SPECIES COMPOSITION, ECOLOGY AND MANAGEMENT OF LEAFHOPPERS (CICADELLIDAE: HEMIPTERA) INFESTING POTATO

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CERTIFICATE

This is to certify that the thesis entitled **'SPECIES COMPOSITION, ECOLOGY AND MANAGEMENT OF LEAFHOPPERS (CICADELLIDAE: HEMIPTERA) INFESTING POTATO'** submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in AGRICULTURAL ENTOMOLOGY** to the College of Agriculture, Shivamogga, University of Agricultural and Horticultural Sciences, Shivamogga is a bonafide record of research work carried out by **Miss. MEGHANA, N., ID. No. MA1TAF0149** (meghana.dvg109@gmail.com) during the period of study in this university under my guidance and supervision and no part of this thesis has previously formed the basis for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

Shivamogga
August, 2018

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SPECIES COMPOSITION, ECOLOGY AND MANAGEMENT OF LEAFHOPPERS (CICADELLIDAE: HEMIPTERA) INFESTING POTATO

(MEGHANA, N.)

ABSTRACT

Investigations on **Species composition, ecology and management of leafhoppers (Cicadellidae: Hemiptera) infesting potato** was carried out in major potato growing regions of Karnataka during 2017-18. During the study, nineteen species of leafhoppers were recorded. Molecular technique using 650-base pair region of the mitochondrial cytochrome oxidase I (COI) gene was employed to identify different *Empoasca* spp. A total of nine sequences were submitted to NCBI-Gene Bank and accession numbers were obtained. The population of potato leafhoppers attained peak during harvesting stage in both seasons *i.e., Kharif* and *Rabi* 2017-18. Incidence of potato leafhoppers had significant negative correlation with wind speed, non-significant positive correlation with minimum temperature, relative humidity and sunshine hours. A non-significant negative correlation was observed with maximum temperature and total rainfall during *Kharif* 2017. Leafhoppers incidence in *Rabi* 2017-18, showed a non-significant positive correlation with maximum temperature, minimum temperature, total rainfall and wind speed and non-significant negative correlation with relative humidity and sunshine hours. Highest number of trichomes were found in Kufri Jyothi (218.00) followed by FL-5 (79.00) and lowest in S-6 (39.30) followed by FC-3 (72.50). Lower TSS, higher phenol and higher potassium content was noticed in Kufri Jyothi and FL-5 followed by FC-3 and S-6. Among the selected insecticides Imidacloprid 17.8 SL @ 0.3 ml/lit recorded significantly lower number of leafhoppers. The economic analysis of different insecticides indicated imidacloprid 17.8 SL @ 0.3 ml/lit was most effective by recording highest C:B ratio of (1:3.90).

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ಆಲೂಗಡ್ಡೆ ಬೆಳೆಯಲ್ಲಿನ ಜಿಗಿಹುಳಗಳ ವರ್ಗಗಳು, ಸಂಖ್ಯಾಸ್ಥಿತಿ ಮತ್ತು ನಿರ್ವಹಣೆ

(ಮೇಘನಾ, ಎನ್.)

ಸಾರಾಂಶ

ಆಲೂಗಡ್ಡೆ ಬೆಳೆಯಲ್ಲಿನ ಜಿಗಿಹುಳಗಳ ವರ್ಗಗಳು, ಸಂಖ್ಯಾಸ್ಥಿತಿ ಮತ್ತು ನಿರ್ವಹಣೆಯ ಪರಿಣಾಮಗಳನ್ನು ೨೦೧೭-೨೦೧೮ ರಲ್ಲಿ ಕರ್ನಾಟಕದ ಪ್ರಮುಖ ಆಲೂಗಡ್ಡೆ ಬೆಳೆಯು ಪ್ರದೇಶಗಳಲ್ಲಿ ಕೈಗೊಳ್ಳಲಾಯಿತು. ಒಟ್ಟಾರೆ ಹತ್ತೊಂಬತ್ತು ಆಲೂಗಡ್ಡೆ ಜಿಗಿಹುಳಗಳ ವರ್ಗಗಳನ್ನು ದಾಖಲಿಸಲಾಯಿತು. ಮೈಟೊಕಾಂಡ್ರಿಯದ ಸೈಟೋಕ್ರೋಮ್ ಆಕ್ಸಿಡೇಸ್ ವಂಶವಾಹಿಗಳ ೬೫೦ ಬೇಸ್‌ಜೋಡಿ ಪ್ರದೇಶವನ್ನು ಬಳಸಿಕೊಂಡು ಆಣ್ವಿಕತಂತ್ರವನ್ನು ವಿವಿಧ ಎಂಪೋಸ್ಕಾ ಜಾತಿಗಳನ್ನು ಗುರುತಿಸಲಾಯಿತು. ಎನ್ಸಿಬಿಐ ಜೀನ್ ಬ್ಯಾಂಕ್ ಗೆ ಒಂಬತ್ತು ಸೀಕ್ವೆನ್ಸಗಳನ್ನು ಸಲ್ಲಿಸಲಾಯಿತು ಮತ್ತು ಪ್ರವೇಶ ಸಂಖ್ಯೆಗಳನ್ನು ಪಡೆಯಲಾಯಿತು. ಮುಂಗಾರು ಮತ್ತು ಹಿಂಗಾರು ೨೦೧೭-೧೮ ಋತುವಿನಲ್ಲಿ ಕೊಯ್ಲು ಹಂತದಲ್ಲಿಜಿಗಿಹುಳಗಳ ಸಂಖ್ಯೆ ಅತ್ಯಧಿಕವಾಗಿ ಕಂಡು ಬಂದಿತು. ಜಿಗಿಹುಳಗಳ ಸಂಖ್ಯೆಯು ಗಾಳಿಯ ವೇಗದೊಂದಿಗೆ ಗಮನಾರ್ಹವಾಗಿ ಪರಸ್ಪರ ನಾಕಾರಾತ್ಮಕ ಸಂಬಂಧವನ್ನು ಹೊಂದಿದೆ, ಕನಿಷ್ಠ ತಾಪಾಮಾನ, ಸಾಪೇಕ್ಷೆಆದ್ರತೆ ಮತ್ತು ಸನ್ಶೈನ್ ಸಮಯದೊಂದಿಗೆ ಗಮನಾರ್ಹವಾಗಿ ಪರಸ್ಪರ ನಾಕಾರಾತ್ಮಕ ಸಂಬಂಧವನ್ನು ಹೊಂದಿದೆ. ೨೦೧೭ ರ ಮುಂಗಾರುಋತುವಿನಲ್ಲಿ ಗರಿಷ್ಠ ತಾಪಾಮಾನ ಮತ್ತು ಒಟ್ಟು ಮಳೆಗೆ ಗಮನಾರ್ಹವಾದ ಪರಸ್ಪರ ನಾಕಾರಾತ್ಮಕ ಸಂಬಂಧವನ್ನು ಹೊಂದಿದೆ. ೨೦೧೭ ರ ಹಿಂಗಾರು ಋತುವಿನಲ್ಲಿ ಜಿಗಿಹುಳಗಳ ಗರಿಷ್ಠ ತಾಪಾಮಾನ ಕನಿಷ್ಠ ತಾಪಾಮಾನ, ಒಟ್ಟು ಮಳೆ, ಗಾಳಿಯ ವೇಗ, ಸಾಪೇಕ್ಷೆಆದ್ರತೆ ಮತ್ತು ಸನ್ಶೈನ್ ಸಮಯದೊಂದಿಗೆ ಗಮನಾರ್ಹವಾಗಿ ಪರಸ್ಪರ ನಾಕಾರಾತ್ಮಕ ಸಂಬಂಧವನ್ನು ಹೊಂದಿದೆ. ಅತ್ಯಧಿಕ ಸಂಖ್ಯೆಯಎಲೆಯ ರೋಮಗಳು ಕುಫ್ರಿಜ್ಯೋತಿ ಮತ್ತು ಎಫ್‌ಲ್-೫ ತಳಿಗಳಲ್ಲಿ ಕಂಡುಬಂದವು. ನಂತರದಲ್ಲಿ ಎಫ್‌ಸಿ-೩ ಮತ್ತು ಎಸ್-೬ ತಳಿಗಳಲ್ಲಿ ಕಂಡುಬಂದವು. ಕಡಿಮೆ ಟಿಎಸ್‌ಎಸ್, ಹೆಚ್ಚಿನ ಫಿನಾಲ್ ಮತ್ತು ಹೆಚ್ಚಿನ ಪೊಟ್ಯಾಶಿಯಮ್ ಅಂಶವನ್ನು ಕುಫ್ರಿಜ್ಯೋತಿ ಮತ್ತು ಎಫ್‌ಲ್-೫ ತಳಿಗಳಲ್ಲಿ ಗಮನಿಸಲಾಯಿತು. ನಂತರದಲ್ಲಿ ಎಫ್‌ಸಿ-೩ ಮತ್ತು ಎಸ್-೬ ತಳಿಗಳಲ್ಲಿ ಗಮನಿಸಲಾಯಿತು. ಆಯ್ದ ಕೀಟನಾಶಕಗಳ ಪೈಕಿ ಇಮಿಡಾಕ್ಲೋಪ್ರಿಡ್‌ಗಮನಾರ್ಹವಾಗಿಕಡಿಮೆ ಸಂಖ್ಯೆಯ ಜಿಗಿಹುಳಗಳನ್ನು ದಾಖಾಲಿಸಿದೆ. ವಿಭಿನ್ನ ಕೀಟನಾಶಕಗಳ ಆರ್ಥಿಕ ವಿಶ್ಲೇಷಣೆ ಸೂಚಿಸಿದಂತೆ ಇಮಿಡಾಕ್ಲೋಪ್ರಿಡ್ (೧: ೩.೯೦) ರ ಗರಿಷ್ಠ ವೆಚ್ಚ: ಲಾಭದ ಅನುಪಾತವನ್ನು ದಾಖಾಲಿಸುವ ಮೂಲಕ ಹೆಚ್ಚು ಪರಿಣಾಮಕಾರಿಯಾಗಿದೆ.

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CONTENTS

LIST OF TABLES

LIST OF PLATES

INTRODUCTION

I. INTRODUCTION

Potato (*Solanum tuberosum* L.; Solanaceae), known as "king of vegetables" is one of the most important crops in the world (Rao, 1977). It is world's most important food crop with annual production of 374.4 million tonnes, with an area of 12 million ha and average productivity of 13.3 metric tonnes ha^{-1}. India produced 43.88 Mt from 2.12 million ha with an average productivity of 21.51 tonnes ha^{-1} (FAO, 2016).

In India, potato is cultivated in almost all states under diverse agro-climatic conditions. The states of Uttar Pradesh, West Bengal, Punjab, Bihar and Gujarat accounted for more than 80 per cent share in total production. During the year 2016, Uttar Pradesh was the major Potato producing state with 31.26 per cent of production share, followed by West Bengal, Bihar, Gujarat and Madhya Pradesh with 23.29 per cent, 13.22 per cent, 7.43 per cent and 6.20 per cent share, respectively.

Karnataka is one of the important potato growing states in peninsular India. Karnataka produces 1.3 per cent of total production from 2.41 per cent of area. Karnataka ranks poorly at 10th, 12th, and 22nd position among Indian states in relation to area, production and productivity, respectively. Hassan district stands first in area followed by Belgaum and Kolar districts (FAO, 2016). It is widely cultivated in six districts *viz.*, Chikkaballapur, Belgaum, Chikkamagaluru, Dharwad, Hassan and Kolar. It is mainly grown as *Kharif* crop in Belgaum, Chikkamagaluru, Dharwad and Hassan districts, whereas, it is grown as a *Rabi* crop in Kolar and Bangalore districts. Potatoes produced in *Kharif* account for the major proportion of 70 per cent (FAO, 2016).

Potato production is seriously affected by the pests and diseases. The major pests of potato are leafhoppers, aphids, white flies, potato tuber moth, white grubs and thrips. Among these, leafhoppers serve as an economically important pests both by direct and indirect damage (Demirel and Yildirim, 2008).

Leafhoppers belong to the family cicadellidae of the superfamily membracoidea. There are more than 22600 species of leafhoppers known from the world and around 1500 species are known from the Indian subcontinent. They are serious pests of various crops including potatoes. They suck the sap from leaves leading to curling and yellowing of leaves (Byrne and Draeger, 1995) besides transmitting phytoplasma diseases in various crops.

The potato leafhoppers have diverse host range of over 200 plant species. Species diversity and host specificity of leafhoppers infesting potato have been underestimated. Considering the pest potential and vectoring ability, it is necessary to identify them rapidly and accurately. Documentation of species diversity of leafhoppers in potato ecosystem play a major role in understanding their damage potential, vectoring ability and designing management strategies.

Among the leafhoppers reported from many countries, *Empoasca* spp. are dominant (Delong, 1931). Their body size and morphological similarity hinders the correct identification to species level (Ross and Moore, 1957). They are by far the most complex and widespread species attacking wide range of hosts. Leafhopper transmitted phytoplasma diseases are an emerging problem for potato and vegetable growers. Potato purple top disease is vectored by leafhoppers *Orosius albicinctus*, *Alebroides dravidanus* and *Seriana equata* and also associated in the genus *Macrosteles* (Munyaneza *et al*., 2007). They are also attributed to transmit other phytoplasma diseases like marginal flavescence (MF) and witch's broom (WB) (Nagaich, 1978).

There is no comprehensive data on the species composition of potato leafhoppers from India in general and Karnataka in particular where potato is an important crop. Among the recommended varieties for Karnataka, Kufri Jyothi accounts for maximum area (Rana *et al*., 2014). However, its succeptibility or resistance to leafhopper is not been evaluated. Efficient identification of these leafhoppers acts as a base for transmission, resistance evaluation and designing management strategies. Considering all these aspects, the present study has been taken up with the following objectives.

1. Species composition of leafhoppers infesting potato in major potato growing regions of Karnataka
2. Population dynamics of leafhoppers infesting potato in Southern Transitional Zone of Karnataka
3. Varietal preference of potato leafhoppers
4. Evaluation of selected insecticides against potato leafhoppers

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

The literature pertaining to "Species composition, ecology and management of leafhoppers (Cicadellidae: Hemiptera) infesting potato" are presented under this chapter.

2.1 Species composition of leafhoppers infesting potato

Literature survey indicated that no studies have been conducted in Karnataka to document species diversity of leafhoppers infesting potato. Hence, efforts were made to collect literature from all over the world and on other crops which could be relevant to present studies.

Empoasca is a large and complex genus of leafhoppers with hundreds of described species, many of which are nearly identical in external appearance; other species of *Empoasca* have often been misidentified as *E. fabae* (Delong, 1931a).

DeLong's (1931b) initial studies of the male genitalia of North American *Empoasca* revealed features that distinguish *E. fabae* from other common *Empoasca* species. However, later studies (Ross and Moore 1957; Ross 1959) revealed that "*E. fabae*" of various authors is a complex of at least 27 closely related species. Thus, positive identification of species belonging to this complex requires examination not only of the male terminalia, but also the internal apodemes of the first two male abdominal segments.

Potato leafhoppers inspite of causing damage, they are also attributed to transmit various phytoplasma diseases, which include purple top roll (PTR), marginal flavescence (MF) and witch's broom (WB) (Nagaich, 1978).

Singh *et al.* (1983) repoted about potato purple top roll disease transmitted by leafhoppers, *Orosius albicinctus* Distant which results in 40-70 per cent yield losses and plants show rolling of basal parts of younger leaves accompanied by pink or purple pigmentation.

Walgenbach *et al.* (1985) sampled leafhopper to develop and evaluate the precision of potato leafhopper (PLH), *Empoasca fabae* (Harris). Sampling programs concluded that midplant leaf samples provided a more reliable estimate of nymphal density than did top or bottom leaf samples.

Similarly, Harper *et al*. (1993) conducted studies to determine different sampling methods (sweepnet sampling and suction sampling) to manage leafhopper. They suggested leafhopper sampling can be adopted for monitoring.

Pantoja *et al*. (2006) conducted insect surveys in commercial and experimental potato fields in Palmer, Delta Junction, Fairbanks and Nenana, Alaska. Tile trap pans and yellow sticky traps were used to collect aphids and leafhoppers, respectively, from April to October, 2003-2005. Insects collected were identified to genera and species. Ten aphid species were detected; *Rhopalosiphum nigrum* represented 86 per cent of the aphids collected. The green peach aphid, which is considered to be the most effective vector of potato viruses was collected in pan traps and found colonizing potatoes. Yellow sticky traps captured six genera of leafhoppers including important vectors of phytoplasmas to potatoes. *Macrosteles* spp. was the most abundant leafhopper collected.

Munyaneza *et al*. (2007) reported the outbreak of potato purple top disease in Columbia Basin of Washington and Oregon. They also reported that this outbreak caused significant yield losses and reduced tuber quality.

Yellow sticky traps are widely used for monitoring leafhoppers. Demirel and Yildirim (2008) conducted study to evaluate the relative attraction of thrips and leafhopper species to various colour traps in cotton growing areas. They concluded that yellow sticky traps were significantly attractive for leafhopper species and orange sticky traps were second attractive.

Zohdi *et al.* (2010) reported 11 species of potato leafhoppers from three potato growing areas (Lalehzar, Bardsir and Mahan) of Iran during 2005–2007. They are: *Empoasca decipiens, Psammotettix striatus, Unkanodes tanasijevici, Sogatella vibix, Exitianus fasciolatus, Euscelis alsius, Reptalus lindbergi, Pseudophlepsius binotatus, Circulifer* sp., *Macrosteles* sp., *Idiocerus* sp.

Pantoja *et al*. (2009) identified 41 leafhopper species associated with potato in Alaska region of United States. Twenty species were identified from sweep net samples and adhesive cards. Two species, *Davisonia snowi* and *Macrosteles fascifrons*, made up approximately 60% of total number of individuals collected, representing 34 & 26 % respectively.

Cullen *et al*. (2012) reported pest management strategy in alfalfa for the potato leafhopper is to monitor the pest throughout the season with a sweep net and treat with foliar insecticide when economic threshold populations are reached.

Henne and Munyaneza (2012) reported that leafhoppers and psyllids are important pests of the potato worldwide. These insects cause damage by direct feeding or by acting as vectors of potato pathogens. Economically important leafhoppers that attack potatoes include *Empoasca fabae, Macrosteles fascifrons, Circulifer tenellus* and *E. fabae* is considered one of the most destructive potato pests in North America and feeds on phloem or mesophyll, resulting in a damaging condition referred to as "hopperburn". *Macrosteles* spp. and *C. tenellus* are known to transmit phytoplasmas that cause potato purple top disease, with serious epidemics having occurred in the Americas, Europe, and Asia.

Casey *et al*. (2014) used sweep net to collect leafhoppers populations in grain cover and vegetable crops. They found that *Empoasca* spp. was more common in potato.

2.2 Population dynamics of leafhoppers infesting potato

Saugstad *et al*. (1967) revealed that sweep net sampling is simple and quick method that can be done frequently to allow timely management decisions and obtained a high positive correlation between leafhopper counts from sweep net sampling and the height of the alfalfa.

Cherry *et al.* (1977) found that wind and temperature were the two most important factors in sweep net estimates of adult leafhopper populations in alfalfa.

The important abiotic factor influencing population dynamics of leafhoppers is temperature. Potato leafhopper development stops when temperatures drop below a lower developmental threshold of 45°F (7.6°C) and the rate begins to decline when temperatures consistently exceed an upper developmental threshold of 86°F (30°C) (Hogg, 1985).

Flinn *et al.* (1986) studied population dynamics for potato leafhopper, *Empoasca fabae* (Harris). Highest population of leafhoppers were present at harvest. The increase of population is due to increases in initial immigrant density.

On moisture-stressed alfalfa, development time of potato leafhopper eggs, nymphs and adults slows, mortality increases and fecundity decreases; hopperburn seems to appear more frequently during summer droughts (Hoffman *et al.,* 1990).

Byrne and Draeger (1995) reported the seasonal abundance of potato leafhoppers. Yellow sticky traps and D-Vac vacuum sampler was used. During both years of sampling peak populations occured near mid-April. This was correlated with a drop in relative humidity and a rise in ambient air temperature.

Degooyer (1997) reported effect on weather, natural enemies, host plants and crop harvesting on the population dynamics of potato leafhopper.

Further, Degooyer *et al.* (1999) similarly observed significantly fewer leafhoppers in alfalfa stands intercropped with smooth bromegrass or orchardgrass compared to alfalfa monocultures. These patterns may be due to higher leafhopper emigration out of plots containing grass.

Raupach *et al.* (2002) reported that temperature had a significant effect on the larval development time of *Empoasca decipiens* Paoli, with a three-fold longer development time at 15°C compared to temperatures >28°C. Shortest larval development time was recorded at 30°C. The host plant species had a significant effect on the larval development time.

Emmen *et al.* (2004) described the dynamics of potato leafhopper, *Empoasca fabae* (Harris). They reported higher leafhopper populations during harvesting time due to immigrants and adults generated from in-field reproduction.

Naseri *et al.* (2009) reported population density and spatial distribution pattern of *Empoasca decipiens* Paoli in Tehran area, Iran, during 2004-2005 on four species of common bean *Phaseolus vulgaris* (L.) var. Talash, lima bean *P. lunatus* (L.) Savi ex Hassk. var. Sadaf, rice bean *P. calcaratus* Roxb. var. Goli and cowpea *Vigna sinensis* (L.) var. Parastoo. The higher and lower mean population densities of *E. decipiens* per leaf were observed on Parastoo cowpea (18.85 in 2004 and 29.94 in 2005) and Talash common bean (1.08 in 2004 and 0.37 in 2005), respectively. It is concluded that bean species influence the population density and spatial distribution pattern of *E. decipiens.*

2.3 Varietal preference of potato leafhoppers

It has been reported that both morphological and biochemical factors of host plant influence the insect defensive responses.

