INVESTIGATIONS ON LEAF CRINKLE VIRUS DISEASE IN GREENGRAM

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CERTIFICATE

This is to certify that the thesis entitled **"INVESTIGATIONS ON LEAF CRINKLE VIRUS DISEASE IN GREENGRAM"** submitted by **Mr. PUNITH KUMAR C.H.** for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **PLANT PATHOLOGY** to the University of Agricultural Sciences, Raichur, is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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(VENKATESHALU)

Affectionately Dedicated

To

My Beloved Parents
Smt. Shivamma and Sri. Hiriyanna,
Brother Shivakumar and
Sister, Vinutha

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Raichur

July, 2013 ***(PUNITH KUMAR, C.H.)***

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LIST OF ABBREVIATIONS

%	:	Percentage
<	:	Lesser than
µg	:	microgram
µl	:	micro litre
^{o}C	:	degree celcius
a.i.	:	active ingredient
AAP	:	Acquisition access period
B:C	:	Benefit Cost ratio
bp	:	Base pair
CD	:	Critical difference
cm	:	centimeter
cv.	:	Cultivar
DAS	:	Days after sowing
DBS	:	Day before spraying
DAS	:	Day after spraying
EC	:	Emulsifiable concentration
et al.	:	and others
Fig.	:	Figure
FS	:	Flowable solution
g	:	gram
hr	:	hours
ha	:	hectare
IAP	:	Inoculation access period
kg	:	kilograms
kb	:	kilo base pair
l	:	litre
m	:	meter
M	:	Molar

ml	:	miligram
mm	:	milimolar
mm	:	milimeter
min	:	minute
No.	:	Number
N	:	North
ppm	:	Parts per million
q	:	quintal
Sl	:	Soluble liquid
SEm	:	Standard error of means
Sl.	:	Serial
rpm	:	Rotation per minute
Rs.	:	Rupees
WS	:	Water soluble
v/v	:	volume by volume
viz.,	:	Namely
X	:	Magnification
±	:	Plus or minus

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Introduction

I. INTRODUCTION

The legumes are popularly called pulses. Pulses are actually seeds of leguminous plants. They are grown throughout the world, especially in tropical and sub-tropical countries, where the annual rainfall is about 300 to 350 mm per annum. The pulses play an important role in preventing malnutrition, as they are the major protein sources in the vegetarian diet. They contain about 24 to 25 per cent protein, 58 per cent carbohydrates, 2 to 5 per cent oil and micronutrients (Calcium and iron). The sprouted seeds are rich in vitamins (A, B and C). Besides being rich sources of protein, they help in maintaining soil fertility through biological nitrogen fixation by the bacteria present in their root nodules. Apart from this, green plants of pulses are used as cattle feed and unripened pods are used as vegetables.

India ranks second in pulse production in the world, sharing about 35 per cent of cultivated land with a productivity of 27 per cent. India has 22.5 million ha under pulse cultivation with an annual production of 14.85 million tons. The total production of pulse is usually in the range of 12 to 14 million tons (Anon., 2012). The important pulse crops grown in India are bengalgram, lentil, greengram, blackgram, cowpea, redgram and pea.

Greengram [*Vigna radiata* (L.) Wilczek, Syn, *Phaseolus aureus* Roxb., *Phaseolus radiataus* L.] is one of the thirteen food legumes grown in India and third most important pulse crop of India after chickpea and pigeonpea. Greengram has many common names *viz*., mung, moong, mungo, greengram, goldengram, chickasawpea and oreganpea. In India, the name greengram is more commonly used than mungbean. The mungbean is native to Indo-Burma region of South-East Asia and is cultivated extensively in the Indo-Burma-Thailand region of Asia.

It is an ancient and well known leguminous crop of Asia. It is popular because of its nutritive value, restoring the fertility of the soil by way of addition of the nitrogen to the soil. It also helps in soil conservation through thick canopy, relatively more tolerant to moisture stress conditions, fits well in double, mixed and relay cropping systems due to their short duration besides providing nutritious fodder and very good green manure.

Presently, the per capita share of pulses in nutrition supply in India with respect to energy, protein and fat is 117.4 K cal, 6.9 g and 1.0 g per day respectively. An adult male

and female requires 80 and 70 g per capita per day, respectively for balanced diet (Anon., 2004).

Greengram is highly relished pulse rich in proteins (23-24%), carbohydrates (54-56%), minerals and vitamins, particularly lysine which is deficit in cereals. So, it is rightly called the poor man's meat. It has high digestibility due to which it is fed to babies, convalescents and elders. Unlike other pulses, it is free from flatulent effects in stomach.

Greengram is successfully tested and found to be the most suitable pulse for the preparation of pharmaceutically important molecules (Kumaraswamy and Ramesh, 2003). The seeds are used for treating rheumatism, a kind of nervous system ailments and liver afflictions, while the roots are narcotic used for relieving bone pains. It is consumed in many forms including the grains (whole or split). The whole grains are eaten after germination parched, salted with sugar or boiled with condiments. It is also consumed as a boiled dal, bean cakes, noodles and pudding.

Greengram is grown mainly as a *kharif* crop. However, its cultivation in *Rabi* season is restricted to the eastern and southern parts of the country. The major greengram growing states are Orissa, Maharashtra, Andhra Pradesh, Rajasthan, Karnataka and Gujarat. It ranks third among all pulses grown in India after chickpea and pigeonpea. In India the total production of greengram is 10.34 lakh tons from an area of 28.19 lakh ha with a productivity of 420 kg ha^{-1} (Anon., 2012). The Hyderabad Karnataka area particularly Bidar, Yadgir and Gulbarga districts have an extensive area with greengram, pigeonpea and bengalgram. Hence, these regions are called "Pulse bowl" of Karnataka. In Karnataka, it occupies an area of 3.98 lakh ha with a production of 0.85 lakh tons with average an yield of 206 kg per ha (Anon., 2012).

The greengram suffers from several diseases caused by both fungi and viruses. Among the viral diseases, leaf crinkle is an important disease that infects the crop at various stages of the crop growth which reduces both quantity and quality of the seed. This disease has become one of the major production constraints in greengram especially during *kharif* and *rabi* seasons. The disease could cause crop losses to an extent of 94 per cent depending on season and variety cultivated (Kadian, 1980).

Williams *et al.* (1968) first reported the occurrence of the leaf crinkle on blackgram and greengram from the states of Delhi and Uttar Pradesh in India. Leaf

crinkle virus is an unclassified virus, seed borne, with narrow host range and aphid transmitted ssRNA viruses (Ashfaq *et al*., 2007). The symptoms of disease appear in the form of extreme crinkling, curling, puckering and rugosity of leaves, stunting of plants, malformation of floral organs and also pollen fertility and pod formation is also reduced severely in infected plants (Nene, 1972).

It is uncommon that one virus known to be transmitted by one group of insects is also transmitted by several other groups of insects. Perusal of the literature on leaf crinkle virus disease indicated that, the disease is reported to be transmitted by aphids (*Aphis craccivora* and *Myzus persicae*) in Delhi (Dhingra, 1975), West Bengal (Nath *et al.,*1986) and Himachal Pradesh (Bhardwaj and Dubey,1984), by leaf feeding beetles (*Henosepilachna dodecastigma*) in Pantnagar, Uttar Pradesh (Beniwal and Bharatan,1980 and Bharatan and Beniwal,1984) and by Whiteflies (*Bemisia tabaci*) in Tamil Nadu and Meghalaya (Narayanaswamy and Jaganathan,1973, Prasad *et al*.,1998 and Sahay *et al*.,1999).

Survey can be conducted either when the incidence has occurred or later. Survey can also be conducted to establish the magnitude of loss caused by a disease, pathogens that occur and their dynamics as described by Nagarajan (1983). Hence, a survey was undertaken.

Some of the varieties are resistant, tolerant or susceptible to leaf crinkle virus disease. The screening of greengram genotypes against the disease would be of great help to identify resistant source. Growing resistant varieties is the cheapest way of combating the disease. Hence, the resistant source can be used in breeding programme.

Effective management of insect vectors of plant pathogens is of crucial importance to minimizing vector-borne diseases in crops. Insecticides play an important role in managing vector populations by reducing the number of individuals that can acquire and transmit a virus, thereby potentially lowering disease incidence. Certain insecticides also play a role in protecting crop plants by virtue of anti-feedant properties that interfere with virus transmission. Studies on efficacy of these insecticides help to know which insecticides could effectively control the vector population and disease incidence of leaf crinkle virus, its effect on grain yield and cost benefit ratio as compared to other ones. These studies would help to control the disease with less expenses.

Many methods have been developed for the detection and identification of plant viruses. A single diagnostic test or assay may provide adequate information on the identity of a virus, but a combination of methods is generally needed for unequivocal diagnosis. Recent advances in techniques for the detection of proteins and nucleic acids have provided an opportunity to develop methods for the diagnosis of plant virus diseases.

A scan through literature revealed that there is meagre information on leaf crinkle virus disease on greengram from India. However, the virus is causing severe yield loss to the crop. Therefore, investigations were taken up with the aim of assessing the status of disease, in Karnataka and understanding the causal nature of the pathogen with the following objectives.

1. To undertake survey and surveillance for leaf crinkle virus disease of greengram.
2. To investigation on transmission of leaf crinkle virus disease of greengram.
3. To detect the leaf crinkle virus disease of greengram by electron microscopy.
4. To screen genotypes against the leaf crinkle virus disease of greengram.
5. To undertake management of leaf crinkle virus disease in greengram.

Review of Literature

II. REVIEW OF LITERATURE

Several viruses are known to infect greengram under natural condition in different parts of India, of which mungbean yellow mosaic, urdbean leaf crinkle, mottle and leaf curl disease are of serious concern. Cowpea mild mottle, southern bean mosaic, tomato spotted wilt, mung and urdbean mosaic-1 and mung and urdbean mosaic- 2 viruses may also pose serious problems under certain conditions (Krishna Reddy and Varma, 1988).

Among the viral diseases, leaf crinkle virus (LCV) is considered to be the most serious constraints causing considerable damage to the crop depending on season and variety being cultivated. During past few years the disease has been economically important and destructive posing severe threat on growth and yield of greengram crop. The literature pertaining to leaf crinkle virus disease, its occurrence, symptoms, causal virus, transmission nature and vector, economic importance, identification of resistant sources to LCV in greengram and other related literature are reviewed briefly under the following headings.

2.1 Occurrence of the disease

Leaf crinkle is an important virus disease on greengram and blackgram crop in tropics. In India, Chohan and Kalia (1967) for the first time reported a new disease from Indian Punjab under the name ‘curly top’ on *Phaseolus mungo* (L.) but William *et al.* (1968) observed this new viral disease on urdbean and mungbean in the states of Uttar Pradesh and Delhi.

Kolte and Nene (1972) named the virus associated with the disease as ‘Urdbean leaf crinkle virus’. Since then, it has reported from different parts of the country *viz*., Gorkhpur, Pratapgarh, Almora, Meerut, Nainital and Bulandshahr districts of Uttar Pradesh (Nene, 1972), Tamil Nadu (Narayanswamy and Jaganathan, 1973a), Harayana (Varma *et al*., 1973; Kadian 1982a and 1983b), Himachal Pradesh (Singh *et al*., 1979 and Bansal *et al*., 1984), Punjab (Bansal *et al*., 1984) Andhra Pradesh (Subba Rao, 1984, Vijay Kumar and Subba Rao, 1994a and Reddy *et al*., 1996), West Bengal (Chowdhury and Saha, 1985), Gujarat (Ashok Mishra *et al*., 1994 and Patel *et al*.,1999), North Eastern Hill region (Prasad *et al*.,1998 and Sahay *et al*.,1999) and Maharasthra (Mahajan and Joi,1999).

2.2 Economic Importance

Diseased plants remain partially or completely sterile resulting in heavy loss in yield was observed by Kolte and Nene (1972). Similar results of Yield loss of 62 to 100 per cent was recorded in blackgram cv.T9 due to urdbean leaf crinkle virus (ULCV) infection under natural conditions has been reported (Nene 1973; Kolte and Nene, 1979).

Beniwal and Chaubey (1979) has reported maximum yield reduction of 70.7 and 83.8 per cent when the blackgram cultivars Pant U- 30 and Pant U-26 were inoculated 10 days after sowing respectively.

The decrease in seed yield in the diseased blackgram plants was due to reduction in number of pods per plant (Singh, 1980, Kadian, 1982a and Ravinder Reddy, 1988).

Singh (1980) studied the effect of urdbean leaf crinkle virus disease on growth components and yield loss of some varieties of urdbean under natural conditions of infection and they reported 86 to 100 per cent loss in grain yield of urdbean cultivars T_9, Mash-1 and Mash 48. Loss in the grain yield was due to leaf crinkle virus disease at Hissar ranged from 2.12 to 93.98 per cent in mung bean cv. Varsha and 2.8 to 95.17 per cent in urdbean cultivar T_9. A direct correlation between stage of plant growth at which infection occurred and loss in grain yield was observed and stated earlier the infection, greater was the loss which was mainly attributed to reduction in number of pods (Kadian, 1982a).

Urdbean leaf crinkle virus disease have greater effect on reducing the plant height, root length, nodulation, pods per plant, length of the pods, seeds per pod, seed weight and yield losses of about 41.1 to 69.3 per cent in different cultivars of urdbean (Reddy *et al*., 1996).

Subba Rao (1984) reported that leaf crinkle has been considered as one of the major constraints for profitable cultivation of blackgram during various seasons especially in *rabi* and rice fallows conditions.

Leaf crinkle virus infection at early stages of the plants was known to reduce yield and affected almost all the components of urdbean and there is a reduction of 90.8 per cent in number of pods, 18.4 per cent in pod length, 26.5 per cent in seed weight and 81 per cent in yield of diseased plants was recorded (Bashir *et al*., 1991).

Ashok Mishra *et al*. (1994) reported that yield loss was mainly due to the decrease in size of the pods, number of seeds per pod and test weight due to leaf crinkle infection in urdbean cv.T9. Similarly, plants that are infected very early failed to produce any pods in susceptible blackgram cultivars T9, Buttaminumu, Chikkuduminumu, LBG 17, 20, 402, 611, 623, 648 and 685 (Ramanamurthy, 1997 and Suneela, 1996).

The least germination, highest mortality and percentage of infected plants from seeds collected from urdbean (*Vigna mungo*) cv. PU 19 showing systemic urdbean leaf crinkle virus symptoms. These seeds also recorded the lowest values for 1000 seed weight (31.64-33.1 per cent) and yield reduction from 21.20 - 23.50 per cent and also confirms that the seed transmission of leaf crinkle virus disease (Negi and Vishunavat, 2004).

2.3 Etiology

Evidence has been presented to show that urdbean leaf crinkle was caused by the sap transmissible urdbean leaf crinkle virus (ULCV) was first reported by Kolte and Nene (1972).

Dubey *et al*. (1983) studied the virus morphology with the partially purified preparation and also in ultra-thin section of infected leaves by leaf dip method and found that the spherical virus particles were with an average diameter of 320 A^{0}.

Ultra structural studies made with the crinkle disease affected urdbean leaves revealed for the first time and shown that the association of virus–like particles (VLP) in the nucleus, cytoplasm and chloroplasts. The VLP appeared to be spherical with a diameter of 25 to 30 nm which were absent in healthy cells (Bhaktavatasalam *et al*., 1983a).

2.4 Symptomatology

Williams *et al*. (1968) observed a new disease in urdbean germplasm in India that was caused by leaf crinkle virus with symptoms of puckering and crinkling of leaves, stunting of plants and rugosity.

Symptoms of blackgram leaf crinkle disease were studied in detail by several workers both under natural and artificial conditions (Kolte and Nene, 1970, Beniwal *et al*., 1979, Subba Rao, 1984, Krishnaveni, 1988, Vijay Kumar, 1993 and Ashok Mishra *et al*., 1994). Affected blackgram plants expressed various symptoms depending on the

stage of the crop at which the plants were infected. In naturally infected plants, the first recognisable symptoms usually appear 3 to 4 weeks after sowing on third trifoliate leaves which shows increase in size with light green colour. The conspicuous crinkling of leaves appear one week after the appearance of initial symptoms. First and second trifoliate did not show any increase in size and enlargement of leaflets is evident from third trifoliate onwards. With the age, crinkling and rugosity on older trifoliate decrease and crinkling will be more distinct on younger trifoliate. A month after appearance of initial symptoms, the tips of leaflets in the affected trifoliate particularly in the 4^{th}, 5^{th} and 6^{th} trifoliates curve downwards. In the later stages of the crop, the stipules at the base of the affected trifoliate become thicker and broader than the healthy ones. Another conspicuous symptom is that the petiole behind the pulvinus of the terminal leaflet become short so that the basal portion of its lamina closely touches the surface of the side two leaflets, thus giving the infected plant a bushy appearance.

The flowering in the affected plant is delayed by 8 to 10 days as compared to healthy plants. The delay in flowering however, depend upon the severity of the disease. The inflorescence produced from the axil of the affected trifoliate bear large number of small sized flower buds. The sepals of the flower bud are thick, greener than normal and cover half or all of the buds giving a bushy appearance to the inflorescence. The virus also affects the pollen fertility.

The symptoms on blackgram under artificial inoculated conditions correspond in all the aspects to conditions observed on plants naturally infected in field except that the symptoms first become evident on the second trifoliate instead of third trifoliate.

Brar and Rataul (1986) stated that the most characteristics symptoms were a wavy appearance on the third trifoliate leaf followed by crinkling of lamina, shortening of the petioles resulting in crowding of leaves. The sepals of flower became thicker and greener than normal. The leaf area of the diseased trifoliate was greater than that of healthy ones. The distribution of symptoms was not uniform in infected plants i.e. crinkling was observed in some branches while others remained apparently healthy.

Ravinder Reddy *et al*. (2006) made studies on the influence of plant age on infection of urdbean leaf crinkle virus (ULCV) and reported that inoculation at primary leaf stage (7 and 10 days after sowing) gave 85 and 72 per cent infection. The incubation period was short and symptoms were 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. Significant enlargement of leaflets was observed in infected plants from third trifoliate onwards. Reduction in rachis length of terminal leaflets of infected trifoliate and thickening of stem and petiole were evident in infected plant. Besides this, in all the infected plants the size of stipules also increased prior to the symptoms development.

2.5 Survey of the leaf crinkle virus disease of greengram

Nene (1972) conducted a survey in different districts of Uttar Pradesh and he reported that the yellow mosaic incidence in mungbean in different districts of Uttar Pradesh ranged from 5-100 per cent and depending upon the stage at which the plants were infected, the yield loss varied from 10-100 per cent. From his results, he also concluded that the virus causing urdbean mosaic was the same as the one causing mungbean yellow mosaic.

During investigations on the nature and rate of spread of ULCV under field conditions and the results revealed that slow spread of ULCV was attributed to absence of whitefly and aphid vectors with percentage infection recording 2.75 per cent (Beniwal *et al*., 1979)

A survey conducted in Haryana during 1975, 1976 and 1979 showed increased ULCV incidence and effect of the diseases was mild (Kadian, 1980).

Bansal *et al.* (1984) conducted a survey for the incidence of yellow mosaic in different districts of Punjab. The results revealed that yellow mosaic was more prevalent on mung bean and incidence was fairly less in districts of Bhatinda (4.50%), Ferozepur (1.25%) and Jalandhar (4.60%) whereas, its incidence was more in Sangrur (13.85%) and Gurdaspur (20.00%).

Patel *et al.* (1999) reported that 1 to 8.3% field incidence of ULCV was recorded in Gujarat. While, Mahajan and Joi (1999) surveyed 143 fields of blackgram and 78 fields of greengram during 1989 and 1990 in Maharashtra had recorded 12 to 30 per cent of ULCV incidence in both the crops.

An extensive survey conducted on viral diseases of mungbean and mashbean in Pakistan during 2004 and reported that, the incidence of mungbean yellow mosaic virus (MYMV) disease ranged from 4 to 40 per cent in mungbean and the incidence of urdbean

leaf crinkle virus (ULCV) has ranged from 5 to 28 per cent in mashbean were recorded respectively. (Muhammad Bashir *et al.*, 2006)

2.6 Transmission studies

2.6.1 Sap transmission

Early research workers (Chohan and Kaliha, 1967 and Williams *et al*., 1968) failed to transmit the blackgram leaf crinkle disease through sap inoculation.

Kolte and Nene (1972) first reported sap transmission of the virus by using 0.1 M potassium phosphate buffer of P^H 7.6 as extracting medium and carborundum as an abrasive. Subsequently similar studies conducted by different workers succeeded in transmission of the causal virus through sap inoculation with phosphate buffer of varying concentrations and P^H *viz*., 0.1M phosphate buffer at P^H 7.8 (Bhaktavatsalam *et al*., 1983b), similarly 0.1 M phosphate buffer at p^H 7.6 (Subba Rao, 1984, Krishnaveni, 1988, Vijaykumar, 1993 and Suneela, 1996), 0.1 M phosphate buffer at P^H 7.0 (Kadian, 1994) and 0.1 M phosphate buffer at P^H 7.4 (Patel *et al*., 1999).

Higher per cent infection was obtained by using potassium phosphate buffer (p^H 8.0) as compared to sodium phosphate buffer and citrate phosphate buffer (Chowdhary and Chowdhury, 1983).

Bhaktavatsalam *et al*. (1983b) reported that addition of 5 per cent sucrose and 1 per cent mercaptoethnol to the inoculum increased infectivity and longevity of ULCV.

2.6.2 Pollen transmission

Kolte (1971) reported that blackgram leaf crinkle virus was not pollen transmitted but it has adversely affected the pollen fertility. He observed 100 per cent pollen sterility in 30 per cent of the flower buds in the infected plant, while it was 38 per cent in the rest of the 70 per cent of the flower buds.

Narayansamy and Jaganathan (1975a) reported that ULCV induced pollen sterility ranging between 13.64 to 72.09 per cent.

Degeneration of androecium and gynoecium, incomplete development of pollen grain in pollen tetrads, mal-formation of ovule and abnormal ovarian cavity in the infected flowers was observed by Sharma and Dubey (1983).

Pollen sterility ranged between 15.15 to 30.61 in leaf crinkle infected plants while the range was 5.15 to 10.38 per cent in healthy plants (Krshnaveni, 1988)

2.6.3 Seed transmission

The virus is also transmitted and the initial occurrence of the disease in the field is through sowing of seeds from the seeds of affected plants in the previous season (Kolte and Nene, 1970).

In the studies conducted with the seeds collected from infected blackgram cv. T9 plants produced 18.39 per cent diseased plants when grown on test (Kolte and Nene, 1972).

Narayansamy and Jaganathan (1975b) studied the effect of age of plant at infection on seed transmission of urdbean leaf crinkle virus disease. when the young seedlings of 5 days old were inoculated, the seed transmission was highest recording 41.86 per cent, but the seed transmission was reduced gradually to 2.56 per cent when the seeds were sown from plants which were infected at 45 days after sowing.

Kadian (1980) reported that increase in disease incidence from year after year in different cultivars was due to spread of virus through infected seeds.

Urdbean leaf crinkle virus was seed borne in 3 out of 49 germplasm collections of mungbean tested and the rate of seed transmission varied from 6 to 75 per cent was recorded (Beniwal *et al*., 1980)

Seed transmission of leaf crinkle virus in different blackgram varieties and germplasm lines varied from 0 to 15 per cent. Blackgram cv. Pant U-26 plants infected at 10 days after sowing gave 10.97 per cent infection compared to 1.2 per cent from the seed collected from plants infected 60 days after sowing (Beniwal *et al*., 1980).

Chowdhury and Nath (1983) studied on seed transmission of the virus and was reported to be maximum (87.5 per cent) when the seeds of blackgram cv.T9 were kept in a moisture chamber for 48 hours and shaking in a vertical shaker for 30 seconds.

Subba Rao (1984) collected seeds from naturally infected blackgram cultivars of LBG 17, T9, Nethiminumu and buttaminumu yielded 10.14, 12.50, 17.45 and 17.45 per cent seed transmission in growing-on test respectively.

Leaf crinkle virus was found in 20 to 30 per cent seeds collected from infected plants (Bansal *et al*., 1984). Similarly 14.95 per cent in seeds collected from blackgram cv. Pant U-26 (Beniwal *et al*., 1984).

Dubey and Sharma (1985) conducted growing on tests with seed collected from naturally infected blackgram cv.kulu-1 indicated that 17.6 per cent seeds had ULCV infection, out of this 5, 11, 1 and 0.55 per cent plants were infected at first, second, third and forth trifoliate stages respectively. Per cent seed transmission was 68, 46, 22 and 10 per cent when the plants were infected at 10, 20, 30 and 40 days after sowing respectively.

