STUDIES ON *URDBEAN* LEAF CRINKLE DISEASE ON*URDBEAN* [*Vigna mungo* (L.) HEPPER]

L. USHA RANI
B.Sc. (Ag.)

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(PLANT PATHOLOGY)

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STUDIES ON *URDBEAN* LEAF CRINKLE DISEASE ON *URDBEAN* [*Vigna mungo* (L.) HEPPER]

BY

L. USHA RANI

B.Sc. (Ag.)

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CHAIRPERSON: Dr. V. MANOJ KUMAR

DEPARTMENT OF PLANT PATHOLOGY

AGRICULTURAL COLLEGE, BAPATLA
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
GUNTUR – 522 509

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DECLARATION

I, **L. Usha Rani,** hereby declare that the thesis entitled **"STUDIES ON *URDBEAN* LEAF CRINKLE DISEASE ON *URDBEAN* [*Vigna mungo* (L.) HEPPER]"**submitted to the **Acharya N. G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Bapatla

Date:

(L. USHA RANI)
ID. No. BAM-17-46

CERTIFICATE

Ms. L. USHA RANI has satisfactorily prosecuted the course of research and that thesis entitled **"STUDIES ON URDBEAN LEAF CRINKLE DISEASE ON *URDBEAN* [*Vigna mungo* (L.) HEPPER]"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by her for a degree of any University.

Date:

(V. MANOJ KUMAR)
Chairperson
Associate Professor
Department of Plant Pathology
Agricultural College
Bapatla-522 101.

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON URDBEAN LEAF CRINKLE DISEASE ON *URDBEAN* [*Vigna mungo* (L.) HEPPER]"** submitted in partial fulfilment of the requirements for the degree of '**Master of Science in Agriculture'** of the Acharya N. G. Ranga Agricultural University, Lam, Guntur is a record of the bonafide original research work carried out by **Ms. L. USHA RANI** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student's Advisory Committee

Chairperson — **Dr. V. MANOJ KUMAR** _____
Associate Professor
Department of Plant Pathology
Agricultural College, Bapatla.

Member — **Dr. V. PRASANNAKUMARI** _____
Associate Professor
Department of Plant Pathology
Agricultural College, Bapatla.

Member — **Dr. C. SANDHYA RANI** _____
Professor
Department of Entomology
Agricultural College, Bapatla.

Date of final viva-voce:

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Place:

Date: **(L. USHA RANI)**

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LIST OF SYMBOLS /ABBREVIATIONS

%	:	Per cent
^{0}C	:	Degree Celsius
CD (P=0.05%)	:	Critical difference at 5 per cent level
CV	:	Coefficient of variation
et al.	:	and other co-workers
Fig.	:	Figure
g	:	Gram (s)
h	:	Hour (s)
i.e.	:	that is
kg	:	Kilogram
ac	:	Acre
kgha^{-1}	:	Kilogram per hectare
q ha^{-1}	:	Quintal per hectare
mg	:	Milligram
min	:	Minute
ml	:	Millilitre
M	:	Molarity
mM	:	Milli molar
nm	:	Nanometre
No.	:	Number
ppm	:	parts per million
RBD	:	Randomized Block Design
rpm	:	Revolutions per minute
sec	:	Second

SEm	:	Standard Error of mean
sp., spp.	:	Species (singular or plural)
M t	:	Million tonnes
µg	:	Microgram
viz.	:	Namely
µl	:	Microlitre
l	:	Litre

ABSTRACT

Name of the author	:	**L. USHA RANI**
Title of the thesis	:	**STUDIES ON *URDBEAN* LEAF CRINKLE DISEASE ON *URDBEAN* [*Vigna mungo* (L.) Hepper]**
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University	:	**ACHARYA N. G. RANGA AGRICULTURAL UNIVERSITY**
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The present investigation on "Studies on *urdbean* leaf crinkle disease on *urdbean* [*Vigna mungo* (L.) Hepper]" was carried out at Agricultural College, Bapatla, Guntur district, Andhra Pradesh during 2018-19.

Blackgram cv LBG 752 belonging to Leguminosae tested for their reaction to *Urdbean* leaf crinkle disease(ULCD) by sap incoulation method, the disease symptoms with crinkling and curling of infected leaves .

Seed treatment with hot water (50^0C for ten min) significantly lowered the incidence (10%) of ULCD followed by seed treatment with 16% trisodium orthophosphate (20.00%) when compared to untreated control (28.33%).

Aphid (*Aphis craccivora*) transmitted ULCD with an acquisition access period of ten minutes (10.00%) and inoculation access period of 24 h (20.00%). Maximum ULCD incidence was noticed with 20 aphids per plant (13.33%).

Beetle (*Henosepilachna dodecastigma*) transmitted ULCD with an acquisition access period of six hours (3.33%) and inoculation access period of 12 hours (6.67%).

ULCD infected sample showed the highest protein content when compared to healthy samples. Phenol content was also the highest in ULCD infected plants when compared to healthy plants.

When the healthy leaf tissues were assessed, the highest protein content was observed at 40, 55 and 70 DAS in $MgSO_4$ @ 0.2% + $ZnSO_4$ @ 0.2% with 1.43, 1.71 and 2.35 mg/100mg of leaf tissue respectively and the lowest was in check 1.34, 1.41and 1.63 mg/100 mg of leaf tissue at the respective interval 15 days from 40 DAS and in infected maximum was observed in $ZnSO_4$ @ 0.2% (1.97, 2.18 and 2.34 mg/100 mg at 40, 55 and 70 DAS) of leaf tissue and minimum was observed in check (1.44, 1.69 and 1.85 at 40, 55 and 70 DAS).

The maximum phenol content in healthy leaf tissue at 40, 55 and 70 DAS in $MgSO_4$ @ 0.2% + $ZnSO_4$ @ 0.2% with 0.60, 0.66 and 0.72 mg/100mg of leaf tissue sprayed plots while minimum was in check (0.34, 0.40 and 0.45 mg/100mg respectively). In infected tissues the highest phenol was observed at 40, 55 and 70 DAS in$MgSO_4$ @ 0.2% + $ZnSO_4$ @ 0.2% with 0.89, 0.92 and 0.97 mg/100mg and the lowest was observed in check (0.51, 0.55 and 0.59 mg/100mg).

Presence of auxins was tested using TLC plates and the R_f values are recorded as 0.92, 0.896 and 0.901 in stranded, infected and healthy leaf samples respectively.

Among the micronutrients tested, spraying of $MgSO_4$ @ 0.2% + $ZnSO_4$ @ 0.2% recorded significantly lowest AUDPC value (466.97) of ULCD followed by $ZnSO_4$ @ 0.2% (567.84) as against unsprayed control (955.92). The highest B:C ratio was found in $MgSO_4$ @ 0.2% + $ZnSO_4$ @ 0.2%(2.11) and lowest in control (1.42).

Chapter – I

Introduction

Chapter I

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] is also called as *urdbean* belongs to the family Fabaceae. It is one of the important short duration pulse crop of India which is grown throughout the year under different agro climatic conditions (Jayasekhar and Ebenezar,2016). As per the descriptions in vedic scriptures of Kautilya's "Arthasasthra" and "Charak Samhita", it is believed that the crop has originated in India.

Urdbean seed contains 24% protein and is the richest source of phosphoric acid among pulses (Duffus and Slaughter, 1980) which is available at low price, hence as it is rightly called as the poor man's meat. *Urdbean* crop is considered as mini-fertilizer factory as it restores soil fertility by fixing nitrogen in the atmosphere equivalent of around 22 kg ha^{-1}(Rachie and Roberts, 1974). It is also an importantgreen manure and as a fodder crop.

India is contributing 28 per cent to the global pulse market (Masood Ali and Shivkumar, 2000). In India, *urdbean* is the third important pulse crop and occupies an area of 44.78 lakh ha producing 28.32 lakh tons with a productivity of 632 kg ha^{-1}(www.Indiastat.com, 2016-17).

Major *urdbean* growing states in India are Andhra Pradesh (A.P.), Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Sikkim, Tamil Nadu and Uttar Pradesh. In Andhra Pradesh, *urdbean* is cultivated in an area of five lakh ha, with a production and productivity of 3.29 lakh tonnes and 658 $kgha^{-1}$, respectively (www.Indiastat.com, 2016-17). The crop is of special significance in Andhra Pradesh as it fits well in rice – pulse cropping system as a relay crop particularly in Krishna – Godavari and North Coastal zones.

Urdbean crop is infected by several viral diseases such as yellow mosaic virus, leaf curl and Urdbean leaf crinkle disease (ULCD). Among the viral diseases, ULCD is one of the most serious disease in all *urdbean* growing areas (Reddy *et al.*, 2005a) causing an yield loss of 35-81% depending on the season and variety (Nene, 1972).

Williams *et al*. (1968) observed the disease showing the symptoms of puckering, crinkling and curling of leaves, malformation of flowers and stunted growth. Kolte and Nene (1972) described the symptoms of ULCD as increase in the size and light green colour followed by conspicuous crinkling of third trifoliate leaf.

In India, ULCD was reported to be transmitted by important insect pests of *urdbean viz*., whiteflies (Narayanasamy and Jaganathan, 1973b), aphids (Bindra, 1971) and beetles (Beniwal and Bharathan, 1980). Seed transmission was also reported (Kolte and Nene, 1972; Narayanasamy and Jaganathan, 1975b).

The causal entity is classified under unassigned group due to ambiguity in identifying the casual agent and ascertaining the vector relationship.

However, several attempts were made to control the disease through the plant defence related mineral nutrients like zinc, manganese, magnesium, iron, copper and silicon. Accumulation of phenolic compounds and flavonoids were detected at the site of infection has indicated the possible role of these compounds on reducing the plant diseases (www.spectrumanalytic.com). Manganese (Mn) content was found to be low in infected plant than healthy due to the reduced ability to synthesize lignin, which in turn reduces plants ability to resist infection. Visible symptoms will be lessen or even disappear when sprayed with Mn (www.spectrumanalytic.com).

Major constraints in management of ULCD were lack of resistant cultivars of *urdbean*, obscurity regarding the mode of transmission and lack of appropriate control measures. The present investigation was undertaken to identify the mode of transmission and management of ULCD by micronutrients with the following objectives

Objectives of research work

1) To study different modes of transmission of ULCD.

2) To study the effect of micronutrients on ULCD under field conditions.

3) To study the biochemical changes in blackgram infected with ULCD

Chapter – II

Review of Literature

Chapter II

REVIEW OF LITERATURE

The literature pertinent to *Urdbean* leaf crinkle disease (ULCD) relevant to the present investigation is presented in this chapter.

2.1 HISTORY AND DISTRIBUTION OF THE DISEASE

The occurrence of leaf crinkle disease of *urdbean* was first reported by Williams *et al*. (1968) from Delhi and Uttar Pradesh.Chohan and Kalia (1967) reported similar disease from Punjab and named it as 'curly top'. Later it has been reported from Uttar Pradesh (Nene, 1968), Punjab (Khatri *et al*., 1971), Tamil Nadu (Narayanasamy and Jaganathan, 1973a), Himachal Pradesh (Gupta, 1974), Delhi (Amin *et al*., 1978), Haryana (Singh *et al*., 1979) and West Bengal (Chowdhury and Saha, 1985). Mean while Kolte and Nene (1970) and Nene (1970, 1971) reported that the causative agent of the disease as virus.

2.2 IMPORTANCE OF THE DISEASE

Among all the viral diseases of *urdbean*, ULCD is said to be the major threat for the cultivation as most of all high yielding varieties of *urdbean* are susceptible to the virus. Leaf crinkle is well thought-out to be one of the foremost constraints for cost-effective cultivation of *urdbean* during *rabi* and in rice fallows of Andhra Pradesh. Depending on the stage of the crop at which the infection occurs, yield loss may vary between 50 to 76%. Plants that are infected very early failed to produce any pods than later infected plants (100% loss) in susceptible cultivars T-9 and Buttaminumu (Kolte, 1971; Subbarao, 1984; Bhagavan, 1985) due to which decreased seed yield per plant was reported (Subbarao, 1984; Bhagavan, 1985).

2.3SYMPTOMATOLOGY

The first recognizable symptoms of ULCD usually appear three weeks after sowing in field condition (Kolte and Nene, 1972). Generally the third trifoliate leaf showed the first symptom characterized by an increase in the size and a lighter green colour (Kolte and Nene, 1972; Brar and Rataul, 1986; Patel *et al.*, 1999). In about a week after initial symptoms, the typical crinkling becomes conspicuous. Late infected

lower leaves were devoid of any symptoms (Kolte and Nene, 1972; Brar and Rataul, 1986). Shortening of petioles result in crowding of leaves (Brar and Rataul, 1986; Ravandar *et al.*, 2006; Janaki Ratnam, 2015). Other prominent symptoms were puckering, crinkling, curling, (Williams *et al.*, 1968; Janaki Ratnam 2015), rugosity (Williams *et al.*, 1968), increase in the leaf area and number of stomata (Patel *et al*., 1999), stunted growth, malformation of flowers (Williams *et al.*, 1968; Janaki Ratnam, 2015), with sepals of flower being thicker and greener than normal, some branches are affected while others remain apparently healthy (Brar and Rataul, 1986). Anatomical changes in stem, petioles and leaves of ULCD infected plant parts, indicated an increase in the number of layers and size of parenchyma cells, cambial cells, epidermal cells and also diameter of phloem parenchyma Ravandar *et al*. (2006).

Further, symptoms varied with the type of host species attacked according to Kolte and Nene (1975) *Vigna mungo, Vigna radiate, Vigna unguiculata* shows rugosity, leaf malformation and Beniwal *et al.* (1983a) reported that in cucurbits, *i.e*., *Cucumis sativus* and *Lagenaria cylindrical* systemic mosaic, rugosity, leaf malformation and systemic rugosity, leaf malformation, yellowing were observed.

2.4 THE PATHOGEN

2.4.1 Morphology of the Virion

Virus particles were observed in the cytoplasm, chloroplast and nucleus of the ULCD infected plant parts. The virus is isometric and measures of about 25-30 nm in diameter, rounded in profile and without a conspicuous capsomere arrangement (Bhaktavatsalam *et al*., 1983).

Serological studies indicated that ULCD and *Cowpea leaf crinkle virus* are serologically related (Singh, 1984) while Bromo, Cucumo, Tymo and Potyvirus groups are unrelated (Beniwal and Bharathan, 1980; Dubey *et al*., 1983).

2.4.2 Physical and Biochemical Properties of the Virus

Different studies indicated that the Thermal Inactivation Point (TIP) ranged between 60-75^{0}C, at 7.8 pH, 1: 10,000 to 1: 1, 00,000 dilution end point, 3-5 days of longevity *invitro* at room temperature, infectivity of sap not changed by diethyl ether (Dubey *et al*., 1983).

2.5 HOST RANGE

S.No.	Crop species	Reported by
1	Greengram, Cowpea, Mothbean	Kolte and Nene (1975)
2	Cucumber and Bottle gourd	Beniwal *et al*. (1983a)
3	Cluster bean, Groundnut and Pigeonpea	Narayanasamy and Jaganathan(1975b)
4	*Vigna radiata*, *Vigna unguiculata*, *Glycine max*, *Luffa acutangula*, *Cucumis sativus* and *Lagenaria siceraria*	Krishnaveni (1988)
5	Leguminosae plants:- *Vigna sinensis* *V. radiata* *Phaseolus acontifolius* Cucurbitaceous plants:- *C. sativus* *L. leucantha*	Reddy (1988)
6	Cucurbitaceous :- *C. sativus* *L. siceraria* *L. acutangu* Fabaceae :- *Dolichos lablab* *V. radiata* *P. vulgaris* *V. unguiculata* Malvaceae :- *Gossypium hirsutum* *Hibiscus cannabinus*	Vijaykumar (1993)
7	Cowpea and cucumber	Pushpalatha *et al*. (1999)
8	Cucurbitaceae:- *C. sativus* (cv. Japanese Long Green) *L. siceraria* (cv. Local) *L. acutangula* (cv. Local) Fabaceae :- *Canavalia ensiformis* (cv. Local) *D. lablab* (cv. Local) *G. max* (cv. Hardee) *P. vulgaris*"*Rajmash*" (cv. Local) *P. vulgaris* (cv. Arka komal) *V. radiata* (cv. ML-267) *V. unguiculata* (cv. Local)	Rao and Reddy (2004)

2.5.1 Weeds as host plants

S .No.	Weed plants	Reported by
1	*Convolvulus arvensis* *Datura stramonium* *D. metal* *D. metaloides* *D. incrimis*	Kadian (1983)
2	*Leptadenia reticulate*	Bhagavan (1985)
3	*V. radiate*	Suneela (1996)

2.6 EPIDEMIOLOGY

Kadian (1989) reported that leaf crinkle disease developed at maximum temperature of 35±2^{0}C, minimum temperature of 25±2^{0}C, minimum relative humidity above 70% and evening relative humidity more than 50%. In summer the symptoms remained masked at 38-45^{0}C when morning and evening relative humidity remained 60 and 40% respectively. The disease symptoms did not appear even at 35^{0}C for a week or above 47^{0}C for a day with morning and evening relative humidity of 45 and 20% respectively.

Ashfaq *et al*. (2008) evaluated 20 *urdbean* genotypes in order to see the impact of environmental conditions on leaf crinkle disease development for four seasons (spring and summer) during 2005-2006 and found that maximum ULCD disease severity was recorded at 35-42^{0}C (maximum temperature) and at 21-29^{0}C (minimum temperature). There was no correlation with weekly relative humidity, rainfall and wind movement.

Binyamin *et al*. (2011) screened six *urdbean* lines against ULCD during summer 2009. Correlation studies between environmental factors (temperatures and relative humidity) and ULCD infection in selected lines revealed positive interaction for maximum and minimum temperatures but a negative one for relative humidity. One degree increase in minimum temperature increased the rate of ULCD infection by 5.60, 5.42, 2.69, 2.13, 2.08 and 1.70% in ES-1, 6036-14, AARIM-32, Mash- 189, 6036-14 and IAM 382-1, respectively. One per cent decrease in relative humidity increased the infection rate by 2.82, 2.49, 1.30, 1.08, 1.03 and 0.87%, in 62027, ES-1, AARIM-32, Mash-189, 6036-14 and IAM 382-1, respectively.

2.7TRANSMISSION

2.7.1 Mechanical Transmission

Earlier research workers (Williams *et al*., 1968; Chohan and Kalia, 1967) efforts failed to transmit ULCD through sap inoculation. Later, Kolte (1971) succeeded in the mechanical transmission of the causal virus using potassium phosphate buffer (pH 7.6) as extracting medium and carborundum as abrasive. The incubation period of virus ranged between 11-19 days in the plant for symptom expression. Chowdhury and Chowdhury (1983) reported that the highest per cent infection of ULCD was obtained by using potassium phosphate buffer (pH 8.0) as compared to sodium phosphate buffer and citrate phosphate buffer. Kadian (1994) reported that efficiency of mechanical transmission of ULCD was increased when inoculum was prepared in 0.1M phosphate buffer, pH 7.0 and when carborandum was used as an abrasive, and also reported that younger plants of *urdbean* cv. Krishna (1 week old) were more sensitive to transmission than older ones.

Kolte (1971) observed that inoculation on primary leaves, first and second trifoliate leaves resulted in 88.88, 71.11 and 66.66% of success of infection respectively. Kolte and Nene (1972) carried out mechanical transmission of ULCD by inoculating six-day old seedlings and transmission was successful to the extent of 100%.

Prasad *et al*. (1998) reported that ULCD was found to be sap transmissible up to 80% with an incubation period of 21 days. Karthikeyan, (2002) stated that among the tested 66 plant species belonging to 18 families for ULCD transmission by sap inoculation, only the Leguminaceae crop plants *viz*., greengram, cowpea, horsegram and soybean were infected by the ULCD.

Rao and Reddy (2004) observed that all the plant species tested for the reaction of plants to ULCD showed systemic symptoms and absolutely no species produced local lesions on sap inoculation of ULCD.

Rao and Reddy (2005a) reported that artificially inoculated ULCD in *urdbean* plants at primary leaf stage showed incubation period of 10 to 12 days after inoculation and the highest susceptibility (80%) was observed at primary leaf stage.

Reddy *et al*. (2005a) reported influence related to plant age on infection of ULCD and given that inoculation at primary leaf stages (7 and 10 DAS) gave 85% and 72% infection respectively and the initial symptom developed on second trifoliate leaf stage onwards when the plants were inoculated at younger stage as compared to older plants. There was no infection when plants were inoculated prior to flowering stage.

Himanshu and Karuna (2006) concluded that the susceptibility of *urdbean* plants to leaf crinkle infection has significantly decreased, with the age, while the incubation period showed positively correlation. A maximum of 86.5% infection was recorded when plants were inoculated at 7 days after sowing (DAS) while the minimum of 15.0% infection was recorded in plants inoculated at 49 DAS. Incubation period ranged from 13 to 38 days and was minimum in plants inoculated at seven DAS and maximum in plants inoculated at 49 DAS, respectively.

2.7.2 Graft Transmission

Chohan and Kalia (1967) from Punjab and Gupta (1974) from Himachal Pradesh recorded graft transmission ofULCD.

2.7.3 Seed Transmission

Kolte and Nene (1972) reported the seed borne nature of the virus and later by Narayanasamy and Jaganathan (1975b). Kolte and Nene (1972) recorded 18.39% of seed transmission.

ULCD is reported as internally seed borne because it was reported in plumule, radical, cotyledons, primary axis and embryo but not in seed coat as per the infectivity tests (Narayanaswamy and Jaganathan, 1975a; Beniwal *et al*., 1980; Dubey and Sharma, 1985).

Narayanaswamy and Jaganathan (1975b) reported that the percentage of seed transmission of ULCD was reduced as the age of the plants increased. Higher percentage (41.86%) of seed transmission was observed in young infected plants (5 days old) than later infected plants (0.00-29.17%). The incubation period of ULCD increased with the age of the plants (13-37) showing a negative linear relationship with the percentage of seed transmission of ULCD.

Beniwal *et al*. (1980) recorded ULCD as seed borne in three out of 49 germplasm collections tested. The rate of its transmission in seed varied from 6 to 15%. Beniwal *et al*. (1983b) reported that seed transmission of ULCD in germplasm and varieties of *urdbean* ranged from nil to 15% and higher percentage of seed transmission occurred in plants infected early (10.97%) than those infected later (1.20 to 4.71%) in the season. Seed transmission of ULCD was not affected by morphological abnormalities in seed, different stages of seed maturation and presence or absence of seed coat. Heat treatment affected ULCD transmission in seed from infected plants as no seed transmission occurred in seed receiving a hot water treatment at 60^0C and 70^0C for 10, 20 and 30 minutes and dry heat treatment at 70^0C for 10, 20 and 30 minutes.

Beniwal and Chaubey (1984) stated that the ULCD is internally seed borne and located within the embryo of the *urdbean* seed. He observed that ULCD to be distributed in all the flower (epicalyx, calyx, corolla, androecium, gynoecium) and seed (seed coat, cotyledons, primary axis) parts by the indicator-inoculation test. The per cent transmission in seeds receiving sodium phosphate treatment was 13 as compared to 14 in untreated control seeds but removal of seed coat did not have any effect on seed-transmission as the per cent transmission in seeds with and without seed coats was 14 and 13 respectively. Seed surface washings when inoculated on indicator plants could not produce any symptoms in them.

Sharma and Dubey (1984) reported that seed-borne infection may be removed by treating the seeds in water bath at 55^0C for 30 minutes. Soaking seeds in water for 3-4 hrs and then exposure to solar heat from 12 to 4 pm in May and June was found effective in eliminating the seed borne infection of ULCD.

Dubey and Sharma (1985) reported that ULCD was carried in 17.6% *urdbean* seeds from naturally field infected plants. It survived in cotyledons and embryos of infected seeds. Cotyledon extract resulted in a higher transmission percentage (56%) than embryo extract (48%) but this virus did not affect seed germination. The location of pods on diseased plants and of seed in the pods had no influence on seed transmission and the highest seed transmission of 68% occured in seeds from 10 day old artificially inoculated plants. No transmission was obtained through seeds collected from 50 day old inoculated plants.

Reddy (1988) recorded ULCD in embryo and cotyledons of seeds harvested from infected seeds and observed high transmission percentage from embryo, while the extracts from seed coat and seed washings did not transmit the virus.

Kadian (1994) recorded twenty one percent seed transmission of ULCD later transmission increased when infected seeds were continuously reused for sowing purpose up to three generations and also reported that younger plants *i.e*. one week old *urdbean* plants were more sensitive to transmission than older ones.

Mishra *et al*. (1994) reported 31.25% ULCD seed transmission at one field test site, but only 4.4 and 2.0% at the other two test sites in Gujarat. Mahajan and Joi (1999) recorded that the rate of ULCD transmission through seeds in succeeding crops of *mungbean* and *urdbean* was 2.0 to 16.0% and 1.16 to 11.0% during 1989 and 1990 respectively.

Pushpalatha *et al*. (1999) observed that 24 out of 29 seed samples collected from different states of India were found to transmit ULCD in the growing-on test. Seed transmission of ULCD ranged from 1 to 83%. The highest percentage of infected seedlings was recorded at first trifoliate leaf stage. However, clear leaf crinkle symptoms were observed at third trifoliate leaf stage.

Karthikeyan (2002) recorded considerably higher per cent (25%) of seed transmission in *urdbean* cultivar, CO 5 followed by cultivars CO 4, CO 2 and T9 (each 20%). The *urdbean* cultivars *viz*., CO 1, CO 3 and VBN 1 were recorded with seed transmission of 16%, 16% and 10% respectively. The per cent transmission was maximum when the sap containing the inoculum was extracted from whole seed (30%) followed by embryo extract (25%), cotyledon and seed coat extracts (5%). The seed washing did not contain any virus.