Trichomes play a role in plant defense, especially with regard to phytophagous insects. In numerous species there is a negative correlation between trichome density and insect feeding and oviposition responses and the nutrition of larvae. Specialized hooked trichomes may impale adults or larvae as well. Trichome may also complement the chemical defense of a plant by possessing glands which exude terpenes, phenolics, alkaloids or other substances which are olfactory or gustatory repellents. In essence, glandular trichomes afford an outer line of chemical defense by advertising the presence of "noxious" compounds (Levin, 1973).

Bhat *et al*. (1981) inferred that peroxidase activity or cumulative index of peroxidase activity could be taken as an index to test genotypes for resistance to jassids.

Riaz *et al.* (1987) studied the physico-chemical aspects of resistance like phenol and other aspects in several cotton cultivars to insect pest complex like both sucking pests and boll worms.

Singh and Agarwal (1988) studied the ovipositional preference of cotton leafhopper, *Amrasca biguttula biguttula* (Ishida) on resistant and susceptible genotypes of cotton and okra and found it to be under the influence of chemical components. The reducing sugars, tannins, silica and free gossypol in the leaves of cotton genotypes showed significant and negative correlation with the number of eggs and also reported that highly susceptible genotype of Cotton Acala-4-42 had a higher amount of reducing sugars as compared to the highly resistant, BJR-741.

Singh and Agarwal (1988) discussed physical and biochemical factors including leaf thickness and hairyness to resistance against insect pests. According to them, hairyness is the characteristic of resistance against jassids. Similarly, Ambekar and Kalbhor (1981) also found that length of hair and hair density on midrib and leaf lamina of cotton were found to contribute towards resistance against jassids.

Rana and Manzoor (1990) investigated sixteen varieties of cotton for their relative resistance against insect pests complex of cotton with reference to physico-chemical factors, *viz.*, number of hairs on leaf vein and leaf lamina, length of hair, number of gossypol glands on various plant parts, total minerals, Ca, Fe, K, Mg, protein, fats and reducing sugars in the leaf tissues and their correlation to pest complexes of cotton.

Sivasubramanian *et al*. (1991) screened a total of 24 entries against leafhoppers and concluded that hairiness as indicated by the length and density of trichomes on leaf lamina as well as on veins was considered responsible for conferring resistance. The trichome density on the ventral surface of the leaves, hair length and hair density of mid vein on ventral surface of the leaves exhibited significant negative association with leafhopper damage and oviposition.

Fourteen plant lectins from a total of 13 species of plants were studied in artificial diet for effect on survival time of adult female Empoasca fabae (Harris). Plant lectins significantly reduced leafhopper survival (Habibi *et al*., 1993).

Ahmad *et al.* (1999) found that hairiness on the leaf veins was a reliable character for resistance against cotton jassid. They opined that incorporating hairiness in the commercial varieties will help in decreasing at least one spray from the spraying schedule.

Bollworm tolerant genotypes *viz.*, SCHH-2, Sahana and CPD-428 possessed higher tannin, phenols and orthodihydroxy pheonls. An increase in gossypol and phenol contents was evident with increase in gland density in leaves (r = 0.57) with a negative correlation (r =-0.18) to square damage. The tolerant lines were characterized with higher levels of condensed tannin and gossypol, total and orthodihydroxy phenolics in squares. Tolerant lines had higher constitutive tannin and less sugar content than susceptible checks (Vamadevaiah, 1999).

Plant use many biochemical components to defend against hundreds of pests in their environment. Some characters physically retard penetration of the plant or passively limit growth and development of the pest (Bell and Stipanovic *et al.*, 2000). Host plant resistance is the result of interactions between two biological entities, the plant and the insect under the influence of various environmental factors (Dhaliwal and Singh, 2004).

Mansoor and Waqas (2000) studied five cotton cultivars, and tested their relative resistance to jassids. Low levels of nitrogen, potassium, zinc and copper were found to contribute susceptibility to jassids. Chu *et al.* (2003) analysed the cotton leaf characteristics for both physiological and biochemical components on jassids in Arizona and California.

Ranger and Hower (2001) conducted experiment to elucidate resistance of glandular-haired alfalfa, *Medicago sativa* L., to the potato leafhopper, *E. fabae* (Harris) and observed nymphal potato leafhoppers showed higher levels of mortality associated with the glandular-haired clone FGplh13 and no nymphs survived after 48 h. Mortality of nymphs and adults decreased after removal of the glandular trichomes from FGplh13. Shahida *et al.* (2001), observed a direct correlation between insect attack and phenol production, says that resistant cotton variety Ravi showed 1.00 to 1.20 mg more phenolics than susceptible varieties at all growth stages.

Chakrabarty *et al.* (2002) opined that rapid accumulation of phenolic compounds occur in incompatible (resistant) host pest interaction than the compatible (susceptible).

Laboratory studies have shown greater potato leafhopper mortality and reduced reproduction on glandular-haired *Medicago* spp. as well as leafhopper preference for smooth stem alfalfa varieties in choice tests; both physical and chemical traits associated with the glandular hairs have been reported as resistance mechanisms (Ranger and Hower, 2002).

Eight proprietary genotypes of glandular-haired alfalfa were compared for the degree and types of resistance to the potato leafhopper, *E. fabae*. A tube cage no-choice bioassay was developed to test leafhopper mortality, feeding, settling preferences, severity of hopperburn symptoms and trichome density and type on feeding sites. Leafhopper mortality was both strongly and significantly associated with feeding and leaf trichome density (Shockley and Backus, 2002).

Mansoor *et al.* (2003) studied the five Cotton cultivars and were tested for their relative resistance against whitefly (*Bemisia tabaci*), jassid (Cicadellidae), aphid and thrips. Whitefly population was found to be positively correlated with nitrogen, potassium, zinc and copper content of the plants. Low levels of nitrogen, potassium, zinc and copper were found to contribute susceptibility against jassid.

Sajjad *et al.* (2004) evaluated eighteen cotton cultivars against *A. devastans*. The cultivars, CRIS-82 and MNH-536 were highly infested by *A. devastans*. The lowest seasonal infestation was recorded for CRIS-467 and CRIS-134. The cultivars with the greatest hair density were resistant to jassid, whereas the cultivars with the lowest hair density were susceptible to jassid. Hair length had no significant effect on the population of jassid.

Gulzar *et al*. (2005) selected ten genotypes of cotton, catalogued in relation to resistance against jassid, *A. devastans* (Dist.). All the tested genotypes of cotton significantly differed in relation to all morphological plant traits. The length of hair on midrib, vein of middle leaves, gossypol glands on lamina of middle leaf resulted in significant and negative correlation with jassid nymph population while all the other morphological traits showed non-significant correlation to jassid population.

Dellinger *et al*. (2006) repoted on potato leafhopper (PLH) resistance the five commercial alfalfa varieties. These varieties display, in addition to simple pubescence, erect glandular hairs which PLH resistance has been associated with. The actual mechanism of resistance was not known, but may involve toxic or repellent compounds released from the glandular hairs. These compounds either kill the leafhoppers or cause them to avoid glandular-haired alfalfa.

The influence of foliar glandular trichomes on developmental biology of the potato leafhopper, *E. fabae* (Harris) (Homoptera: Cicadellidae), was examined by selective removal of trichomes on resistant and susceptible potato germplasm comprising two glandular trichomebearing genotypes (PI 473331 and PI 473334) of wild potato, Solanum berthaultii (Hawkes), two interspecific hybrids of *S. berthaultii* with *S. tuberosum* L. (Q174-2 and NY123), and two commercial *S. tuberosum* cultivars (Elba and Allegany). Adult mortality was affected, whereas nymphal emergence was unaffected by removal of glandular trichome exudates. No nymphs were observed emerging from leaves of PI 473331 or PI 473334 and only a small percentage of nymphs survived to adulthood on NY123 and PI 473334 (Medeiros and Tingey, 2006).

Nam *et al.* (2006) reported the variable effects of K on pest and disease incidence could be affected by the amount and source of K, in strawberry plant. Adequate K increases phenol concentrations, which play a critical role in plant resistance.

Vanitha *et al.* (2007) studied biophysical parameters like trichome density and gossypol glands. Five tetraploid and two triploid cotton species were tested for their influence on the growth and development of leaf hoppers.

Vanitha *et al.* (2007) tested leaf hopper resistant cotton genotypes which includes five tetraploid and two triploid cotton species for their influence on the growth and development of leaf hoppers. Biochemical aspects of resistance were studied among genotypes and they concluded that there was no significant influence of phenols and tannins on the growth and development of leafhoppers.

Ali *et al.* (2009) investigated the biological and chemical factors affecting the resistance in cotton against jassid, *Amrasca devastans* (Dist.). Hair density on plant and biochemical components imparting resistance were determined in different cotton genotypes.

Fathi (2009) reported that Diamant and Cosmos were resistant and Omidbakhsh was tolerant to *E. decipiens* damage. The results of this study also confirmed that the density of glandular trichomes may have more effect on life cycle of *E. decipiens* than the density of simple trichomes by restricting larvae and adult feeding.

Irfan *et al.* (2010) studied four genotypes of Bt cotton viz, BT-121, BT-456, FH-160, FH-216 to assess the effect of leaf pubescence on the incidence of sucking insect pests. Jassid adult had negative correlation, with number of hair on midrib (r=-0.277), vein (r=-0.051) and lamina (r=-0.207) and length of hair (r=-0.023). Jassid nymph was negatively correlated with number of hairs on midrib (r=-0.114) and vein (r=-0.160) and length of hair (r=-0.202) while it had positive correlation with number of hairs on lamina (0.082). This indicates that incorporation of hairiness in Bt cotton genotypes will greatly reduce the sucking pest damage due to jassids and thereby helps to reduce the number of sprays in Bt cotton.

Murugesan and Kavitha (2010) screened twenty six cotton accessions against the leafhopper, *A. devastans* (Distant) by following ICCC grades.The susceptiblity of cotton entries varied significantly. Based on the resistance index the entries were grouped under five categories as highly resistant, resistant, intermediate, susceptible and highly susceptible. Chlorophyll content, moisture content and protein content were estimated and correlated with leafhopper ovipostion and damage. Protein and moisture were positively correlated and chlorophyll negatively associated with jassids oviposition and damage.

Study was conducted on nine selected genotypes of okra to determine the role of chemical components of plant on population of jassid. Crude protein, nitrogen, lignin, reducing sugar, phosphorus and copper showed positive correlation whereas neutral detergent fiber, acid detergent fiber, cellulose, silica, total ether, non-reducing sugars, total sugars, calcium and magnesium had negative correlation with the population of jassid on okra. Crude protein showed the positive and significant impact (69 per cent) on the jassid population fluctuation on okra which was followed by neutral detergent fibre with 21 per cent contribution. When computed together, all the chemical components showed 99.7 per cent role on jassid population fluctuation (Iqbal, 2011)

Kaplan *et al.* (2009) investigated the role of trichomes as a resistance mechanism against a sap-feeding insect (the leafhopper, *Empoasca fabae*) on potato. Natural variation in trichome density among 17 potato cultivars was used to test for the role of trichomes as a putative defense against leafhoppers. Although leafhopper abundance did not decrease as trichome density increased, leafhopper injury to potato plants (foliar necrosis) was negatively correlated with trichome density.

According to Sarwar (2012) less rate of rice borer infestation in higher K plants can be attributed to a lack of pest preference under sufficient nutrient concentrations, as well as the synthesis of defensive compounds leading to higher pest mortality, but infestation decreased rapidly as the K concentration increased.

2.4 Evaluation of insecticides against potato leafhoppers

Insecticides are by far widely used for management of sucking pests of potato, which includes aphids and leafhoppers.

Fenton and Hartzel (1923) reported on the management of potato leafhopper *Empoasca mali* by using 7-8 per cent solution of kerosene emulsion and by use of nicotine sulphate diluted with soapy water or bordeaux mixture, reduced the population of leafhopper.

Imidacloprid is a systemic and contact insecticide exhibiting low mammalian toxicity, with primary activity on sucking insects such as aphids, leafhoppers and planthoppers, thrips and whiteflies. Imidacloprid has a novel mode of action and no cross resistance from any resistant species has been detected (Mullins, 1993).

Bullas *et al*. (2003) reported that economic injury levels for the potato leafhopper vary with susceptibility of infested crop. Resistant cultivars, trap crops and seed treatments were discussed as viable alternatives to conventional insecticide applications for potato leafhopper control.

Thiamethoxam- and imidacloprid-treated seed were evaluated for controlling infestations of potato leafhopper, *E. fabae* (Harris), in snap bean. Efficacy of these seed treatments was assessed by rating leafhopper damage to foliage or by monitoring densities of nymphs through the season in small field plots. Thiamethoxam provided longer and more consistent protection of the crop from leafhoppers than imidacloprid (Nault *et al*., 2004).

Neonicotinoid insecticides comprise seven commercially marketed active ingredients: imidacloprid, acetamiprid, nitenpyram, thiamethoxam, thiacloprid, clothianidin and dinotefuran. The technical profiles and main differences between neonicotinoid insecticides, including their spectrum of efficacy, are described: use for vector control, systemic properties and versatile application forms, especially seed treatment (Elbert *et al.*, 2008).

Neonicotinoid insecticides have been the fastest growing class of insecticides in modern crop protection, with widespread use against a broad spectrum of sucking and certain chewing pests. As potent agonists, they act selectively on insect nicotinic acetylcholine receptors (nAChRs), their molecular target site. Because of the relatively low risk for nontarget organisms and high target specificity this important class has to be maintained globally for integrated pest management strategies and insect resistance management programs (Jeschke *et al.*, 2011).

Foliar insecticides registered for potato leafhopper control on alfalfa are effective against nymphs and adults. There are a limited number of insecticide active ingredients in the organophospate chemical class registered for potato leafhopper control in alfalfa. In addition, insecticide premix products are registered that combine two insecticide classes (e.g., organophosphate + pyrethroid; chlorantraniliprole + pyrethroid; neonicotinoid + pyrethroid). Because potato leafhopper populations vary from year to year, and field to field, populations within a given year cannot be predicted and fields must be monitored weekly to accurately determine damage potential before insecticides are applied (Cullen *et al.*, 2012).

Chasen *et al*. (2014) reported about management options in alfalfa against potato leafhopper, *E. fabae* (Harris) and also the host plant most frequently incurring economically damaging populations of potato leafhopper. Alfalfa scouting and economic thresholds were discussed along with cultural controls and host plant resistance.

MATERIAL AND METHODS

III MATERIAL AND METHODS

The materials used and methodologies adopted during course of studies on species composition, population dynamics, varietal preference and the effect of different insecticides against potato leafhoppers as envisaged in plan of work has been described in this chapter.

3.1 Species composition of leafhoppers infesting potato in major potato growing regions of Karnataka

3.1.1 Collection of leafhoppers

The present investigation was carried out during *Kharif* 2017 and *Rabi* 2017-18 to study the species composition of potato leafhoppers in major potato growing regions of Karnataka which includes Chikkamagaluru, Hassan, Dharwad, Belgaum, Bengaluru and Chikkaballapur. Leafhoppers were collected using sweep net and aspirator (Plate 1) irrespective of potato varieties. The leafhoppers collected in each representative area were preserved separately to know the percentage composition of different leafhoppers from each locality. Further, the collected leafhoppers in both seasons were identified to species level based on morphological characteristics and documented. Further an illustrated identification keys was developed for all the collected leafhoppers.

3.1.2 Processing of leafhopper specimens for preservation

The collected leafhoppers were mounted singly on a triangular thick paper point mounted on an insect pin. For mounting, an adhesive (quickfix diluted with ethyl acetate) was used. The tip of the paper point was bent at an angle, so that when the insect was in upright position, the bent tip of the point conformed to the angle of the thoracic pleuron. The bent point with minimum adhesive was attached to the right side of the thoracic plueron of the insect and then it was manipulated so that it was exactly horizontal to the paper point. The specimen was then labelled. The sex of the leafhopper was indicated on right hand side of the point by code colouring it with green for male and red for female.

3.1.2.1 Male genitilia dissection and preparation for study

Male genitilia preparations were made following the method of Oman (1949). The abdomen was separated from the rest of the body with a bent minute pin mounted on a thin plastic handle. The detached abdomen was placed in 10 per cent KOH solution in a test tube and heated slowly to a temperature just short of boiling till all the internal soft tissue was digested.

Then the abdomen was taken out from the KOH and placed in water in cavity block and the dissolved internal contents was removed by pressing the abdomen with the help of a pair of bent minute pins. After washing in water, the abdomen was transferred to glycerine in a cavity block and the water content in the abdomen was removed by pressing it gently with the pins. Dissection of genitilia was made in glycerine. To accomplish this, a bent minute pins was introduced into the abdomen was gently held in position, while the other bent pin was used to sever the connection between the male plates and connective and the dorsal apodeme and the tenth segment. The whole complex *i.e.*, aedeagus, connective and style was drawn out of the genital capsule. Head, thorax and genitalia diagrams were prepared by using camera lucida attached to stereoscopic microscope. Photographs were made using Leica M205C stereo microscope. After studying and making illustrations, the aedeagus and its accompanying structure were placed in the abdomen which served as a storage organ and the abdomen was stored in a drop of glycerine in a genitalia vial with a plastic stopper. The microvial, containing the prepared genitalia was attached to the pin carrying the specimen from which the abdomen was removed.

For making illustrations, the genital parts were kept in place by placing a very small quantity of beewax at the center of the bottom of a cavity slide before pouring glycerine on it. The genital structures were gently pressed after arranging them in desired orientation.

For studying wing venation, dry wing mounts were used. For this purpose, wings from one side (left) were carefully removed from the specimen and placed on a clean microscope glass slide, the hind wing was then spread and a cover slip was placed carefully over it after applying a minute quantity of adhesive on the margin. Care was taken not to use too much adhesive so that it will not come in contact with the wings.

Leafhoppers collected in major potato growing areas were identified to species level, but there was problem in identification of *Empoasca* spp. The morphological similarity of *Empoasca* spp. posed a great challenge for identification. Body size (<4mm) and morphological similarity hampered species identification of *Empoasca* spp. Hence molecular technique was employed to identify different *Empoasca* spp.

3.1.3 Molecular identification of selected leafhoppers

The work was carried out at Division of Biotechnology, Indian Institute of Horticultural research (IIHR), Bengaluru. The technique employed for molecular identification is described hereunder.

3.1.3.1 Isolation of genomic DNA (gDNA)

Total genomic DNA of twenty six different *Empoasca* spp was isolated using the modified CTAB method (Rugman-Jones *et al.*, 2006) and the procedure is outlined here as follows.

1. Individual leafhopper specimen was grinded in 30 µl of preheated CTAB lysis buffer and makes it final volume of 170 µl of CTAB buffer to grinded sample.
2. The samples were incubated at 56 °C for 2 hours followed by slight spinning at 3000 rpm for 30 seconds.
3. Added 2 µl of Proteinase K and incubated at 37 °C for 10 min.
4. Added equal volume of Chloroform and Isoamyl alcohol (24:1) and mixed by inverting.
5. Kept for 5-10 minutes at room temperature and centrifuged at 8000 rpm for 10 min. Collected and transferred the supernatant to new tube.
6. Added one volume of chilled Iso proponal and 10 µl of 0.5M NaCl. Incubated the tube at -20 °C for 20 min. Then centrifuged at 10000 rpm for 15 min.
7. Discarded the supernatant, washed the pellet with 70 per cent ethanol. Dry the pellet and dissolved in 20 µl TE buffer.
8. DNA was kept at -20°C for long term storage until further use.

3.1.3.2 Quantification and qualification of genomic DNA

The concentration of DNA was determined by Nanodrop (qualitative and quantitative) and further it was confirmed through 0.8% agarose gel. The concentration of the fragment of interest was estimated by comparing the intensity of ethidium bromide fluorescence to that of the known DNA concentration standards. Two µl of DNA sample was loaded on 0.8 per cent agarose gel. The gel was run at 90 volts for 30min. Thereafter, the DNA bands were visualized under ultraviolet light and photographed.

Two µl of genomic DNA was qualified and quantified by measuring absorbances at 260 nm (OD260) and 280 nm (OD280) using a spectrophotometer (NanoDrop, Thermo Scientific). The quantity of DNA was directly recorded from spectrophotometer. DNA quality was indicated by the ratio of OD260/OD280, which should be in the range of 1.8 to 2.0.

3.1.3.3 PCR assay of mtCOI gene

Genomic DNA of twenty six different *Empoasca* spp was diluted to a working concentration of 25ng/µl. Following reaction and cycle optimisation, the PCR products were run on agarose gel and visualised under UV light.

The genomic DNA of twenty six different *Empoasca* spp was amplified by PCR using universal mitochondrial COI gene primer pairs such as forward primer LCO (5'-GGTCAAC AAATCATAAAGATATTGG-3') and reverse primer HCO (5'-TAAACTTCAGGG TGACCAAAAAATCA-3') (Folmer et al., 1994). The mtDNA amplification reactions were carried out using a thermal cycler (Eppendorf, CA) in 25 µl volume containing 3 ul genomic DNA, 1 ul of 10 mM dNTP, 0.7 ul of 10pM primer, 2.5 ul of 10× reaction buffer, 0.7 ul of 25mM $Mgcl_2$, 0.3 ul of DMSO, 1 ul of 2 unit of Taq DNA polymerase (Genie) with the following conditions: initial denaturation at 94°C for 5 min, 35 cycles of (94°C, 40s; 47°C , 45s; 72°C, 60s) and a final extension of 72°C for 10 min. The PCR negative control contained the identical amount of PCR mixture with 5 µl distilled water instead of DNA template. A PCR positive control was also included, containing the PCR mixture plus DNA that had been successfully put through the PCR reaction on previous studies.

3.1.3.4 Agarose gel electrophoresis

PCR amplification products were separated by 0.8% agarose gel electrophoresis in TAE buffer (40mM Tris-acetate [pH 8.0], 1 mM EDTA) at 100 V for 1 hour. The gel was then visualised under UV light.

