NUTRIENT BASED MANAGEMENT OF *Chilli leaf curl virus* IN CHILLI (*Capsicum annuum* L.)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Nutrient based management of *Chilli leaf curl virus* in Chilli (*Capsicum annuum* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Nutrient based management of *Chilli leaf curl virus* in Chilli (*Capsicum annuum* L.)"** is a record of research work done independently by Ms. Shilpa Sankar under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
ha	Hectare
@	At the rate of
Fig.	Figure
CD	Critical difference
SE	Standard error
SE (m) ±	Standard error of mean
et al.	And other co workers
viz.	Namely
i.e.	That is
cm	Centimetre
m	Metre
g	Gram
kg	Kilogram
dSm^{-1}	Deci siemens per metre
mg kg^{-1}	Milligram per kilogram
kg ha^{-1}	Kilogram per hectare
L	Litre
mL	Milllilitre
µl	Microlitre
µg	Microgram
mM	Milli molar
min.	Minute
nm	Nano meter
ELISA	Enzyme linked immunosorbent assay
TAS-ELISA	Triple antibody sandwich-Enzyme linked immunosorbent assay
DIBA	Dot immunobinding assay
NCM	Nitrocellulose membrane
SLCMV	*Sri Lankan cassava mosaic virus*
PCR	Polymerase chain reaction

MAb	Monoclonal antibody
PBS	Phosphate-buffered saline
TBS	Tris buffer saline
TAE	Tris acetate buffer
bp	Base pair
CP	Coat protein
EDTA	Ethylene diamine tetra acetic acid
rpm	Rotations per minute
KAU	Kerala Agricultural University
POP	Package of Practices
N	Nitrogen
P	Phosphorus
K	Potassium
Ca	Calcium
Mg	Magnesium
S	Sulphur
Mn	Manganese
Zn	Zinc
Fe	Iron
Cu	Copper
B	Boron
Si	Silicon
pH	Negative logarithm of hydro carbon ions
°C	Degree Celsius
h	Hour
DNA	Deoxy ribonucleic acid
T	Treatment
CRD	Completely randomized design
OD	Optical density
CI	Coefficient of infection
SAR	Systemic acquired resistance

NSKE	Neem seed kernel extract
ChiLCV	*Chilli leaf curl virus*
ChiLCD	Chilli leaf curl disease
CLCuMV	*Cotton leaf curl Multan virus*
PepYLCIV	*Pepper yellow leaf curl Indonesia virus*
ToLCNDV	*Tomato leaf curl New Delhi virus*
ChiLCVeV	*Chilli leaf curl Vellanad virus*
ToLCV	*Tomato leaf curl virus*
ToLCBV	*Tomato leaf curl Bangalore virus*
TYLCTHV	*Tobacco yellow leaf curl Thailand virus*
TbLCYnV	*Tobacco leaf curl Yunnan virus*
SLCCNV	*Squash leaf curl China virus*
PepLCBV	*Pepper leaf curl Bangladesh virus*
ChiLCMV	*Chilli leaf curl Multan virus*
PepLCLV	*Pepper leaf curl Lahore virus*
ChiLCSLV	*Chilli leaf curl Sri Lankan virus*
ChiLCSLB	*Chilli leaf curl Sri Lankan betasatellite*
ChiLCSV	*Chilli leaf curl Salem virus*
TMV	*Tobacco mosaic virus*
MYMV	*Mungbean yellow mosaic virus*
ULCV	*Urdbean leaf curl virus*
PRSV	*Papaya ring spot virus*

Introduction

1. INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important vegetable cum spice crop belonging to family Solanaceae and originated in Mexico, Southern Peru and Bolivia (Villalon, 1981). India is the world leader in chilli production followed by China and Pakistan. India has about 366 thousand million hectare area and production of 3737 thousand metric tonnes during 2018-19, taking the major share (43 %) of global production. India is not only the largest producer but also the largest consumer of chilli in the world. Karnataka is the largest producer of chilli in India that contributes about 45.91 thousand ha of the total area under chilli followed by Madhya Pradesh (41.29 thousand ha area) (NHB, 2018). In India, Kerala occupies an area of about 85207 ha and produces 34065 tonnes. In Kerala, Palakkad occupies an area of 272 ha and produces 275 tonnes (Farm Guide, 2019).

Some varieties of chillies are famous for red colour because of the pigment capsanthin while others are known for biting pungency attributed by capsaicin. It is known for its flavour and pungency due to capsicum and having a medicinal value. The enzyme isolated from chilli is used in cancer treatment. Chilli crop suffers from many viral, bacterial and fungal diseases. Among the diseases, virus infected chilli crops with symptoms like mosaic, curling, yellowing etc. resulted in heavy yield and economic losses (Villalon, 1981).

Viral diseases acts as the main factor contributing to low yield, reduced fruit quality and 100 percent loss of marketable fruit (Kang *et al.*, 1973). Chilli leaf curl disease (ChiLCD) has been a major problem to chilli in both tropical and subtropical parts of India (Marte and Wetter, 1986).

Chilli leaf curl virus (ChiLCV) is caused by genus begomovirus, the largest genus of family Geminiviridae which infects only dicotyledonous plants and transmitted by whitefly (*Bemisia tabaci* (*Genn.*)). Chilli leaf curl etiology was established in 1960s in India. Whitefly transmitted ChiLCD has been reported in India from different states with more significance in terms of incidence and yield loss (Muniyappa and Veeresh, 1984).

Leaf curl symptoms of chilli were found to be caused by *Cotton leaf curl Multan virus* (CLCuMV) and *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) in Pakistan and Indonesia respectively (Tsai *et al.*, 2008). In India, *Tomato leaf curl New Delhi virus* (ToLCNDV) showed close relation with ChiLCV in Lucknow (Khan *et al.*, 2006). Polymerase chain reaction (PCR) and DNA sequencing have complemented the detection, identification and classification of begomoviruses.

Singh and Singh (1989) reported that this disease spreads more during summer due to the favourable temperature suited for building-up of both vector population and disease incidence. Many indirect approaches like checking the vector population, application of insecticides etc. may reduce ChiLCV infection marginally but it is not possible to ensure complete control. Unselective use of pesticides to control the vector causes environmental pollution and health hazards. The farmers often use a number of chemical pesticides indiscriminately to protect the crop without proper diagnosis of the causal organisms which results in resurgence of the pests, phytotoxicity and presence of high amount of pesticide residue in harvested fruits.

Integrative plant nutrition is a necessary component in sustainable agriculture because of its cost-effectiveness and environmentally friendly reaction to control plant disease with sufficient amount of nutrients and with no pesticides. Nutrients can reduce disease to an acceptable level to which further control by other cultural practices or conventional organic biocides could be achieved. Some nutrients have a greater impact on plant diseases by inhibiting the feeding ability of sucking pests that acts as vector for the transmission of virus (Dordas, 2009).

Hence it is important to study the effect of nutrient management in reducing the leaf curl virus disease incidence. Keeping the above facts and seriousness of the disease, the present study on nutrient based management of Chilli leaf curl disease in chilli (*Capsicum annuum* L.) was undertaken with the following objectives;

- To serologically and molecularly characterize the virus causing leaf curl in chilli and
- To study the role of nutrient application in the management of the disease.

Review of Literature

2. REVIEW OF LITERATURE

Chilli (*Capsicum annuum* L.) is an important vegetable cum spice crop cultivated throughout the country. Leaf curl disease of chilli has emerged as a serious problem and the destructive disease is caused by whitefly (*Bemisia tabaci*) transmitted geminivirus. This disease affecting chilli has been first reported in India by Senanayake *et al.*, 2007.

Amin (1979) concluded that feeding of whitefly, thrips and mites results in leaf curl in chilli and not by an infectious agent; such formed leaf curl is locally called as Murda complex in Karnataka. Economically, chilli leaf curl complex leads to substantial yield losses, often leads to complete failure of the crop.

Chilli leaf curl virus (ChiLCV) became a wide spread disease and caused a great loss of production, which may range from 80 to 100 per cent in case of early infection (Singh and Singh, 1979). According to Sarkar *et al.* (2013), chilli leaf curl disease cause a havoc economic loss (100 %) every year especially in the southern districts of West Bengal, India, and has become a threat to chilli growers.

2.1. COLLECTION OF BEGOMOVIRUS INFECTED CHILLI FROM DIFFERENT CULTIVATED AREAS

2.1.1. Disease incidence

A preliminary survey of viral diseases in the major chilli producing districts of Sri Lanka *viz.*, Jaffna, Puttalam, Vavuniya and Anuradhapura districts were carried out by Sugiura *et al.* (1975) from 1969 to 1970. In Jaffna and Puttalam districts, it was affected by mosaic disease followed by leaf curl disease. While in Vavuniya and Anuradhapura districts, leaf curl severely affected the crops. These differences in incidences of mosaic and leaf curl disease may be due to differences in the kind and number of vectors and inoculum of source plants.

Raju (2010) conducted a roving and fixed plot survey in the chilli growing tracts of Belguam, Bellary, Dharwad, Gadag, Gulburga, Haveri, Koppal and Raichur districts during 2002 to 2004 to assess the distribution of leaf curl complex disease in Northern parts of Karnataka. The results of roving survey confirmed the

presence of leaf curl complex disease in all the surveyed fields with an incidence ranging from 22 to 56 per cent. While in fixed plot survey, the results revealed that a maximum percent disease incidence (82.13) was observed in 2003 at Belguam compared to other locations.

The prevalence of ChiLCD was recorded by Senanayake *et al.* (2012) in Jodhpur district of Rajasthan, India. Five fields each of 0.4 to 0.8 ha were surveyed and diseased samples were collected. The disease incidence varied with different surveyed locations (14 to 100 %) where 100 per cent of incidence was observed in Tiwari region in chilli var. Haripur Raipur.

A field survey was conducted by Senanayake *et al.* (2013) in different areas of Anuradhapura district of Sri Lanka *i.e.*, Mahailluppallama, Rajanganaya and Nochchiyagama. Four samples with severe symptoms were collected from each area which recorded 60 to 100 per cent disease incidence at the fruit-bearing stage of crop.

Olawale *et al.* (2015) conducted surveys in six states of Nigeria in 2010 and 2011 to determine the disease incidence and distribution of viruses infecting pepper. Both healthy and diseased leaf samples were collected and analysed using antigen coated plate enzyme-linked immunosorbent assay (ACP-ELISA) to confirm the presence of viruses. An average disease incidence of 79 per cent was observed in 2010 and 76 per cent in 2011.

A roving survey was conducted in five villages each in two major chilli growing districts of Madhya Pradesh during November 2014. The symptomatology was recorded by selecting 50 random plants from each field. The survey showed that the chilli crop was severely infected by ChiLCV with disease incidence found to be 100 per cent in Khargone district while Ratlam district recorded 88 to 100 per cent with severe yield loss (Kumar *et al.*, 2016).

Field survey was conducted by Chaubey and Mishra (2017) from 2014 and 2015 in 150 fields of 30 villages in Faizabad and Sultanpur districts of Uttar Pradesh. It was revealed that most fields were infected with more than one type of

viral symptoms. The infection of ChiLCV was found in almost all fields with disease incidence ranging from 36.86 to 67.70 per cent in 2014 and 25.53 to 67.39 per cent in 2015.

A survey was carried out by Thakur and Sharma (2017) in major chilli growing areas of Punjab in 2013 to 2014 for assessing the prevalence of the virus. A maximum disease incidence of 79.4 per cent was observed in Ludhiana followed by Tarn Taran, Sangrur, Patiala and Ferozepur (77, 72.2, 68.6 and 57.5 % respectively).

Mishra and Chauvey (2018) surveyed all the chilli fields in different districts of Uttar Pradesh. They observed more occurrence of ChiLCV in all the fields due to the susceptible local cultivars prevailing in the districts. Chilli plants with small pungent fruits in Tarai region were found resistant against the virus.

A survey was carried out in randomly selected 54 fields of 18 villages in six districts of West Bengal during July-August, 2015. More incidence was noticed in Cooch Behar district (30 to 90 %) followed by Burdwan (20 to 85 %) and North 24 Parganas (45 to 75 %). While the lowest incidence was observed at Purulia (18 to 43 %) district. The results of survey revealed mixed infection of virus, mites and thrips in most of the fields (Oraon and Tarafdar, 2018).

2.2. SYMPTOMATOLOGY

Sugiura *et al.* (1975) characterized the disease by curling, puckering and vein swelling symptoms on young leaves at early stage of infection, coupled with upward or downward curling and stunting in young and old leaves. Reduced leaf and branch size were also observed in severely affected plants that resulted in a bushy appearance.

Moghe (1977) reported that the affected plants became erect and bushy and the leaves were dark green, puckered and oval to round in shape. He further observed that there was a pronounced vein thickening and leafy outgrowths or enations on the under surface of the leaves. Fewer flowers and fruits were produced by the diseased plants.

Abaxial curling of the leaves accompanied with yellowing, setting and enlargement of the veins were observed in the ChiLCV affected chilli plants by Muniyappa and Veeresh (1984).

The natural manifestation of severe leaf curl disease was observed in different chilli cultivars growing areas in Bahraich, Lucknow and Kanpur in Uttar Pradesh. The symptoms were upward and downward curling with puckering, scorching of intervenial areas and vein thickening. Infected plants expressed stunted growth with less flowering and deformed fruits (Raj *et al.*, 2005).

The ChiLCV affected leaves had curling, puckering and distortion with blistering of interveinal areas. The affected plants were stunted with shortening of internodes. Flower buds were abscised leading either to failure or development of small fruits (Kumar, 2006).

Senanayake *et al.* (2007) reported that the symptoms developed as curling, puckering and yellowing along with vein clearance. Rigorously affected plants were stunted with no fruits. Symptoms of slight yellowing, unadorned leaf curling, arrested growth and scorching were observed in chilli fields of Ludhiana (Shih *et al.*, 2007).

Chattopadyay *et al.* (2008) observed yellowing, severe leaf curling, leaf distortion, stunting, blistering and distortion busting and shortening of internodes. Characteristic curling, puckering and dwindled leaf area along with arresting of whole plants were observed by Kumar *et al.* (2011). The plants with *Chilli leaf curl Vellanad virus* (ChiLCVeV) symptoms exhibited typical upward leaf crinkling, yellowing, interveinal chlorosis and stunted growth (Kumar *et al.*, 2012). Sinha *et al.* (2013) reported the virus infected plants exhibited typical symptoms such as twisting and puckering of leaves, abnormal petioles, vein yellowing and stunting of plants.

Kumar *et al.* (2015) reported the symptoms of ChiLCV infected plants were curling, rolling, crumpling of leaves and compact leaf size. The more infected plants remained stunted with less fruit that resulted in crop failure. Kumar *et al.* (2016)

reported the virus infected plants with young leaves showing upward leaf curling, vein clearing and veinal thickening.

According to Kaur *et al.* (2016), infected chilli crop under natural viral disease incidence produced viral symptoms including vein-banding, mosaic, puckering, mottling of leaves, chlorotic or necrotic patterns or ring-spots on leaves and curling of leaves with reduced leaf size. The plants showed these viral symptoms at most of the locations except in Tarn Taran and Ferozepur district of Punjab.

Chaubey and Mishra (2017) reported virus symptoms such as vein clearing with dark green mottling, necrotic bands on veins with a bushy appearance. The disease was characterised by Kumar and Kumar (2017) as puckering, reduction in leaf size, curling, swelling of veins and petioles, bushy with reduced growth. Zehra *et al.* (2017) classified the symptoms of ChiLCV into three types such as vein yellowing, yellow mosaic and leaf curl. Curled leaf margin, yellowing, mottling and mosaic were also observed.

The most noticed field symptoms of ChiLCV were upward curling, blistering and bushy appearance with few or no fruits (Chaubey and Mishra, 2018). According to Chauhan *et al.* (2018), ChiLCV infected plants developed symptoms of leaf curling followed by cupping, stunting and curling at a later stage.

The severely infected plants showed crinkling, narrowing, curling of leaves which produced no fruit; however, less infected plants produced fruits of significantly reduced sizes (Oraon and Tarafdar, 2018). The symptoms of ChiLCV observed by Thakur *et al.* (2018) consisted of upward curling and puckering of the leaves accompanied with deformation and yellowing. Stunted plants had less flowers and malformed fruits.

2.3. SEROLOGICAL DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF THE VIRUSES CAUSING CHILLI LEAF CURL

According to Brown *et al.* (2001), enzyme-linked immunosorbent assay (ELISA) is currently employed for the virus detection. The virus identification by

outdated and traditional methods became tough. Hence, a steady and dependable method for the detection of ChiLCV remained as a difficult task. Serological diagnosis alone is not appropriate, since the high virus titre value is challenging to prepare with the infected samples.

2.3.1. Enzyme linked immunosorbent assay (ELISA)

ELISA methods have been performed for the virus detection. Since the development of this method by Clark and Adams (1977), it has become a predictable method for diagnosis, assay of plant viruses using enzyme labelled antibodies and quantification of virus.

ChiLCV is detected by direct antibody sandwich ELISA (DAS-ELISA). In DAS-ELISA, polyclonal antibody of *Sri Lankan cassava mosaic virus* (SLCMV) reacted positively with the crude leaf extracts of chilli infected by ChiLCV (Venkatesh, 1996). Chilli plants with leaf curl symptoms were observed at experimental fields of National Agriculture Research Centre, Islamabad. Leaves were collected from 12 symptomatic plants and analyzed by triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) using the monoclonal antibody (Harrison, 1997).

Devaraja *et al.* (2003) successfully produced monoclonal antibodies (MAb) to *Tomato leaf curl virus* (ToLCV) and detected begomovirus infections in tomato, okra, pepper, beans, tobacco etc. Devaraja *et al.* (2005) reported that DAS-ELISA and TAS-ELISA were used for the detection of *Tomato leaf curl Bangalore virus* (ToLCBV). Perveen *et al.* (2010) detected the presence of begomoviruses in shoe flower, okra, brinjal and chilli using TAS-ELISA.

Seepiban *et al.* (2017) generated two MAbs (M1 and D2) for begomovirus and detected by TAS-ELISA. The results of begomovirus detection indicated that MAb M1 reacted with two begomovirus species, *Tobacco yellow leaf curl Thailand virus* (TYLCTHV) and *Tobacco leaf curl Yunnan virus* (TbLCYnV), whereas MAb D2 reacted with 4 begomovirus species, TYLCTHV, TbLCYnV, ToLCNDV and *Squash leaf curl China virus* (SLCCNV).

Whitefly transmitted geminiviruses were initially detected by TAS-ELISA using panel of 10 MAbs. MAbs indicated the presence of begomoviruses. Serological results revealed great diversity of begomoviruses as all monoclonal antibodies reacted from moderate to high level intensity. For detection and identification of begomoviruses associated with leaf curl disease, degenerate primer pair for begomoviruses was used (Yasmin *et al.*, 2018).

2.3.2. Dot Immunobinding Assay (DIBA)

The principle of DIBA is same as that of ELISA except that the nitrocellulose membrane (NCM) to which the antigen and antibodies are bound and also insoluble enzyme reaction product. DIBA had been used in the detection of different viruses in a number of studies with suitable result which showed higher sensitivity by avoiding non-specific reaction and requiring fewer antibodies (Hajimorad *et al.*, 1996). DIBA was used as an efficient technique for the detection of geminiviruses as well as other viruses. Unlike ELISA, DIBA was a less insensitive method used for virus detection (Abdel-Salam *et al.*, 1998).

2.3.3. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is the routine assay for plant viral genome detection. PCR technique allows rapid, sensitive, and accurate detection of a diverse array in cultivated and weed hosts, with minimal sample preparation (Deng *et al.*, 1994).

Raj *et al.* (2005) stated that the ChiLCV was identified by using the total DNA of infected leaf samples and a geminivirus specific probe. The DNA fragment of expected size (~750 bp) was amplified by PCR with geminivirus specific primers in the selected samples. Four samples from Lucknow and Kanpur were selected for PCR amplification using specific primers which showed similar size of DNA fragments in both the samples.

Khan *et al.* (2006) characterized the virus related to leaf curl in chilli from Lucknow as ToLCNDV. The Blast search analysis showed 88 per cent identity with various ToLCNDV isolates related to *Pepper leaf curl Bangladesh virus*

(PepLCBV) and ChiLCV and showed 85 and 82 per cent identity, respectively and identified as an isolate of ToLCNDV. Senanayake *et al.* (2007) reported ChiLCV from Jodhpur district of Rajasthan state that shared 96.5 per cent identity with *Chilli leaf curl Multan virus* (ChiLCMV) and hence considered to be ChiLCMV.

The sequence evaluations of ChiLCV among the four isolates from Varanasi, Mirzapur, Gorakhpur and Maharajganj revealed 87 to 99 per cent similarity. The maximum coat protein sequence similarity of 92 per cent was observed between isolates of Gorakhpur and Mahrajganj showing close relation with Mirzapur isolate (Rai *et al.*, 2010). Chilli plants with ChiLCV was found to have 99.1 per cent sequence identity with *Pepper leaf curl Lahore virus* (PepLCLV) (Shafiq *et al.*, 2010).

Kumar *et al.* (2012) reported that total DNA extracted from ChiLCV symptomatic leaves and PCR amplification with primers for DNA-A, DNA-B and betasatellite indicated the association of begomoviruses with the field samples. PCR-based amplification of full-length viral components were done. Analysis showed that 35 out of the 41 molecules were 2.8 kb in size with a DNA-A/DNA-A-like begomovirus genome arrangement, whereas 6 molecules had a DNA-B-like genomic organization. Betasatellites were present in all the samples irrespective of whether the begomovirus genome detected was either monopartite or bipartite.

Senanayake *et al.* (2012) identified that ChiLCV affecting Jodhpur shared close sequence identity (96.1 %) with an isolate of ChiLCV from potato in Pakistan. Senanayake *et al.* (2013) conducted the molecular characterization of ChiLCV which shared 89 and 78 per cent sequence identities. The results showed presence of a new begomovirus and betasatellite species in leaf curl disease of chilli in Sri Lanka, which were named as *Chilli leaf curl Sri Lanka virus* (ChiLCSLV) and *Chilli leaf curl Sri Lanka betasatellite* (ChiLCSLB).

Sinha *et al.* (2013) was the first to report the molecular characterization of coat protein sequence of ChiLCV and chilli plant which is affected by radish leaf curl disease in India. Senanayake *et al.* (2015) confirmed ChiLCSLV presence with different symptoms in chilli plants. The primers were used based on the DNA-A of

a monopartite begomovirus and were capable of detecting the presence of ChiLCSLV in plant samples. Except three samples, all the others samples showed positive reaction in PCR with ChiLCSLV specific primers giving the correct amplicon size (501 bp). It confirmed the presence of ChiLCSLV in those samples.

The nucleotide sequence identity of ChiLCD originating from Bijnour, Uttar Pradesh shared less than 91 per cent identity with DNA-A segment of ToLCNDV and highest nucleotide identity (90 %) with *Tomato leaf curl Bangladesh betasatellite* (ToLCBDB). *Chilli leaf curl Bijnour virus* (ChiLCBV) was reported as a new begomovirus species infecting chilli plant in natural conditions (Kumar *et al*., 2016).

Sahu *et al*. (2016) reported the occurrence of ChiLCV and *Chilli vein mottle virus* (CVMV) in the mixed infected chilli plants. The mixed infection of potyvirus and begomovirus was confirmed by the amplicon and the sequence analysis. Menike and Costa (2017) identified the ChiLCV infected chilli plants in Sri Lanka over two successive seasons with highest homology to *Chilli leaf curl Salem virus* (ChiLCSV). Phylogenetic analysis confirmed the genetic divergence of the CL-14 and CL-15 isolates, making them more genetically closer to ChiLCSV, respectively.

2.4. NUTRIENT BASED MANAGEMENT OF CHILLI LEAF CURL

Bawden and Pirie (1937) found that the strains of *Tobacco mosaic virus* (TMV) were denatured by urea. It was known that application of urea leads to a reduction in infectivity and to some other changes which have been attributed to the linear combination of virus particles. According to Park and Fernando (1938), direct insect injury is responsible for ChiLCD. The disease was caused by an insect-borne vector localized in infected leaves. Whitefly, thrips, mites and aphids have been detected on leaves affected with leaf curl disease. Eradication by rogueing was used as the common control method.

According to Clarkson and Hanson (1980), 38.2 per cent of plant height was reduced due to *Mungbean yellow mosaic virus* (MYMV). The percentage of growth

and yield parameters of mungbean minimises with lower percentage of disease severity due to yellow mosaic disease. Thus, lower disease severity was confirmed by plant nutrients. Huber and Arny (1985) reported that the plants which were applied with potassium (K) showed reduction in the disease incidence on account of increased resistance of host plant for the development and spread of pathogens.

Graham *et al.* (1987) reported that the application of zinc (Zn) in soil results in plant absorption which plays important role in the maintenance of membrane integrity and immobilization of the virus particles, making them lesser active. Tomilson and Hunt (1987) reported the application of Zn in greater amount than the required dosage was essential for growth, without going to the level of inducing toxicity was beneficial for controlling *Chlorotic leaf spot virus* (CLSV) in water cress plants. The efficacy of neem seed kernel extract (NSKE) had been reported by Singh *et al.* (1988) in reducing the transmission of ChiLCV by controlling the whitefly population. Marschner and Cakmak (1989) reported that the mineral nutrients helps in build-up of resistance within plants and thereby protects the plant from pathogen attack. The application of micronutrients like Zn, iron (Fe), copper (Cu) and manganese (Mn) helps the plant by inducing protection against oxidative damage.