Cotyledon extract resulted in a higher transmission (56 per cent) than the embryo extract (50%) and the virus was not detected either in seed coat or seed surface washings (Dubey and Sharma, 1985 and Patel *et al*., 1999).

Pushpalatha *et al*. (1999) detected ULCV in *Vigna mungo* seed samples from Gujarat, Maharashtra and Indian Punjab. Twenty four samples out of twenty nine screened were infected and the disease incidence ranging from 1 to 83 per cent.

Patel *et al*. (2001a) attempted to locate ULCV in different plant organs of *Vigna* spp and detected the virus in root, stem, leaf, floral parts, green pod and seeds of urdbean plants by infectivity and enzyme linked immune sorbent assay (ELISA) tests. The virus was also detected from different floral parts and seeds of mungbean and cowpea by infectivity tests and ELISA.

Ravinder Reddy *et al*. (2005a) studied on the seed transmission of urdbean leaf crinkle virus on blackgram and the results revealed that seed transmission of ULCV infection were maximum in pods located at the base of plant rather than pods produced subsequently, whereas the seed lots showing 2.0 to 3.6 per cent of seed transmission.

2.6.4 Vector transmission

The contradicting reports in literature on the transmission of greengram leaf crinkle virus disease by various insects which include aphids, leaf hoppers, whiteflies and beetles.

The investigations carried by Kolte (1971) and Nene (1972) failed to transmit the virus by black cowpea aphid (*Aphis craccivora*) and the cotton whitefly (*Bemisia tabaci*).

Dhingra (1975) and Dubey *et al*. (1983) reported non transmission of ULCV by the aphids, *Rhopalosiphum maidis*, *Brevicornyne brassicae*. *Aphis fabae* and *Macrosiphum rosaeformis* failed to transmit the disease at Pantnagar, Uttar Pradesh.

Evidence against the transmission of ULCV in *Vigna mungo* through four species of aphids *viz*., *Aphis craccivora, Aphis gossypii, Myzus persicae* and *Rhopalosiphum maidis,* a whitefly (*Bemisia tabaci*), leaf hopper, (*Empoasca motti*), beetle, (*Henosephilachna dodecastigma*) and mite, *Tetranychus telarius* was reported by Brar and Rataul (1987).

Reports on transmission of urdbean leaf crinkle virus by whitefly, *Bemisia tabaci* from Tamil Nadu was given by Narayanasamy and Jaganathan,(1973c) and in Meghalaya by Prasad *et al*. (1998) and Sahay *et al*. (1999). Aphids, *Aphis craccivora*, and *Aphis gossypii* from Delhi (Dhingra, 1975); *Myzus persicae* was found to be additional vector of ULCV in Delhi (Dhingra and Chenulu,1981); two aphid species *viz*., *Lipaphis erysime* and *Hysteroneura setariae* are capable to transmit ULCV from Kalyani, West Bengal (Nath *et al*., 1986). Whereas, Bhardwaj and Dubey (1984) reported transmission of ULCV by two aphid vectors, *Aphis craccivora* and *Acyrthosiphon pisum* from Solan, Himachal Pradesh.

In Andhra Pradesh, the transmission of blackgram leaf crinkle disease by *Aphis craccivora* was reported by Vijaykumar (1993) and Suneela (1996). Whereas, non transmission of blackgram leaf crinkle disease in Andhra Pradesh by thrips (*Frakelinella schultzei*) and whitefly (*Bemisia tabaci*) was reported by Vijay Kumar (1993).

Patel *et al*. (1999) reported positive transmission of urdbean leaf crinkle virus disease by aphids, *Aphis gossypii* from Gujarat. There were also reports on transmission by leafhopper, *Circulifer tenellus* from Punjab (Khatri *et al*., 1971) and beetle *Henosephilachna dodecastigma* from Pantnagar, Uttar Pradesh (Beniwal and Bharatan, 1980).

2.6.5 Whiteflies

Transmission of leaf crinkle disease of blackgram by the whitefly, *Bemisia tabaci* was reported by Narayansamy and Jaganathan (1973c) from Tamil Nadu. Success of transmission was 20 to 60 per cent when acquisition access and inoculation access period of one hour each were given.

2.6.6 Beetles

Beniwal and Bharatan (1980) conducted transmission study through Beetle (*Henosephilachna dodecastigma)* and reported that an acquisition feeding of 48 hours and inoculation access of 24 hours at the rate of one beetle per plant and transmission was 36.6 per cent in 10 day old blackgram cv. Pant U-19.

Adult beetles required an optimum of 24 hours acquisition access, 48 hours of inoculation access periods and 5 or more beetles per plant are required for 100 per cent transmission and the virus was detected in mouth parts, legs, eggs and faeces of viruliferous adults (Bharatan and Beniwal, 1984).

2.6.7 Aphids

Dhingra (1975) reported that a short acquisition feeding period of 30 seconds to 2 minutes was sufficient for *Aphis craccivora* and *Aphis gossypii* to transmit the former being more efficient vector which gave 80 per cent as against 66 per cent transmission with *Aphis gossypii.* The virus was not transmitted by aphids when they were allowed to feed on diseased plants for a longer period of 2 to 6 hours.

Dhingra and Chenulu (1981) reported maximum percentage of transmission was obtained by *Aphis craccivora* when the vector allowed acquisition access for 30 seconds. Pre-acquistion fasting for 2 to 4 hours was found necessary for successful transmission of the virus by *Aphis craccivora.* However, there was no transmission when acquisition access period was increased above 5 minutes.

Pre and post-virus-acquisition starvation of *Aphis craccivora* and *Acyrthosiphon pisum* resulted in appreciable increase in percentage of transmission of ULCV. Highest transmission occurred when aphids were starved for 90 minutes prior to virus acquisition. Pre-acquisition fasting of 90 minutes, acquisition feeding period of 20 minutes, Post virus acquisition fasting of 80 minutes, inoculation access of 24 hr and ten aphids per plant are required to achieve 60 to 100 per cent success in transmission with *Acyrthosiphon pisum.* whereas, with *Aphis craccivora* 90 minutes of pre-acquisition fasting, 20 minutes each of virus-acquistion feeding and post-virus-acquistion fasting periods, 24 hours of inoculation access and ten aphids per plant recorded an infection between 80 to 100 per cent (Dubey *et al*.,1983, Bhardwaj and Dubey,1984).

Nath *et al*. (1986) found highest percentage of transmission of 85 and 64 when the vectors *Lipaphis erysime* and *Hysteroneura setariae* respectively were allowed for one

minute acquisition access period. The percentage of transmission gradually decreased with the increase in acquisition access period of 10 minutes, while, *Lipaphis erysime* and *Hysteroneura setariae* transmitted only 16 and 20 per cent disease.

Vijay Kumar (1998) and Vijay Kumar and Subba Rao (1994) studies conducted on the relationship of blackgram leaf crinkle disease and its vector, *Aphis craccivora.* They reported 73.3 and 80 per cent transmission with one minute and two minutes acquisition access period respectively. As the number of aphids increased to ten, the per cent success of transmission was also increased (80%). The minimum inoculation feeding period required for transmission of the virus was found to be 6 hours. However, highest per cent transmission was obtained with the inoculation feeding period of 24 hours.

2.7 Detection of causal virus

Bhaktavatsalam (1976) worked out a method of urdbean leaf crinkle virus purification by celite adsorption preliminary clarification, Polyethylene glycol, differential centrifugation followed by cellulose column chromatography and found E260/280 and maximum /minimum ratios of final purified virus were to be 1.63 and 1.51 respectively. The final purified virus retained its infectivity. However, he could not process the purified virus preparation through electron microscopy.

Studies were conducted on ultra structure of leaf crinkle disease affected leaves of blackgram cv.T9 and the results revealed that the association of virus-like particles in the nucleus, cytoplasm and chloroplasts in the infected leaves. Virus-like particles were observed in 12 day old infected leaves but not in cells of corresponding healthy leaves. However, virus-like particles in 6 and 25 day old infected leaves could not be clearly seen as in 12 day old leaves. The virus-like particles were seen scattered throughout the cytoplasm as dense particles. The shape of such particles appeared to be spherical and the diameter was found to be 25 to 30 nm. (Bhaktavatsalam *et al.,* 1983a)

Dubey *et al*. (1983) studied the virus morphology with the partially purified preparation and also in the ultra thin sections of leaves by leaf dip method and found that the particles were isometric with an average diameter of 320 A^0 .Similar type of virus particles were also seen in the grids prepared from the diseases plants obtained from inoculation by viruliferous *Aphis craccivora* and *Acrythosiphon pisum* following leaf dip method. However, no such particles were observed in the grids prepared from healthy

urdbean leaves. The virus was purified by the modification of butanol-centrifugation method and which yielded partially purified ULCV preparation with milky white in colour and contained no visible impurity by inoculation to 10 day old blackgram cv. Kulu-1 produced crinkled leaves and malformed inflorescence.

2.8 Screening for resistance

Since cultivation of resistant varieties is the most economical way of countering plant diseases in general and virus diseases in particular, as the control of vector borne virus diseases are very difficult to contemplate. Out of 42 urdbean germplasm lines screened for resistance to blackgram leaf crinkle disease under artificial inoculated conditions, none of the lines were found to be resistant to blackgram leaf crinkle disease (Kotle, 1971).

All the 42 urdbean germplasm lines tested for ULCV resistance under glasshouse conditions were infected to the extent of 100 per cent (Nene, 1972).

Of the 119 blackgram germplasm or lines from Lam, Guntur and NBPGR, screened under field conditions at Rajendranagar and the results shows that 61 entries are free from the disease, thirty five lines reacted as resistant (0.1 – 5.0%), twenty three lines as moderately susceptible (5.1 to 10.0%) and none of the lines reacted as either susceptible (25% - 50.0%) or highly susceptible showing incidence of 50.01 to 100.0 per cent incidence (Subba Rao, 1984).

Sharma and Dubey (1984) screened 28 urdbean cultivars and germplasm among them HPU 27, 102, 164 and 315 were highly resistant (infection <10%) while HPU-19, 33, 35, 55, 56, 72, 75, 91, 109, 167, 200, 232, 240, 246, 252, 264, 269 and 277 were moderately resistant (infection 10 to 30%) and none of the entries were minimum (with no plants infected) under artificial inoculated conditions.

Vijay Kumar (1993) screened 40 genotypes against ULCV and reported that four were moderately resistant, eight were highly susceptible and the remaining was highly susceptible.

The experiment conducted for ULCV disease screening in urdbean and which included 32 different test lines and the lines were planted in a double row sub plot having row length 4 m, spacing 30 cm and plant to plant distance 10 cm. A row of susceptible local check is planted after every two rows to serve as spreader and the results shows that,

none of the cultivars was found to be resistant, cultivars S 118 and S 132 are moderately resistant, cultivars S 10 was moderately susceptible, three were susceptible and remaining were highly susceptible. This shows that different cultivars or lines vary in their genetic response and also different genes or gene combination. (Haq *et al*., 1991)

Iqbal *et al*. (1991) screened nineteen genotypes of urdbean (*Vigna mungo*) selected from local races against leaf crinkle disease for two consecutive years (1988-1989) under natural infection conditions. Genotypes varied greatly in their reaction to the disease. Disease intensity was high during second year which might be due to seed transmission of the virus. Four genotypes S 210, MM 5-60, S-250 and Mash- Sialkot remained resistant while the others showed a moderate reaction to leaf crinkle virus disease.

One hundred fifty blackgram germplasm lines screened in rice fallows under field conditions at Agriculture research station, Ghantasa, Andhra Pradesh of which seventy three lines as resistant, nine lines as moderately resistant, two (SV-2115 and BGP-216) were moderately susceptible, and the rest of sixty six germplasm lines reacted as susceptible to leaf crinkle virus disease (Suneela, 1996). Similarly, Sixteen blackgram genotypes from Lam, Guntur were screened under artificial inoculated conditions and all the genotypes reacted as either susceptible or highly susceptible (Ramana Murty, 1997).

Lokesh Babu (1997) screened 25 blackgram genotypes from Lam, Guntur under artificial sap inoculation conditions and the results shows that six genotypes reacted as susceptible and 19 genotypes reacted as highly susceptible for urdbean leaf crinkle virus disease.

Prasad *et al.* (1998) observed maximum disease in cv. T-9-150 (39.5%) and no disease was observed in cv.NDU-94-6 upon sap inoculation. Similarly in sap inoculation tests, blackgram germplasm lines GU-90-47, GU-90-61 and GU-90-66 were found resistant and GU-90-44 was found highly resistant (Patel *et al.,* 1999).

Patel *et al*. (2001b) screened different germplasm of urdbean, mungbean and cowpea against ULCV by sap inoculation under glass house condition and reported that urdbean lines GU 90-44 and IC-73306 were highly resistant to LCV. Among 23 mungbean collections EC-390202 was moderately resistance to LCV.

In order to identify source of resistance in urdbean 132 breeding lines were evaluated against the mungbean yellow mosaic virus and urdbean leaf crinkle virus under field conditions and result showed that, 53 urdbean genotypes were found to be highly resistant to MYMV and twenty six to ULCV (Muhammud Bashir and Muhammad Zubir 2002).

Ganapathy *et al*. (2003) conducted on experiment to identifying resistance against urdbean leaf crinkle virus, mungbean yellow mosaic virus and leaf curl virus in urdbean, evaluated 71 entries at NPRC, Vamban, Tamil Nadu. They found that RU 2229, VBG 86, 2KU 54, VBG 89, SU16 were highly resistant to urdbean leaf crinkle virus disease.

Seventy seven blackgram cultivars were screened during two consecutive *rabi* seasons both natural and artificial conditions and the results revealed that, under field condition no cultivar were found to be resistant to ULCV, four entries *viz.,* PLU-257, UM81-7, UM-82-81, VGP-64 were found moderately resistant, where as twenty nine, twenty seven and seventeen entries were showed moderately susceptible, susceptible and highly susceptible reaction respectively, These lines tested under artificially inoculated condition exhibited that none of them of were highly resistant. Resistant and moderately resistant fourteen, forty, and twenty three entries were moderately susceptible, susceptible and highly susceptible respectively (Nageswara Rao *et al*., 2003).

Muhammud Bashir *et al*. (2005) conducted screening experiment against urdbean leaf crinkle virus disease which consists of 16 accessions of mungbean and urdbean respectively and these were evaluated under greenhouse condition by sap inoculation method and the results revealed that, only five genotypes *viz.,* VC-3960(A-88), VC-3960 (A-89), 98-CMH-016, NM-2 and BRM-195 were found highly resistant to ULCV in mungbean, and in urdbean only one genotype VH94400039-3 were found to be highly resistant and one genotype ES-1 resistant to ULCV others were moderately susceptible to highly susceptible.

An experiment conducted on screening of urdbean germplasm for resistance against ULCV, which consist of 87 genotypes of blackgram were screened under field condition and results revealed that, the nine genotypes *viz.,* 703, 15, 19, 01, IAM382-9, IAM382-15 and IAM133 were found highly resistant to ULCV and 19 genotypes were resistant, 29 as moderately resistant, 11 as moderately susceptible, 3 as susceptible and 4 were highly susceptible to the ULCV disease (Ashfaq *et al.,* 2007).

Chaudhry *et al*. (2007) screened 67 urdbean [*Vigna mungo* (L.) Hepper] germplasm lines, originating from various research organizations, were screened against natural infection of urdbean leaf crinkle virus under field trial conducted at Pulses Research Institute, Faisalabad during *kharif* season 2006. Out of 30 test lines originating from Barani Agricultural Research Institute, Chakwal, none was found to be immune or resistant; however, two lines (3 CM-707 and CH-Mash 97) were found to be moderately resistant to urdbean leaf crinkle virus infection while all other test lines were moderately susceptible to susceptible.

To catalogue urdbean genotypes, an urdbean disease screening nursery was established in the Research Area of the Department of Plant Pathology, University of Agriculture Faisalabad (UAF). Each test entry was planted in a row of 3 meter in length and spaced 30 cm apart and replicated three times. One row of the most susceptible check (Kabali mash) was repeated after every two entries in the experiment. The spread of ULCV in the experimental plot was recorded at 7 day intervals until maximum infection was achieved. None of the lines appeared to be highly resistant (HR). Four lines (M-6206, IAM-382-15, IAM-133 and Mash-1) were rated as moderately resistant (MR); eight were rated as moderately susceptible (MS) and 21 as susceptible (S) and the remaining seven lines were highly susceptible (HS) (Baniyamin *et al*., 2011).

2.9 Management of the disease

2.9.1 Seed Treatment

The complete elimination of seed-borne ULCV by treating urdbean seeds (cv. Kulu-1) in a hot water bath for 30 minutes at 55 ^{o}C without affecting seed germination and found that the treatment was more effective at 55 ^{o}C for 30 minutes in eliminating the seed-borne infection of the virus (Sharma and Dubey, 1980).

The studies were conducted on the effect of heat treatment on the transmission of urdbean leaf crinkle virus through urdbean seed and found that hot water treatment at 60^{o}C for 10 20 and 30 minutes and 70^{o}C for 10, 20 and 30 minutes. In untreated seed 14.1 per cent seed transmission was recorded in blackgram cv. Pant U-26 (Beniwal *et al*., 1983).

2.9.2 Use of botanicals

Chowdhury and Shah (1985) studied the Pre inoculation spray of onion extract exhibited maximum inhibition of ULCV *in vivo* and *in vitro,* while the extracts of ginger and turmeric inhibited the ULCV to an extent of more than 50 per cent.

Bhardwaj and Dubey (1988) tested different plant oils for their effectiveness to prevent transmission of ULCV by *Aphis craccivora*, mustard, rapeseed, sesame and groundnut oils at 1.0 per cent reduced transmission of the virus significantly and 2.0 per cent emulsion of rapeseed and sesame oils completely prevented transmission of the virus. The oils showed a marked effect on virus acquisition, the aphids failed to acquire the virus from diseased plants sprayed with mustard, rapeseed, sesame and castor oils at 2.5 per cent.

Field trials were conducted with 2, 4 dixohexahydro 1, 3, 5-triazine (DHT) at 0.1 per cent, 2 per cent Thuja, 10 per cent Fresh butter milk and leaf extracts of *Mirabilis jalapa* were given on 7 and 22 days after planting and the control plants were sprayed with water. The number of diseased plants was counted at weekly intervals from 20 to 60 DAS. The disease incidence recorded in different treatments in the field showed that DHT reduced the disease spread to the maximum extent (9.01%) compared to control (1.47%).However other materials tested were also effective in reducing the disease spread significantly over control (Ravinder Reddy *et al.*, 2005b).

Thirumalaisamy and Rathi (2007) screened 21 plant extracts against the ULCV, the fresh plant materials were washed frozen and homogenized in distilled water using sterilized pestle and mortor. Homogenised plant materials were passed through sterilized muslin cloth and applied as pre inoculation application. The observation for the appearance of symptoms and inhibitory action of extracts were taken after one month. Inhibition per cent was calculated and the results showed that nine plants extracts given inhibitory effect against ULCV as pre inoculation treatment. Extracts of *Zingiber officinale*, *Prosopis julifera* and *Piper longum* gave higher inhibitory activity 56, 49 and 47 per cent respectively.

Karthikeyan *et al.* (2009) evaluated two specific strains of the plant growth promoting rhizobacterium (PGPR) and *Pseudomonas fluorescens* for induced systemic resistance against ULCV in blackgram under controlled condition and results revealed that pre-inoculation of blackgram plants with strains of *P. fluorescens viz*., Pf1 and CHAO were found to reduce ULCV infection significantly.

A field experiment of urdbean leaf crinkle virus management using plant extracts like neem, akk and garlic at two per cent concentrations and resulted minimum disease severity with least vector population was observed on plants that were sprayed with neem (2.0%) followed by akk (2.52%) (Baniyamin *et al*., 2011).

2.9.3 Use of oils

Suppressive effect of oils on virus transmission was demonstrated by several workers but their usage has not been popular. Mineral oils are most frequently used in agriculture but the use of neem oil is of recent origin for the management of plant diseases. Successful demonstration of control with oils is reported for CMV, PVY, watermelon mosaic and many other diseases. Oils have no effect on virus particles, but they limit virus acquisition and transmission by aphids. Acquisition is more strongly affected by the use of oils.

Ravindra Babu (1987) reported yellow mosaic disease incidence of 23.38 and 23.06 per cent in plot sprayed with neem oil 0.5 and 1 per cent respectively compared to 34.81 per cent in control.

An experiment was conducted on the effect of neem oil on leaf crinkle virus disease incidence and the results showed that ULCV incidence of 3.51 per cent in plots treated with neem oil when compared to 5.85 per cent in untreated control plot (Ravindra Reddy, 1988).

A study was conducted on the effect of neem oil sprays on aphids and results revealed that neem oil is more toxic to nymphs causing 100 per cent mortality than to the adult stages where it caused 60 to 98 per cent mortality. The toxicity of neem oil to nymphs was on par with phosphomidon an systemic organophosphorus insecticide (Roychoudhary and Jain, 1996).

The efficacy of botanicals and insecticides were evaluated against sucking pests, *viz*., aphid, *Aphis craccivora* Koch and whitefly, *Bemisia tabaci* on greengram. The results showed that among the treatments, acephate 75 SP @ 0.075 per cent and TNAU neem oil (C) 60 EC at 3.0 per cent were found significantly superior by recording higher percentage of reduction in aphid population and yellow mosaic virus (YMV) incidence due to whitefly and also with grain yield recording 8.5 and 7.4 q/ha respectively (Chandrasekharan and Balasubramanian, 2002).

2.9.4 Chemical control

The insecticidal control of spread of plant viruses has been reviewed by Broadbent (1957). Thereafter, a number of reports pertaining to the control of viral diseases by the use of different groups of insecticides have been published in literature. The aim of

insecticide application in the field is mainly to destroy insect pests or to check their build up below the economic injury levels. Most of these insects which serve as virus vectors, the use of pesticides can achieve the additional objective of checking their spread to a certain extent. Among vectors, the most efficient and dangerous are the aphids which transmit in a stylet borne manner. Both systemic and contact insecticides are effective on aphids but there is not a single compound that causes instant kill before inoculating the plant with the virus. Inspite of such a phenomenon, there are instances where noticeable decrease in the incidence of viral diseases with the use of pesticides has been observed.

Kousalya Gangadharan *et al*. (1977) reported complete mortality of aphids (*Aphis craccivora*) in a metasystox (0.1%) ekatin (0.1%) and diptterex (0.1%) sprayed plots in all insecticidal treatments (metasystox 0.1% ekatin 0.1% and diptterex 0.1%), folidol 0.025%, endrex 0.02%, sevin 10% dust and DDT 5% dust) were effective in controlling aphid infestation on cowpea compared to control and water spray. The extent of mosaic disease incidence closely followed the extent of aphid infestation in the trial *i.e*., ekatin treated plots recorded least disease incidence followed by metasystox treated plots compared to highest disease incidence in control plots.

Evaluation different insecticides in managing sucking pests of blackgram *viz*., aphids, jassids, thrips and whiteflies and the results showed that imidacloprid seed treatment (10 g/kg) effectively checked aphid and jassid population up to 60 days but for thrips and whiteflies a higher dose of 15 g/kg was found effective. Plant growth characters like plant height, number of leaves, leaf area and yield were quite superior in imidacloprid treated plots (Mote *et al*., 1993).

Naik *et al*. (1993) reported that the number of aphids (*Lipaphis erysime*) per shoot was only 41 in Monocrotophos treated plots as against 70 and 96 per shoot in plots treated with phosalone and endosulfan respectively as against 148 aphids per shoot in control plots. Maximum seed yield (18.32 q/ha) was recorded in Monocrotophos treated plots followed by phosalone (16.91 q/ha) and endosulfan (16.0q/ha) compared to 9.73 q/ha in untreated control.

Imidacloprid 70 WS seed treatment @ 15 g/kg seeds gave excellent control of sucking pests and also recorded a yield of 63.47 q/ha as against 37.12 q/ha in untreated crop (Jarande and Dethe, 1994).

Mote *et al*. (1994a) used imidacloprid 70 WS as seed dresser on cowpea @ 5, 7.5, 10 and 15 g/kg seed for the control of sucking pests of cowpea. Seed treatment @ 15 g/kg consistently recorded least number of whiteflies at all observations (*i.e*., 20, 35 and 50 days) and also recorded least percentage of mosaic incidence (0.8%) compared to untreated control (10%).

Walunj and Mote (1995) tested the efficacy of imidacloprid as seed treatment against sucking pests of blackgram during *kharif* 1993and reported that imidacloprid seed treatment (10 g/kg) recording with only 4.00 whiteflies/plant, 3.00 thrips/plant and highest yield of 74.70 q/ha as against 7.00 whiteflies/plant, 15.00 thrips/plant and 26.00 q/ha yield in untreated control.