Himanshu and Karuna (2004) reported that seeds collected from systemically infected young *urdbean* plants with ULCD showed the lowest germination (74.6%) compared to infection in older stages of crop growth (88.3%).

Studies of Singh and Awasthi (2004) indicated that ULCD infection prevailed in *urdbean* seed and continuous use of such seeds possibly accounted for the appearance of leaf crinkle disease in next generations under field conditions.

Rao and Reddy (2005b) reported that flowering in ULCD infected plants was delayed by 10-12 days in comparison with healthy plants. The infected seeds were shrivelled, crinkled and small sized and the mean seed transmission was 17.70%. They recorded 50% seed transmission with the cotyledon and embryo extracts.

Reddy *et al*. (2005b) observed that seeds collected from the first and second nodes of ULCD infected plants expressed symptoms earlier than seeds from other nodes. The degree of transmission gradually decreased as the position of the pod increased from the base. The highest mean seed transmission (19.7%) was recorded for pods collected from plants showing first symptoms on the third leaf. The virus was detected only in the embryo and cotyledons.

Himanshu and Karuna (2006) reported that *urdbean* plants infected with ULCD at 7 DAS exhibited maximum number of shrivelled seeds (25.4%) while in plants infected at 49 DAS a total of 15.9% seeds were found shrivelled. A maximum of 18.3% off-coloured seeds and 20.1% over sized seeds were recorded from plants infected at 7 DAS while plants infected at 49 DAS exhibited 5.0% off-coloured seeds and 7.5% over sized seeds. Seeds collected from plants inoculated at 7 DAS showed minimum 76.0% germination while the maximum germination percentage (88.0%) was analysed in seeds collected from plants inoculated at 49 DAS. Transmission of leaf crinkle infection in seeds recovered from plants inoculated at 7 DAS was 26.2% while it was 2.2% in seeds obtained from plants inoculated at 49 DAS.

Kanimozhi *et al.* (2009) stated that seed transmission of ULCD in *mungbean* and *urdbean* was 26.25 and 34.50%, respectively, whereas, in seeds collected from plants infected with both MYMV and ULCD, the seed transmission of ULCD in *mungbean* was 21.13% and in *urdbean* 26.25%.

2.7.4 Vector Transmission

Kolte (1971) during his insect transmission studies of the virus under glasshouse conditions reported that the ULCD could not be transmitted by *Aphis craccivora* Koch and cotton whitefly, *Bemisia tabaci* Genn.

Bindra (1971) and Khatri *et al*. (1971) reported that ULCD is transmissible through the aphid, *A. craccivora* Koch. and also the leaf-hopper *Circulifer tenellus* Baker. Later, Nene and Kolte (1972) found that ULCD could not be transmitted by *A. craccivora* Koch.

Narayanasamy and Jaganathan (1973b) reported that the ULCD was transmitted by whitefly, *B. tabaci* (25-67.5%) in Tamil Nadu.

Dhingra (1976) reported that ULCD was found to be transmitted by *A. craccivora* Koch. And *Aphis gossypii* Glov. with in very short acquisition feeding period of 30 seconds to 2 minutes preceded by a pre-acquisition fasting was found necessary for the successful transmission of ULCD.

Beniwal and Bharathan (1980) could not transmit ULCD with aphids, whitefly and leaf hopper but with beetle (*Henosepilachna dodecastigma*).

Kadian (1980) reported from Haryana that the virus was readily transmitted through *A. craccivora* and *A. gossypii* in non-persistant manner.

Dhingra and Chenulu (1981) reported that *Myzus persicae* was an efficient vector for ULCD when 30 seconds acquisition access time was given preceeding fasting for four hours. Dubey *et al*. (1983) transmitted ULCD successfully by *A. craccivora* and *Acyrthosiphon pisum* to the extent of 60 and 80% respectively. *Aphis fabae*, *Rhopalosiphum maidis*, *Brevicoryne brassicae* and *Macrosiphum rosaeformis* failed to transmit the virus.

Bhardwaj and Dubey (1986) reported that the highest transmission of ULCD by *A. craccivora* and *A. pisum* fasting period of 90 minutes prior to virus acquisition.

Nath *et al*. (1986) reported that the ULCV was transmitted by the aphids, *Lipahis erysime* and *Hysteroneura setariae* in a non-persistant manner. The transmission percentage was highest with an acquisition access period of one minute.

The insect biotypes *A. craccivora* of Andhra Pradesh transmitted ULCD (Vijaykumar, 1993 and Suneela, 1996). The non-transmission of ULCD by thrips (*Frankliniella schultzei*) and whitefly (*B. tabaci*) was reported by Vijaykumar (1993). Patel *et al*. (1999) reported 40% of ULCD transmission through aphids (*A. gossypii*).

Karthikeyan (2002) reported that the ULCD was effectively transmitted by whitefly (*B. tabaci*), aphids (*A. craccivora*) and Epilachna beetle (*H. dodecastigma*) to the extent of 78, 33 and 25% respectively. The other vectors like thrips and leaf hoppers failed to transmit the ULCD.

Nageswararao (2002) stated that ULCV was transmitted through seed, sap and by aphid vector, *A. craccivora* in a non-persistant manner. However, the disease was not transmitted through grafting, whitefly (*B. tabaci*), melon aphid (*A. gossypii*) and galerucid beetle (*Madurasia obscurella*). Virus-vector relationship revealed that a single viruliferous adult of *A. craccivora*with an acquisition access period of two minutes and inoculation access period of 24 hours could transmit ULCD. However, maximum transmission (80%) was obtained by 10 adults per plant with an acquisition access period of two minutes, inoculation access period of 24 hours and pre-acquisition fasting period of one hour.

2.7.5 Pollen Transmission

ULCD is not pollen transmitted but had an adverse effect on pollen fertility. Thirty per cent of flower buds in an affected plant had 100 per cent sterile pollen and the remaining seventy per cent had 38 per cent sterile pollen (Kolte, 1971). Narayanasamy and Jaganathan (1975a) reported theULCD induced pollen sterility range between 13.64% - 72.09%. Sharma and Dubey (1983) noticed that changes in the ULCD infected *urdbean* plants such as degeneration of androecium and gynoecium, incomplete development of pollen grain in pollen tetrads, non-formation of ovule and abnormal ovarian cavity.

2.8 EFFECT OF MICRONUTRIENTS ON ULCD INFECTION

The ability of the plant to express its induced resistance to a particular disease is affected by mineral nutrition (Huber and Graham, 1999). Therefore, it has been reported that, nutrient elements either single or combined application of boron and molybdenum had significant effect in reducing viral diseases in *mungbean* (Ahmad *et al*.,1987).

Murthy (1996) reported that pre-inoculation spraying of micronutrients like zinc sulphate, ferrous sulphate, magnesium sulphate, manganese sulphate and calcium sulphate showed no effect on ULCD infection.

Lokeshbabu (1997) reported that kinetin and manganese sulphate were found to be highly effective in reducing the ULCD infection with 35.66 and 38.43% incidence respectively followed by sodium sulphate and potassium dichromate

treatments with per cent infection of 46.1 and 46.6% respectively as against 91.63% infection in unsprayed control. With respect to incubation period, maximum increase in incubation period was recorded in kinetin (13.33 days) and manganese sulphate (13.17 days) sprayed plants as against 8.40 days in unsprayed control. Other chemicals tested also increased the incubation period to an extent of 10.61 to 12.58 days.

Islam *et al*. (2002) studied the effect of nutrients on the incidence of yellow mosaic disease on *mungbean* and concluded that the disease incidence, severity and mosaic leaves were lowest in B + Zn + S applied plots.

Simoglou and Dordas(2006) observed that foliar application of boron for 2 years on winter durum wheattan spot disease caused by *Drechslera triticirepentis*significantly reduced the number of lesions per leaf at booting stage. There was no difference in the number of lesions per leaf between Mn and Zn treatments in the first year,But in second year Zn and Mn showed lowest number of lesions at the booting,heading stage and milk stage respectively.

Dordas (2008) studied effect of nutrients such as N, K, P, Mn, Zn, B, Cl and Si on disease resistance and tolerance.Mn can control many diseases as it plays important role in lignin biosynthesis and other function and Zn shows different effects as it effect the pathogen directly through toxic effect.

Bobade *et al*. (2009) reported that application of micronutrients *viz*., magnesium sulphate, manganese sulphate, lime, zinc sulphate and ferrous sulphate were effective in French bean against chlorotic mottle disease by soil application and spray application during both *kharif* and *rabi* seasons 2002-03 and also stated that application of micro nutrients such as ferrous sulphate, magnesium sulphate, lime, and zinc sulphate on french bean (*Phaseolus vulgaris L.)* cultivar ArkaKomal and Contender was found more effective in *rabi* and *kharif* seasons against chlorotic mottle (*Geminivirus*).

Basavaraj (2012) observed significance in the disease and showed the effect on MYMV using the salicylic acid (SA@3mM), aliphatic alcohol, tricontanol (tria@10ppm) and mineral nutrients like potassium as potassium nitrate (KNO_3@ 0.5%),magnesium sulphate ($MgSO_4$ @ 0.5%) and manganese sulphate ($MnSO_4$@

0.2%) either sprayed alone successively or alternated with each other at weekly interval, during *kharif* and *rabi*, 2011-12 in field and pot culture among all the micronutrients $MgSO_4$ was observed to be effective.

Farooq *et al*. (2012) stated that application of micronutrients as seed treatment performed better or similar to other application method but as seed treatment is easy and cost effective method for poor farmers.

Irshad *et al*. (2012) reported significant reduction of *Cotton leaf curl virus* incidence under field conditions by spraying with K_2SO_4 @ 0.1 kg/ha + $MgSO_4$ @ 0.7 kg/ha + Borax @ 0.5 kg/ha + $ZnSO_4$ @ 0.5 kg/ha at 60 and 90 DAS, during *kharif* 2010.

Gooding (1975) reported that 1% trisodium orthophosphate for 15 min followed by sodium hypochlorite 0.525% for 30 min treated TMV infected tomato seeds was proved to be more effective in inactivation of the virus than either chemical alone.

Zeshan *et al*. (2012) reported that NPK (classic) was more effective against ULCV and reduced the disease severity up to 65%. Zn + B was found second more effective treatment and reduced the disease severity up to 62.57%. Naphthalene acetic acid (NAA) reduced the disease severity to 60.33% followed by the urea that reduced the disease severity to 58.57%, whereas in control, maximum viral infection was observed (48.93%).

Tengoua *et al*. (2014) reported that micronutrients such as copper, boron and manganese when applied as a soil treatment to basal stem rot of oil palm (*Ganodermaboninense*) can reduce the disease incidence and severity when applied as a single nutrient rather than as combinations.

Bilqees*et al*. (2016) observed that the application of 1% silicon, 1.5% silicon, 2% multi-nutrients, and 2% garlic extract, multi-nutrients and garlic extract gave the best result by minimizing the Okra yellow vein mosaic disease incidence up to 7.42% and 9.05% respectively.

2.9BIOCHEMICAL ANALYSIS

2.9.1 Total Proteins

Decreased protein content was seen in both young and old BLCV infected plants (Brar and Rataul, 1990).

Ashfaq (2010) reported that ULCD infection resulted in significant increases in total soluble protein content in the leaves of susceptible (Mash-88) and resistant (CM-2002) varieties of *urdbean.*

Srivastava and Singh (2010) stated that effect of ULCD infection on protein content in the T-9 and IUP 94-1(Uttara) varieties of *urdbean* that there is a increase in protein content after 10, 20, 30 days of virus inoculation.

Basavaraj (2012) reported increased protein content in infected plants compared to healthy plants and the highest protein content was observed in plants treated with $MgSO_4$ diseased plant when compared with healthy plant of $MgSO_4$ in *Urdbean*.

2.9.2 Total Phenols

Karthikeyan *et al*. (2009)reported atwo-fold increase in accumulation of total phenol in ULCD plants of *urdbean* which are pre-inoculated with salicylic acid and benzothiadiazole.

Basavaraj (2012) reported that the highest phenol content in diseased plant of *Mungbean yellow mosaic virus* in *urdbean* plants treated with KNO_3, $MgSO_4$, $MnSO_4$, more than unsprayed healthy plants and the lowest phenol content was observed in unsprayed diseased plants.

Ashfaq *et al*. (2014) reported that ULCD infection lead to significant increase in total soluble phenols in susceptible *urdbean* genotype (Mash-88) when compared to resistant one (CM-2002).

2.9.3 Indole-3-Acetic Acid (IAA)

Cauliflower mosaic virus and Tomato mosaic virus infection in tomato causes tomato shoestring mosaic diseases due to increase of auxin level (Andrade *et al.,* 1981; Pratap *et al.,*2012).

The pathogen infections have found to cause upregulation of the expression of genes which are encoding the enzymes involved in auxin biosynthesis as well as of auxin signalling. This finally results in the disruption of AUX/ IAA repressor proteins and enhanced plant susceptibility by increased auxin responses in *Arabidopsis*. (O'Donnell *et al.*, 2003; Dharmasiri *et al.*, 2005; Thilmony *et al.*, 2006; Kazan and Lyons, 2014).

Kazan and Manners (2009) reported that some of the pathogens (*Pseudomonas solanaceraum*) have the ability to produce the auxins and in addition to the auxins initially produced by tomato plant.

Chapter – III

Material and Methods

Chapter III

MATERIAL AND METHODS

The present investigation on "Studies on *urdbean* leaf crinkle disease on urdbean [*Vigna mungo* (L.) Hepper]"was carried out at the College Farm, Agricultural College, Bapatla, Guntur district, Andhra Pradesh. Pot culture experiments were conducted at Department of Plant Pathology, Agricultural College, Bapatla. The field experiments were carried out during *kharif* 2018. The materials used and methods followed are described in this chapter.

3.1 MODE OF TRANSMISSIONS

Mechanical sap transmission, seed transmission and vector transmission by using aphids and beetles were carried out on potted plants kept under insect proof cages in greenhouse of the Department of Plant Pathology, Agriculture College, Bapatla during *rabi* 2018-19.

3.1.1 Mechanical Transmission of ULCD

Urdbean (*Vigna mungo*) belongs to the family Leguminosae. The cultivar LBG 752 used in the present investigation and this cultivar is popularly grown, which showed high incidence of ULCD in field condition and could take the virus successfully during pilot trails of mechanical inoculation.

3.1.1.1 Raising of seedlings

Earthen pots of 10" dia accommodating eight kg of black soil were used to raise seedlings of cultivar LBG 752 maintained in insect proof cages under greenhouse conditions.

3.1.1.2 Collection of ULCD infected material

Based on the visual symptoms given by Nene (1972), plants suspected to be infected with ULCD were collected from the College Farm, Bapatla. Samples were enclosed in polyethylene bags and were carried to the laboratory by placing them in ice packs in a thermocol.

3.1.1.3 Preparation of potassium phosphate buffer

Potassium phosphate buffer at 0.05 M and pH 7.0 was prepared in the laboratory with the following reagents for extraction of virus from the infected samples.

Dipotassium hydrogen phosphate (K_2HPO_4)	: 5.4 g
Potassium dihydrogen phosphate (KH_2PO_4)	: 2.4 g
Sodium thiosulphate ($Na_2S_2O_3$)	: 1.26 g
Distilled water	: 1000 ml

3.1.1.4Preparation of inoculum

For mechanical inoculation, young leaves were collected showing typical symptoms of ULCD and washed properly in running tap water until the dirt is removed and the leaves were blot dried. The infected leaves were uniformly ground in a pre chilled, clean and sterilised mortar and pestle with 0.05 M potassium phosphate buffer with pH 7.0 (1 g of leaf sample / 9 ml of potassium phosphate buffer). The ground leaf tissue was strained through a muslin cloth and the extract was used as viral extract for mechanical transmission.

3.1.1.5 Method of inoculation

Well established, actively growing young seedlings of *urdbean* plants were raised in insect proof cages in greenhouse for virus inoculation. Inoculations were done on fully expanded two leaf stage healthy *urdbean* (LBG-752) seedlings. A small quantity of celite was dusted on test plants. The inoculum was then applied on the upper surface of primary leaves with the help of a sterilised cotton swab dipped in the inoculum from base to tip. The inoculated leaves were washed immediately with a fine jet of distilled water to remove excess inoculum and superfluous material. The plants were labelled and maintained in insect proof cages for observations. The un-inoculated plants of test genotype, also maintained in cages, were kept as control.

3.1.1.6Collectionof experimental data

The numbers of plants expressing ULCD symptoms were counted and per cent disease incidence was calculated. The type of symptoms produced and the time taken for symptom expression on the host plants were recorded.

Plate 3.1 Healthy seeds and ULCD infected seeds of *Urdbean* (LBG 752).

3.1.2Seed Transmission of ULCD

Seed treatment studies were carried out under greenhouse conditions during *rabi* 2018-19. Seeds were collected from plants showing clear symptoms of ULCD (Plate 3.1) and they were tested for seed transmission by sowing them in pots enclosed in insect proof cages. One set of 20 ULCD infected seeds were treated with trisodium orthophosphate (16%). Untreated seeds were maintained as pathogen control. Seeds from disease free plant were maintained as absolute control. Four replications were maintained for each treatment and per cent seed transmission of ULCD was recorded at 20 DAS and 27 DAS.

3.1.3 Vector Transmission of ULCD

Suspected insect vectors *viz.,* aphids (*Aphis craccivora*) and beetles (*Henosepilachna dodecastigma)*collected from the field were tested for ULCD transmission with different acquisition access periods and inoculation access periods in insect proof cages under greenhouse conditions.

3.1.3.1 Aphid transmission

3.1.3.1.1 Determination of acquisition access period for the transmission of ULCD by *A. craccivora*

Aphids were collected from the field (Table 3.1) and they were reared on healthy *urdbean* (LBG-752) in insect proof cages (Plate 3.2). Non-viruliferous adults of*A. craccivora*were collected from the insect culture with the help of a camel hair brush very smoothly without disturbing their stylet and were given a fasting period of about 30 minutes on a moist blotting paper in a Petri-dish. A diseased plant with succulent leaves showing prominent ULCD symptoms was collected and placed in a water tub containing glucose as aphids has a tendency to prefer plants growing in solution. Then aphids were allowed to probe on infected plant with an acquisition access period of 10, 20, 30, 60, 360, 720 minutes. Then, these aphids were released at ten per plant on to healthy *urdbean* seedlings (seven days old) raised in glasshouse and plants were caged. All the aphids were then allowed to probe with an inoculation access period of 24 hours after which aphids were killed by spraying dimethoate (0.2%. Plants were kept under observation till the symptom expression. A batch of ten plants was also inoculated with non-viruliferous insects to maintain as check. Per cent transmission of ULCDwas recorded. The best acquisition access period was used to test inoculation access period.

Table 3.1. Key representation from order to family of *Aphis craccivora* (Aphididae:Hemiptera) (Triplehorn and Johnson, 2005)

S. No.	Keys of *Aphis craccivora*	Characteristic feature
1	1	Beak arising from back of the head or apparently from between front wings of uniform texture throughout, held roof like over abdomen, tips not or but slightly overlapping.
2	2'	Antenna usually filliform, with evident segmentation, beak when present arising between front coxi, tarsi, often not active insect(sub order sternorrhyncha).
3	81(2')	Tarsi 2 segmented with 2 claws. Winged forms with 4 wings, mouthparts usually well developed in both sexes with beak long.
4	82'	Antenna with 3-7 segments, wings membranous or opaque whitish not jumping insects.
5	83'	Wings membranous and not covered with whitish powder; high wings. Cornicles often present (super family Aphidoidea)
6	84(83')	Front wings with 4 or 5 (rarely 6) vein behind stigma extending to cornicles usually present; antenna generally 6-segmented sexual female oviparous, parthenogenetic, female viviparous

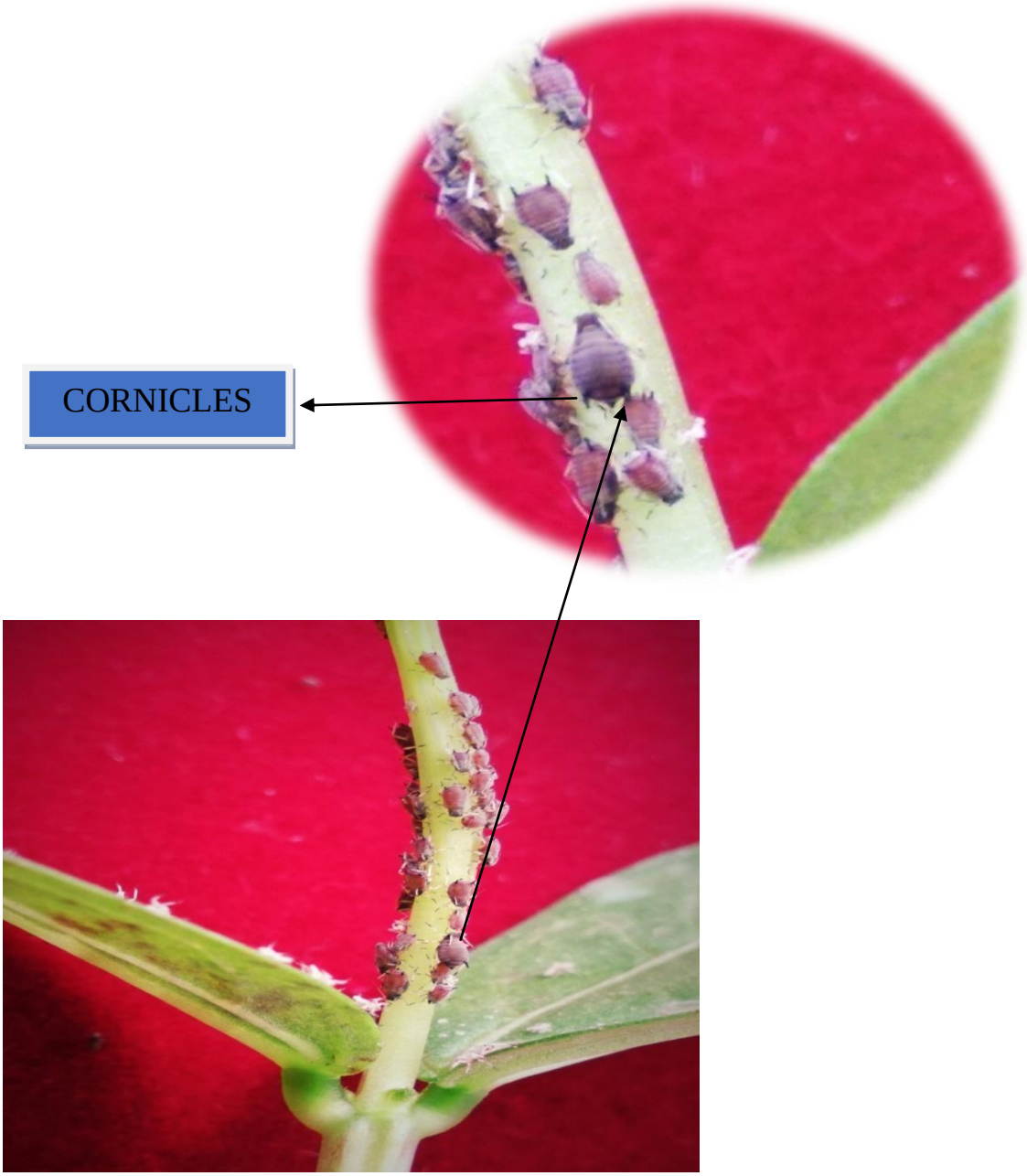


Plate 3.2.Aphid's culture (*Aphis craccivora*) maintained on *Urdbean*.

3.1.3.1.2 Determination of inoculation access period for the transmission of ULCDby *A. craccivora*

After 30 min of pre acquisition fasting and 10 min of acquisition access period, the viruliferous aphids were collected and allowed to feed on seven day old *urdbean* seedlings with different inoculation access periods of 6, 12 and 24 hours using ten aphids per plant. After the required period of inoculation, the plants were sprayed with dimethoate (0.2%) to kill aphids. The test plants were kept for observation in insect proof cages for symptom expression. A batch of plants inoculated with non-viruliferous insects served as check. Per cent transmission of ULCDwas recorded. The best ULCD inoculation access period was used to test number of aphids required for successful transmission.

3.1.3.1.3 Determination of number of insects for the transmission of ULCDby *A. craccivora*

Different number of aphids *viz*., 10, 20 and 30 per plant were allowed to feed on young *urdbean* seedlings with acquisition access period and inoculation access period of 24 hours, respectively after pre-acquisition fasting period of 30 minutes. After 24 hours of inoculation, the plants were sprayed with 0.2 per cent dimethoate to kill the aphids. The test plants were kept under observation in insect proof cages for symptom expression. Per cent transmission of ULCD was recorded.

3.1.3.1.3 Determination of pre acquisition access period for the transmission of ULCDby *A. craccivora*

Non-viruliferous aphids collected from the culture maintained in the greenhouse were given a pre-acquisition fasting period of 10, 20, 30, 60, 120 and 180 min and allowed to feed on ULCD infected *urdbean* plants, with acquisition access period of 10 minutes at the rate of 20 insects per plant. After 10 min they were transferred to healthy *urdbean* seedlings and allowed to feed for 24 hours. After 24 hours of inoculation, the plants were sprayed with 0.2 per cent dimethoate to kill the aphids. The test plants were kept for observation in insect proof cages for symptom expression. A batch of plants was inoculated with non-viruliferous insects to maintain as check. Per cent transmission of ULCD was recorded.

3.1.3.2 Beetle transmission

3.1.3.2.1 Collection and rearing of beetles (*Henosepilachna dodecastigma*)

Beetles (10) were collected from the surrounding brinjal plants (Table 3.2)(maintainer plant) were used for transmission studies (Plate 3.3).

Eggs laid by the beetles were kept in the incubator at 27^0 C for 3-4 days. Grubs emerged from the eggs were allowed to feed on *urdbean* leaves for 2 days, few of them were collected gently by using the camel hair brush without disturbing any of its mouth parts with gentle touch. As they started to move and grub stages were kept for further metamorphosis to obtain the adults and these were collected by using a small plastic container and used for ULCD transmission.