3.1.3.5 Cloning, sequencing and sequence analysis of the mtCOI gene

PCR products observed as single sharp and clear bands on agarose gel were excised using sharp blade and purified using a NucleoSpin® Gel and PCR clean up kit (Macherey-Nagel) according to the manufacturer's instructions. The standard recombinant DNA techniques used in cloning were, according to the Sambrook and Russell (2001). The *E. coli* DH5α cells were transformed with PCR-amplified ~750bp mtCOI gene ligated in pTZ57R (T/A cloning vector) using Fermentas DNA ligation kit (#K1214). The transformed cells (20µl) were spread on LB agar plates containing X-gal (270 µg/ml), IPTG (120 µg/ml) and ampicillin (100 µg/ml). The plates were then incubated at 370C for 24 h to screen blue and white colonies. Cloning was confirmed by colony PCR, plasmid mobility check and restriction analysis of recombinant plasmid DNA containing mtCOI gene. The mtCOI gene was sequenced, to determine the homology with the known sequences in the NCBI GenBank database.

Sequencing was repeated three times for each fragment and the sequences were aligned in a sequence alignment editor, 'Bioedit' to look for any introduced errors while sequencing, if any.

3.1.3.6 Cloning Principle

The InsTAclone PCR Cloning Kit takes advantage of the terminal transferase activity of Taq DNA polymerase and other non-proofreading thermostable DNA polymerases. Such enzymes add a single 3'-A overhang to both ends of the PCR product. The structure of these PCR products favours direct cloning into a linearized cloning vector with single 3'-ddT overhangs. Such overhangs at the vector cloning site not only facilitate cloning, but also prevent the recircularization of the vector. As a result, more than 90 per cent of recombinant clones contain the vector with an insert. Recombinant clones are selected based on blue or white screening.

3.1.3.7 Transformation

1. The day before the transformation, overnight culture was seeded by inoculating 2 ml of C-medium with a single bacterial colony. The culture was incubated overnight at 37°C in a shaker.
2. 150 µl of the overnight bacterial culture was added to 1.5 ml of pre-warmed C-medium and incubated for 20 min at 37°C in a shaker.
3. Bacterial cells were pelleted out by 1 min centrifugation and the supernatant was discarded.
4. The pelleted cells were resuspended in 120 µl of T-solution (1 TA: 1TB mixture) and incubated on ice for 5 minutes.
5. Centrifugation was performed for 1 min in a microcentrifuge and the supernatant was discarded.
6. The pelleted cells were resuspended in 120 µl of T-solution and incubated on ice for 5 minutes.
7. 2.5 µl of ligation mixture was added to new microcentrifuge tubes and chilled on ice for 2 minutes.
8. 50 µl of the prepared cells were added to each tube containing ligated sample mixed and incubated on ice for 5 minutes.
9. Plated immediately on pre-warmed LB-amphicillin agar plates and incubated overnight at 37°C.

3.1.3.8 Colony selection

a) Blue/White colony selection

The transformation mixture was plated immediately on pre-warmed LBampicillin agar plates with X-gal and IPTG and spread thoroughly on LB-ampicillin agar plates using a spreader. The plates were wrapped with the parafilm and incubated overnight at 37°C. Transformed white colonies and untransformed blue colonies were formed. Transformed (white) colonies were selected and sub-streaked on pre-warmed LBampicillin agar plates with X-gal and IPTG and incubated overnight at 37°C.

3.1.3.9 Analysis of recombinant clones

White colonies were verified for the presence of DNA insert using colony PCR.

a) Colony PCR

Colony PCR was the screening of bacterial (*E. coli*) clones for correct ligation or plasmid products. White colonies of bacteria were picked from substreaked plates with a sterile toothpick or pipette tip from a LB-ampicillin agar plates with X-gal and IPTG growth plate. This was then inserted into the PCR master mix or pre-inserted into autoclaved water. PCR was then conducted to determine if the colony contains the DNA fragment of interest.

The following protocol for colony screening by PCR was used. Enough PCR master mix was prepared for the number of colonies analyzed. For each 20 µl reaction, the following reagents were mixed:

Component	Volume
10X *Taq* Buffer	2.0 µl
dNTP mix, 2 mm each	2.0 µl
25 mM MgCl2	1.2 µl
LCO forward primer, 10 µM	0.6 µl
HCO reverse primer, 10 µM	0.6 µl
*Taq*DNA polymerase 5 u/µl	0.1 µl
Water, nuclease-free	13.5 µl
Total volume	**20 µl**

1. 20µl of the mix was aliquated into the PCR tubes on ice
2. Substreaked individual white colony was picked and resuspended in 20 µl of the PCR master mix
3. PCR was performed: 94°C, 2 min; 94°C, 30 s, 45°C, 30 s, 72°C 1 min/kb; 30 cycles
4. Presence of the PCR product of the expected length was analysed on the gel

3.1.3.10 Plasmid isolation

Plasmid purification was carried out from incubated transformed colonies multiplying in LB broth vials with the help of Gene JET™ Plasmid Miniprep Kit (Thermo Scientific, Fermentas, Lithuania) according to manufacturer's protocol.

1. The colony PCR confirmed (white) colonies were restreaked on LB broth and then next day inoculated into 15 ml Falcon tubes containing 5 ml LB-ampicillin broth with the help of autoclaved plastic tooth-pick sand wrapped with the parafilm.
2. The tubes were incubated in a shaker incubator overnight at 37°C at the speed of 220 rpm.
3. The bacterial culture was harvested by centrifugation at 8,000 rpm in a 2 ml microcentrifuge for 2 minutes at room temperature. The supernatant was discarded and the remaining medium was removed. This step was repeated twice.
4. The pellet was resuspended in a microcentrifuge tube contain 250 µl of the resuspension solution by vortexing or pipetting up and down until no cell clumps remained.
5. 250 µl of the Lysis solution was added and mixed thoroughly by inverting the tube 4-6 times until the solution became viscous and slightly clear.
6. 350 µl of the neutralization solution was added and mixed immediately and thoroughly by inverting the tube 4-6 times. The neutralized bacterial lysate should become cloudy.
7. Centrifuge at 9500 rpm for 5 min. Supernatant was transferred to the GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate. Centrifuge for 1 min. Flow-through was discarded and the column was placed back into the same collection tube.

8. 500 µl of the wash solution (diluted with ethanol prior to first use) was added to the GeneJET spin column. Centrifuged for 30-60 seconds and discarded the flowthrough. Column was placed back into the same collection tube.

9. Wash procedure (step 8) was repeated using 500 µl of the wash solution.The flowthrough was discarded and centrifuged for an additional 1 min to remove residual wash solution. This step was essential to avoid residual ethanol in plasmid preps.

10. The Gene JET spin column was transfered into a fresh 1.5 ml microcentrifuge tube. 50 µl of the elution buffer was added to the center of Gene JET spin column membrane to elute the plasmid DNA. Care was taken not to contact the membrane with the pipette tip. Column was incubated for 2 min at room temperature and centrifuged for 2 min.

11. The column was discarded and the purified plasmid DNA was stored at -20°C until further use.

3.1.3.11 Sequence analysis and Data interpretation

Chromatograms were edited to discard ambiguous bases, and edited sequences were aligned by using the Basic Local Alignment Search Tool (BLAST), with the sequences of same or related genera retrieved from the nucleotide database (PUBMED) of National Centre for Biotechnology Information (NCBI). Sequences containing insertions, deletions, nonsense or stop codons were considered as having resulted from PCR or sequencing errors or represented pseudogenes, and were thus excluded from the analyses. In addition to verifying whether a sequence had been derived from the true mitochondrial COI gene and not a nuclear mitochondrial pseudogene (numt), the BLAST program was used for testing in the National Center of Biotechnology Information (NCBI). The existence of any double peak (i.e. identical to or at least clustered with each other) in the chromatogram of the fragments was determined. Sequences were aligned in a sequence alignment editor, 'Bioedit' to look for any introduced errors while sequencing, if any. Sequences were edited and aligned using BioEdit 7.0.5.2 (Hall, 1999). The nBLAST program (http://www.ncbi.nlm.nih.gov/blast/) was employed to identify similarities between the sequences obtained in this work and previously published data.

3.2 Population dynamics of leafhoppers infesting potato in Southern Transitional Zone of Karnataka

3.2.1 Experimental site

This experiment was conducted in Malligenahalli village (13°36' N latitude and 75°248' E longitude with an altitude of 798 meter from MSL), of Tarikere Taluk, Chikkamagaluru District in Karnataka during *Kharif* 2017 and *Rabi* 2017-18 seasons. Observations were made on Kufri Jyothi variety from 15 days after sowing on the incidence of leafhoppers at fortnight intervals. During *Kharif* 2017-18, observations were taken from first fortnight of July 2017 to first fortnight of October 2017. During *Rabi* 2018, observations were taken from first fortnight of December 2017 to second fortnight of March 2018 by using fixed plot survey.

3.2.2 Leafhopper monitoring

3.2.2.1 Leafhopper monitoring on potato plants

Observations on number of leafhoppers (both nymphs and adults) on 3 compound leaves were recorded from upper, middle and lower part in a plant at forthnightly interval (Plate 2). A total of 34 plants were observed each time for presence of leafhoppers and were counted. The data was averaged to number of leafhoppers per plant per 3 compound leaves.

3.2.2.2 Leafhopper monitoring using sweep net collection

Number of leafhoppers were counted in sweep net samples by taking 20 sweeps in 5 representative sites (Plate 3). A sweep net with a mouth diameter of 45cm was used for this purpose. Sweep net collections were transformed to an aspirator and then to test tube and were counted. The data was averaged to number of leafhoppers per 20 sweeps in five representative sites.

3.2.2.3 Leafhopper monitoring using yellow sticky traps

The observations were recorded at fortnightly interval on average of leafhoppers caught per two yellow sticky traps (Plate 3). Yellow sticky trap was made using yellow acrylic fibre board of 5mm thickness and its size was 60X40 cm and each location had two yellow sticky traps. The trapped leafhoppers were counted and averaged to number per trap.

During leafhopper monitoring, potato plants showing phytoplasma symptoms were also observed (Plate 4).

The observations on number of leafhoppers per three compound leaves per plant, number of leafhoppers per 20 sweeps and number of leafhoppers per yellow sticky trap (Y) were correlated with six weather parameters like maximum temperature (X1), minimum temperature (X2), relative humidity (X3), total rainfall (X4), Wind speed (X5) and sunshine hour (X6). Multiple regression and step wise regression analysis was also performed with the same. The weekly meteorological data on weather parameters was collected from the nearest station *i.e.*, Agricultural and Horticultural

Research Station (AHRS), Bavikere. The mean fortnightly weather parameters of *Kharif* 2017 and *Rabi* 2017-18 are given in Appendix I and II, respectively.

3.3 Varietal preference of potato leafhoppers

In order to evaluate varietal preference of potato leafhopper, morphological (trichomes) and biochemical components were estimated by selecting commonly grown varieties in Southern Transitional Zone of Karnataka to understand population buildup of leafhoppers.

3.3.1. Population build up of leafhoppers on different potato varieties

Four different potato varieties were selected (Kufri Jyothi, S-6, FC-3, FL-5) and sowing was done in pots (4 plants per variety) (Plate 5). The varieties selected were meant for table purpose (Kufri Jyothi, S-6) and chips making (FC-3, FL-5). Leafhopper adults were bought live from potato fields and released at five per plant (three males and two females) when the plants were 30 days old. Number of leafhoppers were counted and averaged from a total of four plants at forthnightly interval from the day of release.

3.3.2 Influence of trichomes on incidence of potato leafhoppers

Number of trichomes were counted by following the procedure described by Maiti *et al.* (1980), the detailed protocol is mentioned below;

Fifth leaf was selected, from different potato varieties, the collected leaf segments was preserved in 70 per cent alcohol. Later the leaf segment was heated in water for 15 minutes in an incubator at 85^{o}C. The water was poured off and ethyl alcohol 95 per cent was added to the bottle containing leaves and boiled for 20 minutes at 85^{o}C in incubator. The alcohol was removed and fresh alcohol was added, the boiling procedure was repeated till the chlorophyll content of leaf cleared off completely. Then the alcohol was poured out and 90 per cent lactic acid was added. The bottles was stoppered and was heated again at 80^{o}C for 45 minutes till the leaf segments became clear and bottles were cooled and preserved for observation. The processed leaves were mounted on a glass slide with a drop of lactic acid and observed under simple microscope (Plate 6).

Number of trichomes were counted in each of the varieties selected and expressed as number of trichomes per centimeter square leaf area. Correlation analysis was performed to know the influence of trichomes on the development of leafhoppers.

Plate 1: Collection of leafhoppers in potato fields

Plate 2: Leafhopper monitoring on potato plants

Plate 3: Sweep net and yellow sticky traps sampling for studying population dynamics of potato leafhoppers

Plate 4: Purple top roll symptoms on potato plant

Plate 5: Potted plants (15 days old) for varietal preference of potato leafhoppers

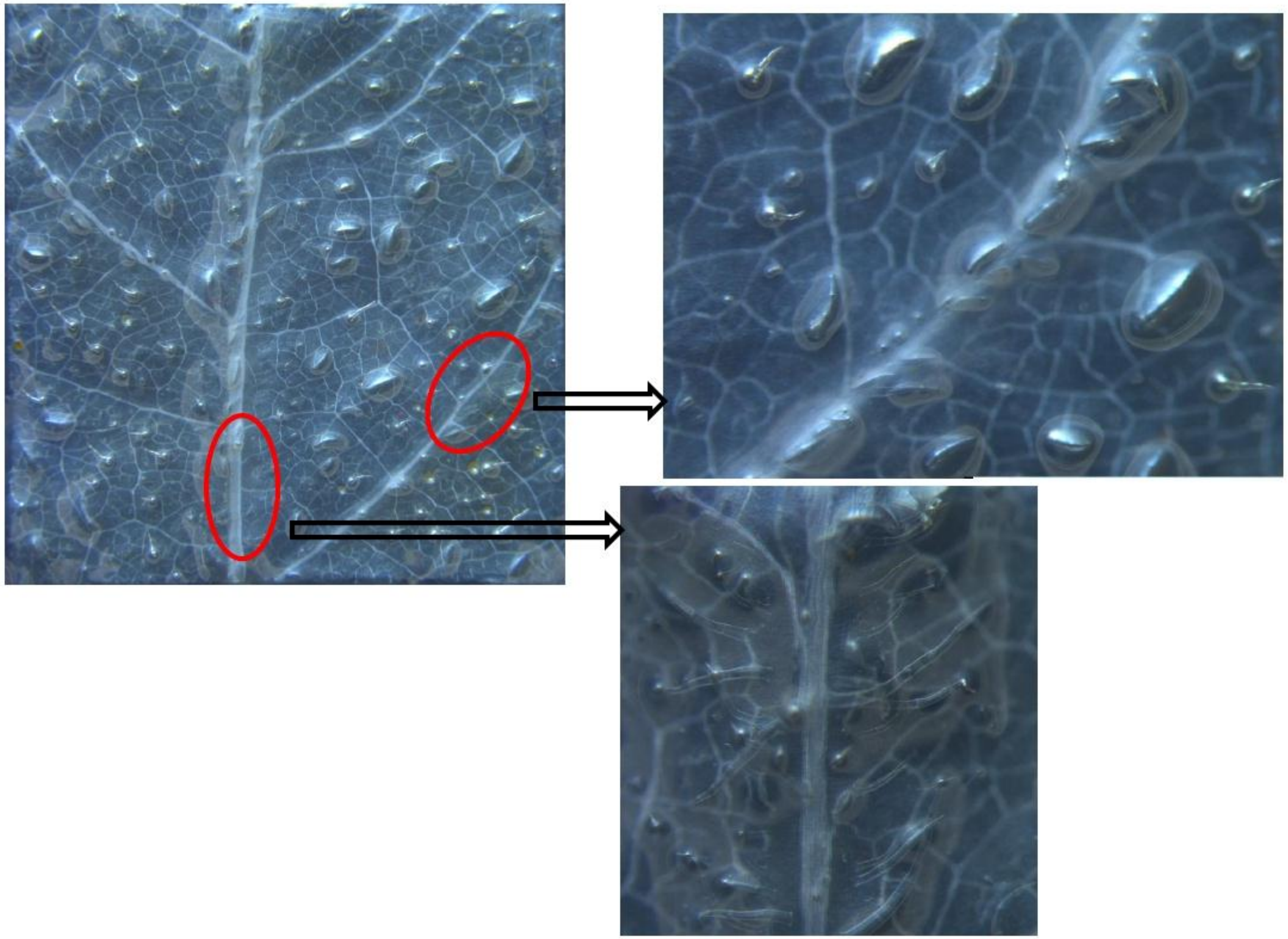


Plate 6: Trichomes of potato leaves (Kufri Jyothi)

3.3.3. Estimation of biochemical components of different potato varieties

The estimation of biochemical constituents *viz.*, free phenols, total sugars and potasium in potato leaves collected from peak vegetative stage (30 days old plants) of different varieties was carried out to establish the relationship between various biochemicals with resistance or susceptibility by following the standard procedure as mentioned below.

Plant material

Second and third leaves of a potato plant was sampled from different potato varieties. Metabolites were extracted by using 80 per cent alcohol. This extraction was stored in cool place for further analysis.

3.3.3.1 Estimation of total sugars by Nelson Somgys method

Reducing sugars was estimated using Dinitrosalicylic acid (DNSA) reagent. Fresh shoots (5 g) of plants extract was homogenized in hot 80 per cent ethanol. Sugars was dissolved by adding 10 ml distilled water (Plate 7). Reducing sugars was estimated by using DNSA reagent calorimetrically at 510 nm wavelength and calculated from graph plotted using glucose as a standard.

Non-reducing sugars was estimated using anthrone reagent sample extractant was hydrolysed separately by keeping in boiling water bath for 3 hr with 2.5 N HCl (5 ml) and was neutralized with Na_2CO_3 after cooling it to room temperature. Volume was made up to 100 ml and non-reducing sugars was estimated spectrophotometrically at 610 nm wavelength on UV-visible spectrophotometer and calculated from graph plotted using glucose as a standard.

Total sugar was calculated using

Total sugar = Non reducing sugar - Reducing sugar and expressed in mg/100g sample.

3.3.3.2 Estimation of free phenols by Folin - Ciocoltuave reagent

The total phenolic content of potato plant extract was determined by the spectrophotometric method using Folin-Ciocoltuave phenol reagent. The crude extract (0.5 ml) was diluted to 5.0 ml with distilled water. Folin-Ciocoltuave reagent 5 ml was added mixed thoroughly. After 3.0 min, 5 ml of 10 per cent sodium carbonate solution was added and the mixture was allowed to stand for 1hr with intermittent shaking (Plate 8). The mixture of solution was measured at 510 nm using spectrophotometre. The total phenolic content was calculated by using standard graph of catechol curve. This analysis was carried out in triplicate and averaged the values of content.

3.3.3.3 Estimation of potasium by Jackson method

3.3.3.3.1 Digestion of plant samples with di-acid mixture

One gram of powdered plant samples (leaves) was pre-digested with HNO_3 and then digested with di-acid mixture containing HNO_3 and $HClO_4$ in the proportion of 10:4 as described by the Jackson, 1973. The volume of the digest was made up to 100 ml with distilled water and used it for total elemental analysis (Plate 9).

3.3.3.3.2 Total Potassium

Total potassium content in plant sample was determined by flame photometric method after diluting one ml of the di-acid digest to 25 ml with distilled water. Comparing the flame photometer reading of the sample with calibration curve of potassium, per cent potassium in plant sample was calculated (Jackson, 1973).

Correlation analysis was performed to know the influence of biochemical components on developmental behavior of potato leafhoppers.

3.4 Evaluation of selected insecticides against potato leafhoppers

3.4.1 Experimental site

Two field experiments was carried out during *Kharif* 2017 and *Rabi* 2017-18 at Lingadahalli village (13°37' N latitude and 75°49' E longitude with an altitude of 753 meter from MSL), of Chikkamagaluru district on Kufri Jyothi variety in order to study the effect of different insecticides against leafhoppers. Field experiments were laid out in randomized block design (RBD) with three replication and nine treatments with the plot size of 4 m x 4 m (Plate 10). In each plot five plants were selected and tagged. First spray was given on 16^{th} July-2018 and second spray on 31^{st} July during *Kharif*. Similarly, first spray was given on 26^{th} December-2018 and second spray on 10^{th} January during *Rabi*. The treatment details of the experiments are presented in Table 1. Layout of field experimental plot is presented in Table 2.

3.4.2 Observations

Observations were made on the number of leafhoppers per three compound leaves per plant from five tagged plants of each replication and also in twenty sweeps using sweep nets. Leafhopper population was recorded at one day before, seventh and fifteenth days after application of insecticides. The observations on number of leafhoppers in both seasons was analyzed separately as well as pooled *i.e. Kharif* 2017 and *Rabi* 2017-18.