The systemic acquired resistance (SAR) in the plant system was triggered by the presence and solicitation of micronutrients such as Mn, Cu and boron (B) which initiate the interchange of ions within cell resulting in exchange of calcium ions (Ca^{2+}) from the cell wall. These ions interact with salicylic acid and activate the SAR mechanism in plants which results in suppression of plant diseases (Reuveni and Reuveni, 1998).

Pramanik and Ali (2001) observed that the plant nutrients (B, Zn and sulphur (S)) made notable effect on reducing the disease severity due to the induced resistance, and vigorous growth by plant nutrients. Bertamini *et al.* (2002) observed poor and less development of chlorophyll in diseased and virus affected plants due to the lower levels of magnesium (Mg) found in such plants. Thus, the application of Mg source reduces disease incidence in plants.

Chandramani *et al.* (2002) demonstrated the effectiveness of organic amendments such as farm yard manure, neem cake and biofertilizers (azosphos and silica solubilising bacteria) in reducing the incidence of sucking pests of chilli like aphid (*Myzus persicae*) and thrips (*Scirtothrips dorsalis*). Islam *et al.* (2002) studied the effects of B and Zn on disease incidence and severity of yellow mosaic and leaf crinkle disease of mungbean. They reported that the application of B and Zn reduced the disease severity by 18.31 per cent.

Oborn *et al.* (2003) reported that the use of different types of fertilizers provided direct means of supplying nutrient ions to plants which finally provided the internal resistance and reduced the severity of many diseases. George (2006) recorded a reduction in disease incidence with the application of vermicompost and neem cake at 500 g m^{-2} and 100 g m^{-2}, respectively. The seedling vigour was enhanced using vermicompost that contains both major and micro plant nutrients in available forms and neem cake that contains 2.0 to 2.5 per cent of organic nitrogen (N).

The deficiency of elements like Zn, B, Mn, Cu and Fe in plant tissue remain as a precursor for various disease incidence on plants. The micronutrient deficient tissues were more vulnerable to the attack of pest and diseases (Giraddi and Verghese, 2007).

Dordas (2009) found that silicon (Si), although it is not a plant nutrient, inhibits the feeding ability of sucking pests like aphids and thereby reduce the viral disease incidence. The addition of nutrients or application of fertilizers has decreased the incidence of disease in crop plants. This is probably because these nutrients are involved in the tolerance or resistance mechanisms of the host plant.

Pandey *et al.* (2010) reported that NSKE was most effective in reduction of 27.78 per cent of disease incidence of ChiLCV when compared to tumba and karanj seed extracts. According to Marschner (2012), beneficial microorganisms like mycorrhizal fungi enhance nutrient availability to plants by improve uptake of immobile nutrients such as phosphorus (P) and Zn through hyphal network

extending into the surrounding soils. Thus the increased nutrient content in plants increased tolerance to heavy metals, drought and disease resistance.

Zeshan *et al.* (2012) reported that N, P and K application was more effective against *Urdbean leaf curl virus* (ULCV) that reduced the disease severity up to 65 per cent followed by zinc and boron which reduced the disease severity up to 62.57 per cent. Marodin *et al.* (2014) reported that Si fertilization increases the marketable productivity of tomato plants by reducing the cracked fruits.

Samuel *et al.* (2015) observed the symptoms of *Papaya ring spot virus* (PRSV) infection were reduced with the combination of soluble B (1 g L^{-1}), zinc sulphate (2 g L^{-1}) and micro nutrient spray (1 ml L^{-1}) at fortnightly intervals (0.5 L $plant^{-1}$). They noticed that there was a significant increase in both fruit number (14.02 fruits) and fruit yield (15.94) in response to the micro nutrient sprays. Micronutrient applied at triple dose to kokkan affected banana plants reduced symptoms and virus titre values; and increased yield and yield characters compared to diseased plants (Sangeetha, 2015).

Ali *et al.* (2016) assessed the treatments in managing purple blotch disease in onion. Rovral 50 WP (0.2 %) along with micronutrients recorded more reduction of plant infection, leaf infection and leaf area diseased (41.18 %, 20.92 % and 44.88 %, respectively). It proved that micronutrients along with fungicides had significant effect in decreasing the disease incidence. Zeeshan (2017) stated that among plant products, NSKE (5 %) was found more effective in reducing ChiLCV. The disease was minimized by one-time soil application of carbofuran 3G (30 Kg ha^{-1}) and two sprays of imidacloprid (0.003 %) and was found to be very effective.

Materials and Methods

3. MATERIALS AND METHODS

The present research work entitled 'Nutrient based management of *Chilli leaf curl virus* in Chilli (*Capsicum annuum* L.)' was carried out at the Department of Plant Pathology, College of Agriculture, Vellayani during 2017-2019, with the objectives to serologically and molecularly characterize the virus causing leaf curl in chilli and to study the role of nutrient application in the management of the disease. The materials used, and methods employed in this study are presented systematically under appropriate headings.

3.1 COLLECTION OF BEGOMOVIRUS INFECTED CHILLI FROM DIFFERENT CULTIVATED AREAS

3.1.1. Disease incidence

The study was conducted in two districts *viz.*, Palakkad and Thiruvananthapuram during the year 2017-2019. In Palakkad, the samples expressing the typical symptoms of the disease were collected from major chilli growing areas *viz.*, Vadakarapathy and Kozhinjanpara. In Thiruvananthapuram, the samples were collected from College of Agriculture, Vellayani (Plate 1, 2). Five fields of chilli from each area were selected in consultation with the extension workers of Krishi Vigyan Kendra (KVK), Palakkad and Vegetable and Fruit Promotion Council Keralam (VFPCK), Palakkad. Random sampling was adopted. Fifty plants were selected at random and the symptoms were observed from the collected samples. Disease incidence (DI) was calculated as,

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

Plate 1. Map of Kerala showing two districts of sample collection

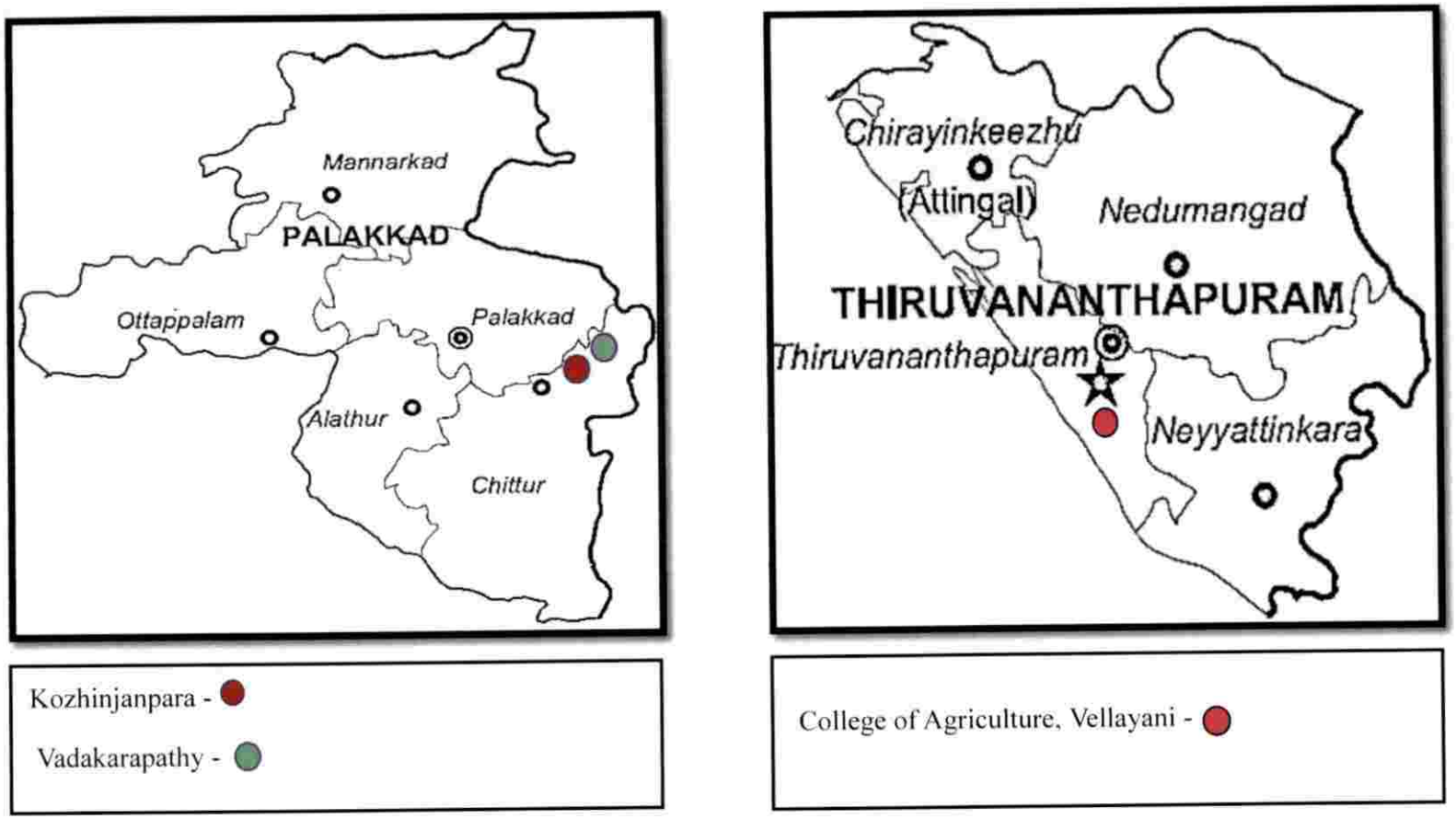

Plate 2. Map of Palakkad and Thiruvananthapuram district showing locations of sample collection

3.2. SYMPTOMATOLOGY

Chilli plants expressing the symptoms of the disease were collected from the field and observed. Symptomatology of infection at initial and later stages of crop growth was also studied.

3.3. SEROLOGICAL DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF THE VIRUSES CAUSING CHILLI LEAF CURL

3.3.1. Enzyme linked immunosorbent assay (ELISA)

Serological diagnosis of the infected chilli leaf samples was carried using Triple antibody sandwich - Enzyme linked immunosorbent assay (TAS-ELISA). Antisera specific to begomovirus, *Sri Lankan cassava mosaic virus* (SLCMV) was used to detect the presence of virus. TAS-ELISA was performed by the protocol described by DSMZ, Germany with three replications each. The details of antibodies used are given in Table 1. Buffers used for the work are detailed in Appendix I. The protocol followed was,

1. The specific antibody (IgG) was prepared in coating buffer i.e., 20 µl in 20 ml buffer at the recommended dilution of 1:1000. 200 µl was dispensed into each well of the ELISA plate.

2. The plate was covered with aluminium foil and incubated for 2-4 h at 37 °C.

3. The plate was washed with wash buffer i.e., Phosphate-buffered saline-Tween (PBS-Tween) using a wash bottle and soaked for a few minutes. The same procedure of washing was repeated twice. The plate was blotted dry by tapping upside down on tissue paper.

4. Blocking solution of 2 % skimmed milk in PBS-Tween at concentration of 200 µl was added into each well. The plate was covered and incubated at 37°C for 30 min.

5. The plate was washed with wash buffer i.e., PBS-Tween using a wash bottle and then soaked for a few minutes. The same procedure of washing was repeated twice. The plate was blotted dry by tapping upside down on tissue paper.

6. The samples were prepared by homogenising the plant material in extraction buffer in the ratio of 1:5 (w/v). 200 µl of aliquots of the test sample was dispensed into each well.

7. The plate was covered and incubated overnight at 4°C.

8. The plate was washed with wash buffer i.e., PBS-Tween using a wash bottle and then soaked for a few minutes. The same procedure of washing was repeated twice. The plate was blotted dry by tapping upside down on tissue paper.

9. 200 µl of MAb added in appropriate conjugate buffer was dispensed into each well at 1:1000 dilution.

10. The plate was covered and incubated for 2-4 h at 37°C.

11. The plate was washed with wash buffer i.e., PBS-Tween using a wash bottle and then soaked for a few minutes. The same procedure of washing was repeated twice. The plate was blotted dry by tapping upside down on tissue paper.

12. RAM-AP (anti-mouse IgG conjugated with alkaline phosphatase) was diluted in conjugate buffer at concentration of 1:1000 ratio, i.e., 20 µl in 20 ml buffer. 200 µl was added into each well.

13. The plate was covered and incubated for 1 h at 37°C.

14. The plate was washed with wash buffer i.e., PBS-Tween using a wash bottle and then soaked for a few minutes. The same procedure of washing was repeated twice. The plate was blotted dry by tapping upside down on tissue paper.

15. 1 mg/mL of substrate was prepared in substrate buffer (1 mg/ml para-nitrophenyl-phosphate in substrate buffer) and 200 µl aliquots of freshly prepared substrate was dispensed into each well.

16. The plate was covered and incubated for 30 min at 37°C to obtain clear reactions.

17. The intensity of colour development (Optical Density (OD) value) was measured in ELISA reader (Microplate Reader 680, BIORAD) at 405 nm.

Table 1. Details of TAS-ELISA

	Dilution	Volume used (µl)
Antigen	1:5	200
Specific Antibody (IgG)	1:1000	200
Monoclonal Antibody (MAb)	1:1000	200
Conjugate (RAM-AP)	1:1000	200

3.3.2. Dot Immunobinding Assay (DIBA)

DIBA was carried out with the infected chilli leaves using polyclonal antisera of SLCMV. Buffers used are detailed in Appendix II. The procedure followed was,

1. The sap was extracted from the infected tissue in antigen extraction buffer in the ratio of 1:10 (w/v), filtered and 0.8 ml was taken in eppendorf tube.

2. 0.4 ml chloroform was added to it, vortexed and centrifuged at 12,000 rpm for 2 min. The clarified sap was mixed with buffer (1:4) and again vortexed.

3. NCM marked with squares of 1 cm × 1 cm was kept in tris buffer saline (TBS), air dried and spotted with the sample (10 µl) and again dried.

4. Treated NCM was submerged in blocking solution with gentle oscillation for 1 h at room temperature and was treated with TBS for 10 min and incubated overnight in antiserum diluted in TBS-Spray Dried Milk (SDM).

5. After rinsing for 10 min in TBS, NCM was incubated for 1 h at room temperature in secondary antibody and again incubated in a solution of nitro blue tetrazolium (NBT) and bromo chloro indolyl phosphate (BCIP) at room temperature in dark for colour development. After this, NCM was rinsed in fixing solution for 10 min. The membrane was air dried between Whatman filter paper and stored. The colour development was analysed in Gel Doc System (Gel DOC ™ XR+).

3.3.3. Polymerase chain reaction (PCR)

3.3.3.1. Isolation of genomic DNA

The genomic DNA of chilli was isolated using modified CTAB method (Lodhi *et al.*, 1994) as well as by using DNeasy plant mini kit (Qiagen). Protocol for genomic DNA isolation using modified CTAB method is given below.

1. Three to four young leaves (100 mg) of fresh leaf tissue with leaf curl symptoms were collected from chilli fields and powdered in pre-chilled pestle and mortar using liquid nitrogen. The finely ground powder was transferred to the labelled 2.0 ml centrifuge tubes.

2. 1 ml of pre-heated (65°C) CTAB extraction buffer was added into the powdered sample and mixed thoroughly.

3. Transferred 750 µl of sample to a 1.5 ml centrifuge tube. The tubes were incubated at 60°C for 30-45 min in a hot water bath with regular shaking in every 10 min.

4. The mixture was then centrifuged at 10000 rpm for 10 min.

5. The supernatant was transferred into a new 1.5 ml eppendorf tube. 10 µl of RNAase was added to the supernatant and incubated for 1 h at 37°C.

6. After incubation period, equal volume (500 µl) of chloroform: isoamyl alcohol (24:1 mixture) was added to each tube and mixed well to form an emulsion by inverting the tube. The mixture was then centrifuged at 15000 rpm for 10-15 min.

7. Supernatant was carefully pipetted out and transferred to a new 1.5 ml micro centrifuge tube. 600-700 µl of ice-cold isopropanol was added to the supernatant, mixed and kept at -20°C for 1 h for the DNA to precipitate.

8. The mixture was then centrifuged at 15000 rpm at 4°C for 10 min.

9. The supernatant was then decanted, and the pellet was washed with 1 ml of 70 per cent ethanol two times.

10. The mixture was then centrifuged at 12000 rpm for 5 min.

11. Ethanol was removed, and pellet was air dried at room temperature.

12. The dried pellet was re-suspended in 50 µl of 1X Tris EDTA buffer (pH 8.0) and stored at - 20°C (Appendix III).

3.3.3.2. Agarose gel electrophoresis

Confirmation of presence of DNA was done by running the samples in 0.8 % agarose gel prepared in Tris-acetate-buffer (TAE-buffer) with 1 µl of ethidium bromide and casted in a horizontal gel electrophoresis unit (Biorad Power Pack). 100 bp ladder (GeNei) was used as molecular marker. 2 µl of DNA was mixed with 1 µl of 6 X loading dye and was dispensed into wells of the gel. The gel was run at 5 V cm^{-1} in TAE-buffer. Gel was removed when the dye had run three fourth of the total distance, visualized in a UV transilluminator system (Bio-Rad) and documented in Gel Doc system (Gel DOC ^{TM} XR+) (Appendix IV).

3.3.3.3. PCR amplification

The DNA isolated was subjected to PCR amplification with the two universal Begomovirus primers specific to coat protein of Begomovirus *viz.*, AV-AC (Wyatt and Brown, 1996) and Deng (Deng *et al.*, 1994) primers, and DNA extracted was used as the template in the reaction component.

The PCR amplification was carried out in a 20 µl PCR reaction mixture. PCR reaction mixture of volume 20 µl contained: 10x PCR buffer 2.0 µl, 1.5 mM $MgCl_2$ 1.5 µl, 10*mM* dNTPs (dATP, dGTP, dCTP and dTTP) 1.0 µl, forward primer (5 pM) 1 µl, reverse primer (5 pM) 1 µl, Taq polymerase enzyme (2.5 Units) 0.5 µl and sterile water 13 µl. The details of PCR primers are given in Table 2.

Before inserting into the wells of PCR, spin the reaction mix for 1 min and the amplification was carried out in the thermoycler. The reaction mixture was run in a thermocycler (Veriti 96 well Thermal cycler, Applied Biosystems) under the specified conditions (Table 3).

Table 2. Sequences of PCR primers specific to coat protein region of begomovirus

Primers	Primer sequences (5'-3') (Forward)	Primer sequences (3'-5') (Reverse)	Product size (bp)
AV494-F AC1048-R	GCCHATRTAYAGRA AGCCMAGRAT	GGRTTDGARGCATG HGTACANGCC	~575
DENG 541-F DENG 540-R	TAATATTACCKGW KGVCCSC	TGGACYTTRCAWG GBCCTTCACA	~520

Table 3. Conditions of PCR

Stage	Steps	Temperature	Time	No. of cycles
I	Preheating	95°C	1 min	1
II	Denaturation	95°C	1 min	35
	Annealing	58°C	1 min	
	Extension	72°C	1 min	
III	Final extension	72°C	10 min	1
	Incubation	4°C Infinite		

3.3.3.4. Agarose gel electrophoresis of PCR products

The PCR products were run in 1.2 per cent agarose gel, which was prepared in 0.5X TAE buffer that containing 0.5 µg / ml ethidium bromide. 2 µl of 6X loading dye was mixed with 10 µl of PCR products and electrophoresis was performed at 75V power supply with 0.5X TAE as electrophoresis buffer for about 1-2 h. 100 bp DNA ladder (GeNei) was used as the molecular standard. The gels were visualized and imaged under UV light using Gel documentation system (Bio-Rad Gel DOC ™ XR+) (Appendix V).

3.3.3.5. Sequencing

Sequencing of PCR products of chilli isolates was done in Agri Genome Pvt. Ltd, Kochi.

3.3.3.6. Characterization

The molecular characterization of the Begomovirus infecting chilli was confirmed by performing a similarity search using Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI) database and the sequences were aligned with existing NCBI database. Nucleotide sequences based on the coat protein region of the Begomovirus were retrieved from NCBI Genbank data base (USA) and compared. Multiple sequence alignment tool was used to compare sequences and phylogeny related studies were conducted in MEGA 6.0 (Tamura *et al.*, 2013).

3.4. NUTRIENT BASED MANAGEMENT OF CHILLI LEAFCURL

3.4.1. Plant nutrient analysis (major and micronutrients) of healthy and diseased samples

Plant samples were collected from major chilli growing areas of Palakkad and Thiruvananthapuram districts. An appropriate quantity of dried plant samples was weighed and ground for further analysis. Various parameters were analysed and estimated as per the standard procedures outlined in Table 4.

3.4.2. Soil analysis for major and micronutrients of collected soil samples from major chilli growing areas of Palakkad and Thiruvananthapuram districts

Soil samples were also collected from the major chilli growing areas of Palakkad and Thiruvananthapuram districts. The samples were air dried in shade, weighed, ground and taken for analysis of various parameters as per the standard analytical procedures outlined in Table 5.

Table 4. Analytical methods used for determining different major and micro nutrients in the symptom expressed and healthy plant samples

Sl. No.	Parameters	Methods	References
1	Total nitrogen	Micro Kjeldahl	Jackson (1973)
2	Total phosphorus	Nitric perchloric acid (9:4) digestion and spectrophotometry by using vanadomolybdo phosphoric yellow colour	Jackson (1973)
3	Total potassium	Nitric perchloric acid (9:4) digestion and flame photometry	Jackson (1973)
4	Total calcium and magnesium	Neutral normal ammonium acetate and titration with EDTA (versenate titration)	Piper (1967)
5	Iron, manganese, zinc, copper	Nitric perchloric acid (9:4) digestion and atomic absorption spectrophotometry	Jackson (1973)
6	Sulphur	Nitric perchloric acid (9:4) digestion and turbidimetry	Chesnin and Yien (1950)
7	Boron	Azomethine-H colorimetry	Gupta (1967)

Table 5. Analytical methods followed for determining different major and micro nutrients in the soil

Sl. No.	Parameters	Methods	References
1	Available nitrogen	Alkaline potassium permanganate method	Subbiah and Asija (1956)
2	Available phosphorus	Bray No 1 extraction and spectrophotometry	Jackson (1973)
3	Available potassium	Neutral normal ammonium acetate and flame photometry	Stanford and English (1949)
4	Exchangeable calcium and magnesium	Neutral normal ammonium acetate and atomic absorption spectrophotometry	Hesse (1971)
5	Iron, manganese, zinc, copper	0.5 N HCl extraction and atomic absorption spectrophotometry	O'Cannor (1988)
6	Sulphur	Turbidimetry	Chesnin and Yien (1950)
7	Boron	Hot water extraction and Azomethine-H colorimetry	Gupta (1967)

3.4.3. Pot culture studies for nutrient based chilli leaf curl management

3.4.3.1. Disease incidence

A pot culture study for nutrient based chilli leaf curl management was conducted in Department of Plant Pathology using completely randomized design (CRD) with ten treatments and three replications in the chilli variety Vellayani Athulya during May 2019 to August 2019. Plants were allowed to take up natural

infection but to increase the infection they were surrounded by infected chilli plants and whiteflies were released to increase the disease incidence.

The details of the treatments used in the study are given below in Table 6.

Table 6. Details of treatments imposed for management of chilli leaf curl

Treatments	Treatment details
T1	Absolute control
T2	Fertilizer and insecticide schedule as per package of practice (KAU, 2016)
T3	Organic POP recommendation
T4	POP modified based on soil test (STBR)
T5	POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2kg ha^{-1} + B @ 10 kg ha^{-1}) + Silicon @ 400 kg ha^{-1}
T6	POP + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application
T7	POP + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1})
T8	POP + Micronutrient (B @ 10 kg ha^{-1})
T9	Basal (1/2 N + full P+1/2 K) followed by foliar application (19:19:19) 0.5% at fortnightly
T10	POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) + Application of Sampoorna (KAU formulation (0.5 g L^{-1}) as foliar

The disease incidence (DI) was calculated for the treated chilli plants.

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

3.4.3.2. Coefficient of infection

The coefficient of infection (CI) was calculated by multiplying the disease incidence with the response value (RV) assigned to each severity grade. The overall disease reaction was assigned to the coefficient of infection (CI) range as given in the table below (Kumar *et al.*, 2006.) (Table 7).

3.4.3.3. Preliminary soil analysis for major and micronutrients

Preliminarily, soil sample was analysed for major and micronutrients. Based on the value of pre-treated soil analysis, the treatments were imposed for the management of chilli leaf curl disease.

3.4.3.4. Plant nutrient analysis of the index leaf stage

The plant nutrient analysis for major and micronutrients of chilli leaf curl infected samples was carried out in the index leaf *i.e.*, just fully developed leaves at middle of the growing period.

3.4.3.5. Yield data

Yield was taken at 70-75 days after sowing. Five harvests per plant were taken and the data was analysed statistically.

3.4.3.6. Enzyme linked immunosorbent assay (ELISA)

ELISA was done as per the procedure of DSMZ, Germany to study the influence of various treatments on virus titre.