Imidacloprid (Gaucho 70 WS) has tested as seed treatment chemical for the control of sucking pests of greengram. And the results revealed that a lower dose of 5 g/kg seed recorded 1.20 whiteflies/3 leaves/plant which was on par with control (0.77 whiteflies/3 leaves/plant). The yield was highest in imidacloprid seed treatment at 10 g/kg followed by seed treatment at 5 g/kg (Dandale *et al*., 2001).

Kotreshe (2002) reported a combination of seed treatment with confidar followed by two sprays of confidar followed by sorghum leaf extract along with one spray of confidar were found effective in reducing incidence of mosaic virus in soybean by recording 6.22 per cent incidence as against 39.85 per cent in control.

Field trials were conducted for two years at Millet breeding station, Tamil Nadu Agricultural University, Coimbatore. The susceptible mungbean variety CO 4 was sown with various treatments and the results revealed that lowest leaf crinkle disease incidence was observed on Cypermethrin treated plots followed by Thiamethoxam 0.2 g /lit spray at 15 DAS, seeds treated with imidachloprid (1.7%) and Thiamethoxam (2.1%) compared to control (5.65%) at 30 DAS. But 60 DAS the ULCV incidence was minimum in Thiamethoxam 0.2 g /lit spray at 15 DAS followed by Cypermethrin spray at 15 DAS @ 0.5 ml /lit and the incidence was 5.8 and 6.0 per cent respectively. Thiamethoxam gave longer protection compared to other treatments (Ganapathy and Karuppiah, 2004).

Studies on integrated disease management of blackeye cowpea mosaic virus (BlCMV) showed the treating the seeds with imidacloprid 60 FS 5 ml/kg with one spray of dimethoate 1.75 ml/lit at 30 days and one spray of nimbicidine 5 ml/lit spray at 45 days

recorded the least number of aphid population with reduced per cent disease incidence and brought out significant increase in all the growth and yield parameters of cowpea (Shilpa Shree, 2006).

Rathore (2009) conducted experiment on efficacy of methyl dematon and plants extracts against the leaf crinkle virus disease of greengram and the results on incidence of leaf crinkle virus disease of greengram was significantly reduced (9.15%) in plots sprayed with methyl dematon when compared to the margosa extract or azadiractin spray plot (17.7-22.8%) recorded high incidence.

The experiment was laid in Randomized Complete Block Design with three replications and five treatments including one untreated check. Nutrients *i.e*., Classic (NPK) @ 500 ml/acre, Fashion (Zn and B) @ 500 ml/acre and Urea @ 840 g/acre and a growth regulator naphthalene acetic acid (NAA) @ 70 ml/acre were used against the disease. The crop was sprayed by above mentioned nutrients at 7 days interval. The results revealed that maximum disease was found in control where no treatment was applied. The minimum disease was found in plants treated with NPK which was 17.04 per cent. Planofix (NAA) significantly reduced the disease with 19.41 per cent disease severity. The fourth treatment applied for the management of ULCV was urea which exhibited 20.27 per cent disease severity (Zeshan *et al*., 2012).

2.9.5 Growth attributes and losses

Since this disease is known to cause partial or complete sterility in infected plants resulting in considerable reduction on growth components and yield of the plants.

Kolte (1971) studied the effect of ULCV on yield and yield parameters in urdbean cv.T9 under natural conditions. The extent of loss on per plant basis was estimated to be between 62 to 100 per cent depending on the stage at which the plant became infected.

Studies on the effect of ULCV on yield and yield parameters of urdbean plants and the results revealed that the plants infected early failed to produce any pods. The reduction in number of pods appears to be the most important single factor which brings about differences in yield. The extent of loss on per plant basis was estimated to be between 62 to 100 per cent depending upon the stage at which the plant became infected in urdbean cv.T9 (Nene, 1972).

Beniwal and Chaubey (1979) made comprehensive studies on the effect of ULCV infection at different crop growth stages on yield contributing factors, total yield and seed characters in two susceptible urdbean cultivars viz., Pant U-30 and Pant U-26 under artificially inoculated conditions. Infection by ULCV significantly and adversely affected number of pods per plant and number of seeds per pod in urdbean. The total seed yield was also significantly affected by ULCV infection at different stages of growth in both the varieties. Maximum yield reduction of 70.70 and 83.80 per cent was caused in infection occurring at 10 days after planting in Pant U-30 and Pant U-26 cultivars respectively which was progressively and significantly reduced with the delay in infection upto 60days after planting and caused only 18 and 13.2 per cent loss in yield. The ULCV infection resulted in production of shrivelled and light brown coloured seeds which were maximum in the early infected plants.

The experiment was conducted on the effect of various virus diseases on growth components and yield of some varieties of mungbean and urdbean under natural conditions of infection from Hissar, Haryana. There was a decrease of 4, 88 and 86 per cent in shoot length, pod setting and grain respectively in blackgram T9 variety. Same trend was observed in shoot length and pod setting. However, the loss in grain yield was 96 to 100 per cent in Mash-1 and Mash-48 respectively (Singh, 1980).

Subba Rao (1984) studied the effect of blackgram leaf crinkle disease in blackgram cv.T9 under natural infection. Significant differences in number of pods per plant, number of seeds per pod and grain weight between the diseased and healthy plants was observed and the loss in yield was about 50 per cent which was attributed mainly due to the reduction in number of pods per plant due to ULCV infection.

Studies on the effect of ULCV infection at different stages of growth, yield and yield attributing characters under artificial inoculated conditions using blackgram cv. LBG17. The plants inoculated at 15 days after sowing were severely stunted and completely sterile and survived for about 20 to 25 days after symptom expression. The plants inoculated at 30, 45 and 60 days after sowing recorded a reduction of 59.40, 35.60, and 5.4 per cent pods per plant respectively as compared to the healthy plants. The test weight differed significantly among the treatments where in maximum test weight (3.37 g) was recorded in healthy plants and minimum (3.18 g) test weight was recorded in plants inoculated at 30 days after sowing. Maximum grain yield (6.15 g) per plant was

recorded in healthy plants followed by the plants inoculated at 45 DAS (5.17 g) and 30 DAS (4.52 g) which differed significantly among themselves (Vijay Kumar, 1993).

Manadhare *et al*. (1999) studied the effect of leaf crinkle infection on seed yield of mungbean under field conditions at Maharashtra and the results shows that 47.3, 31.9, 21.8, 12.5 and 15.84 per cent reduction in yield per plant , pods per plant, seeds per pod, pod length and seed weight were recorded in diseased plants respectively.

Material and Methods

III. MATERIAL AND METHODS

The present field investigations were carried out at College of Agriculture Bheemarayanagudi, University of Agricultural Sciences, Raichur and laboratory studies were undertaken at Main Agricultural Research Station (MARS) and Department of Plant Pathology, College of Agriculture, Raichur during the year 2012-13.

Bheemarayanagudi is situated in North Eastern dry zone (Zone-2) of Karnataka state at 16^{o} 43' N latitude and 76^{o} 51' E longitude with an altitude of 412 meters above mean sea level. The data on daily rainfall, maximum and minimum temperature and relative humidity from May (2012) to April (2013) are presented in (Table 1 and Fig.1). It was seen that during the period of study, the highest maximum temperature of 40.32 °C was recorded in the month of May, 2012 and the lowest minimum temperature (31.93.66 °C) was recorded in the month of November, 2012. The highest minimum temperature (24.19 °C) was recorded in the month of May, 2012 and the lowest minimum temperature (16.29 °C) was recorded in the month of January, 2013. The maximum average relative humidity (77%) was recorded in the month of July, 2012 and the minimum average relative humidity of 48 per cent in the month of April, 2012. The mean monthly maximum and minimum temperature, rainfall and relative humidity is shown in Fig.1.

In the present investigation, the following work has been carried out *viz*., survey, identification of virus through transmission studies, detection of virus through electron microscopy, screening of germplasm for resistance and disease management against disease incidence.

3.1 Symptomatology

Various types of symptoms of leaf crinkle disease in greengram, Chinamung were studied both in naturally and artificially inoculated plants and were described as per the sequence of occurrence.

3.1.1 Symptoms under field conditions

Symptoms of leaf crinkle virus disease in greengram under field conditions were studied in the experimental plot at Bheemarayanagudi and the symptoms were recorded at every 15 days intervals.

Table 1. Monthly meteorological data for the year 2012-13 and mean of the last four years at Agricultural Research Station, Bheemarayanagudi

Month	Rainfall (mm)		Temperature (^{o}C)				Relative Humidity (%)	
			Maximum		Minimum			
	2009-2012	2012-2013	2009-2012	2012-2013	2009-2012	2012-2013	2009-2012	2012-2013
April	2.08	8.30	40.86	39.00	21.81	21.77	52.73	48.00
May	32.38	27.00	40.98	40.32	24.35	24.19	54.77	54.00
June	72.13	127.50	36.56	36.93	23.24	23.97	63.18	65.00
July	95.98	95.00	33.26	32.42	22.75	22.81	77.57	77.00
August	33.63	125.50	32.97	32.81	23.66	22.94	75.80	75.22
September	118.00	63.00	31.32	32.00	22.62	22.17	73.77	71.31
October	124.40	83.50	30.92	32.35	19.74	19.39	70.30	68.51
November	30.23	16.00	31.01	31.93	18.39	17.07	69.37	70.73
December	14.38	0.00	30.54	32.10	17.44	16.40	68.81	68.31
January	4.13	0.00	29.79	32.41	15.77	16.29	62.21	67.15
February	.75	0.00	33.19	33.67	16.37	18.39	53.17	63.57
March	00	0.00	37.42	37.00	20.42	19.93	50.49	55.58
Total	**628.05**	**545.80**						

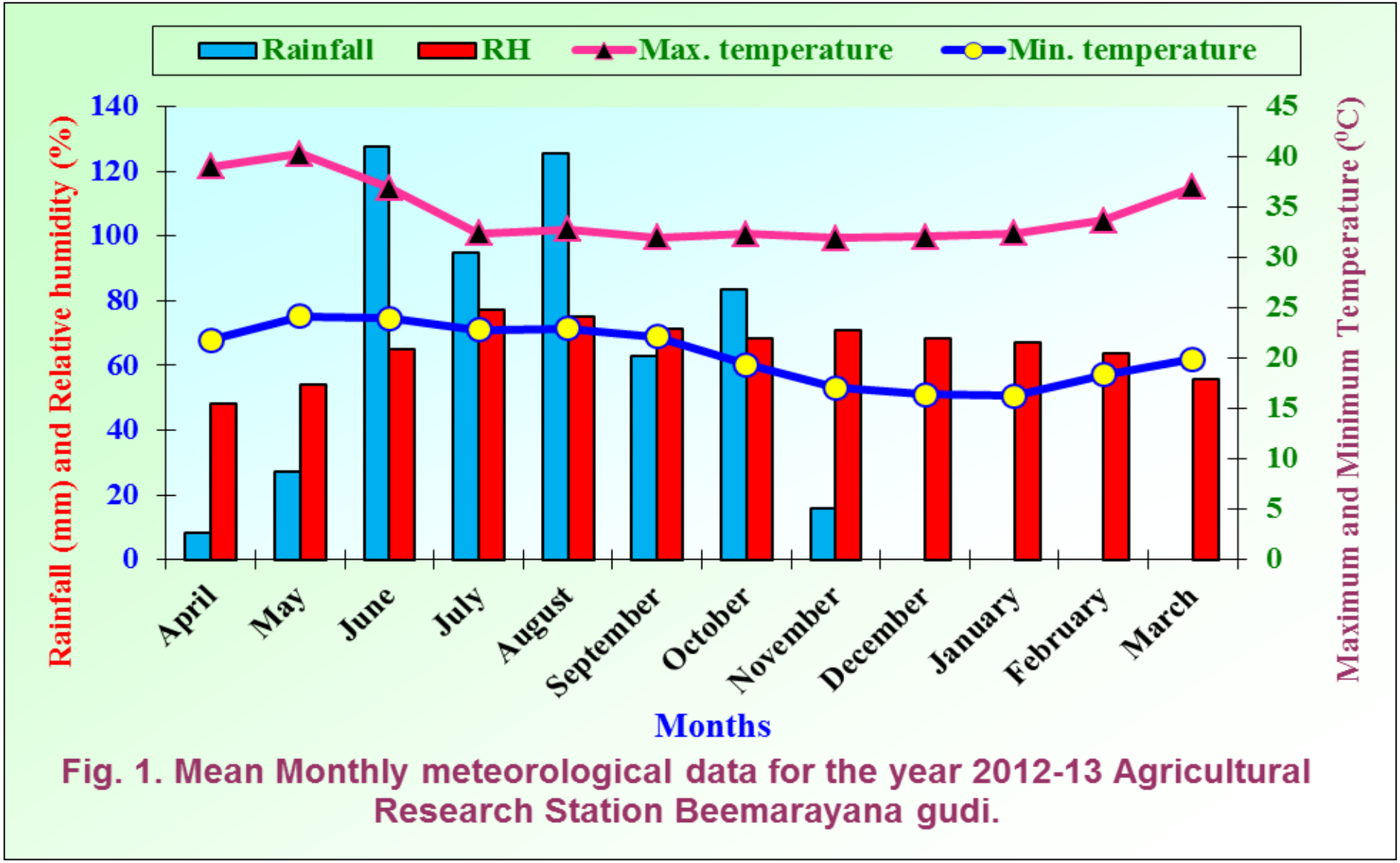

Fig. 1. Mean Monthly meteorological data for the year 2012-13 Agricultural Research Station Beemarayana gudi.

3.1.2 Symptoms on artificially inoculated plants

To study the symptoms, greengram seeds of variety Chinamung were sown in polybags of size 6" x 4" filled with soil and farmyard manure. When the plants were of 10-14 days old healthy seedlings were inoculated by 0.1 M Phosphate buffer at P^H 7.0 from diseased plants and the plants were maintained in glasshouse for symptom development.

3.2 Survey for the leaf crinkle virus disease incidence

Roving survey for incidence of leaf crinkle virus disease of greengram was carried out in July-August (first fortnight of July) during *kharif* 2012. For the survey, four districts *viz*., Raichur, Gulbarga, Yadgir and Bidar were selected from the point of greengram growing status. In each district three talukas were selected with two villages. In each field, five blocks of each of 5 sq mt areas were selected randomly at four corners and one at centre (Fig. 2). Observation was recorded on incidence of leaf crinkle disease based on number of diseased plants among the total plants observed in each block. Finally average incidence of five blocks was calculated for each field and presented with the table. During survey, data on variety grown, irrigated or rainfed, stage of the crop, insect vectors involved, plant protection measures taken and also type of symptoms produced were recorded. The disease was scored by using 0-5 scale given by Ashfaq *et al*. (2007) and described in (Plate 1). Per cent disease incidence was calculated by using the formula.

$$\text{Per cent disease incidence (\%)} = \frac{\text{Number of plants showing LCV symptoms}}{\text{Total number of plants observed}} \times 100$$

3.3 Transmission studies

3.3.1 Maintenance of culture

3.3.1.1 Raising healthy greengram seedlings

Greengram seeds of disease susceptible variety, Chinamung were sown individually in polythene bags of size 6 x 4 inches filled with soil + sand + FYM (1:2:1). Seedlings were protected using insect proof nylon net (40 mesh) when the seedlings were of 10-15 days old, they were used for transmission studies.

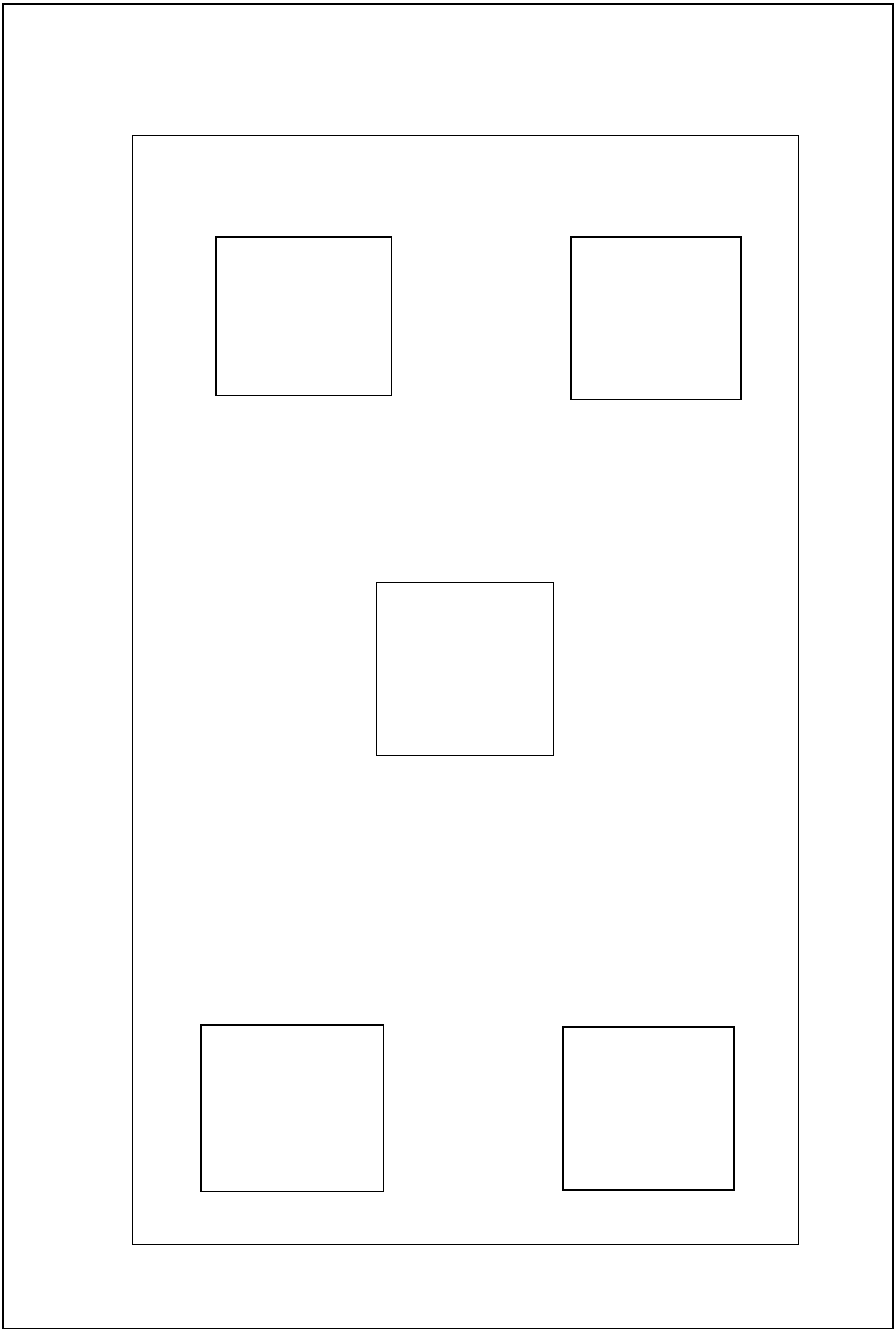

Fig. 2. Sampling procedure to record the incidence of greengram leaf crinkle virus disease

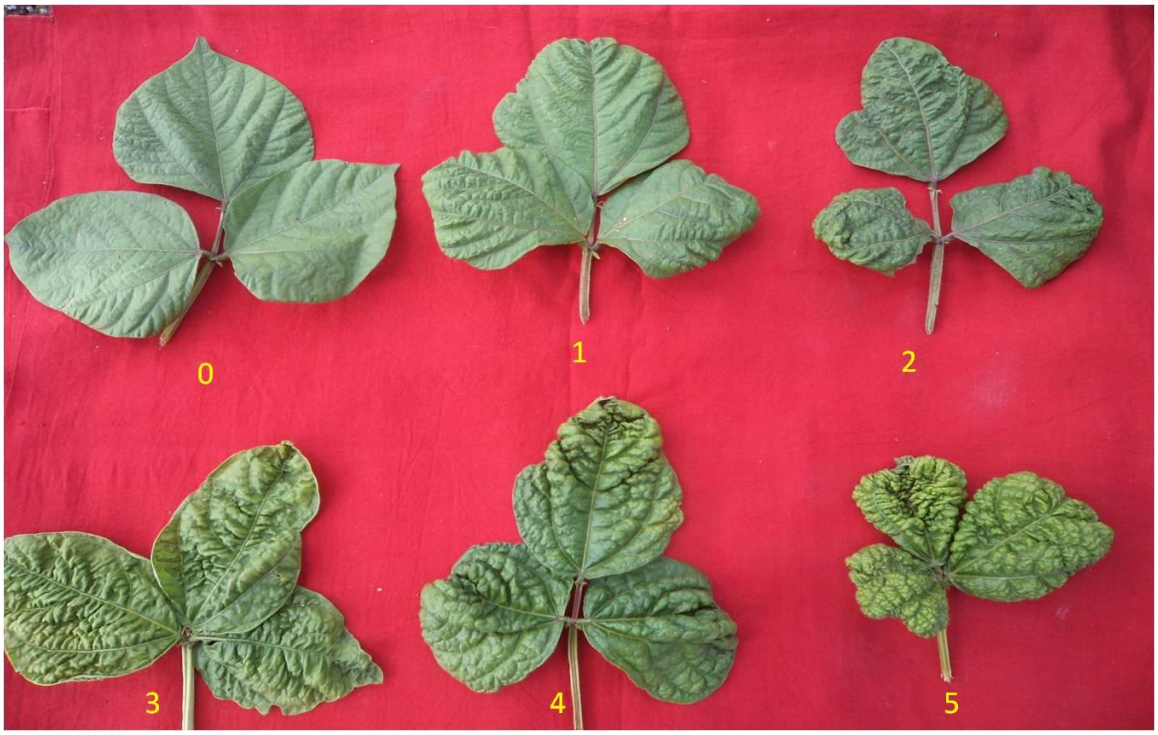

Plate 1. 0-5 scale for leaf crinkle virus disease of greengram

3.3.1.2 Leaf crinkle virus culture

The culture of leaf crinkle virus (LCV) disease showing symptoms was obtained from the experimental plots at Bheemarayanagudi. Such culture was brought to the greenhouse at MARS, Raichur. Later the culture was mechanically inoculated to the healthy seedlings using 0.1M Phosphate buffer at P^H 7.0. Culture developed was maintained in an insect proof cage by following frequent time of inoculations, culture was used for experimental purpose for future studies.

3.3.2 Mechanical transmission

The leaf crinkle virus infected greengram samples were mechanically inoculated on to healthy greengram plants.

3.3.2.1 Materials

- Pestle and mortor
- Muslin cloth
- Celite
- Chemicals for appropriate inoculation buffer

3.3.2.2 Inoculation buffer (Phosphate buffer- 0.1M)

Phosphate buffer was prepared by adding the following components accurately in to a sterilized glass beaker and stirred thoroughly by keeping it on magnetic stirrer and stored in chilled condition.

- Di potassium hydrogen phosphate (K_2HPO_4) - 12.37 g
- Potassium dihydrogen phosphate (KH_2PO_4) - 3.93 g
- pH - 7.0
- Distilled water - 1 l

3.3.2.3 Procedure for mechanical transmission

The virus infected leaves were collected and macerated in a clean and sterilized pestle and mortar by adding different concentrations of 0.05, 0.075, 0.1, 0.125, 0.15 M phosphate buffer at pH 7.0 @ 2 ml/ l g of fresh leaf tissue. The resulting extract was

strained through a muslin cloth. Celite was dusted on to first and second trifoliate leaves of test plants for making microscopic punctures in epidermis. Then, the inoculation was made by rubbing the surface of healthy plant leaves with cotton swab dipped in extract (inoculums) gently, unidirectional (Plate 2). The leaves to be inoculated were supported by a card sheet from below to ensure uniform pressure during inoculation. Excess inoculum was washed with water using a wash bottle and the inoculated plants are kept in insect proof cages under glasshouse conditions for symptoms production.

3.3.3 Aphid (*Aphis craccivora*) culture

3.3.3.1 Maintenance of insect culture

Aphid, *Aphis craccivora* was multiplied from single viviparous wingless female insect on suitable host plants cowpea *viz*., *Vigna unguiculata* (L) Walp. Healthy seedlings were raised from seeds of C-152 variety in the glasshouse and used for maintenance of the aphid culture. (Plate 3) These 15 to 20 days old plants were kept inside small insect proof wooden cages, covered with thin muslin cloth on three sides with a glass shutter on one side. These cages were previously sprayed with dimethoate @ 0.25 per cent to ensure insect free condition of cages. Then aphids were transferred on to these cages.