7.1: Potato sample crush with pestle mortar

7.2: Preparation of potato leaf sample for analysis

Plate 7: Analysis of sugars in potato leaves by Nelson Somgys method

Plate 8: Analysis of phenols in potato leaves by Folin Ciocoltuave method

Plate 9: Analysis of potassium in potato leaves by Jackson method

Plate 10: General view of insecticide trail plot showing replication separated by wooden pegs at Lingadahalli, Chikkamagaluru

Table 1. List of insecticides evaluated against potato leafhoppers

SL. NO.	Treatment details	Brand name	Manufacturer	Dose/ l
T_1	Imidacloprid 17.8 SL	Confidor	Bayer Crop Science	0.3 ml
T_2	Acetamiprid 20 SP	Prias	Indogulf Crop Sciences Limited	0.3 g
T_3	Acephate 75 SP	Hilphate	Hindustan Insecticides Limited	1.5 g
T_4	Thiamethoxam 25 WG	Actara	Syngenta India Limited	0.5 g
T_5	Dinotefuran 20 SG	Token	Indofil Insecticides Limited	0.3 g
T_6	Acephate 50 SP + Imidacloprid 1.8 SP	Lancer gold	UPL Limited	2.0 g
T_7	Dimethoate 30 EC	Tafgor	Rallis India Limited	1.7 ml
T_8	NSKE 5%	-	-	-
T_9	Control	-	-	-

Table 2. Layout of field experimental plot

4 m 30 cm N

T_1R_1		$T_2\ R_2$		$T_7\ R_3$
$T_3\ R_1$		$T_4\ R_2$		$T_9\ R_3$
$T_5\ R_1$		$T_6\ R_2$		$T_2\ R_3$
$T_7\ R_1$		$T_8\ R_2$		$T_3\ R_3$
$T_9\ R_1$		$T_1\ R_2$		$T_4\ R_3$
$T_2\ R_1$		$T_3\ R_2$		$T_6\ R_3$
$T_4\ R_1$		$T_5\ R_2$		$T_8\ R_3$
$T_6\ R_1$		$T_7\ R_2$		$T_1\ R_3$
$T_8\ R_1$		$T_9\ R_2$		$T_5\ R_3$

Obtained data were analyzed using ANOVA to compare the efficacy of treatments. Observations of number of leafhopper per three compound leaves per plant and also sweep net counts were analyzed after square root transformation.

3.4.3 Cost economics of various treatments used against leafhoppers

Yield data was recorded and computed on hectare basis. C:B ratio was calculated for both the seasons *i.e., Kharif* 2017 and *Rabi* 2017-18. Economics of insecticides sprays of both seasons was pooled *i.e., Kharif* 2017 and *Rabi* 2017-18 and analyzed.

3.4.3.1 Cost of cultivation

The prevailing cost of input materials and labour cost were considered for computing the cost of cultivation which is expressed in Rs/ha.

3.4.3.2 Gross return

The price of potato in the market at the time of harvest was used for the calculation of gross return (Rs/ha).

Gross return = Marketable yield (ton. /ha) x Market price (Rs /ha)

3.4.3.3 Net return

Net return (Rs/ha) was calculated by subtracting the cost of cultivation (Rs /ha) from the gross return (Rs/ha).

Net return = Gross return – Total cost of cultivation

3.4.3.4 Benefit cost ratio

B: C ratio was calculated by dividing the gross returns (Rs/ha) by cost of cultivation (Rs/ha).

$$\text{B: C ratio} = \frac{\text{Gross returns}}{\text{Total cost of cultivation}}$$

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The results of the experiments conducted during 2017-18 on species composition, population dynamics, varietal preference and the effect of different insecticides against potato leafhoppers are presented in this chapter.

4.1 Species composition of leafhoppers infesting potato in major potato growing regions of Karnataka

The results of the investigations on the species composition of potato leafhoppers in major potato growing regions of Karnataka are presented in Table 3 and 4.

Potato leafhoppers were collected from major potato growing regions of Karnataka *i.e.* Chikkamagaluru (Malligenahalli, Lingadahalli and Lakshmipura), Hassan (Bageshpura and Shankarapura), Dharwad (Anandanagara and Navalur) and Belgaum (Usagaum, Kodoli and Bombarga) during *Kharif* 2017. Chikkamagaluru (Lingadahalli and Lakshmipura), Bengaluru (Devanahalli) and Chikkaballapur (Yaluvahalli, Aruru and Doddapayalagurki) during *Rabi* 2017-18.

4.1.1 Collection of potato leafhoppers in major potato growing regions of Karnataka during Kharif 2017and Rabi 2017-18

During *Kharif* 2017, leafhopper incidence was recorded in Chikkamagaluru (nine species), Hassan (two species), Dharwad (four species) and Belgaum (four species) during the peak vegetative growth of the crop. Among them *Empoasca* spp. recorded 81.74 per cent in Chikkamagaluru, 97.87 per cent in Hassan, 65.51 per cent in Dharwad and 92.75 per cent in Belgaum respectively. Whereas in all places other leafhopper species recorded on potato is below 20 per cent during the peak vegetative growth of the crop. This indicates that Empoasca spp. is dominant species causing more damage to crop compared to other species of leafhoppers (Table 3).

Similarly during *Rabi* 2017-18, leafhoppers collected in Chikkamagaluru (five species), Bengaluru (three species), Chikkaballapur (six species) during the peak vegetative growth of the crop. Among them, *Empoasca* spp. recorded 57.57 per cent in Chikkamagaluru, 54.23 per cent in Bengaluru and 44.27 per cent in Chikkaballapur respectively. Whereas in all places other leafhopper species recorded on potato were below 32 per cent during the peak vegetative growth of the crop. This indicates the *Empoasca* spp. is dominant causing more damage to crops as compared to other species of leafhoppers (Table 4).

In both *Kharif* and *Rabi* seasons, the *Empoasca* spp. is dominant species. Whereas in case of *Rabi* other leaf hopper species is more (> 32%) as compared to

Table 3. Species composition of leafhoppers infesting potato in major potato growing regions of Karnataka during *Kharif* 2017

Major potato areas	Places selected	GPS	Species recorded	Per cent of each species observed
Chikkamagaluru	Malligenahalli Lingadahalli Lakshmipura	13°36' N75°48' E798 m 13°37'N75°49' E,753m 13°17'N75°47' E,1000m	*Empoasca* spp. *Bactromorpha angustatus* (Osborn) *Ianagallia bifurcata* (Sawai Singh & Gill) *Cicadulina bipunctata* (Melichar) *Cenedius horvathi* Distant *Kolla ceylonica* (Melichar) *Maiestas pruthi* (Metcalf) *Exitianus indicus* (Distant) *Seriana* sp.	81.74 2.38 7.14 1.58 0.79 2.38 1.58 0.78 2.38
Hassan	Bageshpura, Shankarapura	12°27'N76°11' E,912m 12°58'N76°21' E,926m	*Empoasca* spp. *Bactromorpha angustatus* (Osborn) *Orosius albicinctus* Distant	97.87 1.41 0.70
Dharwad	Anandanagara, Navalur	15°26'N75°01' E,712m 15°26'N75°03' E,702m	*Empoasca* spp. *Nirvana pallida* Melichar *Amrasca biguttula biguttula* (Ishida) *Cicadulina bipunctata* (Melichar)	65.51 3.44 19.82 11.20
Belgaum	Usagaum , Kodoli, Bombarga	15°52'N74°26' E,770m 15°57'N74°30' E,770m 15°57'N74°29' E,770m	*Empoasca* spp. *Monobazus dissimilis* (Distant) *Cicadulina chinai* Ghauri *Exitianus indicus* (Distant)	92.75 2.89 1.44 2.89

Table 4. Species composition of leafhoppers infesting potato in major potato growing regions of Karnataka during *Rabi* 2017-18

Major potato areas	Places selected	GPS	Species recorded	Per cent of each species observed
Chikkamagaluru	Lingadahalli, Lakshmipura	13°37'N75°49' E,753m 13°17'N75°47' E,1000m	*Empoasca* spp. *Cicadulina bipunctata* (Melichar) *Amrasca biguttula biguttula* (Ishida) *Orosius albicinctus* Distant *Nirvana pallida* Melichar	57.57 25.00 10.60 1.51 5.30
Bengaluru	Devanahalli	15°15'N77°43',872m	*Empoasca* spp. *Balclutha incise* (Matsumura) *Nirvana pallida* Melichar	54.23 27.11 18.64
Chikkaballapur	Yaluvahalli, Aruru, Doddapayalagurki	13°21'N77°43',934m 13°37'N77°46',848m 13°32'N77°46',1004m	*Empoasca* spp. *Amrasca biguttula biguttula* (Ishida) *Nirvana pallida* Melichar *Balclutha incisa* (Matsumura) *Balclutha saltuella* (Kirschbaum) *Stirellus* sp.	44.27 31.29 6.87 9.92 0.76 6.87

Kharif season (<20%). This indicates environment play a major role in deciding the species compositions of leafhoppers under potato ecosystem.

4.1.3 Identification of potato leafhoppers

A total of 19 species representing six subfamilies (Evacanthinae, Cicadellinae, Iassinae, Megaphthalminae, Deltocephalinae and Typhlocybinae) were identified (Table 5), based on morphological characters of adult leafhoppers (Plate 11-13).

Deltocephalinae included nine tribes *i.e.* Macrostelini, Opsiini, Deltocephalini, Stenometopiini, Athysini, Chiasmini, Paralimnini. In which Macrostelini included *Cicadulina chinai* Ghauri (Plate 11H), *C. bipunctata* (Melichar) (Plate 11G), *Balclutha incisa* (Matsumura) (Plate 12A), *B. saltuella* (Kirschbaum) (Plate 12B); Opsiini included *Orosius albicinctus* Distant (Plate 11B); Deltocephalini included *Maiestas pruthi* (Metcalf) (Plate 11C); Stenometopiini included *Stirellus* sp. (Plate 11E); Athysini included *Monobazus dissimilis* (Distant) (Plate 11A); Chiasmini included *Exitianus indicus* (Distant) (Plate 11D); Paralimnini included *Cenedaeus horvathi* Distant (Plate 11F).

Megaphthalminae included tribe Agalliini. In Agalliini, *Ianagallia bifurcata* (Sawai Singh & Gill) (Plate 13A) and *Austroagallia* sp. (Plate 13B) were recorded. Evacanthinae included tribe Nirvanini. In Nirvanini, single species *Nirvana pallida* Melichar (Plate 13C) was recorded. Cicadellinae included tribe Cicadellini. In Cicadellini, single species *Kolla ceylonica* (Melichar) (Plate 13D) was recorded. Iassinae included tribe Iassini. In Iassini, single species *Batracomorphus angustatus* (Osborn) (Plate 13F) was recorded (Table 5).

Typhlocybinae included two tribes Erythroneurini and Empoascini. Erythroneurini included *Seriana* sp. (Plate 12C); Empoascini included three species *Amrasca biguttula biguttula* (Ishida) (Plate 12D), *Empoasca kerri* Singh-Pruthi (Plate 12E), *Empoasca simbava* Dworakowska (Plate 12F) along with *Empoasca* spp. complex (Plate 14).

In all major potato growing areas of Karnataka, *Empoasca* spp. was the dominant species which contributed high percentage.

4.1.1. Taxonomical key based on adult morphological characteristics

4.1.1.2 Keys to leafhoppers associated with potato in major potato growing regions of Karnataka

1. Face in lateral view almost horizontal (Plate 15) with a median ridge dorsally on frontoclypeus (Plate 16)………...Evacanthinae: Nirvanini: *Nirvana pallida*

Deltocephalinae


11A *Monobazus dissimilis*


11B *Orosius albicinctus*


11C *Maiestas pruthi*


11D *Exitianus indicus*


11E *Stirellus* sp.


11F *Cenedaeus horvathi*


11G *Cicadulina bipunctata*


11H *Cicadulina chinai*

Plate 11: Different species of potato leafhoppers recorded during study

12A *Balclutha incisa*

12B *Balclutha saltuella*

Typhlocybinae

12C Erythroneurini: *Seriana* sp.

12D Empoascini: *Amrasca biguttula biguttula*

12E Empoascini: *Empoasca kerri*

12F Empoascini: *Empoasca simbava*

Plate 12: Different species of potato leafhoppers recorded during study

Agallinae

13A *Ianagallia bifurcata*

13B *Austroagallia* sp.

Evacanthinae

13C Nirvanini: *Nirvana pallida*

Cicadellinae

13D Cicadellini: *Kolla ceylonica*

Iassinae

13F Iassini: *Batracomorphus angustatus*

Plate 13: Different species of potato leafhoppers recorded during study

Plate 14: *Empoasca* species complex

Table 5: Species diversity of leafhoppers in major potato growing regions of Karnataka

Subfamily	Tribe	Species
Deltocephalinae	Macrostelini	1. *Cicadulina chinai* Ghauri
		2. *Cicadulina bipunctata* (Melichar)
		3. *Balclutha incisa* (Matsumura)
		4. *Balclutha saltuella* (Kirschbaum)
	Opsiini	5. *Orosius albicinctus* Distant
	Deltocephalini	6. *Maiestas pruthi* (Metcalf)
	Stenometopiini	7. *Stirellus* sp.
	Athysini	8. *Monobazus dissimilis* (Distant)
	Chiasmini	9. *Exitianus indicus* (Distant)
	Paralimnini	10. *Cenedaeus horvathi* Distant
Megaphthalminae	Agalliini	11. *Ianagallia bifurcata* (Sawai Singh & Gill)
		12. *Austroagallia* sp.
Evacanthinae	Nirvanini	13. *Nirvana pallida* Melichar
Cicadellinae	Cicadellini	14. *Kolla ceylonica* (Melichar)
Iassinae	Iassini	15. *Batracomorphus angustatus* (Osborn)
Typhlocybinae	Erythroneurini	16. *Seriana* sp.
	Empoascini	17. *Amrasca biguttula biguttula* (Ishida)
		18. *Empoasca kerri* Singh-Pruthi
		19. *Empoasca simbava* Dworakowska
		Empoasca spp. (complex)

- Face in lateral view oblique (Plate 17); without median ridge dorsally on frontoclypeus(Plate 18)……..…………………………………………….....2

- Ocelli on crown of head (Plate 19); face swollen (Plate 20)….…………...……………………………...Cicadellinae: Cicadellini: *Kolla ceylonica* Ocelli either on face (Plate 21) or on anterior margin of head (Plate 22)……………………………..3

2. Crown and pronotum transversely striated (Plate 23)………………………………Iassinae: Iassini: *Batracomorphus angustatus*

- Crown not transversely striated…….…......……………………………......4

3. Ocelli on face; crown short, sinuately expanded behind eyes (Plate 24)…...…Megaphthalminae: Agalliini...................**I**

- Ocelli either absent, when present on anterior margin of head; crown atleast as long medially as against eye margin (Plate 25)…………...……………..…….5

4. Forewing with crossveins before apical cells (Plate 26); hind basitarsus truncate apically (Plate 27)…………...……….…Deltocephalinae……….…**II**

- Forewing without crossveins before apical cells (Plate 28); hind basitarsus acutely produced(Plate 29)……………………..…Typhlocybinae………...**III**

I. Agallinae

1. Face with one large black spot at base of antenna occupying entire depressed area(Plate 30)………….……….....………………….*Ianagallia bifurcata*

- Face with one small black spot at the base of antenna but not occupying the entire depressed area (Plate 31)………… ……...…………...*Austroagallia* sp.

II. Deltocephalinae

1. Forewing with two subapical cells (Plate 32)..…………..…………….………...2

- Forewing with three subapical cells (Plate 33)….………….……………........5

2. Crown with two black spots (Plate 34)…………………..…...Cicadulina……….3

- Crown without black spots (Plate 35)………………….……Balclutha………4

3. Forewing with a lateral longitudinal fuscous fascia (Plate 36)…………………………………………………….....*Cicadulina chinai*

- Forewing without such a fascia (Plate 37)..…………….*Cicadulina bipunctata*

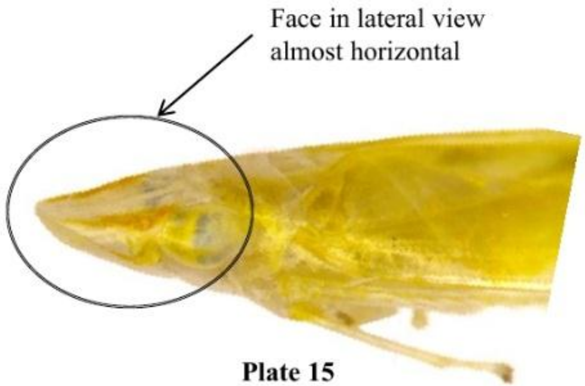


Plate 15

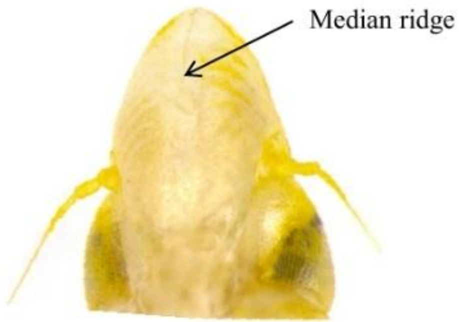
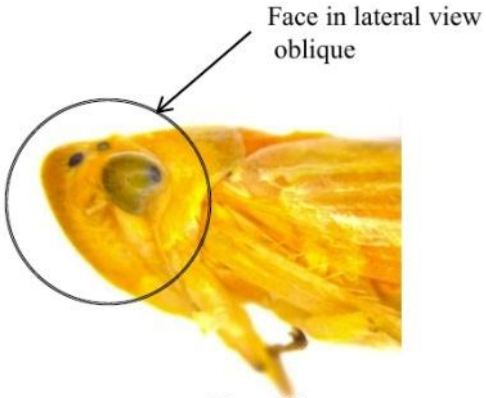


Plate 16



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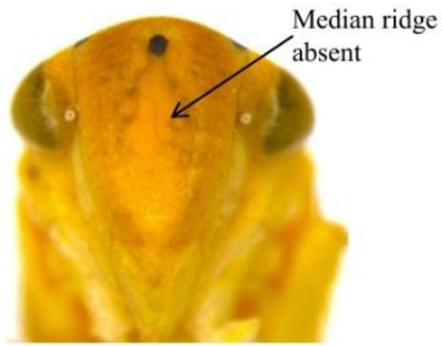

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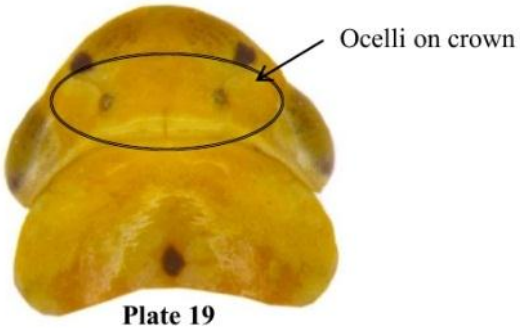

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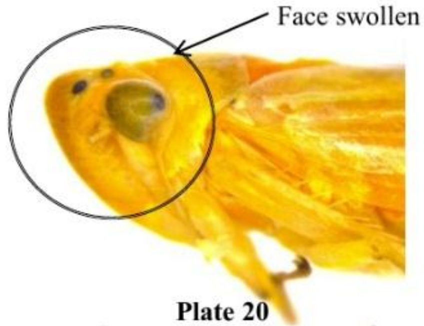

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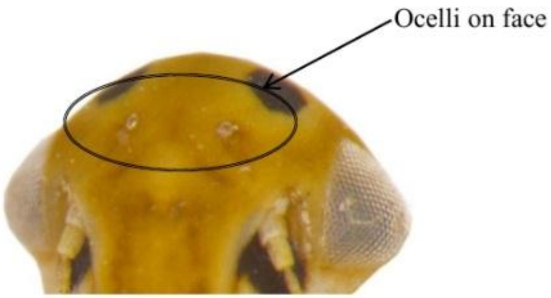

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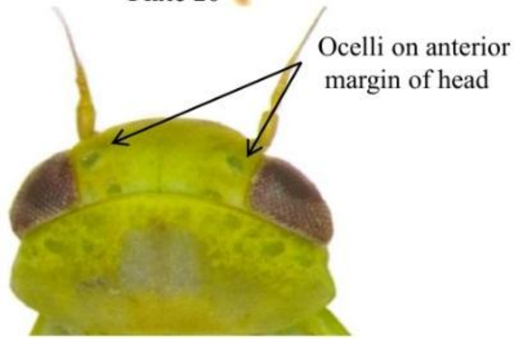

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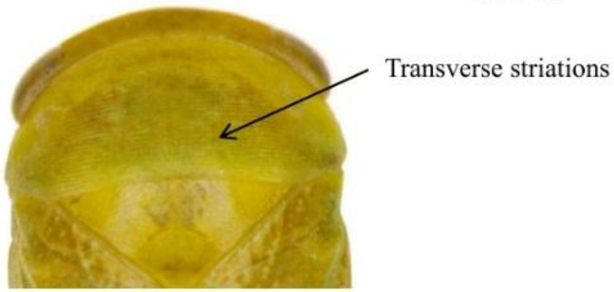

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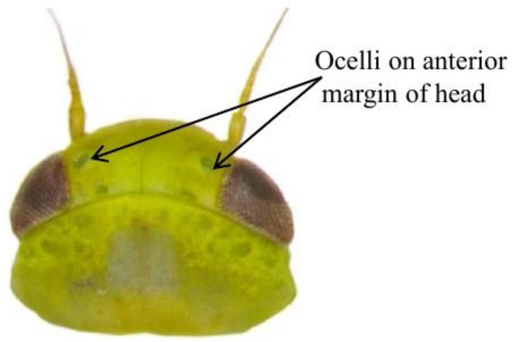
Plate 24



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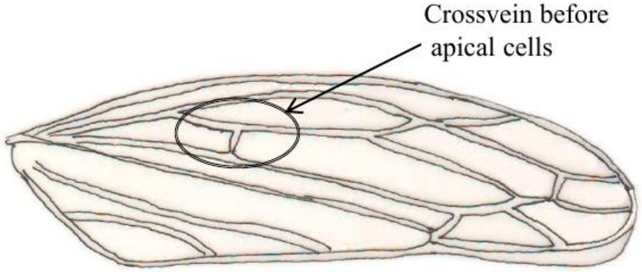

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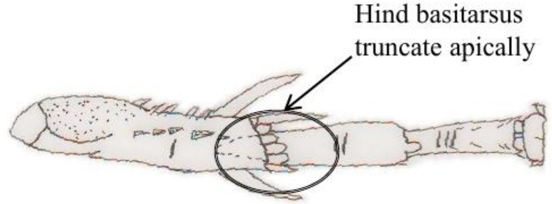