3.1.3.2.3 Determination of acquisition period for the transmission of ULCD by *Henosepilachna dodecastigma*

Adult beetles were given the acquisition feeding period of 30min, 6 and 24 hrs on ULCD infected plant with ten numbers of beetles under each AAP. Subsequently, they were released (one adult per test plant) on to healthy test plant (LBG 752) of 10 days old for common IAP of 24 hrs. Post IAP, beetles were killed by spraying 0.2 % triazophos. Caged test plants were observed for the symptom expression.

3.1.3.1.2. Determination of inoculation access period for the transmission of ULCDby *Henosepilachna dodecastigma*

30 adult beetles were given a common acquisition feeding period of six hrs. and were released (one adult per test plant) on to the ten day old test plants (LBG 752) for IAP of 6, 12 and 24 hrs. 10 test plants were used for each IAP. Beetles were killed post IAP by spraying with 0.2 % triazophos.

Table 3.2 Key representation from order to family of *Henosepilachna dodecastigma* (Coccinilidae: Coleoptera)(Triplehorn and Johnson, 2005).

S. No.	Keys of *Henosepilachna dodecastigma*	Characteristic feature
1	1	Elytra present, complete, short (or) reduced to flap like stubs on mesothorax.
2	2'	Notopleural suture absent.
3	12'	Antennae not lamellate or coxa, tibia or tarsi not as in preceding entry
4	24(12')	Hind tarsus with 2-5 tarsomeres, but never pseudotarsomeres (third of 5 tarsomeres on hind legs are not lobed beneath and enclosing small fourth, any other configuration possible).
5	25'	Palps longer, flexible and usually evident. Head not usually prolonged into a beak. But if rostrate or antenna elobowed and club compact, than palps longer and flexible.
6	38'	Length variable, antenna not as in preceding entry, wings rarely with fringe longer than width of the wings.
7	39'	Head without paired ocelli.
8	42'	Elytra longer, leaving no more than one or two abdominal tergite exposed.
9	64'	Antenna usually lacking periaurticular gutters on antennal clubs. Other characters variable. If complete periaurticular gutters than four trochantin hidden, antenna with 11 antennomere 8 not samaller than 7,9.
10	66(64')	Mid tarsus with 3 apparent tarsomeres either clearly with 3 tarsomeres (or) 2nd tarsomere strongly lobed, hiding small pen ultimate tarsomere
11	67(66)	Mid tarsus pseudotrimerous with 2nd tarsomeres strongly lobed, hiding small pen ultimate (3rd tarsomere)
12	68'	Four coxal cavity open. Head visiable from above infront of pronotum, size variable up to 11 mm.
13	69'	Frontoclypeal suture absent; 2 Basal ventrites connate, first ventrite with post coxal lines; pronotum lacking sublateral lines (family coccinelidae)

Hatching of beetles

3.3. a

Newly emerged grub of (*H. dodecastigma*)

Grubs feeding on the lower surface of brinjal

Adult beetle with 24 spots on*urdbean.*

Plate 3.3 Stages of *H. dodecastigma*on brinjal (3.3. a and 3.3. b) and adult feeding on LBG 752 (3.3. c)

3.2 EFFECT OF MICRONUTRIENTS ON INCIDENCE OF ULCD UNDER FIELD CONDITIONS

The effect of micronutrients on ULCV was evaluated under field condition at Agriculture College Farm, Bapatla during *kharif* 2018.

Date of sowing: *kharif* 01-09-2018

Design: Randomized Block Design

Plot size: 6 x 4m

Spacing: 30 x 10cm

Replications: three

Variety: LBG 752

3.2.1Methodology

Ten day old seedlings of *urdbean* (LBG 752) were inoculated by spraying the ULCD infected leaves extract mixed with ceilite during evening hours of the day, 24 hours later the following treatments were applied in field at 20, 27 and 34 DAS.

3.2.2 Treatment Details

T1: Manganese sulphate @ 0.2 %
T2: Magnesium sulphate @ 0.2%
T3: Zinc sulphate @ 0.2 %
T4: Manganese sulphate @ 0.2 %+Magnesium sulphate @ 0.2%
T5: Manganese sulphate @ 0.2 %+Zinc sulphate @ 0.2 %
T6: Magnesium sulphate @ 0.2%+Zinc sulphate @ 0.2 %
T7: Seed treatment with trisodium orthophosphate @ 16%
T8: Dimethoate @ 0.2 % spray
T9: Control

3.2.3 Land Preparation

The land was prepared to a fine tilth and levelled using cultivator and rotovator.

3.2.4 Fertilizer Application

Nitrogen @ 20 kg ha^{-1} and phosphorus @ 50 kg ha^{-1} were applied as basal dose before sowing.

3.2.5 Weed Management

Pendimethalin @ 2 l ac^{-1} was sprayed as pre-emergence application.

3.2.6 After Care

Weeding was carried out regularly, irrigation was given at 15 and 45 DAS.

3.2.7 Harvesting

The crop was harvested at physiological maturity in all plots by uprooting of the plants. The plants were dried in the sun and later threshed for separation of seeds from the pods.

3.2.8 Collection of Experimental Data

3.2.8.1ULCD incidence

ULCD incidence was scored at seven days intervals from the date of spraying by counting the total number plants infected in each treatment and per cent disease incidence was calculated by following formula.

$$\text{Per cent Disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

3.3STATISTICAL ANALYSIS

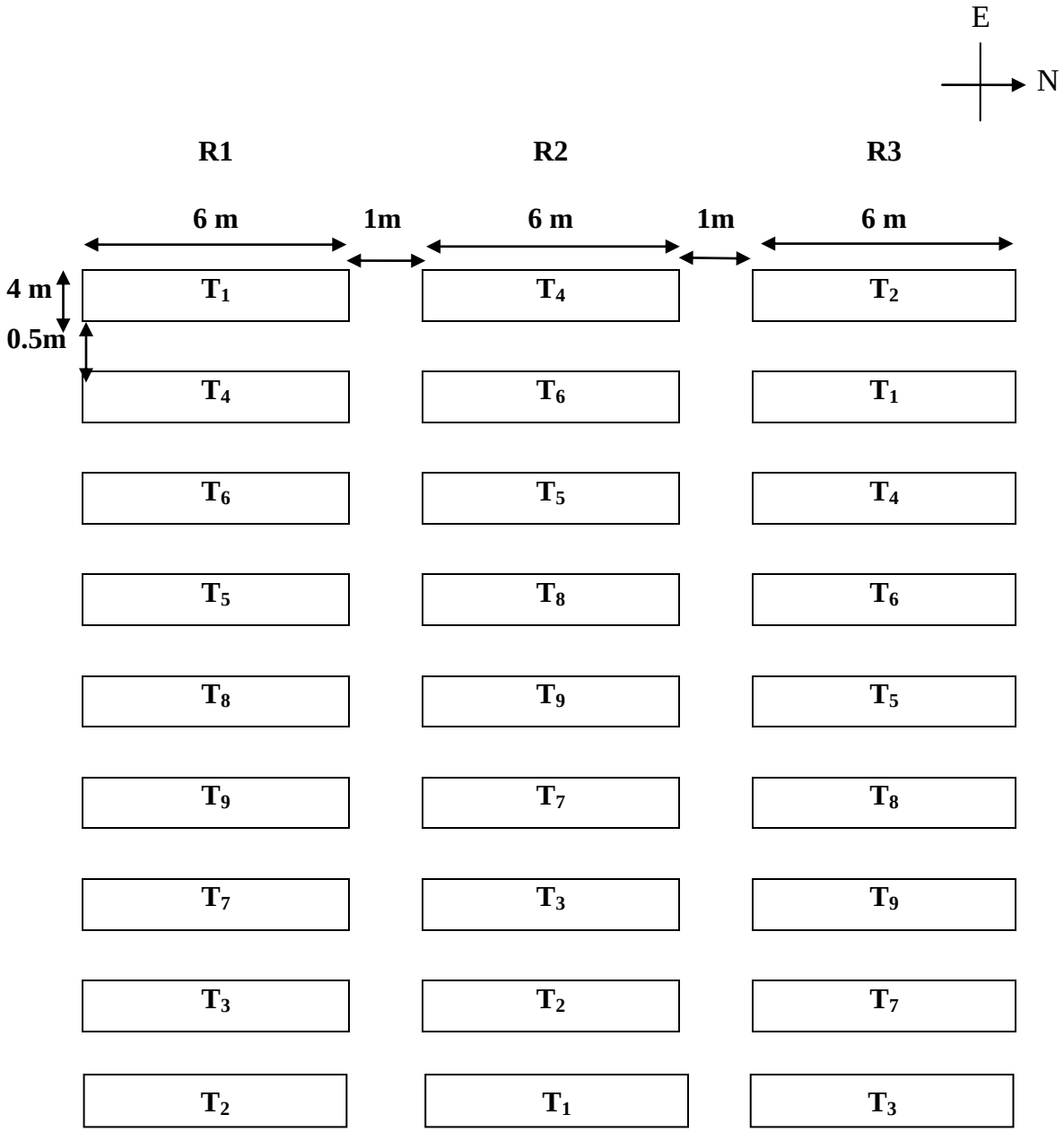
The data obtained from all the experiments was statistically analyzed following the standard procedures (Gomez and Gomez, 1984).

1a. Crinkled leaves with shortened petioles **1b. Reduction in intermodal length**

1c. Puckered leaves, **1d. Elongated leaves with malformed inflorescence**

1e. Poor pod formation and malformed pods

Plate 3.4. Observed field symptoms of ULCD



T_1: Manganese sulphate @ 0.2 %

T_2: Magnesium sulphate @ 0.2%

T_3: Zinc sulphate @ 0.2 %

T_4: Manganese sulphate @ 0.2 % + Magnesium sulphate @ 0.2%

T_5: Manganese sulphate @ 0.2 %+ Zinc sulphate @ 0.2 %

T_6: Magnesium sulphate @ 0.2%+ Zinc sulphate @ 0.2 %

T_7: Seed treatment with trisodium orthophosphate@ 16%

T_8: Dimethoate @ 0.2 % T_9: control

Fig.3.1. Layout of field trial on management of *urdbean* leaf crinkle disease of *urdbean* with above treatments.

3.4 BIOCHEMICAL ANALYSIS

Biochemical analysis of total phenols, total proteins was done in variety LBG 752 at 15 days period of interval *i.e.,* 40 DAS, 55DAS, 70DAS along with testing the presence of auxins in healthy and infected samples.

3.4.1 Estimation of Total Proteins

Total protein estimation was carried out according toLowry *et al.* (1951).

3.4.1.1 Reagents

1. 2.0% sodium carbonate in 0.1 N NaOH (Reagent A).
2. 0.5% copper sulphate in 1% potassium sodium tartrate (Reagent B).
3. Alkaline copper sulphate solution: 50 ml of Reagent A and 1 ml of Reagent B were mixed prior to use (Reagent C).
4. Folin- Ciocalteau reagent (Reagent D).
5. Protein solution (stock standard): Bovine serum albumin 50 mg was dissolved in distilled water to make up the volume to 50 ml in standard flask.

10 ml of the stock solution was diluted to 50 ml with distilled water in a standard flask so that one ml of this solution contains 200 µg of protein.

6. Extraction was carried out with 0.1 M phosphate buffer.

3.4.1.2 Extraction procedure

1) 500 mgofhealthy or diseased *urdbean* leaves was taken. Ground well using pestle and mortar in 5-10 ml of buffer, centrifuged and the supernatant was used for protein estimation.

3.4.1.3 Procedure for estimation

1) 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standards weretaken using micropipette into a series of test tubes.
2) 0.2 ml of the sample extract was taken using pipette in to other test tube.

3) Then the volume was made up to 1 ml in all the test tubes and a tube with 1 ml of distilled water served as blank.

4) Five ml of Reagent C was added to each test tube including the blank, mixed well and was allowed to stand for 10 min.

5) Then, 0.5 ml of Reagent D was added.

6) After thorough mixing, reaction mixture was incubated at room temperature in dark for 30 min.

7) After the blue colour was developed the absorbance values were taken at 660 nm.

8) Standard graph was drawn and the amount of protein in the sample was calculated and expressed as mg per g of sample.

3.4.2 Estimation of Total Phenols

Total phenols were estimated by Folin-Ciocalteau Reagent method (Malick and Singh, 1980).

Reagents

1. 80% ethanol
2. Folin-Ciocalteau Reagent
3. 20% Na_2CO_3
4. Standard:

   Stock Solution : 100 mg of Catechol was dissolved in 100 ml water

   Working standard: 10 ml of stock diluted to 100 ml with water.

Procedure for estimation

1. 0.5 g of infected or healthy leaf samples were taken and was ground with pestle and mortar with10 ml of 80% ethanol.
2. This homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was collected in a test tube. The residue was re-extracted with 5 ml of 80% ethanol and centrifuged. The supernatants were pooled and then evaporated to dry

3. The residue was dissolved in a 5 ml of distilled water.
4. Different aliquots (0.2 to 2 ml) were pipetted into tubes
5. The volume in each tube was made up to 3 ml with distilled water.
6. 0.5 ml of Folin-Ciocalteau reagent was added to each tube and kept for three minutes and then 2 ml of 20% Na_2CO_3 was added.
7. The contents in the test tubes were mixed thoroughly, the tubes were placed in boiling water for exactly one min, cooled and absorbancewas measured at 650 nm against a reagent blank.
8. A standard curve was prepared using different concentrations of catechol using working standard.
9. Amount of total phenols present in the sample tube were calculated from the graph.

$$\text{Total phenols (µg)} = \frac{\text{Total volume of aliquot x Phenol value from graph}}{\text{Used aliquot}}$$

3.4.3 Estimation of Indole-3-acetic Acid

Auxins are the compounds with low molecular weight involveed in the control of plant growth and its development. Indole-3-aceticacid(IAA) is one of the most important hormone. Sample preparation for estimating IAA involved three steps *i.e* extraction, purification and detection.

3.4.3.1 Extration of auxins

The plants were collected from the field using the ice packets in a thermocol box and brought immediately and kept at low temperature and take 10 grams of infected and the healthy plant samples grind them (we have to take large sample as possible because the auxin content is very less) by using the organic solvent (e.g. methanol, ethanol, acetone, diethyletheranddimethylsulfoxide). The solvents 80% methnol was taken duringextraction.

3.4.3.2 Purification of auxins

This procedure is very crucial step in the extraction of the auxins. QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe). 10 ml of homogenized sample were weighed in a 50mL polypropylene centrifuge tube and10 ml of 1% v/v of acetic acid in acetonitrile were added.Thenthis mixture was shaken by hand for one min. After that, four g of anhydrous magnesium sulphate, one g of sodium chloride, 1 g of sodium citrate dehydrate and 0.5 g of disodium citratewere added and the tubes were shaken immediately for one min. The mixture was centrifuged at 5000 rpm for five min. About 0.1ml was taken for chromatographic analysis.

3.4.3.3 Detection of auxin

Thin layer chromato-graphictechniques have been used for analysis of auxin hormones.

3.4.3.3.1 Reagents

1. IAA standard solution: 10^{-3}
2. Aqueous ammonium hydroxide (NH_4OH)
3. Isopropanal
4. Salkowski reagent :
   Mix 2 ml of 0.05M $FeCl_3$ and 100 ml of 5% $HClO_4$

3.4.3.3.2 TLC plate preparation

Silica gel G, Glass plate, micropipette, micro oven (110^0C for 30 minutes), TLC pouring equipment.

Procedure

1. Isopropanol - ammonia – water (80:10:10) solvent was prepared.
2. TLC plate was taken, where silica gel G which is activated at 110^0C for 30 minutes.

3. The points in equi distant were marked without touching the plate.
4. Then the standard, infected and healthy samples were spotted on the plate.
5. This plate was kept in a glass jar containing solvent and this solvent was kept 30 min before placing the plate.
6. Care was taken to avoid contact between the spot and solvent while placing the plate.
7. The plates were then allowed to 6 hours so that the solvent have reached ¾ length of the silica.
8. Plates were then taken out of TLC chamber and allowed to dry.
9. Reagent was then sprayed on the plates using hand atomizer.
10. Plate was then observed for the development of color (pink) and R_f value were calculated.

    R_f= distance travelled by solute/ distance travelled by solvent

Chapter – IV

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

Results obtained in the "Studies on *Urdbean* leaf crinkle disease on *urdbean* [*Vigna mungo* (L.) Hepper]" are presented and discussed in this chapter.

4.1 DESCRIPTION OF SYMPTOMS

Symptoms were observed in the field at 20 DAS on the third trifoliate leaf which showed an increase in size and light green colour, crinkling became conspicuous at seven days after the occurrence of initial symptoms. Crowding of leaves became apparent due to reduction in the petiole length, subsequently puckering, crinkling, curling, increase in leaf area, stunted growth of plant and malformation inflorescence was observed. The observed field symptoms were in conformity with the symptoms described by Williams *et al.* (1968); Nene (1972); Kolte and Nene (1972); Brar and Rataul (1986); Ravander *et al.* (2006) and Janaki Ratnam (2015).

4.2ULCD TRANSMISSION STUDIES

4.2.1 Sap Transmission of ULCD

LBG 752 was used for the sap or mechanical transmission studies under green house conditions. Buffered sap prepared as mentioned in materials and methods was used for inoculating the seven day old glass house raised healthy seedling 30 numbers. Inoculated seedlings were observed daily for the symptoms expression. All the inoculated plants showed symptoms as described in 4.1. The first recognizable symptoms appeared between 13-17 days after inoculation with the first trifoliate leaves exhibiting mild crinkling and curling followed by expansion of leaves with consequent light green colour (Plate 4.1). Within a week after appearance of initial symptoms, typical crinkling of leaves with downward curling was observed. Subsequently produced leaves also exhibited crinkling symptoms indicating the systemic movement of virus. Out of 30 test plants two plants shows sap transmission (6.67%). The observed symptoms were in consonance with the field symptoms described by Nene (1972); on *urdbean*. Kolte and Nene (1972); Krishnaveni

Field infected ULCD leaves used for inoculation

Mechanical sap inoculation

Uninoculated (Control) leaves at 17 days after sowing

Plate 4.1. Inoculated leaves showing ULCD symptoms at 10 days after inoculation (17 Days after sowing)

Suneela (1996); Kartikeyan (2002) reports on the symptoms of *urdbean* inoculated with ULCD during their ULCD host range studies were also in conformity with the present investigation symptoms.

4.2.2 Effect of Seed Transmission of ULCD by Seed Treatment

The observations for potential seed transmission of ULCD were first recorded on 20 DAS. The highest ULCD incidence of 26.67% was observed in untreated control and significantly the lowest mean disease incidence (8.33%) was observed in hot water treatment *i.e.,* 50°C for 10 min. 16% trisodium orthophosphate seed treatment was found with 20% disease incidence. No disease was recorded in plants raised with seeds collected from disease free healthy plants (Table 4.1; Fig. 4.1).

At 27 DAS the highest ULCD incidence was observed in untreated control indicating that ULCD is transmitted as seed borne to an extent of 28.33% and the lowest was (10.00%) recorded in hot water treatment (50^0C for ten min) followed by trisodium orthophosphate seed treatment (20.00%). No disease incidence was observed in plants raised from disease free seeds (Table 4.1).

This indicated that out of 60 plants sown; only in 5 plants (17 in untreated and 12 in trisodium orthophosphate treated) virus is present as internally seed borne inoculum. Therefore it is clear that ULCD exists as externally seed borne inoculum to an extent of 8.3%. Further, hot water treatment at 50^0C for ten min (effective in denaturing internally seed borne virus) has effectively denatured virus only with 35.3 % of total infected seeds. The hot water treatment takes care of denaturing internally seed borne of ULCD (64.7%) of total infected seeds. So, the best treatment to get rid of internally seed borne virus is to treat the seeds with either wet or dry heat, which has the property of penetrating to the core of seed as observed by Smith (2011).

Heat treatment was normally used for elimination of viruses from plant materials and seed. The main lethal principle involved in heat treatment is attenuation or inactivation of virus by heating the material for specific duration at specific temperature. Among the treatments hot water treatment was found more effective than trisodium orthophosphate for controlling ULCD. Reduction in the incidence of ULCD by 70.60% was observed in present investigation when seeds were exposed to 50^0C for ten min than the trisodium orthophosphate (16%).

The ULCD was inactivated from the crude sap at 60^0C to 70^0C temperature for 10 min. and similar reports are given by Kolte, (1971); Narayanasamy and Jaganathan, (1975a). Beniwal *et al*. (1983b) observed that hot water treatment of the infected seeds at a temperature of 60^0C for 10, 20 and 30 min and 70^0C at the interval of 10 and 20 min to be effective in controlling ULCD.

Earlier workers reported complete abolition or maximum reduction of disease but in the present study the per cent disease reduction over control was only 70.60%. It reduced the incidence of the virus but could not eliminate it. This variation may be due to the difference in the strains of ULCD or the insufficient temperature or exposure period required for the complete elimination of disease.

The result of the present study indicated that the virus is internally seed-borne which was also reported by Narayanasamy and Jaganathan (1975a);Beniwal and Chaubey (1984); Karthikeyan (2002); Rao and Reddy (2005b).

Table 4.1. Determination of effect of seed transmission on incidence of ULCD in the urdbean

S. No.	Treatment	Total No. of plants tested	No. of plants infected		Per cent transmission(%)		Disease reduction over control
			20 DAS	27 DAS	20 DAS	27 DAS	
1	T1: Seed treatment with Na_3PO_4(16%)	60	12	12	20.00 (4.58)	20.00 (4.58)	29.40
2	T2: Hot water treatment (50 °C for 10 minutes)	60	5	6	8.33 (3.05)	10.00 (3.32)	70.60
3	T3: Untreated seed	60	16	17	26.67 (5.26)	28.33 (5.42)	-
4	T4: Seeds collected from disease free plants	60	0	0	0.00 (1.00)	0.00 (1.00)	-
SEM					0.06	0.05	
CD($P \leq 0.05$)					0.18	0.15	
CV(%)					8.54	5.39	

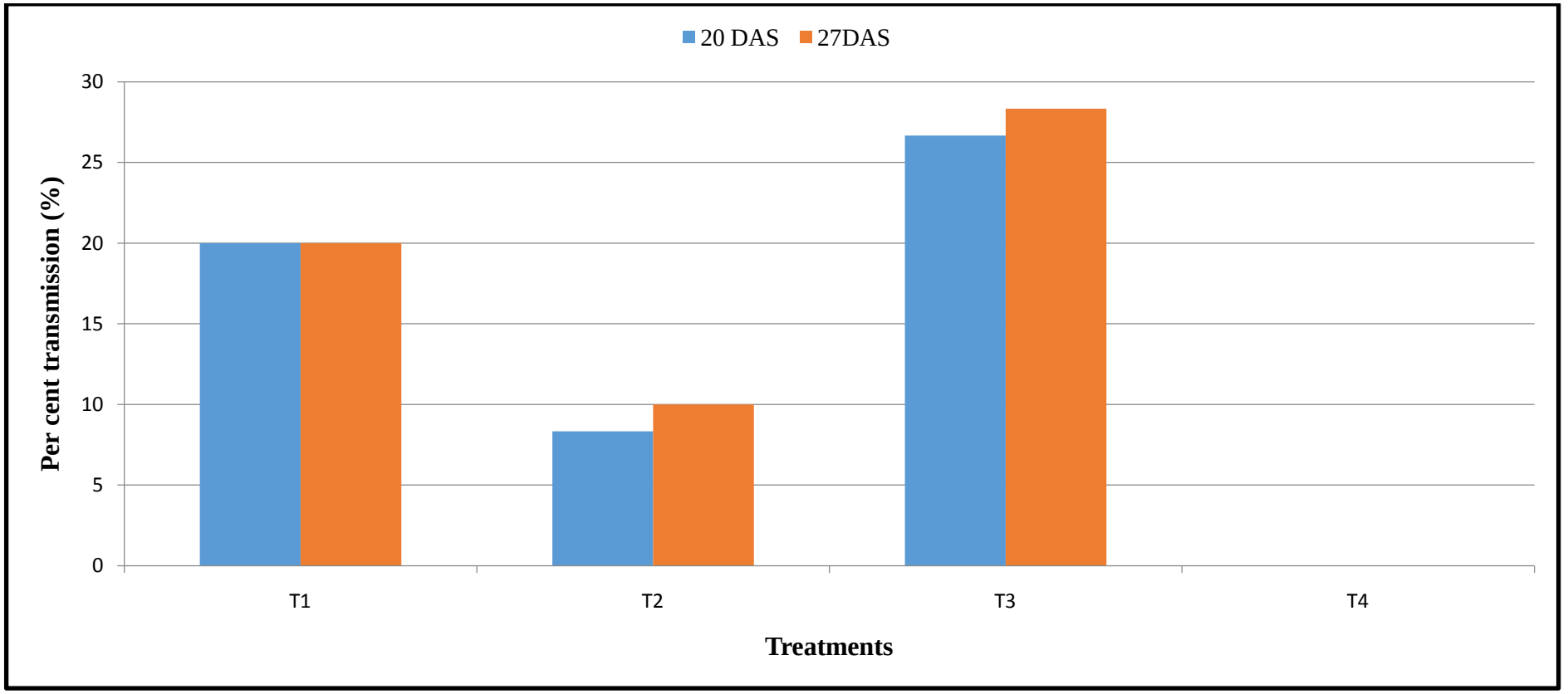


Fig. 4.1.Effect of seed treatment on incidence of ULCD.

T_1: Seed treatment with trisodium orthophosphate (Na_3PO_4)

T_2: Hot water treatment (50 °C for 10 minutes)

T_3: Untreated seed

T_4: Seeds from disease free plant

4.2.3 Vector Transmission studies of ULCD

Transmission studies of ULCD by means of aphid (*Aphis craccivora*) and beetles (*Henosepilachna dodecastigma*) were carried out to prove the efficiency of the insect in transmitting ULCD.