Table 7. Coefficient of infection (CI) ranges with specific disease reaction of *Chilli leaf curl virus* in chilli

Symptom	Symptom severity grade	Response value	Coefficient of infection	Disease reaction
No symptom	0	0	0	Symptom-less
0–5 per cent curling and clearing of upper leaves	1	0.05	0.1–5	Highly resistant
6-25 per cent curling, clearing of leaves and swelling of veins	2	0.25	5.1–10	Resistant
26–50 per cent leaf curling, puckering, yellowing of leaves and swelling of veins	3	0.50	10.1–20	Moderately resistant
51–75 per cent leaf curling and stunted plant growth and blistering of internodes	4	0.75	20.1–40	Moderately susceptible
More than 75 per cent curling and deformed small leaves, stunted plant growth with small flowers and no or small fruit set	5	1.00	40.1–70	Susceptible
100% curling and deformed small leaves, stunted plant growth without flowering			70.1–100	Highly susceptible

3.4.3.7. Statistical Analysis

The data generated from the experiment were statistically analysed using Analysis of Variance (ANOVA) for CRD and the significance was determined using 't' values. Wherever the t-values were found to be significant, critical difference was calculated at five per cent level of significance. Significant treatments were compared with critical difference value. All the treatments were analysed in WASP 2.0 software.

Results

4. RESULTS

The present study on "Nutrient based management of *Chilli leaf curl virus* in Chilli (*Capsicum annuum* L.)" was conducted during 2017-2019 at the Department of Plant Pathology, College of Agriculture, Vellayani with the objectives to serologically and molecularly characterise the virus causing leaf curl in chilli and to study the role of nutrient application in the management of the disease. The results of the study are detailed here.

4.1. COLLECTION OF BEGOMOVIRUS INFECTED CHILLI FROM DIFFERENT CULTIVATED AREAS

4.1.1. Disease incidence

Survey was conducted to assess the incidence of *Chilli leaf curl virus* (ChiLCV) in major chilli growing areas of Palakkad and Thiruvananthapuram districts during December 2018 to March 2019. In Palakkad district, the survey was conducted in the major chilli growing areas of Vadakarapathy and Kozhinjanpara. In Thiruvananthapuram, the incidence was recorded from fields in College of Agriculture, Vellayani.

The major symptoms of the infection included upward curling, puckering, yellowing and stunting of leaves (Plate 3, 4 and 5). In Palakkad district, the disease incidence ranged from 55.71 to 73.33 per cent. The least incidence of 55.71 per cent was recorded in Kozhinjanpara, whereas the highest disease incidence (73.33%) was recorded in Vadakarapathy (Table 8 and 9). The variety cultivated in the district was local variety. In Thiruvananthapuram district, disease incidence ranged from 69.33 to 80.00 per cent (Table 10). The variety cultivated was Vellayani Athulya. In all the locations, whiteflies (*Bemisia tabaci*) were found associated with the crop (Plate 3, 4 and 5).

Table 8. Disease incidence of Chilli leaf curl disease in Vadakarapathy, Palakkad

Location	Disease incidence (%)
Vadakarapathy 1	71.00
Vadakarapathy 2	70.00
Vadakarapathy 3	73.33
Vadakarapathy 4	63.71
Vadakarapathy 5	68.00

Table 9. Disease incidence of Chilli leaf curl disease in Kozhinjanpara, Palakkad

Location	Disease incidence (%)
Kozhinjanpara 1	55.71
Kozhinjanpara 2	65.55
Kozhinjanpara 3	71.70
Kozhinjanpara 4	68.57
Kozhinjanpara 5	67.50

Table 10. Disease incidence of Chilli leaf curl disease in College of Agriculture Vellayani, Thiruvananthapuram

Location	Disease incidence (%)
Thiruvananthapuram1	74.00
Thiruvananthapuram 2	69.33
Thiruvananthapuram 3	75.71
Thiruvananthapuram 4	80.00
Thiruvananthapuram 5	76.66

Plate 3a. Upward curling and puckering of leaves

Plate 3b. Yellowing of leaves

Plate 3c. Incidence of whitefly

Plate 3. Different symptoms of chilli leaf curl disease collected from Vadakarapathy, Palakkad

Plate 4a. Upward curling of leaves

Plate 4b. Yellowing of leaves

Plate 4c. Incidence of whitefly

Plate 4. Different symptoms of chilli leaf curl disease collected from Kozhinjanpara, Palakkad

Plate 5a. Upward curling of leaves

Plate 5b. Puckering of leaves

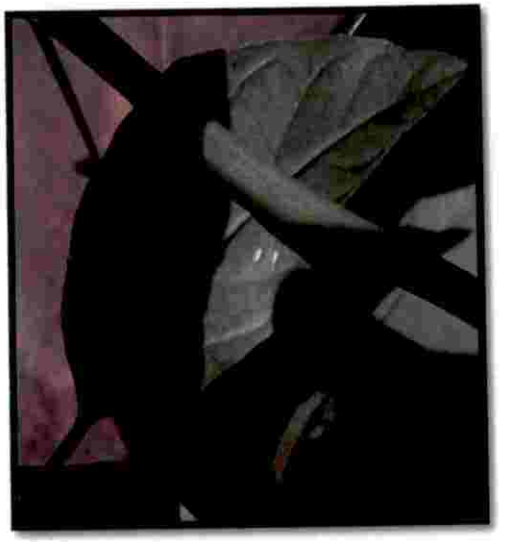

Plate 5c. Incidence of whitefly

Plate 5. Different symptoms of chilli leaf curl disease collected from College of Agriculture, Vellayani, Thiruvananthapuram

4.2. SYMPTOMATOLOGY

The symptoms collected from different surveyed area were upward curling, puckering of leaves, reduced leaf size, yellowing, petiole elongation, crinkling and mottling with no or less fruits and stunting of whole plant (Plate 6).

During early stages of infection, the symptoms observed were upward curling, puckering and yellowing. While at later stages of growth, the infected plant expressed reduced leaf size, petiole elongation, lesser flowers, smaller fruits and stunted growth.

4.3. SEROLOGICAL DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF THE VIRUSES CAUSING CHILLI LEAF CURL

4.3.1. Enzyme linked immunosorbent assay (ELISA)

Serological diagnosis was carried out using Triple Antibody Sandwich - Enzyme linked immunosorbent assay (TAS-ELISA) using polyclonal antisera specific to Begomovirus, *Sri Lankan cassava mosaic virus* (SLCMV) which was considered as the only Geminivirus antiserum available to prove the presence of the respective virus in the collected sample and were obtained from DSMZ, Germany. The absorbance was read at 405 nm in an ELISA reader (Microplate Reader 680, BIORAD).

Diseased chilli samples collected from the fields of Palakkad recorded a negative reaction with the OD value of 1.1 and healthy of 0.2 indicating that there was no difference between the absorbance value of healthy and diseased. In case of samples collected from College of Agriculture, Vellayani, all of them recorded a positive reaction with highest OD value of 4.2 and healthy was 0.2 (Table 11 and Plate 7).

4.3.2. Dot Immunobinding Assay (DIBA)

All the samples collected were subjected to Dot Immunobinding Assay (DIBA) with *Sri Lankan cassava mosaic virus* (SLCMV). But no colour development was noticed in any of the samples (Table 12 and Plate 8).

Plate 6a. Upward curling of leaves

Plate 6b. Puckering of leaves

Plate 6c. Stunting of whole plant

Plate 6d. Reduced leaf size

Plate 6e. Yellowing

Plate 6f. Petiole elongation

Plate 6g. Crinkling and mottling

Plate 6h. No or less-small fruits

Plate 6. Symptoms of chilli leaf curl disease observed under field conditions

Table 11. Detection of *Chilli leaf curl virus* in the infected plant samples through TAS-ELISA using polyclonal antibody of SLCMV

Samples	Isolates	OD value at 405 nm (*)	Increase in OD value	Reaction (+/-)
Healthy		0.254		
Diseased 1	Thiruvananthapuram	1.050	4.2	+
Diseased 2		0.848	3.3	+
Diseased 3		0.676	2.7	+
Diseased 4		0.972	3.8	+
Diseased 5		0.949	3.7	+
Healthy	Palakkad	0.254		
Diseased 6		0.277	1.1	-
Diseased 7		0.248	1.0	-
Diseased 8		0.262	1.0	-
Diseased 9		0.252	1.0	-
Diseased 10		0.292	1.1	-

+ = Positive reaction, - = Negative reaction

* OD value are mean of 3 replications

Table 12. Immunodetection of the chilli samples for *Chilli leaf curl virus* through DIBA using SLCMV polyclonal antibody

Sl. No.	Label	Type	Volume (Int.)	Minimum value (Int.)	Maximum value (Int.)	Mean value (Int.)	Standard deviation	Area (mm^2)
1	U1	Healthy	7,270,669	1056	3135	1261	235.3	24.9
2	U2	Diseased	7,344,389	986	2686	1250	162.0	27.4
3	B1	Background	6,214,309	1010	2758	1286	210.2	22.5

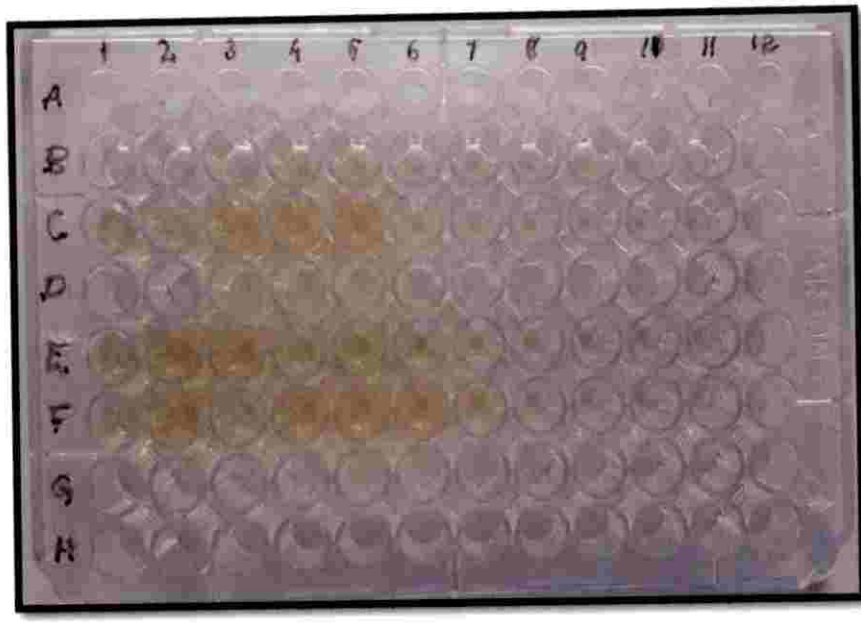

Plate 7. Colour development in ELISA plate by interaction of the Begomovirus infected samples with the polyclonal antiserum SLCMV. C1, C2, C3, C4 and C5 - Healthy; D1, D2, D3, D4 and D5 - Palakkad isolates; E1, E2, E3, E4, E5, F2, F3, F4, F5 and F6 - Thiruvananthapuram isolates and G1 - Blank.

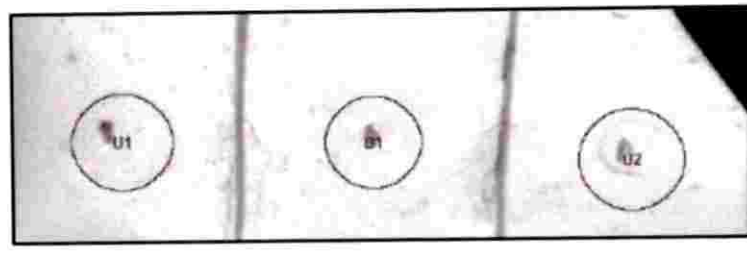

Plate 8. Colorimetric analysis by DIBA for chilli leaf curl infected samples using polyclonal antiserum SLCMV. U1 - Healthy, U2 - Diseased and B1- Background.

4.3.3. Polymerase chain reaction (PCR)

Molecular characterization for the detection of virus was done in PCR using specific primers for amplification of coat protein of the virus. DNA isolated from the samples collected from different districts was subjected to PCR amplification using AV/AC and DENG primers. No PCR product was obtained for any of the samples collected from Palakkad district (Plate 9), while an amplicon size of~550 bp (AV/AC) and~500 bp (DENG) were obtained for the samples collected from College of Agriculture, Vellayani (Plate 10 and 11).

Sequencing of these coat protein amplicons at Agri Genome Private Limited, Kochi, and BLAST analysis in NCBI indicated 96.63 per cent similarity of Thiruvananthapuram isolate with *Chilli leaf curl Vellanad virus* (Table 13).

The phylogenetic tree was constructed with related sequences from NCBI in Mega 6.0 software which established clustering of *Chilli leaf curl virus* isolate Thiruvananthapuram and *Chilli leaf curl Vellanad virus* (Fig. 1).

4.4. NUTRIENT BASED MANAGEMENT OF CHILLI LEAFCURL

4.4.1. Plant nutrient analysis (major and micronutrients) of healthy and diseased samples

The primary major nutrients status of healthy and diseased plant samples revealed that the per cent of N, P and K content in the infected leaves were low when compared to the healthy leaves but in sufficient level. High level of N in leaves was recorded from Thiruvananthapuram 2 and 3 (3.70 %) among the infected samples while the N value (4.00 %) was recorded in healthy sample. In the case of P, values ranged from 0.13-0.15 for diseased leaf samples while the P in healthy recorded (0.20 %). With respect to K, highest (4.00) per cent was recorded from diseased sample collected from the location Palakkad 2. Healthy leaves recorded a value of (4.90 %) (Table 14).

The secondary major nutrients such as Ca and Mg content in the infected leaves were low but S remained high compared to the healthy leaves that too in sufficient level. Ca in the diseased leaves collected from Palakkad ranged between

0.20 % - 0.70 % while those collected from Thiruvananthapuram recorded a value between 0.50 % - 0.90 %. Healthy leaves recorded a value of 0.90 %. The secondary nutrient Mg was high in the sample collected from the location Palakkad 3 (0.36 %) and least in the location Palakkad 4 (0.22 %) while healthy recorded (0.40 %). S in the leaf of healthy (0.40 %) was low while highest S content was recorded from the location Palakkad 2 (1.16 %) (Table 15).

The micronutrient content of chilli samples were estimated and it was found that only Fe content in the infected leaves was high and in a toxic level when compared to healthy leaves. Mn, Zn, Cu and B content in infected leaves were low and in sufficient level compared to healthy leaves. Highest Fe content was recorded from the location Palakkad 4 (875 mg kg^{-1}), highest Mn was recorded from the location Thiruvananthapuram 2 (200 mg kg^{-1}) and Zn was highest in diseased leaves collected from the location Palakkad 3 (118 mg kg^{-1}). Status of Cu in diseased leaves was highest in sample collected from location Palakkad 4 (21.00 mg kg^{-1}) while B was highest in the leaf sample collected from location Thiruvananthapuram 5 (14.40 mg kg^{-1}) (Table 16).

4.4.2. Soil analysis for major and micronutrients of collected soil samples from major chilli growing areas of Palakkad and Thiruvananthapuram districts

Analysis of major soil nutrients from disease infected fields of Palakkad and Thiruvananthapuram revealed that the status of all nutrients were high in soils collected from the infected field. N was highest (338.69 kg ha^{-1}) from the soil collected from diseased plot in location Palakkad 4. While the healthy recorded 282.05 kg ha^{-1}. Available P was highest in soil collected from the location 2 of Thiruvananthapuram district (24.7 kg ha^{-1}) K was highest in soil sample collected from location Palakkad 2 (273 kg ha^{-1}). Available Ca was lowest in soil sample collected from healthy plants and Mg was highest in diseased soil from Thiruvananthapuram 5 (166 mg kg^{-1}). Available S was highest in sample collected from Thiruvananthapuram 3 (9.5 mg kg^{-1}) (Table 17 and 18).

Table 13. BLAST analysis of *Chilli leaf curl virus* isolate of Thiruvananthapuram

Similarity to other begomovirus sequences	Per cent identification	Accession number
Chilli leaf curl Vellanad virus (India / Vellayani / 2008) clone pChVelK52 segment DNA-A, complete sequence	96.63%	NC038442.1
Chilli leaf curl Multan virus isolate Nagpur segment DNA-A, complete sequence	85.47%	KY420149.1
Chilli leaf curl virus isolate 58SA DNA-A, complete sequence	85.21%	JN135234.2
Pepper leaf curl Lahore virus- (Pakistan: Lahore 2: 2004) segment A, complete sequence, clone PeAL2	85.28%	KR074212.1
Chilli leaf curl virus -India isolate SZ78 segment DNA-A, complete sequence	85.39%	KM923995.1
Chilli leaf curl virus complete sequence, clone Tom 86	85.88%	FM210475.1
Chilli leaf curl virus complete sequence, clone Tom 84	85.69%	FM210477.1
Chilli leaf curl virus, isolate Tom 85, complete sequence	85.50%	FM210474.1
Chilli leaf curl virus, isolate Tom 87, complete sequence	85.20%	MF488985.1
Chilli leaf curl virus, isolate Tom 88, complete sequence	85.35%	FJ9685255.1
Chilli leaf curl virus complete sequence, clone HP-12	90.13%	HG969257.1
Chilli leaf curl virus complete sequence, clone HP-5	90.13%	HG969255.1
Chilli leaf curl virus genomic DNA containing Barka region, strain Tom-85, isolate Tom-85, clone Tw10	90.13%	LN650708.1
Pepper leaf curl Bangladesh virus isolate India / Coimbatore / Chilli / 2008, segment DNA-A, complete sequence	90.13%	HM007096.1
Chilli leaf curl virus complete sequence, clone Tom 85	85.54%	FM210476.1

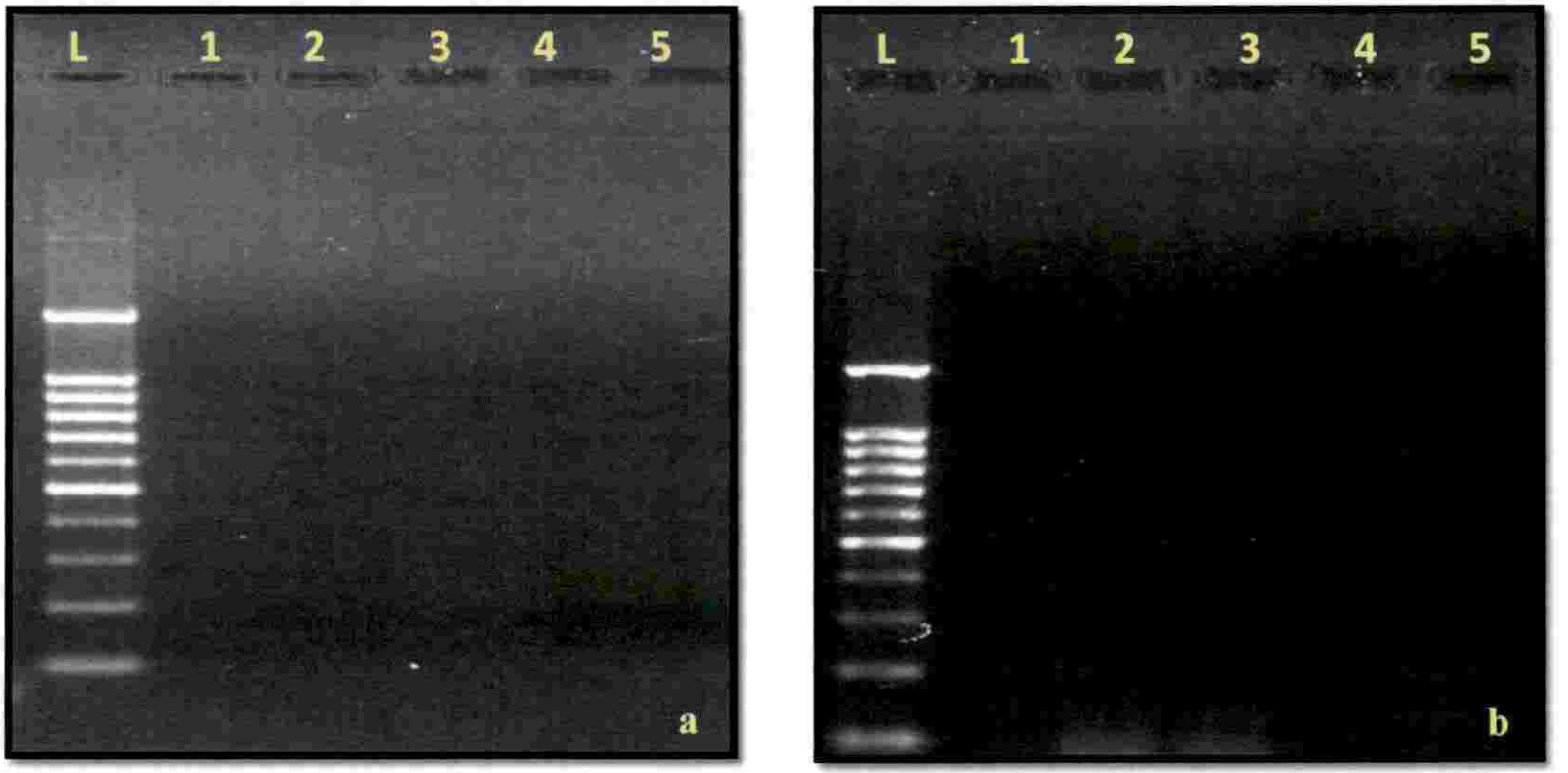

Plate 9. Electrophoresis gel image of PCR product of amplified DNA from chilli samples collected from Palakkad using AV/AC and DENG primer. (a) L - 100 bp DNA ladder and 1, 2, 3, 4, 5 - Palakkad isolates; (b) L - 100 bp DNA ladder and 1, 2, 3, 4, 5 - ChiLCV Palakkad isolates.

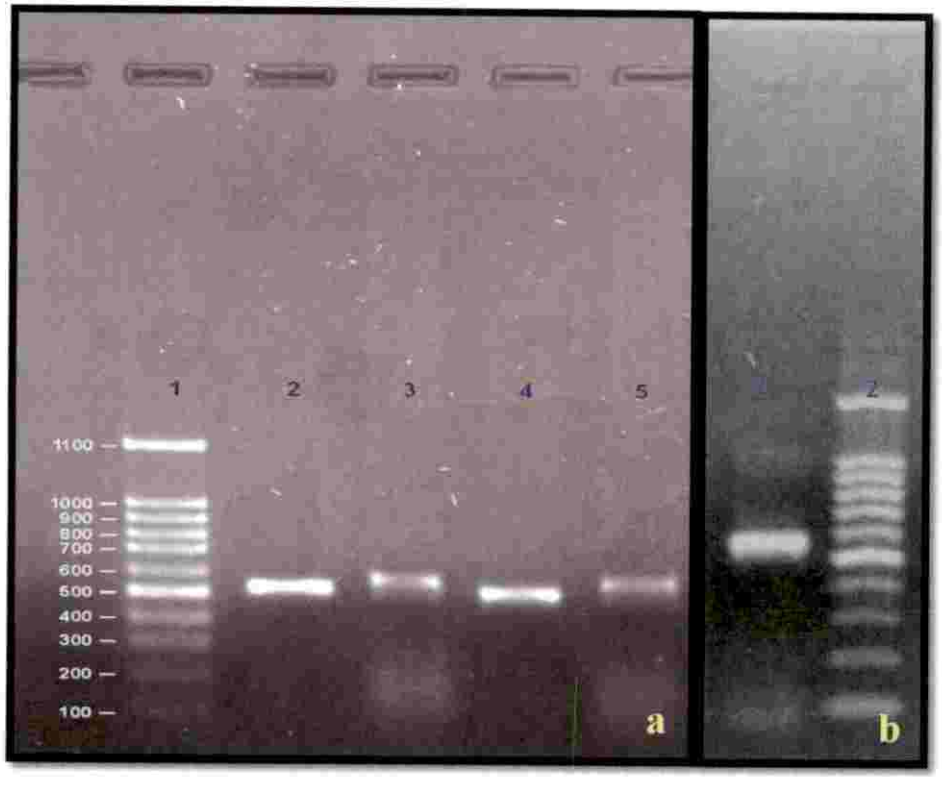

Plate 10. Electrophoresis gel image of PCR product of amplified DNA of chilli samples collected from Thiruvananthapuram using DENG primer (500 bp). (a) 1 - 100 bp DNA ladder and 2, 3, 4, 5 - ChiLCV Thiruvananthapuram isolates; (b) 1 - ChiLCV Thiruvananthapuram isolates and 2 - 100 bp DNA ladder.