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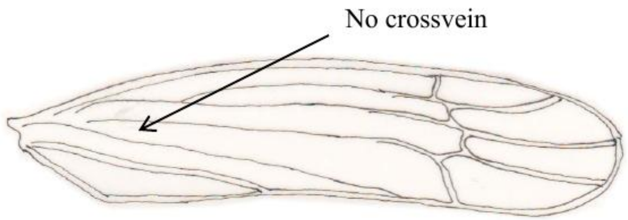

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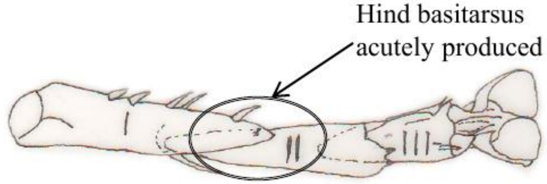

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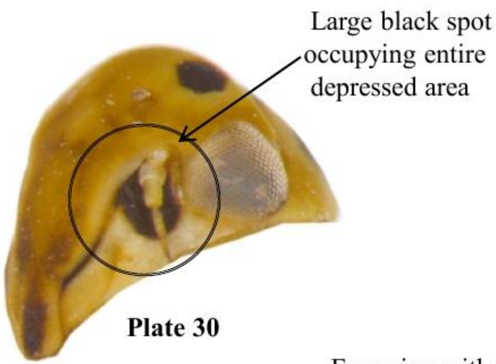

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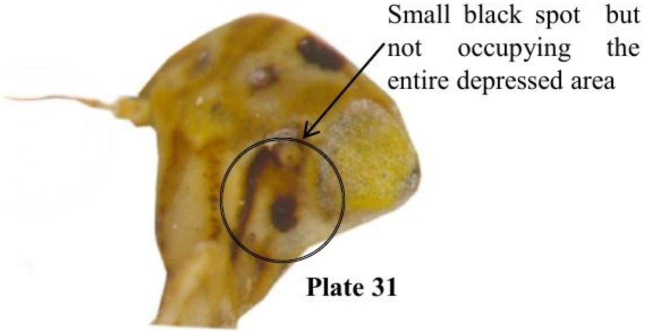

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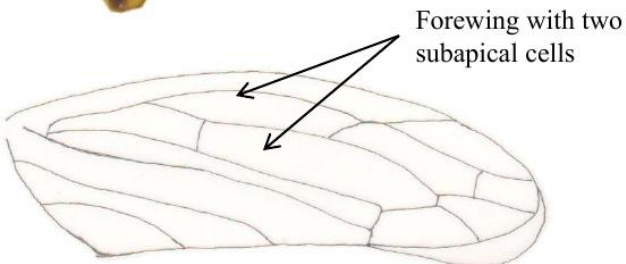

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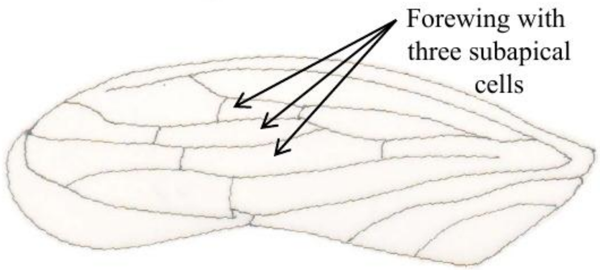

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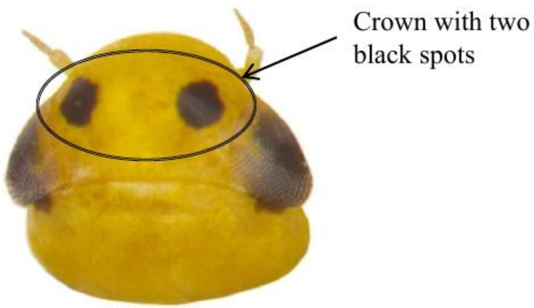

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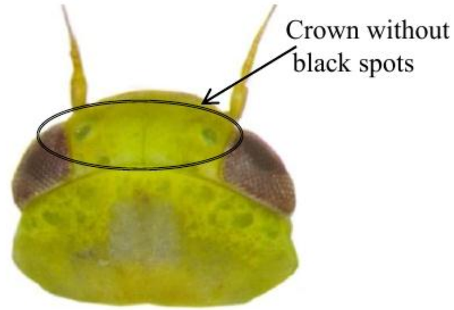


Plate 35

4. Aedeagus robust with three or more pairs of basal processes (Plate 38)……………………………………………………*Balclutha incisa*

- Aedeagus without processes (Plate 39)……..…….………*Balclutha saltuella*

5. Forewing claval veins fused for some distance; head, thorax and forewings with dark brown irregular spots on creamy background (Plate 40); aedeagus with paired shafts (Plate 41)….…..………………………......*Orosius albicinctus*

- Forewing claval veins atmost connected by a crossvein; aedeagus with one shaft……..6

6. Head in dorsal view distinctly longer medially than against eye margin, so that the crown is not parallel sided (Plate 42)..…..………………………….……..7

- Head in dorsal view about as long medially as against eye margin so that crown more or less parallel sided (Plate 43)..……………………….……...9

7. Aedeagus and connective fused and linear, gonopore not well defined (Plate 44)…………………………………………….…........*Maiestas pruthi*

- Aedeagus and connective articulated, gonopore well defined (Plate 45)...........8

8. Aedeagus long, strongly anteriorly recurved at base; subgenital plate without membranous appendage covered with hair like setae (Plate 46)..….*Stirellus* sp.

- Aedeagus not as above but with ventral processes; subgenital plate with membranous appendage covered with hair like setae (Plate 47)……………………………………………………*Monobazus dissimilis*

9. Forewing appendix going round the outer of wing margin; male pygofer with 1-4 black stout setae (Plate 48)...……..……………….……......*Exitianus indicus*

- Forewing appendix short, not going round the outer wing margin; male pygofer without black stout setae but apical ventral margin serrate (Plate 49).………...*Cenedaeus horvathi*

III. Typhlocybinae

1. Hind wing with submarginal vein present at wing apex (Plate 50); green species (Plate 51)……….…………………………....……….Empoascini

- Hind wing submarginal vein absent at wing apex (Plate 52); creamy white species (Plate 53)… ……………………………..Erythroneurini: *Seriana* sp.

2. Forewing with black spot near apex (Plate 54)…...*Amrasca biguttula biguttula*

- Forewing without black spot (Plate 55)……..…………..……..………………3

3. Pygofer process attenuated, spine like (Plate 56)……..………*Empoasca kerri*

- Pygofer ventral appendage lobe like, rounded (Plate 57).....*Empoasca simbava*

4.1.2 DNA analysis

Total genomic DNA of twenty six different *Empoasca* spp was isolated and the concentration DNA was checked using Nandrop. The concentration of DNA varied from 111.1 to 136.2 ng/µL in twenty six different *Empoasca* spp. samples (Table 7). In most of the cases, the 260:280 absorbance ratios satisfied for pure DNA requirements and varied between 1.71-1.88 (Table 7).

Total genomic DNA of twenty six different *Empoasca* spp was amplified by PCR using universal mitochondrial COI gene primer corresponds to DNA bar coding region of the MtCOI gene. A single fragment of approximately 690 bp of COI was successfully amplified in nine samples and remaining seventeen samples were not amplified (Plate 58). The PCR amplified product (~690 bp) was eluted from the agarose gel using Nucleospin Extract II as per manufacture's protocol (Machery Nagal, Germany).

4.1.2.1 Cloning and Transformation

PCR products obtained from amplification of COI gene were gel eluted and ligated to TA cloning vector pTZ57R/T. For the efficient cloning process, the *Escherichia coli* (DH5α) cells were prepared by growing the *E. coli* culture in Luria-Bertani (LB) broth (containing Nalidixic acid) for overnight, incubated in C-media for 1 hour in 37°C incubator and stored in liquid nitrogen. The ligated PCR products were incubated along with competent DH5α cells. Transformed and non-transformed cells were selected by Blue-white screening (Plate 59) which is a rapid and efficient technique for the identification of recombinant bacteria. It relies on the activity of β-galactosidase, an enzyme occurring in *E. coli*, which cleaves lactose into glucose and galactose. The presence of lactose in the surrounding environment triggers the lacZ operon in *E. coli*. The operon activity results in the production of β-galactoisdase enzyme that metabolizes the lactose. Most plasmid vectors carry a short segment of lacZ gene that contains coding information for the first 146 amino acids of β-galactosisdase. The host *E. coli* strains used are competent cells containing lacZΔM15 deletion mutation. When the plasmid vector is taken up by such cells, due to α-complementation process, a functional β-galatosidase enzyme is produced. The plasmid vectors used in cloning are manipulated in such a way that this α- complementation

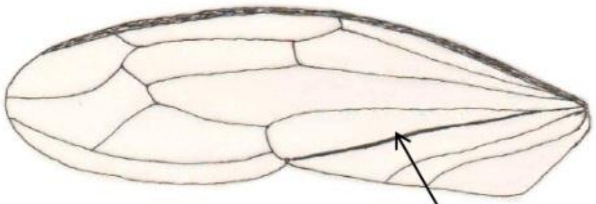
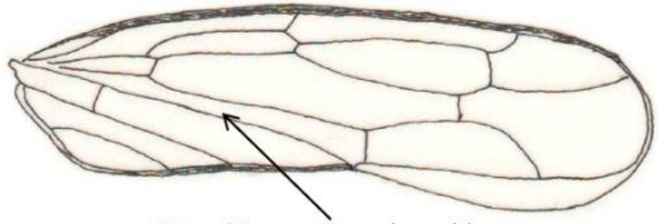


Plate 36



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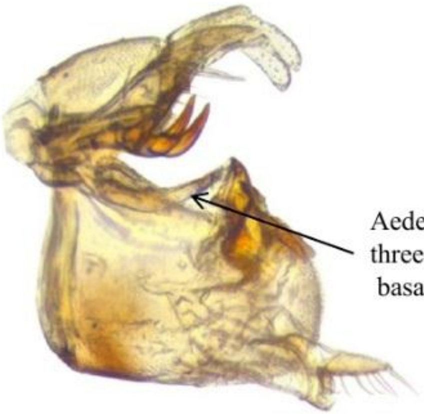

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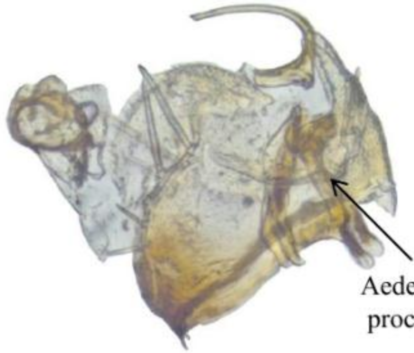
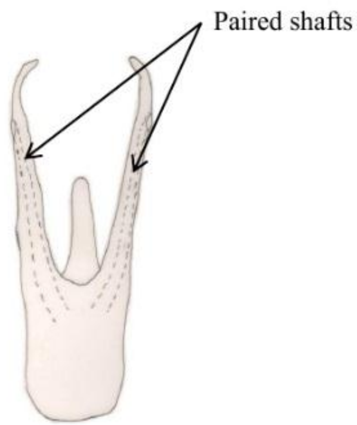


Plate 39

Plate 40



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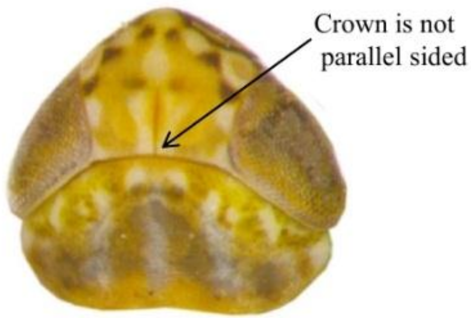

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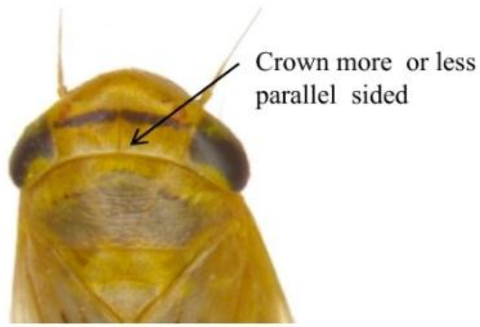

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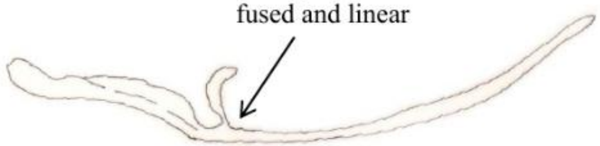


Plate 44

Plate 45

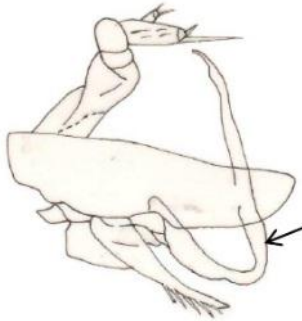


Plate 46

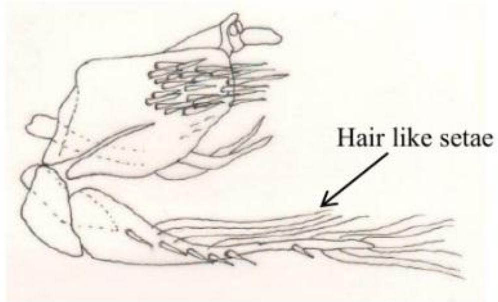
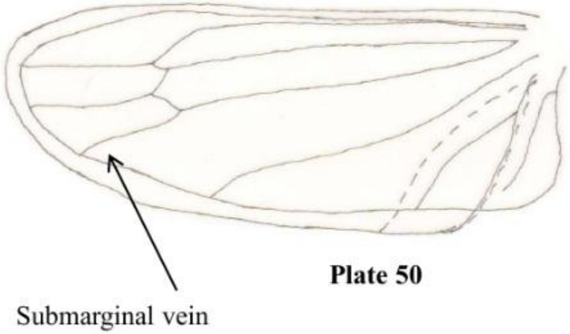


Plate 47

Plate 48

Plate 49



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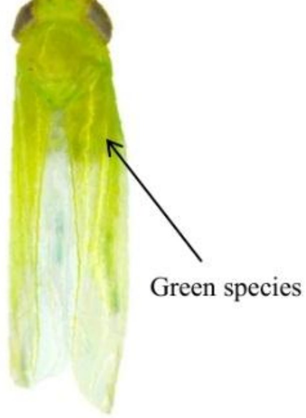

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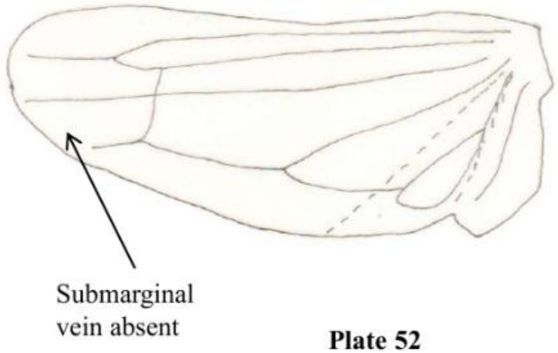

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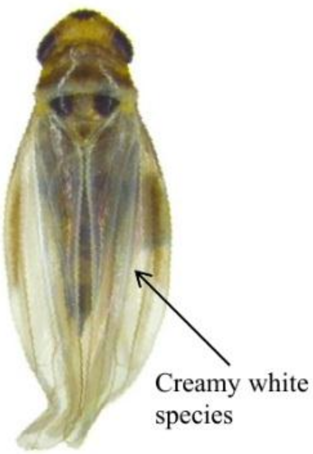

Plate 53

Plate 54

Plate 55

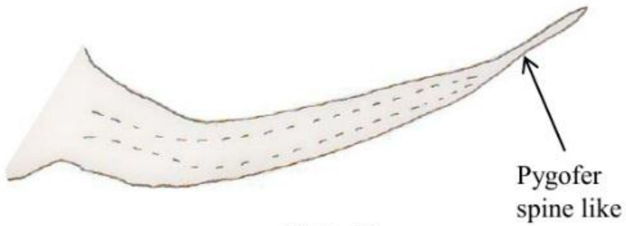

Plate 56

Plate 57

Table 6. *Empoasca* specimens collected from major potato growing regions of Karnataka and their NCBI accession number

Sl. No.	Species	Place of collection	Coordinates	Date of collection	NCBI Accession number
1	*Empoasca* sp. 1	Chikkamagaluru-Lingadahalli	13°37’N75°49’,753m	19.xi.17	MH182662
2	*Empoasca* sp. 2	Belgaum-Kodoli	15°57’N74°30’,741m	14.ix.17	MH182663
3	*Empoasca* sp. 3	Belgaum	15°57’N74°29’,758m	14.ix.17	MH182664
4	*Empoasca* sp. 4	Belgaum-Bambarga	15°57’N74°29’,758m	14.ix.17	MH182665
5	*Empoasca* sp. 5	Belgaum	15°57’N74°29’,758m	14.ix.17	MH182666
6	*Empoasca* sp. 6	Belgaum	15°57’N74°29’,758m	14.ix.17	MH182667
7	*Empoasca* sp. 7	Dharwad-Navloor	15°26’N75°03’,702m	15.ix.17	MH182668
8	*Empoasca* sp. 8	Chikkaballapur-Yaluvahalli	13°21’N77°43’,934m	17.i.18	MH182669
9	*Empoasca* sp. 9	Chikkaballapur	13°21’N77°43’,934m	17.i.18	MH182670

Table 7. DNA yield of *Empoasca* samples and absorption ratio

Sl. No.	Sample	Concentration (ng/µl)	Ratio (260/280 ηm)*
1	*Empoasca* sp. 1	112.9	1.72
2	*Empoasca* sp. 2	122.7	1.76
3	*Empoasca* sp. 3	120.7	1.83
4	*Empoasca* sp. 4	111.1	1.85
5	*Empoasca* sp. 5	136.2	1.81
6	*Empoasca* sp. 6	118.5	1.87
7	*Empoasca* sp. 7	114.4	1.73
8	*Empoasca* sp. 8	135.4	1.88
9	*Empoasca* sp. 9	126.7	1.77

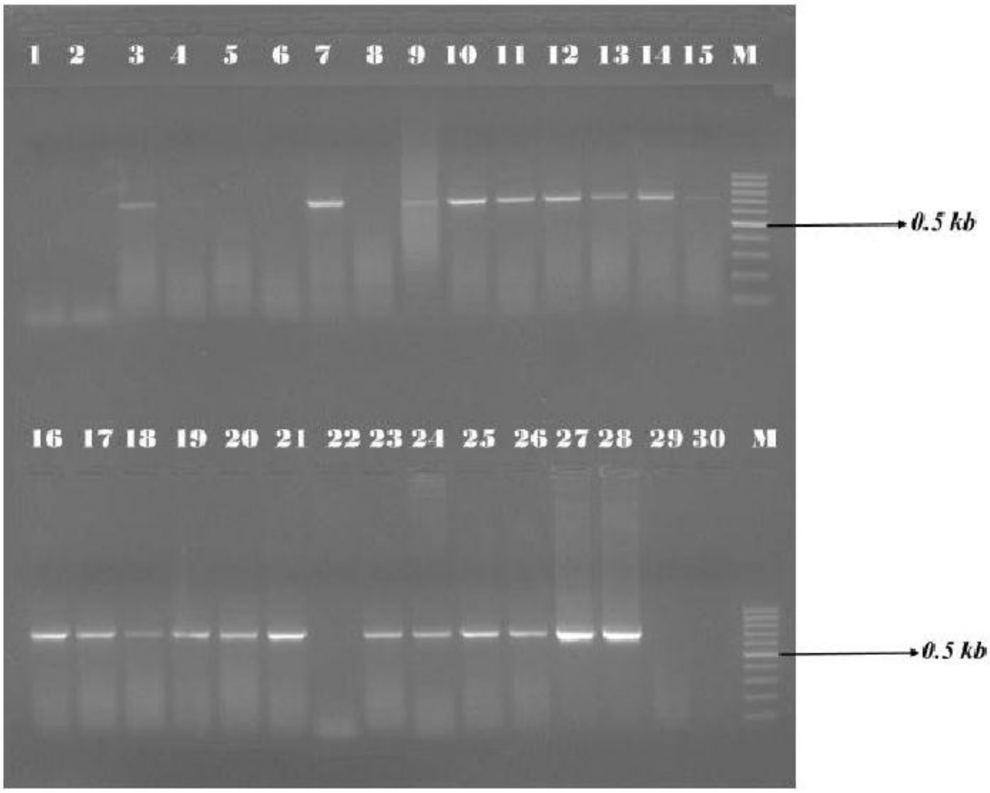

Plate 58: Agarose (1.5%) gel electrophoresis of PCR using Cytochrome oxidase subunit I (COI) gene products of leafhopper samples

Legend: **M**-Molecular weight marker (100 bp; Thermo Scientific USA); **Legends: 1**- *Empoasca* sp. Chikkamagaluru (Lingadahalli); **2**- *Empoasca* sp. Chikkamagaluru (Lingadahalli); **3**- *Empoasca* sp. Chikkamagaluru (Lingadahalli); **4**- *Empoasca* sp. Chikkamagaluru (Lingadahalli); **5**- *Empoasca* sp. Chikkamagaluru (Malligenahalli); **6**- *Empoasca* sp. Chikkamagaluru (Lingadahalli); **7**- *Empoasca* sp. Chikkamagaluru (Malligenahalli); **8**- *Empoasca* sp. Belgaum (Kodoli); **9**- *Empoasca* sp. Belgaum; **10**- *Empoasca* sp. Belgaum (Kodoli); **11**- *Empoasca* sp. Belgaum (Kodoli); **12**- *Empoasca* sp. Belgaum (Bambarga); **13**- *Empoasca* sp. Belgaum; **14**- *Empoasca* sp. Belgaum; **15**- *Empoasca* sp. Belgaum (Bambarga); **16**- *Empoasca* sp. Belgaum (Kodoli); **17**- *Empoasca* sp. Belgaum (Bambarga); **18**- *Empoasca* sp. Dharwad (Navloor); **19**- *Empoasca* sp. Dharwad (Navloor); **20**- *Empoasca* sp. Dharwad (Navloor); **21**- *Empoasca* sp. Dharwad (Navloor); **22**- *Empoasca* sp. Chikkaballapur; **23**- *Empoasca* sp. Chikkaballapur; **24**- *Empoasca* sp. Chikkaballapur; **25**- *Empoasca* sp. Chikkaballapur (Yaluvahalli); **26** *Empoasca* sp. Chikkaballapur (Yaluvahalli), Leafhopper samples; **27** & **28**- Genomic Positive control; **29**-Genomic negative control; **30**-Water negative control.