3.3.3.2 Handling of insects for transmission study

The aphids were transferred from rearing cage onto infected leaves with the help of camel hairbrush. The tip of the brush was slightly moistened with water to facilitate easy transfer of aphids. At the time of collection, aphids were disturbed by gentle touching with camel hairbrush to ensure withdrawal of their stylet from plant tissue. Later, a gentle tap was given to the twig and the aphids were collected in petriplates covered with black paper to provide dark condition to arrest the movement of aphids, while transferring aphids individually on to test plants, each aphid was disturbed by a gentle touch with camel hair brush and carefully lifted by its posterior end to avoid damage to the stylets.

3.3.3.3 Aphids transmission

Apterous adult aphids were collected from the culture and are transferred to petriplates carefully using moistened camel hair brush. Young virus infected leaves were taken in another petriplate (Plate 4a). Petioles of the leaves were embedded in moist cotton to keep them fresh and to prevent drying. The aphids starved for one hour were

Plate 2. Photo showing procedure for sap transmission of leaf crinkle virus

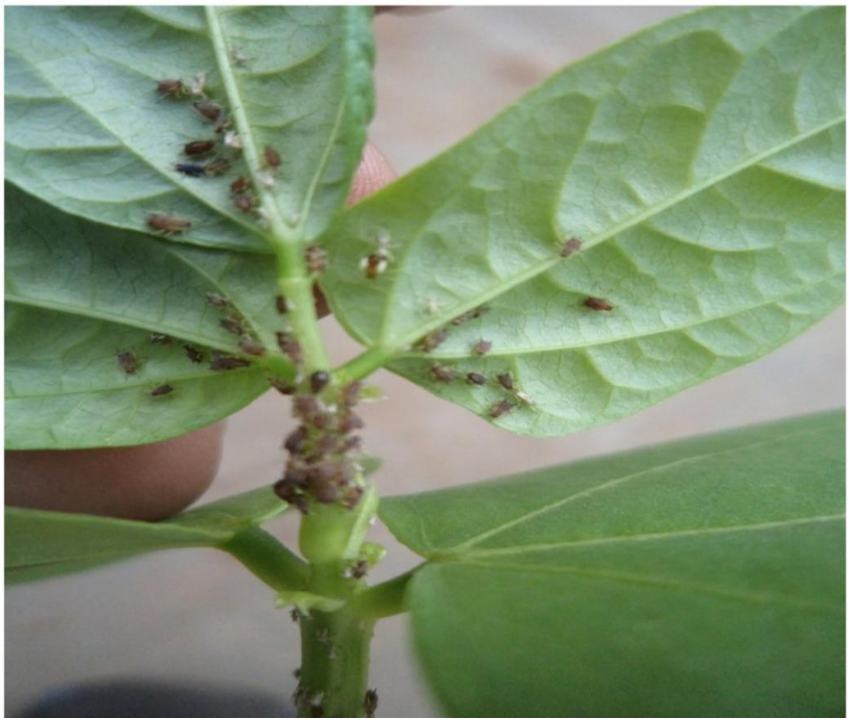

Plate 3. Maintenance of aphid (*Aphis craccivora*) culture on cowpea plants

Plate 4a. Procedure for acquisition of virus by aphids for transmission studies

Plate 4b. Photo showing viruliferous aphids inoculated on the greengram plant

transferred on to these leaves and allowed to feed for 30 minutes. After the acquisition feeding period, aphids were transferred to 15 days old greengram (cv. Chinamung) plants at the rate of ten aphids per plant for 24 hours inoculation feeding (Plate 4b).The aphids were confined to the test plants by using plastic chimneys. After allowing for 24 hours of inoculation feeding, the aphids were killed by spraying plants with 0.075 per cent acephate. The inoculated plants were kept in the insect proof glass house for one month for symptom expression. Ten plants in four replications were used to study the aphid transmission.

3.3.4 Whitefly (*Bemisia tabaci*) culture

3.3.4.1 Maintenance of whitefly

The whiteflies were collected from cotton field by gently turning the leaves slightly upwards and sucking with an aspirator. Such were released on cotton seedlings cv. DCH 32 kept in insect rearing net house. Subsequently, culture was maintained by frequently introducing the young cotton plants into the net (Plate 5).

3.3.4.2 Preparation of cages for acquisition access feeding by *B. tabaci*

Plastic or Polyvinyl chloride (PVC) bottles (20 x 7.5 cm) tapering towards the narrow mouth were taken, the bottom portion of the bottles was removed with the help of a soldering rod and they were covered with muslin cloth. The narrow mouth of the bottle was cut upto few centimetres above the screw cap and plugged with cotton to prevent flies escaping from the bottle during usage (Plate 6a).

3.3.4.3 Preparation of cages for inoculation of plants

Plastic tubes (7.5 x 2.5 cm) were taken and the bottom of the tube was removed with the help of a soldering rod. The bottom ends were sealed with a black muslin cloth to avoid accumulation of excess moisture inside the cage and also to provide aeration. A small hole (0.5 cm) was made in the middle portion of the tube to facilitate release of whiteflies. The open end of the tube was plugged with cotton after inserting young leaflets into the tube.

3.3.4.4 Collection of whiteflies

An aspirator made of a glass tube (30 x 0.5 x 40 cm) and a rubber tube of 40 cm length was used for the collection of whiteflies. The leaves colonized with healthy

Plate 5. Maintenance of whitefly (*B. tabaci*) culture on cotton plants

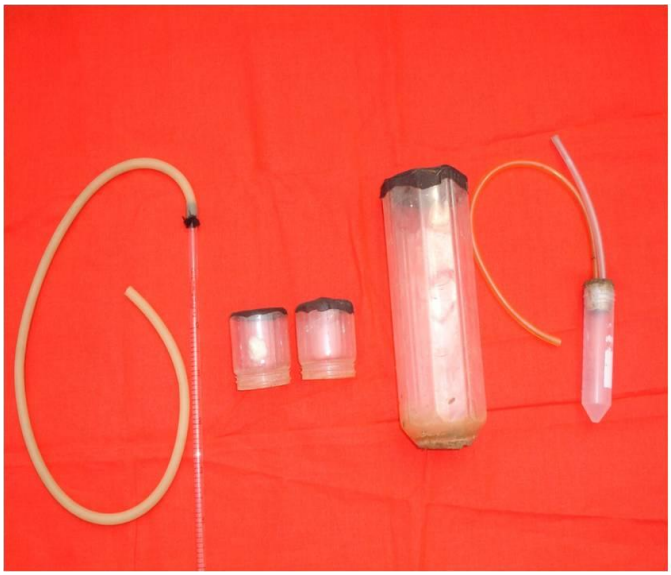

Plate 6: a) Different sizes of tubes used for inoculation, acquisition access and aspirator

b) Inoculation of leaf crinkle virus to greengram seedlings by whitefly *B.tabaci*

whiteflies were turned slightly upwards and the flies were sucked in to the aspirator. Later, they were gently blown in to the plastic tubes. Such collected virus free whiteflies were used in this investigation.

3.3.4.5 Whitefly transmission

Whiteflies were collected from rearing net and released into poly vinyl chloride (PVC) tubes, in which leaf crinkle virus infected branches were inserted previously and allowed to feed for 6 hr (acquisition access period). Then, the viruliferous whiteflies were released on to 15 days old healthy greengram plants (cv. Chinamung) at 10 whiteflies per plant by using aspirator and were allowed to feed for 24 hr for Inoculation access period (Plate 6b).

After inoculation, the plants were sprayed with 0.1 per cent dimethoate to kill all the whiteflies. The inoculated plants were kept under insect proof cages in glasshouse for symptom development. Healthy greengram plants fed with non viruliferous whiteflies served as check.

3.4 Detection of leaf crinkle virus disease of greengram through electron microscopy

3.4.1 Collection of virus isolates

Young shoots of greengram plants infected by LCV were collected from infected field in polythene covers and fresh leaf samples from LCV infected greengram plants maintained by vector transmission in glasshouse were collected separately and stored at freezer – 85 ^{o}C and used in molecular analyses.

3.4.2 Electron microscopy

The work on electron microscopy was carried out at the Division of Plant Pathology, Indian Institute of Horticulture Research, Bengaluru.

3.4.2.1 Leaf dip preparation

The virus was examined under electron microscope by modified Brande's leaf dip method (Gibbs *et al*., 1996). Small 2 to 3 mm diameter bits of infected leaf tissue were crushed on a clean glass slide in a drop of phosphate buffer (0.07 M, pH 7.5) and 10 µl of sap homogenate was placed on parafilm or waxed slide. An EM carbon-coated grid (film side down) was placed on the surface of the droplet, ensuring that the grid

surface becomes wet. After 5 minutes the grid was washed from the filmed surface, using a continuous flow of 10 to 15 drops of double distilled water to remove the sap. Staining was done using 2 to 4 drops of uranyl acetate (pH 6.5) on the filmed surface of the grid. Excess stain was removed with the help of a filter paper. After drying, it was examined under JEOL-100 EX-II transmission electron microscope at 80 K.V.

3.5 Confirmation of leaf crinkle virus disease of greengram for non begomovirus infection through PCR

3.5.1 DNA extraction from host plants

Total genomic DNA of the virus was extracted by following CTAB (Cetyl Trimethyl Ammonium Bromide) method of Lodhi *et al*. (1994) modified by Maruthi *et al*. (2002a).

DNA extraction buffer

Reagents

2% (w/v) CTAB

1.4 M NaCl

20 mM EDTA

100 mM Tris- HCl (pH 8.0)

1% Sodium sulphite*

2% PVP-40*

0.2 % (v/v) ß -mercapto ethanol (add after autoclaving)

***** PVP and Sodium sulphite added fresh to aliquot of stock buffer (containing first four reagents immediately prior to extraction)

Procedure

Approximately 100 mg of diseased leaf tissue was placed into a thick-gauge plastic bag. The tissue was ground using a roller and mixed with 10 volumes (1 ml) of CTAB extraction buffer.

1. About 750 µl of the sample was poured into a 1.5 ml Eppendorf tube and the samples were heated at 60°C for 30 min.

2. The samples were mixed with an equal volume (750 µl) of chloroform: isoamylalcohol (24:1) and centrifuged at 13000 rpm for 10 min.
3. The top aqueous phase was transferred into a new 1.5 ml eppendorf tube and DNA was precipitated by adding 0.6 volumes (300 µl) of cold (-20°C) isopropanol and incubated at –20°C for at least 1 h.
4. The samples were centrifuged at 13000 rpm at 4°C for 10 min and the supernatant was discarded.
5. The pellet was washed in 0.5 ml 70 per cent ethanol by vertexing and then centrifuged for 5 min at 13000 rpm.
6. The ethanol was removed and the pellet was vacuum dried for 5 min and the dried pellet was suspended in 100 µl 1x TE buffer and stored at –20°C.
7. All the DNA extracts were further diluted 10-fold in single distilled water (SDW) before using for PCR amplifications.

PCR procedure

Reagents

a. PCR buffer

200 mM Tris (pH 8.3)	1.0 ml
500 mM KCI	2.5 ml
0.01% Gelatin	0.5 mg
H_20 (SDW)	1.0 ml

b. dNTP mixture

Each 25µl of dATP, dCTP, dGTP and dTTP from a 100mM stock was mixed. The concentration of each dNTP in this mixture was 25 mM. Further the final concentration of each dNTP was made to 2.5 mM by diluting it by ten times.

c. Primers:

Two sets of degenerated oligonucleotide primers were used for amplification of the core region of DNA-A coat protein fragment are as follows

Primer	Oligonucleotides	Reference
AV494	5'GCCCATGTATAGAAAGCC 3'	(Wyatt and Brown,1996)
AC1048	5'GGATTAGAGGCATGTGTA 3'	
Primer (F)	5'TAATATTACCGGAGGACC 3'	(Deng *et al*., 1994)
Primer (R)	5'TGGACCTTACAAAGGCCCT 3'	

d. Procedure

1. 0.5 ml Eppendorf tubes were selected, labelled and kept on ice crystals.
2. Samples were taken for PCR along with positive control (CTAB extracted LCV DNA) and negative control (distilled water).
3. 25 µl PCR mixture was prepared by adding the following ingredients into the eppendorf tube.

Sterile distilled water	13.4 µl
10 x PCR buffer (Supplied with the enzyme)	2.5 µl
25 mM $MgCl_2$	1.0 µl
2.5 mM dNTP mixture	2.0 µl
Primer CP-F (20 mM)	2.0 µl
Primer CP-R (20 mM)	2.0 µl
Taq polymerase	0.1 µl
Viral DNA	2.0 µl

After preparing the cocktail, the DNA template was added and tubes were spun briefly. The PCR was performed in a thermal cycler (Techne Genius/ Eppendorf) using the following parameters:

Stage	Step	Temperature	Duration	No. of cycles
1.	Pre denaturation	94 ^{o}C	2 min	One
2.	Denatuartion	94 ^{o}C	1min	Thirty five
3.	Annealing	61 ^{o}C	1 min	
4.	Extension	72 ^{o}C	2 min	
5.	Final extension	72 ^{o}C	10 min 30 sec	One

After the completion of the reaction the products were kept at 4 ^{o}C prior to gel analysis (Wyatt and Brown, 1996).

3.5.2 Analysis of PCR products

Twenty microlitres of PCR product was analyzed in 1.5% agarose gels were prepared by melting 1.0g of Agarose in 100 ml of 1X TBE in microwave oven (BPL 700T) until a clear transparent solution was obtained. The edges of the gel tray were sealed with a tape and the comb was placed at one end of the tray surface. The agarose solution was cooled to about 50^{o}C and poured into the gel tray to a thickness of 4-5 mm and allowed the gel to set. After gel hardening the tape was removed and platform was kept in electrophoresis tank. Sufficient electrophoresis buffer (1X TBE) was filled into the tank to cover the gel to a depth of 10 mm and then the comb was removed carefully. Each lane of the gel was dispensed with 10 µl PCR product along with 5 µl loading dye. One lane was added with one kb molecular weight marker (MBI fermentas). The electrophoresis unit was connected to the power pack (Biometra) and the power supply was turned on until the orange G dye reached the bottom of the gel. Agarose gel with migrated DNA was stained with ethidium bromide (0.5 µg/ml of water) for about 30 min at room temperature. DNA fragments were visualized on gel documentation unit (B & L Image system).

3.6 Screening of germplasm against leaf crinkle virus disease incidence

Studies were undertaken to test the reaction of local greengram germplasm against leaf crinkle virus disease. Field experiments were conducted under rainfed conditions at College of Agriculture. Bheemarayanagudi.

A total of thirteen genotypes were collected from Department of Genetics and Plant Breeding, College of Agriculture. Bheemarayanagudi were used for screening studies. An infector row of greengram cv. Chinamung was planted in a row of five meter as a susceptible check after every four test entries and the whole field was surrounded by an infector row to create natural epiphytotic conditions in the field (Plate 7). The recommended agronomic practices were followed and plots were irrigated whenever necessary. The initial disease plant count was recorded in all genotypes starting from fifteen days after sowing (DAS) at every fifteen days intervals. The per cent disease incidence was calculated by using following formula

Plate 7. Screening of greengram entries against leaf crinkle virus disease by infector row method

$$\text{Per cent disease incidence (\%)} = \frac{\text{Number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

The genotypes were later grouped into different categories based on 0 to 5 scale from highly resistant to highly susceptible according to (Ashfaq *et al*., 2007).

Disease grade	Disease reaction	Reaction
0	All plants free of symptoms.	HR
1	1-10 per cent plants infected showing mild crinkling at the top and pods normal	R
2	11-20 per cent plants infected showing crinkling and curling of top leaves with pods are normal	MR
3	21-30 per cent plants infected with crinkling, puckering, malformation and shortening of pods	MS
4	31-40 per cent plants infected showing all the typical disease symptoms	S
5	More than 40 per cent plants infected showing all the plants with severe symptoms with few pods containing only few seeds	HS

3.7 Management of leaf crinkle virus disease of greengram

To know the effectiveness of different management practices against LCV, a field experiment was conducted at College of Agriculture. Bheemarayanagudi during 2012-13 in a randomized block design (RBD) with three replications. (Plate 8).The sowing was taken up first week of July with a spacing of 30 x 10 cm during *kharif* season. The layout of the experimental field is presented in Fig. 3. The trail was laid with different treatments and their combinations in the field under natural epiphytotic condition. Recommended agronomic practices were followed. The plots were irrigated when required and the weeding was done manually twice, first weeding was done 15 days after sowing and the second one 30 days after sowing. In all, the 11 treatments were tested and the particulars of each treatment and other details are as follows

Plate 8. Field view of greengram for the management of leaf crinkle virus disease

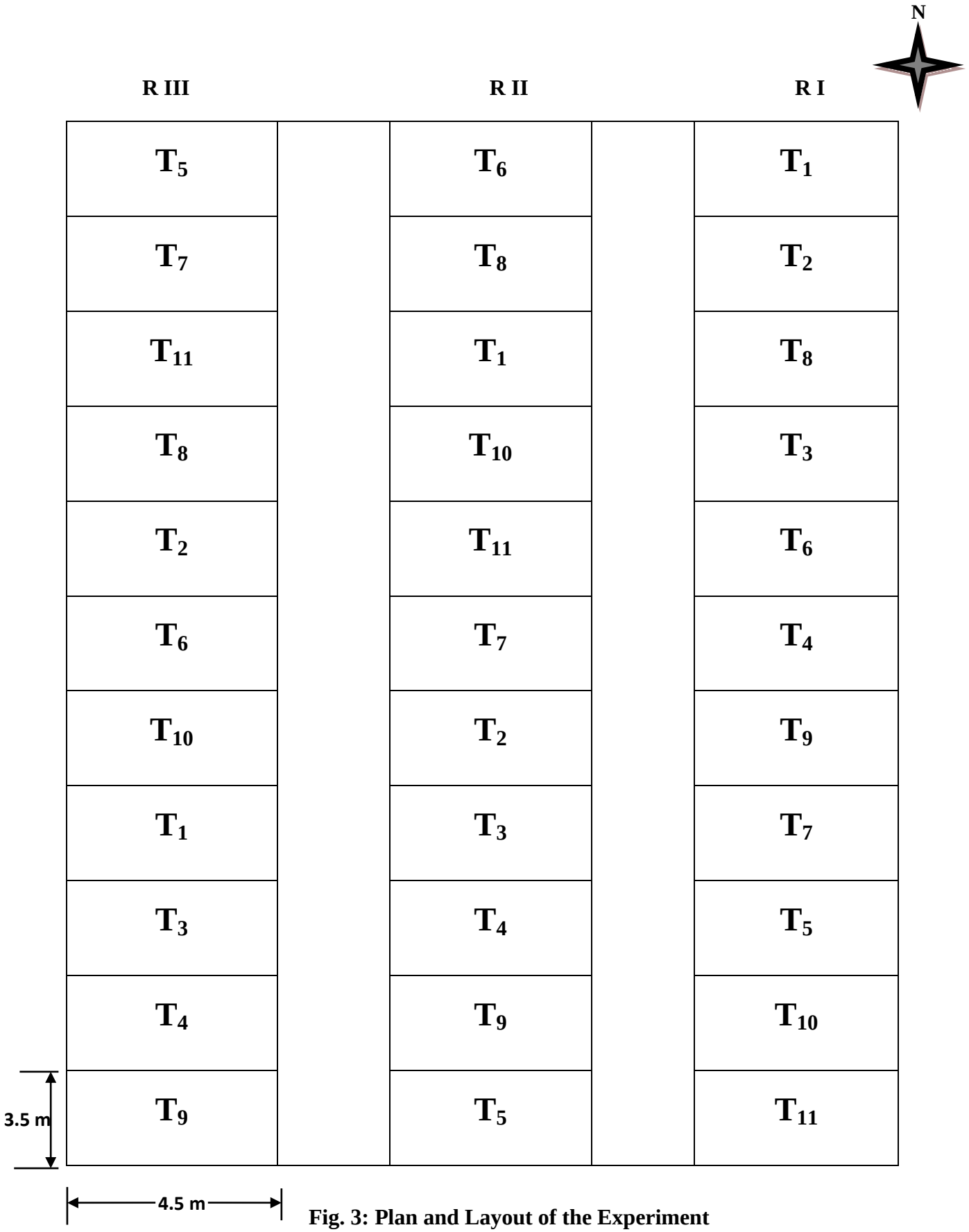

Fig. 3: Plan and Layout of the Experiment

Treatments	:	11
Replication	:	3
Plot size	:	4.5 m × 3.5 m
Spacing	:	30 × 10 cm
Season	:	*Kharif*

3.7.1 Treatment details:

T_1	Seed treatment with imidacloprid 60 FS (Gaucho ® at 5 ml /kg of seeds)
T_2	Seed soaking with cow urine @ 2.0%
T_3	T_1 + Two sprays with imidacloprid 17.8 SL @ 0.03%
T_4	T_1 + Two sprays with cow urine @ 2.0%
T_5	T_1+ Two sprays with azadiractin 1500 ppm @ 3 ml/lit
T_6	T_1 + Two sprays with profenophos 50EC @ 2 ml /lit
T_7	T_2 + Two sprays with imidachloprid 17.8 SL @ 0.03%
T_8	T_2 + Two sprays with cow urine @ 2.0%
T_9	T_2 + Two sprays with azadiractin 1500 ppm @ 3 ml/lit
T_{10}	T_2 + Two sprays with profenophos 50 EC @ 2 ml/lit
T_{11}	Control

3.7.2 Application and schedule of insecticide

The imidachloprid, azadiractin, and profenophos were used for foliar application at the concentration of 0.03 per cent (0.3 ml per one litre of water), 3 ml per litre and 2 ml per litre respectively. The application was done as per the schedule in each treatment where in first application was done at 25 days after sowing followed by second application at 40 days after sowing. Foliar application of the insecticide was done with help of a knapsack sprayer. Care was taken to ensure complete drenching of the treatment and drift avoided.

3.7.3 Application of cow urine

Cow urine was used at a concentration of two per cent (20 ml per one litre of water) and sprayed with the help of a knapsack sprayer. Care was taken to ensure complete drenching of the treatment and drift avoided. The application was done as per the schedule in each treatment where in first application was done at 25 days after sowing followed by second application at 40 days after sowing.

3.7.4 Per cent disease incidence

Incidence of leaf crinkle virus disease was calculated by counting the number of plants infected and total number of plants in a plot by using following formula.

$$\text{Per cent disease incidence (\%)} = \frac{\text{Number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

3.7.5 Vector population

3.7.5.1 Population of aphids

The number of aphids on top 3 trifoliate leaves per plant from each of five randomly selected plants at one day before and 5 days after sprays were recorded in each treatment. Per cent reduction over control was calculated by using formula.

$$\text{Per cent reduction over control} = \frac{\text{Number of aphids in control} - \text{Number of aphids in treatment}}{\text{Number of aphids in control}} \times 100$$

3.7.6 Growth and yield parameters

Randomly selected five plants from each treatment was collected at harvesting stage for assessing growth and yield parameters. The effect of leaf crinkle virus disease on plant height, pods per plant, 100 seed weight and yield per hectare was studied and the five plant average data was analysed statistically.

3.7.7 Yield and economics

Net returns from each treatment was calculated by taking into account of yield obtained and cost of treatment application on hectare basis. Benefit: cost ratio was

calculated to compare the feasibility of various treatments economically and whatever the increased yield obtained over control was because of the plant protection measures applied only.

Experimental Results

IV. EXPERIMENTAL RESULTS

The results pertaining to the thesis comprising the survey and surveillance of leaf crinkle virus disease of greengram in North Eastern Karnataka, transmission studies on LCV of greengram, detection of LCV by electron microscopy, screening of greengram genotypes and management of LCV disease of greengram by chemicals and botanicals are presented in this chapter under different headings.

4.1 Symptomatology

The symptoms were studied in detail both under natural and artificial conditions.

4.1.2 Field symptoms

Under field conditions, the symptoms displayed by diseased plants were variable. The first recognisable symptoms of the disease under natural conditions appeared on the second trifoliate leaf of greengram cv. Sel-4, which turned light green at 18 days after sowing (DAS). Around 25 DAS, crinkling appeared in addition to enlargement of trifoliate which became more pronounced with age. First and second trifoliate leaves did not show any enlargement, but crinkling with enlargement of leaves was more in the third succeeding trifoliate. As the infected plants grow older, extreme crinkling and rugosity on the older trifoliate appear to diminish, crinkling on younger trifoliate remain (Plate 9a).

Around 30 days after the first appearance of the symptoms, tips of the affected leaflets especially in 4^{th}, 5^{th} and 6^{th} trifoliate curve downwards. The petiole of the lamina touched the surface of the lower leaf lets on either side. Thus the affected plants remain stunted giving a bushy appearance (Plate 9b).

