaf crinkle virus in blackgram (Vigna mungo L. Hepper). *Annl. PI. Protec. Sci.* **6**(2): 189-219.

Prasad, H. P., Shankar, U. A. C., Kumar, B. H., Shetty, S. H. and Prakash, H. S. 2007. Management of bean common mosaic virus strain black eye cowpea mosaic (BCMV-BlCM) in cowpea using plant extracts. *Archives of Phytopath. and Pl. Prot.* **40**: 139–147.

Reddy, C. R., Topani, V. A., Varanavasiappan, S., Navi, S. S. and Jayarajan, R. 2005. Studies on seed transmission of Urdbean leaf crinkle virus on urdbean. *Indian J. Pl. Protection*, Vol.**33**: 241-245.

Reddy, B. A., Patil, M. S. and Rajasekaram, T. 2010. Effect of plant extracts on the incidence of tomato leaf curl

virus and whitefly population under field conditions. *Karnataka J. Agric. Sci.* **23**: 807–808.

Sahay, G., Sarma, B.K., Gupta, H.S., Pathak, K.A. and Prasad, M.S. 1999. Biotic stresses in pulses in North Eastern Hill region of India. *Indian J. Hill Farming*, **12**(1-2): 8-16.

Sharma, I. and Dubey, G.S. 1984. Control of urdbean leaf crinkle virus through heat treatment and resistance. *Indian Phytopath.* **37**(1): 26-30.

Sharma, L. and Dubey, G.S. 1985. Anatomy of urdbean leaf crinkle virus infected urdbean root. *Indian J. Plant Patho*. **3**(2): 236-237.

Shivanathan, P. 1980. Legume Pathology of Maha Illupallama. *Tropical Agriculturist*, **136**: 405-408.

Singh, J.P. 1980. Effect of virus diseases on growth components and yield of mungbean (*Vigna radiata*) and urdbean (*Vigna. mungo*). *Indian Phytopath*. **33**(3): 405-408.

Singh, B.B. and Singh, D.B. 1994. Narendra Urd 1- a high yielding and disease resistant variety of urdbean. Indian Farming, **43**: 3-4.

Singh, I. and Varma, J. P. 1981. Virus inhibitor from Datura metel. *Indian Phytopath.* **34**: 452–457.

Singh, S. and Awasti, L. P. 2009. Plant products for the management of yellow mosaic disease of mungbean and urdbean. *The J. Plant Protect. Sci.* **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. *Current Botany* **2**: 08–11.

Srivastava, G., Verma, A. and Tewari, J. P. 2010. Management of watermelon mosaic virus disease by use of leaf extracts of some medicinal plants. *Int. J. Plant Protect.* **2**: 224–226.

Srivastava, S. and Singh, A.K. (2010) Changes in catalase activity and total protein content in urdbean [Vigna mungo (L) Hepper] plants as a result of ULCV infection. *Indian J. Sci. Res.* **1**:67–69.

Tewari, J. P., Singh, K. and Srivastava, P. 2001. Inhibition of cucumber green mottle mosaic virus by extract of some ferns. *J. Living World* **8**: 28–32.

Thirumalaisamy, P.P., Rathi, Y.P.S. and Tripathi, H.S. 2003. Screening of some plant extracts inhibitory to urdbean leaf crinkle virus. *Indian Phytopath.* **56**(2): 233-235.

Tomlinson, J. A., Walker, V. M., Flewett, T. H. and Barclay, G. R. 1974. The inhibition of infection by cucumber

mosaic virus and influenza virus by extracts from *Phytolacca americana*. *J. Gen. Virol.***22**: 225–232.

Verma, H. N. and Baranwal, V. K. 1983. Antiviral activity and the physical properties of the leaf extract of Chenopodium ambrosoides L. Proceedings: Plant Sciences **92**: 461–465.

Verma, H. N. and Mukherjee, K. 1975. Brinjal leaf extract induced resistance to virus infection in plants. *Indian J. Exp. Bio.* **13**: 416–417.

Williams, F. J., Grewal, J. S. and Amin, K. S. 1968. Serious and new diseases of pulse crops in India in 1966. *Plant Dis. Reptr*. **52**(4): 300-304.

Zaidi, Z. B., Gupta, V. P., Samad, A. and Naqvi, Q. A. 1988. Inhibition of spinach mosaic virus by extracts of some medicinal plants. *Current Science* **57**: 151–152.

Zukovskij, P.M. 1962. Cultivated Plants and their Wild relatives. *C.A.B. London*.

8. VITA

Mr. TIKHE DNYANDEV ASHOK

A candidate for the degree
of
MASTER OF SCIENCE (AGRICULTURE)
in
PLANT PATHOLOGY AND AGRICULRURAL MICROBIOLOGY
2017

Title of Thesis : Effect of Botanicals on Seed Borne Leaf Crinkle Virus in Black Gram (*Vigna mungo* L).

Major field : Plant Pathology

Biographical information

Personal : Born At. Belgaon, Tal. Karjat, Dist. Ahmednagar on 10th November, 1992. Son of Mr. Ashok Nana Tikhe and Mrs. Indubai Ashok Tikhe.

Educational : Passed S.S.C. examination from Jawaharlal Vidyalaya, Kedgaon Tal. Daund, Dist. Pune with First class.

Passed H.S.C. examination from Nutan Junior College Mirajgaon Tal. Karjat, Dist. Ahmednagar with First class.

Completed Bachelor of Science (Agriculture) degree from College of Agriculture, Kolhapur, with First Class.

Extra-curricular Activities	:	Participated in NSS camp of 240 hrs.
Address	:	At. Belgaon, Post. Kokangaon, Tal. Karjat, Dist. Ahmednagar Pincode- 414 401 Mob- 9011057586 Email-dnyandevtikhe@gmail.com