4.2.3.1 Aphid transmission of ULCD: Efficiency of Aphids to act as ULCD vector was found positive in successful transmission of the virus under the greenhouse conditions and the results are presented here in Tables 4.3, 4.4 and 4.5; Plate 4.2. Control with non viruliferous insects and pure ULCD infected plants were also maintained under greenhouse conditions.

4.2.3.1.1Studies on acquisition access period required for the transmission of ULCD: Pre-acquisition fasting period for 30 min was given to *Aphis craccivora* population that were collected from the culture plant. Batches of 10 aphids each were given an acquisition feeding period of 10,20,30,60, 360 and 720 min respectively on ULCD infected plants and aphids were allowed to feed on healthy test plants with inoculation access period of 24 hours. The test plants were kept under observation in an insect proof cage. Test plant started showing the symptoms between 15-20 days after acquisition feeding. Maximum per cent transmission of 10 per cent transmission was observed with acquisition access period of 10 min and minimum transmission of 3.33% was observed with acquisition access period of 20 min. With increase in acquisition access period over and above 10 min *i.e.,* with 20, 30 min, the success of transmission decreased (10.00, 3.33 and 0.00 per cent respectively). The aphids failed to transmit beyond 20 min of acquisition access period (Table 4.2, Fig. 4.2)

4.2.3.1.2Determination of inoculation access period required for the transmission of ULCD: Inoculation access period of 6, 12 and 24 hours were given to the viruliferous aphids which were subjected to the acquisition access period of 10 min. the test plants were kept under insect proof cages and were observed daily for the symptom expression. Symptoms started appering between 20-25 days after inoculation access. Maximum transmission was observed at 24 hrs of inoculation access period (20%) and the transmission was minimum with 12 h (6.67%)(Table 4.3; Fig. 4.3).

Plate 4.2 Crinkling and puckering symptoms produced by aphid transmission of ULCD on *Urdbean* (LBG 752).

Table 4.2. Determination of acquisition access period for the transmission of ULCD in the urdbean by *Aphis craccivora*

S. No.	Acquisition access period (min)	No. of plants inoculated	No. of plants expressed symptoms	Per cent transmission (%)
1	10	30	3	10.00
2	20	30	1	3.33
3	30	30	0	0.00
4	60	30	0	0.00
5	360	30	0	0.00
6	720	30	0	0.00
7	Control(aphids without virus)	30	0	0.00

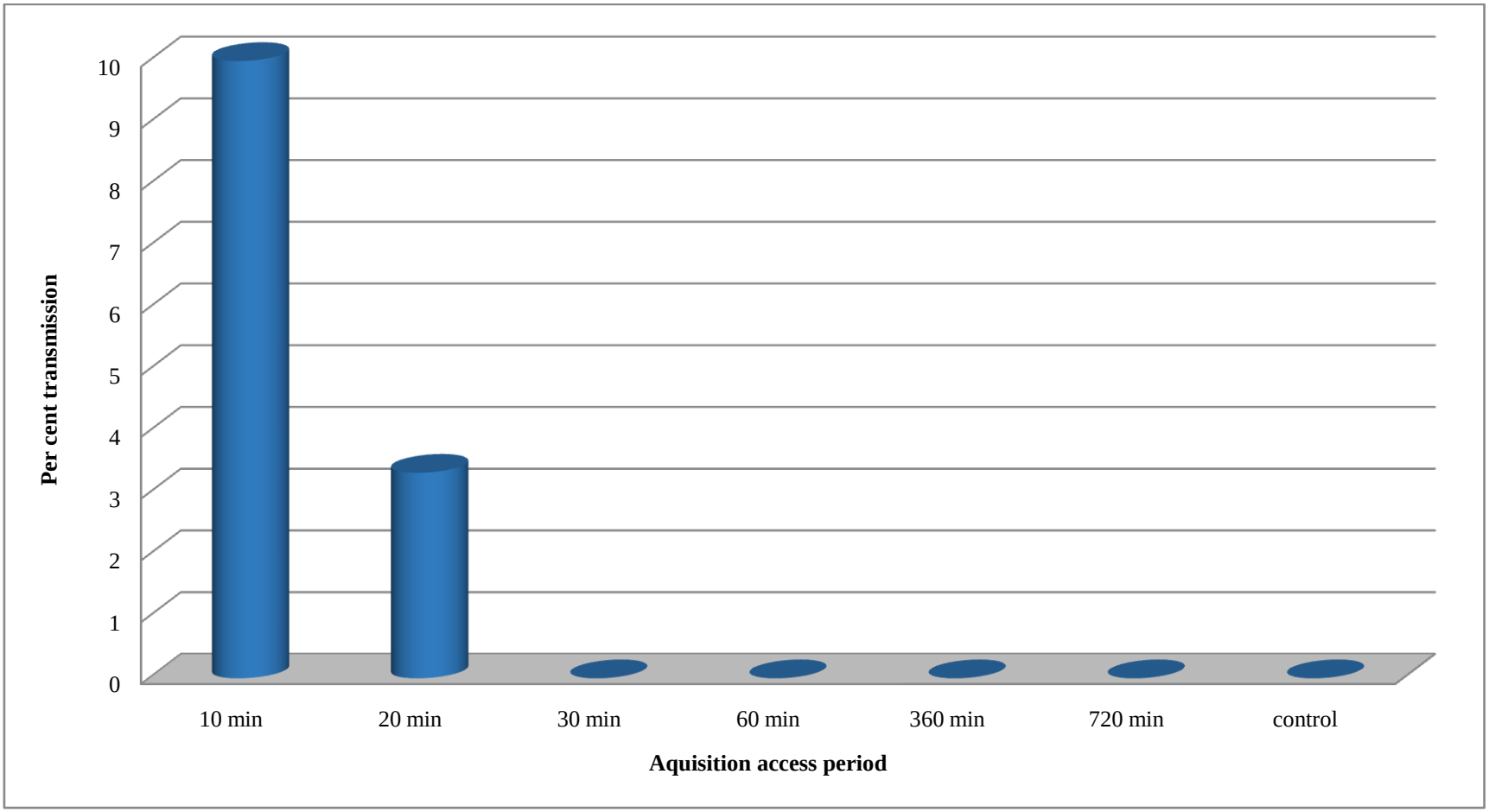


Fig. 4.2. Effect of varying acquisition access periods on the transmission of ULCD by *Aphis craccivora*

4.2.3.1.3Determination of minimum number of aphids required for the transmission of ULCD: Transmission studies were carried out by *A. craccivora* under greenhouse condition by using different number of insects *viz.,* 10, 20 and 30. Aphids under pre-acquisition fasting period of 30 min, acquisition access period of 10 min were released on to the test plant and given inoculation access period of 24 hrs. the test plants were kept in insect proof cages and were observed daily for the symptoms expression. Symptoms started appearing between 20-25 days after inoculation access. Maximum transmission was observed with 20 aphids (13.33%) and minimum with 10 aphids (6.67%)(Table 4.4; Fig. 4.4).

The present investigation has revealed that the ULCD can be successfully transmitted by *A. craccivora* with the pre-acquisition fasting period of 30 min, acquisition access period of 10 min, inoculation access period of 24 hours and with 20 aphid number. Therefore, it can be reiterated that the ULCD occurring on *urdbean* is transmitted by transmitted by aphids in a non-persistant mode.

4.2.3.1.4Determination of pre-acquisition access period: Transmission studies were conducted with pre-acquisition access periods of 10, 20, 30, 60, 120 and 180 min in a regime of acquisition access period of 10 minutes, inoculation access period of 24 hours and with 20 no. of aphids. The test plants were kept in insect proof cages and were observed daily for the symptoms expression. Symptoms started appearing between 20-25 days after inoculation access. The results have revealed that, pre-acquisition starvation was found to be essential for the efficient transmission of ULCD. However, when the starvation period was increased from 10 to 30 min, transmission increased and beyond this transmission decreased gradually. Maximum transmission was obtained when the vectors was allowed for a pre-acquisition fasting period of 20 (3.33%) and 30 min (10.00%). When the pre-acquisition fasting period was further increased to one, two and three hours, transmission per cent decreased (table 4.5). There was no transmission beyond 4 hrs of fasting before acquisition access period (Table 4.5; Fig. 4.5).

The present investigation has revealed that the ULCD can be successfully transmitted by *A. craccivora* with pre-acquisition fasting period of 30 min, acquisition access period of 10 min, inoculation access period of 24 hrs. Therefore, it can be reiterated that the ULCD occurring on *urdbean* is transmitted by aphids in a non-persistant mode.

Bindra (1971), Khatri *et al.* (1971), Dhingra (1976), Kadian (1980), Dhingra and Chenulu (1981), Dubey *et al*. (1983),Bhardwaj and Dubey (1986), Vijaykumar (1993), Subbarao (1984), Suneela (1996), Nageswararao (2002) and Bhavani (2016) have reported two min. acquisition period for successful transmission is in conformity with the present investigation acquisition period of 10 min. counted from the time of release. As a matter of fact, that, aphids tend to move on the infected material before settling down for feeding activity (insertion of stylet into the plant tissue). Therefore actual from the release of aphid. In this investigation, time period was counted from the insertion of stylet by the aphid. Varying acquisition periods which are well within the range of the present investigation. The marginal differential period of acquisition, inoculation and aphids number and pre-acquisition fasting period across the investigations may be ascribed to the differential stylet probing activity, salivary sheathing activities of the aphids which was well documented by Bhargava, (1951), Sylvester, (1954), Bradley, (1959), Nariani and Sastry, (1962) and Bhardwaj and Dubey, (1986) in case of non-persistantly transmitted viruses by aphids.

However, non-transmission of ULCD by *A. craccivora* as reported by Nene and Kolte (1972), Beniwal and Bharathan (1980), Singh (1982) and Brar and Rataul (1987) may ascribed to the strainal variation among the isolates used in the experiments.

Table 4.3. Determination of inoculation access period for the transmission of ULCD in the urdbean by *Aphis craccivora*

S. No.	Inoculation access period (h)	No. of plants inoculated	No. of plants expressed symptoms	Per cent transmission (%)
1	6	15	0	0.00
2	12	15	1	6.67
3	24	15	3	20.00
4	Control	15	0	0.00

Table 4.4. Determination of number of aphids required for the transmission of ULCD in the urdbean by *Aphis craccivora*

S.No.	No. of insects used for inoculation	No. of plants inoculated	No.of plants expressed symptoms	Per cent transmission(%)
1	10	15	1	6.67
2	20	15	2	13.33
3	30	15	2	13.33

Table 4.5 Determination of pre-acquisition access period for the transmission of ULCD in the urdbean by *Aphis craccivora.*

S.No.	Pre-acquisition fasting period	No. of plants inoculated	No.of plants expressed symptoms	Per cent transmission(%)
1	10 min	30	0	0.00
2	20 min	30	1	3.33
3	30 min	30	3	10.00
4	60 h	30	1	3.33
5	120 h	30	0	0.00
6	180 h	30	0	0.00
7	Control(aphids without virus)	30	0	0.00

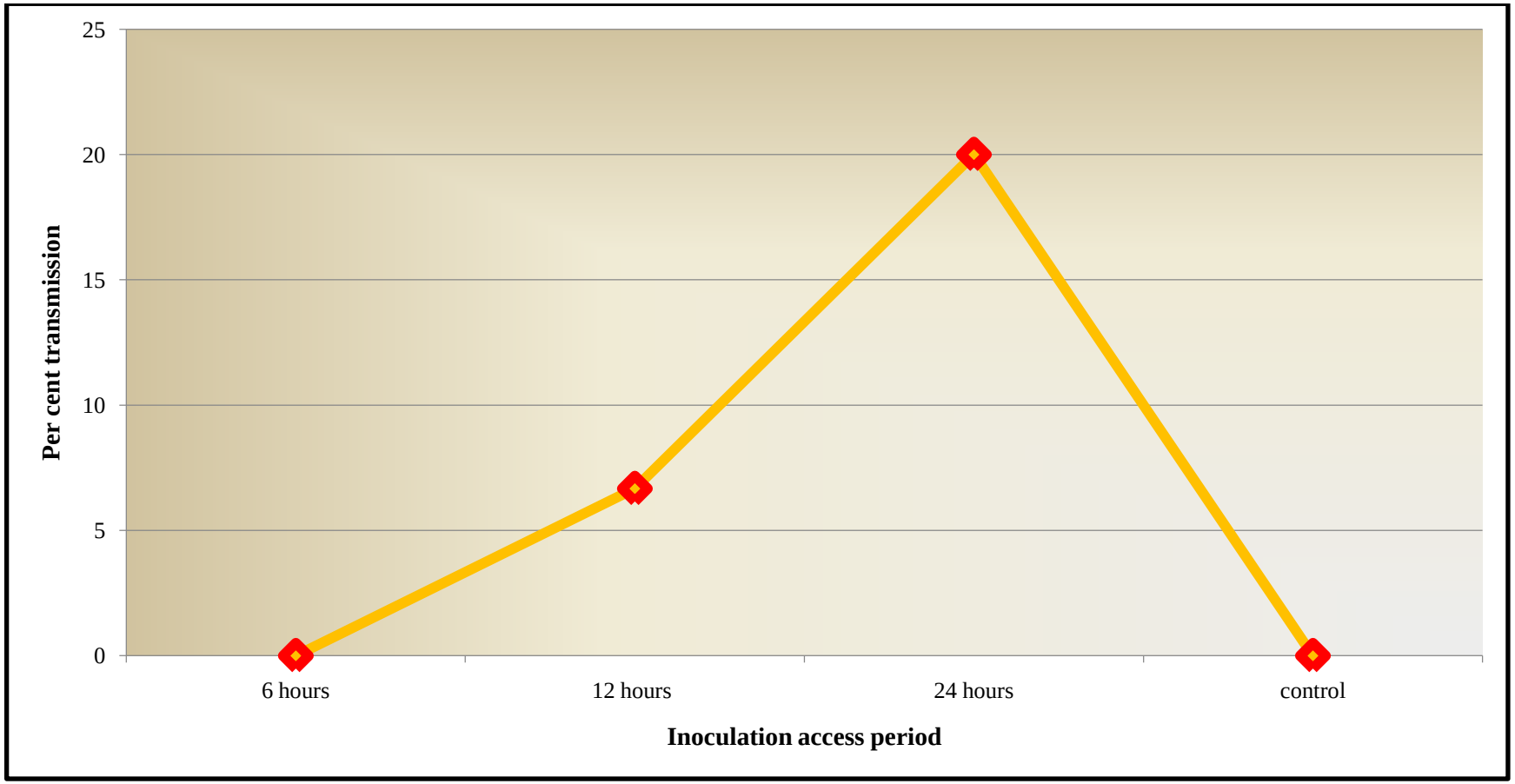


Fig. 4.3. Effect of varying inoculation access period on the transmission of ULCD by *Aphis craccivora*

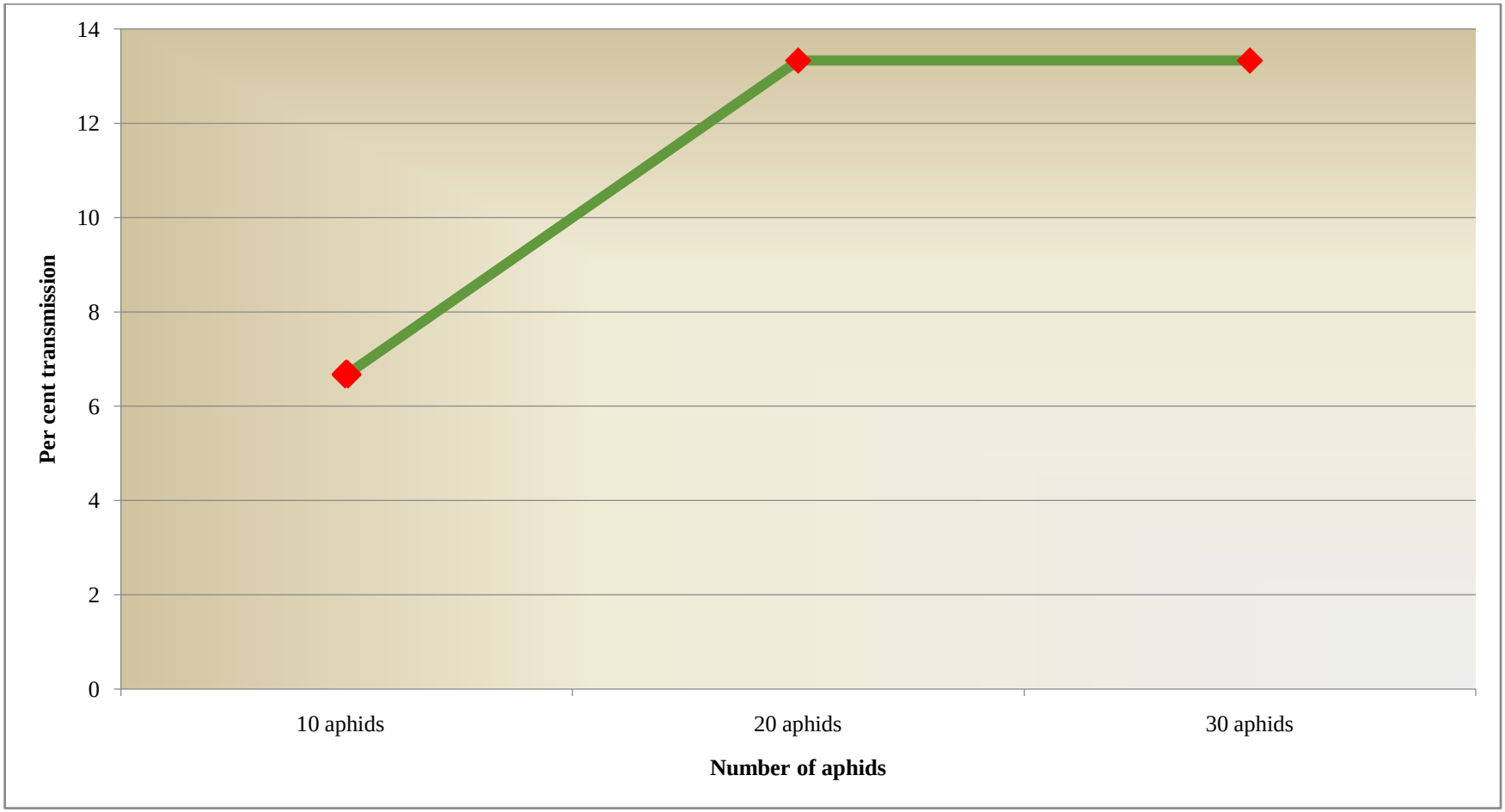


Fig. 4.4.Effect of number of aphids on the transmission of ULCD by *Aphis craccivora.*

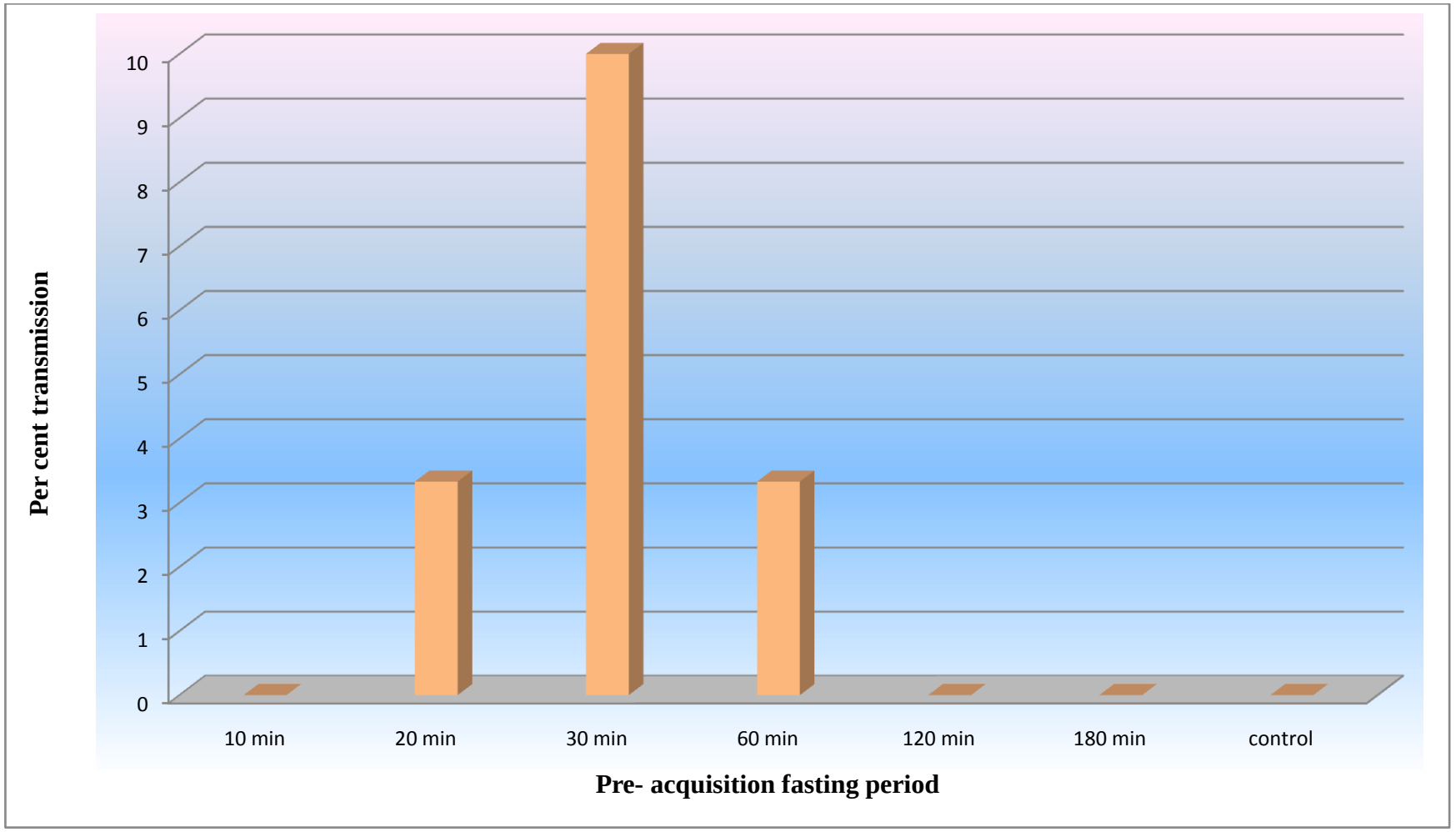


Fig. 4.5.Determination of pre-acquisition access period for the transmission of ULCD in the urdbean by *Aphis craccivora.*

4.2.3.2 Beetle (*H. dodecastigma*) transmission of ULCD under different AAP's and IAP's : Out of 30 plants tested, only one plant which was fed with beetle under AAP of 6 hrs and IAP of 24 hrs. exhibited the ULCD symptoms on 15th day after inoculation (3.33 %).

Only one out of 15 plants tested under AAP of 6 hrs and IAP of 24 hrs. exhibited the ULCD symptoms on 20th and 25th day after inoculation (6.67%) (Table 4.6; Fig. 4.6).

Beetles (*Henosepilachna dodecastigma)* could successfully transmit *urdbean* ULCD with AAP of 6 hrs and IAP of 12 hrs. with transmission per cent ranging between 3.33 and 6.67 as per the present investigation (Table 4.7; Fig. 4.7).

Similar results were published by Beniwal and Bharathan (1980) showed that mungbean ULCD is tranamitted by *H. dodecastigma.* with 24 and 48 hours of acquisition feeding period and inoculation access period of 24 hrs on 10 day old plants (Plate 4.3).

However Brar and Rajaul (1986) could not establish the transmission of ULCD in field and laboratory by *H. dodecastigma*.

Beetle :-
(*Henosepilachna dodecastigma*)
Beetle used for ULCD transmission .

Leaf showing ULCD symptoms due to beetle transmission

Plate 4.3. Beetle (*H. dodecastigma*) transmission of ULCV in *urdbean* (LBG 752).

Table 4.6. Determination of acquisition access period for the transmission of ULCD in the urdbean by beetle (*Henosepilachna dodecastigma*)

S.No.	Acquisition access period	No. of plants inoculated	No.of plants expressed symptoms	Per cent transmission(%)
1	30 min	30	0	0.00
2	6 hours	30	1	3.33
3	24 hours	30	0	0.00
4	Control(aphids without virus)	30	0	0.00

Table 4.7. Determination of inoculation access period for the transmission of ULCD in the urdbean by beetle (*Henosepilachna dodecastigma*).