Plate 59: Overnight (37°C) grown transformed colonies on LB-ampicillin X-Gal/IPTG agar plates of *Empoasca* leafhopper samples

process serves as a marker for recombination. A multiple cloning site (MCS) is present within the lacZ sequence in the plasmid vector. This sequence can be nicked by restriction enzymes to insert the foreign DNA. When a plasmid vector containing foreign DNA is taken up by the host *E. coli*, the α-complementation does not occur, therefore, a functional β-galactosidase enzyme is not produced. If the foreign DNA is not inserted into the vector or if it is inserted at a location other than multiple cloning site, the lacZ gene in the plasmid vector complements the lacZ deletion mutation in the host *E. coli* producing a functional enzyme. For a better selection of transformed cells substreaking was followed on LB agar with ampicillin and smeared with Xgal and IPTG (Plate 60). Later white (transformed colonies with CO-I gene insert) colonies from the sub-streaked plates were selected and subjected to colony PCR for reconfirmation of the correct transformants.

4.1.2.2 Colony PCR for confirmation of correct transformants

Colony PCR is used to screen for plasmids containing a desired insert directly from bacterial colonies. The transformed white colonies were selected to carry out the colony PCR for the confirmation of cloned fragments using COI gene primer. The resulted colony PCR amplicon 690bp was detected in the agarose gel leafhopper (*Empoasca* spp), which corresponds to the original PCR fragments (Plate 61).

4.1.2.3 Plasmid Isolation

The colony PCR confirmed white colonies were inoculated in LB broth (containing ampicillin) for multiplication. Plasmids were isolated from the multiplied bacterial cells and thus isolated plasmids were visualized on 1% agarose gel electrophoresis and compared with control plasmids (without insert) that was provided with ligation kit (Thermo Scientific, Fermentas, Lithuania) (Plate 62). These purified recombinant plasmids containing CO-I gene were dissolved in 30µl of nuclease-free water. From this 10 µl dissolved plasmid were sent for Sanger's sequencing to MWG, Bangalore. Sequencing reactions were performed in both M13 forward and reverse direction. The rest of the plasmids were preserved in -80°C.

4.1.2.4 Sequencing and in silico analysis

DNA sequencing involves the determination of the sequence of nucleotides in a sample of DNA. DNA sequence data was used to identify the specimen from which the DNA was obtained, and to compare sequences to one another. A chromatogram is a type of data file produced by a DNA sequencing instrument. In the DNA chromatogram, each DNA base is represented as a peak (A point where the signal intensity from a fluorescent dye is stronger than the intensity in the surrounding areas. Each colored peak represents a different DNA nucleotide (green for adenine, red for thymine, blue for cytosine, and black for guanine) of a different color (Plate 63). The

DNA sequencing instrument "reads" the concentration measurement for each base and uses that data to determine the most likely identity for each base at each position. Sequencing instruments also produce text files showing the identity and order of all the bases (the DNA sequence).

4.1.2.5 Sequence editing

The raw sequences that were received from Eurofins, Bangalore were analysed using bioinformatics tools such as NCBI-BLAST and BioEdit v.7.0.5.3. The complete datasets were aligned (separately for MtCOI gene region of leafhopper species) employing sequence alignment editor, BioEdit v.7.0.5.3. The sequence data of *Empoasca* species was submitted to NCBI and got Gene Bank accession numbers (Table 6).

They could only be identified till *Empoasca* spp. as there was no prior genomic information. Some specimens showed more than 90 per cent similarity with *Empoasca onukii* Matsuda, *E. fabae* Harris, *E. decipiens* Paoli and *E. vitis* Gothe. Sequencing comparision proves that all *Empoasca* spp. are different (Plate 63).

4.2 Population dynamics of leafhoppers infesting potato in Southern Transitional Zone of Karnataka

Studies on population dynamics of leafhopper in *Kharif* and *Rabi* seasons was conducted, using plant count, sweep net and sticky traps during 2017-18 and data is presented in Table 8.

4.2.1 Leafhopper monitoring on potato plants

During *Kharif*, leafhoppers were first observed in July second fortnight (0.20). Maximum number of leafhoppers were recorded during first fortnight of October (16.00) followed by second fortnight of September (10.30). During *Rabi*, leafhoppers were first observed in second fortnight of January (0.20). Maximum number of leafhoppers were recorded during second fortnight of March (14.00) followed by first fortnight of March (8.60) (Table 8).

4.2.2 Leafhopper monitoring using sweep nets

During *Kharif*, leafhoppers were first observed in July second fortnight (0.60). Maximum number of leafhoppers were recorded during first fortnight of October (19.40) followed by second fortnight of September (13.20). During *Rabi*, leafhoppers were first observed in second fortnight of January (0.50). Maximum number of leafhoppers were recorded during second fortnight of March (17.44) followed by first fortnight of March (9.23) (Table 8).

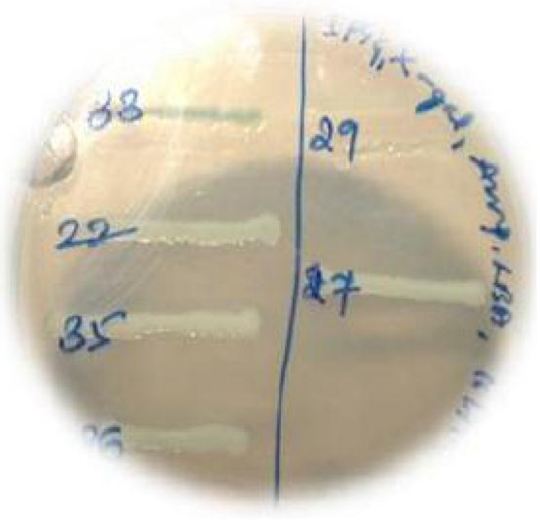

Plate 60: Sub-streaking of Transformed clones on LB-ampicillin X-Gal/IPTG agar

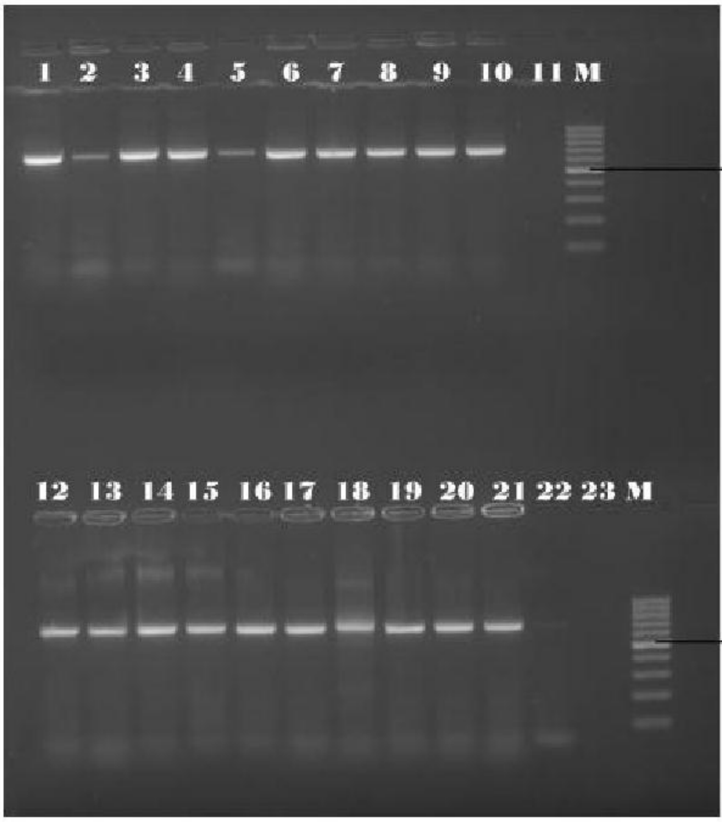

Plate 61: Agarose (1.5%) gel electrophoresis of Colony PCR of transformed clones using Cytochrome oxidase subunit I (COI) gene PCR amplified products of leafhopper samples

Legend: **M**-Molecular weight marker (100 bp; Thermo Scientific, USA); **Legends: 1**- *Empoasca* sp. Chikkamagaluru (Lingadahalli); **2**- *Empoasca* sp. Chikkamagaluru (Malligenahalli); **3**- *Empoasca* sp. Belgaum; **4**- *Empoasca* sp. Belgaum (Kodoli); **5**- *Empoasca* sp. Belgaum (Kodoli); **6**- *Empoasca* sp. Belgaum (Bambarga); **7**- - *Empoasca* sp. Belgaum; **8**- *Empoasca* sp. Belgaum; **9**- *Empoasca* sp. Belgaum (Bambarga); **10**- *Empoasca* sp. Belgaum (Kodoli); **12**- *Empoasca* sp. Belgaum (Bambarga); **13**- *Empoasca* sp. Dharwad (Navloor); **14**- *Empoasca* sp. Dharwad (Navloor); **15**- *Empoasca* sp. Dharwad (Navloor); **16**- *Empoasca* sp. Dharwad (Navloor); **17**- *Empoasca* sp. Chikkaballapur; **18**- *Empoasca* sp. Chikkaballapur; **19**- *Empoasca* sp. Chikkaballapur; **20**- *Empoasca* sp. Chikkaballapur (Yaluvahalli); **21**- *Empoasca* sp. Chikkaballapur (Yaluvahalli); Leafhopper species transformed colonies, **11**-Genomic Negative control. **12-21-** Leafhopper transformed colonies**; 22 & 23-**Water control.

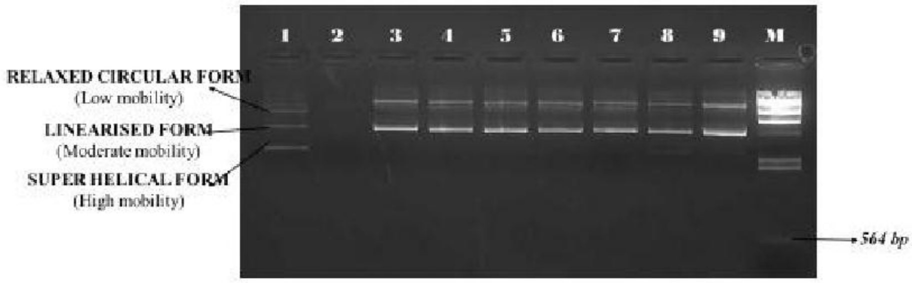

Plate 62: Plasmid mobility profile of the COI genes recombinant clones of leafhopper samples

1- plasmid of blue colony (Colony without insert); 2- Intentionally left blank; **3-9**: Recombinant clones; **M**- Molecular weight marker -Lambda DNA/HindIII Marker (Thermo Scientific, USA).

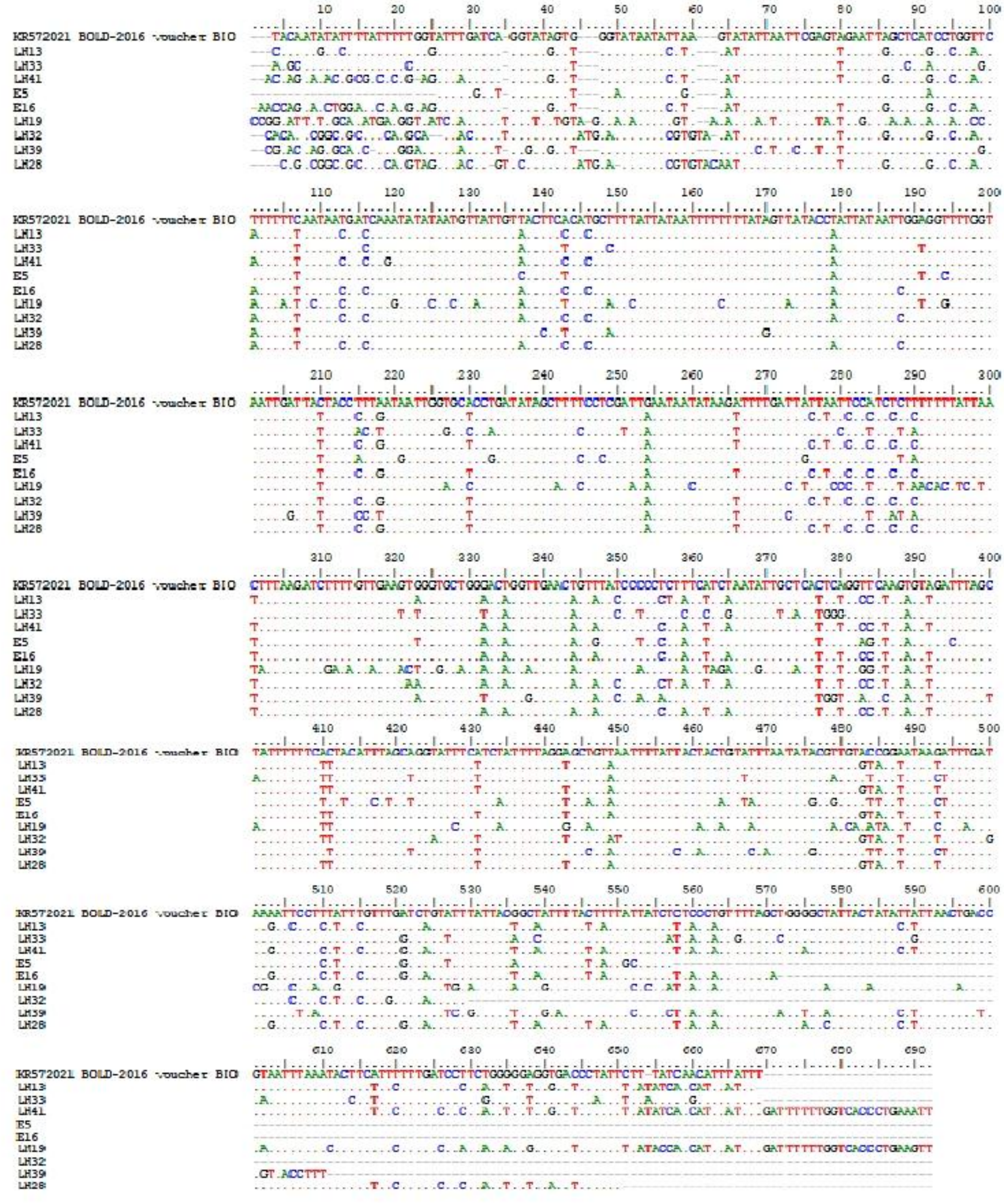

Plate 63: Consensus sequence of 650 bp from the mitochondrial cytochrome oxidase I (COI) gene for *Empoasca* species complex

Table 8. Population dynamics of leafhoppers infesting potato during *Kharif* 2017 and *Rabi* 2017-18

Season	Kharif	Number of leafhoppers		
	Date	**Plantcount***	**Sweepnet#**	**Stickytrap@**
***Kharif*, 2017**	1/7/2017	0.00	0.00	0.00
	15/7/2017	0.20	0.00	0.00
	30/7/2017	1.60	0.60	2.00
	14/8/2017	1.60	2.80	3.50
	28/8/2017	6.00	10.20	7.50
	13/9/2017	8.40	10.00	9.00
	28/9/2017	10.30	13.20	14.50
	13/10/2017	16.00	19.40	19.00
***Rabi*, 2017-18**	17/12/2017	0.00	0.00	0.00
	2/1/2018	0.00	0.00	0.60
	18/1/2018	0.20	0.50	0.80
	2/2/2018	1.60	2.40	2.00
	17/2/2018	4.00	6.20	4.80
	2/3/2018	8.60	9.23	9.10
	17/3/2018	14.00	17.44	16.73

* Number per plant (34 plants-3 compound leaves per plant)

Number per 20 sweeps (average of 20 sweeps in 5 representative sites)

@ Number per yellow sticky trap (average of 2 traps)

4.2.3 Leafhopper monitoring on traps

During *Kharif*, leafhoppers were first observed in July second fortnight (2.00). Maximum number of leafhoppers were recorded during first fortnight of October (19.00) followed by second fortnight of September (14.50). During *Rabi*, leafhoppers were first observed in first fortnight of January (0.60). Maximum number of leafhoppers were recorded during second fortnight of March (16.73) followed by first fortnight of March (9.10) (Table 8).

4.2.4 Correlation and regression between potato leafhoppers with weather parameters (Kharif 2017)

Correlation and regression analysis between potato leafhoppers and weather parameters during *Kharif* (2017) are presented in Table 9. The correlation coefficient between weather parameters and plant count of leafhoppers showed non-significant positive correlation with minimum temperature (r= 0.172), relative humidity (r=0.282) and sunshine hours (r=0.527) a non-significant negative correlation was observed with maximum temperature (r=-0.589) and total rainfall (r=-0.063) and significant negative correlation was observed with wind speed (r=-0.865*). The equation obtained when the data subjected to multiple linear regression analysis was Y= -213.765 -4.627 X1 -2.877 X2+4.996 X3+0.111 X4-10.404 X5-4.163 X6+5.108, with an R2 value of 0.88, which indicated that 88 per cent of total variation in plant count of leafhoppers could be explained by above six weather parameters. The equation obtained when the data subjected to stepwise regression analysis was Y= 24.245 -6.661 X5, with an R2 value of 0.74, which indicated that 74 per cent of total variation in number of leafhoppers could be explained by wind speed parameter.

The correlation coefficient between weather parameters and sweep net count of leafhoppers showed non-significant positive correlation with minimum temperature (r= 0.089), relative humidity (r=0.354) and sunshine hours (r=0.614) and non-significant negative correlation was observed with maximum temperature (r=-0.634) and total rainfall (r=-0.157) and significant negative correlation was observed with wind speed (r=-0.855*). The equation obtained when the data subjected to multiple linear regression analysis was Y= -111.209 -4.851 X1 -3.819 X2 +4.192 X3 -0.064 X4 -11.236 X5 -3.319 X6 +7.404 with an R2 value of 0.85, which indicated that 85 per cent of total variation in plant count of leafhoppers could be explained by above six weather parameters. The equation obtained when the data subjected to stepwise regression analysis was Y= 30.431 -8.322 X5, with an R2 value of 0.73, which indicated that 73 per cent of total variation in number of leafhoppers could be explained by wind speed alone.

The correlation coefficient between weather parameters and sticky trap count of leafhoppers showed non-significant positive correlation with minimum temperature (r= 0.113), relative humidity (r=0.238) and sunshine hours (r=0.517) and non-significant negative correlation was observed with maximum temperature (r=-0.677) and total rainfall (r=-0.033) and significant negative correlation was observed with wind speed (r=-0.840*). The equation obtained when the data subjected to multiple linear regression analysis was Y= -84.829 -7.317 X1 -3.028 X2 +4.272 X3 +0.237 X4 -10.851 X5 -3.883 X6 +6.565 with an R2 value of 0.87, which indicated that 87 per cent of total variation in plant count of leafhoppers could be explained by above six weather parameters. The equation obtained when the data subjected to stepwise regression analysis was Y= 29.028 -7.854 X5, with an R2 value of 0.70, which indicated that 70 per cent of total variation in number of leafhoppers could be explained by wind speed alone.

4.2.5 Correlation and regression between potato leafhoppers with weather parameters (Rabi 2017-18)

Correlation and regression analysis between potato leafhoppers and weather parameters during *Rabi* (2017-18) are presented in Table 9. The correlation coefficient between weather parameters and plant count of leafhoppers showed non-significant positive correlation with maximum temperature (r= 0.172), minimum temperature (r= 0.435), total rainfall (r=0.400) and wind speed (r=0.487) and non-significant negative correlation was observed with relative humidity (r=-0.200) and sunshine hours (r=-0.376).

The correlation coefficient between weather parameters and sweep net count of leafhoppers showed non-significant positive correlation with maximum temperature (r= 0.257), minimum temperature (r= 0. 345), total rainfall (r=0.486) and wind speed (r=0.430) and non-significant negative correlation was observed with relative humidity (r=-0.097) and sunshine hours (r=-0.464).

The correlation coefficient between weather parameters and sticky trap count of leafhoppers showed non-significant positive correlation with maximum temperature (r= 0.196), minimum temperature (r= 0.368), total rainfall (r=0.423) and wind speed (r=0.443) and non-significant negative correlation was observed with relative humidity (r=-0.145) and sunshine hours (r=-0.398).

Since there is no significant relationship of weather parameters with leafhoppers, regression was not performed.

Table 9. Correlation coefficient (r) and coefficient of determination (R_2) between weather parameters and population dynamics of potato leafhoppers, during *Kharif* 2017 and *Rabi* 2017-18

Particulars	Correlation coefficient ('r' value)						Coefficient of determination (R_2)
	Temperature (^0C)		Relative humidity (%) X_3	Total rainfall (mm) X_4	Wind speed (km/h) X_5	Sunshine (hours/day) X_6	
	Maximum (^0C) X_1	Minimum(^0C) X_2					
***Kharif* 2017[@]**							
Plantcount	-0.589	0.172	0.282	-0.063	-0.865*	0.527	0.88
Sweepnet	-0.634	0.089	0.354	-0.157	-0.855*	0.614	0.85
Stickytrap	-0.677	0.113	0.238	-0.033	-0.840*	0.517	0.87
***Rabi* 2017-18[#]**							
Plantcount	0.176	0.435	-0.200	0.400	0.487	-0.376	-
Sweepnet	0.257	0.345	-0.097	0.486	0.430	-0.464	-
Stickytrap	0.196	0.368	-0.145	0.423	0.443	-0.398	-

[@]N = 8; *Significance at p = 0.05; Table r value at p=0.05 is 0.707;

[#]N=7; *Significance at p = 0.05; Table r value at p=0.05 is 0.754

- Plant count- Number per plant (34 plants-3 compound leaves per plant)
- Sweep net count- Number per 20 sweeps (average of 20 sweeps in 5 representative sites)
- Sticky trap count- Number per yellow sticky trap (average of 2 traps)

4.3 Varietal preference of potato leafhoppers

4.3.1 Influence of trichomes on incidence of potato leafhoppers

Highest number of trichomes was found in Kufri Jyothi (218.00) followed by FL-5 (79.00) and lowest was found in S-6 (39.30) followed by FC-3 (72.50). Lowest number of leafhoppers were recorded in Kufri Jyothi followed by FL-5 (Table 10).