Flowering in the affected plants was delayed by 10-12 days compared to healthy plants. At the time of flowering, peduncles produced from axils of affected trifoliate bear large number of small sized flowers and which never opened due to folding of its floral parts. No pods were formed in severely affected plants. Even if they are formed, they are ill filled and small in size. The seeds obtained from virus infected plants when observed carefully in dry state revealed morphological abnormalities in shape, size and colour of seed such as presence of bold, shrivelled and crinkled small sized seeds (Plate 9c).

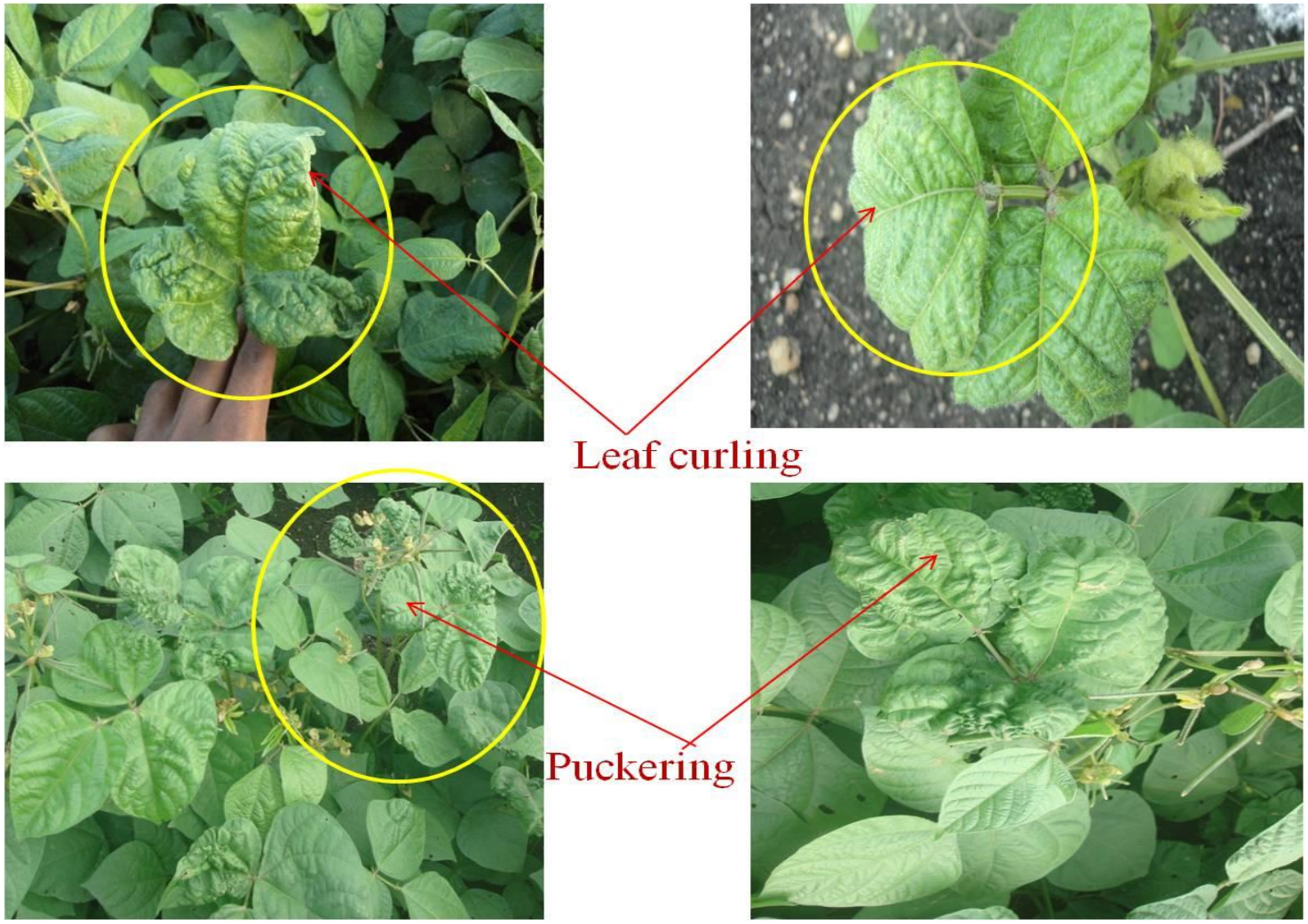

Plate 9a. Different types of symptoms of leaf crinkle virus disease in greengram

Enlargement of leaf

Crinkling of leaves

Extreme crinkling of leaves

Rugosity of leaves

Plate 9b. Different types of symptoms of leaf crinkle virus disease in greengram

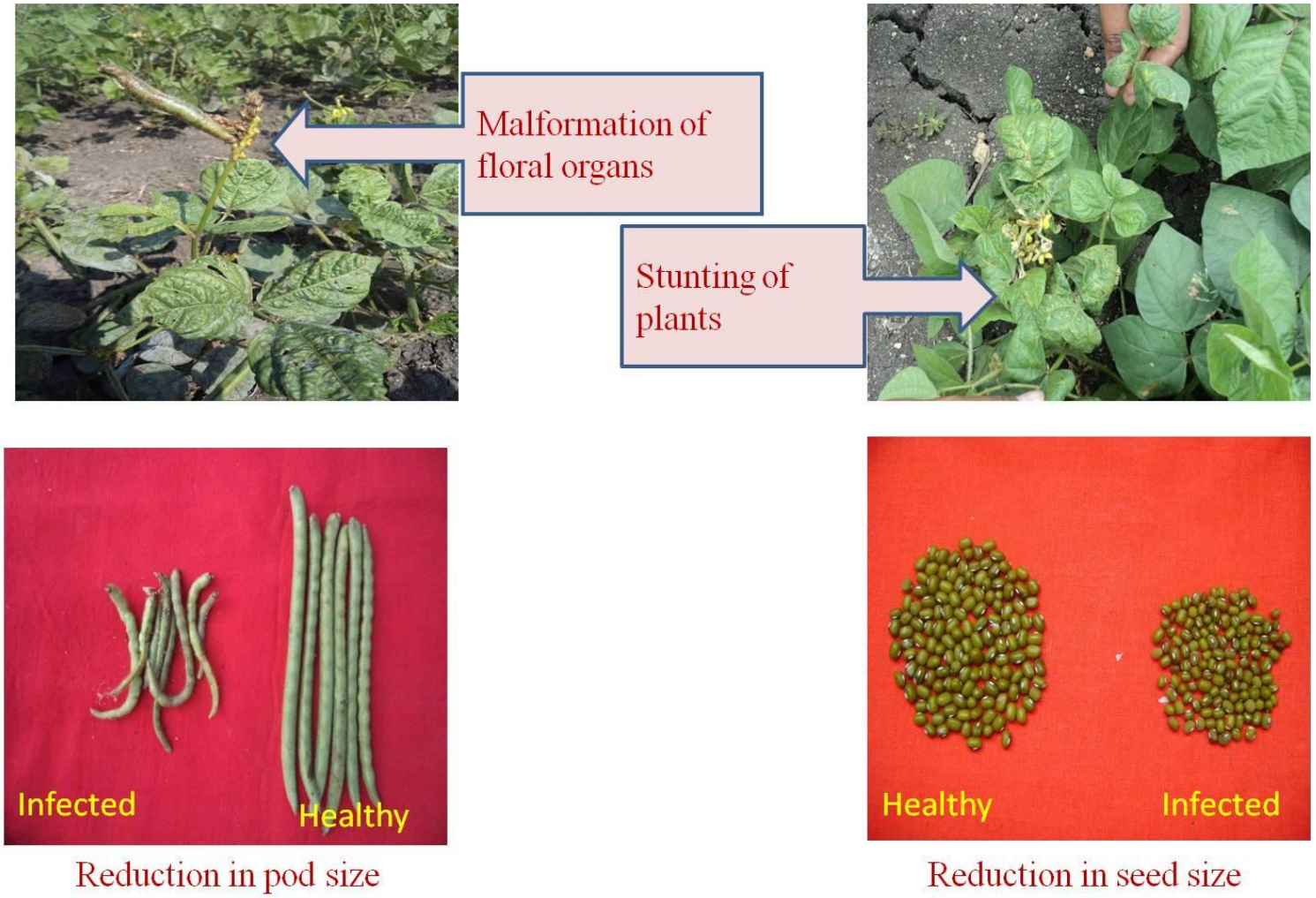

Plate 9c. Different types of symptoms of leaf crinkle virus disease in greengram

4.1.3 Symptoms expression under glass house condition

Proving pathogenicity

The symptoms on greengram under artificial inoculated conditions were similar in all respects to those observed on plants naturally infected in the field. Mechanically sap inoculated plants at primary leaf stage developed systemic symptoms twelve days after inoculation. The first recognisable symptoms appeared on the second trifoliate leaf which showed curling coupled with chlorosis. Typical crinkling symptoms were observed on third trifoliate after a week.

4.2. Survey and surveillance for severity of the leaf crinkle disease of greengram

4.2.1 Disease prevalence and distribution

Roving survey was undertaken to know the incidence of leaf crinkle virus disease of greengram in four districts of North Eastern Karnataka *viz*., Raichur, Yadgir, Gulbarga and Bidar during 2012 when the crop was in vegetative growth and flowering stages. All the plants in the randomly selected area of the field were first counted and then numbers of plants showing the symptoms of Leaf crinkle virus (LCV) were recorded separately to calculate the per cent disease incidence. The survey results are presented in table 2.

The severity of the crinkle disease was maximum in Yadgir district during *kharif* 2012 with the per cent disease incidence of 31.21. In Yadgir district, Adnur village of Shahpur taluk recorded highest per cent disease incidence of 33.96, while least disease incidence (28.76%) was in Balichakra village of Gurumatkal taluk. In Gulbarga district, the highest per cent disease incidence of 24.82 was recorded in Goudnalli village of Sedam taluk. While least (20.19%) was observed in Margola village of Chittapur taluk (Plate 10).

In Bidar district, Ballur village recorded the highest per cent crinkle virus disease incidence of 24.25. While the least was in Janawada village (13.36%) of Bidar taluk. In Raichur district, Kavital village of Manvi taluk recorded highest per cent disease incidence of 14.05, while the least crinkle disease incidence (7.42%) was observed in Jambaldinni village of Raichur taluk.

The survey results of 2012 showed that the highest mean per cent disease incidence of 31.21 was observed in Yadgir district, followed by Gulbarga district with

Table 2. Survey for the incidence of leaf crinkle virus disease of greengram during *kharif* 2012 in four districts of North Eastern Karnataka under rainfed conditions

Sl. No.	District	Taluka	Village	Variety	Per cent Disease Incidence	Mean PDI of districts	Insects recorded	Symptoms observed
1.	Raichur	Raichur	Jambaldinni	Chinamung	7.42	10.04	Aphids, Pod borers	Crinkling, puckering and curling of leaves.
			New area UAS, Raichur	Chinamung	8.72		Aphids	Crinkling, puckering and curling of leaves.
		Deodurga	Sasigera	S-4	12.1		Aphids, Jassids, Pod borers	Crinkling, rugosity of leaves and curling of leaves.
			Vandali	Chinamung	10.45		Aphids	Crinkling, puckering and curling of leaves.
		Manvi	Kavital	S-4	14.05		Aphids	Crinkling, puckering, rugosity of leaves and curling of leaves.
			Aksalapura	S-4	7.55		Aphids, Jassids,	Crinkling, puckering, rugosity of leaves and curling of leaves.
2.	Yadgir	Shahapur	Doornahalli	Chinamung	31.41	31.21	Aphids, Jassids, Pod borers	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
			Adanur	Chinamung	33.96		Aphids, Pod borers	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
		Gurumitkal	Chaptla	Chinamung	29.19		Aphids, Thrips, Jassids	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
			Balichakra	Chinamung	28.76		Aphids	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.

Table 2. Contd….

Sl. No.	District	Taluka	Village	Variety	Per cent Disease Incidence	Mean PDI of districts	Insects recorded	Symptoms observed
		Yadgir	Savoor	Chinamung	30.08		Aphids, Whiteflies, Thrips, Pod bores	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
			Malar	Chinamung	33.86		Aphids, Thrips, Pod bores	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
3.	Gulbarga	Gulbarga	Tavargera	Chinamung	21.74	21.84	Aphids	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
			Patan	Chinamung	20.70		Aphids, Thrips, Pod bores	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
		Sedam	Kodla	Chinamung	22.32		Aphids, Pod bores, Jassids	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
			Goudanalli	S-4	24.82		Aphids, Pod bores, Jassids	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
		Chittapur	Mudbal	Kopergaeon	21.28		Aphids, Pod bores, Jassids	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.
			Margola	Kopergaeon	20.19		Aphids	Crinkling, puckering, rugosity of leaves, curling of leaves and reduced pod formation.

Table 2. Contd….

Sl. No.	District	Taluka	Village	Variety	Per cent Disease Incidence	Mean PDI of districts	Insects recorded	Symptoms observed
4.	Bidar	Bidar	Janawada	Chinamung	13.36	17.10	Aphids, Whitflies, Jassids, Pod borers	Crinkling, puckering, curling of leaves and reduced pod formation.
			Chidri	Chinamung	14.66		Aphids	Crinkling, puckering, curling of leaves and reduced pod formation.
		Aurad	Kawata	Chinamung	14.85		Aphids, Jassids, Pod borers	Crinkling, puckering, curling of leaves and reduced pod formation.
			Ballur	Chinamung	24.25		Aphids, Jassids Pod bores	Crinkling, puckering, curling of leaves and reduced pod formation.
		Humnabad	Hudgi	Chinamung	16.75		Aphids	Crinkling, puckering, curling of leaves and reduced pod formation.
			Manaekhalli	Chinamung	18.75		Aphids	Crinkling, puckering, curling of leaves and reduced pod formation.

Plate 10. Natural infection of greengram plants by LCV under field conditions: (a) Yadgir district and (b) Gulbarga district

21.84 per cent disease incidence. The least disease incidence 10.04 per cent leaf crinkling was recorded in Raichur district (Table 2.). During the survey of leaf crinkle virus disease, the greengram variety Chinamung is the sole variety that occupied 95 per cent of the area in these four districts and the remaining area was occupied by Selection-4 and Koperguan. However, all the varieties are susceptible to LCV infection. It was noticed that the crop infected at early stage of the growth suffered more with symptoms with almost all the leaves showing curling, crinkling and reduction of leaf size, while the severely infected plants showing puckering and rugosity of leaves was observed and the plants showed late maturity giving few flowers and pods with flower stalk was condensed very much. The pod formation was severely reduced and the pod size also reduced giving few under developed and immature seeds.

Invariably aphids were found feeding in most of the greengram fields surveyed along with jassids, whiteflies, thrips and pod borers found sporadically in some of the fields.

4.3 Transmission of leaf crinkle virus disease

4.3.1 Mechanical transmission

Mechanical sap inoculation was carried out in 10 to 12 days old seedlings raised in polyethylene bags under insect proof glass house conditions. The virus was easily transmissible by mechanical means. To see the effect of molarity of phosphate buffer at P^H 7.0 on the efficiency of mechanical transmission, the diseased leaves of greengram cv. China mung was brought from field and were crushed in varied (0.05-0.15) concentration separately. Thereafter, inoculum prepared as above was rubbed with fore fingers and celite powder is sprinkled over the leaves before inoculation.

The results as presented in table 3 indicated that the efficiency of mechanical transmission was maximum when the inoculum (sap) was prepared in 0.1 M phosphate buffer at P^H 7.0 (90%) with exhibition of curling and crinkling symptoms on first trifoliate leaves with in 10 to 12 days after inoculation (Plate 11).

4.3.2 Aphids transmission

The insect transmissibility of the virus was carried out by using aphid species (*Aphis craccivora*) for transmission studies. The aphid was found transmitting the virus to an extent of 67.5 per cent (Table 4). The inoculated plants exhibited symptoms such as

Table 3. Effect of molar concentration of phosphate buffer at pH 7.0 on mechanical transmission of leaf crinkle virus disease

Phosphate buffer (Molar)	No. of plants infected/ inoculated	Percentage of transmission
0.05	1/10	10
0.075	3/10	30
0.10	10/10	100
0.125	2/10	20
0.15	4/10	40

Table 4. Transmission of leaf crinkle virus of greengram by aphids (*Aphis craccivora*)

Experiment No.	No. of plants infected/ inoculated	Percentage of transmission
1	6/10	60
2	7/10	70
3	6/10	60
4	8/10	80
Average	6.75/10	67.5

Variety used - Chinamung;
Pre acquisition starvation - 1 hr
Inoculation access period - 24 hr
Number of aphids inoculated per plant – 10;
Acquisition access period – 30 min;

Plate 11. Symptoms of leaf crinkle virus on greengram plants inoculated through sap

curling and crinkling on the newly formed trifoliate leaves at 15 to 18 days after inoculation (Plate 12).

4.3.3 Whitefly transmission

Whitefly (*Bemisia tabaci)* was used for studies on transmission of leaf crinkle virus of greengram as described under materials and methods. The results obtained are presented in Table 5 indicate that the leaf crinkle virus disease was not transmitted by whitefly (*Bemisia tabaci*), as none of the plant was infected and observed symptom in an inoculated plants even after six weeks after inoculation.

4.4 Detection of leaf crinkle virus disease of greengram

4.4.1 Electron microscopy

The diseased samples that were carried to IIHR, Bengaluru for viral identification tested positive. The partially purified samples showed the presence of spherical particles (Plate 13).

The greengram leaves infected by leaf crinkle virus disease was brought from the field as well as glasshouse and was amplified in PCR using two sets of primers *viz*., Deng (Deng A and Deng B) and CP (AV 494 and AC 1048). The PCR products were visualized through gel documentation unit.

4.4.2 PCR detection of LCV using Deng primers

Leaf crinkle virus of greengram was not detected in the PCR product. But the positive sample of Sunflower leaf curl virus was detected approximately ~520 bp using Deng A and Deng B primers. A band of approximately ~520 bp was consistently amplified from the total DNA extracted from infected samples (Plate 14a)

4.4.3 PCR detection of LCV using CP primers

Again greengram leaf crinkle virus was not detected in the PCR product. A band of approximately ~575 bp was consistently amplified from the total DNA extracted from infected Sunflower leaf curl samples using AV 494 and AC 1048 primers (Plate 14b).

Hence leaf crinkle virus of greengram was not grouped under begmoviruses, it doesn’t contain DNA in the virus particle and also virus was failed to transmit through whitefly (*Bemisia tabaci*).

Table 5. Transmission of leaf crinkle virus of greengram by whitefly (*Bemisia tabaci*)

Experiment No.	No. of plants infected/ inoculated	Percentage of transmission
1	0/10	0
2	0/10	0
3	0/10	0
4	0/10	0
Average	0/10	0

Variety used - Chinamung; Number of whiteflies inoculated per plant – 10;
Acquisition access period – 6 hr; Inoculation access period - 24 hr.

Plate 12. Symptoms of leaf crinkle virus disease on greengram plants inoculated through aphid

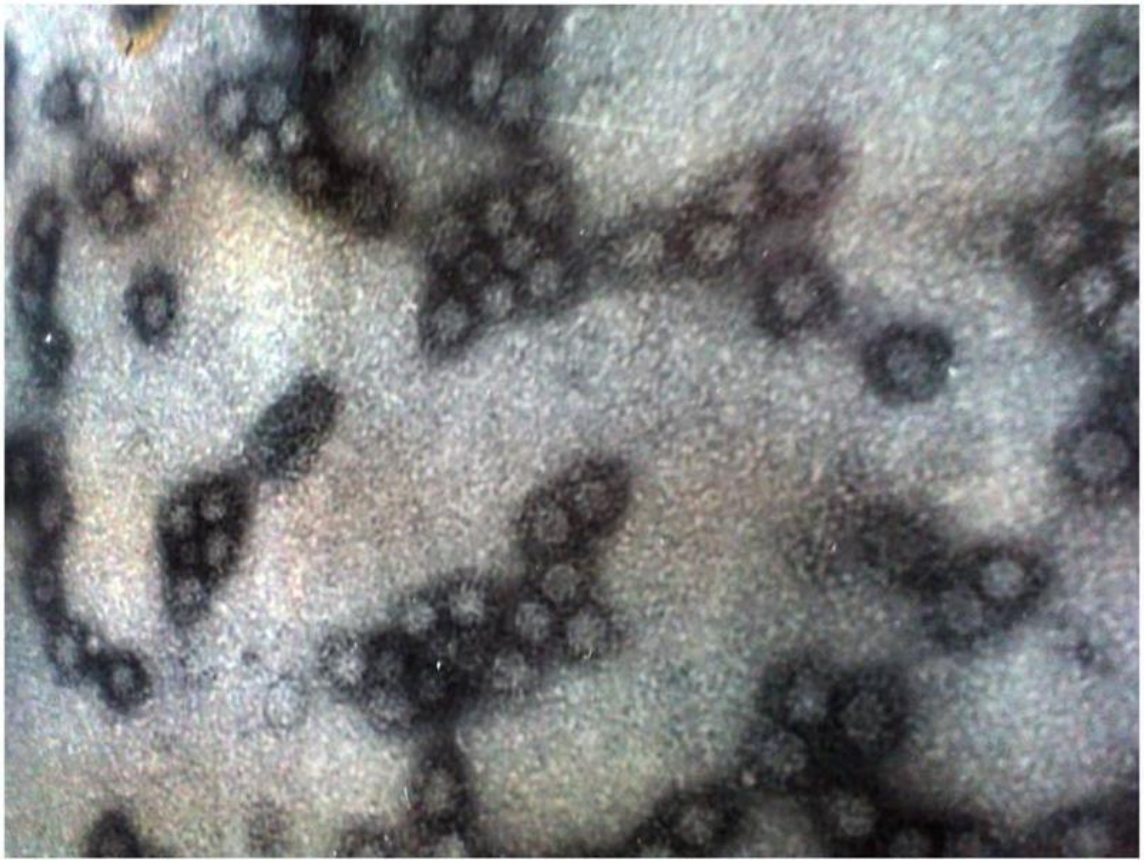

Plate 13: Electron micrograph of leaf crinkle virus showing spherical particles obtained from naturally infected greengram (magnification 10000X)

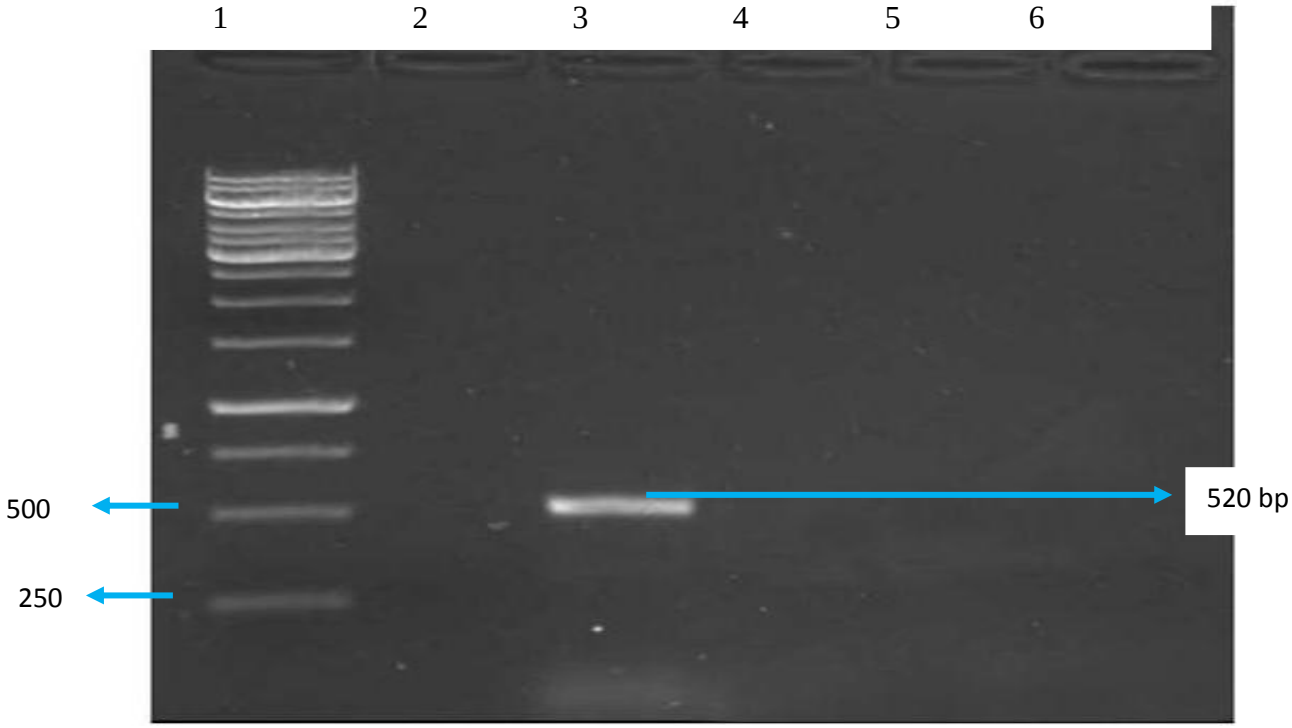

Plate 14 a). Gel showing PCR products obtained with Deng primers: lane 1: 1 kb DNA ladder; lane 2 Healthy Sunflower: lane 3: Field infected SLCV sample;; lane 4: Greengram LCV infected sample ; lane 5: Greengram healthy sample ;lane 6: water blank