S.No.	Inoculation access period	No. of plants inoculated	No.of plants expressed symptoms	Per cent transmission(%)
1	6 hours	15	0	0.00
1	12 hours	15	1	6.67
2	24 hours	15	0	0.00
3	Control	15	0	0.00

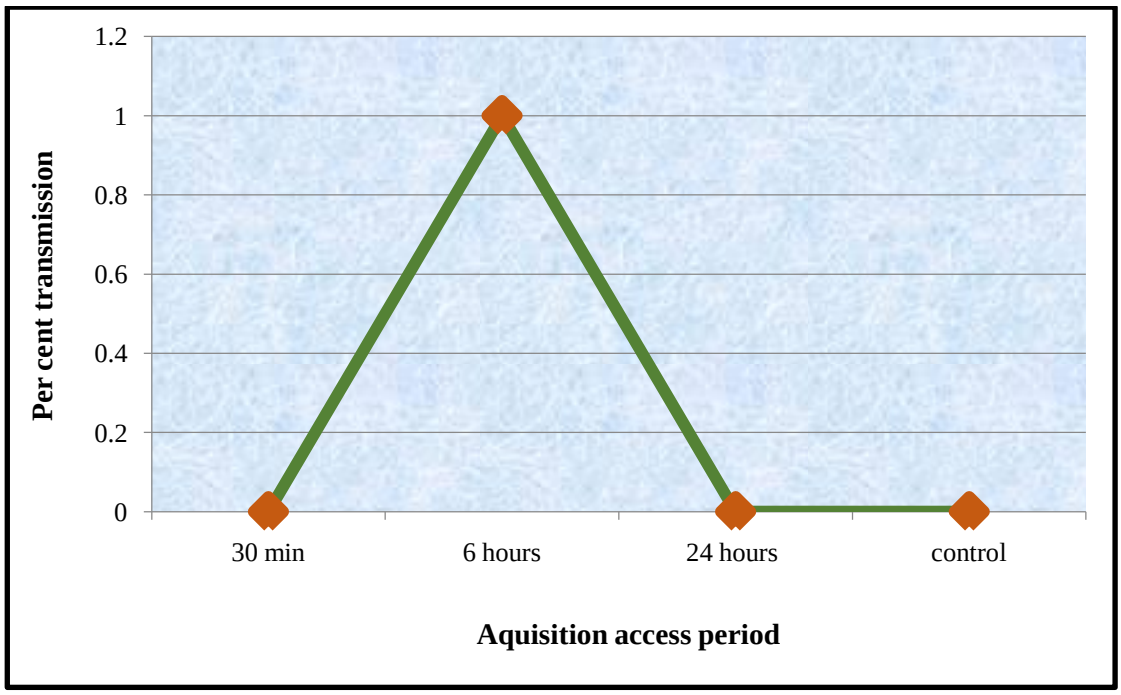


Fig. 4.6. Effect of varying acquisition access periods on the transmission of ULCD by *Henosepilachna dodecastigma.*

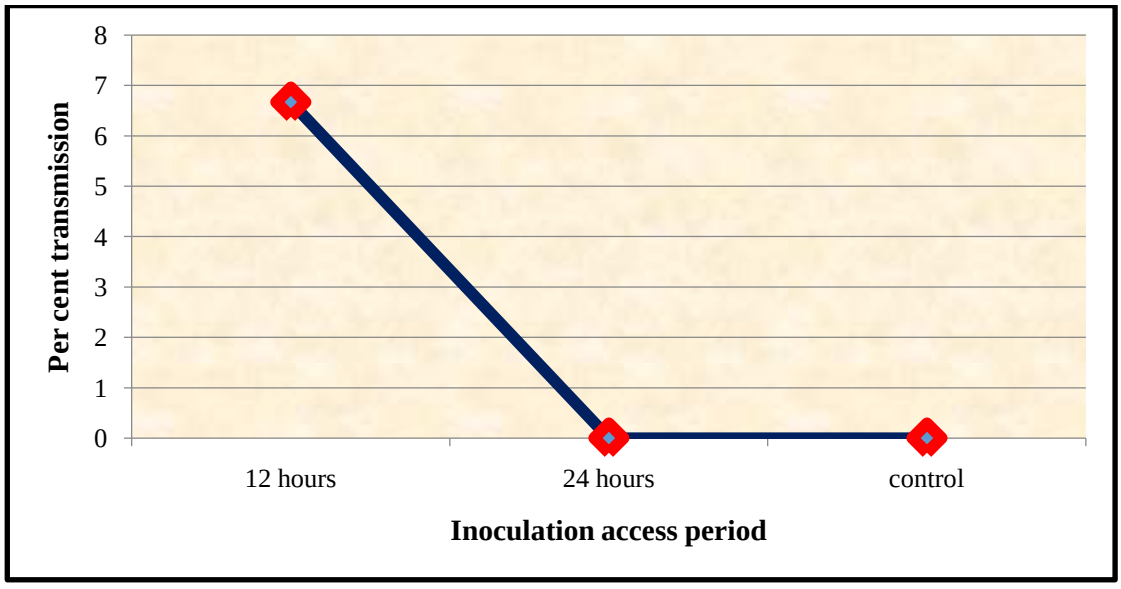


Fig. 4.7. Effect of varying inoculation n access periods on the transmission of ULCD by *Henosepilachna dodecastigma.*

4.3 EFFECT OF MICRONUTRIENTS ON INCIDENCE OF *URDBEAN* LEAF CRINKLE DISEASE

At 19 DAS *i.e.*, a day before the first spraying, ULCD incidence was on a par among the treatments (Table 4.8; Fig. 4.8; Plate 4.4).

At 26 DAS *i.e.*, a week after the first spray,T6 ($MgSO_4$ @ 0.2% + $ZnSO_4$@ 0.2%) (10.56% incidence), T3 ($ZnSO_4$ @ 0.2%) (12.69%incidence), T5 ($MnSO_4$@ 0.2% + $ZnSO_4$@ 0.2%) (12.95% incidence) and T8 (Dimethoate @ 0.2%) (13.55%incidence) treatments showed statistically equal efficacy in reducing the incidence. The treatment T3 has reduced the disease incidence (12.69%) and exhibited on a par reduction of disease with T5 (12.95%), T8 (13.55%), T4 (14.70%), T1 (14.96%), and T2 (15.17%). T8 has reduced the disease incidence and found to be on a par with T1(14.96%), T2 (15.17%), T7 (16.50%) and has reduced the ULCD incidence significantly than T9 (17.78%).

At 33 DAS *i.e.*, a week after the second spray, T6 (11.11% incidence), T3 (13.63%) and T5 (13.72%) treatments showed statistical parity in decreasing the incidence. The treatment T3 has reduced the disease incidence of 13.63%, which is on a par with T5 (13.72%), T8 (14.83%), T4 (15.38%), T1 (15.64%), T2 (15.85%) and T7 (17.44%) and has reduced the ULCD incidence significantly than T9 (17.78%).

When the incidence of ULCD was recorded on a week after the third spray (40 DAS) T6 was observed to remain effective over the rest showing minimum incidence (11.71%) and was statistically comparable with T3 ($ZnSO_4$ @ 0.2%) (13.93% incidence) and T5 ($MnSO_4$ @ 0.2% + $ZnSO_4$ @ 0.2%) (14.32% incidence).T2 (16.92%) and T7 (18.5%) showed on par disease incidences and were significantly superior than control (23.55%) in reduction of the disease.

From the three consecutive observations taken after the third spray at weekly intervals, it was noticed that the T6 ($MgSO_4$ @ 0.2% + $ZnSO_4$@ 0.2%) was significantly most effective over all other treatments followed by T3($ZnSO_4$ @ 0.2%) and T5 ($MnSO_4$@ 0.2% + $ZnSO_4$@ 0.2%) which were on par. The ULCD incidence on the last observation (61DAS) was recorded to range between 12.26% (T6) to 27.22% (control). However, T7 (ST with 16% trisodium orthophosphate), T1 ($MnSO_4$ @ 0.2%), T2 ($MgSO_4$@ 0.2%), T8 (Dimethoate @ 0.2%)and T4 ($MnSO_4$ @ 0.2% + $MgSO_4$ @ 0.2%) treatments were least effective and were statistically on a par.

Table 4.8. Effect of micronutrients, dimethoate and trisodium orthophosphate on incidence of ULCD in *urdbean*

		Disease incidence at								
Tr. No.	**Treatment**	**19DAS**	**26 DAS (1st spray)**	**33 DAS (2nd spray)**	**40 DAS (3rd spray)**	**47 DAS**	**54 DAS**	**61 DAS**	**AUDPC**	**B: C ratio**
T_1	$MnSO_4$ (0.2%)	13.16 *(3.62)	14.96 *(3.85)^{bcd}	15.64 *(3.94)^{b}	16.75 *(4.08)^{bc}	17.31 *(4.15)^{bc}	17.39 *(4.16)^{bc}	17.39 *(4.16)^{bc}	681.28	2.39
T_2	$MgSO_4$ (0.2%)	13.21 (3.63)	15.17 (3.89) ^{bcd}	15.85 (3.98)^{b}	16.92 (4.11)^{bc}	17.26 (4.15)^{bc}	17.31 (4.16)^{bc}	17.31 (4.16)^{bc}	684.39	2.69
T_3	$ZnSO_4$ (0.2%)	10.90 (3.30)	12.69 (3.56)^{ab}	13.63 (3.69)^{ab}	13.93 (3.73)^{ab}	14.19 (3.77)^{ab}	14.32 (3.78)^{ab}	14.32 (3.78)^{ab}	567.84	3.16
T_4	$MnSO_4$ (0.2%) + $MgSO_4$ (0.2%)	13.03 (3.61)	14.70 (3.83)^{bcd}	15.38 (3.92)^{b}	15.85 (3.98)^{bc}	16.15 (4.02)^{bc}	16.24 (4.03)^{abc}	16.24 (4.03)^{abc}	648.31	2.52
T_5	$MnSO_4$ (0.2%) + $ZnSO_4$ (0.2%)	12.39 (3.50)	12.95 (3.58)^{ab}	13.72 (3.69)^{ab}	14.32 (3.77)^{ab}	14.44 (3.79)^{ab}	14.53 (3.80)^{ab}	14.53 (3.80)^{ab}	583.94	2.70
T_6	$MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%)	10.04 (3.17)	10.56 (3.25)^{a}	11.11 (3.33)^{a}	11.71 (3.41)^{a}	12.05 (3.46)^{a}	12.26 (3.50)^{a}	12.26 (3.50)^{a}	561.75	3.12
T_7	Seed treatment with trisodium orthophosphate (16%)	14.36 (3.79)	16.50 (4.06)^{cd}	17.44 (4.17)^{bc}	18.50 (4.30)^{c}	18.97 (4.36)^{c}	19.10 (4.37)^{c}	19.10 (4.37)^{c}	750.68	2.47
T_8	Dimethoate (0.2%)	12.09 (3.47)	13.55 (3.67)^{abc}	14.83 (3.84)^{b}	16.37 (4.04)^{bc}	16.84 (4.09)^{bc}	16.92 (4.10)^{bc}	16.92 (4.10)^{bc}	651.11	3.11
T_9	Control	15.98 (3.99)	17.78 (4.21)^{d}	20.30 (4.49)^{c}	23.55 (4.83)^{d}	26.11 (5.09)^{d}	27.22 (5.19)^{d}	27.22 (5.19)^{d}	955.92	2.33
	SEm ±	0.15	0.15	0.16	0.16	0.17	0.18	0.18		
	CD	NS	0.45	0.49	0.49	0.52	0.53	0.53		
	CV (%)	7.48	6.91	7.31	7.06	7.28	7.47	7.47		

* Figures in parenthesis are square root transformed values

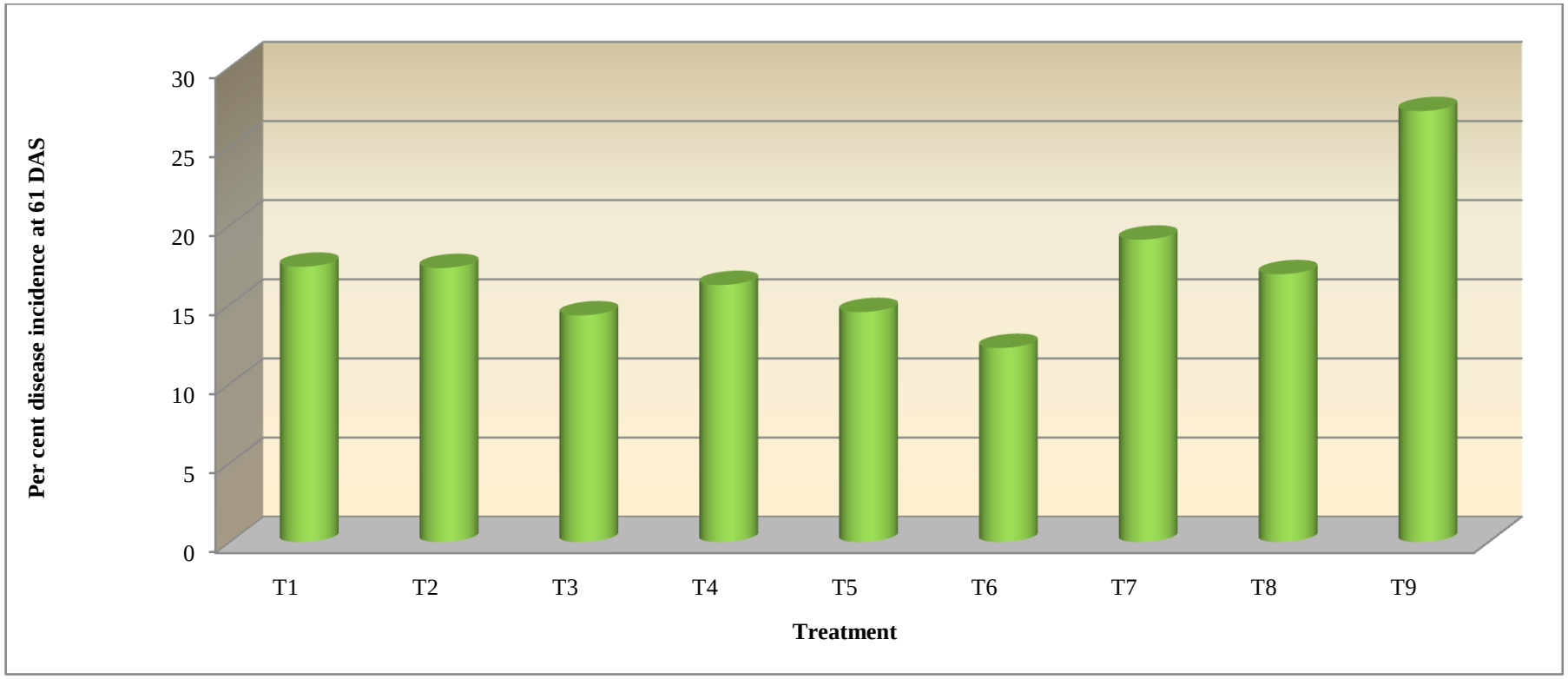


Fig. 4.8Evaluation of micronutrients, dimethoate and seed treatment with TSOP on incidence of ULCD in *urdbeankharif*2018-19.

T_1: $MnSO_4$(0.2%) T_4: $MnSO_4$ (0.2%)+ $MgSO_4$(0.2%) T_7: Seed treatment with trisodium orthophosphate (16%)

T_2: $MgSO_4$(0.2%) T_5: $MnSO_4$(0.2%)+ $ZnSO_4$(0.2%) T_8: Dimethoate (0.2%)

T_3: $ZnSO_4$(0.2%) T_6: $MgSO_4$(0.2%)+ $ZnSO_4$(0.2%) T_9: Control

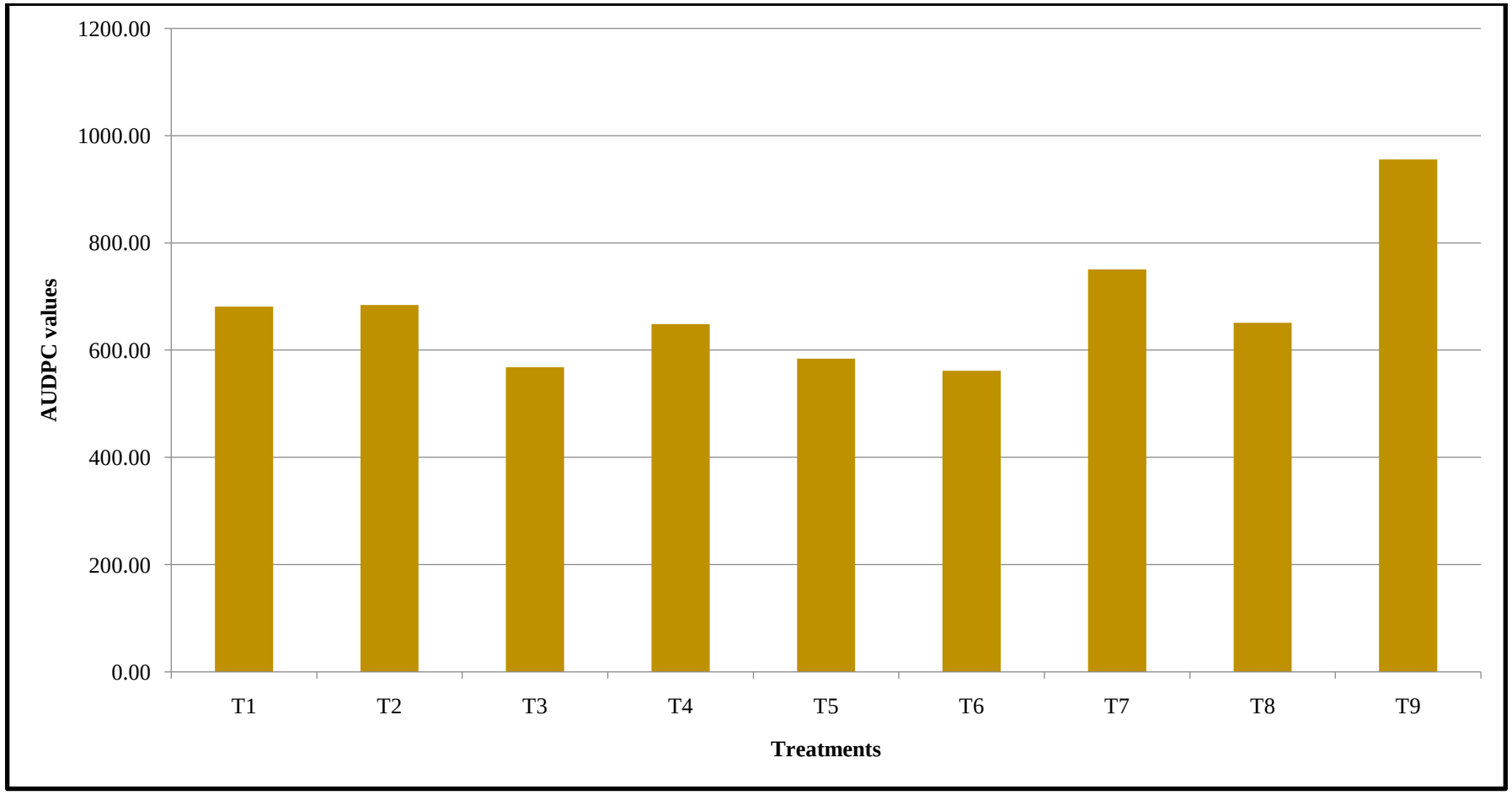


Fig.4.9.AUDPC trend in respective treatments during *kharif* 2018-19.

Among the treatments, T6 had lowest AUDPC value (561.75) followed by T3 (567.84) and T5 (583.94). These treatments were observed as best treatments in reducing the incidence.

Therefore, spraying with $MgSO_4$ (0.2%)+$ZnSO_4$(0.2%) at 20^{th}, 27^{th} and 34^{th}days after sowing has reduced the ULCD incidence significatly.

It may be noted from the table 4.5, disease progression in $MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%) was 10.04% incidence to 12.26% from 19 DAS to 40DAS with three sprays at 7 days interval and observed upon 61 DAS which was immediately followed by $ZnSO_4$(0.2%) alone with 567.84 AUDPC value and $MgSO_4$ (0.2%) alone was 684.39 AUDPC value. In the present investigation $MgSO_4$ (0.2%) + $ZnSO_4$(0.2%) was effective with low AUDPC value might be due to synergistic effect of $ZnSO_4$ (0.2%) alone and $MgSO_4$ (0.2%) alone or might be due to improper distribution of virus in seeds (Table 4.8; Fig. 4.9).

Thus the present investigation revealed that $ZnSO_4$ @ 0.2% thrice at 7 days interval yielded maximum and was on par with dimethoate @ 0.2% spray. This indicated that $ZnSO_4$ is equally effective to that of dimethoate @ 0.2%. Further, insignificant difference between $ZnSO_4$ alone and its combination with either $MgSO_4$ $MnSO_4$ indicated that $ZnSO_4$ alone was sufficient to manage ULCD and there by obtaining better yields. $MgSO_4$or $MnSO_4$ or their combinations was inferior to $ZnSO_4$.

It may be noted here that seed treatment with trisodium orthophosphate did not yield better than untreated control indicating its inefficacy in managing ULCD which is found to be mainly internally seed borne in present investigation. The effect of micronutrients is either due to the sub- expressional deficiencies in plants, thereby restoring the harmony in the plant physiological processes and making the plants to resist the ill effects of virus or it might be due to direct toxic effect of micronutrients.

Lokeshbabu (1997) reported that post–inoculation spraying of all the micronutrients were found effective in reducing ULCD through increased incubation period in the plant through sap inoculation. Among all the micronutrients, magnesium sulphate was observed to be effective. Bobade *et al*. (2009) reported that soil and spray application of magnesium sulphate, manganese sulphate, zinc sulphate, ferrous sulphate, boron, copper sulphate reduced *Geminivirus* infection in frenchbean.

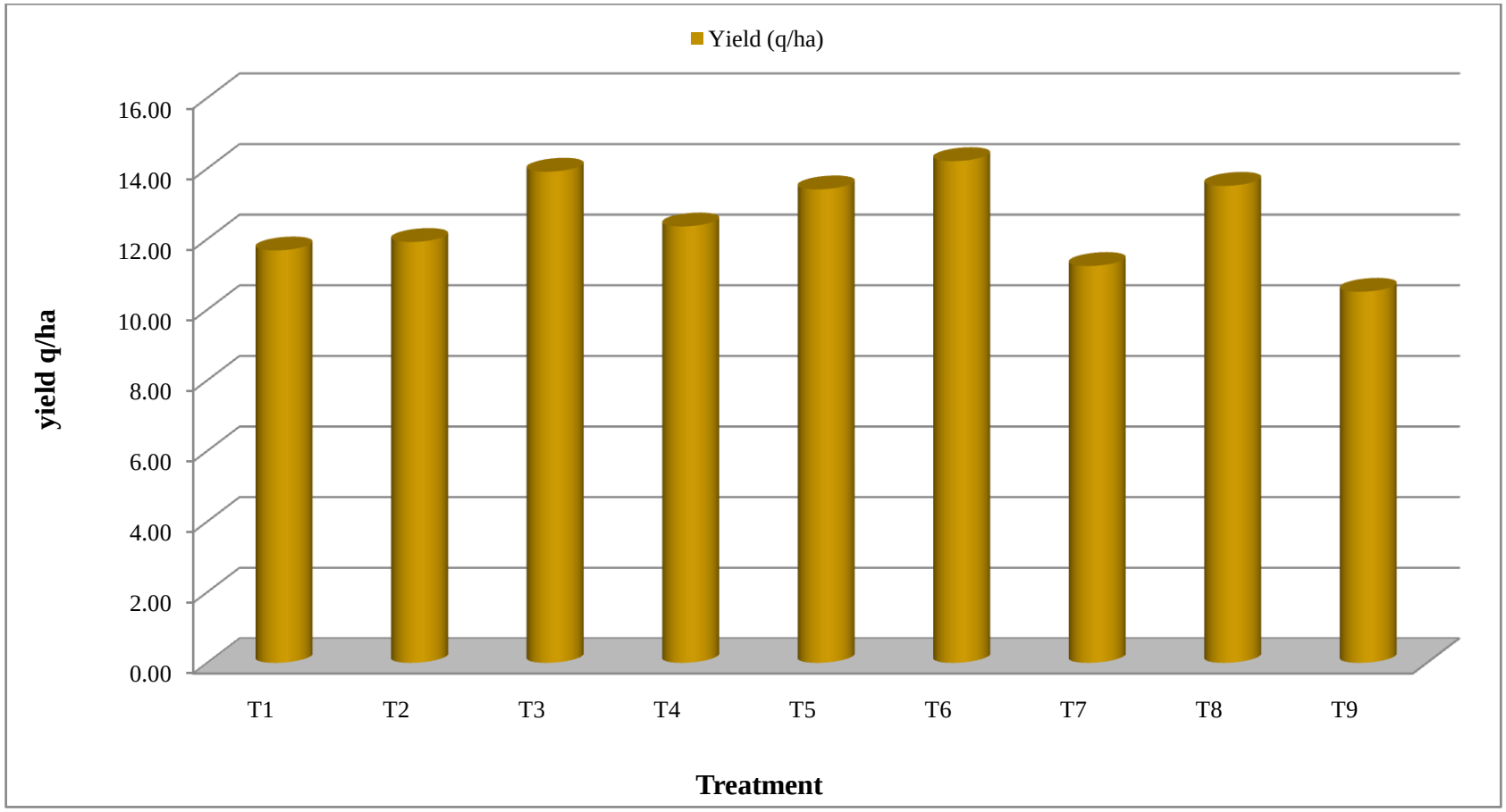


Fig. 4.10.Efficacyof micronutrients, insecticide and seed treatment with TSOP in*urdbean*on yield.

Irshad *et al*., 2012 observed that foliar application of K_2SO_4 0.7 kg ha^{-1}+ $MgSO_4$ 0.7 kg ha^{-1}+ Borax 0.5 kg ha^{-1} + $ZnSO_4$ 0.5kg ha^{-1}showed the significant reduction of *Cotton leaf curl virus* under field conditions. Zeshan *et al*. (2012) reported that spraying of Zn and B (Fashion) was found to be effective against ULCD under field conditions on *mungbean* varieties.

The efficacy of Zinc may be ascribed to the its functions as an activator of Cu/Zn-SOD, Zn is also involved in membrane protection against oxidative damage through the detoxification of superoxide radicals. Zinc deficiency may lead to impairment in membrane structure caused by free radicals leading to increased membrane leakage of low-molecular-weight compounds that favour pathogenesis (Marschner, 1995; Graham and Webb, 1991).Thus efficacy of $ZnSO_4$ relied upon its action on plant (activator of Cu/Zn-SOD), vector and virus itself.

On contrary to the above reports, Murthy (1996) reported that pre-inoculation spraying of micronutrients like zinc sulphate, ferrous sulphate, magnesium sulphate, manganese sulphate and calcium sulphate showed no effect on ULCD infection.