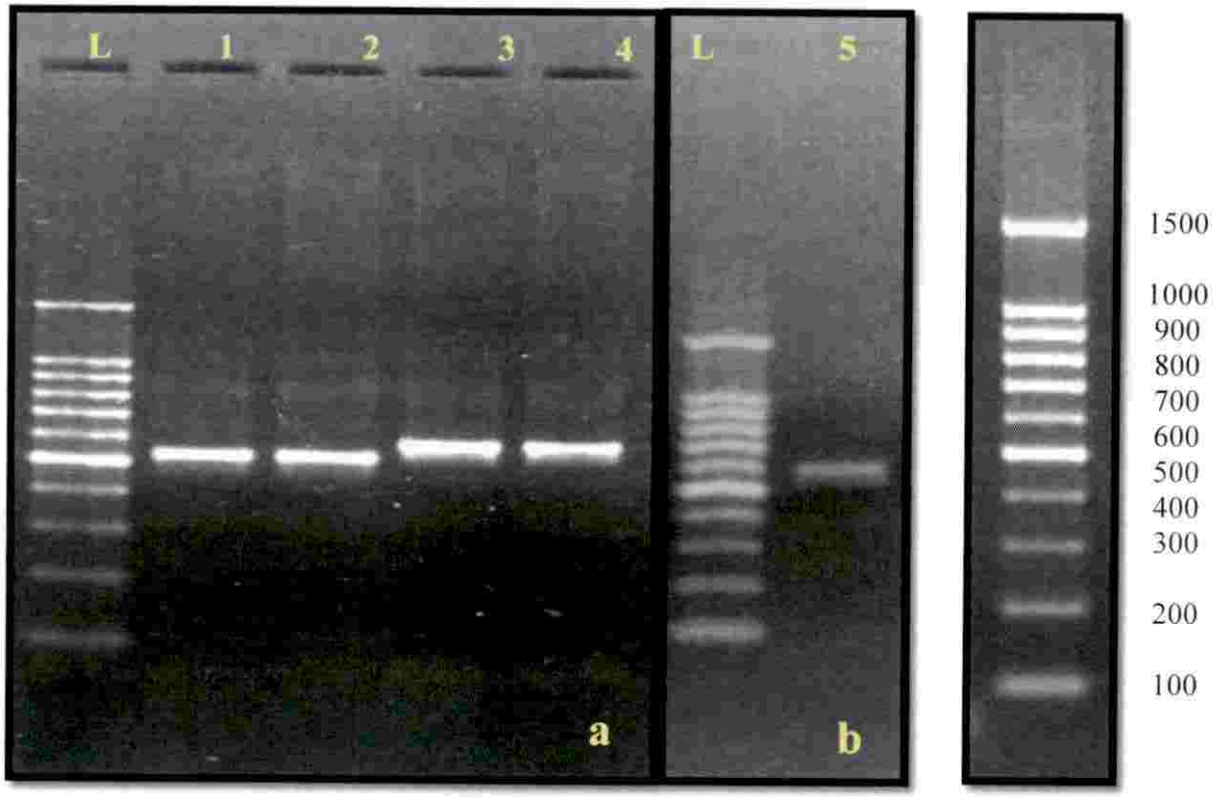

Plate 11. Electrophoresis gel image of PCR product of amplified DNA from chilli samples collected from Thiruvananthapuram using AV/AC primer (550 bp). (a) L - 100 bp DNA ladder and 1, 2, 3, 4 - ChiLCV Thiruvananthapuram isolates; B) L - 100 bp DNA ladder and 5 - ChiLCV Thiruvananthapuram isolates.

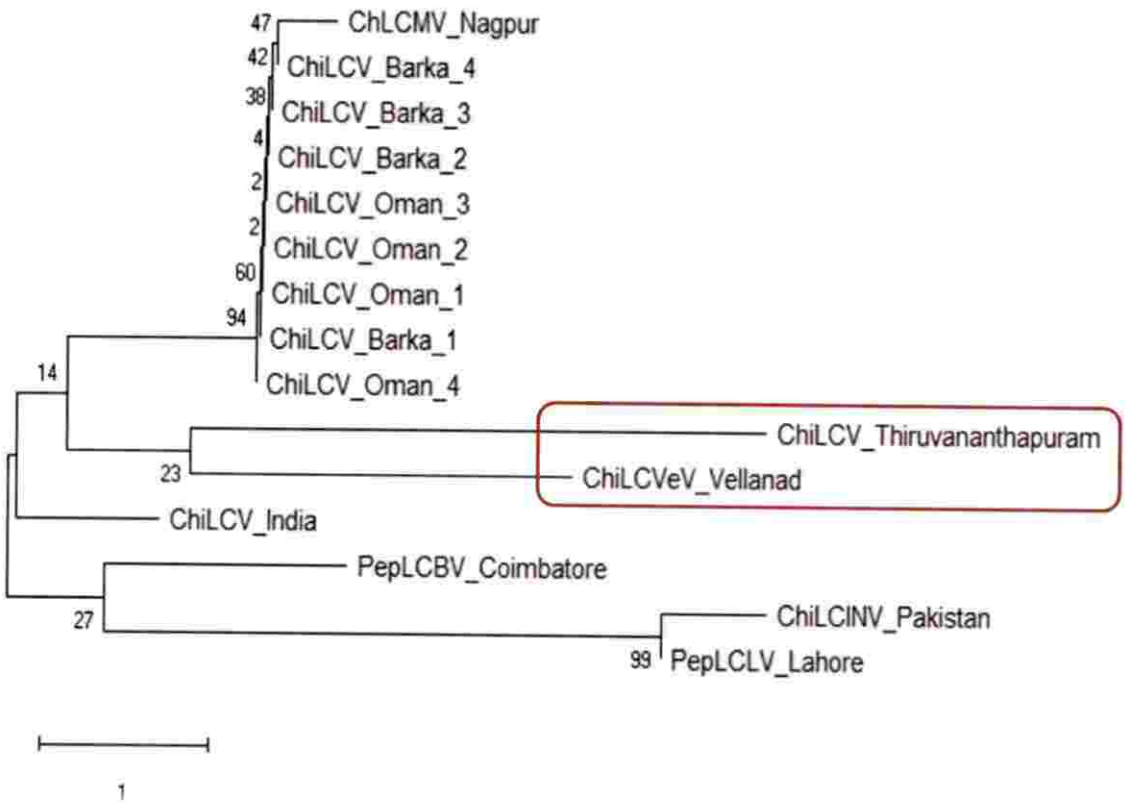

Fig. 1. Phylogenetic relationship among coat protein sequences of begomovirus isolates constructed using neighbor-joining method in MEGA 7.0.26 software.

Table 14. Primary major nutrient content of healthy and the infected chilli leaf samples

Location	N (%)	P (%)	K (%)
Palakkad 1	3.2	0.14	3.5
Palakkad 2	3.3	0.15	4.2
Palakkad 3	2.6	0.14	3.3
Palakkad 4	2.9	0.14	2.4
Palakkad 5	3.3	0.15	4.0
Thiruvananthapuram 1	3.2	0.15	3.9
Thiruvananthapuram 2	3.7	0.14	3.3
Thiruvananthapuram 3	3.7	0.13	3.4
Thiruvananthapuram 4	3.2	0.13	3.1
Thiruvananthapuram 5	3.2	0.13	3.0
Healthy	4.0	0.20	4.9

Range of sufficiency in index leaf: N: 1-5%, P: 0.1-0.4%, K: 1-5%

Table 15. Secondary major nutrient content of healthy and infected chilli leaf samples

Location	Ca (%)	Mg (%)	S (%)
Palakkad 1	0.2	0.30	0.98
Palakkad 2	0.4	0.30	1.16
Palakkad 3	0.2	0.36	0.87
Palakkad 4	0.4	0.22	0.88
Palakkad 5	0.7	0.30	0.94
Thiruvananthapuram 1	0.6	0.29	0.87
Thiruvananthapuram 2	0.9	0.32	0.83
Thiruvananthapuram 3	0.5	0.29	0.79
Thiruvananthapuram 4	0.6	0.28	0.73
Thiruvananthapuram 5	0.5	0.35	0.78
Healthy	0.9	0.40	0.40

Range of sufficiency in index leaf: Ca: 0.2-1%, Mg: 0.1-0.4%, S: 0.1-0.4%

Table 16. Foliar micronutrient concentration of healthy and infected chilli plants

Location	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})
Palakkad 1	595.00	130.50	113.50	15.00	10.10
Palakkad 2	595.00	141.00	112.00	18.00	10.50
Palakkad 3	645.00	144.50	118.00	12.00	11.20
Palakkad 4	875.00	131.50	75.50	21.00	12.50
Palakkad 5	700.00	151.00	101.50	17.50	12.00
Thiruvananthapuram 1	595.00	139.00	82.00	14.00	10.30
Thiruvananthapuram 2	800.00	200.00	91.00	12.50	10.30
Thiruvananthapuram 3	765.00	140.00	82.00	16.00	12.80
Thiruvananthapuram 4	800.00	102.00	74.00	19.50	13.20
Thiruvananthapuram 5	560.00	112.50	78.50	18.00	14.40
Healthy	448.50	280.00	148.50	29.50	18.60

Range of sufficiency in index leaf: Fe: 100-500 mg kg^{-1}, Mn: 20-300 mg kg^{-1}, Zn: 20-150 mg kg^{-1}, Cu: 5-30 mg kg^{-1}, B: 10-20 mg kg^{-1}

Table 17. Primary major nutrient status of the soil samples collected during the survey

Location	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
Palakkad 1	313.60	13.3	272
Palakkad 2	301.05	19.8	273
Palakkad 3	338.69	15.8	267
Palakkad 4	383.24	19.9	249
Palakkad 5	288.51	17.7	269
Thiruvananthapuram 1	298.88	21.0	270
Thiruvananthapuram 2	299.42	24.7	255
Thiruvananthapuram 3	295.79	24.4	242
Thiruvananthapuram 4	288.33	23.9	231
Thiruvananthapuram 5	296.14	24.0	229
Healthy	282.05	11.9	201

Sufficiency ratings: N: 280-560 kg ha^{-1}, P: 10-25 kg ha^{-1}, K: 140-280 kg ha^{-1}

Table 18. Secondary major nutrient status of the soil samples collected during the survey

Location	Available Ca ($mg\ kg^{-1}$)	Available Mg ($mg\ kg^{-1}$)	Available S ($mg\ kg^{-1}$)
Palakkad 1	500	148	7.0
Palakkad 2	520	138	7.5
Palakkad 3	570	142	8.5
Palakkad 4	400	156	6.0
Palakkad 5	400	134	6.0
Thiruvananthapuram 1	500	132	7.5
Thiruvananthapuram 2	500	140	6.0
Thiruvananthapuram 3	400	152	9.5
Thiruvananthapuram 4	360	160	8.0
Thiruvananthapuram 5	450	166	8.0
Healthy	310	127	5.5

Sufficiency ratings: Ca: > 300 $mg\ kg^{-1}$, Mg: > 120 $mg\ kg^{-1}$, S: 5-10 $mg\ kg^{-1}$

In case of micronutrients, all of them had a high status in soils collected from the infected field. The status of soil Fe was very high in all the areas of sample collection and highest was in Thiruvananthapuram 5 (557.60 mg kg^{-1}), Mn was high in all the locations, Zn was also high in all the locations, Cu was highest at the location Thiruvananthapuram 1 (6.41 mg kg^{-1}) and B was found slightly high in the soil collected from Palakkad location (Table 19).

4.4.3. Pot culture studies for nutrient based chilli leaf curl management

4.4.3.1. Disease incidence

A pot culture study was undertaken in the Department of Plant Pathology using CRD with ten treatments and three replications in the chilli variety Vellayani Athulya during May 2019 to August 2019 (Plate 12). Cent per cent incidence of leaf curl was recorded in all the treatments. Plants were allowed to take up natural infection but to increase the infection they were surrounded by infected chilli plants and whiteflies were released to increase the disease incidence.

4.4.3.2. Coefficient of infection

The response value for various treatments ranged between 0.25-1. Lowest response value of 0.25 was recorded for three treatments namely, POP and $ZnSO_4$, POP and B, and basal application of N, P, K followed by foliar application of NPK 19:19:19 at 0.5 per cent. Highest response value (1.00) was recorded in absolute control. Highest response values of 0.75 was recorded for three treatments viz., fertilizer and insecticide schedule as per POP, organic POP recommendation and POP recommendation with secondary nutrients and application of sampoorna (Plate 13 and 14).

The effect of treatments on the coefficient of infection of chilli leaf curl disease was also calculated. After imposing the treatments, it was observed that the highest coefficient of infection (75.00) was recorded in untreated virus infected plants whereas, the plants supplemented with nutrients as per POP supplemented with B, POP supplemented with $ZnSO_4$ and basal application of ½ N + ½ P + ½ K

followed by 0.5 per cent foliar application of NPK (19:19:19) at fortnightly interval recorded the least C. I (25.00) (Table 20).

Table 19. Micronutrient status of the soil samples collected during the survey

Location	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})
Palakkad 1	265.20	63.70	11.14	5.71	1.0
Palakkad 2	300.10	63.40	15.58	5.52	1.4
Palakkad 3	337.50	63.70	12.20	5.24	1.4
Palakkad 4	303.30	63.70	13.46	6.28	1.3
Palakkad 5	401.10	63.20	17.06	5.37	1.2
Thiruvananthapuram 1	258.30	63.40	18.24	6.41	0.9
Thiruvananthapuram 2	292.50	63.20	17.59	4.95	0.7
Thiruvananthapuram 3	374.70	51.20	19.41	5.51	0.6
Thiruvananthapuram 4	152.40	63.70	13.21	4.42	0.6
Thiruvananthapuram 5	557.60	63.60	7.82	4.70	0.6
Healthy	125.20	42.20	3.01	2.30	0.5

Sufficiency ratings: Fe: > 5 mg kg^{-1}, Mn: > 1 mg kg^{-1}, Zn: > 1 mg kg^{-1}, Cu: > 1 mg kg^{-1}, B: > 0.5 mg kg^{-1}

Plate 12. Overall view of experiment plot

Table 20. Effect of treatments on the coefficient of infection of chilli leaf curl disease

Treatments	Response value	CI
Absolute control	1.00	100
Fertilizer and insecticide schedule as per package of practice (KAU, 2016)	0.75	75
Organic POP recommendation	0.75	75
POP modified based on soil test	0.50	50
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) + Silicon @ 400 kg ha^{-1}	0.50	50
POP + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application	0.50	50
POP + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1})	0.25	25
POP + Micronutrient (B @ 10 kg ha^{-1})	0.25	25
Basal (1/2 N + full P + 1/2 K) followed by foliar application (19:19:19) 0.5% at fortnightly	0.25	25
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Application of Sampoorna (KAU formulation (0.5 g L^{-1})) as foliar	0.75	75

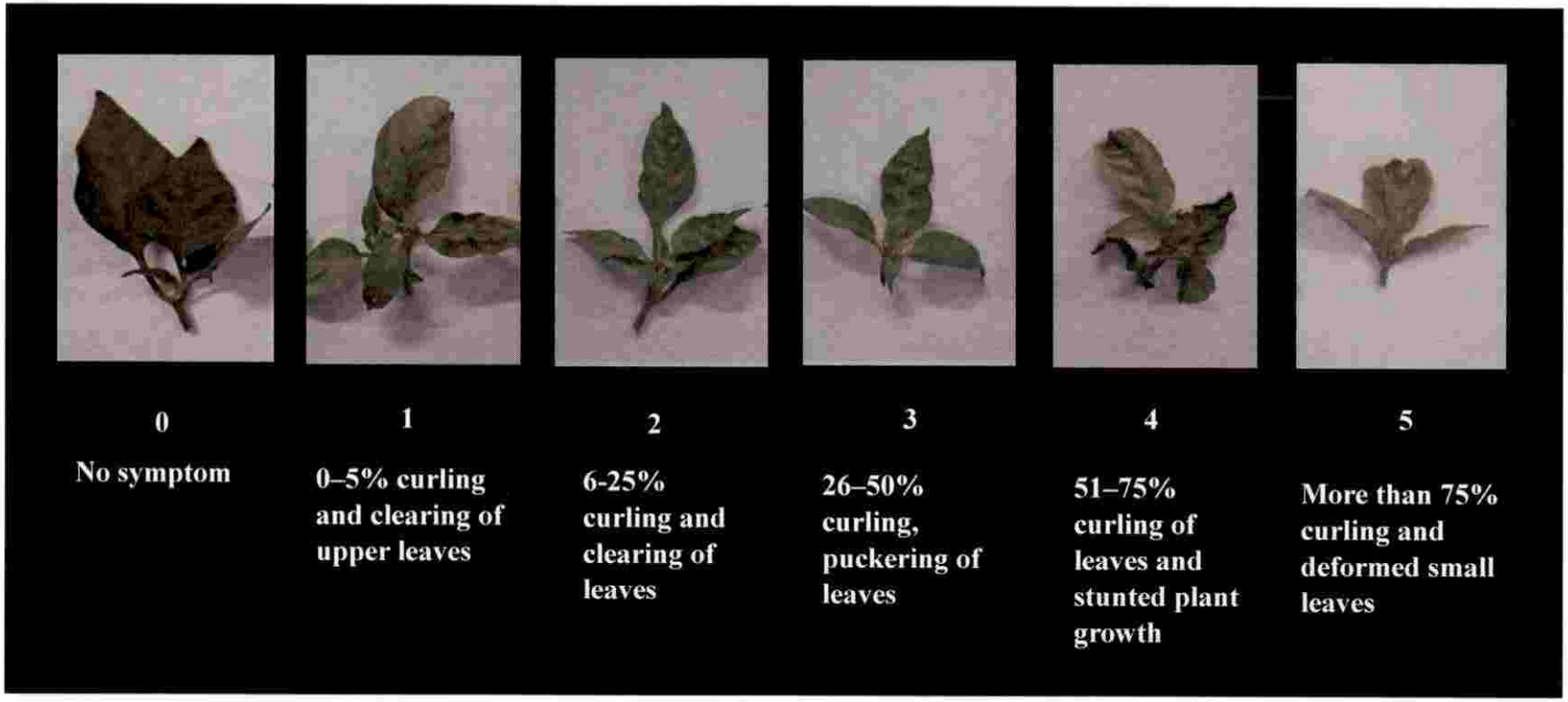

Plate 13. Scoring for coefficient of infection

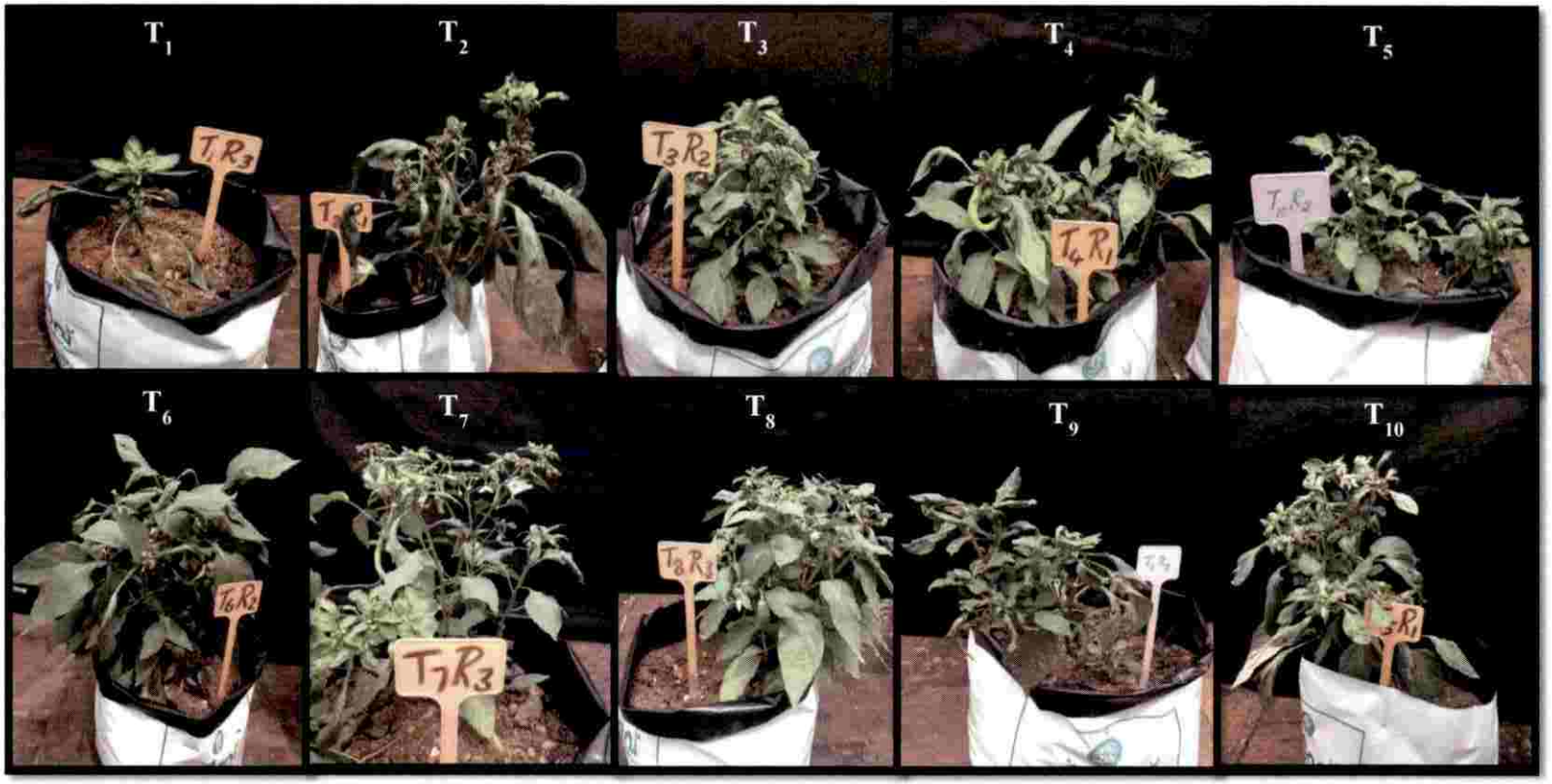

Plate 14. Effect of treatments on coefficient of infection on chilli leaf curl disease

4.4.3.3. Preliminary soil analysis for major and micronutrients

The soil samples were collected from individual treatments and analysed for major and micronutrients before imposing treatments. The data indicated that the pH of the soil sample of the experimental plot was 6.16 and is rated as slightly acidic. The electrolytic conductivity of the soil was observed to be normal (0.81 dS m^{-1}). The organic carbon status (1.6 %) indicated a high value. The primary major nutrient status in soil indicated that the available N (401.41 kg ha^{-1}), available P (21.77 kg ha^{-1}) and available K (261 kg ha^{-1}) content of soil were in the medium range. The secondary major nutrients status of soil, particularly available Ca (310 mg kg^{-1}), available Mg (150 mg kg^{-1}) and available S (9.5 mg kg^{-1}) status remained sufficient. The micronutrient status of the soil revealed that Fe (370 mg kg^{-1}), Cu (25 mg kg^{-1}), Zn (62.83 mg kg^{-1}) and Mn (25.57 mg kg^{-1}) indicated a sufficient level whereas B (0.02 mg kg^{-1}) remained extremely deficient (Table 21).

4.4.3.4. Plant nutrient analysis of the index leaf stage

Plant nutrient analysis was performed in the index leaf of chilli *i.e.*, just completely or fully developed mature leaves at growth phase (flowering stage). In case of primary major nutrients, the treatment with fertilizer and insecticide schedule as per POP gave significant N, P, K content (4.89 %, 0.20 % and 4.91 %) and was on par with the treatment POP recommendation supplemented with B (4.79 %, 0.18 % and 4.69 % N, P and K respectively). This was followed by the treatment POP recommendations with secondary nutrient lime and micronutrient Zn, B and Si (4.61 %, 0.15 % and 4.53 % N, P and K respectively) (Table 22).

In case of secondary major nutrients, maximum nutrient status of secondary nutrients was recorded in POP application supplemented with secondary nutrients (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) and micronutrients ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application which remained significantly on par with the treatment POP recommendation supplemented with secondary nutrients (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) and micronutrients ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) and silicon @ 400 kg ha^{-1} (Table 23).

The micronutrient concentration in the index leaf is represented in Table 24 and it was observed that highest value of Fe, Mn and B was recorded in the treatment POP supplemented with B (434.33 mg kg^{-1}, 66.35 mg kg^{-1} and 18.66 mg kg^{-1} respectively). While Zn recorded highest value with the treatment POP application supplemented with $ZnSO_4$ (112.60 mg kg^{-1}), Cu recorded maximum value with the treatment POP recommendation along with secondary nutrients (lime and $MgSO_4$), micronutrients (Zn, Cu and B) as foliar and soil application (26.60 mg kg^{-1}).

4.4.3.5. Yield data

The number of fruits per plant (g $plant^{-1}$) and fruit yield of chilli (kg) was recorded from different treatments (Table 25 and Plate 15). Among the treatments, the highest number of fruits per plant was recorded in the treatment POP and B application (8.83 per plant) which was on par with POP and $ZnSO_4$ (7.16 per plant). The highest fruit yield of 48.85 g $plant^{-1}$ was recorded from the treatment supplemented with nutrients as per POP and B @10 kg ha^{-1} which was found to be on par with the treatment POP and $ZnSO_4$ application (31.77 g $plant^{-1}$). This was followed by POP recommendation along with lime, micronutrient Zn Cu and B along with Si (20.60 g $plant^{-1}$) which is on par with POP modified based on soil test (19.42 g plant $^{-1}$), fertilizer and insecticide schedule as per POP (17.90 g plant $^{-1}$) and all other treatments. The untreated virus infected plant yielded the least fruit yield of 3.10 g $plant^{-1}$.