4.3.2 Estimation of biochemical components of different potato varieties

4.3.2.1 Estimation of total sugars by Nelson Somgys method

Highest amount of total sugars (mg/g) was recorded in S-6 (7.65 mg/g) followed by FC-3 (7.10 mg/g) and lowest was found in Kufri Jyothi (5.01 mg/g) followed by FL-5 (6.20 mg/g) (Table 11).

4.3.2.2 Estimation of free phenols by Folin - Ciocoltuave reagent

Highest amount of phenols (mg/g) was recorded in Kufri Jyothi (1.08 mg/g) followed by FL-5 (0.80 mg/g) and lowest was found in S-6 (0.36 mg/g) followed by FC-3 (0.43 mg/g) (Table 11).

4.3.2.3 Estimation of potasium by Jackson method

Highest amount of potassium (%K) was recorded in Kufri Jyothi (7.80%) followed by FL-5 (5.20%) and lowest was found in S-6 (3.30%) followed by FC-3 (3.80%) (Table 11).

The correlation coefficient between trichomes, biochemical components and incidence of potato leafhoppers in different varieties are presented in Table 11. Leafhoppers showed significant positive correlation with TSS (r= 0.983*), significant negative correlation with phenols (r=-0.965*) and non-significant negative correlation was observed with trichomes (r=-0.878) and potassium (r=-0.946).

4.4 Evaluation of selected insecticides against potato leafhoppers

Different insecticides were evaluated against leafhoppers infesting potato at Lingadahalli village of Chikkamagaluru district during *Kharif* 2017 and *Rabi* 2017-18 seasons.

4.4.1 Kharif 2017

One day before imposition of treatments, the number of leafhoppers in plant count ranged from 5.93 to 3.73 and in sweep net count (20 sweeps) ranged from 6.00 to 3.66. Differences in number of leafhoppers in plant count and in sweep net between the treatments were statistically non-significant (Table 12 and 13).

Table 10. Trichomes density and leafhoppers incidence on different potato varieties

Variety	Trichomes#	Number of Leafhoppers*			Mean number of leafhoppers
		16-08-17	30-08-17	15-09-17	
Kufri Jyothi	218.00	4.40	4.7	6.3	5.12
FL-5	79.00	7.62	7.3	9	7.98
FC-3	72.50	9.40	11	12	10.78
S-6	39.80	13.10	14	15	13.96

*Number of leafhoppers per plant

#Number of trichomes per cm^2

Table 11. Relationship between morphological and biochemical components with population of leafhoppers

Variety	Mean number of leafhoppers	Trichomes	TSS (mg/g)	Phenols (mg/g)	Potassium (%K)
Kufri Jyothi	5.12	218.00	5.01	1.08	7.80
FL-5	7.98	79.00	6.20	0.80	5.20
FC-3	10.78	72.50	7.10	0.43	3.80
S-6	13.96	39.80	7.65	0.36	3.30

Correlation coefficient values

	Trichomes	TSS (mg/g)	Phenols (mg/g)	Potassium (%K)
Leafhoppers	-0.878	0.983*	-0.965*	-0.946

#N=3; *Significance at p = 0.05; Table r value at p=0.05 is 0.950

I spray

Observation after 7 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population in plant count was recorded with imidacloprid17.8 SL @ 0.3ml/l (0.86), which was followed by dinotefuran 20 SG @ 0.3g/l (1.47) and higher leafhopper population per plant was recorded in acephate + imidacloprid 51.8 SP @ 2g/l (2.40) followed by dimethoate 30 EC @ 1.7ml/l (2.00). In control, leafhopper population was 3.40 per plant which was significantly higher compared to other treatments (Table 12).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (0.67), which was followed by dimethoate 30 EC @ 1.7ml/l (1.00) and high leafhopper population per plant was recorded in acephate + imidacloprid 51.8 SP @ 2g/l (2.33) followed by thiamethoxam 25 WG @ 0.5g/l and NSKE 5% (2.00). In control, leafhopper population was 4.33 per 20 sweeps which was significantly higher compared to other treatments (Table 13).

Observation after 15 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (0.93), which was followed by acetamiprid20 SP @ 0.3g/l (2.40) and higher leafhopper population per plant was recorded in acephate 75 SP @ 1.5g/l (4.20) followed by thiamethoxam 25 WG @ 0.5g/l (3.80). In control, leafhopper population was 4.66 per plant which was significantly higher compared to other treatments (Table 12).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (1.33), which was followed by acephate 75 SP @ 1.5g/l (2.33) and high leafhopper population per plant was recorded in acephate + imidacloprid 51.8 SP @ 2g/l, dimethoate 30 EC @ 1.7ml/l, acetamiprid 20 SP @ 0.3g/l (4.67) followed by dinotefuran 20 SG @ 0.3g/l (4.00). In control, leafhopper population was 5.66 per 20 sweeps which was significantly higher compared to other treatments (Table 13).

II spray

Observation after 7 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (0.40), which was followed by thiamethoxam 25 WG @ 0.5g/l (0.867) and higher leafhopper population per plant was recorded in dimethoate 30 EC @ 1.7ml/l (1.40) followed by acephate + imidacloprid 51.8 SP @ 2g/l (1.27). In control, leafhopper population was 3.13 per plant which was significantly higher compared to other treatments (Table 12).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (0.33), which was followed by thiamethoxam 25 WG @ 0.5g/l and dinotefuran 20 SG @ 0.3g/l (1.00). High leafhopper population per plant was recorded in NSKE 5% (2.00) followed by acephate 75 SP @ 1.5g/l and dimethoate 30 EC @ 1.7ml/l (1.66). In control, leafhopper population was 3.33 per 20 sweeps which was significantly higher compared to other treatments (Table 13).

Observation after 15 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (1.00), which was followed by acetamiprid 20 SP @ 0.3g/l (3.06). Higher leafhopper population per plant was recorded in dimethoate 30 EC @ 1.7ml/l (3.86) followed by dinotefuran 20 SG @ 0.3g/l (3.46). In control, leafhopper population was 4.73 per plant which was significantly higher compared to other treatments (Table 12).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (1.00), which was followed by acetamiprid 20 SP @ 0.3g/l, thiamethoxam 25 WG @ 0.5g/l and dinotefuran 20 SG @ 0.3g/l (2.00) and high leafhopper population per plant was recorded in acephate + imidacloprid 51.8 SP @ 2g/l (3.33) followed by dimethoate 30 EC @ 1.7ml/l (3.00). In control, leafhopper population was 4.33 per 20 sweeps which was significantly higher compared to other treatments (Table 13).

4.4.2 Rabi 2017-18

One day before imposition of treatments, the number of leafhoppers in plant count ranged from 12.66 to 5.40 and in sweep net count ranged from 8.66 to 6.66. Differences in number of leafhoppers in plant count and in sweep net between the treatments were statistically non-significant (Table 14 and 15).

I spray

Observation after 7 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (1.27), which was followed by acephate + imidacloprid 51.8 SP @ 2g/l (1.67) and higher leafhopper population per plant was recorded in acetamiprid 20 SP @ 0.3g/l (2.53) followed by NSKE 5% (2.47). In control, leafhopper population was 3.80 per plant which was significantly higher compared to other treatments (Table 14).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (0.66), which was followed by acetamiprid 20 SP

Table 12. Evaluation of selected insecticides against potato leafhoppers during *Kharif* 2017 (Plant count)

Treatments	Number of leafhoppers/ 3 compound leaves/ plant[#]				
	1DBS	1^{st} Spray		2^{nd} Spray	
		7DAS	15DAS	7DAS	15DAS
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	4.06 (1.996)	0.86 $(1.16)^{d}$	0.93 $(0.95)^{d}$	0.40 $(0.93)^{c}$	1.00 $(0.98)^{c}$
T_2 - Acetamiprid 20 SP @ 0.3g/l	3.73 (1.91)	1.73 $(1.49)^{bcd}$	2.40 $(1.54)^{c}$	1.13 $(1.27)^{b}$	3.06 $(1.74)^{b}$
T_3 - Acephate75 SP @ 1.5g/l	5.93 (2.43)	1.60 $(1.44)^{bcd}$	4.20 $(2.04)^{ab}$	1.13 $(1.27)^{b}$	3.20 $(1.78)^{b}$
T_4 - Thiamethoxam25 WG @ 0.5g/l	5.67 (2.37)	1.66 $(1.46)^{bcd}$	3.80 $(1.92)^{ab}$	0.86 $(1.13)^{bc}$	3.33 $(1.82)^{b}$
T_5 - Dinotefuran 20 SG @ 0.3g/l	4.86 (2.18)	1.46 $(1.33)^{cd}$	3.40 $(1.83)^{abc}$	1.06 $(1.24)^{bc}$	3.46 $(1.85)^{b}$
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	4.33 (2.06)	2.40 $(1.70)^{ab}$	3.13 $(1.76)^{bc}$	1.26 $(1.32)^{b}$	3.20 $(1.78)^{b}$
T_7 - Dimethoate 30 EC @ 1.7ml/l	4.60 (2.09)	2.00 $(1.58)^{bc}$	3.26 $(1.79)^{bc}$	1.40 $(1.37)^{b}$	3.86 $(1.96)^{ab}$
T_8 - NSKE 5%	4.13 (1.96)	1.86 $(1.53)^{bc}$	3.26 $(1.80)^{abc}$	1.06 $(1.25)^{bc}$	3.40 $(1.82)^{b}$
T_9 - Control	5.26 (2.27)	3.40 $(1.97)^{a}$	4.66 $(2.15)^{a}$	3.13 $(1.89)^{a}$	4.73 $(2.17)^{a}$
SEm ±	NS	0.11	0.11	0.10	0.08
CD (P= 0.05)	NS	0.34	0.35	0.32	0.26
CV %	-	13.01	11.67	14.51	8.71

[#]- Mean number of leafhoppers per three compound leaves per plant; Figures in parenthesis are $\sqrt{X+0.5}$ are transformed values;
DBS- Day before spray: DAS- Days after spray

Table 13. Evaluation of selected insecticides against potato leafhoppers during *Kharif* 2017 (Sweep net count)

Treatments	Number of leafhoppers/ 20 sweeps#				
	1DBS	1st Spray		2nd Spray	
		7DAS	15DAS	7DAS	15DAS
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	4.33 (2.07)	0.66 $(1.05)^{c}$	1.33 $(1.13)^{d}$	0.33 $(0.88)^{c}$	1.00 $(1.17)^{c}$
T_2 - Acetamiprid 20 SP @ 0.3g/l	4.00 (1.98)	1.66 $(1.46)^{bc}$	4.66 $(2.15)^{ab}$	1.33 $(1.34)^{ab}$	2.00 $(1.58)^{bc}$
T_3 - Acephate75 SP @ 1.5g/l	3.66 (1.91)	1.33 $(1.29)^{bc}$	2.33 $(1.52)^{cd}$	1.66 $(1.46)^{ab}$	2.66 $(1.77)^{ab}$
T_4 - Thiamethoxam25 WG @ 0.5g/l	5.33 (2.30)	2.00 $(1.55)^{bc}$	3.33 $(1.82)^{bc}$	1.00 $(1.22)^{bc}$	2.00 $(1.55)^{bc}$
T_5 - Dinotefuran 20 SG @ 0.3g/l	6.00 (2.40)	1.00 $(1.17)^{bc}$	4.00 $(1.98)^{ab}$	1.00 $(1.17)^{bc}$	2.00 $(1.55)^{bc}$
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	5.00 (2.21)	2.33 $(1.67)^{ab}$	4.66 $(2.13)^{ab}$	1.33 $(1.34)^{bc}$	3.33 $(1.95)^{ab}$
T_7 - Dimethoate 30 EC @ 1.7ml/l	5.66 (2.37)	1.33 $(1.34)^{bc}$	4.66 $(2.15)^{ab}$	1.66 $(1.46)^{bc}$	3.00 $(1.85)^{ab}$
T_8 - NSKE 5%	4.66 (2.15)	2.00 $(1.58)^{b}$	3.33 $(1.79)^{bc}$	2.00 $(1.55)^{ab}$	2.33 $(1.64)^{b}$
T_9 - Control	5.00 (2.22)	4.33 $(2.19)^{a}$	5.66 $(2.37)^{a}$	3.33 $(1.95)^{a}$	4.33 $(2.19)^{a}$
SEm ±	NS	0.17	0.14	0.13	0.14
CD (P= 0.05)	NS	0.52	0.44	0.41	0.43
CV %	-	20.41	13.64	17.47	14.64

#- a total of 20 sweeps were made in each treatment; Figures in parenthesis are $\sqrt{X+0.5}$ are transformed values;
DBS- Day before spray: DAS- Days after spray

@ 0.3g/l, NSKE 5% and acephate 75 SP @ 1.5g/l (1.33) and high leafhopper population per plant was recorded in thiamethoxam 25 WG @ 0.5g/l, dinotefuran 20 SG @ 0.3g/l and dimethoate 30 EC @ 1.7ml/l (2.00). In control, leafhopper population was 3.33 per 20 sweeps which was significantly higher compared to other treatments (Table 15).

Observation after 15 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (1.60), which was followed by acephate + imidacloprid 51.8 SP @ 2g/l (2.53) and higher leafhopper population per plant was recorded in dimethoate 30 EC @ 1.7ml/l (3.07) followed by thiamethoxam 25 WG @ 0.5g/l (3.00). In control, leafhopper population was 4.33 per plant which was significantly higher compared to other treatments (Table 14).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid (1.33), which was followed by acephate + imidacloprid 51.8 SP @ 2g/l (1.67) and high leafhopper population per plant was recorded in NSKE 5% (3.00) followed by acetamiprid 20 SP @ 0.3g/l (2.66). In control, leafhopper population was 4.67 per 20 sweeps which was significantly higher compared to other treatments (Table 15).

II spray

Observation after 7 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (0.33), which was followed by acetamiprid 20 SP @ 0.3g/l and acephate + imidacloprid 51.8 SP @ 2g/l (1.33) and higher leafhopper population per plant was recorded in thiamethoxam 25 WG @ 0.5g/l and dimethoate 30 EC @ 1.7ml/l (2.33) followed by acephate 75 SP @ 1.5g/l and dinotefuran 20 SG @ 0.3g/l (2.00). In control, leafhopper population was 3.33 per plant which was significantly higher compared to other treatments (Table 14).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (0.33), which was followed by thiamethoxam 25 WG @ 0.5g/l (0.66) and high leafhopper population per plant was recorded in acephate 75 SP @ 1.5g/l (1.66) followed by acetamiprid 20 SP @ 0.3g/l, dinotefuran 20 SG @ 0.3g/l and NSKE 5% (1.33). In control, leafhopper population was 3.00 per 20 sweeps which was significantly higher compared to other treatments (Table 15).

Observation after 15 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (1.00), which was followed by acephate 75 SP @ 1.5g/l (2.33) and higher leafhopper population per plant was recorded in thiamethoxam and acephate + imidacloprid 51.8 SP @ 2g/l (3.33) followed by dinotefuran 20 SG @ 0.3g/l (3.00). In control, leafhopper population was 5.66 per plant which was significantly higher compared to other treatments (Table 14).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (1.00), which was followed by acetamiprid 20 SP @ 0.3g/l (1.67). High leafhopper population per plant was recorded in thiamethoxam 25 WG @ 0.5g/l and dinotefuran 20 SG @ 0.3g/l (3.00) followed by dimethoate 30 EC @ 1.7ml/l (2.66). In control, leafhopper population was 5.00 per 20 sweeps which was significantly higher compared to other treatments (Table 15).

4.4.3 Pooled data on evaluation of selected insecticides against potato leafhoppers

The observations on number of leafhoppers in both seasons was pooled *i.e. Kharif* 2017 and *Rabi* 2017-18.

One day before imposition of treatments, the number of leafhoppers in plant count ranged from 8.30 to 4.33 and in sweep net count (20 sweeps) ranged from 6.52 to 3.71. Differences in number of leafhoppers in plant count and in sweep net between the treatments were statistically non-significant (Table 16 and 17).

I spray

Observation after 7 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population in plant count was recorded with imidacloprid17.8 SL @ 0.3ml/l (1.36), which was followed by dinotefuran 20 SG @ 0.3g/l (1.53) and higher leafhopper population per plant was recorded in acetamiprid20 SP @ 0.3g/l (2.67) followed by NSKE 5% (2.42). In control, leafhopper population was 3.28 per plant which was significantly higher compared to other treatments (Table 16).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (0.68), which was followed by acephate 75 SP @ 1.5g/l (1.69) and high leafhopper population per plant was recorded in NSKE 5% (2.53) followed by acephate + imidacloprid 51.8 SP @ 2g/l (2.43). In control, leafhopper population was 3.69 per 20 sweeps which was significantly higher compared to other treatments (Table 17).

Observation after 15 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (1.09), which was followed by acetamiprid20 SP @ 0.3g/l (2.00) and higher leafhopper population per plant was

Table 14. Evaluation of selected insecticides against potato leafhoppers during *Rabi* 2017-18 (Plant count)

Treatments	Number of leafhoppers/ 3 compound leaves/ plant[#]				
	1DBS	1st Spray		2nd Spray	
		7DAS	15DAS	7DAS	15DAS
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	7.13 (2.64)	1.26 $(1.12)^{e}$	1.60 $(1.26)^{e}$	0.33 $(0.88)^{c}$	1.00 $(1.171)^{c}$
T_2 - Acetamiprid 20 SP @ 0.3g/l	7.86 (2.73)	2.53 $(1.59)^{b}$	2.60 $(1.61)^{cd}$	1.33 $(1.34)^{b}$	2.66 $(1.76)^{b}$
T_3 - Acephate75 SP @ 1.5g/l	6.00 (2.43)	2.13 $(1.45)^{bcd}$	2.86 $(1.69)^{bc}$	2.00 $(1.58)^{ab}$	2.33 $(1.67)^{b}$
T_4 - Thiamethoxam25 WG @ 0.5g/l	5.40 (2.31)	1.86 $(1.36)^{cd}$	3.00 $(1.73)^{b}$	2.33 $(1.67)^{ab}$	3.33 $(1.95)^{b}$
T_5 - Dinotefuran 20 SG @ 0.3g/l	12.66 (2.53)	2.33 $(1.52)^{bc}$	2.73 $(1.65)^{bcd}$	2.00 $(1.55)^{ab}$	3.00 $(1.85)^{b}$
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	7.13 (2.65)	1.66 $(1.29)^{de}$	2.53 $(1.58)^{d}$	1.33 $(1.34)^{b}$	3.33 $(1.95)^{b}$
T_7 - Dimethoate 30 EC @ 1.7ml/l	5.86 (2.40)	1.93 $(1.37)^{cd}$	3.06 $(1.75)^{b}$	2.33 $(1.678)^{ab}$	2.66 $(1.76)^{b}$
T_8 - NSKE 5%	6.46 (2.53)	2.46 $(1.56)^{b}$	2.73 $(1.65)^{bcd}$	1.66 $(1.44)^{b}$	2.66 $(1.77)^{b}$
T_9 - Control	8.60 (2.90)	3.80 $(1.94)^{a}$	4.33 $(2.08)^{a}$	3.33 $(1.95)^{a}$	5.66 $(2.84)^{a}$
SEm ±	NS	0.06	0.03	0.14	0.14
CD (P= 0.05)	NS	0.18	0.10	0.43	0.42
CV %	-	7.21	3.64	16.29	13.56

[#]- Mean number of leafhoppers per three compound leaves per plant; Figures in parenthesis are $\sqrt{X+0.5}$ are transformed values;
DBS- Day before spray: DAS- Days after spray

Table 15. Evaluation of selected insecticides against potato leafhoppers during *Rabi* 2017-18 (Sweep net count)

Treatments	Number of leafhoppers/ 20 sweeps[#]				
	1DBS	1st Spray		2nd Spray	
		7DAS	15DAS	7DAS	15DAS
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	8.66 (2.94)	0.66 $(1.05)^{c}$	1.33 $(1.13)^{d}$	0.33 $(0.88)^{c}$	1.00 $(1.00)^{d}$
T_2 - Acetamiprid 20 SP @ 0.3g/l	7.33 (2.69)	1.33 $(1.34)^{bc}$	2.66 $(1.62)^{bc}$	1.33 $(1.34)^{ab}$	1.66 $(1.27)^{cd}$
T_3 - Acephate75 SP @ 1.5g/l	6.66 (2.57)	1.33 $(1.29)^{bc}$	2.00 $(1.38)^{bcd}$	1.66 $(1.46)^{ab}$	2.00 $(1.38)^{bcd}$
T_4 - Thiamethoxam25 WG @ 0.5g/l	7.33 (2.70)	2.00 $(1.55)^{b}$	2.00 $(1.41)^{bcd}$	0.66 $(1.05)^{bc}$	3.00 $(1.71)^{b}$
T_5 - Dinotefuran 20 SG @ 0.3g/l	7.33 (2.69)	2.00 $(1.58)^{ab}$	2.33 $(1.52)^{bc}$	1.33 $(1.34)^{bc}$	3.00 $(1.73)^{b}$
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	8.00 (2.79)	1.66 $(1.46)^{b}$	1.66 $(1.27)^{cd}$	1.00 $(1.17)^{bc}$	2.33 $(1.52)^{bc}$
T_7 - Dimethoate 30 EC @ 1.7ml/l	7.66 (2.76)	2.00 $(1.55)^{b}$	2.33 $(1.52)^{bc}$	1.00 $(1.22)^{bc}$	2.66 $(1.62)^{bc}$
T_8 - NSKE 5%	7.00 (2.63)	1.33 $(1.34)^{bc}$	3.00 $(1.71)^{b}$	1.33 $(1.29)^{bc}$	2.33 $(1.48)^{bc}$
T_9 - Control	7.67 (2.764)	3.33 $(1.95)^{a}$	4.66 $(2.15)^{a}$	3.00 $(1.85)^{a}$	5.00 $(2.22)^{a}$
SEm ±	NS	0.12	0.12	0.16	0.14
CD (P= 0.05)	NS	0.38	0.36	0.49	0.43
CV %	-	15.35	13.65	22.01	16.29

[#]- a total of 20 sweeps were made in each treatment; Figures in parenthesis are $\sqrt{X+0.5}$ are transformed values;
DBS- Day before spray: DAS- Days after spray

recorded in acephate 75 SP @ 1.5g/l (3.67) followed by dimethoate 30 EC @ 1.7ml/l (3.62). In control, leafhopper population was 4.96 per plant which was significantly higher compared to other treatments (Table 16).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (1.07), which was followed by acephate 75 SP @ 1.5g/l (1.49) and high leafhopper population per plant was recorded in dinotefuran 20 SG @ 0.3g/l (3.64) followed by NSKE 5% (3.39). In control, leafhopper population was 5.39 per 20 sweeps which was significantly higher compared to other treatments (Table 17).