4.3.1 Seed Yield and Benefit Cost Ratio

Seed yield (q ha^{-1}) among the treatments ranged from 10.51 q ha^{-1} (Unsprayed check) to 14.21 q $ha^{-1}$with $MgSO_4$ (0.2%)+$ZnSO_4$(0.2%). The seed yield in unsprayed check was least (10.51 q ha^{-1}) compared to all other treatments. All the test chemicals evaluated were effective in increasing seed yield compared to check. The highest seed yield (14.21q ha^{-1}) was obtained with $MgSO_4$(0.2%)+$ZnSO_4$(0.2%)sprayed plots. However the yield in $MgSO_4$(0.2%)+$ZnSO_4$(0.2%) was on a par with yield in 0.2% ZnSO4 (13.91 q ha^{-1}), Dimethoate (0.2%)(13.51 q ha^{-1}) and 0.2% $MnSO_4$+ 0.2% $ZnSO_4$ (13.41 q ha^{-1}). The least seed yield (10.51 q ha^{-1}) was obtained with unsprayed plots which was found to be on a par with yield in 16% seed treatment with trisodium orthophosphate (11.24 q ha^{-1}), 0.2%$MnSO_4$ (11.68 q ha^{-1}), 0.2% $MgSO_4$ (11.92 q ha^{-1}). Spraying 0.2% $MnSO_4$ + $MgSO_4$(12.36 q ha^{-1}) gave on par yields with 0.2% $MnSO_4$+ 0.2% $ZnSO_4$ (13.41 q ha^{-1}) (Fig. 4.10; Table 4.9).

The maximum B:C ratio was recorded with $MgSO_4$(0.2%)+$ZnSO_4$(0.2%) (2.11).The minimum was observed in control (1.42). $ZnSO_4$(0.2%) and dimethoate (0.2%) was with same B:C ratio (2.09) and remaining all other are mid values

Plate 4.4. View of field experiment on micronutrient spray in *Urdbean* (LBG 752) during*kharif* 2018.

Table 4.9.Benefit: Cost ratio of urdbean leaf crinkle disease management in *urdbean*during *kharif*2018-19.

Treatment	Cost of treatment (Rs)	Cost of cultivation (Rs/ha)	Yield (q/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C Ratio
T1: $MnSO_4$ @ 0.2%	1626	25961	11.68	65408	39447	1.52
T2: $MgSO_4$ @ 0.2%	336	24671	11.92	66752	42081	1.71
T3: $ZnSO_4$ @ 0.2%	900	25235	13.91	77896	52661	2.09
T4: $MnSO_4$ @ 0.2% + $MgSO_4$ @ 0.2%	981	25316	12.36	69216	43900	1.73
T5: $MnSO_4$ @ 0.2% + $ZnSO_4$ @ 0.2%	1263	25598	13.41	75096	49498	1.93
T6: $MgSO_4$ @ 0.2% + $ZnSO_4$ @ 0.2%	1236	25571	14.21	79576	54005	2.11
T7: Seed trearment with tri sodium orthophosphate (16%)	1648	25983	11.24	62944	36961	1.42
T8: Dimethoate @ 0.2%	145	24480	13.51	75656	51176	2.09
T9:Control	-	24335	10.51	58856	34521	1.42

Note: Cost of Cultivation per hectare

Tractor- 2000/-
Seed cost-2500/-
Fertilizers: Urea-44kg x 5/- = 220/-
SSP-313kg x 10/- = 3130/-
Insecticide : Coragen@ 0.2ml/L – 100ml- 1660/-
Labour requirement: Sowing- 10women x 250=2500/-
Harrowing = 700/-
Weeding- 8women x 250=2000/- and herbicide = 625/-
Spraying- 2men =2000/-
Irrigation - 2men = 2000/-
Harvesting and threshing- 20women x 250=5000/-

Total – 24,335/-

T1- $MnSO_4$ @ 0.2% (1kg) Rs. 1626/-
T2-$MgSO_4$ @ 0.2% (1 kg) Rs. 336/-
T3- $ZnSO_4$ @ 0.2% (1 kg) Rs. 900/-
T4- $MnSO_4$ @ 0.2% (500g)+ $MgSO_4$ @ 0.2 (500g) Rs. 981/-
T5- $MnSO_4$ @ 0.2% (500 g) + $ZnSO_4$ @ 0.2% (500g) Rs. 618/-
T6- $MgSO_4$ @ 0.2% (500g)+$ZnSO_4$ @ 0.2% (500g) Rs 1236/-
T7- Seed trearment with tri sodium orthophosphate (16%)Rs. 1648/- (1 kg)
T8- Dimethoate @ 0.2% Rs. 145/-
Selling price was then existing market price Rs. 5600/- /q

between the maximum and minmum B:C ratio, are in the order of 16% seed treatment with trisodium orthophosphate (1.42), 0.2%$MnSO_4$ (1.52), 0.2%$MnSO_4$ + 0.2% 0.2% $MgSO_4$ (1.71), $MgSO_4$ (1.73) and 0.2% $MgSO_4$ + 0.2% $ZnSO_4$ (1.93)(Table 4.9).

Ghazvineh and Yousefi (2012) stated that highest grain of maize was obtained with foliar spray of complete fertilizer in stem and cob elongation stage followed by Zn fertilizer.

Reddy *et. al.*(2018) observed that the foliar application of micronutrients on tomato cultivars showed maximum growth rate (85.7%) by application of Zn on Arka sourabh and in Arka vikas maximum branches per plant was increased with application of manganese. Singh and Kathayat (2018) reported that high yields can be obtained with optimal application of NPK in balanced ratio and efficiency of NPK fertilizers can be increased by application of micronutrients (B, S, Zn, Mn) which ultimately increases the potato tuber yield.

4.4 BIOCHEMICAL ANALYSIS

In order to assess the impact of mineral nutrient spray on the physiology of plant tissues, *urdbean* leaf sample from different treatment were collected and analysed for total proteins, total phenols, and auxins.

4.4.1 Determination of Protein Content in the Infected ULCD Plants and Healthy plants

At 40 DAS, in healthy plant from unsprayed control plot, protein content was 1.34 mg/100 mg of leaf tissue. In trisodium orthophosphate, protein content was 1.35mg with insignificant difference compared to check. In dimethoate sprayed plot the protein content in leaf tissue was 1.36mg/100mg. Among the nutrients sprayed treatment, the protein content in healthy plant was highest in $MgSO_4$ +$ZnSO_4$ sprayed plot (1.43mg/100mg) and lowest in $MnSO_4$ alone sprayed plot (1.37mg/100mg). Maximum per cent increase over control (%INOC) in healthy plant tissue was observed in $MgSO_4$ +$ZnSO_4$ (6.89) followed by $MnSO_4$ +$ZnSO_4$ (6.11) and $ZnSO_4$ (5.47) (Table 4.10; Fig. 4.11, 4.12; Plate 4.5).

When protein content was analysed in 40 days old diseased plants, protein content in unsprayed control was 1.44, in trisodium orthophosphate treated 1.45mg and dimethoate sprayed 1.51mg/100mg. Among the mineral nutrients sprayed plants

highest protein was observed in $ZnSO_4$ alone sprayed plot (1.97mg/100mg) and the least protein content was in $MnSO_4$ alone sprayed plot (1.68mg/100mg). When the per cent increase in protein content over untreated control in infected sample was analysed, at 40 DAS, maximum increase was noticed with $ZnSO_4$ (36.29%) followed by $MgSO_4$ +$ZnSO_4$ (30.23%). Least increase in protein content over control was observed in trisodium orthophosphate (0.26%) followed by dimethoate (4.61%) indicating no impact of trisodium orthophosphate or dimethoate on ULCD. Per cent increase of infected over healthy was maximum in$ZnSO_4$ (0.2%) (39.41) followed by $MgSO_4$ +$ZnSO_4$ (31.44).

Protein content at 55 DAS in healthy plant from unsprayed control plot was recorded to be 1.41 mg/100 mg of leaf tissue. In trisodium orthophosphate, protein content was 1.43mg with significant difference compared to check. In dimethoate spray plot the protein content in leaf tissue was 1.46mg/100mg. In nutrient sprayed treatment, the protein content in healthy plant was highest in $MgSO_4$ +$ZnSO_4$ sprayed plot (1.71 mg/100 mg) and lowest in $MnSO_4$ alone sprayed plot (1.47mg/100mg). When compared to control per cent increase of protein was maximum in $MgSO_4$ +$ZnSO_4$ (21.03) followed by $MnSO_4$ +$ZnSO_4$ (14.09) and $ZnSO_4$ (11.46).

When protein content was analysed in diseased plants of 55 days old, protein content in unsprayed control was 1.69mg/100mg of leaf tissue, in trisodium orthophosphate treated 1.72mg/100mg and dimethoate sprayed 1.76mg/100mg. In mineral nutrients sprayed plots, highest protein was observed in $ZnSO_4$ alone sprayed plot (2.18mg/100mg) and the least protein content was in $MnSO_4$ alone sprayed plot (1.86mg/100mg).At 55 DAS, per cent increase in protein content over untreated control in infected was maximum observed to be maximum in $ZnSO_4$ (28.81%) followed by $MgSO_4$ +$ZnSO_4$ (20.16%). Minimum increase in percent protein content over control in infected was observed in 1.46% in trisodium orthophosphate followed by dimethoate (4.10%),which indicate that there was no impact of trisodium orthophosphate or dimethoate on ULCD multiplication or disease development. Per cent increase of infected over healthy was maximum in$ZnSO_4$ (0.2%) (38.71) followed by $MgSO_4$ (29.28) and $MnSO_4$ +$MgSO_4$ (28.27).

Biochemical analysis for protein content was done in infected and healthy test plants at 70 DAS. The highest in healthy plants from unsprayed control plot protein content was 1.63 mg/100 mg of leaf tissue and followed by trisodium orthophosphate,

protein content was 1.70mg with significant difference compared to check. In dimethoate spray plot the protein content in leaf tissue was 1.75mg/100mg. In nutrient sprayed treatment, the protein content in healthy plant was highest in $MgSO_4$ +$ZnSO_4$ sprayed plot (2.35mg/100mg) and lowest in $MnSO_4$ alone sprayed plot (1.78mg/100mg). Per cent increase over control was maximum in $MgSO_4$ +$ZnSO_4$ (43.79) which was followed by $MnSO_4$ +$ZnSO_4$ (32.14) and $ZnSO_4$ (21.95)

When protein content was analysed in diseased plants of 70 days old, protein content in unsprayed control was 1.85mg/100mg leaf tissue, in trisodium orthophosphate treated 1.91mg/100mg of leaf tissue and dimethoate sprayed 1.94mg/100mg. In mineral nutrients sprayed plots, highest protein was observed in $ZnSO_4$ alone sprayed plot (2.34mg/100mg) and the least protein content was in $MnSO_4$ alone sprayed plot (1.97mg/100mg).Per cent increase over control was maximum in$ZnSO_4$ (26.02) which was followed by $MgSO_4$ +$ZnSO_4$ (20.03) and $MnSO_4$ +$ZnSO_4$ (16.95).Per cent increase in protein content over untreated control in infected plant at 70 DAS was observed maximum in $ZnSO_4$ with 26.02% followed by $MgSO_4$ +$ZnSO_4$ (20.03%). Least was observed in trisodium orthophosphate (3.07%) followed by dimethoate (4.61%). Per cent increase of infected over healthy was maximum in$ZnSO_4$ @ 0.2% (17.47) followed by $MnSO_4$ +$MgSO_4$ (14.54) and seed treatment with trisodium orthophosphate (12.59) (Table 4.10; Fig. 4.11, 4.12).

It may be seen from the present investigation (Fig. 4.11) that the protein content was higher in diseased plants compared to healthy plants irrespective of whether sprayed or not. This increase in protein content may be attributed to viral multiplication and host reactions of all the treatments in untreated control, dimethoate sprayed and trisodium orthophosphate seed treated plots had low protein content.

Table 4.10. Estimation of total soluble protein content mg/100mg in infected and healthy leaves of ULCD

Treatment	Total soluble protein content mg/100mg														
	40 DAS					55 DAS					70 DAS				
	H	% INOC	D	% INOC	% INOH	H	% INOC	D	% INOC	% INOH	H	% INOC	D	% INOC	% INOH
T1 : $MnSO_4$(0.2%)	1.37	2.77	1.68	16.73	22.54	1.47	4.58	1.86	10.05	26.30	1.78	9.26	1.97	6.15	-10.45
T2 : $MgSO_4$(0.2%)	1.40	4.62	1.77	22.39	26.22	1.51	6.81	1.95	15.05	29.28	1.90	16.30	2.04	9.83	7.36
T3 : $ZnSO_4$(0.2%)	1.41	5.47	1.97	36.29	39.41	1.57	11.46	2.18	28.81	38.71	1.99	21.95	2.34	26.02	17.47
T4:$MnSO_4$(0.2%)+$MgSO_4$(0.2%)	1.39	4.26	1.72	19.36	23.51	1.49	5.86	1.91	13.14	28.27	1.82	11.76	2.09	12.60	14.54
T5:$MnSO_4$(0.2%)+$ZnSO_4$(0.2%)	1.42	6.11	1.76	22.26	24.31	1.61	14.09	1.97	16.74	22.81	2.16	32.14	2.17	16.95	0.62
T6:$MgSO_4$(0.2%)+$ZnSO_4$(0.2%)	1.43	6.89	1.88	30.23	31.44	1.71	21.03	2.03	20.16	19.16	2.35	43.79	2.23	20.03	5.10
T7 : Seed treatment with trisodium orthophosphate(16%)	1.35	1.07	1.45	0.26	7.03	1.43	1.35	1.72	1.46	20.15	1.70	4.08	1.91	3.07	12.59
T8: Dimethoate (0.2%)	1.36	1.49	1.51	4.61	11.20	1.46	3.71	1.76	4.10	20.48	1.75	7.16	1.94	4.61	10.98
T9: Control	1.34	-	1.44	-	7.89	1.41	-	1.69	-	20.02	1.63	-	1.85	-	13.68
SEM	0.004		0.005			0.005		0.004			0.006		0.006		
CD($P \leq 0.05$)	0.010		0.015			0.016		0.012			0.017		0.018		
CV(%)	1.016		1.197			1.435		0.921			1.247		1.186		

H=Healthy leaf tissue D=Diseased leaf tissue %INOC= Per cent increase over control %INOH= Per cent increase over healthy

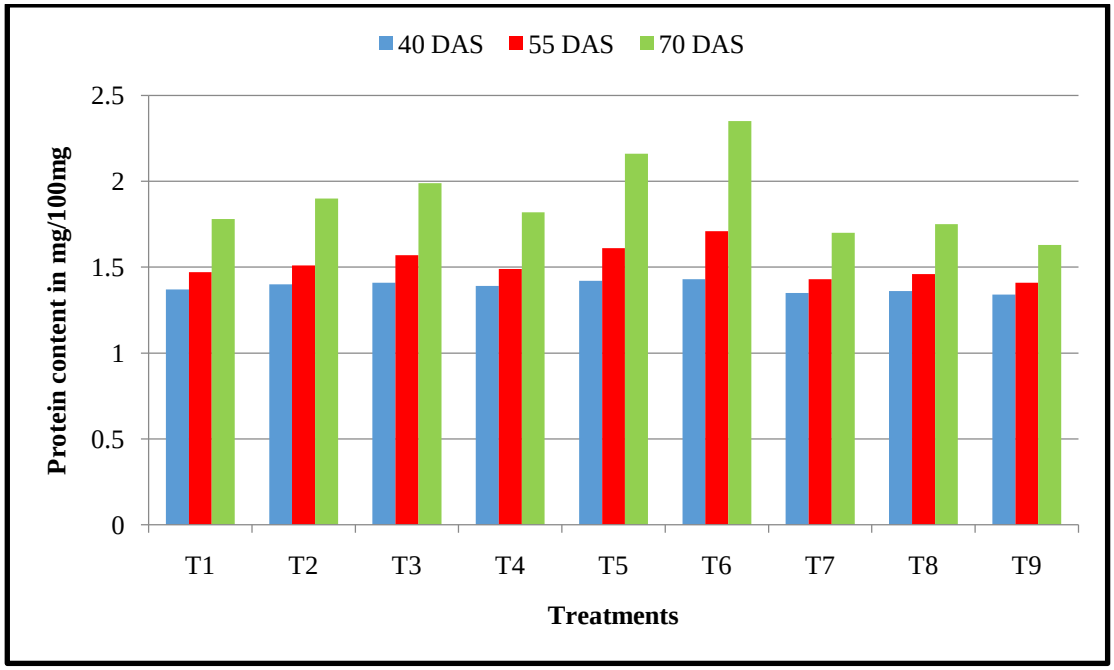


Fig. 4.11. Estimated protein content in mg/100mg of leaf tissue in healthy plants at 40, 55 and 70 DAS in treated plots for management of ULCD in field.

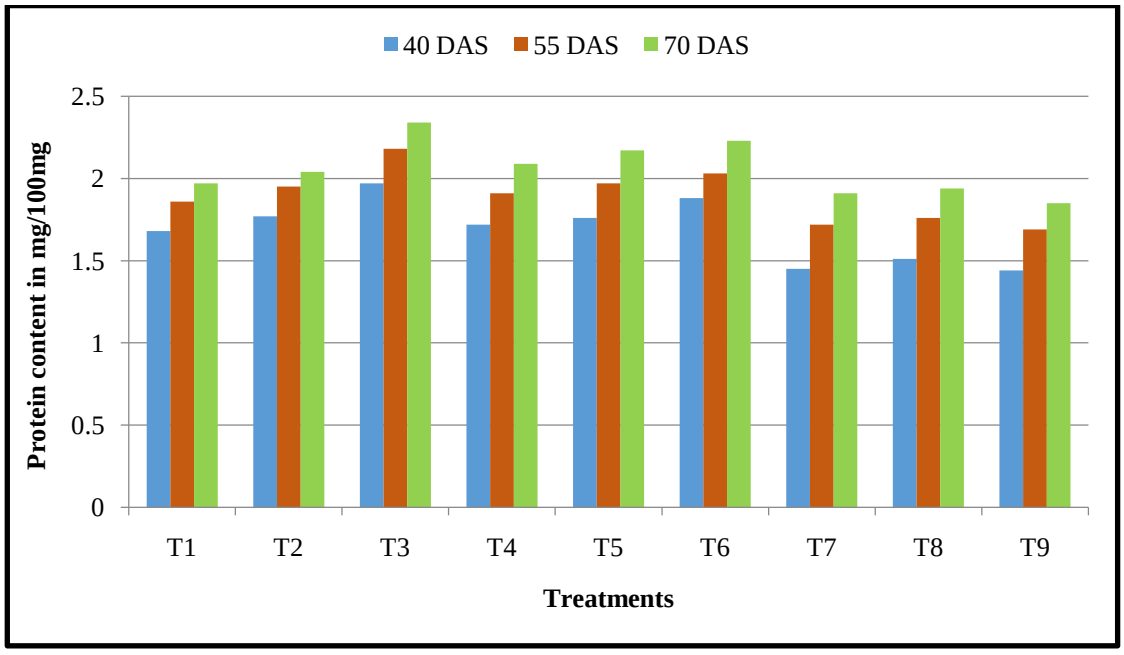


Fig. 4.12. Estimated protein content in mg/100mg of leaf tissue in infected plants at 40, 55 and 70 DAS in treated plots for management of ULCD in field.

In present analysis, the protein content was highest in infected ULCD sample when compared to healthy treated test plants at 40DAS, 55DAS, 70DAS in all the treatments as reported by Brar and Rataul, 1990; Ashfaq, 2010 and Srivastava and Singh, 2010).

Among all the treatments $MgSO_4$ (0.2%) +$ZnSO_4$ (0.2%) was with highest total protein in the healthy which is on par with $ZnSO_4$ (0.2%) at every 15 days interval after sowing and this may be due to the combained effect of Zn and Mg. Zn which indirectly effect the virus load by reducing the feeding intensity and reproduction in sucking pest like aphids.www.spectrumanalytic.com). Similar result was reported in yellow vein mosaic virus (Basavaraj, 2012) that, highest protein content was observed in diseased plant when compared with healthy plant in *Urdbean*.

4.4.2 Determination of Phenol Content in the Infected ULCD Plants and Healthy Plants

At 40 DAS, in healthy plant from unsprayed control plot phenol content was 0.34mg/100 mg of leaf tissue which is on a par with trisodium orthophosphate treated phenol content (0.36mg). In dimethoate sprayed plots, the phenol content in leaf tissue was 0.38mg/100mg with no significant difference with trisodium orthophosphate. In nutrient sprayed treatment, the phenol content in healthy plant was highest in $MgSO_4$ +$ZnSO_4$ sprayed plot (0.60mg/100mg) which is on a par with $MnSO_4$ +$ZnSO_4$ (0.58mg/100mg). Lowest in $MgSO_4$ alone sprayed plot (0.41mg/100mg).When the per cent increase in phenol content over untreated control in infected sample was analysed, at 40 DAS, maximum increase was noticed with $MgSO_4$ +$ZnSO_4$ (77.69%). Least increase in protein content over control was observed in trisodium orthophosphate (5.38%) followed by dimethoate (12.31%) indicating no impact of trisodium orthophosphate or dimethoate on ULCD intraction with host plant (table 4.11; fig. 4.13, 4.14; plate 4.6).

When phenol content was analysed in diseased plants of 40 days old, phenol content in unsprayed control was 0.51mg/100mg, in trisodium orthophosphate treated 0.59mg/100mg and dimethoate sprayed 0.66mg/100mg. In mineral nutrients sprayed plots, highest phenol content was observed in $MgSO_4$ +$ZnSO_4$ sprayed plot (0.89mg/100mg) and the least phenol content was in $MnSO_4$ alone sprayed plot

(0.69mg/100mg).Maximum increase was noticed with $MgSO_4$ +$ZnSO_4$ (74.87%). Least increase in phenol content over control was observed in trisodium orthophosphate (16.92%) followed by dimethoate (30.77%) indicating no impact of trisodium orthophosphate or dimethoate on ULCD intraction with host plant. Per cent increase of infected over healthy was maximum in dimethoate @ 0.2% (74.66) and followed by $MgSO_4$ (72.61%) and seed treatment with trisodium orthophosphate (66.42).

Phenol content at 55 DAS in healthy plant from unsprayed control plot was recorded to be 0.40mg/100 mg of leaf tissue. In trisodium orthophosphate, phenol content was 0.43mg/100 mg of leaf tissue with significant difference compared to check and followed next to control. In dimethoate spray plot the phenol content in leaf tissue was 0.47mg/100mg. In nutrient sprayed treatment, the phenol content in healthy plant was highest in $MgSO_4$ +$ZnSO_4$ sprayed plot (0.66mg/100mg) and lowest in $MgSO_4$ alone sprayed plot (0.51mg/100mg). At 55 DAS, per cent increase in phenol content over untreated control in infected was observed to be maximum in $MgSO_4$ +$ZnSO_4$ (64.94%). Minimum increase in per cent phenol content over control in infected was observed to be 7.14% in trisodium orthophosphate followed by dimethoate (16.23%) which indicate that there was no impact of trisodium orthophosphate or dimethoate on ULCD interaction with host plant defence mechanisms.(table 4.11; plate 4.6).

When phenol content was analysed in diseased plants of 55 days old, phenol content in unsprayed control was 0.55mg/100mg of leaf tissue, in trisodium orthophosphate treated 0.63mg/100mg and dimethoate sprayed 0.72mg/100mg. In mineral nutrients sprayed plots, highest phenols was observed in $MgSO_4$ +$ZnSO_4$ sprayed plot (0.92mg/100mg) and the least phenol content was in $MnSO_4$ alone sprayed plot (0.74mg/100mg) and $MnSO_4$ +$MgSO_4$. Minimum increase in per cent phenol content over control in infected was observed in 15.17% in trisodium orthophosphate followed by dimethoate (30.81%) which indicate that there was no impact of trisodium orthophosphate or dimethoate on ULCD interaction with host plant defence mechanisms. Per cent increase of infected over healthy was maximum in dimethoate (0.2%) (54.19) and followed by $MgSO_4$ (49.74) and seed treatment with trisodium orthophosphate (47.27)

Biochemical analysis for phenol content was done in infected and healthy test plants at 70 DAS. The highest phenol content in healthy plants was in unsprayed control plot with 0.45 mg/100 mg of leaf tissue and followed by trisodium orthophosphate, phenol content was 0.49mg with significant difference compared to check which was next to the control. In dimethoate spray plot the phenol content in leaf tissue was 0.51mg/100mg. In nutrient sprayed treatment, the phenol content in healthy plant was highest in $MgSO_4$ +$ZnSO_4$ sprayed plot (0.72mg/100mg) and lowest in$MgSO_4$ alone sprayed plot (0.57mg/100mg). Per cent increase in phenol content over untreated control in infected plant at 70 DAS was observed maximum in $MgSO_4$ +$ZnSO_4$ with 61.40%. Least was observed in trisodium orthophosphate (9.94%) followed by $MgSO_4$ (27.49%).

When phenol content was analysed in diseased plants of 70 days old, phenol content in unsprayed control was 0.59mg/100mg leaf tissue, in trisodium orthophosphate treated 0.68mg/100mg of leaf tissue and dimethoate sprayed 0.77mg/100mg. In mineral nutrients sprayed plots, highest phenol was observed in $MgSO_4$ +$ZnSO_4$ sprayed plot (0.97mg/100mg) and the least phenol content was in $MnSO_4$ alone sprayed plot (0.76mg/100mg).Per cent increase in phenol content over untreated control in infected plant at 70 DAS was observed maximum in $MgSO_4$ +$ZnSO_4$ with 63.88%. Least was observed in trisodium orthophosphate (14.98%) followed by $MnSO_4$ (29.07%).Per cent increase of infected over healthy was maximum in dimethoate @ 0.2%) (50.77) and followed by $MgSO_4$ (41.28) and seed treatment with trisodium orthophosphate (38.83)

The present study says that the total phenol content is highest in infected ULCD test plants when compared to healthy plants. This results are in agreement with many reporters (Karthikeyan *et al*., 2009 and Ashfaq *et al*., 2014) they reported that healthy plants shows less phenol content than infected plants. Total phenol content is highest in $MgSO_4$ (0.2%)+$ZnSO_4$(0.2%) in infected ULCD and healthy leaves. This result were similar with Basavaraj (2012) highest phenol content in diseased plant of *MYMV* in *Urdbean* treated plants with $MgSO_4$, and $MnSO_4$ more than unsprayed healthy plants and lowest phenol content was observed in unsprayed diseased plants.