4.4.3.6. Enzyme linked immunosorbent assay (ELISA)

Leaf samples collected from all the treatments were subjected to ELISA to know the changes in the OD value in the treated and untreated plants. Lower OD value (0.212) was observed in the plants supplemented with the nutrients as per POP and B @ 10 kg ha^{-1}, while the highest was in untreated control (0.590). POP with micronutrient Zn gave an OD value of 0.240 which was followed by POP along with secondary nutrient lime, and micronutrients Zn, Cu, B and Si (0.262). Healthy gave an OD value of 0.090 (Table 26).

Table 21. Chemical properties including major and micronutrients of the soil before imposing treatments

Sl. No.	Chemical properties	Parameters
1	Organic carbon (%)	1.6
2	pH	6.16
3	Electrical conductivity (dS m^{-1})	0.81
4	Available N (kg ha^{-1})	401.41
5	Available P (kg ha^{-1})	81.77
6	Available K kg ha^{-1})	261
7	Available Ca (mg kg^{-1})	310
8	Available Mg (mg kg^{-1})	150
9	Available S (mg kg^{-1})	9.5
10	Available B (mg kg^{-1})	0.02
11	Available Fe (mg kg^{-1})	370.00
12	Available Cu (mg kg^{-1})	25.00
13	Available Zn (mg kg^{-1})	62.83
14	Available Mn (mg kg^{-1})	25.57

Table 22. Effect of treatments on primary macronutrient content in the index leaf of chilli

Treatments	N (%)	P (%)	K (%)
Absolute control	2.31	0.09	4.17
Fertilizer and insecticide schedule as per package of practice (KAU, 2016)	4.89	0.20	4.91
Organic POP recommendation (KAU, 2016)	3.31	0.13	4.52
POP modified based on soil test	3.76	0.14	4.66
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) + Silicon @ 400 kg ha^{-1}	4.61	0.15	4.53
POP + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application	3.68	0.10	4.56
POP + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1})	3.93	0.17	4.19
POP + Micronutrient (B @ 10 kg ha^{-1})	4.79	0.18	4.69
Basal (1/2 N + full P + 1/2 K) followed by foliar application (19:19:19) 0.5% at fortnightly	3.45	0.09	4.40
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Application of Sampoorna (KAU formulation (0.5 g L^{-1})) as foliar	3.12	0.11	4.50
SE (m±)	0.02	0.02	0.07
C. D.	0.129	0.026	0.371

Table 23. Effect of treatments on secondary macronutrient analysis in the index leaf of chilli

Treatments	Ca (%)	Mg (%)	S (%)
Absolute control	0.60	1.28	0.15
Fertilizer and insecticide schedule as per package of practice (KAU, 2016)	0.85	1.52	0.42
Organic POP recommendation	0.99	1.38	0.30
POP modified based on soil test	0.88	1.66	0.25
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) + Silicon @ 400 kg ha^{-1}	1.26	1.62	3.27
POP + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application	1.65	1.73	3.29
POP + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1})	1.14	1.25	2.98
POP + Micronutrient (B @ 10 kg ha^{-1})	1.14	1.43	0.36
Basal (1/2 N + full P + 1/2 K) followed by foliar application (19:19:19) 0.5% at fortnightly	1.04	1.47	0.51
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Application of Sampoorna (KAU formulation (0.5 g L^{-1})) as foliar	0.83	1.41	3.21
SE (m±)	0.18	0.16	0.11
C. D.	0.545	0.398 (*)	0.336

* Significant at 1%

Table 24. Micronutrient concentration in the index leaf of chilli, mg kg^{-1}

Treatments	Fe	Mn	Zn	Cu	B
Absolute control	116.40	24.66	46.00	13.63	10.93
Fertilizer and insecticide schedule as per package of practice (KAU, 2016)	164.43	43.00	76.43	11.46	12.23
Organic POP recommendation	221.20	41.33	59.73	14.60	13.80
POP modified based on soil test	183.96	35.00	58.56	13.13	13.10
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @)80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) + Silicon @ 400 kg ha^{-1}	342.66	36.50	103.00	26.30	18.40
POP + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application	404.66	33.16	111.40	26.60	17.73
POP + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1})	379.83	46.76	112.60	17.03	14.93
POP + Micronutrient (B @ 10 kg ha^{-1})	434.33	66.35	79.66	13.43	18.66
Basal (1/2 N + full P + 1/2 K) followed by foliar application (19:19:19) 0.5% at fortnightly	355.40	34.00	64.80	9.73	15.06
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{1} + $MgSO_4$ @) 80 kg ha^{-1}) + Application of Sampoorna (KAU formulation (0.5 g L^{-1})) as foliar	120.66	24.70	35.50	7.73	11.56
SE (m±)	20.88	4.326	4.59	1.15	0.73
C. D.	62.042	12.852	13.650	3.436	2.176

Table 25. Effect of treatments on number of fruits per plant and fruit yield

Treatments	Number of fruits per plant	Fruit yield (g plant^{-1})
Absolute control	2.66	3.10
Fertilizer and insecticide schedule as per package of practice (KAU, 2016)	1.50	17.90
Organic POP recommendation	2.83	14.56
POP modified based on soil test	4.00	19.42
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) + Silicon @ 400 kg ha^{-1}	4.00	20.60
POP + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application	3.00	16.88
POP + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1})	7.16	31.77
POP + Micronutrient (B @ 10 kg ha^{-1})	8.83	48.85
Basal (1/2 N + full P + 1/2 K) followed by foliar application (19:19:19) 0.5% at fortnightly	3.00	14.36
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{1} + $MgSO_4$ @) 80 kg ha^{-1}) + Application of Sampoorna (KAU formulation (0.5 g L^{-1})) as foliar	1.50	9.53
SE (m±)	1.42	7.89
C. D.	4.20	23.442

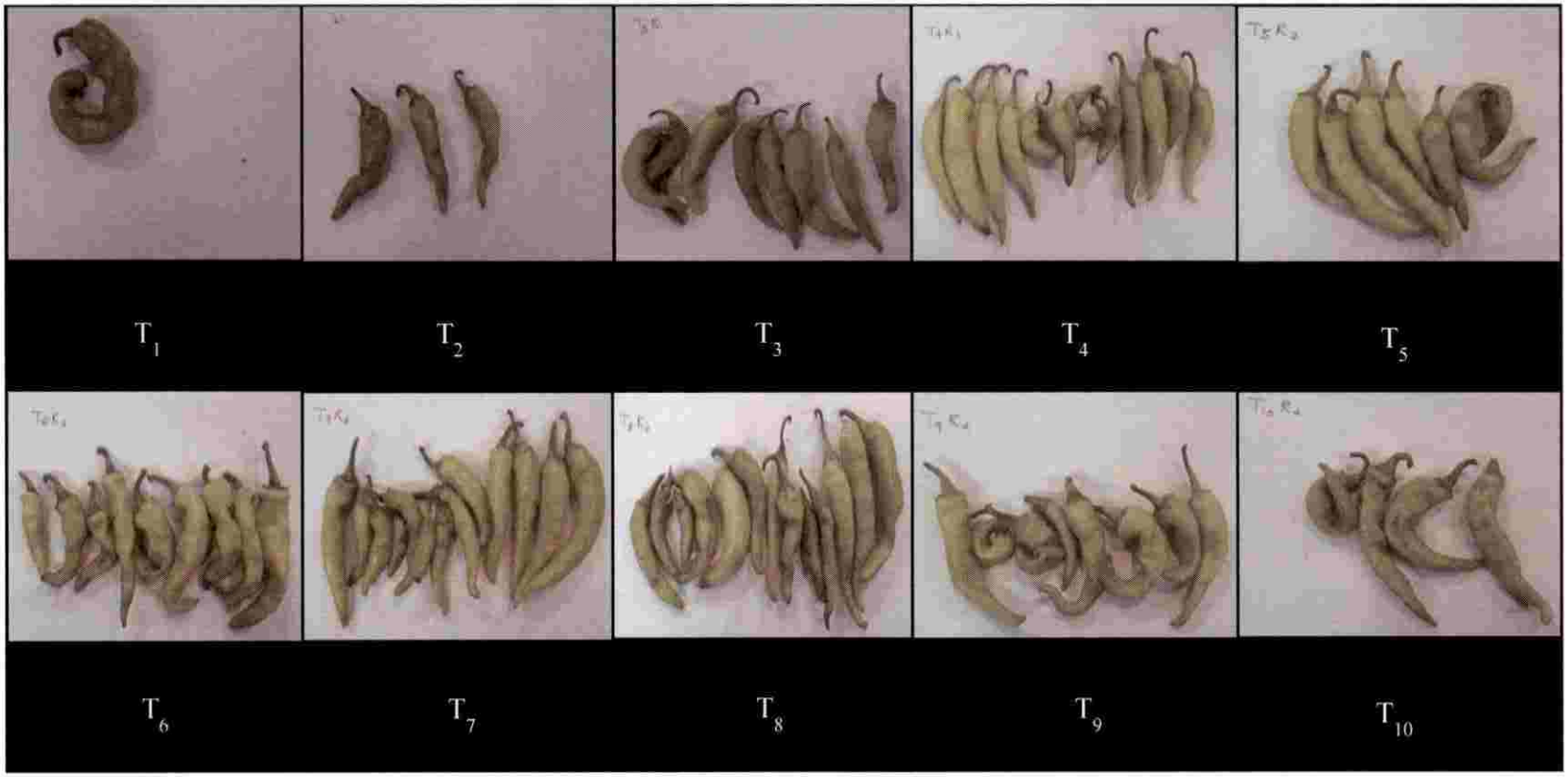

Plate 15. Effect of treatments on fruit yield during 5th harvest (number of fruits per plant)

Table 26. Effect of treatments on OD value of ELISA

Treatments	OD value (*)	Increase in OD value	Reaction
Healthy	0.090	-	
Absolute control	0.590	6.5	+
Fertilizer and insecticide schedule as per package of practice (KAU, 2016)	0.303	3.4	+
Organic POP recommendation	0.339	3.8	+
POP modified based on soil test	0.353	3.9	+
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @) 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) + Silicon @ 400 kg ha^{-1}	0.262	2.9	+
POP + Secondary nutrient (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application	0.318	3.5	+
POP + Micronutrient ($ZnSO_4$ @ 20 kg ha^{-1})	0.240	2.7	+
POP + Micronutrient (B @ 10 kg ha^{-1})	0.212	2.3	+
Basal (1/2 N + full P + 1/2 K) followed by foliar application (19:19:19) 0.5% at fortnightly	0.293	3.2	+
POP recommendation + Secondary nutrient (lime @ 350 kg ha^{1} + $MgSO_4$ @) 80 kg ha^{-1}) + Application of Sampoorna (KAU formulation (0.5 g L^{-1})) as foliar	0.343	3.8	+

* Absorbance at 405 nm

Discussion

5. DISCUSSION

Chilli is one of the chief commercial vegetable grown worldwide for its green immature fruits and also as dried spice. Among the diseases affecting chilli, viral diseases are of great importance due to severe loss in quality and yield. ChiLCD is a devastating disease of chilli in India which is predominant because of lack of awareness and their proper management. Because of its complexity in symptomology, ChiLCV and vectors involved and unavailability of nutrients, it is difficult to manage ChiLCD. Hence the present study was undertaken to investigate on the complexity of symptoms, detection of the virus and management of the disease by supplementing nutrients.

Specific techniques are essential to identify ChiLCV in chilli to attain the ultimate objective of viral management. In addition, survey for the disease incidence was conducted in traditional chilli growing tracts of Kerala. The disease occurring under natural conditions in two districts such as Palakkad and Thiruvananthapuram have been studied in detail with respect to disease incidence. Serological diagnosis was done to test the presence of this virus through different virus diagnostic tools like ELISA and molecular characterization of virus was performed through PCR based techniques by sequencing of coat protein gene.Studies were conducted for the management of leaf curl using various combinations of nutrients which was undertaken in a pot culture experiment in Department of Plant Pathology.

Based on the findings described in the preceding parts of study, results obtained on collection of begomovirus infected chilli from different cultivated areas, symptomatology, serological diagnosis and molecular characterization of the viruses causing chilli leaf curl and nutrient based management of chilli leaf curl are discussed here with relevant literature pertaining to findings of other workers.

5.1. COLLECTION OF BEGOMOVIRUS INFECTED CHILLI FROM DIFFERENT CULTIVATED AREAS

5.1.1. Disease incidence

Surveys were conducted in major chilli growing areas of Palakkad and Thiruvananthapuram districts during 2017 to 2019 to determine the distribution of ChiLCV in two districts of Kerala. The results clearly indicated the presence of ChiLCV in all the surveyed fields with the disease incidence ranging from 55.71 to 73.33 per cent in Palakkad and 69.33 to 80 per cent in Thiruvananthapuram district. The disease incidence was more in Thiruvananthapuram district compared to Palakkad district (Fig. 2, 3 and 4).

Surveys had been conducted by many research workers to study the disease incidence and distribution of viruses. Senanayake *et al.* (2007) had reported a very high disease incidence (up to 100 %) of ChiLCV in Narwa and Tinwari villages at Jodhpur District, Rajasthan. Reduction of plant growth and absence of fruits were noticed in severely affected plants. Raju (2010) had also reported the presence of ChiLCV with maximum disease incidence of 22 to 56 per cent in all the surveyed fields during early stage of infection. Both roving and fixed plot survey were conducted during 2002 to 2004 in the chilli growing areas of Belguam, Bellary, Dharwad, Gadag, Gulburga, Haveri, Koppal and Raichur districts in Northern parts of Karnataka. A progressive increase in disease intensity and a progressive decrease in number of pods and pod weight were observed during the later stage of the disease.

Senanayake *et al.* (2012) recorded an epidemic of ChiLCD in 2004 in Jodhpur district of Rajasthan. Disease incidence (14 to 100 %) varied with field to field and village to village. The disease incidence was maximum in Tiwari where 100 per cent of chilli variety Haripur Raipur showed severe leaf curl, whereas the disease incidence in the same cultivar varied from 14 to 44 per cent in Narwan.

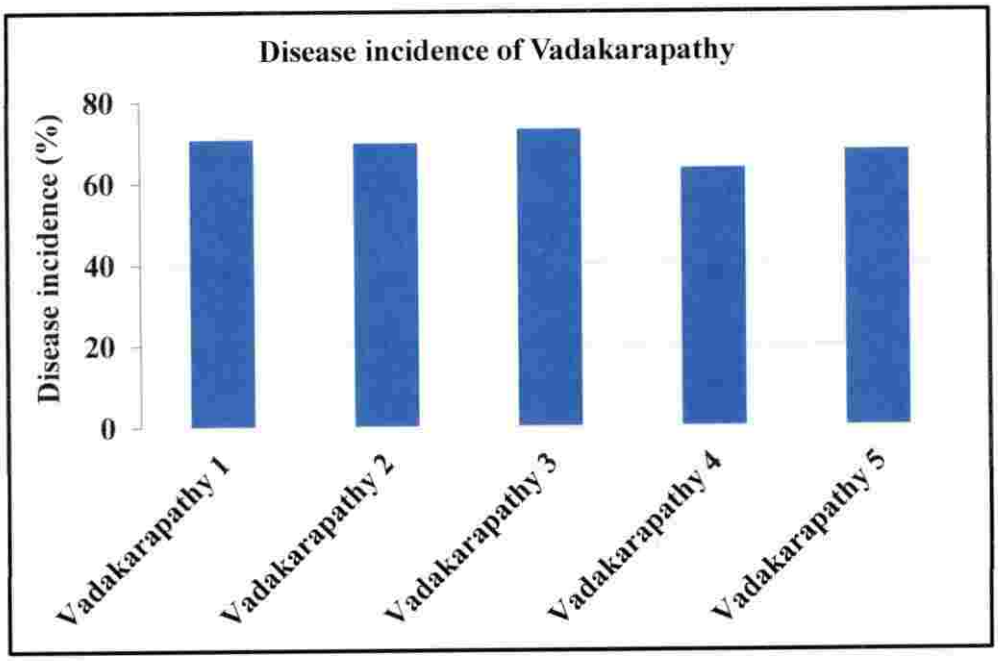

Fig. 2. Disease incidence of chilli leaf curl disease in different fields of Vadakarapathy, Palakkad

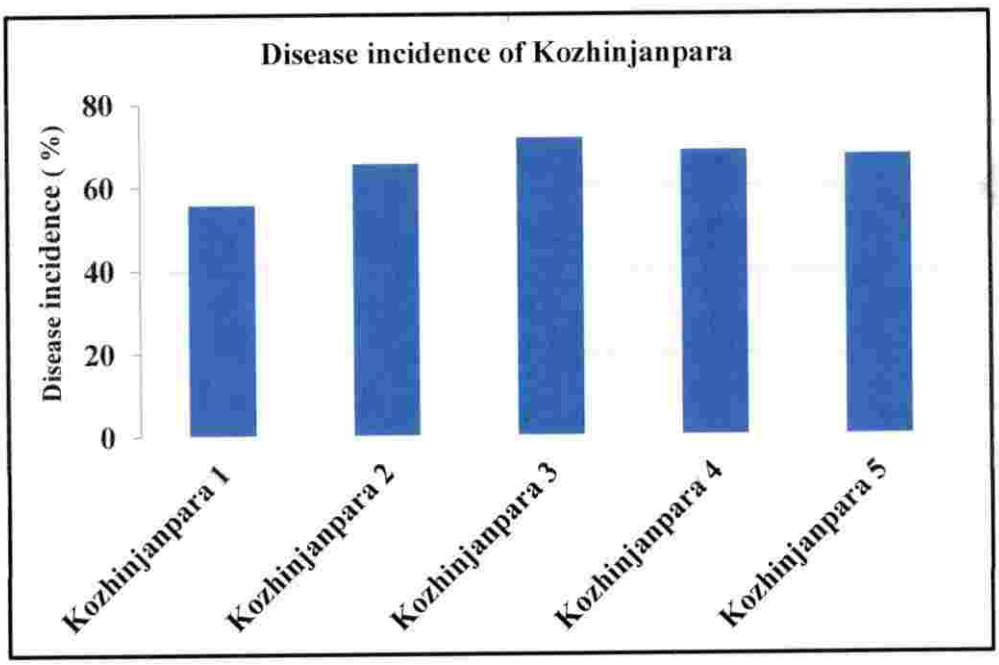

Fig. 3. Disease incidence of chilli leaf curl disease in different fields of Kozhinjanpara, Palakkad

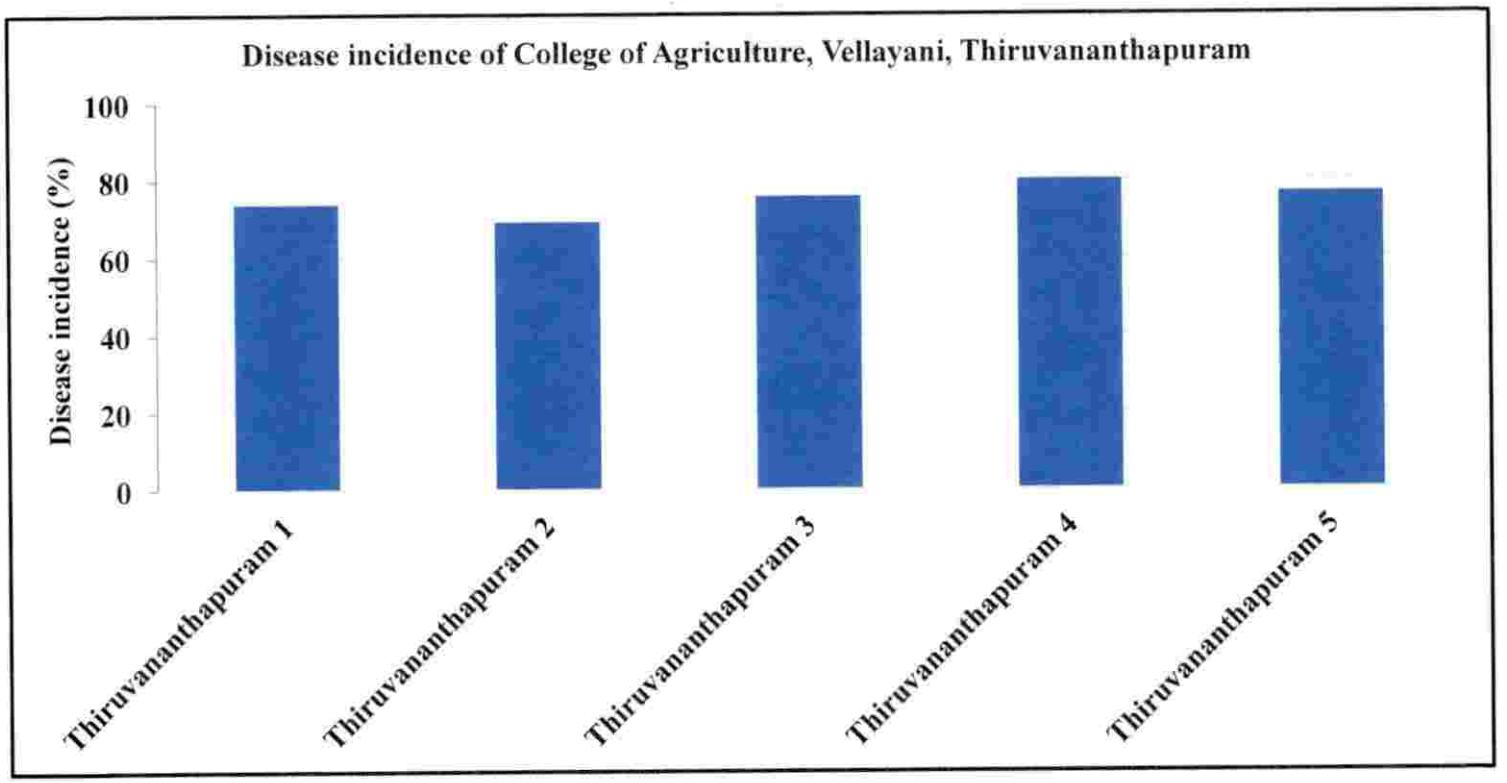

Fig. 4. Disease incidence of chilli leaf curl disease in different fields of College of Agriculture, Vellayani, Thiruvananthapuram

Senanayake *et al.* (2013) recorded 60 to 100 per cent of disease incidence in the surveyed areas of Anuradhapura district in Sri Lanka at the bearing stage of crop. Olawale *et al.* (2015) conducted surveys to determine the disease incidence, and diversity and distribution of the viruses infecting pepper in South-West Nigeria during 2010 and 2011. The average disease incidence was 79 per cent in 2010 and 76 per cent in 2011. A roving survey was conducted in two major chilli growing districts of Madhya Pradesh in five villages of each district during 2014. The survey revealed severe yield losses due to ChiLCV infected chilli fields with 100 per cent disease incidence in Khargone district but the disease incidence ranged from 88-100 per cent in Ratlam district (Kumar *et al.*, 2016).

Thakur and Sharma (2017) also reported high incidence of chilli leaf curl disease ranging from 57.5 to 79.4 per cent in different parts of Punjab whereas the disease incidence ranged from 25.53 to 67.70 per cent in Faizabad and Sultanpur districts of eastern Uttar Pradesh in 2014 and 2015 (Olawale *et al.*, 2015), and 52.53 to 56.70 per cent in 2009-2011 in Sehore, Madhya Pradesh (Kumar and Kumar, 2017). Maximum disease incidence of 27.50 per cent and 23.50 per cent was recorded in Etawah and Lucknow districts of Uttar Pradesh respectively (Singh and Awasthi, 2017).

Oraon and Tarafdar (2018) conducted surveys in 18 villages and 54 fields in six districts of West Bengal during 2015 to study the distribution of virus and disease incidence of ChiLCD. The maximum chilli leaf curl disease incidence was noticed in Cooch Behar district (30-90 %) followed by Burdwan (20-85 %) and North 24 parganas (45-75 %) in 2015. The lowest incidence was observed at Purulia district (18-43 %).

5.2. SYMPTOMATOLOGY

The results have evidently shown that disease became severe after early stage of crop growth and started expressing symptoms such as upward curling, puckering, yellowing, reduced leaf size, petiole elongation, crinkling and mottling with no or less fruit set. During early stages of infection, the symptoms observed were upward curling, puckering and yellowing. While at later stages of growth, the

infected plant expressed reduced leaf size, petiole elongation, lesser flowers, smaller fruits and stunted growth. In Palakkad and Thiruvananthapuram district, the fields surveyed showed severe upward curling with puckering of leaves with yellowing and stunted growth. These results were similar to the findings of Muniyappa (1980), and Sakia and Muniyappa (1989) who described the similar symptoms such as upward curling, puckering, petiole elongation, yellowing, stunting of plants etc.

Osaki and Inouye (1981) and Sinha *et al.* (2011) also recorded similar symptoms in the chilli field *viz.*, leathery and brittle leaves along with vein clearing, puckering, crinkling and stunting of the plants.

Different symptoms produced by insects were also observed in all the fields visited such as upward curling, puckering and internal eruptions of leaves produced by thrips which were also reported by Reddy and Puttaswamy (1983) and symptoms such as curling of leaves and elongation of petiole caused by mites which were also noticed by Karupachamy *et al.* (1993). A wide range of symptom variability was observed in chilli by Shivanathan (1983) and Shih *et al.* (2003) where the symptoms observed were downward curling of leaves, puckering of leaf lamina, vein clearing, plant stunting and bushy appearance due to reduced internodal length with reduced flowers and fruits.