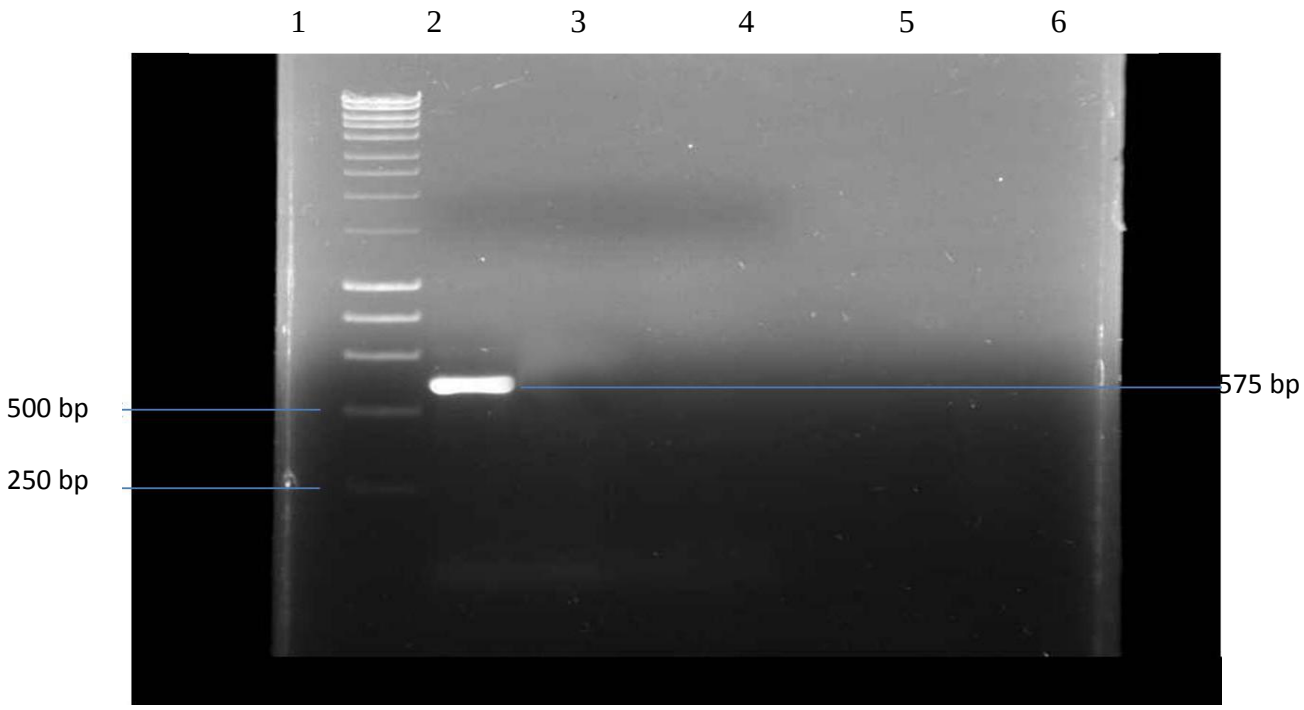

Plate 14 b. Gel showing PCR products obtained with CP primers: lane 1: 1 kb DNA ladder; lane 2: Field infected sunflower sample; lane 3: Healthy SLCV; lane 4: Greengram LCV infected sample ; lane 5: Greengram healthy sample ; lane 6: water blank

4.5 Screening of germplasm against leaf crinkle virus disease of greengram

To identify the source of resistance, a total of thirteen greengram genotypes were screened against LCV at the Farm of College of Agriculture, Bheemarayanagudi. during *kharif* 2012 under rainfed conditions. The per cent disease incidence was recorded at every 15 days interval from 15 days after sowing. The results are presented in Table 6 and 7.

During *kharif* 2012, LCV disease incidence varied from 14.6 to 42 per cent, 14.6 per cent and 15.0 per cent in DGGV-04 and LGG 460 respectively. Chinamung and S-401 varieties recorded 38.7 per cent and 42.0 per cent incidence respectively. Moderately susceptible genotypes were Co-16, KMM-3306, Pusa-9072 and MH 969 with the per cent crinkle disease incidence of 24.32, 28.05, 21.4 and 23.3 per cent respectively (Table 6 and Plate 15)

Further, these genotypes were grouped into different categories based on 0-5 scale. None of the genotypes showed highly resistant and resistant reaction. Whereas the genotypes BGS-9, Pusa Baisaki, CGG 973, LGG 460 and DGGV-04 were showed 17.64, 20.0, 16.6, 15.0 and 14.6 per cent disease incidence respectively and were grouped under moderately resistant. While, Sel-4, MH-564 and China mung were found to be susceptible for crinkle virus disease with PDI of 32.3, 37.5 and 38.7 respectively. The variety S-401 was found highly susceptible with 42.0 PDI.

4.6. Management of leaf crinkle virus disease of greengram using chemicals and botanicals

An experiment was conducted for the management of greengram leaf crinkle virus disease by using various chemicals and botanicals during *kharif* 2012 at College of Agriculture, Bheemarayanagudi. Per cent crinkle disease incidence, vector population, growth and yields parameters and economics in each treatment were computed by statistical analysis.

4.6.1 Per cent disease incidence

During the studies, the initial crinkle disease incidence was varied from 7.83 to 9.08 per cent before the imposition of first sprays (Table 8).

The experimental results revealed that after first spray, the plot imposed with imidacloprid 60 FS as seed treatment (5ml/kg of seeds) along with imidacloprid 17.8 SL

Table 6. Screening of greengram genotypes against leaf crinkle virus disease during *kharif* 2012

Sl. No.	Genotypes	PDI at Pre flowering stage
1.	BGS-9	17.64
2.	Pusa Baisaki	20.00
3.	Co-16	24.32
4.	CGG -973	16.60
5.	KM-3306	28.50
6.	S-4	32.30
7.	Pusa-9072	21.40
8.	MH-969	23.30
9.	S-401	42.00
10.	MH-564	37.50
11.	LGG-460	15.00
12.	DGGV-04	14.60
13.	China mung	38.70

*****PDI - Per cent Disease Incidence

Table 7. Grouping of greengram genotypes screened against leaf crinkle virus disease during *kharif* 2012

Genotypes	Description	Category
- Nil -	All plants free of symptoms.	Highly Resistant
- Nil-	1-10 per cent plants infected showing mild crinkling at the top and pods are normal	Resistant
LGG-460, DGGV-04, BGS-9, Pusa Baisaki, and CGG-973,	11-20 per cent plants infected showing crinkling and curling of top leaves with pods are normal	Moderately Resistant
CO-16, KMM-3306, Pusa-9072 and MH-969	21-30 per cent plants infected with crinkling, puckering, malformation and shortening of pods	Moderately Susceptible
Sel-4, MH-564 and China mung	31-40 per cent plants infected showing all the typical disease symptoms	Susceptible
S-401	More than 40 per cent plants infected showing severe symptoms with few pods containing only few seeds	Highly Susceptible

Moderately Resistant genotypes

Moderately susceptible genotypes

Susceptible (check)

Highly susceptible genotype

Plate 15. Greengram genotypes showing reaction against leaf crinkle virus disease during *kharif* 2012

Table 8. Effect of different chemicals and botanicals on leaf crinkle virus disease incidence of greengram during *karif* 2012

Treatment No.	Treatment details	Before sprays	15 days after first spray	15 days after Second spray	Mean	Percent reduction over control
T_1	Seed treatment with Imidacloprid 60 FS (Gaucho ® at 5 ml/ kg of seeds)	8.51 (16.97)	30.22 (33.36)	41.03 (39.85)	26.59	12.79
T_2	Seed soaking with Cow urine @ 2.0%	8.22 (16.66)	31.33 (34.05)	44.67 (41.96)	28.07	7.93
T_3	T_1 + Two sprays with Imidacloprid 17.8 SL @ 0.03%	8.05 (16.48)	15.43 (23.12)	19.84 (26.46)	14.44	52.64
T_4	T_1 + Two sprays with Cow urine @ 2.0%	8.14 (16.58)	29.91 (33.17)	39.45 (38.93)	25.84	15.25
T_5	T_1 + Two sprays with Azadiractin 1500 ppm @ 3 ml/lit	8.33 (16.78)	27.05 (31.36)	34.30 (35.87)	23.23	23.81
T_6	T_1 + Two sprays with Profenophos 50EC @ 2 ml /lit	8.40 (16.86)	21.44 (27.60)	25.43 (30.30)	18.43	39.55
T_7	T_2+ Two sprays with Imidachloprid 17.8 SL @ 0.03%	8.05 (16.49)	16.74 (24.15)	21.70 (27.77)	15.50	49.16
T_8	T_2 + Two sprays with Cow urine @ 2.0%	8.14 (16.57)	30.51 (33.55)	39.98 (39.24)	26.21	14.03
T_9	T_2 + Two sprays with Azadiractin 1500 ppm @ 3 ml/lit	8.19 (16.63)	28.49 (32.27)	36.69 (37.30)	24.45	19.8
T_{10}	T_2 + Two sprays with Profenophos 50 EC @ 2 ml/lit	7.83 (16.25)	22.60 (28.40)	27.38 (31.56)	19.27	36.79
T_{11}	Control	9.08 (17.54)	33.63 (35.46)	48.77 (44.32)	30.49	
	S.m±	0.36	0.35	0.38		
	C.D. at 5%	NS	1.04	1.11		

*Figures in parentheses indicate angular transformed values *Sprays were given at 25 and 40 days after sowing

(0.03%) spray at 25 and 40 DAS (T_3) recorded significantly lower disease incidence (15.43%) followed by (T_7) cow urine seed treatment with two sprays of imidacloprid 17.8 SL at 25 and 40 DAS showed lower (16.74%) disease incidence. The next best treatments were seed treatment with imidacloprid along with two sprays of profenophos 50 EC @ 2 ml/lit (T_6) which was followed by cow urine seed treatment with two sprays of profenophos @ 2 ml/lit (T_{10}). In control plot disease incidence was 33.63 per cent.

Fifteen days after second spray Imidacloprid seed treatment plot with two sprays of imidacloprid 17.8 SL at 25 and 40 days (T_3) showed lowest per cent disease incidence of 19.84, followed by cow urine seed treatment along with two sprays of imidacloprid 17.8 SL with 21.70 per cent disease incidence. The seed treatment with imidacloprid along with two sprays of profenophos 50EC @ 2 ml/lit (T_6) emerged as next best treatment with the per cent disease incidence of 25.43, when compare to control plot was recorded 48.77 per cent crinkle disease incidence.

At the end of the experimental period there was significantly lowest mean disease incidence of 14.44 per cent was recorded in imidacloprid (5 ml/kg of seeds) seed treatment plot along with two sprays of imidacloprid 17.8 SL (0.03%) at 25 and 40 DAS (T_3) which is followed by (T_7) cow urine seed treatment with two sprays of imidacloprid 17.8 SL, seed treatment with imidacloprid along with two sprays of profenophos (T_6), cow urine seed treatment along with two sprays of profenophos (T_{10}), seed treatment with imidacloprid along with two sprays of azadiractin 1500 ppm @ 3 ml/lit (T_5) and cow urine seed treatment with two sprays of azadiractin (T_9) has recorded with a mean incidence of 15.50, 18.43, 19.27, 23.23 and 24.45 per cent in order of their effectiveness, whereas in control plot disease incidence was 30.49 per cent (Table 8 and Plate 16).

Seed treatment with imidacloprid along with two sprays of imidacloprid at 25 and 40 DAS (T_3) showed highest 52.64 per cent reduction over control followed by seed treatment with cow urine along with imidacloprid two sprays at 25 and 40 DAS (T_7) with 49.16 per cent reduction. Least reduction of 7.93 per cent of crinkle disease incidence over control was found in seed soaking with cow urine @ 2.0 per cent.

4.6.2 Vector population

The mean vector, *Aphis craccivora* population varied from 14.20 to 16.23 per plant was observed at one day before the first spray (Table 9). The plot imposed

a) T_1 + Two sprays with Imidacloprid 17.8 SL @ 0.03%
b) T_2+ Two sprays with Imidachloprid 17.8 SL @ 0.03%
c) T_1 + Two sprays with Profenophos 50EC @ 2 ml /lit
d) T_2 + Two sprays with Profenophos 50EC @ 2 ml /lit
e) Control

Plate 16: Effect of chemicals and botanicals on leaf crinkle virus disease incidence of greengram during *Kharif* 2012

Table 9. Effect of different chemicals and botanicals on vector population in greengram during *kharif* 2012 under field condition

Treatment No.	Treatment details	Average number of aphids on three top leaves/Plant					
		First spray at 25 DAS			Second spray at 40 DAS		
		1 DBS	5 DAS	Per cent reduction over control	1 DBS	5 DAS	Per cent reduction over control
T_1	Seed treatment with Imidacloprid 60 FS (Gaucho ® at 5 ml /kg of seeds)	14.53	15.87	31.97	24.67	30.3	17.43
T_2	Seed soaking with Cow urine @ 2.0%	16.10	15.07	35.40	30.33	36.0	1.90
T_3	T_1 + Two sprays with Imidacloprid 17.8 SL @ 0.03%	15.17	7.00	69.99	14.33	5.7	84.4
T_4	T_1 + Two sprays with Cow urine @ 2.0%	15.33	16.00	31.41	25.00	23.0	37.3
T_5	T_1 + Two sprays with Azadiractin 1500 ppm @ 3 ml/lit	14.23	9.67	58.55	20.33	16.7	54.4
T_6	T_1 + Two sprays with Profenophos 50EC @ 2 ml /lit	15.83	9.50	59.27	18.00	10.0	72.75
T_7	T_2 + Two sprays with Imidachloprid 17.8 SL @ 0.03%	14.20	9.00	61.42	14.67	6.7	81.74
T_8	T_2 + Two sprays with Cow urine @ 2.0%	15.90	17.00	27.13	28.00	28.0	23.70
T_9	T_2 + Two sprays with Azadiractin 1500 ppm @ 3 ml/lit	15.47	11.33	51.43	21.00	18.3	50.13
T_{10}	T_2 + Two sprays with Profenophos 50 EC @ 2 ml/lit	15.10	11.00	52.85	19.00	11.0	70.02
T_{11}	Control	16.23	23.33		31.00	36.7	
	S.Em±	1.11	0.34		0.49	0.23	
	C D at 5%	3.27	1.0		1.47	0.67	

*DBS – Day before spraying, DAS – Days after spraying

combination of seed treatment with imidacloprid along with two sprays of imidacloprid 17.8 SL at 25 and 40 DAS has recorded the least vector population per plant giving 69.99 percent reduction over control at five days after first spray followed by (T_7) seed treatment with cow urine followed by two sprays of imidacloprid 17.8 SL at 25 and 40 DAS recorded the vector population of 9.00 per plant with 61.42 per cent reduction over control.

At one day before second spray, plot imposed with the seed treatment by imidacloprid with two sprays of imidacloprid (T_3) showed a least of vector population of 14.0 per plant followed by (T_7) seed treatment with cow urine followed by two sprays of imidacloprid 17.8 SL and plots with (T_6) seed treatment by imidacloprid along with two sprays of profenophos had 16.00 and18.00 aphids per plant respectively. Whereas the plot received seed treatment with imidacloprid followed by two spray of azadiractin 1500 ppm has recorded 20.0 aphids per plant. The highest 32 aphid population per plant was recorded in untreated control plot.

The vector population at five days second spray ranged from 5 to 36 per plant in (T_3) imposed with seed treatment by imidacloprid followed by two sprays with imidachloprid which recorded lowest vector population of 5.00 per plant indicating highest per cent reduction over control (84.4%). The treatment (T_7) recorded 6.00 aphids per plant and 81.74 per cent reduction over control, because of seed treatment with cow urine followed by two sprays of imidacloprid 17.8 SL. The plot combined with seed treatment by imidacloprid followed by two sprays of profenophos (T_6) at five days after second spray recorded 10.00 aphids per plant found to be next best treatment and has given 72.75 per cent reduction over control plot when compare to control plot 36 aphids per plant was recorded.

4.6.3 Growth and yield parameters

4.6.3.1 Plant height

At the end of the experiment (60 DAS), there was a significant differences between various treatments with regard to plant height. However, in the present study the plant height varied from 29.1 to 35.1 cm. The seed treatment plot by imidacloprid 60 FS along with two sprays of imidacloprid at 25 and 40 DAS (T_3) showed 35.1 cm plant height followed by plot seed treatment with cow urine along with imidacloprid sprays

(T_7) which showed 31.8 cm plant height when compare to control plot observed with 29.1 cm plant height (Table 10).

4.6.3.2 Pods per plant

The treatment T_3 (Imidacloprid seed treatment @ 5 ml kg^{-1} along with two sprays of imidacloprid @ 0.03%) was found to be the best with maximum pod production giving 11.7 pods per plant and which was on par with seed treatment by cow urine along with two sprays of imidacloprid which recorded 10.7 pods per plant respectively. Where as seed treatment with imidacloprid along with two sprays of profenophos (T_6), seed treatment with cow urine along with two sprays of profenophos (T_{10}), seed treatment with imidacloprid along with two sprays of azadiractin @ 3 ml/ lit (T_5), seed treatment with cow urine along with two sprays of azadiractin @ 3 ml/ lit (T_9), seed treatment with imidacloprid 60 FS alone @ 5 ml kg^{-1} of seeds (T_1) were found to be the next best treatments recording 10.1, 9.5, 9.3, 8.5 and 8.6 pods per plant respectively. Seed soaking with cow urine (T_2) recorded significantly lowest pods per plant (7.9) and was on par with untreated control plot (7.5) (Table 10).

The treatment imposed with imidacloprid seed treatment @ 5 ml kg^{-1} along with two sprays of imidacloprid @ 0.03% (T_3) and plot having seed treatment with cow urine along with two sprays of imidachloprid (T_7) was found most effective recording 56.0 and 42.66 per cent increased number of pods per plant over control.

4.6.3.3 100 seed weight

The plots which received Imidacloprid seed treatment @ 5 ml kg^{-1} of seed along with two sprays of imidachloprid @ 0.03 per cent (T_3) recorded significantly highest 100 seed weight of 5.07g followed by the seed treatment plot with cow urine along with two sprays of imidachloprid @ 0.03 per cent (T_7) recorded 3.85 g of 100 seed weight. Seed soaking with cow urine @ 2.0 per cent (T_2) were least effective recording 100 seed weight of 3.20 g and was on par with control plot (3.18g).

4.6.3.4 Yield

The plot with imidacloprid seed treatment @ 5 ml kg^{-1} of seed along with two sprays of imidachloprid @ 0.03 per cent recorded highest yield of 11.57 q ha^{-1} which was on par with seed treatment with cow urine along with two sprays of imidachloprid @ 0.03 per cent (11.08 q ha^{-1}) while the treatment seed treated with imidachloprid along with two

Table 10. Effect of leaf crinkle virus disease on growth and yield parameters of greengram under field conditions during *kharif* 2012

Treatment No.	Treatment details	Plant height (cm)	Pods/ plant	% increase of pods over control	100 seed weight (g)	Yield (q/ha)	% increase in yield over control
T_1	Seed treatment with Imidacloprid 60 FS (Gaucho ® at 5 ml /kg of seeds)	29.7	8.6	14.66	3.48	8.12	32.46
T_2	Seed soaking with Cow urine @ 2.0%	29.5	7.9	5.33	3.20	6.53	6.52
T_3	T_1 + Two sprays with Imidacloprid 17.8 SL @ 0.03%	35.1	11.7	56.0	5.07	11.57	88.74
T_4	T_1 + Two sprays with Cow urine @ 2.0%	30.0	9.1	21.33	3.42	7.63	24.46
T_5	T_1+ Two sprays with Azadiractin 1500 ppm @ 3 ml/lit	29.9	9.3	24.0	3.58	9.54	55.62
T_6	T_1 + Two sprays with Profenophos 50EC @ 2 ml /lit	32.1	10.1	34.66	3.73	10.63	73.40
T_7	T_2 + Two sprays with Imidachloprid 17.8 SL @ 0.03%	31.8	10.7	42.66	3.85	11.08	80.75
T_8	T_2 + Two sprays with Cow urine @ 2.0%	28.7	8.2	9.33	3.31	7.02	14.51
T_9	T_2 + Two sprays with Azadiractin 1500 ppm @ 3 ml/lit	29.8	8.5	13.33	3.50	8.60	40.29
T_{10}	T_2 + Two sprays with Profenophos 50 EC @ 2 ml/lit	30.3	9.5	26.66	3.64	10.23	66.88
T_{11}	Control	29.1	7.5		3.18	6.13	
	S.Em±	0.78	0.34		0.30	0.85	
	C D at 5%	2.30	1.01		0.89	2.51	

sprays of profenophos @ 2 ml/lit has given 10.63 q ha^{-1} followed by seed treatment with cow urine along with two sprays of profenophos @ 2ml/lit (10.23 q ha^{-1}) and the plot with seed treatment by imidachloprid along with two sprays of azadiractin (9.54 q ha^{-1}) recorded next best yields and were on par. Seed treatment with imidachloprid 60 FS @ 5 ml kg^{-1} of seed and seed treatment with imidachloprid and two sprays of cow urine at two per cent were next best in order of their effectiveness recording yields of 8.12 and 7.63 q ha^{-1} respectively. Similarly the treatment with seed soaking by cow urine at two per cent recorded lower yield of 6.53 q ha^{-1}, whereas lowest yield lowest yield of 6.13 q ha^{-1} was recorded in untreated control.

The highest per cent increase in yield over control was recorded in imidachloprid seed treatment plot along with two sprays of imidachloprid @ 0.03 per cent (88.74%) whereas, the least yield of 6.52 per cent was recorded in seed soaking with cow urine plot (Table 10).

4.6.4 Economics

Data on cost of cultivation, gross returns and net returns as influenced by chemicals and botanicals on leaf crinkle virus disease and their combinations is presented in Table 11.

Seed treatment with imidacloprid @ 5 ml kg^{-1} along with two sprays of imidacloprid @ 0.03 per cent resulted in higher cost of cultivation (16,536 Rs ha^{-1}) while the lowest cost of cultivation was incurred with plot seed treatment by cow urine along with two sprays of cow urine and control (14,911 Rs ha^{-1}).

Higher gross returns were obtained from the plot seed treatment by imidacloprid @ 5 ml kg^{-1} along with two sprays of imidacloprid @ 0.03 per cent (Rs 40,483 ha^{-1}) followed by seed treatment by cow urine @ 2 per cent along with two sprays of imidacloprid @ 0.03per cent (Rs. 38,768 ha^{-1}). The lowest gross return was obtained in control plot (Rs. 21,467 ha^{-1}).

Highest net returns was recorded with seed treatment by imidachloprid @ 5 ml kg^{-1} along with two sprays of imidacloprid @ 0.03 per cent (Rs. 23,947 ha^{-1}) and the lowest net returns was obtained with control plot (Rs. 6,556 ha^{-1}).