Table 4.11.Estimation of total phenol content mg/100mg in infected and healthy leaves of ULCD.

Treatment	Total phenol content mg/100mg														
	40 DAS					55 DAS					70 DAS				
	H	% INOC	D	% INOC	% INOH	H	% INOC	D	% INOC	% INOH	H	% INOC	D	% INOC	% INOH
T1 : $MnSO_4$(0.2%)	0.46	36.15	0.69	35.90	49.72	0.56	38.96	0.74	34.12	32.24	0.63	42.11	0.76	29.07	20.58
T2 : $MgSO_4$(0.2%)	0.41	20.77	0.71	38.97	72.61	0.51	26.62	0.76	38.39	49.74	0.57	27.49	0.80	35.68	41.28
T3 : $ZnSO_4$(0.2%)	0.50	47.69	0.80	58.46	60.94	0.60	49.35	0.85	54.03	41.30	0.64	43.86	0.88	49.34	37.80
T4:$MnSO_4$(0.2%)+$MgSO_4$ (0.2%)	0.45	31.54	0.70	37.44	56.73	0.53	33.12	0.74	35.07	39.02	0.60	35.09	0.79	33.48	31.17
T5:$MnSO_4$(0.2%)+$ZnSO_4$(0.2%)	0.58	72.31	0.83	62.56	41.52	0.64	59.74	0.87	57.82	35.37	0.69	54.39	0.91	53.30	31.82
T6:$MgSO_4$(0.2%)+$ZnSO_4$(0.2%)	0.60	77.69	0.89	74.87	47.62	0.66	64.94	0.92	68.25	39.76	0.72	61.40	0.97	63.88	34.78
T7 : Seed treatment with trisodiumorthophosphate(16%)	0.36	5.38	0.59	16.92	66.42	0.43	7.14	0.63	15.17	47.27	0.49	9.94	0.68	14.98	38.83
T8: Dimethoate (0.2%)	0.38	12.31	0.66	30.77	74.66	0.47	16.23	0.72	30.81	54.19	0.51	14.04	0.77	29.52	50.77
T9: Control	0.34	-	0.51	-	50.00	0.40	-	0.55	-	37.01	0.45	-	0.59	-	32.75
SEM	0.01		0.010			0.01		0.004			0.01		0.003		
CD($P \leq 0.05$)	0.02		0.012			0.02		0.013			0.03		0.009		
CV(%)	7.13		2.225			4.91		2.361			5.92		1.639		

H=Healthy leaf tissue D=Diseased leaf tissue %INOC= Per cent increase over control %INOH= Per cent increase over healthy

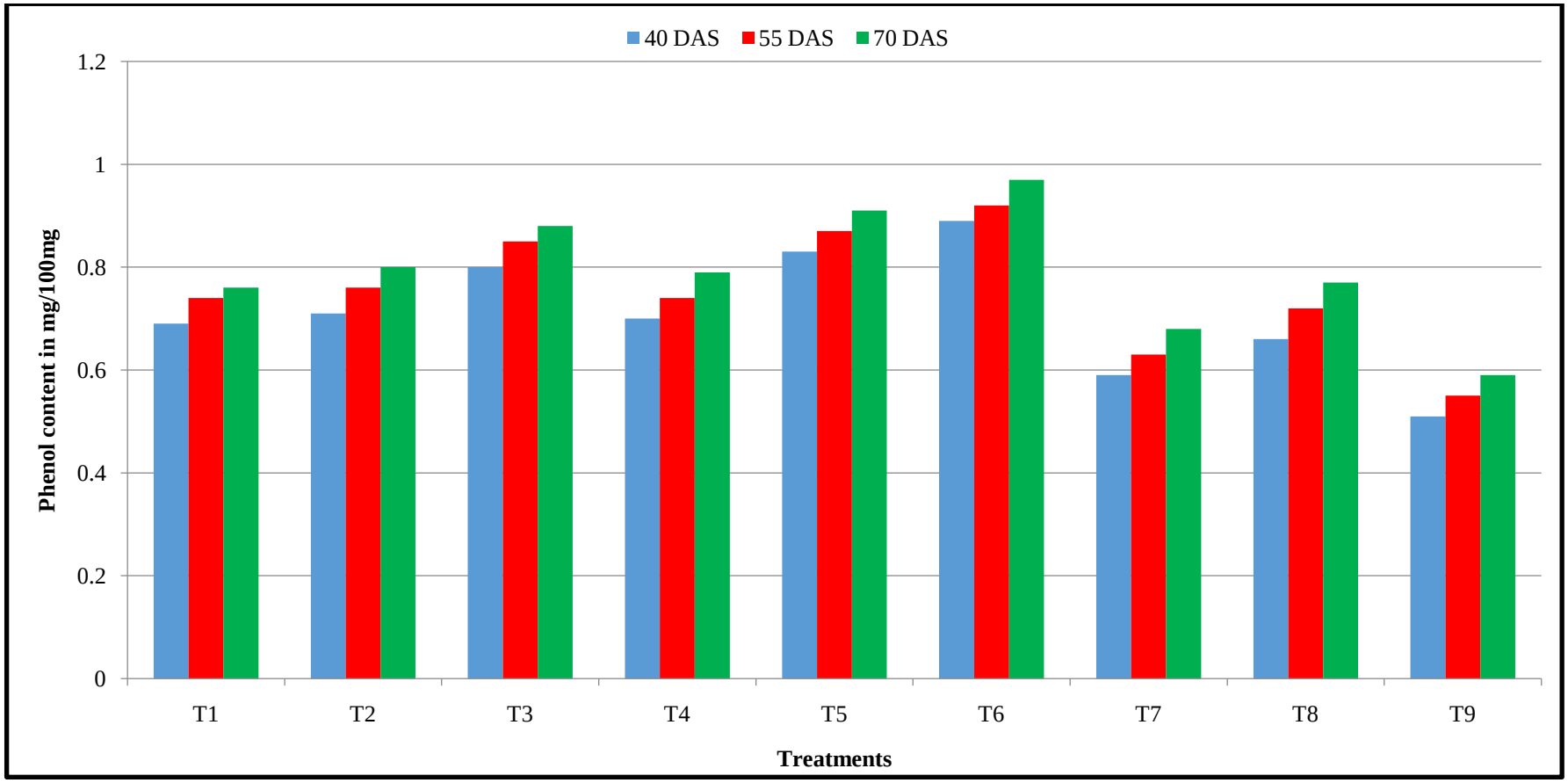


Fig. 4.13 Estimated Phenol content in mg/100mg of leaf tissue in infected plants at 40, 55 and 70 DAS in treated plots for management of ULCD.

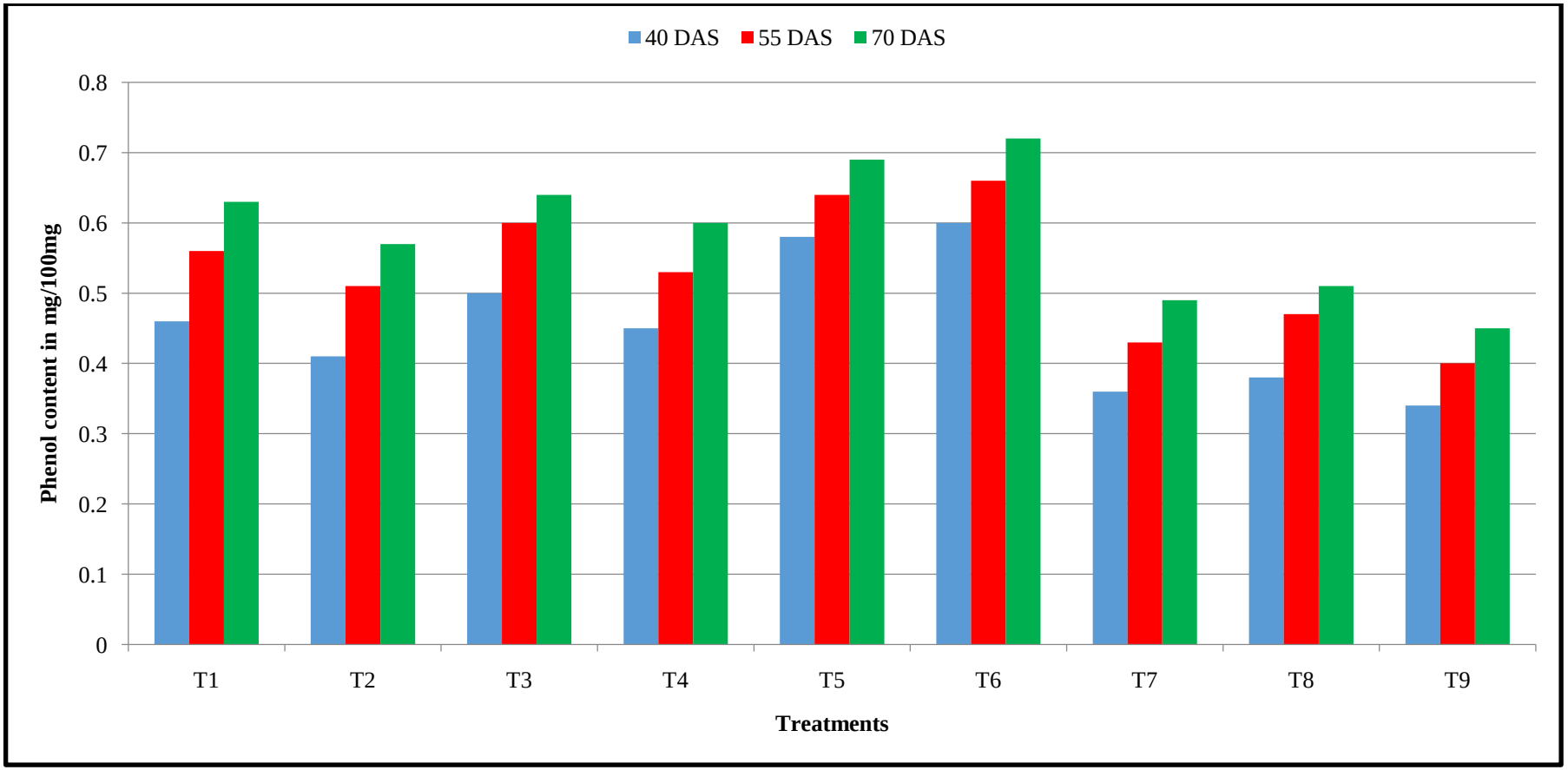


Fig. 4.14 Estimated phenol content in mg/100mg of leaf tissue in healthy plants at 40, 55 and 70 DAS in treated plots for management of ULCD .

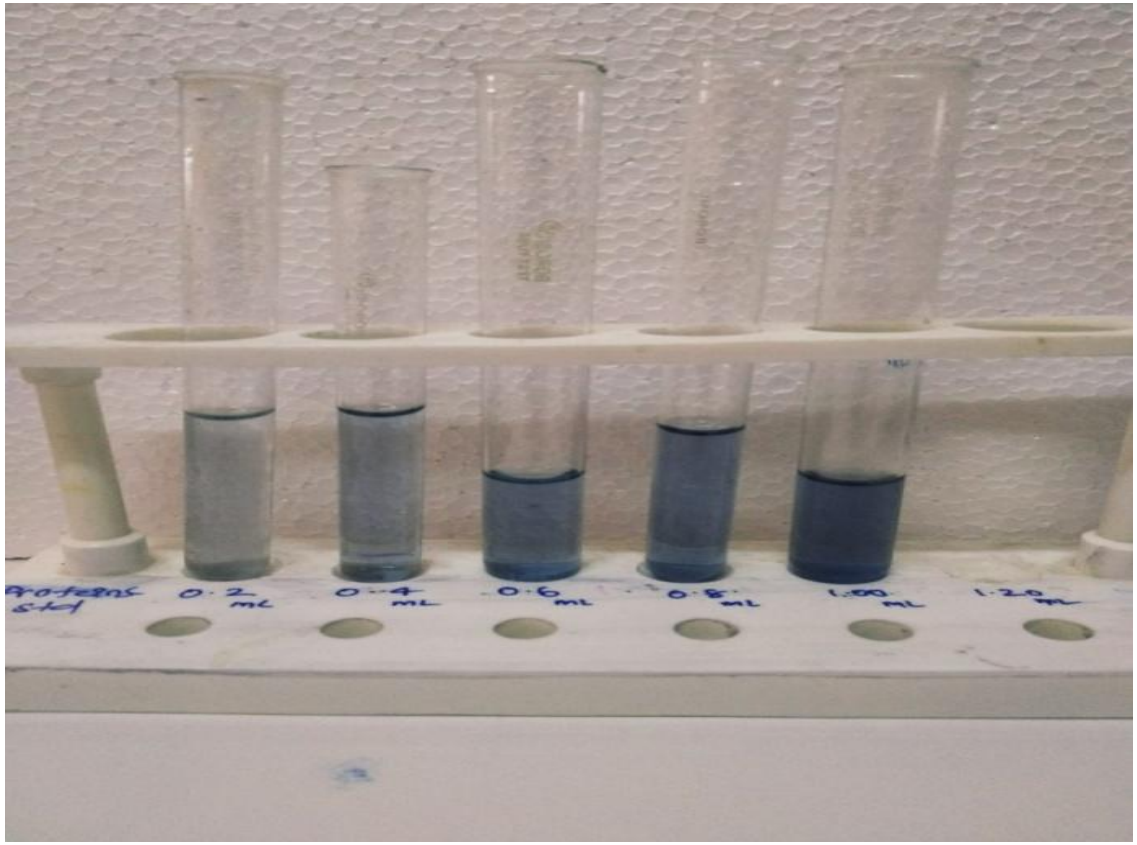


Plate 4.5. Biochemical analysis for estimation of protein content

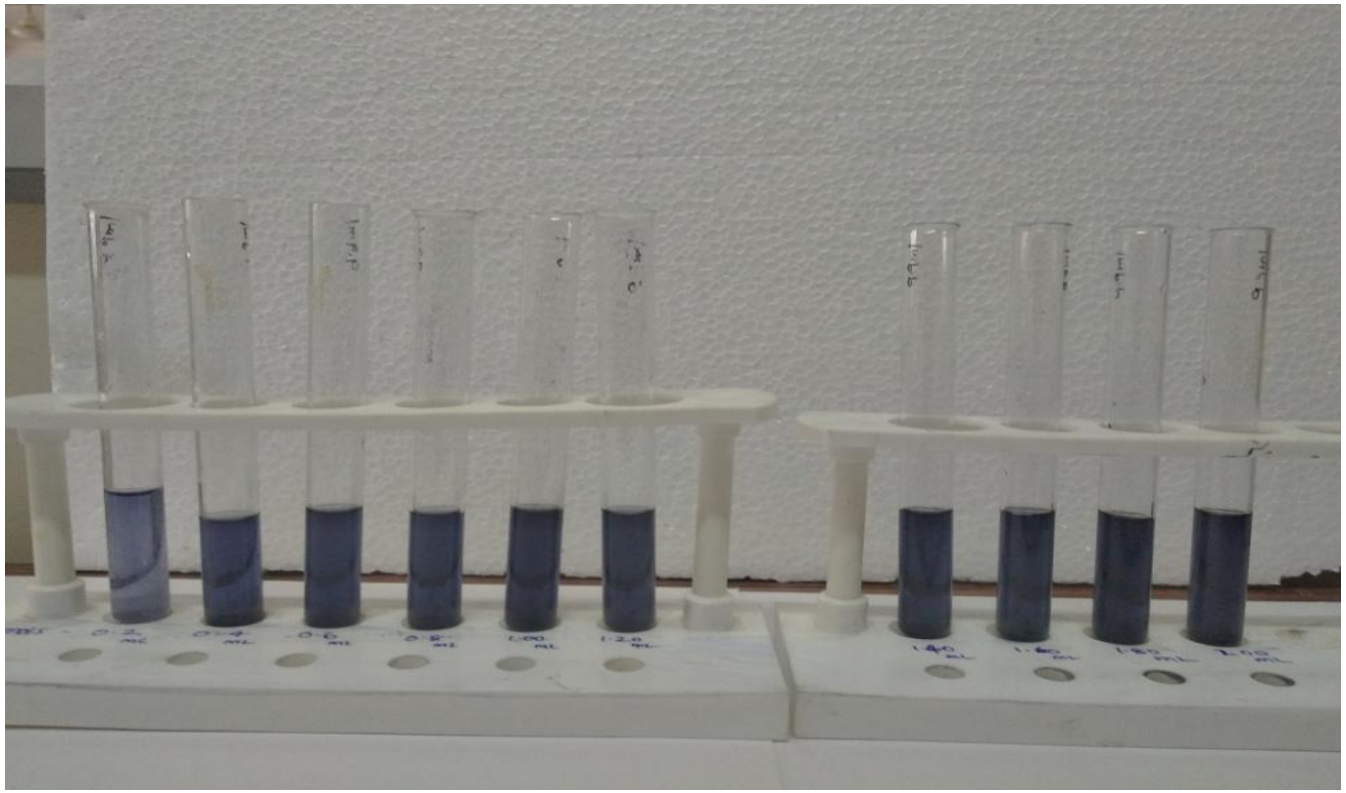


Plate 4.6. Biochemical analysis for estimation of phenol content

4.4.3 Evaluation of presence of auxins in the infected ULCD plants and healthy plants

Auxin plays an important role in plant growth and development and by mutations in Aux signalling pathways or a responsive factor which shows peculiar growth phenotypes. So the presence of auxins is to be known for understanding the involvement of auxins in host plant interactions. For this, the qualitative analysis of auxins was done *i.e.,* extraction, purification and detection. For purification QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) methodology was followed and for detection Salkowski reagent (Mix 2 ml of 0.05M $FeCl_3$ and 100 ml of 5% $HClO_4$) was used which gives pink color on reaction with auxins in the test samples. Thin layer chromatography (TLC) plates was spotted equi distantly with standard, infected and healthy test samples of 100µl each and after 6 hrs the R_f values were recorded as 0.92,0.896 and 0.901 respectively (table 4.12).

Table 4.12. Calculation of R_f values for infected and healthy ULCD leaves.

S.No.	Test sample plants	Distance travelled by solvent	Distance travelled by solute	R_f values
1	Standard	12.5	11.5	0.920
2	Infected	12.5	11.2	0.896
3	Healthy	12.2	11	0.902

The amount of auxin on the TLC plate was indicated by the amount of reaction that was seen between the auxin and the reagent. Color development in infected ULCD plant was found to be more when compared to healthy plant (plate 4.7).

These results are supported with the explanation that the pathogen infections were observed to cause up regulation in the expression of genes which are encoding the enzymes involved in auxin biosynthesis as well as of auxin signalling. This finally results in the disruption of AUX / IAA repressor proteins and enhanced plant susceptibility by increased auxin responses in *Arabidopsis*. (O'Donnell *et al.,* 2003; Dharmasiri *et al.,* 2005; Thilmony *et al.,* 2006; Kazan and Lyons, 2014).

Kazan and Manners (2009) reported some of the pathogen (*Pseudomonas solanaceraum)* have the ability to produce the auxins and in addition to the auxins initially produced by tomato plant.

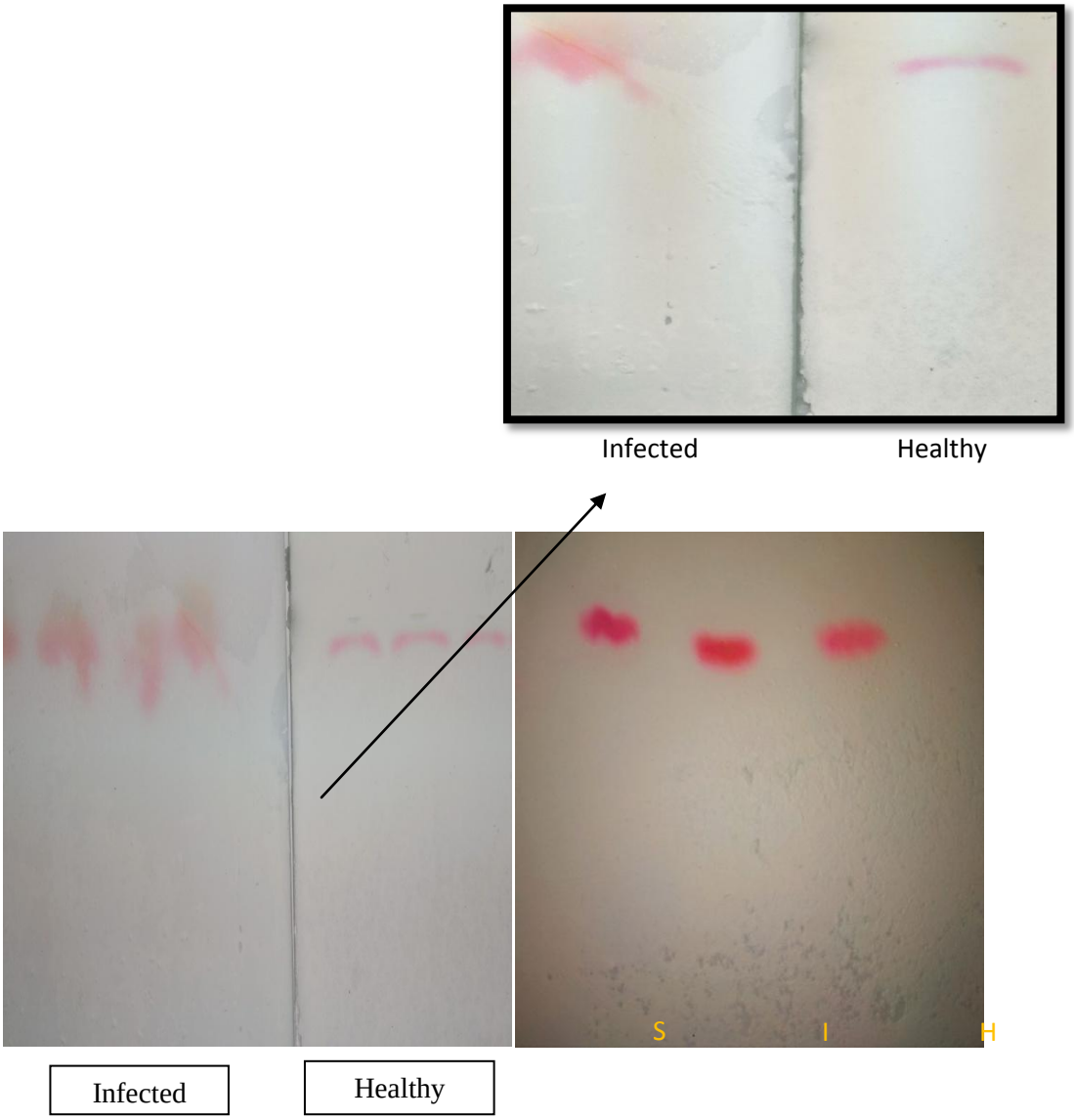


Plate 4.7. Estimation of auxins in healthy and infected plants of ULCD

Chapter – V

Summary and Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

The present investigation on "Studies on *urdbean* leaf crinkle disease on *urdbean* [*Vigna mungo* (L.) Hepper]" was carried out in order to study different modes of transmission of ULCD, the biochemical changes in *urdbean* infected with ULCD and to evaluate micronutrients effect on ULCD incidence in field. The results obtained are summarized below:

Urdbean cv LBG 752 when tested for reaction to ULCD by sap inoculation method, it produced crinkling and curling symptoms.

Seed transmission studies on ULCD revealed that hot water treatment (50^0C for ten min) was significantly efficient in reducing (70.60%) the disease incidence than seed treatment with trisodium orthophosphate at the rate of 16% (29.40%) over untreated control.

The studies on virus-vector relationship of ULCD and aphids (*A. craccivora*) revealed that the ULCD transmission was maximum (10%) with an acquisition access period (AAP) of ten min. There was a decrease in transmission of ULCD with further increase in AAP. Beyond 20 min of AAP the insects failed to transmit the disease. The inoculation access period was found to be 12 h (6.67%) and a maximum of 24 h (20.00%) for the successful transmission of the disease. 10 viruliferous aphids could able to transmit the ULCD. With increase in number of insects, per cent transmission was also increased, but the maximum transmission was observed with 20 aphids (13.33%).

Transmission studies of ULCD with beetle, *Henosepilachna dodecastigma* under greenhouse conditions with different acquisition access periods of 30 min, 6 and 24 h revealed that transmission of ULCD at 6 h found to be more successful (3.33%) and inoculation access periods of 12 h and 24 h while beetle transmitted ULCD only at 12 h with 6.67%.

Biochemical analysis for total protein, total phenol and auxins were carried out using the leaf samples obtained from the micronutrients, insecticides and trisodium orthophosphate treated management plots for ULCD and the analysis revealed that

infected leaf tissue contains more proteins when compared to healthy leaf tissue. At 40 DAS the protein content in healthy leaf tissues ranged from 1.43 to1.34 mg/100 mg of leaf tissue, whereas maximum was observed in $MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%) and minimum was observed in check. In case of infected leaf tissue, it ranged from 1.97 to 1.44 mg/100 mg of leaf tissue and highest was observed in $ZnSO_4$ (0.2%) and lowest was check. At 55 DAS the protein content in treatments of healthy leaf tissue ranged from 1.71 to 1.41 mg/100 mg of leaf tissue. Maximum was observed in $MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%) and minimum was in check. In infected leaf tissue the highest protein content was observed in $ZnSO_4$ (2.18 mg/100 mg of leaf tissue) and lowest was in check 1.69 mg/100 mg of leaf tissue. At 70 DAS the highest protein content (2.35mg/100 mg of leaf tissue) in healthy leaf tissue was observed in $MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%) and the lowest (1.63mg/100mg of leaf tissue) was in check. In infected leaf tissue the highest protein content was observed in $ZnSO_4$ (2.18 mg/100 mg of leaf tissue) and lowest of 1.69 mg/100 mg of leaf tissue.