Senanayake *et al.* (2007) also observed abaxial curling, puckering, swelling of veins, reduced size of leaves with stunted growth and no fruit development. Similar observations were made by Krishnareddy *et al.* (2008) where the individual plants showed chlorotic, necrotic spots symptoms on leaves with typical apical necrosis. Chattopadhyay *et al.* (2008) and Shafiq *et al.* (2010) identified the virus infected leaves with leaf curl, yellowing, leaf rolling and distortion. These symptoms were confirmed as the characteristic of begomovirus in chilli. Hence, confirmation of virus association was carried out with these characteristic symptoms.

Patel and Mondal (2013) described the nature of symptoms of leaf curl complex as upward curling, thickening and crinkling of leaves. In severely infested

plants, margin of the leaves became burned and dried up. Elongation of leaves and petioles were found. Leaves become hardened, twisted and in advance stages, got scorched. Infested plants produced very small sized leaves with cracked and deformed fruits. Similarly, Senanayake *et al.* (2013) also observed varied disease symptoms such as chlorosis, puckering, mottling, dark greenish small leaves, boat shape leaves with mosaic appearance.

The symptoms of the leaf curl infected chilli plants were observed as yellowing, curling, puckering followed by mild to severe mosaic, necrotic streaks on vein and petiole with stunted and bushy appearance (Talukdar *et al.*, 2015). A wide array of chilli leaf curl symptoms was shown by chilli plants. It included leaf curling, interveinal chlorosis and puckering. To a lesser extent leaf mosaic, mottling and vein clearing were also observed (Menike and Costa, 2017).

Thakur and Sharma (2017) described the symptoms of ChiLCD as puckering of leaves, blistering of interveinal areas and thickening of veins. In advanced stage, axillary buds produced clusters of reduced sized leaves. The whole plant gave a bushy appearance and stunted growth.

According to Kumar *et al.* (2018), the leaves of symptomatic plants were curled upwardly, deformed, and small in size than the leaves of asymptomatic plants. Oraon and Tarafdar (2018) observed different kinds of ChiLCD symptoms like mild to severe leaf curling, leaf blistering, leaf crinkling and leaf narrowing of leaves with stunted growth and bushy appearance in the field conditions. The severely infected plants produced no fruit. However, less infected plants produced fruits of significantly reduced sizes.

5.3. SEROLOGICAL DIAGNOSIS AND MOLECULAR CHARACTERIZATION OF THE VIRUSES CAUSING CHILLI LEAF CURL

5.3.1. Enzyme linked immunosorbent assay (ELISA)

Zaidi *et al.* (1993) reported ELISA as one of the most popularly used techniques for detection of viruses. It has been found to be best way for large scale routine testing of viruses due to its rapid, cost effective, simple and fast assay

properties. TAS-ELISA was performed for checking the presence of chilli viruses (Begomovirus). TAS-ELISA was carried out using polyclonal antiserum SLCMV (another begomvirus) for the chilli samples collected during survey. Diseased chilli plant samples were collected from the fields of Palakkad and Thiruvananthapuram districts. All the samples collected from Thiruvananthapuram were serologically related to the SLCMV. Polyclonal antiserum of SLCMV was serologically related to chilli isolates of ChiLCV. These results were found to be correlated with the data given by Haq *et al.* (1996) and Aglave *et al.* (2007). Similar results were established by Fargette *et al.* (1996) who conducted TAS-ELISA on three ToLCV in susceptible *Lycopersicon* species and tomato cultivars; and Tiendrebeogo *et al.* (2008) who conducted TAS-ELISA for the characterization of *Pepper yellow vein mali virus* (PepYVMV) in *Capsicum* spp. The results indicated that the symptoms observed were associated with same as that of begomovirus infections.

Valizadeh *et al.* (2011) also conducted TAS-ELISA for the detection of *Tomato yellow leaf curl virus* (ToYLCV) in the symptomatic leaf samples of tomato and cucurbits. The results clearly indicated close relation of leaf curl of tomato and cucurbit isolates with ToYLCV infection.

The virus infecting chilli samples that surveyed in Palakkad and Thiruvananthapuram district had been identified as geminivirus showing a serological relation to SLCMV. Similarly, Xiang *et al.* (2012) conducted TAS-ELISA with five begomoviruses infected tomato samples. The results showed that only two isolates mainly reacted with ToYLCV. Hence, TAS-ELISA was set up as the established method could successfully detect virus in plants. Yasmin *et al.* (2017) analysed twelve symptomatic chilli pepper plants by TAS-ELISA using the MAbs. The results proved the infection of begomovirus in the chilli leaf samples. Yasmin *et al.* (2018) conducted the detection and characterization of begomovirus infecting chill pepper using TAS-ELISA. Whitefly transmitted geminivirus were initially detected by TAS-ELISA using a panel of ten monoclonal antibodies in the infected chilli plants in Pakistan.

5.3.2. Dot Immunobinding Assay (DIBA)

DIBA was conducted to detect the presence of the virus causing leaf curl in chilli using polyclonal antibody SLCMV. But no colour development was noticed in any of the samples indicating a negative result.

5.3.3. Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is considered as the basic tool for the molecular characterization of virus by analysing the presence or absence of virus in plant samples. Randomly selected infected chilli samples from Palakkad and Thiruvananthapuram district were used to isolate total DNA. DNA isolated from the samples were subjected to PCR using specific primers for amplification of coat protein of the virus *viz*., AV/AC and Deng primers. No PCR amplicon was obtained for any of the samples collected from Palakkad district while an amplicon size of~ 550 bp (AV/AC) and~500 bp (DENG) was obtained for the samples collected from Thiruvananthapuram. It was clear from PCR amplification that the infected chilli samples collected from Thiruvananthapuram district harbours viral genome. Sequencing of these coat protein amplicons and BLAST analysis in NCBI indicated 96.63 per cent similarity of Thiruvananthapuram isolate with ChiLCVeV. Shih *et al*. (2003) was the first who reported ChiLCV in Pakistan along with sequence information. DNA was extracted from three affected plants and tested for the presence of begomoviral DNA-A, DNA-B and associate satellite DNA by PCR using Beta01/Beta02 primers. Highest full-length DNA-A with 90.8 per cent sequence identity was found with a newly described begomovirus from Pakistan.

Senanayake *et al.* (2007) used the primer pair specific to ChiLCV for checking the presence of virus particles in India. Severely affected chilli plants were collected from Narwa village in Rajasthan and subjected to PCR using degenerate primes (AVF28 5'-GCCCACATYGTC TTYCCNGT-3' and AV29R 5'-GGCTTYCTTTACATRGG-3') to detect the presence of virus. Sequence analysis showed that the virus isolated from Jodhpur was distantly related (59.1-67.9 % identity) to CLCuMV, PepYLCIV and ToLCNDV. However, it shared 96.5 per

cent identity with *Chilli leaf curl virus*-[Pakistan: Multan] (ChiLCuV-[Pak:Mul]) and gave the close sequence identity with ChiLCuV-[Pak:Mul].

The results of the present study for molecular characterization of the virus were found to be in tandem with the findings of Kumar *et al.* (2012) who confirmed the association of a new begomovirus species, *Chilli leaf curl Vellanad virus* (ChiLCVeV) along with two distinct betasatellites (*Radish leaf curl betasatellite* (RaLCB) and ToLCBDB) with ChiLCD in Kerala.

5.4. NUTRIENT BASED MANAGEMENT OF CHILLI LEAFCURL

5.4.1. Plant nutrient analysis (major and micronutrients) of healthy and diseased samples

The virus in plant must have weakened the entire working system of the plant through changes in basic functions such as absorbtion of water and minerals, exchange of gases, photosynthesis etc. Therefore, the availability of a nutrient in any tissue must be reflection of the health and stage of the crop. The results showed that all the major and micro nutrients except S and Fe content in the infected leaves were low compared to healthy leaves (Fig. 5, 6 and 7). There was significant difference in N, P, K, Ca, Mg status in tissues arising from all the treatments receiving these nutrients as source in the form of urea, rajphos, murate of potash, lime, magnesium sulphate, respectively. Under diseased condition, effective utilization of absorbed primary major nutrients (N, P and K) within the plant tissues may not take place properly which could be the reason for low content of these nutrients in diseased tissues. Jeyarajan (1965) also reported the low content of primary major nutrients in diseased plant tissue than the healthy ones. Muqit *et al.* (2007) reported that the increased P content in PRSV infected leaves of papaya crop might be due to phosphorus containing polypeptide of the virus particles. Similarly, K content was also reported significantly decreased in leaves infected with PRSV when compared to healthy leaves (Vijayalakshmi *et al.*, 2018).

Marschner (1995) reported that deficiency of Ca is common in the virus infected plants and under deficiency plants are more susceptible to infection.

According to Singh and Singh (1972), there was a reduction in the Mg concentration upon infection by a *Potato virus X* and application of the same has been shown to reduce disease to certain extent. The high content of S in leaves must be due to the presence of any addition of S sources such as sulphur containing fungicides for the control of fungal diseases in chilli.

Upon infection by *Alfalfa mosaic virus* in alfalfa, there was a decrease in B, Zn, Mn and Cu (Yardimci *et al*., 2007). The higher level of Fe content must be due to the inherently high Fe content in the soil. The variation of nutrients with different levels could be due to the utilization of these elements in different ways for plant growth and development which was also reported by Islam (1987).

5.4.2. Soil analysis for major and micronutrients of collected soil samples from major chilli growing areas of Palakkad and Thiruvananthapuram districts

All the nutrients were high in soils collected from infected field compared to healthy (Fig. 8, 9 and 10) which could be due to the varied interaction of nutrients in soil restraining their availability. There was increase in nitrogen content in PRSV infected papaya field, PRSV infected ash gourd field, MYMV infected mungbean field. This observation was due to the variations in absorption of major nutrients such as N, P and K by plants or through possible losses of nutrients from individual plots since the soil prone to leaching (Muqit *et al*., 2007).

Similarly, Sharma and Yadav (1987) and Santral *et al*. (1989) reported the different availability of nutrients in soil where higher value of available Ca might be due to the total absence of fixation reactions occurring in the plot particularly between Ca and P. Takkar *et al*. (1989) reported the availability of Mg in different levels in soil which might result in two fold increase on the improvement of plant health. Marschner (2012) also reported the higher level of S in experimental plots must be due to the lesser utilization of S by plants which in turn decreased the health of the virus-infected plants.

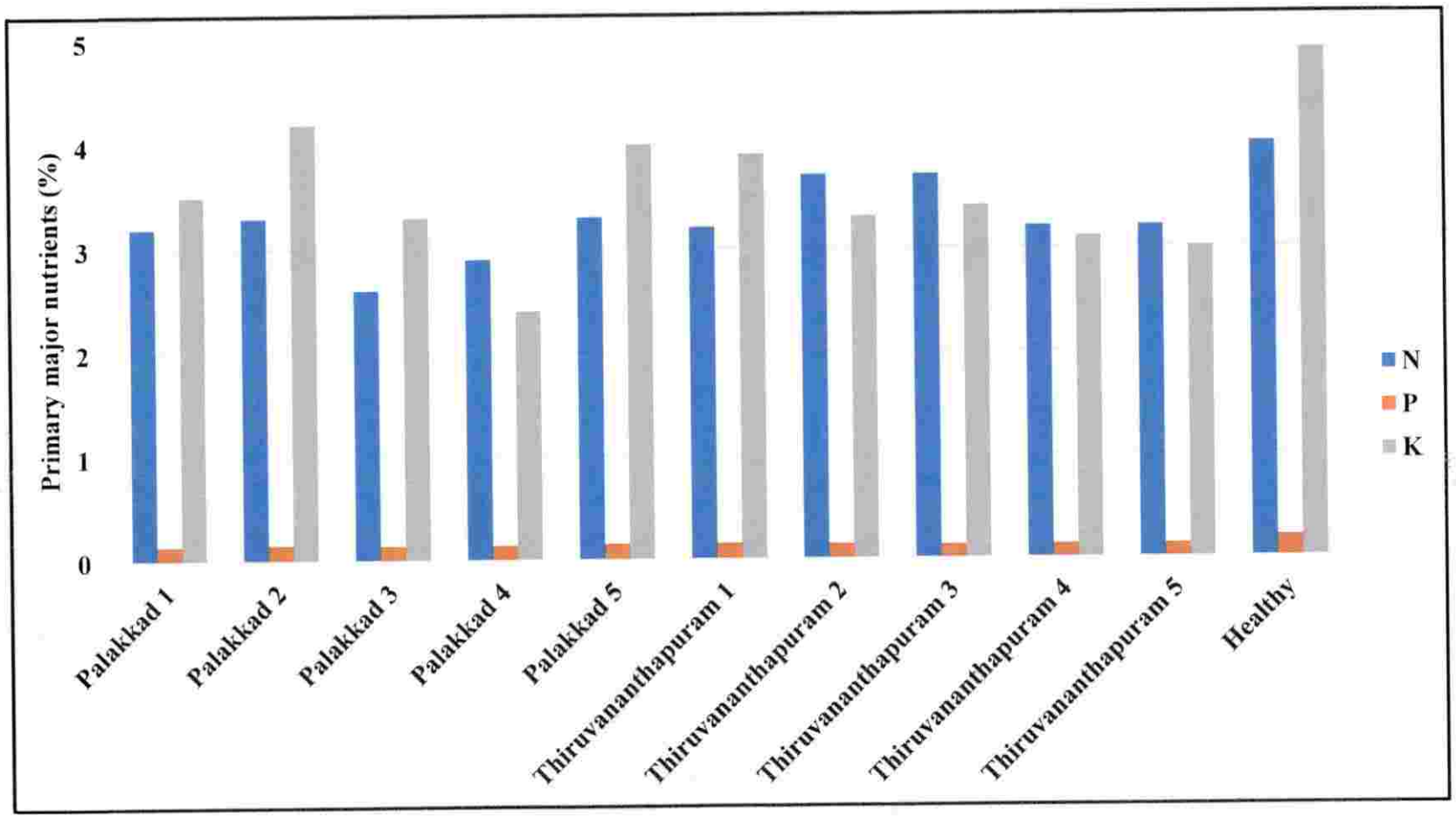

Fig. 5. Primary nutrient contents of healthy and diseased chilli leaf from the survey locations

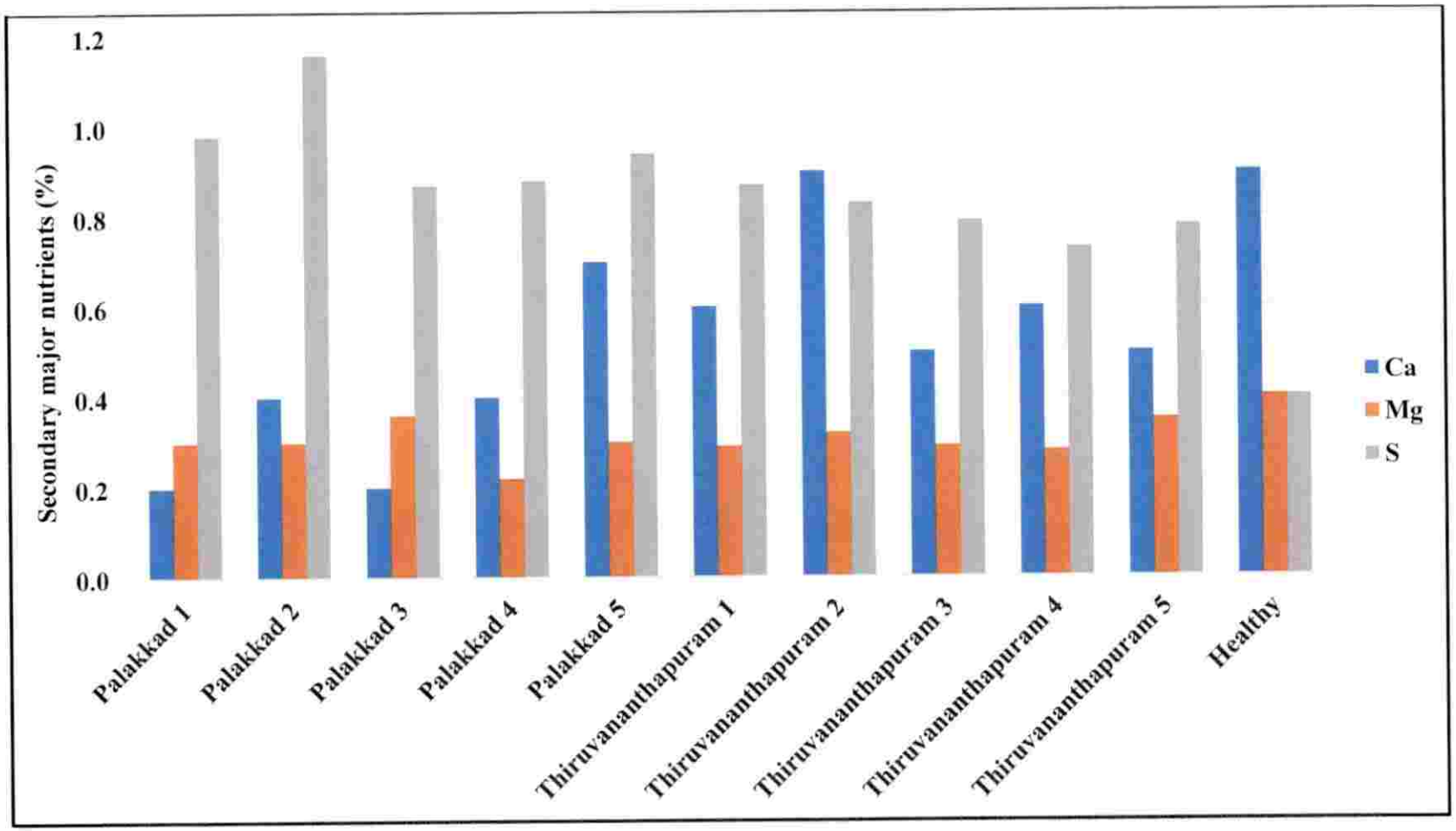

Fig. 6. Secondary major nutrient contents of healthy and diseased chilli leaf from the survey locations

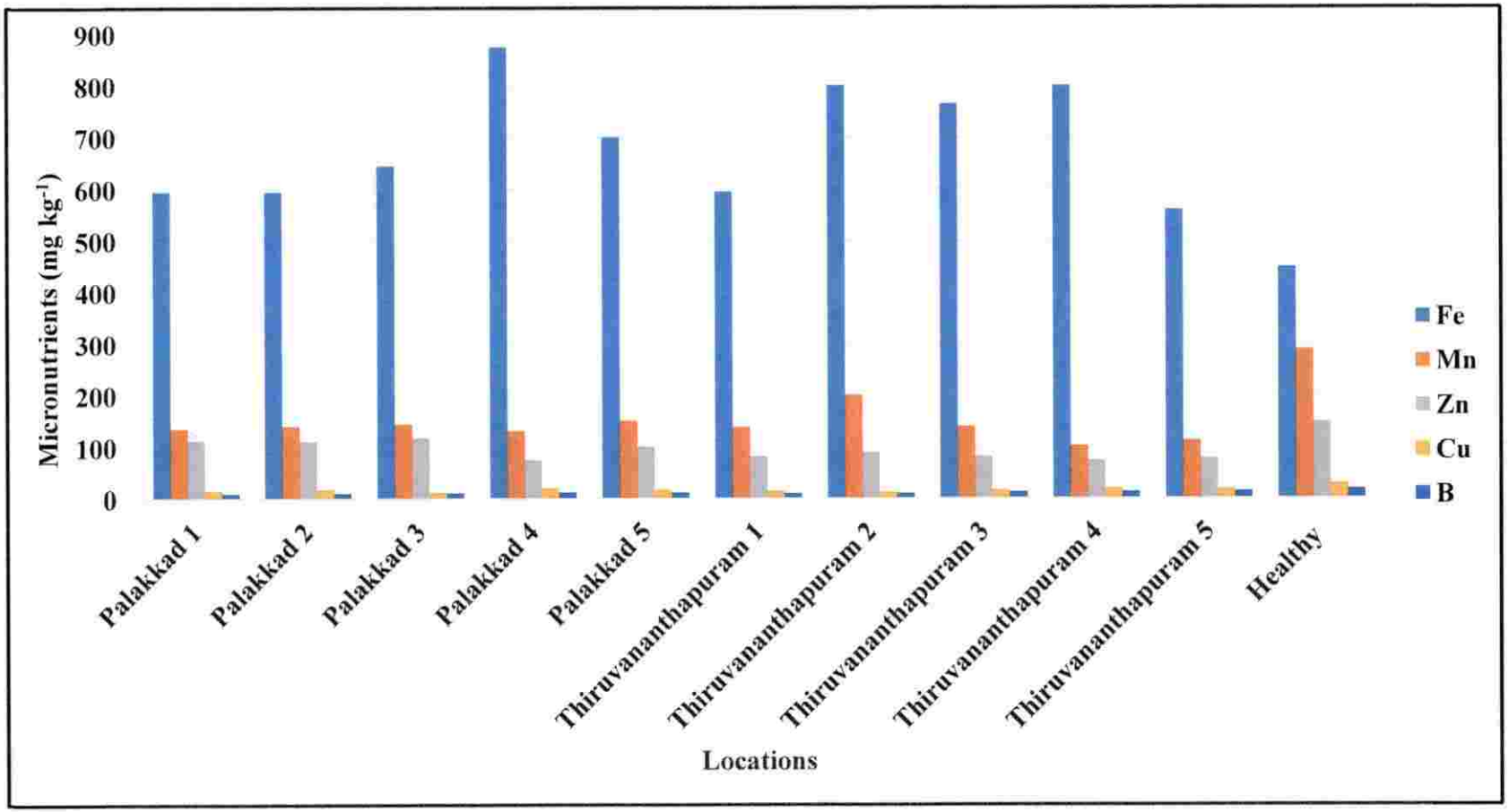

Fig. 7. Micronutrient concentrations of healthy and diseased chilli leaf from the survey locations

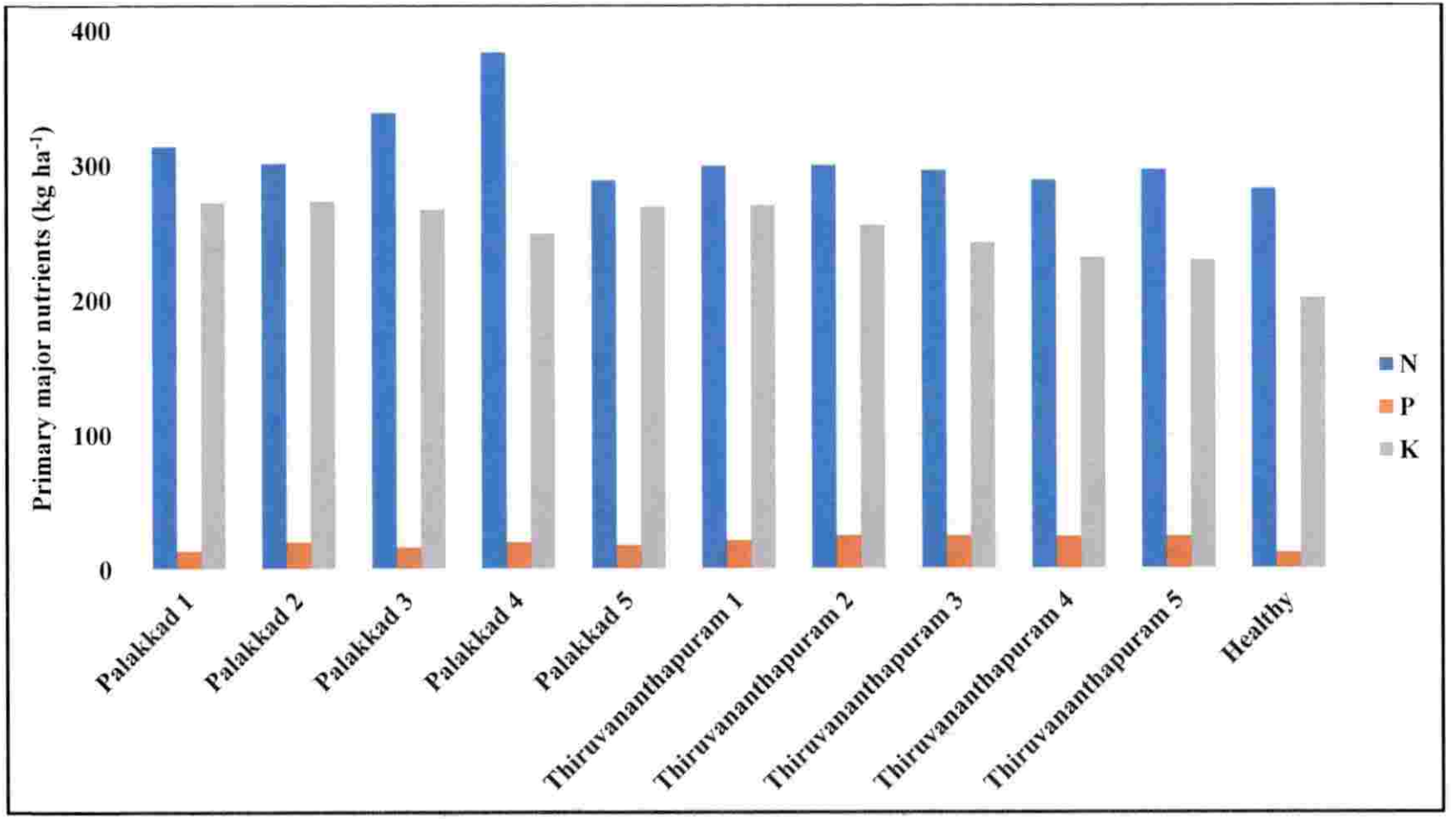

Fig. 8. Primary major nutrient status of soil samples collected from different survey locations