Table 11. Benefit Cost (B:C) ratio for the management of leaf crinkle virus disease of greengram under field conditions during *kharif* 2012

Treatment No.	Treatment details	Yield (q/ha)	Cost of production (Rs.)	Treatment cost (Rs.)	Total cost (Rs.)	Gross return (Rs.)	Net profit (Rs.)	B : C ratio
T_1	Seed treatment with Imidacloprid 60 FS (Gaucho ® at 5 ml /kg of seeds)	8.12	14911	1125	16036	28420	12384	1.77
T_2	Seed soaking with Cow urine @ 2.0%	6.53	14911	-	14911	22867	7956	1.53
T_3	T_1 + Two sprays with Imidacloprid 17.8 SL @ 0.03%	11.57	14911	1625	16536	40483	23947	2.45
T_4	T_1 + Two sprays with Cow urine @ 2.0%	7.63	14911	1125	16036	26717	10681	1.67
T_5	T_1+ Two sprays with Azadiractin 1500 ppm @ 3 ml/lit	9.54	14911	1885	16796	33390	16594	1.99
T_6	T_1 + Two sprays with Profenophos 50EC @ 2 ml /lit	10.63	14911	1725	16636	37217	20581	2.24
T_7	T_2 + Two sprays with Imidachloprid 17.8 SL @ 0.03%	11.08	14911	500	15411	38768	23357	2.52
T_8	T_2 + Two sprays with Cow urine @ 2.0%	7.02	14911	-	14911	24570	9659	1.65
T_9	T_2 + Two sprays with of Azadiractin 1500 ppm @ 3 ml/lit	8.60	14911	760	15671	30100	14429	1.92
T_{10}	T_2 + Two sprays with Profenophos 50 EC @ 2 ml/lit	10.23	14911	600	15511	35817	20306	2.31
T_{11}	Control	6.13	14911	-	14911	21467	6556	1.44
	S.Em±	0.85						
	C.D. at 5%	2.51						

Benefit cost ratio for management of greengram crinkle virus disease was significantly influenced by combination of botanicals and chemicals. The plot seed treatment by cow urine @ 2 per cent along with two sprays of imidacloprid @ 0.03 per cent recorded significantly higher B:C ratio (2.52), however, it was found to be on par with the seed treatment with imidacloprid @ 5 ml kg^{-1} along with two sprays of imidacloprid @ 0.03 per cent (2.45), which was followed by seed treatment with imidacloprid @ 5 ml kg^{-1} along with two sprays of profenophos @ 2 ml/lit (2.24) and the lowest benefit cost ratio was recorded in untreated control (1.44).

Discussion

V. DISCUSSION

Greengram crop is a victim of a large number of diseases caused by fungi, bacteria and viruses. Among the virus diseases, leaf crinkle is an important disease in India causing considerable yield losses depending on the season and type of cultivar. The disease was first reported from India by Williams *et al*. (1968). The results obtained in the present study on survey and surveillance for leaf crinkle virus, transmission of crinkle virus disease, detection of virus, screening of genotypes, management of disease against leaf crinkle virus in greengram are interpreted in detail here under.

5.1 Symptomatology

The leaf crinkle virus disease in greengram produced similar symptoms in natural and artificial infection conditions with variations in severity. The major prominent symptoms of the disease on greengram were enlargement of trifoliate leaves, crinkling of leaf lamina and shortening of petiole of terminal leaflets. Infected plants were stunted and showed bushy appearance with sparse or no flowering, reduced pod formation and pods contain few small discoloured and shrivelled seeds. Kolte and Nene (1970) reported typical symptoms as in the present study such as enlargement of leaf lamina, leaf crinkling, sterility and stunting of the plant. Similar symptoms of the disease were also reported in Delhi and Uttar Pradesh (Williams *et al.*, 1968).

Ravinder Reddy *et al*. (2005a) reported similar range of symptoms with significant enlargement of leaflets on trifoliate leaves, crinkling, puckering and reduction in rachis length to terminal leaflets. Enlargement of leaflets was evident from the third trifoliate leaflets. The possible reason for increase in size may be due to higher auxin content in diseased leaves.

Bhaktavatsalam *et al*. (1982) also reported increase in indole acetic acid (IAA) content in ULCV infected urdbean leaves than the healthy leaves and attributed this to be pronounced expansion and distinct crinkling of diseased leaves.

5.2 Survey and surveillance for severity of the leaf crinkle disease of greengram

Roving survey was conducted to know the distribution of leaf crinkle virus on greengram in North Eastern parts of Karnataka during *kharif* 2012 in major greengram growing areas of Raichur, Yadgir, Gulbarga and Bidar districts. During the survey, the

mean crinkle disease incidence ranged from 10.04 to 31.21 per cent. Maximum disease incidence was recorded in Yadgir district with 31.21 per cent. At Adanur village, it was recorded 33.96 per cent disease incidence and least incidence of 28.76 per cent was recorded at Balichakra. It was clear that the disease incidence was noticed in all localities (Fig. 4). Muhammad Bashir *et al.* (2006) reported the disease ranging from 5 to 28 per cent in blackgram, Mahajan and Joi (1999) also reported 12 to 30 per cent crinkle disease incidence in greengram in Maharashtra.

The variation in disease incidence is due to the variations in temperature and relative humidity that have direct influence on vector population and its migration. Similarly, the nature and rate of spread of urdbean leaf crinkle virus under field conditions was earlier reported by Beniwal *et al*. (1979).

Aphids (*Aphis craccivora*) were invariably found in every infected field surveyed. However, jassids, thrips and pod borers were also the other insects noticed.

5.3 Transmission studies

5.3.1 Sap transmission

The efficiency of sap transmission of leaf crinkle virus was greatly in sap prepared with 0.1 M phosphate buffer at P^H 7.0. Changing the molarity of the phosphate buffer did not enhance the efficiency of mechanical transmission. The results are in confirmation with the studies made by other previous workers. [Kolte and Nene 1972; Bhaktavatsalam *et al*., 1983b; Subbarao 1984; Krishnaveni, 1988; Vijaykumar, 1993; Suneela, 1996; Kadian 1994 and Patel *et al*., 1999] who also achieved maximum systemic infection at 0.1 M of phosphate buffer with varying P^H of 7.6, 7.8, 7.0 and 7.4 respectively (Fig. 5).

5.3.2 Vector transmission

In the present study, the virus causing leaf crinkle disease was successfully transmitted by *Aphis craccivora* to an extent of 60 to 80 per cent, which confirm the previous findings [Dhingra, 1975, Dhingra and Chenulu, 1981, Dubey *et al.*, 1983, Vijay Kumar and Subba Rao 1994a and Vijaykumar, 1993] (Fig. 6).

Present isolate of the virus could not be transmitted by whitefly (*Bemisia tabaci*) and confirms the results of Vijaykumar (1993) pertaining to the non transmission of virus by whitefly. In contrast, urdbean leaf crinkle virus isolate from Tamil Nadu was

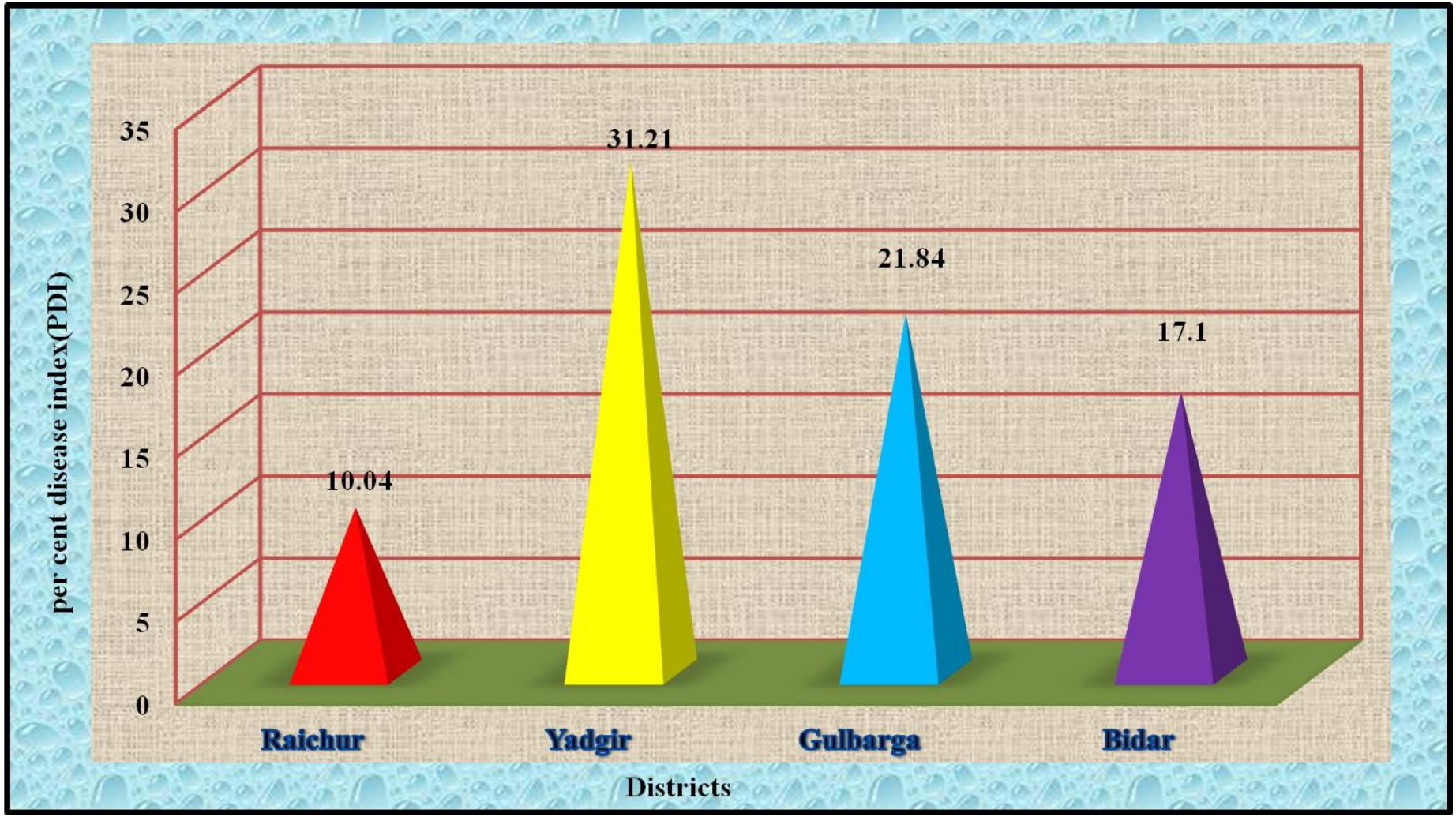

Fig. 4. Survey for the incidence of leaf crinkle virus disease of greengram during *Kharif* 2012 in four districts of North Eastern Karnataka under rainfed conditions

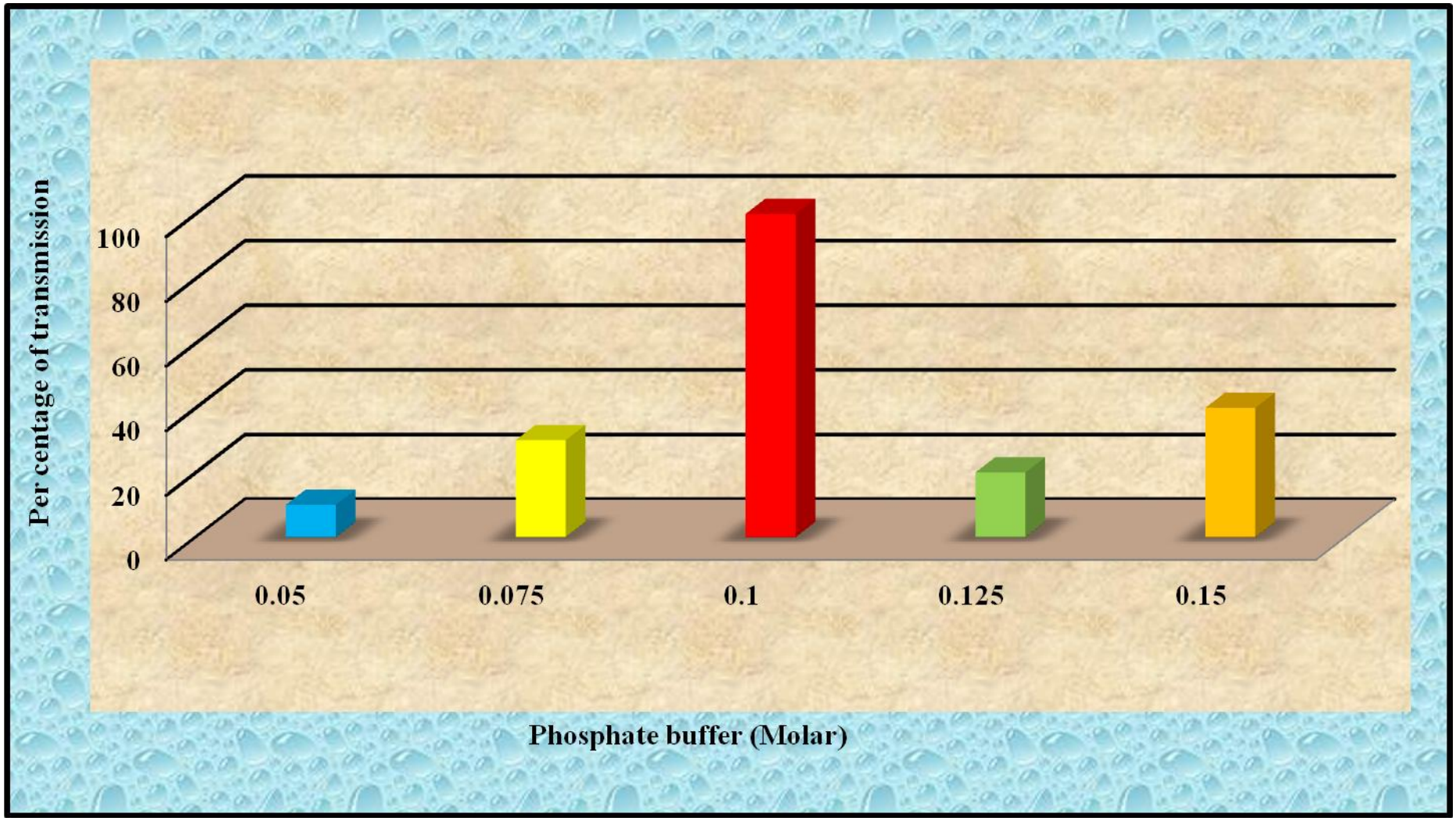

Fig. 5. Effect of molar concentration of phosphate buffer at P^H 7.0 on mechanical transmission of leaf crinkle virus disease of greengram

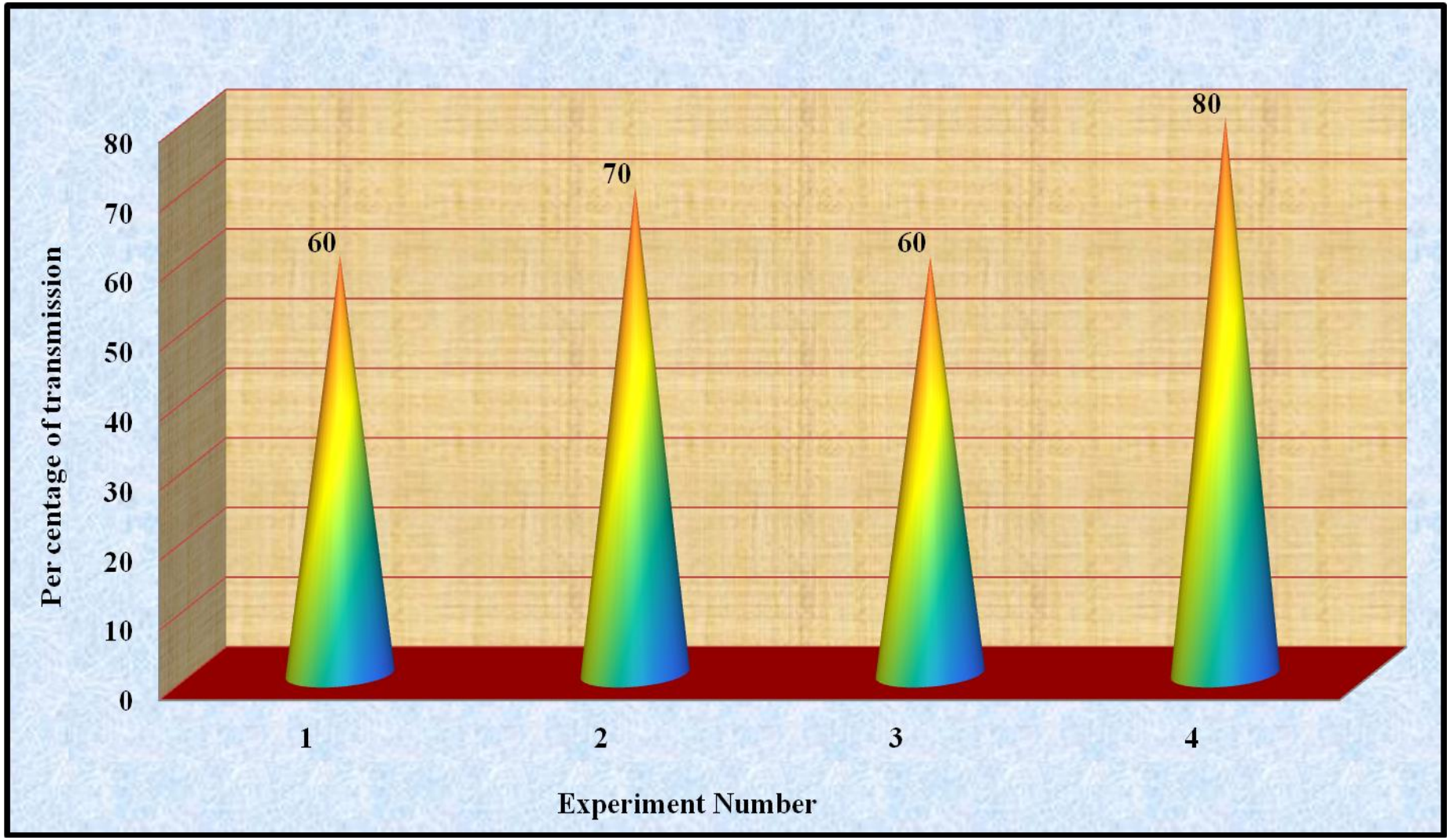

Fig. 6. Transmission of leaf crinkle virus of greengram by aphids (*Aphis craccivora*)

transmited by whitefly, *Bemisia tabaci* in a non persistent manner as reported by Narayansamy and Jaganathan (1973c) and Prasad *et al.* (1998) and Sahay *et al.*(1999) from Meghalaya.

Acythosiphan pisum is an additional vector for ULCV from Pantnagar and Uttar Pradesh as reported by Dubey *et al.* (1983) and Bhardwaj and Dubey (1984) other insect vectors *Henosepilanchna dodecastigma* (Beniwal and Bharatan,1980), *Hyseteroneura setariae* and *Liphaphis erysime* (Nath *et al*., 1986) reported to transmission of leaf crinkle virus disease and were not found on this crop in Karnataka.

These studies indicated that the vector specificity in transmission of various isolates of the virus causing leaf crinkle virus from different parts of the country. There is need to work on the insect transmission of leaf crinkle virus to know the virus isolate vector specificity pertaining to the leaf crinkle isolates reported from different parts of the country.

5.4 Detection of leaf crinkle virus disease of greengram

Electron microscopic studies revealed the presence of typical spherical particles in diseased samples of greengram. Similar types of spherical particles were earlier reported by Bhakthavatsalam (1976) and Dubey *et al*. (1983).

In the present study, leaf crinkle virus was not detected in the greengram infected samples by PCR using two sets of primers (Deng *et al*., 1994) and (Wyatt and Brown, 1996).

It was confirmed by PCR that the leaf crinkle virus was not a DNA virus and the virus was not transmitted by whiteflies. Hence leaf crinkle virus would not come under begmoviruses group.

5.5 Screening of genotypes against leaf crinkle virus disease of greengram

Identification of resistant genotypes is one of the most important aspects in the management of viral diseases and which could be the best possible solution in certain crops.In the present investigations a total of 13 genotypes were screened during *Kharif* 2012 under rainfed conditions. The disease incidence varied from 14.6 to 42.0 per cent in various genotypes. None of the genotypes of greengram tested under field conditions were found highly resistant to the disease. While, BGS-9, Pusa Baisaki and DGGV-04

showed moderately resistant reactions with less than 20 per cent incidence. Co -16, KMM-3306, Pusa-9072 and MH-969 genotypes were found moderately susceptible with exhibiting symptoms and the susceptible check Chinamung has showed 38.70 per cent disease incidence.

In general the disease was less and the majority of the genotypes tested have recorded moderately resistant to moderately susceptible reaction against leaf crinkle virus disease during *kharif* 2012.

Similar type of varietal evalutions were previously documented by several workers (Vijay Kumar, 1993; Haq *et al.*, 1991; Ramana Murthy, 1997; Ganapathy *et al*., 2003; Nageswara Rao *et al*., 2003; Ashfaq *et al*., 2007; Chaudary *et al*., 2007 and Baniyamin *et al*., 2011). However, the genotypes now showing some degree of resistance should be tested again by artificial inoculation or at "hot spot" areas like Yadgir and Shahpur taluks. This should be done before including them either in resistance breeding programme or recommending directly as resistant varieties.

5.6 Management of leaf crinkle virus disease of greengram using chemicals and botanicals

Among the various treatments tested for managing the leaf crinkle virus disease, the seed treatment by imidacloprid along with two sprays of imidacloprid was found highly effective and recorded the lowest per cent disease incidence and least number of aphids (7.0 aphids per plant at five days after first spray and 5.7 aphids per plant five days after second spray). This consequently led to light incidence of leaf crinkle virus at 40 days after planting (15.43%) and 55 days after planting (19.84%) (Fig. 7 and 8).

The efficacy of imidacloprid for the management of aphids was earlier reported by Mote *et al*. (1993), Jarante and Dethe (1994) and Dandale *et al*. (2001).

The application of organophosphorous insecticides to kill the aphid vectors has been widely investigated as a method of decreasing spread of aphid borne virus diseases in crop plants. The control of aphids transmitting viruses in persistent manner by using organophosphorous insecticides has been more successful than the control of non-persistent aphid transmitted viruses. In the present study foliar application of the organophosphorous insecticide *i.e*., Profenophos 50 EC at 2 ml/ lit was effective next to the imidachloprid 17.8 SL at 0.03 per cent in reducing the disease incidence and also

LEGEND

Treatment details

T_1: Seed treatment with imidacloprid 60 FS (Gaucho ® at 5 ml /kg of seeds)

T_2: Seed soaking with cow urine @ 2.0%

T_3: T_1 + Two sprays with imidacloprid 17.8 SL @ 0.03%

T_4: T_1 + Two sprays with cow urine @ 2.0%

T_5: T_1 + Two sprays with azadiractin 1500 ppm @ 3 ml/lit

T_6: T_1 + Two sprays with profenophos 50EC @ 2 ml /lit

T_7: T_2+ Two sprays with imidachloprid 17.8 SL @ 0.03%

T_8: T_2 + Two sprays with cow urine @ 2.0%

T_9: T_2 + Two sprays with azadiractin 1500 ppm @ 3 ml/lit

T_{10}: T_2 + Two sprays with profenophos 50 EC @ 2 ml/lit

T_{11}: Control

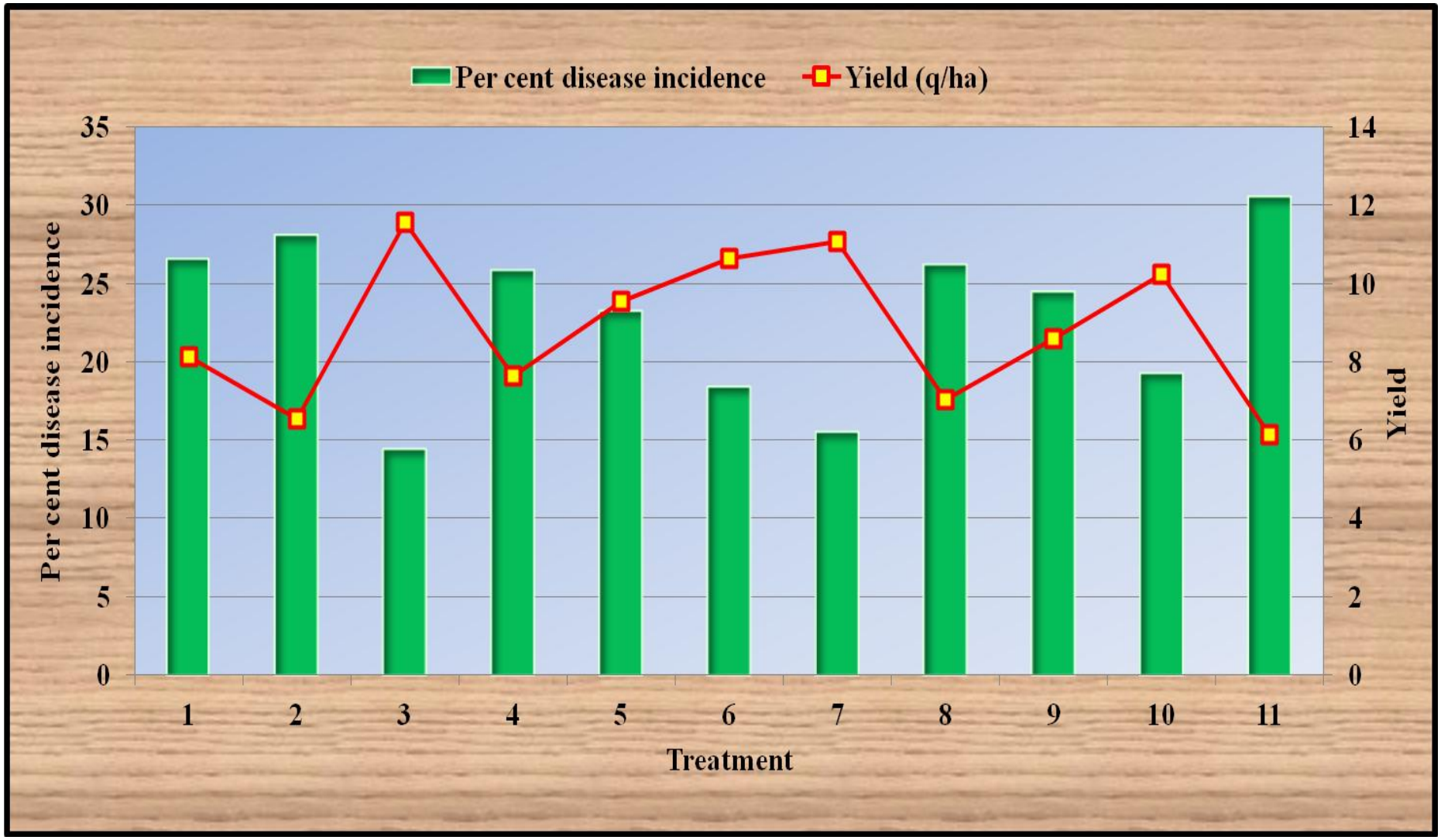

Fig. 7. Effect of different chemicals and botanicals on leaf crinkle virus disease incidence of greengram during *Kharif* 2012