The total phenol content was highest in infected leaf tissue and the lowest in healthy leaf tissue. At 40 DAS the phenols content ranged from 0.60 to 0.34mg/100 mg of leaf tissue. Maximum phenol content was observed in $MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%) and minimum was check and in infected it ranges from 0.89 to 0.51mg/100 mg of leaf tissue and the highest was observed in $MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%) and lowest was check. At 55 DAS the phenols content in treatments of healthy leaf tissue ranged from 0.66 to 0.40 mg/100 mg of leaf tissue. Maximum was observed in $MgSO_4$ + $ZnSO_4$ and minimum was in check. In infected leaf tissue the highest phenol content was observed in$MgSO_4$+$ZnSO_4$ (0.92 mg/100 mg of leaf tissue) and the lowest was in check (0.55 mg/100 mg of leaf tissue). At 70 DAS the phenol content in healthy leaf tissue was observed to be the highest in $MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%) (0.72mg/100 mg of leaf tissue) and lowest was in check (0.45mg/100mg of leaf tissue). In infected leaf tissue the highest phenol content was observed in $MgSO_4$ + $ZnSO_4$ (0.97 mg/100 mg of leaf tissue) and lowest was in check (0.59 mg/100 mg of leaf tissue).

Auxins were present in both the healthy and infected leaves of *urdbean* cv LBG 752, but apparent increase of colour was observed on TLC plates in infected leaf sample with the R_f value of 0.896 than healthy leaf samples with the R_f value of 0.901.

Among the six micronutrient treatments, one systemic insecticide and seed treatment with trisodium orthophosphate tested against ULCD infection, all the treatments were found to be effective in reducing the ULCD infection when compared to check. However, Zinc sulphate @ 0.2% and combination of Magnesium sulphate @ 0.2% + Zinc sulphate @ 0.2% were found to be highly effective in reducing the ULCD infection with lower AUDPC values(567.84 and 466.97 respectively) followed by $MnSO_4$ (0.2%) + $ZnSO_4$ (0.2%), Dimethoate (0.2%) $MnSO_4$ (0.2%) + $MgSO_4$ (0.2%), $MnSO_4$ (0.2%), $MgSO_4$ (0.2%) and Seed treatment with trisodium orthophosphate (16%) treatments with AUDPC value of 583.94, 648.31, 651.11, 681.27, 684.39 and 750.68 respectively as against 955.92 AUDPC value in unsprayed control.

The highest yield (14.21 q ha^{-1}) was observed in $MgSO_4$ (0.2%) + $ZnSO_4$ (0.2%) with the highest BC ratio of 2.11, followed by $ZnSO_4$(0.2%) with the yield of 13.91q ha^{-1} and the BC ratio was 2.09. The lowest yield (10.51q ha^{-1}) was observed in check with the BC ratio of 1.42.

Literature Cited

LITERATURE CITED

Ahmad, B.Y., Sharif, F.M and Sarhan, A.R.T. 1987. Effect of certain micro-nutrients on Fusarium wilt of tomato.*Journal of Agriculture and Water Resistant Plant Protection*. 6: 13-28.

Amin, K.S., Baladev, B and Williams, F.J. 1978. Transmission of *Leaf crinkle virus* of *urdbean* by vaccum infusion of extract into water soaked seeds. *Current Research*. 6: 98-99.

Andrade, O., Latorre, B.A and Escaffi, O.1981. Tomato mosaic-virus associatedwith shoestring symptom in Chilean tomatoes. *Plant Disease.*65: 761–762.

Ashfaq, M., Khan, M.A and Javed, N. 2008.Characterization of environmental factors conducive for *urdbean* leaf crinkle (ULCV) disease development. *Pakistan Journal of Botany*. 40: 2645-2653.

Ashfaq, M., Khan, M.A., Mughal, S.M., Javed, N., Shahid, M and Sahi, S. T. 2010. Effect of urdbean leaf crinkle virus infection on total soluble proteins and antioxidant enzymes in blackgram plants.*Pakisthan Journal of botany*.42(1): 447-45.

Ashfaq, M., Khan, M.A., Mukhtar, T and Sahi, S.T. 2014. Role of mineral metabolism and some physiological factors in resistance against *urdbean leaf crinkle virus* in blackgram genotype. *International journal of agriculture and biology.* 16:189-194.

Basavaraj, K. 2012. Alleviation of the effects of *mungbean yellow mosaic virus* infection in blackgram through foliar application of salicylic acid, triacontanol and mineral nutrients.*M.Sc (Ag.)Thesis.*Acharya N G Ranga Agricultural University, Bapatla.

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

Beniwal, S.P.S and Chaubey, S.N. 1984. Internal seed-borne nature of *Urdbean leaf crinkle virus* in *urdbean* seed.*Seed Research*. 12: 8-10.

Beniwal, S.P.S., Bharathan, N and Chaubey, S.N. 1983a. Two cucurbitaceous hosts of *Urdbean leaf crinkle virus*.*Indian Phytopathology*. 6: 577-579.

Beniwal, S.P.S., Chaubey, S.N and Bharathan, N. 1980.Presence of *Urdbean leaf crinkle virus* in seeds of *mungbean* germplasm.*Indian Phytopathology*. 33: 360-361.

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

Bhagavan, V.B. 1985. Studies on the effect of *Leaf crinkle virus* infection on growth and physiology of blackgram (*Vigna mungo* (L.) Hepper).*M. Sc.* (*Ag.*) *Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India.

Bhagavan, V.B. 1985. Studies on the effect of *leaf crinkle virus* infection on growth and physiology of blackgram (*Vigna mungo* (L.) Hepper).*M. Sc.* (*Ag.*) *Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India.

Bhakthavatsalam, G., Nene, Y.L and Beniwal, S.P.S. 1983. Ultrastructural changes in *urdbean* leaves infected by *Urdbean leaf crinkle virus*. *Indian Phytopathology*. 36: 228-232.

Bhardwaj, S.V and Dubey, G.S. 1986. Studies on the relationship of *Urdbean leaf crinkle virus* and its vectors, *Aphis craccivora* and *Acyrthosiphon pisum*. *Journal of Phytopathology*. 115: 83-88.

Bhavani, G. 2016. Studies on leaf crinkle disease of *Urdbean* (*Vigna mungo* (L.)Hepper).*M.Sc (Ag.)Thesis.*Acharya N G Ranga Agricultural University, Bapatla.

Bilqees, I.,Iftikhar, Y., Kamran, M., Mubeen, M., Ullah, M.I., Tahir, M.A and Hussain, Z. 2016.Management of okra yellow vein mosaic disease (OYVMD) through micronutrients and botanicals in relation to disease progression and environmental factors.*Transylvanian Review*.24(8).

Bindra, O.S. 1971. Studies on arthropods in relation to plant diseases in Punjab. Paper presented at the *Second International Symposium. Plant Pathology. IARI, New Delhi.*27th Jan- 3rd Feb.

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. *International Journal of Agriculture and Biology*. 13: 411-414.

Bobade, A., Chaurasiya, A and Katare, S. 2009. Study on testing the role of micronutrients on chlorotic mottle (*Geminivirus*) disease development in frenchbean (*Phaseolus vulgaris* L.). *International Journal of Plant Protection*. 2: 12-14.

Bradley, R.W.E. 1959. Loss of virus from the stylet of aphid.*Virology*. 8: 308-318.

Brar, J.S and Rataul, H.S. 1986. Some field characteristics of *leafcrinkle virus* of *urdbean, Vigna mungo* (L.) Hepper.*Indian Journal of Virology*. 2: 41-56.

Brar, J.S and Rataul, H.S. 1987. Evidence against the transmission of *Urdbean leaf crinkle virus* (ULCV) in mashbean,*Vigna mungo* (L.) through insects-Laboratory studies.*Indian Journal of Entomology*. 49: 69-72.

Brar, J.S and Rataul, H.S. 1990 Leaf crinkle virus induced biochemical changesin mash bean (*Vigna mungo)* and its effects on *Aphis craccivora* (Koch). *Journal of Insect Sciences.* 3:62–66

Chohan, J.S and Kalia, H.R. 1967. Virus diseases of *Phaseolus mungo* L. and their control through resistant varieties.Proceedings of International Pulse Workshop Conference-IARI, New Delhi.65-67.

Chowdhury, A.K and Chowdhury, S.R. 1983. Effect of buffers of different pH on the infectivity of *Urdbean leaf crinkle virus*. *Geobios*. 10: 34-35.

Chowdhury, A.K and Saha, N.K. 1985. Inhibition of *Urdbean leaf crinkle virus* by different plant extracts. *Indian Phytopathology*. 38: 566-568.

Dharmasiri, N., Dharmasiri, S and Estelle, M. 2005. The F-box protein TIR1 isan auxin receptor. *Nature* 435, 441–445.

Dhingra, K.L and Chenulu, V.V. 1981. Studies on the transmission of leaf crinkle and chickpea leaf reduction viruses. *Indian phytopathology*. 34: 38-42.

Dhingra, K.L. 1976. Transmission of *Urdbean leaf crinkle virus* by two aphid species.*Indian Phytopathology*. 28: 80-82.

Dordas, C 2008. Role of nutrients in controlling plant diseases in sustainable agriculture: a review. *Agronomy for sustainable development.* 28:33-46

Dubey, G.S and Sharma, I. 1985. Seed transmission of *Urdbean leaf crinkle virus*.*Seed Science and Technology*. 13: 149-153.

Dubey, G.S., Sharma, I and Prakash, N. 1983. Some properties of *Urdbean leaf crinkle virus*. *Indian Phytopathology*. 36: 762-764.

Duffus, C.M and Slaughter. 1980. Seed and their uses. *Wiley and sons Chichester*. New York. USA. 60-64.

Farooq, M., Wahid, A and Siddique, K.H.M. 2012.Micronutrients application through seed treatments – A review.*Journal of soil science and plant nutrition*. 12(1): 125-142.

Ghazvineh, S and Yousefi, M. 2012. Study the effect of micronutrients application on yield and yield components of maize. *Journal of Agricultural and Environmental Sciences*. 12(2): 144-147.

Gomez, K.A and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research.*Second edition, John Wiley and Sons Ltd., Singapore. 683.

Gooding, G. V., Jr. 1975. Inactivation of tobacco mosaic virus on tomato seed with trisodiumorthophosphate and sodium hypochlorite.*Plant DiseaseReporter* 59:770-772.

Graham, D.R and Webb, M.J. 1991.Micronutrients and disease resistance and tolerance in plants. In: Mortvedt, J.J., Cox, F.R., Shuman, L.M., Welch, R.M. (Eds.), Micronutrients in Agriculture, 2nd edition, Soil Science Society of America Inc., Madison, Wisconsin, USA.329-370.

Gupta, V.K. 1974. Leaf crinkle, a virus disease of *Phaseolus mungo* L. in Himachal Pradesh. *Indian Journal of Experimental Biology*. 12: 477-478.

Himanshu, N and Karuna, V. 2004.Role of seed borne inoculum of *Leaf Crinkle Virus* in disease development and yield of *urdbean.Annals of Plant Protection Sciences.* 12: 452-453.

Himanshu, N and Karuna, V. 2006.*Urdbean* leaf crinkle infection in relation to plant age, seed quality, seed transmission and yield in *urdbean*. *Annals of Plant Protection Sciences.*14: 169-172.

Huber, D.M and Graham, R.D. 1999. The role of nutrition in crop resistance and tolerance to disease. In: Rengel Z. (Ed). Mineral nutrition of crops, fundamental mechanisms and implications.Food product press, New York.205-226.

Indiastat, 2016-17. https://www.Indiastat.com /agriculture-data/2/stats.aspx

Irshad, M., Abbas, G., Aslam, Z., Abbas, Z., Aslam, M., Bukhash, K.M and Amer, M. 2012. Nutrient management for *Cotton leaf curl virus* (CLCV), *begomovirus*. *Crop and Environment*. 31: 32-6.

Islam, M.R., Ali, M.A., Islam, M.S and Hossain, A.F.M.G.F. 2002.Effect of nutrients and weeding on the incidence of *mungbean* mosaic.*Pakistan Journal of Plant Pathology*. 1: 48-50.

Janaki Ratnam, N. 2015. Studies on viral diseases of *urdbean* [*Vigna mungo* (L.)Hepper].*M. Sc.*(*Ag.*) *Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India.

Jayasekhar M. and Ebenezar E. G. 2016. Management of powdery mildew of black gram (*Vigna mungo*) caused by *Erysiphe polygoni*.*Agricultural Science Digest.*36 (1):72-74.

Kadian, O.P. 1980. Studies on leaf crinkle disease of *urdbean* (*Vigna mungo* (L.) Hepper), *mungbean* (*V. radiata* (L.)Wilczek) and its control.*Ph. D. Thesis*. Haryana Agricultural University, Hisar, India.

Kadian, O.P. 1983. Studies on weed plants as host range of *urdbean leaf crinkle virus*. *Haryana Agricultural UniversityJournal of Research*. 4: 602-603.

Kadian, O.P. 1989. Effect of environment on incidence and development of leaf crinkle disease in *urdbean*. *Indian Phytopathology*.42: 272.

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

Kanimozhi, S., Ganapathy, T. and Rajinimala, N. 2009. Seed transmission of ULCV in *mungbean* and *urdbean* plants infected with both MYMV and ULCV. *Archives of Phytopathology and Plant Protection.* 42: 401-408.

Karthikeyan, G. 2002. Management of *urdbean leaf crinkle virus* through induced systemic resistance in blackgram [*Vigna mungo* (L.) Hepper].*M. Sc.* (*Ag.*) *Thesis*.Tamil Nadu Agricultural University, Coimbatore, India.

Karthikeyan, G., Doraisamy, S and Rabindran, R. 2009.Induction of systemic resistance in blackgram (*Vigna mungo*) against *urdbeanleaf crinkle virus* by chemicals.*Archives of phytopathology and plant protection*.42: 1-15.

Kazan, K and Lyons, R. 2014. Intervention of phytohormone pathways bypathogen effectors.*The Plant Cell*.26: 1–26.

Kazan, K and Manners, J.M. 2009.Linking development to defense: auxin inplant–pathogen interactions.*Trends in Plant Science.*14: 373–382.

*Khatri, H.L., Bhatia, D.S and Chohan, J.S. 1971. Brief account of the work done on diseases of *kharif* pulse crops at the Department of Botany and Plant Pathology PAU, Ludhiana during 1970-71.5th*All India Pulse Improvement Programme Workshop*.Hissar.

Kolte, S.J and Nene, Y.L. 1970. Know the leaf crinkle disease of *urdbean*. *IndianFarmers Digest*. 3: 6-7.

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

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

Kolte, S.J and Nene, Y.L. 1975. Host range and properties of *urdbean leaf crinkle virus*. *Indian Phytopathology*. 28: 430-431.

Krishnaveni, D. 1988. Studies on transmission of *Blackgram leaf crinkle virus*. *M. Sc.* (*Ag.*)*Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India.

Lokeshbabu, A. 1997. Studies on patho-physiology and induction of resistance to leaf crinkle disease in blackgram [*Vigna mungo* (L.) Hepper].*M. Sc.* (*Ag.*)*Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India.

Lowry, O.H., Rosebrough, N.J., Farr, A.Land Randall, R.J. 1951.Protein measurement with the folin-phenol reagent.*Journal of BiologicalChemistry*.193: 265-275.

Mahajan, P.D and Joi, M.B. 1999. Survey and seed transmission of *Leaf crinkle virus* of *mung* and *urdbeans.Seed Research.* 27:131-133.

Malick, C.P and Singh, M.B. 1980. Quantification of total phenol content.*InPlant Enzymology and Histo Enzymology*.Kalyani publishers, New Delhi,286.

Marschner, H. 1995.*Mineral Nutrition of Higher Plants*.2nd edition.Academic press, London. 889.

Masood Ali and Shivkumar. 2000. Problems and prospects of pulses research in India. *IndianFarming*.4-13PP.

Mishra, A., Gohel, V.R and Patel, J.G. 1994. Extent of seed transmission of *urdbean leaf crinkle virus* in Gujarat. *Gujarat Agricultural University Research Journal*. 19: 130-132.

Murthy, B.V.R. 1996. Studies on management of leaf crinkle disease of blackgram [*Vigna mungo* (L.) Hepper].*M. Sc.* (*Ag.*)*Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India.

Nageswararao, G.V. 2002. Studies on leaf crinkle disease of blackgram [*Vigna mungo* (L.) Hepper].*Ph. D. Thesis*. Acharya N G Ranga Agricultural University, Hyderabad, India.

Narayanasamy, P and Jaganathan, T. 1973a. Sources of resistance to blackgram virus diseases.*Madras Agricultural Journal*. 60: 1836-1838.

Narayanasamy, P and Jaganathan, T. 1973b. Vector transmission of *Blackgram leaf crinkle virus*.*Madras Agricultural Journal.* 60: 651-652.

Narayanasamy, P and Jaganathan, T. 1975a. Effect of *Blackgramleaf crinkle virus* infection on seed set and distribution of virus in the seeds. *Madras Agricultural Journal*. 62: 151-154.

Narayanasamy, P and Jaganathan, T. 1975b. Studies on seed transmission of *Blackgram leaf crinkle virus* - effect of age of plants at infection. *Madras Agricultural Journal*. 62: 287-290.

Nariani, T.K and Sastry, K.S.M. 1962. Studies on the relationship of *Chilli mosaic virus* and its vector, *Aphis gossypii* Glover.*Indian Phytopathology*. 15: 173-183.

Nath, P.S., Khan, M.A and Chowdhury, A.K. 1986.Transmission of *Urdbean leaf crinkle virus* by *Lipaphis erysime* and *Hysterneura setariae*.*Indian Journal of Plant Pathology*. 4: 198-199.

Nene, Y.L. 1968. A survey of the viral diseases of pulse crops in Uttar Pradesh. First Annual Report, FG-In-358, Uttar Pradesh Agricultural University.1-25.

Nene, Y.L. 1970. A survey of the viral diseases of pulse crops in Uttar Pradesh. Third Annual Report, F.G-In-358, Uttar Pradesh Agricultural University, Pantnagar.1-26.

Nene, Y.L. 1971. A survey of viral diseases of pulse crops in Uttar Pradesh. Fourth Annual Report, F.G-In-358, Uttar Pradesh Agricultural University.1-41.

Nene, Y.L. 1972. A survey of viral diseases of pulse crops in Uttar Pradesh. Final Technical Report, F.G-In-358, G. B. Pant University of Agriculture and Technology, Pantnagar.1-187.

Nene, Y.L. and Kolte, S.J. 1972.Leaf crinkle.Research Bulliten.G.B. Pant University of Agriculture and Technology. 4: 109-134.

O'Donnell, P.J., Schmelz, E.A., Moussatche, P., Lund, S.T., Jones, J.B and Klee, H.J. 2003. Susceptible to intolerance-a range of hormonal actionsin a susceptible *Arabidopsis* pathogen response.*The Plant Journal*.33: 245–257.

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

Prasad, M.S., Sarma, B.K., Sangit, K and Prasad, M.S.L. 1998. Transmission tests and varietal screening for *urdbean leaf crinkle virus* in blackgram (*Vigna mungo* L. Hepper). *Annals of Plant Protection Sciences.* 6: 205-207.

Pratap, D., Kumar, S., Snehi, S.K and Raj, S.K**.**2012. Biological and molecularcharacterization of *cucumber mosaic virus* isolate causing shoestring disease oftomato in India which has closer affinity to European or East Asian isolates of CMV.*Indian Journal of Virology*.23: 57–63.

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 Research*. 27: 112-115.

Rachie, K.O and Roberts, L.M. 1974. Grain legumes of low land tropics.*Advances in Agronomy*. 26: 1-132.

Rao, G.V.N and Reddy, D.R. 2004. Natural occurrence of leaf crinkle disease on blackgram in north coastal districts of Andhra Pradesh. *Journal of Research, ANGRAU.* 32: 96-98.

Rao, G.V.N and Reddy, D.R. 2005a.Effect of different stages of inoculation of ULCV on yield of blackgram.*Journal of Research, ANGRAU*. 33: 87-90.

Rao, G.V.N and Reddy, D.R. 2005b. Floral malformation in infected flowers and seed transmission of leaf crinkle disease in *urdbean*. *Indian Journal of Pulses Research*. 18: 256-257.

Ravandar, R., Tonapi Vilas, A.C., Varanavasiappn, S., Navi, S.S and Jayarajan, R. 2006. Management of urdbean leaf crinkle disease in urdbean(*Vignamungo* L. Hepper). *International Journal of Agricultural Sciences.* 2:8–10

Reddy, C.R. 1988.Studies on *urdbean* [*Vigna mungo* (L.) Hepper] *leaf crinkle virus*. *Ph. D. Thesis.*Tamil Nadu Agricultural University, Coimbatore, India.

Reddy, C.R.,Vilas, CH., Tonapi, V.A., Navi, S.S and Jayarajan, R. 2005a. Influence of plant age on infection and symptomological studies on Urdbean leaf crinkle virus in *urdbean* (*Vigna mungo* (L.) Hepper).*International Journal of Agricultural Sciences*. 1: 1-6.

Reddy, C.R., Tonapi, V.A., Varanavasiappan, S., Navi,S.S and Jayarajan, R. 2005b. Studies on seed transmission of *Urdbean leaf crinkle virus* on *Vigna mungo*.*Indian Journal of Plant Protection.* 33: 241-245.

Reddy, G.P.D., Reddy, S.S., Reddy, G.C and Sivaram, T.G. 2018. Effect of Foliar Application of Micronutrients on Growth and Yield Parameters in Tomato (*Solanum lycopersicon* L.). *International Journal of Pure and Applied Bioscience*. 6(2): 929-934.

Sharma, I and Dubey, G.S. 1983. Histological changes in *urdbean* flowers due to *urdbean leaf crinkle virus* infection. Zeitschrift fur plannzenkran.Kheiten und planzenschutz.*Journal of Plant Disease and Protection*. 90: 63-67.

Sharma, I and Dubey, G.S. 1984. Control of *leaf crinkle virus* through heat treatment, chemotherapy and resistance. *Indian Phytopathology*.37: 26-30.

Simoglou, K. B and Dordas, C. 2006. Effect of foliar applied born, manganese and zinc on tan spot in winter durum wheat. *Crop Protection*. 25: 657-663.

Singh, R.N. 1982. Natural occurrence of leaf crinkle disease on common bean. *Indian Phytopathology*. 35: 320-321.

Singh, J.P., Kadian, O.P and Varma, J.P. 1979. Survey of virus diseases of *mungbean* and *urdbean* in Haryana.*Haryana Agricultural University Journal Research*. 9: 345-347.

Singh, N and Kathayat, K. 2018. Integrated Application of Micronutrients to Improve Growth, Yield, Quality and Economic Yield in Potato- A Review.*International Journal of Current Microbiology*. 7(8): 2930-2935.

Singh, R.N. 1984. Further studies on leaf crinkle disease of cowpea. *Indian Journal of Plant Pathology*. 2: 199-200.

Singh, S and Awasthi, L.P. 2004.Varietal screening of *urdbean* against *Mungbean yellow mosaic virus* under field conditions.*Annals of Plant Protection Sciences*. 12: 225-226.

*Smith, J.L. 2011. The seed bin- May 2011.http://www.highmowingseeds.com/SB Seed-borne-Disease-and-Its-Control.html.

Srivastava, S and Singh, A.K. 2010. Changes in catalase activity and total protein content in urdbean plants as a result of ulcv infection [(L.) Hepper] *Vigna mungo*. *Indian Journal of Scientific Research*, 1(2): 67-69.

Subbarao, K. 1984. Studies on leaf crinkle disease of blackgram. *M. Sc.* (*Ag.*)*Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India. 2(1): 12-14

Suneela, R. 1996. Studies on *Blackgram leaf crinkle virus*. *M. Sc.* (*Ag.*)*Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India.

*Sylvester, E.S. 1954. Aphid transmission of non-persistant plant viruses with special reference to the *Brassica nigra virus*.*Hilgardia*. 23: 53-98.

Tengoua, F.F., Hanafi, M.M., Idris, A.S., Jugah, K., Azwa, J.N.M., Hasmah, M and Omar, S.R.S. 2014.Effect of micronutrients-enriched fertilizers on basal stem rot disease incidence and severity on oil palm (*Elaeis guineensis* Jacq.) seedlings. *American Journal of Applied Sciences*. 11: 1841-1859.

Thilmony, R., Underwood, W and He, SY. 2006. Genome-wide transcriptionalanalysis of the*Arabidopsis thaliana* interaction with the plant pathogen*Pseudomonas syringae* pv. *tomato*DC3000 and the human pathogen*Escherichia coli* O157:H7. *The Plant Journal*.46: 34–53.

Triplehorn, C.A and Johnson, N.F. 2005.*Borror and Delong's introduction to the study of insects,* Brook/Cole Cengage learning, U.S.A., 103-400

Vijaykumar, S. 1993. Studies on *Blackgram leaf crinkle virus*. *M. Sc.* (*Ag.*)*Thesis*.Acharya N G Ranga Agricultural University, Hyderabad, India.

Williams, F.J., Grewal, J.S and Amin, K.S. 1968. Serious and new diseases of pulse crops in India in 1966.*Plant Disease Reporter*. 52: 300-304.

www.spectrumanalytic.com

Zeshan, M.A., Ali, S., Khan, M.A and Sahi, S.T. 2012. Role of nutrients and naphthalene acetic acid in the management of *urdbean leaf crinkle virus*. *PakistanJournal of Phytopathology*. 24: 79-81.

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