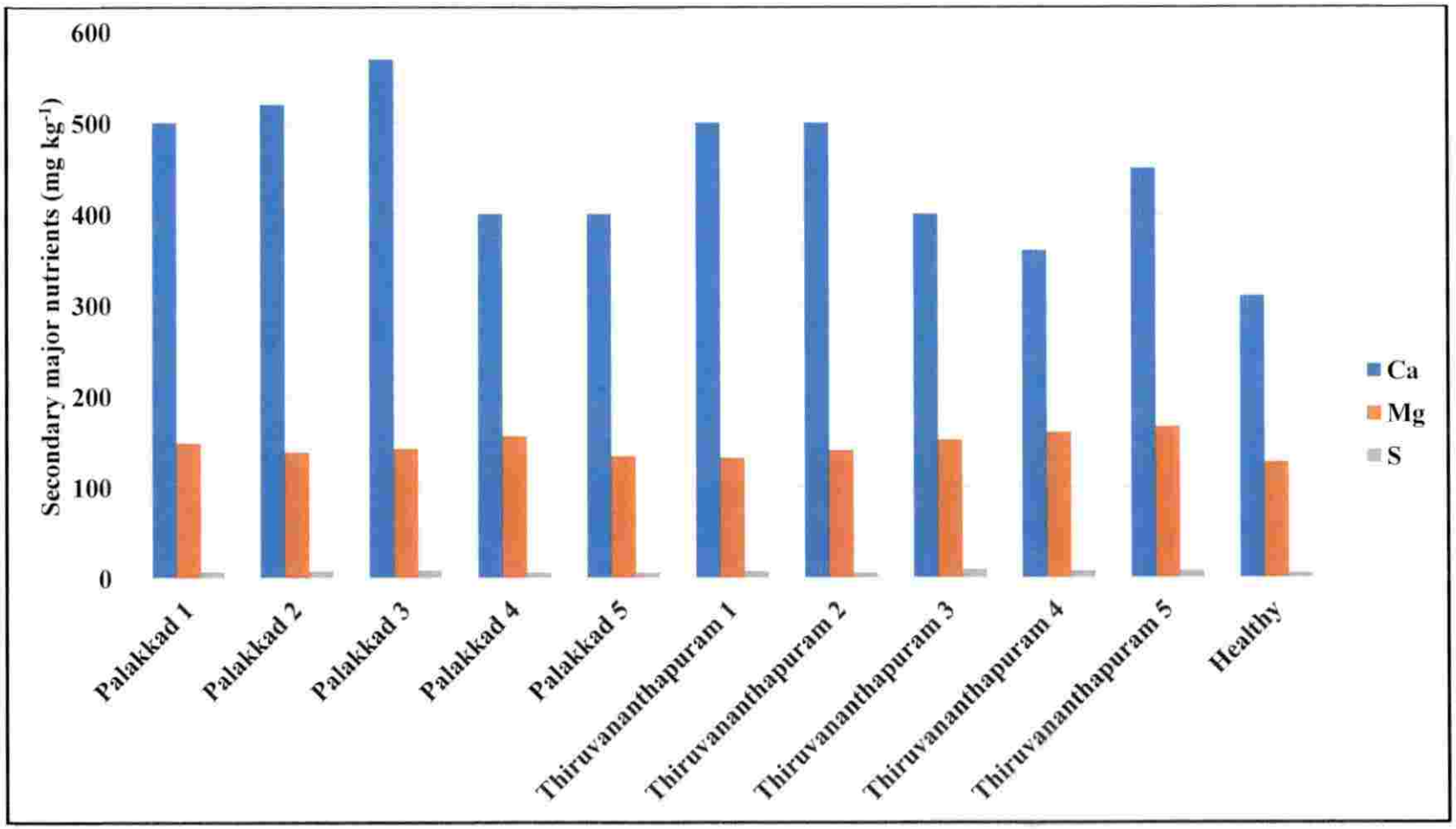

Fig. 9. Secondary major nutrient status of soil samples collected from different survey locations

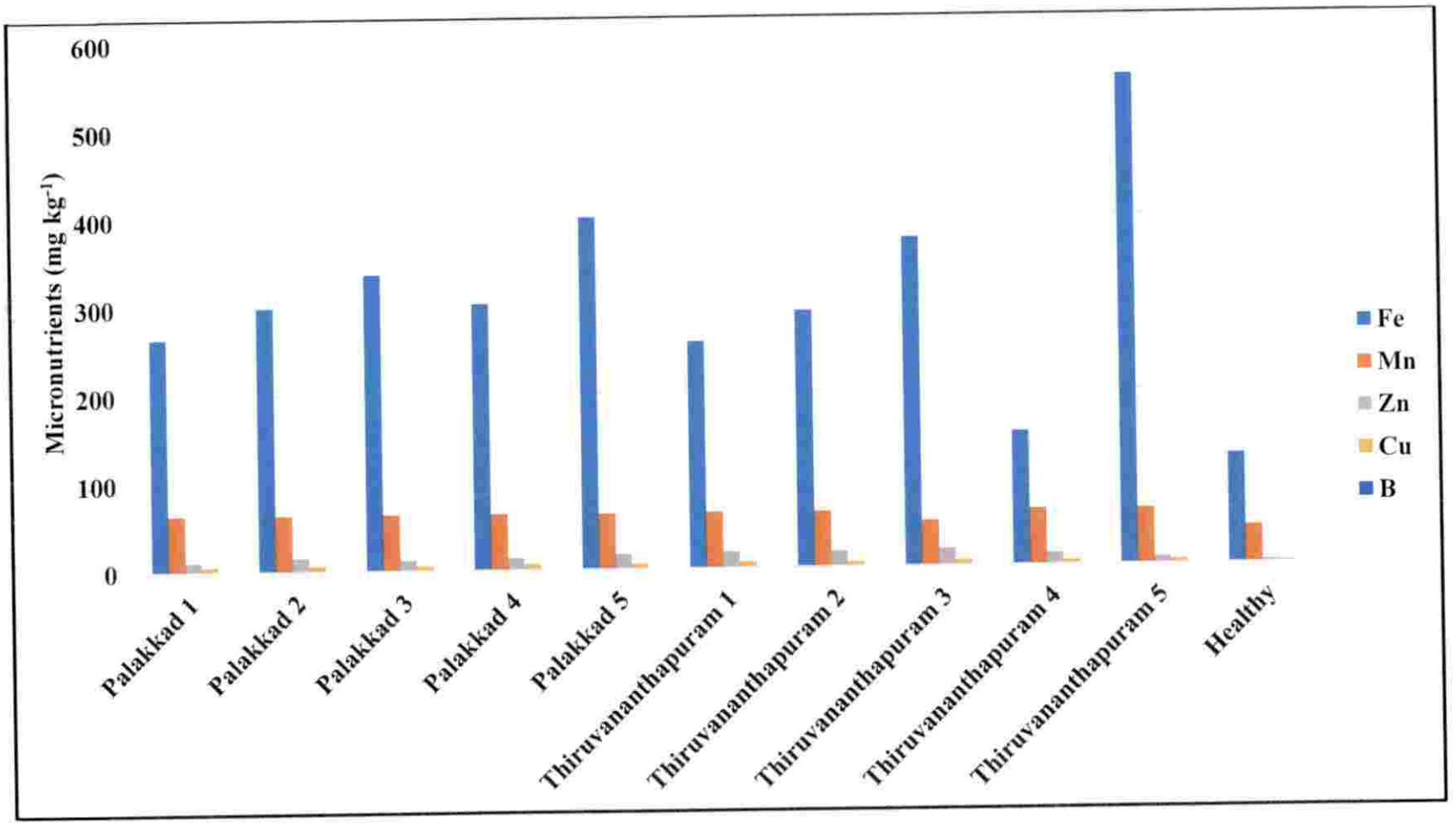

Fig. 10. Micronutrient status of soil samples collected from different survey locations

According to Santral *et al.* (1989), the available micronutrient levels in soil was higher when compared to the control which might be due to the lesser utilization of these nutrients by the plants and higher utilization of nutrients in soil. The interactions of these nutrients with each other enhanced their absorption and availability in the soil.

5.4.3. Pot culture studies for nutrient based chilli leaf curl management

5.4.3.1. Disease incidence

Cent per cent disease incidence was observed for all the treatments confirming the presence of virus followed by natural infection. The study was undertaken during summer season with ChiLCV susceptible variety, Vellayani Athulya. Mishra and Chauvey (2018) observed maximum disease incidence of 100 per cent in in Narwa and Tinwari villages during December. Wet and dry season are favourable for infection of ChiLCV on chilli, MYMV on mungbean and *Okra yellow vein mosaic virus* (OYVMV) on okra (Shivanathan, 1983). Similar studies had been undertaken to study the disease incidence of chilli leaf curl complex disease in chilli by Raju (2010). Consequently, it was reported that the leaf curl incidence increased with increasing temperature and relative humidity. Darshan *et al.* (2017) reported a significant leaf curl virus disease incidence in chilli variety, Vellayani Athulya. According to Anandhi and Khader (2011), both the leaf curl virus and vector population were significantly high in case of Vellayani Athulya variety. The present observation could be justified in view of these reports.

Lower disease incidence was recorded with the application of B spray for the management of ChiLCD in chilli reported by Pandey *et al.* (2010). The disease incidence of 70 to 80 per cent was recorded in chilli for the study of leaf curl by Thriveni (2017). Similarly, a lower incidence of PRSV in papaya was observed due to the combined sprays of soluble B (1 g L^{-1}), $ZnSO_4$ (2 g L^{-1}) and micro nutrient spray (1 ml L^{-1}) given at fortnightly intervals (Samuel *et al.*, 2015).

5.4.3.2. Coefficient of infection

Highest CI was recorded in the untreated virus infected plants when compared to the treated plants. Similar studies were also done by Thakur *et al.* (1987) who studied the CI value for studying the resistance against chilli leaf curl disease. These results were also correlated with results obtained by Kumar *et al.* (2006) for the identification of host plant resistance to *Pepper leaf curl virus* (PeLCV) in chilli. Keerthana *et al.* (2019) observed a higher CI (56.87) during the evaluation of resistance of tomato leaf curl disease in tomato. The more or less similar findings were also reported by Hosamani (2007) where lowest CI (18.4) was observed in case of untreated chilli plants when compared to ChiLCV infected chilli plants. Patel and Mondal (2013) reported similar results with low CI value (23.6) for untreated chilli plants during the study for management of chilli leaf curl complex through conventional chemical insecticides such as profenophos and diafenthiuron.

5.4.3.3. Preliminary soil analysis for major and micronutrients

The soil samples collected for analysis from the experimental plot indicated that the soils were high in organic carbon status, slightly acidic pH and normal electrical conductivity. The primary and secondary major nutrient status was found to be medium. The micronutrients status was found to be sufficient except for boron that was in deficient range. These results indicated that the soils need related alteration and amendment in the nutrient status.

5.4.3.4. Plant nutrient analysis of the index leaf stage

The primary major nutrient content indicated a highest value by the treatment with fertilizer and insecticide schedule as per POP which was on par with the treatment POP recommendation supplemented with B (Fig. 11). This might be due to the regular NPK applications that have improved the overall health of plants allowing a better utilization of the nutrients in the leaves. Similarly, Jeyarajan (1965) reported that diseased plant tissues retain more N, P and K content than the healthy ones. The well-established interaction between these nutrients and

micronutrients in soil may prevent the availability and retard uptake of these nutrients leaving low content in tissues. Certain plant viruses like TMV and *Potato virus X* protein contributes to the total protein of the host plant which resulted in increase of total nitrogen due to virus multiplication that allows virus specific protein synthesis to accumulate and ultimately raise the percentage over healthy plants (Hoffland *et al.*, 2000). Huber and Graham (1999) reported that P and K fertilization showed beneficial effects when applied to control seedlings and fungal diseases, soil borne diseases and virus disease for many economic and ornamental crop plants.

Leaf tissue contents of all the secondary major nutrients recorded highest value in POP application supplemented with secondary nutrients (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) and micronutrients ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil (Fig. 12). This may be due to the effect of the availability of Ca from the added sources and variation in capacity of the plants to contain Ca in the infected plant tissues. Similar interactions were also observed by Cakmak (2000) confirming the results. Hemida (2005) reported similar reasons justifying the Mg content in the virus affected plant tissues. Subsequently, it is possible that the virus affected plants could retain only lesser amount of Mg due to the lack of cellular integrity and poor chlorophyll development in tissues. In case of S, the low level in tissues of the control plant may be due to the absence of the S sources.

Micronutrients such as Fe, Mn and B recorded highest value in application of POP recommendation along with B (as borax) (Fig. 13). It may be due to the interaction of added B source (borax) providing higher yield compared to other treatments. The higher level of B plays important roles in maintenance of cell wall rigidity particularly in virus infected plants. In case of virus infected plants, the porous nature of cell wall remains a common feature that permits leakage of cell contents that leads to weakening of plants. Thus, the addition of B (borax) favours the plant health. Miwa and Fujiwara (2010) reported the higher level of boron ensuring plant growth and development.

Foliar Zn content recorded highest value with POP application supplemented with $ZnSO_4$ (Fig. 13). This might be due to the fair availability of Zn^{2+} ion in the plants provided by the added source. Similar reasons were also reported by Tomilson and Hunt (1987) showing the role of Zn in suppression of viral disease in water cress. Das (2000) reported the role of Zn in providing the tolerance level of plants to pest and diseases and better resistance.

Copper (Cu) recorded highest value with POP application supplemented along with secondary nutrients (lime @ 350 kg ha^{-1} + $MgSO_4$ @ 80 kg ha^{-1}) and micronutrient ($ZnSO_4$ @ 20 kg ha^{-1} + $CuSO_4$ @ 2 kg ha^{-1} + B @ 10 kg ha^{-1}) as foliar and soil application (Fig. 13). This might be due to presence of any source carrying Cu^{2+} ion ($CuSO_4$). Chaudhary *et al.* (2002) reported the synergistic interaction of Zn and Cu in plants. The variation in the Cu levels observed in tissues may be the reason for the result obtained. Vijayalakshmi *et al.* (2018) observed higher level of micronutrients i.e., Fe, B, Zn and Mn in healthy untreated and plants sprayed with different biotic defense inducers at 15 and 45 DAS. Whereas, in PRSV infected untreated and infected treated plants, showed reduced micronutrient content.

5.4.3.5. Yield data

The fruit yield of chilli (kg) was supported by application of POP supplemented with B (borax) (Fig. 14). This might be due to the specific role played by B in maintaining the cell wall rigidity which in turn increases the yield characteristics. While assessing the number of fruits per plant (g $plant^{-1}$) from different treatments, the maximum number of fruits were noticed in the plants treated with POP supplemented with B (borax) (Fig. 14). Huber (2012) reported that the application of micronutrients such as boron has an indirect effect in the management of diseases by modifying some specific physiological functions such as photosynthesis, plant nutrition, etc. thus resulting in enhancement of a greater number of fruits in the plants. Samuel *et al.* (2015) reported a high significant increase in both fruit number and yield with combined sprays of soluble B (1 g L^{-1}), $ZnSO_4$ (2 g L^{-1}) and micro nutrient spray (1 ml L^{-1}) were given at fortnightly intervals at 0.5 L per plant which proves the role of B in increasing yield.

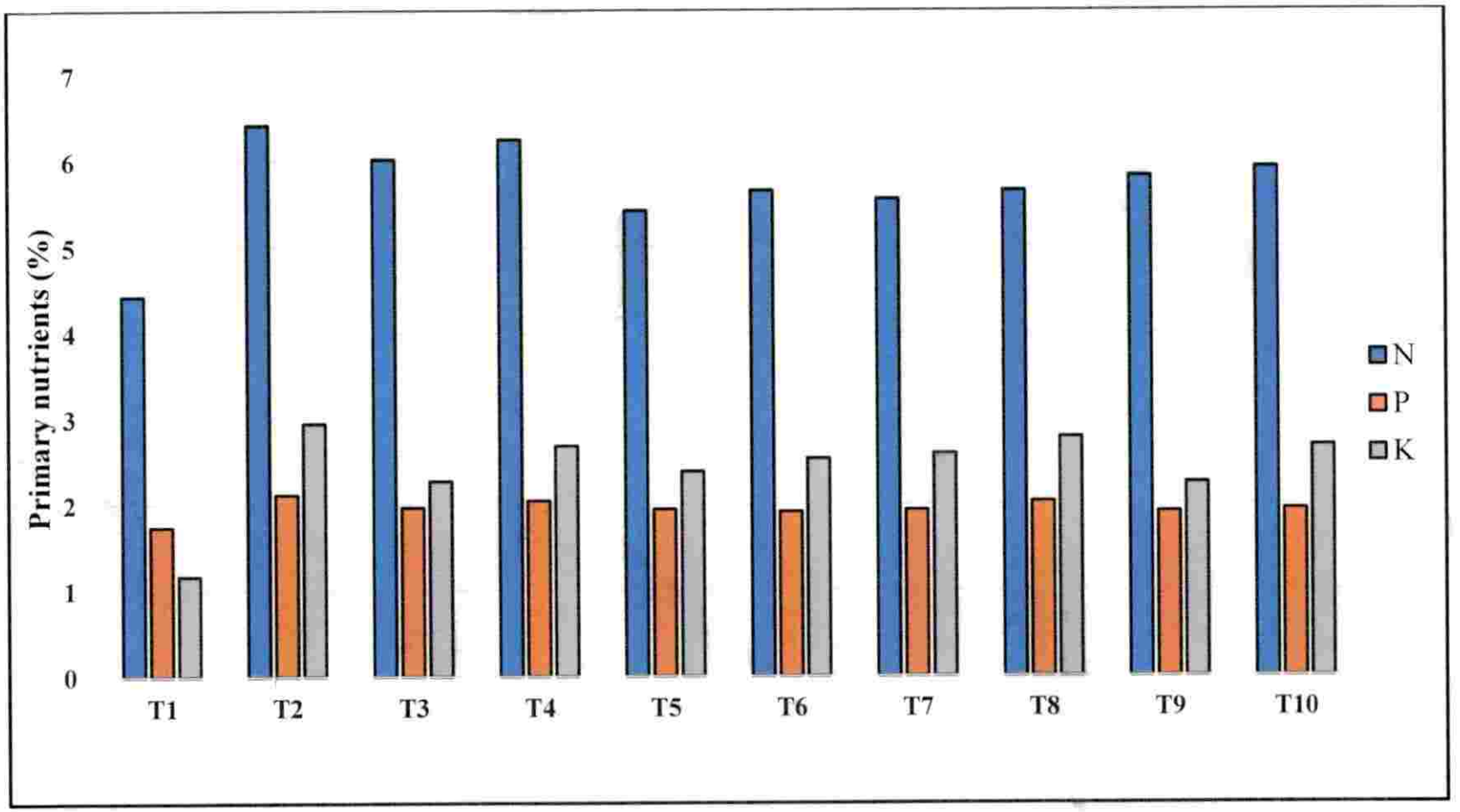

Fig. 11. Effect of different nutrient combinations on primary nutrient status at index leaf stage of chilli due to the infection

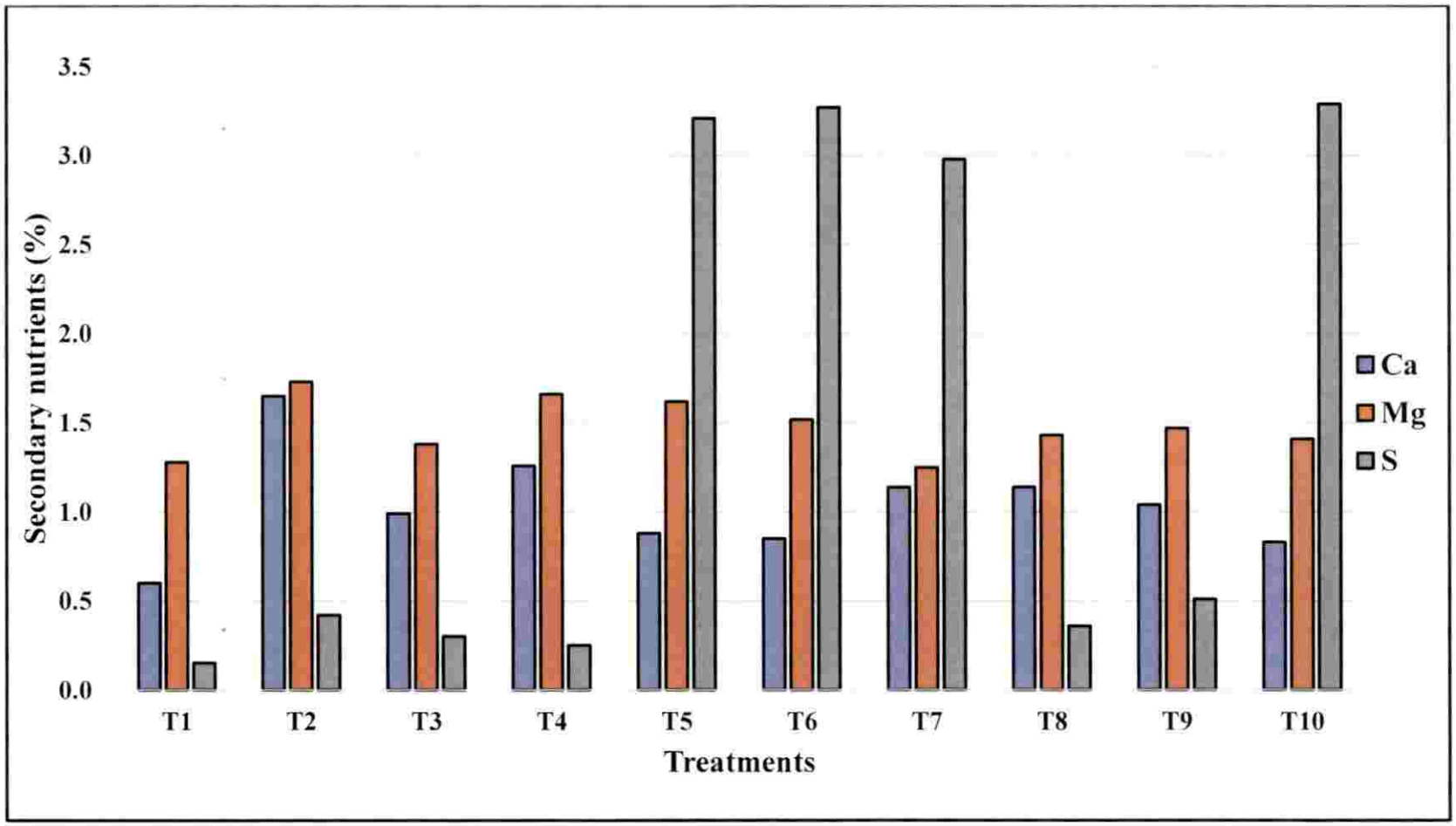

Fig. 12. Effect of different nutrient combinations on secondary nutrient status at index leaf stage of chilli due to the infection