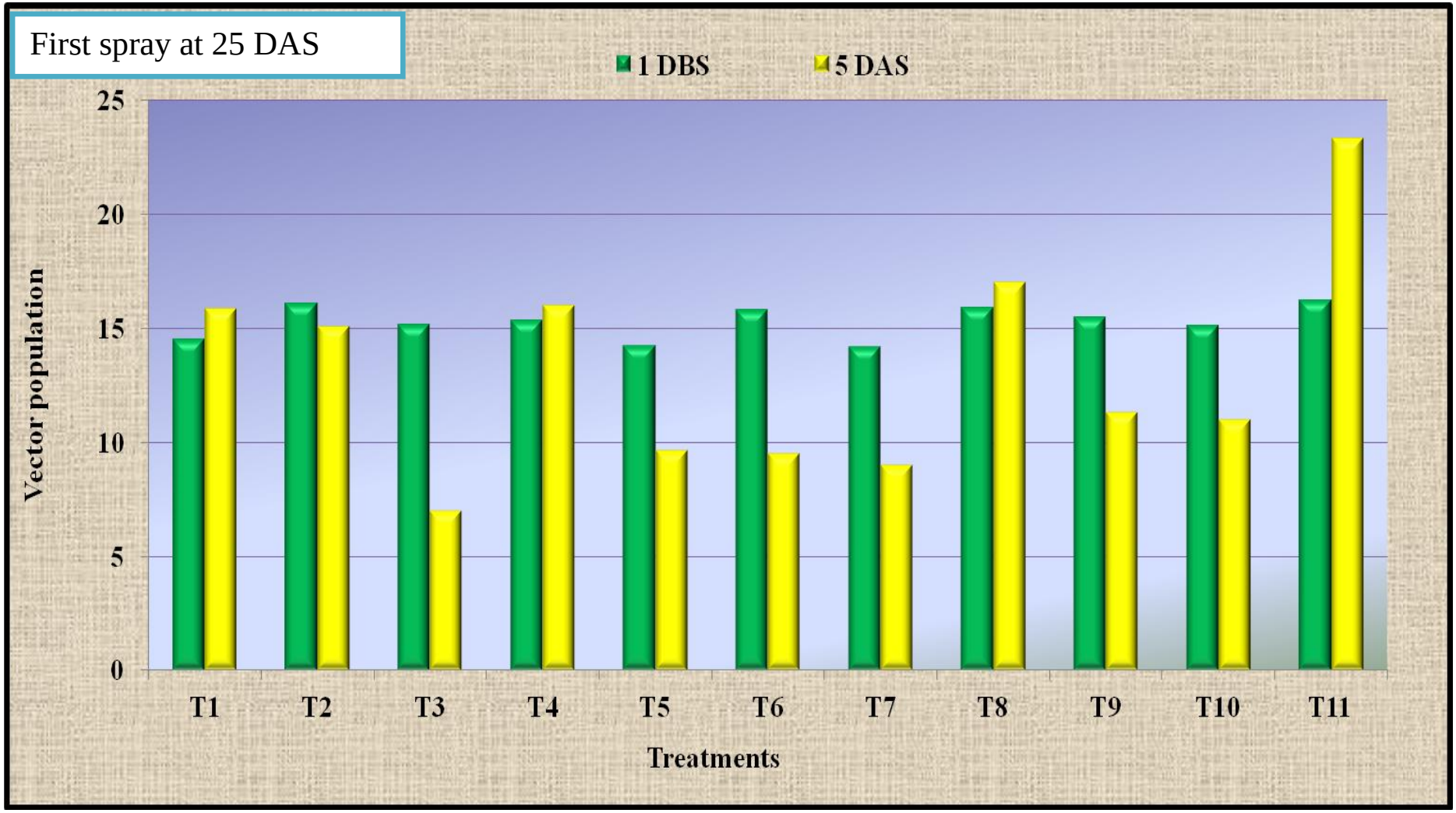

Fig. 8a. Effect of different chemicals and botanicals on vector population in greengram during *Kharif* 2012 under field condition

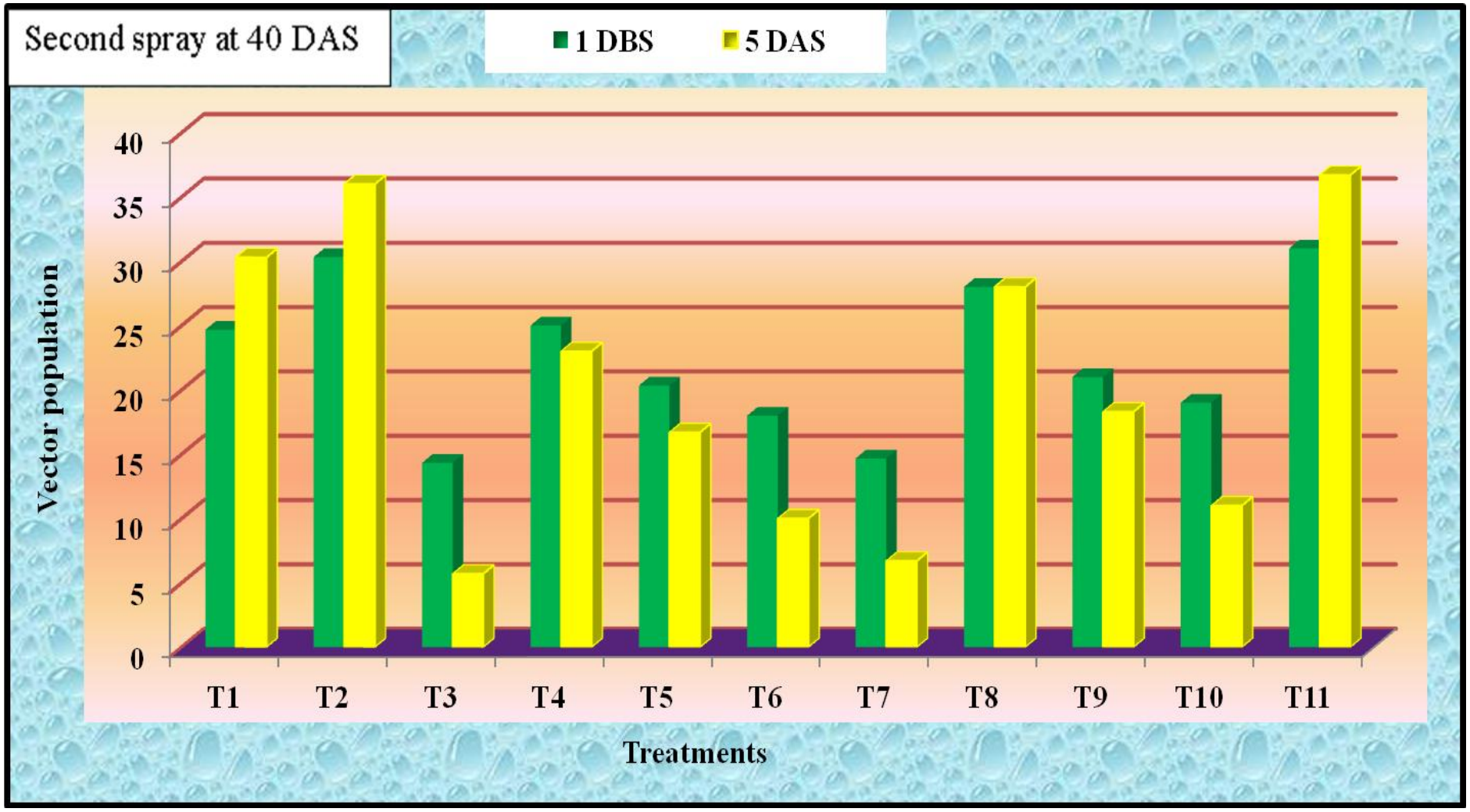

Fig. 8b. Effect of different chemicals and botanicals on vector population in greengram during *kharif* 2012 under field condition

increasing the yield and yield parameters of greengram. The results are in agreement with several researchers who reported organophosphorous insecticides to reduce non persistently aphid transmitted viruses such as PVY in potatoes, tobacco etch and tobacco vein mottling viruses in tobacco, bean yellow mosaic virus in lupins and cucumber mosaic virus in narrow leafed lupins (Broadbent *et al*., 1956; lobenstein and Raccah 1980; Pirane *et al*., 1988 and Bwye *et al*., 1997).

Seed treatment of imidacloprid followed by two sprays of azadiractin 1500 ppm at 3 ml/lit and seed treatment with cow urine followed by two sprays of azadiractin were moderately effective by recording an incidence of 27.05 and 36.69 per cent at 15 days after first spray, while 34.30 and 36.69 per cent of crinkle incidence observed at 15 days after second spray. Lower aphids count of 9.67 and 11.33 per plant at five days after first spray and 16.7 and 18.3 aphids per plant at five days after second spray was observed. The results of the present study were supported by Roychaudhary and Jain (1996) who studied the effect of neem oil sprays on aphids and reported that it is more toxic to nymphs, causing 100 per cent mortality than to the adult stage in which it caused 60 to 98 per cent mortality.

Chandrashekhar and Balsubramanian (2002) reported the higher percentage of reduction in aphid population and yellow mosaic virus disease incidence in greengram due to foliar spray of neem oil 60 EC at 3 per cent. Similar results with regard to ULCV disease incidence was recorded in neem oil treated plot by Ravindra Babu (1987). Similarly Baniyamin *et al*. (2011) reported that the minimum ULCV disease incidence and vector population was observed on plants sprayed with neem (2.0%) followed by akk (2.52%).

Kannan and Doraiswamy (1993) noticed reduction in cowpea mosaic with one percent emulsion of *Azardictina indica* and increased the yield up to 890 kg. Neem oil was presumed to contain antifeedant and repellent properties. Verma (1974) identified two compounds *viz.*, nimbidin and nimbin in neem oil which inhibited local lesion formation.

In the present study, the seed treatment with imidacloprid followed by two sprays of imidacloprid drastically reduced the spread of leaf crinkle virus disease and significantly increased yields compared to control plot. This may be attributed to the superior knockdown activity of imidacloprid which has a systemic as well as contact effect and it is a second generation nicotinic acetylcholine receptor, which acts on the central nervous system of aphids and causes paralysis leading to death of aphids.

5.6.1 Growth and yield Parameters:

The effect of leaf crinkle virus disease on various growth and yield parameters *viz*., Plant height, pods per plant, 100 seed weight and yield per hectare in different treatments were recorded. It is evident from the results that the treatments which recorded least aphid population and per cent disease incidence have shown a significant positive effect on all the growth and yield parameters evaluated.

Among the treatments, imidacloprid 60 FS @ 5 ml kg^{-1} seed treatment followed by two sprays imidacloprid 17.8 SL @ 0.03 per cent has recorded highest number of pods of per plant (11.7), plant height (35.1 cm), 100 seed weight (5.07 g) and yield (11.57 q/ha). The superiority of imidacloprid in controlling sucking pests was earlier documented by several workers like Mote *et al*. (1993), Jarande and Dethe (1994), Mote *et al*. (1994a), Walunj and Mote (1995) and Dandale *et al*. (2001) on various other crops. The treatment with cow urine seed treatment @ 2.0 per cent along with two sprays of imidacloprid @ 0.03 per cent was found the next best treatments with 31.8 cm plant height, 10.7 pods per plant, 3.88 g of 100 seed weight and yield of 11.08 q per ha .

Jhangir Shah *et al*. (2007) reported the imidacloprid treated plots had significantly the highest yield of 1563 kg ha^{-1} while, the lowest yield of 1056 kg ha^{-1} was obtained from the control plots of greengram. The results are in agreement with Singh (1980), Beniwal and Chaubey (1979), Mandhare *et al*. (1999).

Bashir *et al*. (1991) reported that LCV infection at early stages of the plants was known to reduce heavy losses and affected almost all the components of urdbean and a reduction of 90.8 per cent in number of pods, 18.4 per cent in pod length, 26.5 per cent in seed weight and 81 per cent in yield of diseased plants was recorded. Similarly, results of reduction in pod plant height, root length, nodulation, pods per plant, length of the pod, seeds per pod, seed weight and yield losses of 41.1 to 69.3 per cent in different cultivars of urdbean were reported (Reddy *et al*.,1996).

The effectiveness of insecticides was attributed to greater residual activity, high level of protection, quick knock down effect of insecticides on viruliferous vectors compared to botanicals, plant products and animal products that act indirectly by enhancing growth of plant, delaying disease appearance as reported by Baranwal and Ahamed (1997), and by inducing resistance as reported by Verma and Varsha (1995), or by changing feeding behaviour by deterring settling activity of vector or becoming toxic to vector before inoculating virus as reported by Somashekara *et al*. (1997).

5.6.2 Economics

The treatment- wise yield and cost of treatment application was worked out based on the data, net profit and benefit cost ratio were computed and presented in Table 11.

The highest yield of 11.57 q ha^{-1} was recorded imidacloprid seed treatment followed by two sprays of imidacloprid (T_3) followed by the next best yield was recorded to be 11.08 qha^{-1} in the treatment combining cow urine as seed treatment along with two sprays of imidacloprid @ 0.03 per cent (T_7) as against control with 6.13 q ha^{-1}.

The treatment T_3 combining imidacloprid 60 FS @ 5 ml kg^{-1} as seed treatment along with two sprays of imidacloprid recorded highest net returns of Rs. 23,947. The next best to follow were $T_{7,}$ (cow urine seed treatment along with two sprays of imidacloprid 17.8 SL @ 0.03%) and T_6 (imidacloprid 60 FS @ 5 ml kg^{-1} seed treatment along with two sprays profenophos) that gave Rs. 23,357 and 20,581 respectively.

Seed treatment with cow urine along with two sprays of imidacloprid (T_7) recorded highest benefit to cost ratio of 2.52. Although the yield was not highest here but due to less cost of plant protection that showed highest B:C ratio.

The treatment T_3 (combining seed treatment by imidacloprid 60 FS @ 5 ml kg^{-1} along with two sprays of imidacloprid) recorded highest net returns and it gave a B: C ratio of Rs. 2.45 which is next best treatment to $T_{7.}$ Seed treatment with imidacloprid along with two sprays of profenophos @ 2 ml/lit recorded good net returns but showed a meagre B:C ratio because of higher dosage and inturn increased cost of insecticide compared to the treatment T_3. The lowest net returns was realized (Rs. 7956) in seed soaking with cow urine @ 2.0 per cent alone with least B:C ratio of Rs.1.53, this is because of seed treatment alone could protect the crop for a period up to 30 to 35 days and after that it might lost its effectiveness.

Summary and Conclusions

VI. SUMMARY AND CONCLUSIONS

The present investigation was undertaken to study the survey and surveillance of leaf crinkle virus disease, transmission studies on LCV in greengram, detection of the leaf crinkle virus, screening of genotypes against the LCV and management of leaf crinkle virus disease through chemicals and botanicals and the results obtained are summarised here under.

Under field condition, the first recognisable symptoms of the disease appeared as light green in colour on the second trifoliate leaf. Around 25 days after sowing crinkling

of leaves appear in addition to enlargement of trifoliate which become more pronounced with age. As the infected plant grows older extreme crinkling and rugosity on the older trifoliate leaves was observed. Around thirty days after first appearance of the symptoms, tips of the affected leaflets curve downwards and petiole of the lamina touches the surface of the lower leaflets on either side of the infected plants. The affected plants become stunted and gave bushy appearance. Flowering in the affected plants got delayed and peduncles produced from axils of affected trifoliate bear large number of small size flowers which never open due to folding of its floral parts. No pods were formed in severely affected plants even if they were formed, filled poorly, small and under sized, variation in shape and testa colour.

The Survey conducted during *kharif* 2012, revealed that the LCV of greengram in North Eastern Karnataka was severe in vegetative stage of the crop. The severity was more in Yadgir district (31.21%) followed by Gulbarga district (21.84%) and Bidar district (17.10%). The disease severity was lower in Raichur district (10.04%). The maximum disease incidence of 33.26 per cent was observed in Adanur village of Yadgir district on susceptible variety Chinamung. Aphids, whiteflies, jassids and pod borers were the common insects prevalent in almost all the fields surveyed.

Maximum infection (80.0%) was recorded when the plants were inoculated with standard extract of phosphate buffer 0.1 M at P^H 7.0 with the symptoms of curling and crinkling ten to twelve days after inoculation.

The aphid (*Aphis craccivora*) transmitted the virus and the transmission varied from 60 to 80 per cent. Whitefly (*Bemisia tabaci*) failed to transmit the virus to the test plants.

Electron microscopic study from diseased samples revealed the presence of spherical particles in partially purified preparations.

Studies were made on detection of leaf crinkle virus disease through PCR amplification and results showed the absence of viral DNA and also it confirmed that leaf crinkle virus was not grouped under begomovirus genera. Considering all these studies on this curling and crinkling symptoms of virus was identified as leaf crinkle virus.

Among thirteen genotypes evaluated for resistance against leaf crinkle virus disease of greengram during *kharif* 2012 under rainfed conditions, none of the genotypes showed resistance to highly resistant reaction. BGS-9, Pusa Baisaki and LGG 460 were found moderately resistant, whereas the genotypes S-4, MH 564 and Chinamung were

found to exhibit susceptible reaction. Chinamung that covers the most of the greengram growing areas in Karnataka was found susceptible to leaf crinkle virus disease.

The crop could be protected by seed treatment with imidacloprid 60 FS (5 ml/kg) along with two sprays of imidacloprid 17.8 SL (0.03%) at 25 and 40 days after sowing followed by seed treatment with cow urine at 2 per cent along with two sprays of imidacloprid 17.8 SL (0.03%) at 25 and 40 days after sowing which recorded least number of aphids per plant and crinkle virus disease incidence. This brought out significant increase in all the growth and yield parameters assessed. However, seed treatment by cow urine at 2 per cent along with two sprays of imidacloprid 17.8 SL (0.03%) recorded highest benefit cost ratio (Rs. 2.52) it was on par with seed treatment with imidacloprid along with two spray with imidacloprid (Rs. 2.45). Instead of using chemical (imidacloprid) for seed treatment it was advisable to shift to use our farming from chemicals to this type of natural (cow urine) cost effective and eco-friendly formulation provided.

Conclusion

- Among the four districts surveyed for leaf crinkle virus disease incidence in greengram during *kharif* season of 2012 in North Eastern Karnataka, Yadgir (31.21%) and Gulbarga (21.84%) districts were recorded highest incidence leaf crinkle virus disease of greengram.
- Leaf crinkle virus exhibited symptoms of curling, crinkling, enlargement of the trifoliate leaves and rugosity of leaves, stunting of plants, malformation of floral organs and pod formation is reduced severely in infected plants.
- Transmission studies of leaf crinkle virus disease of greengram revealed the virus was readily transmitted through sap at 0.1 M potassium phosphate buffer at P^H 7.0.
- With respect to the transmission of leaf crinkle virus by vectors, aphids showed the positive results by showing the symptoms on the inoculated plants.
- It was confirmed by PCR, that the leaf crinkle virus was not a DNA virus and the virus failed to be transmitted by whiteflies. Findings indicate that the virus may not belong to begomovirus group.
- Among thirteen genotypes screened for disease resistance none of the genotypes showed resistant reaction to leaf crinkle virus disease but five genotypes showed moderately resistant reaction.

- Comparatively lower disease incidence with increase in yield was recorded in plots receiving seed treatment with imidacloprid at 5 ml/kg along with two sprays of imidacloprid 17.8 SL @ 0.03 per cent at 25 and 40 DAS.
- Maximum benefit cost ratio was obtained from the plots receiving cow urine seed treatment at two per cent along with two sprays of imidacloprid 17.8 SL at 0.03 per cent compared to control plot.

Future line of work

- Survey and surveillance of the disease may be continued and extended to other areas to confirm the hot spots to record the situation of the leaf crinkle virus disease.
- Seed transmission study must be focused.
- Detailed studies on Virus -vector relationship through aphid may be required.
- The studies on host range for the leaf crinkle virus disease in greengram are required.
- Screening of genotype which is resistance to disease may be evaluated further to exploit in the resistance breeding programme.
- Complete characterisation of the causal virus with reference to the chemical composition, and its identification is required.
- Synthesis of specific primers for the detection of leaf crinkle virus of greengram has great potentiality.
- Production of antisera and developing serological diagnostic techniques for the virus detection is very much essential.
- Further studies may be carried out on epidemiology with reference to the role of seed transmission and other pulse crops in perpetuation an spread of leaf crinkle virus disease under field conditions is essential.

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* Originals are not seen

Appendix

APPENDIX – I

Prices of inputs and outputs

Sl. No.	Items	Rate
1.	Greengram seeds	40 Rs./ kg
2.	Farmyard manure	600 Rs./ t
3.	Chemical fertilizers	
	Urea	6 Rs./ kg
	DAP	22 Rs./ kg
	MOP	8 Rs./ kg
4	Chemicals	
	Profenophos	560 Rs/lit
	Imidacloprid	2584 Rs/lit
	Azadiractin	380 Rs /lit
	Gaucho	75 Rs /5ml
5.	Preparatory tillage	1000 Rs./ ha
6.	Sowing, fertilizer and manure application	1500 Rs./ ha
7.	Weeding	1000 Rs./ ha
8.	After care	1000 Rs./ ha
9.	Bagging and transportation	800 Rs./ ha
10.	Harvesting and threshing	3400 Rs./ ha
11.	Land rent	100 Rs./ ha
12.	Price of out puts	
	Grain yield	3000 Rs./ q

INVESTIGATIONS ON LEAF CRINKLE VIRUS DISEASE IN GREENGRAM

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ABSTRACT

Greengram (*Vigna radiata* (L) Wilczek) is the third most important pulse crop grown in India. The crop becomes a victim of large number of diseases caused by both fungi and viruses. Among the viral diseases, Leaf crinkle virus (LCV) is considered to be most serious one causing considerable damage to the crop. The roving survey of leaf crinkle virus was carried out in four districts of North Eastern Karnataka *viz*., Raichur, Gulbarga, Yadgir and Bidar during *kharif* season of 2012. Maximum disease severity was recorded in Yadgir (31.21%) followed by Gulbarga (21.84%) and Bidar (17.10%) districts. Maximum infection (80.0%) was recorded when the plants were inoculated with phosphate buffer 0.1 M at p^H 7.0. The aphid (*Aphis craccivora*) transmitted the virus and the transmission varied from 60 to 80 per cent. Whitefly (*Bemisia tabaci*) was failed to transmit the virus to the test plants. Electron microscopic studies showed the presence of spherical particles in partially purified preparations. Detection of leaf crinkle virus disease through PCR amplification showed the absence of viral DNA and confirms that leaf crinkle virus was not grouped under begomovirus genera. Among thirteen genotypes evaluated for resistance against LCV of greengram during *kharif* 2012, none of the genotypes showed resistance to highly resistant reaction. BGS-9, Pusa Baisaki and LGG 460 were found moderately resistant, whereas the genotypes S-4, MH564 and Chinamung were found to be susceptible reaction. Comparatively lower disease incidence with increase in yield was recorded in plots receiving seed treatment with imidacloprid at 5 ml/kg along with two sprays of imidacloprid 17.8 SL @ 0.03 per cent at 25 and 40 DAS.