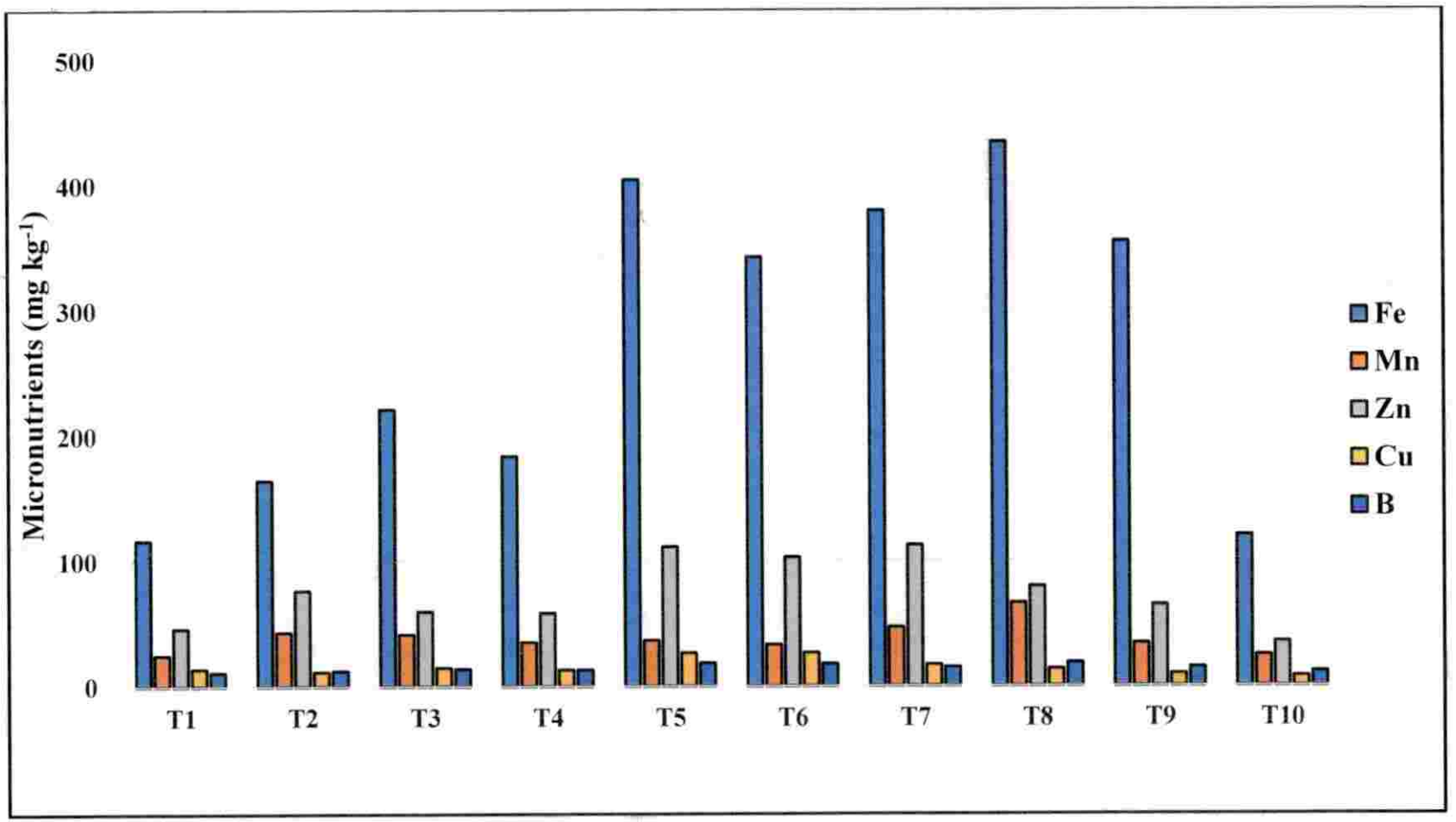

Fig. 13. Effect of different nutrient combinations on micronutrient status at index leaf stage of chilli due to the infection

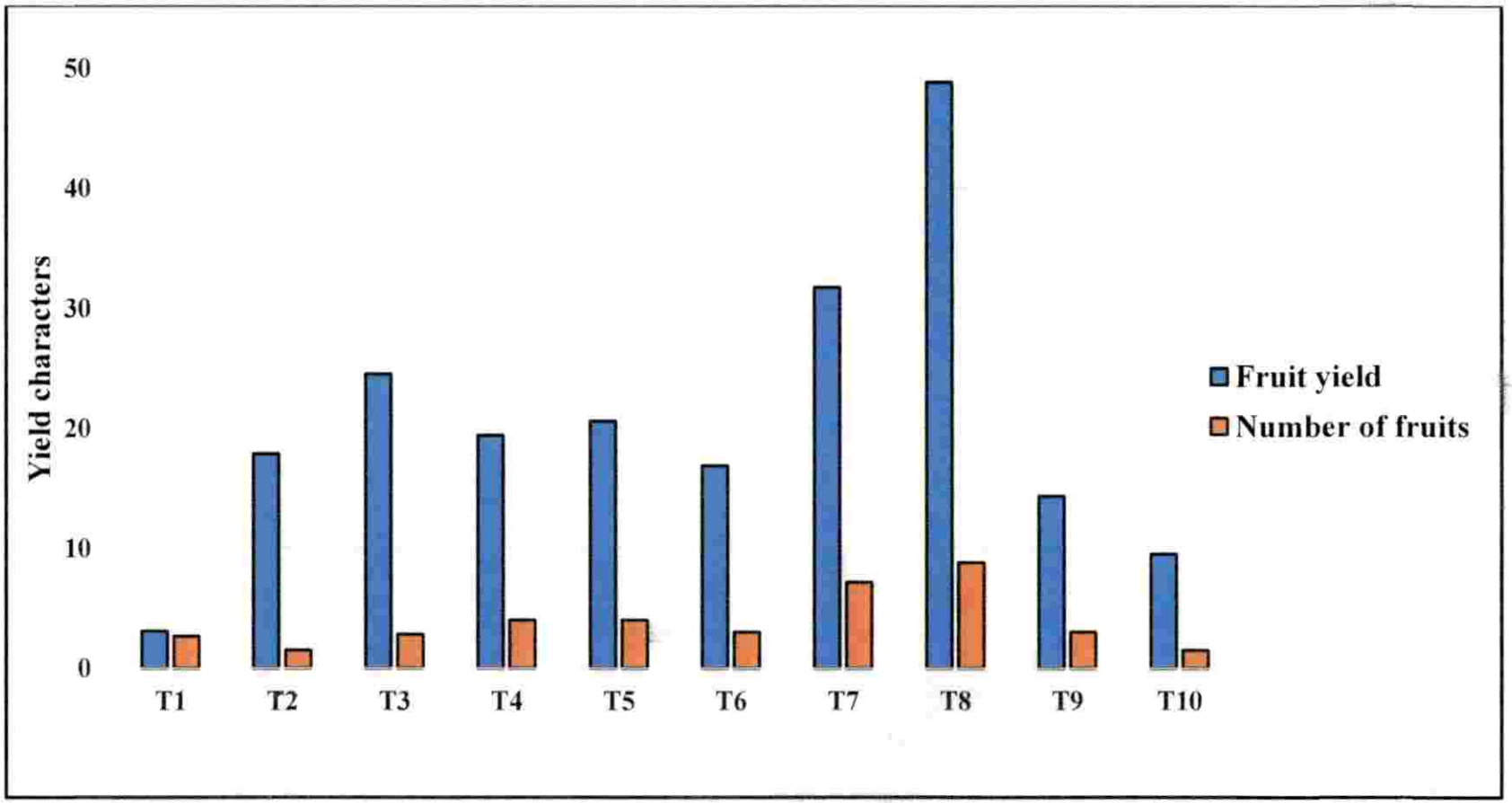

Fig. 14. Effect of different nutrient combinations on number of fruits per plant and fruit yield (g plant^{-1})

5.4.3.6. Enzyme linked immunosorbent assay (ELISA)

TAS-ELISA conducted with all the experimental plants confirmed the presence of virus. The virus titre value from the treated plants exceeded twice the value of healthy plants. Lower OD value (0.212) was observed in the plants supplemented with the nutrients as per POP and B @ 10 kg ha^{-1}. This might be due to the added B source (borax) which favours the plant health by maintaining the cell wall rigidity. The micronutrient application must have provided the plants with varying kinds of resistance at cellular levels in tissues including protection against damages. Cakmak and Marschner (1988) reported similar reasons of protection.

Viruses are obligate in nature. They depend on the host mechanisms for their existence. As soon as the virus enters the host, it unwinds from protein coat and starts replication. During these events, the host nutrition and physiology is also affected. Nutrient based management mainly involves the supplementing of elements that are being used by viruses. This will help to replenish the deficient nutrient and helps the plant to give a better yield. Nutrient management can be integrated with other strategies to evolve eco-friendly and sustainable virus disease management. Hence, as indicated in this study application of B along with other major nutrients could be integrated with other available methods for the management of ChiLCD.

Summary

6. SUMMARY

Chilli (*Capsicum annuum* L.) is the most important and widely grown vegetable cum spice crop in the world belonging to family Solanaceae and originated in Mexico, southern Peru and Bolivia. More than 45 viruses have been reported from chilli which causes substantial losses worldwide every year. Out of these, ChiLCD is the most serious and destructive disease in terms of incidence and yield loss which is caused by geminivirus and transmitted by whitefly (*Bemisia tabaci*). Begomovirus is the largest genus of the family Geminiviridae (ssDNA) that is responsible for significant agro-economic losses. The disease is characterized by upward curling, yellowing, puckering, crinkling of leaves and shortening of internodes with complete sterility. Because of use of same varieties and incidence of different sucking pests such as whitefly, thrips and aphids, the management of the disease has become a major task for both farmers and scientific community.

Keeping this in view, the present investigation was undertaken as four experiments *viz*., collection of Begomovirus infecting chilli from different cultivated areas, symptomatology, serological diagnosis and molecular characterization of the virus causing chilli leaf curl and nutrient based management of chilli leaf curl disease.

The survey for collection of samples were undertaken from December 2018 to March 2019 to investigate the disease incidence in two major chilli growing districts of Kerala i. e., Palakkad and Thiruvananthapuram. In Palakkad, samples were collected from major chilli growing areas *viz*., Vadakarapathy and Kozhinjanpara. In Thiruvananthapuram, samples were collected from College of Agriculture, Vellayani. For the collection of samples, date of observation, location of field, area of field, name of farmer, name of variety grown, season of planting, approximate yield, time of viral disease attack, collateral host and incidence of whitefly like parameters were also recorded. The disease incidence in percentage was recorded at random in different locations in each field of surveyed districts. In Thiruvananthapuram, minimum disease incidence of 69.33 per cent and maximum disease incidence of 80 per cent were recorded. Whereas Palakkad recorded the

maximum incidence of 73.33 per cent in Vadakarapathy village and Kozhinjanpara village recorded the disease incidence of 55.71 to 71.70 per cent. All the chilli varieties grown in different parts of districts surveyed were found susceptible to the disease complex. The results clearly indicated more disease incidence in all the surveyed locations of Thiruvananthapuram district.

With respect to symptomology, majority of the plants showed typical symptoms like severe leaf curling in upward direction with puckering, yellowing of leaves and stunting of whole plants. Reduced leaf size, crinkling and mottling of leaves, petiole elongation, and partial to complete sterility with fewer and smaller fruits were the other characteristic symptoms observed during survey. The prominent symptoms such as upward curling, puckering and yellowing of leaves were observed at early stage of infection. Whereas, petiole elongation, reduced leaf size, stunting of whole plants were noticed at later stage of infection. In all the fields surveyed, association of whitefly was observed.

The chilli samples were serologically diagnosed through TAS-ELISA using polyclonal antisera specific to Begomovirus, SLCMV. Ten symptomatic chilli leaf samples showing leaf curl symptoms were collected and analysed. The highest optical density value (4.2) was recorded in chilli leaf samples collected from College of Agriculture, Vellayani of Thiruvananthapuram district, whereas least optical value (1.0) was recorded in samples collected from Palakkad district. The results proved that all the isolates collected from Thiruvananthapuram district were detected with the virus that is serologically related to SLCMV, whereas none of the samples collected from Palakkad district were positively related to the virus.

Dot immunobinding assay (DIBA) was also conducted for the serological diagnosis of the chilli samples. It was observed that none of the samples collected from both Palakkad and Thiruvananthapuram district showed positive result.

The most sensitive diagnostic tool, polymerase chain reaction (PCR), was performed for molecular characterization of the virus. Universal primers specific to CP of Begomovirus *viz*., AV/AC and DENG were used to amplify the CP gene of ChiLCV in PCR. Ten symptomatic chilli leaf samples showing different leaf curl

symptoms were collected and analysed. It was revealed that the DNA isolated from samples of Vellayani gave an amplicon size of ~550 bp (AV/AC) and ~500 bp (DENG) that confirmed the presence of the virus. Blast analysis with the sequence of CP of Vellayani revealed that the virus showed 96.63 per cent similarity with ChiLCVeV. Phylogenetic analysis revealed that the ChiLCV isolate Thiruvananthapuram and ChiLCVeV were clustered together.

Nutrient status of healthy and diseased leaves for major and micro nutrients was analysed. The results revealed that the content of major nutrients *viz*., N, P, K, Ca, Mg in the infected leaves was low compared to the healthy leaves but in sufficient level. However, the content of S in infected leaves was high and in toxic level. The concentration of micronutrients *viz*., Mn, Zn, Cu and B in the infected leaves was also low compared to the healthy leaves but in sufficient level. Whereas, the per cent of Fe in infected leaves was high and in toxic level. In case of analysis of soil samples, higher levels of both major and micro nutrients were recorded in the soils collected from the infected fields than the soils from the healthy field.

A pot culture study was conducted at Department of Plant Pathology in CRD consisting of ten treatments and three replications with chilli variety Vellayani Athulya from May 2019 to August 2019. Since ChiLCV is whitefly transmitted, the viruliferous whiteflies were released at 20 days after transplanting in all the treatments. Cent per cent disease incidence was recorded in all chilli plants. Soil samples were taken and analysed for major and micro nutrients at pre-treatment stage. Urea, rajphos, murate of potash, lime and magnesium sulphate ($MgSO_4$) were used as the source of major nutrients like N, P, K, Ca and Mg, respectively. Whereas, manganese sulphate ($MnSO_4$), zinc sulphate ($ZnSO_4$), copper sulphate ($CuSO_4$), borax and potassium silicate were used as source for micronutrients such as Mn, Zn, Cu, B and Si, respectively. After imposing the treatments, CI was also calculated. The highest CI (75.0) was recorded in untreated infected plants whereas, the plants supplemented with nutrients as per package of practices (POP) + B @ 10 kg ha^{-1}, POP + ($ZnSO_4$) @ 20 kg ha^{-1} and, basal application of 1/2 N + full P + 1/2

K followed by 0.5 per cent foliar application of NPK 19:19:19 at fortnightly recorded the lowest CI (25.0).

The virus titre values were monitored using TAS-ELISA which confirmed presence of virus in all the treatments. All the experimental plants confirmed the presence of virus. The results showed that lower virus titre was observed in the plants supplemented with nutrients as per package of practices (POP) and recorded maximum level of major nutrients. Plant nutrient analysis was estimated for major and micronutrients at leaf index stage. Yield data were also calculated. Among the treatments, highest fruit yield of 48.85 g $plant^{-1}$ was obtained from plants supplemented with nutrients as per POP + B @ 10 kg ha^{-1} was found to be most effective in reducing the coefficient of infection with better yield.

The present study concluded that the virus causing chilli leaf curl in Thiruvananthapuram district was serologically and molecularly related to *Chilli leaf curl Vellanad virus* (ChiLCVeV) with 96.93 per cent similarity. Nutrient management studies indicate that the disease could be managed by application of nutrients as per package of practices recommendation along with B as borax @ 10 kg ha^{-1}.

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Appendices

APPENDIX-I

BUFFERS FOR TAS-ELISA

1. Coating buffer (pH 9.6)

Sodium carbonate (Na_2CO_3)	- 1.59 g
Sodium bicarbonate ($NaHCO_3$)	- 2.93 g
Sodium azide (NaN_3)	- 0.20 g

Dissolve in 900 ml H_2O, adjust pH to 9.6 with HCl and make up to 1 l.

2. Phosphate buffered saline (PBS (pH 7.4))

Sodium chloride (NaCl)	- 8.0 g
Monobasic potassium phosphate (KH_2PO_4)	- 0.2 g
Dibasic sodium phosphate (Na_2HPO_4)	- 1.15 g
Potassium chloride (KCl)	- 0.2 g
Sodium azide (NaN_3)	- 0.2 g

Dissolve in 900 ml H_2O, adjust pH to 7.4 with NaOH or HCl and make up to 1 l.

3. PBS-Tween (PBST)

PBS + 0.5 ml Tween 20 per litre

4. Sample extraction buffer (pH 7.4)

PBST + 2% PVP (e.g. Serva PVP-15 polyvinyl pyrrolidone)

5. Sample extraction buffer (pH 8.5) for Begomoviruses

0.05 M Tris containing 0.06 M sodium sulphite, pH 8.5

6. Conjugate buffer

PBST + 2 % PVP + 0.2 % egg albumin (e.g. Sigma A-5253)

7. Substrate buffer

Diethanolamine	- 97 ml
Water	- 600 ml
Sodium azide (NaN_3)	- 0.2 g

Adjust to pH 9.8 with HCl and make up to 1 litre with H_2O

Buffers can be stored at 4 ° C for at least 2 months. Warm to room temperature before use.

APPENDIX-II

STOCK SOLUTIONS FOR DIBA

1. Stock solution buffer (Tris-buffer saline, TBS, pH 7.5)

0.02 M Tris	- 4.84 g
0.5 M NaCl	- 58.48 g

Add the pH to 7.5 with 1 N HCl and make up to 2 litres. This is used as used as wash solution.

2. Antigen extraction buffer (TBS - 500 mM DIECA)

Add 11.25 g Diethyl dithiocarbamate (DIECA) to 1 litre TBS.

3. Blocking solution (TBS-SDM)

Add 5.0 g Spray dried milk (SDM) to 100 ml TBS.

4. Antibody and enzyme – conjugate diluent/buffer

Same as TBS-SDM.

5. Substrate buffer (pH 9.5)

0.1 M Tris	- 12.11 g
0.1 M NaCl	- 5.85 g
5 mM $MgCl_2.6H_20$	- 1.01 g

Adjust the pH to 9.5 with 1 N HCl and make up to 1 litre.

6. Substrate solution

Solution A

Nitro blue tetrazolium (NBT)	- 75 mg
Dimethyl formamide (DMFA)	- 1 ml

Solution B

Bromochloroindolyl phosphate (BCIP)	- 50 mg
DMFA	- 1 ml

Store solutions A and B refrigerated in amber coloured bottles. Add 44 µl of NBT and 35 µl of BCIP to 10 ml substrate buffer.

7. Fixing solution (pH 7.5)

10 mM Tris	- 1.21 g
1 mM EDTA	- 0.29 g

Adjust the pH to 7.5 with 1 N HCl and make up to 1 litre. All buffers contain 0.02 % Sodium azide as preservative.

APPENDIX-III

BUFFERS FOR DNA EXTRACTION

1. Extraction buffer (pH 8.0)

2% (W/W) Cetyl Trimethyl Ammonium Bromide (CTAB)

100 mM Tris HCl

1.4 M NaCl

20 mM EDTA

0.2 % (V/V) Mercaptoethanol

2. TE buffer

10 mM Tris HCl

1 mM EDTA (pH 8.0)

APPENDIX-IV

BUFFERS FOR DNA GEL ELECTROPHORESIS

50 X TAE for running the gel

Tris Base	- 242 g
Glacial Acetic Acid	- 57.1 ml
0.5 M EDTA (Ph 8.0)	- 100 ml

Make the volume up to 1 l

0.8% agarose gel was prepared for casting and examination of DNA.

APPENDIX-V

BUFFERS FOR PCR AMPLIFICATION

TBE buffer, pH 8.0 (5 X stock)

0.45 M Tris base	- 54.0 g
0.45 M Boric acid	- 27.5 g
0.5 M EDTA, pH 8.0	- 20 ml
Distilled water	- 500 ml

All the constituents were dissolved and the volume was made up to 500 ml. pH was adjusted to 8.0, sterilized by autoclaving and stored at room temperature.

1.2 % agarose gel was prepared for casting and examination of PCR product.

APPENDIX-VI

SEQUENCES OF VIRUS ISOLATE USED FOR PHYLOGENY STUDIES

>ChiLCV_THIRUVANANTHAPURAM ISOLATE

CCAAATCGTAAGGACGGCGGCAAACATCATGTGTCATTTACAAATAAT
GGTGATATGAAATCATCCAACTGTAAGTTAGTGATTATGGAACAATTC
TACATTGAAAGCGACATGAAATTTACCCAAGAGCTTTCAATTCCTTCC
CCTCTCGTATAGTTTTGATATATTACGACTGCACTCATCCTAACCTTCC
TCTGTTCCCTGTGTATGAATCCTGGACTAAAACTTTTCCTTTATGTGAT
ATGTTTTCAACTATTTTTTAATGTCCGTTACTGAAGGTACTGACTGTGT
ACCGAGCTAGCCTGGAAAAGTCCTTATTAAATACAAATGGGGGTACTT
ATTCATGCTCTCTATCTCTGCCCCTAAAAAACAAATGGCGCCATAGAT
AGGATGACATGAATATTAATTGTCTATGTATACCGAAACGAAACTACC
GATGAAGCAATGTCACAGGAAGTATCCTCGCGCCCTGACACTGAACAA
CGAAACACGCATAAATCATAAATTGGGGAACGAACTGCCAAGACGAT
ATCCCTGAAGAGGGG

Abstract

NUTRIENT BASED MANAGEMENT OF *Chilli leaf curl virus* IN CHILLI (*Capsicum annuum* L.)

by

SHILPA SANKAR
(2017-11-103)

Abstract of the thesis
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Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
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2019

KERALA AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE, VELLAYANI
DEPARTMENT OF PLANT PATHOLOGY

MASTER'S DEFENSE SEMINAR

SHILPA SANKAR
(2017-11-103)

Date: 27-08-2019
Time: 10:00 am

Nutrient based management of *Chilli leaf curl virus* in Chilli (*Capsicum annuum* L.)

ABSTRACT

The study entitled "Nutrient based management of *Chilli leaf curl virus* in Chilli (*Capsicum annuum* L.) was conducted at Department of Plant Pathology, College of Agriculture, Vellayani from 2017 to 2019. The main objectives were to serologically and molecularly characterize the virus causing leaf curl in chilli and to study the role of nutrient application in the management of the disease. The present investigation was carried out in four experiments *viz.*, collection of Begomovirus infecting chilli from different cultivated areas, symptomatology, serological diagnosis and molecular characterization of the virus causing chilli leaf curl and nutrient based management of chilli leaf curl.

The survey was undertaken from December 2018 to March 2019 to investigate the disease incidence in major chilli growing areas of Palakkad (Vadakarapathy and Kozhinjanpara) and Thiruvananthapuram districts (College of Agriculture, Vellayani) of Kerala. In Thiruvananthapuram, the disease incidence ranged from 69.33 to 80 per cent whereas Palakkad recorded maximum incidence of 73.33 per cent in Vadakarapathy village and Kozhinjanpara village recorded the disease incidence of 55.71 to 71.70 per cent.

The common symptoms of the disease were upward curling and puckering of leaves, and stunting of whole plant. Other symptoms *viz.*, reduced leaf size, yellowing, petiole elongation, crinkling and mottling of leaves with few or no fruits were also observed.

Serological diagnosis of the disease was carried out using triple antibody sandwich – enzyme linked immunosorbent assay (TAS-ELISA) using polyclonal antisera specific to Begomovirus, *Sri Lankan cassava mosaic virus* (SLCMV). All the samples collected from College of Agriculture, Vellayani were detected with the virus whereas none of the samples from Palakkad district were positive to the virus.

Molecular characterization of the virus using universal primers specific to coat protein of Begomovirus *viz*., AV-AC and DENG through Polymerase chain reaction (PCR) revealed that the DNA isolated from samples of Vellayani could yield an amplicon size of ~550 bp (AV-AC) and ~500 bp (DENG); thus confirming the presence of the virus. But no PCR amplification was observed in any of the samples from Palakkad district. Blast analysis with the sequence of coat protein of Vellayani revealed that the virus had 96.63 per cent similarity with *Chilli leaf curl Vellanad virus*.

Nutrient status of healthy and diseased leaves revealed that the per cent of major nutrients *viz*., nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg); and micronutrients *viz*., manganese (Mn), zinc (Zn), copper (Cu) and boron (B) in the infected leaves was low compared to the healthy leaves but in sufficient level. Whereas, the per cent of sulphur (S) and iron (Fe) in infected leaves was high and in toxic level. Comparatively higher levels of all the major and micro nutrients were recorded in the soils collected from the infected fields than the soils from the healthy field.

A pot culture study was conducted at Department of Plant Pathology in completely randomized design consisting of ten treatments and three replications with chilli variety Vellayani Athulya from May 2019 to August 2019. Cent per cent disease incidence was recorded in all chilli plants before imposing the treatments. Soil samples were taken and analysed for major and micro nutrients at pre-treatment stage. Urea, rajphos, murate of potash, lime and magnesium sulphate ($MgSO_4$) were used as the source of major nutrients like N, P, K, Ca and Mg, respectively. Whereas, manganese sulphate ($MnSO_4$), zinc sulphate ($ZnSO_4$), copper sulphate ($CuSO_4$), borax and potassium silicate were used as source for micronutrients such as Mn, Zn, Cu, B and Si, respectively. After imposing the treatments, the highest coefficient of infection (75.0) was recorded in untreated infected plants whereas, the plants supplemented with nutrients as per package of practices (POP) + B @ 10 kg ha^{-1}, POP + ($ZnSO_4$) @ 20 kg ha^{-1} and, basal application of 1/2 N + full P + 1/2 K followed by 0.5 per cent foliar application of NPK 19:19:19 at fortnightly recorded the lowest coefficient of infection (25.0). TAS-ELISA conducted with all the experimental plants confirmed the presence of virus. Lower virus titre value was observed in the plants supplemented with nutrients as per POP recorded maximum level of major nutrients. Among the treatments highest fruit yield of 48.85 g $plant^{-1}$ was obtained from plants supplemented with nutrients as per POP + B @ 10 kg ha^{-1} was found to be most effective in reducing the coefficient of infection with better yield.

Thus, the present study revealed that the serological diagnosis of the disease carried out using TAS-ELISA with antisera SLCMV and molecular characterization of the virus using primers AV-AC and DENG through PCR confirmed the presence of virus in Vellayani. The BLAST analysis of *Chilli leaf curl Vellayani* isolate thus showed 96.63 per cent similarity with *Chilli leaf curl Vellanad virus*. It was also indicated that *Chilli leaf curl virus* in chilli could be managed by the application of nutrients as per package of practices recommendation along with boron as borax @ 10 kg ha^{-1} which need to be validated under field conditions.