II spray

Observation after 7 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (0.33), which was followed by acetamiprid20 SP @ 0.3g/l (1.50) and higher leafhopper population per plant was recorded in NSKE 5% (2.54) followed by dimethoate 30 EC @ 1.7ml/l (2.30). In control, leafhopper population was 4.35 per plant which was significantly higher compared to other treatments (Table 16).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (0.35), which was followed by thiamethoxam 25 WG @ 0.5g/l and dinotefuran 20 SG @ 0.3g/l (1.32). High leafhopper population per plant was recorded in acephate 75 SP @ 1.5g/l (1.98) followed by dinotefuran 20 SG @ 0.3g/l (1.97). In control, leafhopper population was 3.98 per 20 sweeps which was significantly higher compared to other treatments (Table 17).

Observation after 15 days of treatment imposition, the results showed significant differences among the treatments. Lowest leafhopper population per plant was recorded with imidacloprid17.8 SL @ 0.3ml/l (0.92), which was followed by acetamiprid 20 SP @ 0.3g/l (2.73). Higher leafhopper population per plant was recorded in acephate + imidacloprid 51.8 SP @ 2g/l (4.40) followed by thiamethoxam 25 WG @ 0.5g/l (3.93). In control, leafhopper population was 6.15 per plant which was significantly higher compared to other treatments (Table 16).

Similarly, in sweep net counts, lowest leafhopper population was recorded in imidacloprid17.8 SL @ 0.3ml/l (1.00), which was followed by acetamiprid 20 SP @ 0.3g/l, (1.72) and high leafhopper population per plant was recorded in NSKE 5% (3.05) followed by dimethoate 30 EC @ 1.7ml/l (2.87). In control, leafhopper population was 4.55 per 20 sweeps which was significantly higher compared to other treatments (Table 17).

Table 16. Evaluation of selected insecticides against potato leafhoppers (Pooled data of plant count in two seasons[@] 2017-18)

Treatments	Number of leafhoppers/ 3 compound leaves/ plant[#]				
	1DBS	1[st] Spray		2[nd] Spray	
		7DAS	15DAS	7DAS	15DAS
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	5.19 (2.27)	1.36 $(1.15)^{d}$	1.09 $(1.04)^{d}$	0.33 $(0.57)^{d}$	0.92 $(0.96)^{c}$
T_2 - Acetamiprid 20 SP @ 0.3g/l	4.33 (2.06)	2.67 $(1.62)^{ab}$	2.00 $(1.40)^{c}$	1.50 $(1.22)^{c}$	2.73 $(1.61)^{b}$
T_3 - Acephate75 SP @ 1.5g/l	5.28 (2.28)	1.82 $(1.34)^{cd}$	3.67 $(1.91)^{ab}$	2.08 $(1.43)^{bc}$	3.19 $(1.77)^{b}$
T_4 - Thiamethoxam25 WG @ 0.5g/l	5.42 (2.32)	1.86 $(1.36)^{cd}$	3.39 $(1.83)^{b}$	2.29 $(1.50)^{bc}$	3.93 $(1.98)^{b}$
T_5 - Dinotefuran 20 SG @ 0.3g/l	8.30 (2.87)	1.53 $(1.22)^{d}$	2.82 $(1.65)^{bc}$	1.85 $(1.35)^{bc}$	3.33 $(1.82)^{b}$
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	4.61 (2.13)	2.38 $(1.54)^{bc}$	3.24 $(1.79)^{b}$	1.96 $(1.39)^{bc}$	4.40 $(2.04)^{ab}$
T_7 - Dimethoate 30 EC @ 1.7ml/l	5.14 (2.26)	1.72 $(1.31)^{cd}$	3.62 $(1.83)^{b}$	2.30 $(1.50)^{bc}$	3.53 $(1.86)^{b}$
T_8 - NSKE 5%	5.43 (2.32)	2.42 $(1.54)^{bc}$	3.18 $(1.78)^{b}$	2.54 $(1.56)^{b}$	3.43 $(1.84)^{b}$
T_9 - Control	5.41 (2.29)	3.28 $(1.81)^{a}$	4.96 $(2.26)^{a}$	4.35 $(2.07)^{a}$	6.15 $(2.47)^{a}$
SEm ±	NS	0.08	0.10	0.10	0.15
CD (P= 0.05)	NS	0.25	0.32	0.30	0.47
CV %	-	10.03	11.38	13.12	15.05

[@]- pooled data of two seasons *i.e. Kharif* (2017) and *Rabi* (2017-18); [#]- Mean number of leafhoppers per three compound leaves per plant;
Figures in parenthesis are $\sqrt{X+0.5}$ are transformed values; DBS- Day before spray: DAS- Days after spray

Table 17. Evaluation of selected insecticides against potato leafhoppers (Pooled data of sweep net count in two seasons[@] 2017-18)

Treatments	Number of leafhoppers/ 20 sweeps[#]				
	1DBS	1^{st} Spray		2^{nd} Spray	
		7DAS	15DAS	7DAS	15DAS
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	5.87 (2.41)	0.68 $(0.82)^{c}$	1.07 $(1.03)^{e}$	0.35 $(0.58)^{d}$	1.00 $(0.99)^{d}$
T_2 - Acetamiprid 20 SP @ 0.3g/l	6.19 (2.47)	1.70 $(1.30)^{b}$	3.12 $(1.76)^{bc}$	1.69 $(1.29)^{bc}$	1.72 $(1.28)^{cd}$
T_3 - Acephate75 SP @ 1.5g/l	3.71 (1.89)	1.69 $(1.26)^{b}$	1.49 $(1.21)^{de}$	1.98 $(1.40)^{b}$	2.74 $(1.65)^{b}$
T_4 - Thiamethoxam25 WG @ 0.5g/l	6.49 (2.54)	2.06 $(1.42)^{b}$	2.58 $(1.60)^{bc}$	1.32 $(1.04)^{c}$	2.30 $(1.51)^{bc}$
T_5 - Dinotefuran 20 SG @ 0.3g/l	6.52 (2.55)	1.70 $(1.30)^{b}$	3.64 $(1.90)^{b}$	1.97 $(1.37)^{bc}$	2.26 $(1.48)^{bc}$
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	5.63 (2.33)	2.43 $(1.51)^{ab}$	2.45 $(1.52)^{b}$	1.62 $(1.26)^{bc}$	2.41 $(1.54^{bc}$
T_7 - Dimethoate 30 EC @ 1.7ml/l	6.18 (2.48)	1.85 $(1.35)^{b}$	3.00 $(1.72)^{bc}$	1.74 $(1.31)^{bc}$	2.87 $(1.69)^{b}$
T_8 - NSKE 5%	5.24 (2.25)	2.53 $(1.56)^{ab}$	3.39 $(1.83)^{bc}$	1.88 $(1.37)^{bc}$	3.05 $(1.73)^{b}$
T_9 - Control	5.57 (2.31)	3.69 $(1.91)^{a}$	5.39 $(2.32)^{a}$	3.98 $(1.98)^{a}$	4.55 $(2.13)^{a}$
SEm ±	NS	0.13	0.11	0.11	0.11
CD (P= 0.05)	NS	0.41	0.34	0.34	0.34
CV %	-	17.32	11.99	15.34	12.73

[@]- pooled data of two seasons *i.e. Kharif* (2017) and *Rabi* (2017-18); [#]- a total of 20 sweeps were made in each treatment;
Figures in parenthesis are $\sqrt{X+0.5}$ are transformed values; DBS- Day before spray: DAS- Days after spray

4.4.3 Cost economics of various treatments used against leafhoppers

Economics of insecticide sprays during the course of study has been presented in Table 18 and 19.

4.4.3.1 Kharif season

Considering the cost effectiveness of various treatments, the maximum net returns were registered in T_1 *i.e.,* imidacloprid17.8 SL @ 0.3ml/l (149812) with C:B ratio of (1:3.62) followed by T_4 *i.e.,* thiamethaxam 25 WG @ 0.5g/l (132010) with C:B ratio of (1:3.34). Lowest C:B ratio was recorded in T_9 *i.e.,* untreated check (1:2.71) (Table 18).

4.4.3.2 Rabi season

Considering the cost effectiveness of various treatments, the maximum net returns were registered in T_1 *i.e.,* imidacloprid17.8 SL @ 0.3ml/l (195140) with C:B ratio of (1:4.15) followed by T_1 *i.e.,* thiamethaxam 25 WG @ 0.5g/l (181010) with ratio of (1:3.96). Lowest C:B ratio was recorded in T_9 *i.e.,* untreated check (1:2.96) (Table 19).

4.4.3.3 Pooled data on Cost economics of various treatments used against leafhoppers

Economics of insecticides sprays of both seasons was pooled *i.e. Kharif* 2017 and *Rabi* 2017-18 and been presented in Table 20.

Considering the cost effectiveness of various treatments, the maximum net returns were registered in T_1 *i.e.,* imidacloprid17.8 SL @ 0.3ml/l (172476) with C: B ratio of (1:3.90) followed by T_4 *i.e.,* thiamethaxam 25 WG @ 0.5g/l (156510) with ratio of (1:3.67). Lowest C: B ratio was recorded in T_9 *i.e.,* untreated check (1:2.84).

Table 18. Effect of selected insecticides on yield and cost economics (*Kharif* 2017)*

Treatments	Yield (t/ha)	Cost of cultivation	Cost of treatment(Rs/ha)	Total cost (Rs/ha)	Gross Returns (Rs/ha)	Net Returns (Rs/ha)	C:B
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	16.08	51300	1920	53220	193032	139812	1:3.62
T_2 - Acetamiprid 20 SP @ 0.3g/l	14.51	51300	3000	54300	174180	119880	1:3.20
T_3 - Acephate75 SP @ 1.5g/l	14.07	51300	600	51900	168852	116952	1:3.25
T_4 - Thiamethoxam25 WG @ 0.5g/l	14.5	51300	750	52050	174060	122010	1:3.34
T_5 - Dinotefuran 20 SG @ 0.3g/l	14.87	51300	2130	53430	178440	125010	1:3.33
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	13.46	51300	3000	54300	161532	107232	1:2.97
T_7 - Dimethoate 30 EC @ 1.7ml/l	14.38	51300	1080	52380	172656	120276	1:3.29
T_8 - NSKE 5%	13.35	51300	500	51800	160200	108400	1:3.09
T_9 - Control	11.62	51300	-	51300	139500	88200	1:2.71

(Price of potato = Rs. 12,000/t); * Variety- Kufri Jyothi

Note: cost of insecticides

T1: Imidacloprid17.8 SL - Rs 1370/500ml
T2: Acetamiprid 20 SP - Rs 750/250g
T3: Acephate75 SP - Rs 185/250g
T4: Thiomethaxam25 WG - Rs 390/100g
Cost of labour: Rs 250/day

T5: Dinutefuron 20 SG - Rs. 452/50g
T6: Acephate + Imidachloprid 51.8 SP - Rs 250/250g
T7: Dimethoate 30 EC - Rs 63/100ml
T8: NSKE 5% - Rs 10/1000ml
Number of labourers required per spray /ha = 2

Table 19. Effect of selected insecticides on yield and cost economics (*Rabi* 2017-18)*

Treatments	Yield (t/ha)	Cost of cultivation	Cost of treatment(Rs/ha)	Total cost (Rs/ha)	Gross Returns (Rs/ha)	Net Returns (Rs/ha)	C:B
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	16.26	56840	1920	58760	243900	185140	1:4.15
T_2 - Acetamiprid 20 SP @ 0.3g/l	14.44	56840	3000	59840	216630	156790	1:3.62
T_3 - Acephate75 SP @ 1.5g/l	14.94	56840	600	57440	224100	166660	1:3.90
T_4 - Thiamethoxam25 WG @ 0.5g/l	15.24	56840	750	57590	228600	171010	1:3.96
T_5 - Dinotefuran 20 SG @ 0.3g/l	14.19	56840	2130	58970	212895	153925	1:3.61
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	13.99	56840	3000	59840	209925	150085	1:3.50
T_7 - Dimethoate 30 EC @ 1.7ml/l	14.72	56840	1080	57920	220815	162895	1:3.81
T_8 - NSKE 5%	13.8	56840	500	57340	207090	149750	1:3.61
T_9 - Control	11.24	56840	-	56840	168615	111775	1:2.96

(Price of potato = Rs. 15,000/t); * Variety- Kufri Jyothi

Note: cost of insecticides

T1: Imidacloprid17.8 SL - Rs 1370/500ml
T2: Acetamiprid 20 SP - Rs 750/250g
T3: Acephate75 SP - Rs 185/250g
T4: Thiomethaxam25 WG - Rs 390/100g
Cost of labour: Rs 250/day

T5: Dinutefuron 20 SG - Rs. 452/50g
T6: Acephate + Imidachloprid 51.8 SP - Rs 250/250g
T7: Dimethoate 30 EC - Rs 63/100ml
T8: NSKE 5% - Rs 10/1000ml
Number of labourers required per spray /ha = 2

Table 20. Effect of selected insecticides on yield and cost economics (Pooled data of two seasons[@] 2017-18)

Treatments	Yield (t/ha)	Cost of cultivation	Cost of treatment(Rs/ha)	Total cost (Rs/ha)	Gross Returns (Rs/ha)	Net Returns (Rs/ha)	C:B
T_1 - Imidacloprid17.8 SL @ 0.3ml/l	16.17	54070	1920	55990	218466	162476	1:3.90
T_2 - Acetamiprid 20 SP @ 0.3g/l	14.47	54070	3000	57070	195405	138335	1:3.42
T_3 - Acephate75 SP @ 1.5g/l	14.5	54070	600	54670	196476	141806	1:3.59
T_4 - Thiamethoxam25 WG @ 0.5g/l	14.87	54070	750	54820	201330	146510	1:3.67
T_5 - Dinotefuron 20 SG @ 0.3g/l	14.53	54070	2130	56200	195668	139468	1:3.48
T_6 - Acephate + Imidacloprid 51.8 SP @ 2g/l	13.72	54070	3000	57070	185729	128659	1:3.25
T_7 - Dimethoate 30 EC @ 1.7ml/l	14.55	54070	1080	55150	196736	141586	1:3.56
T_8 - NSKE 5%	13.57	54070	500	54570	183645	129075	1:3.36
T_9 - Control	11.43	54070	-	54070	154058	99988	1:2.84

(Price of potato = Rs. 13,500/t); * Variety- Kufri Jyothi; [@]- pooled data of two seasons *i.e. Kharif* (2017) and *Rabi* (2017-18).

Note: cost of insecticides

T1: Imidacloprid17.8 SL - Rs 1370/500ml
T2: Acetamiprid 20 SP - Rs 750/250g
T3: Acephate75 SP - Rs 185/250g
T4: Thiomethaxam25 WG - Rs 390/100g
Cost of labour: Rs 250/day

T5: Dinutefuron 20 SG - Rs. 452/50g
T6: Acephate + Imidachloprid 51.8 SP - Rs 250/250g
T7: Dimethoate 30 EC - Rs 63/100ml
T8: NSKE 5% - Rs 10/1000ml
Number of labourers required per spray /ha = 2

DISCUSSION

V DISCUSSION

The potato leafhopper, (Homoptera: Cicadellidae) is an economically significant pest of field legume crops. It is a lacerate and flush feeder causing phytotoxicity expressed as hopperburn. Potato purple top disease vectored by leafhopers has become increasingly important causing severe yield losses and reduction in tuber quality of potato. Considering the pest potential and vectoring ability, it is necessary to identify them rapidly and accurately. In this perspective, present investigation was taken up to study the "Species composition, ecology and management of leafhoppers (Cicadellidae: Hemiptera) infesting potato". The salient findings of these studies presented in the previous chapter are discussed here.

5.1 Species composition of leafhoppers infesting potato in major potato growing regions of Karnataka

The study was mainly aimed at recording species composition of leafhoppers infesting potato in major potato growing regions of Karnataka which includes Chikkamagaluru, Hassan, Dharwad, Belgaum, Bengaluru and Chikkaballapur in 2017-18. A total of 19 species representing six subfamilies (Evacanthinae, cicadellinae, Iassinae, Megaphthalminae, Deltocephalinae, Typhlocybinae) were identified (Table 5), based on morphological characters of adult leafhoppers. This is the first study from South India which documents presence of very high number of species diversity from potato ecosystem. However, Pantoja *et al.* (2009) recorded as many as 41 species of leafhoppers on potato in Alaska region of United States. Nagaich (1978) documented 20 species of potato leafhoppers from North India. He also suggested the role of leafhopper in transmitting phytoplasma diseases such as purple top roll (PTR), marginal flavescence (MF) and witch's broom (WB).

Out of more than 600 specimens collected across potato growing regions of Karnataka, *Empoasca* spp. contributed high percentage. Similarly, Zohdi *et al.* (2010), reported 11 species of leafhoppers from three potato growing areas (Lalehzar, Bardsir and Mahan) of Iran. They also revealed that *Empoasca decipiens* was the most abundant species in potato fields of Iran. The information generated in this study has utility in understanding plant-phytoplasma-vector epidemiology. The study helps to assess the damage caused by each species and assessing vectoring ability of each species.

The present work revealed existence of number of species under genus *Empoasca.* Through morphological approach, only two species of *Empoasca* viz., *E. kerri* and *E. simbava* were identified. However other species of *Empoasca* could not be identified to species level. The problem associated with *Empoasca* species identification is not new (Ross and Moore, 1957) because of their extremely small size (<4mm) and cryptic behavior. All the other species of potato leafhoppers except *Empoasca* collected during the present study were differentiated clearly based on morphological taxonomy. Hence, *Empoasca* were subjected to molecular analysis as an effort to resolve the species complex.

The mitochondrial COI from *Empoasca* samples were successfully sequenced and sequences generated in this study were deposited to NCBI-GenBank. Analysis with BLAST indicated presence of nine different cryptic species which were different from one another and were considered as *Empoasca* sp. 1-9 as they were not matching with any previously submitted sequence. A total of nine sequences were submitted to data base and accession numbers were obtained. These include, MH182662, MH182663, MH182664, MH182665, MH182666, MH182667, MH182668, MH182669 and MH182670. This indicates that the *Empoasca* species 1-9 to which the accession numbers were obtained in the present study are not been previously documented from India or elsewhere and the genomic information is not available. This indicates they may be new to science or the genomic information of these species are not available in public domain. Further morphological and molecular studies are required to correctly characterize those species which probably have implications in leafhopper taxonomy.

5.2 Population dynamics of leafhoppers infesting potato in Southern Transitional Zone of Karnataka

Successful management of leafhoppers depends on understanding the seasonal fluctuation of the population and seasonal incidence in revealing the pest behaviour. This study carried out during the year 2017-18 in Malligenahalli village, of Tarikere Taluk, Chikkamagaluru District in Karnataka during *Kharif* 2017 and *Rabi* 2017-18 seasons using plant count, sweep net and sticky traps. The locality selected is a traditional potato growing region.

5.2.1 Kharif 2017

Leafhoppers were first observed in July second fortnight after 15 days of sowing. Maximum number of leafhoppers were recorded during first fortnight of October followed by second fortnight of November. Number of leafhoppers were found increasing from second fortnight of July 2017 to first fortnight of October 2017 in all three counts. In this part of Karnataka, as the sowing takes place synchronously in large area, colonization may take few days after crop emergence. As most of the leafhoppers are polyphagous, the role of host plants in population dynamics needs further investigation.

5.2.2 Rabi 2017-2018

Observations were made on the incidence of leafhoppers at fortnight intervals starting from second fortnight of December 2017 to second fortnight of March 2018. Leafhoppers were first observed in first fortnight of January. Maximum number of leafhoppers were recorded during second fortnight of March followed by first fortnight of March. Number of leafhoppers were found increasing from second fortnight of December 2017 to second fortnight of March 2018 in all three counts. The results are in line with Degooyer (1997), who reported that number of leafhoppers collected was greatest on the last sampling date before the harvest. The increase in leafhopper numbers may be due to immigrants and adults generated from in-field reproduction (Flinn *et al.,* 1986; Emmen, 2004).

5.2.3 Correlation and regression analysis between potato leafhoppers with weather parameters

Correlation studies revealed that potato leafhoppers incidence in *Kharif* 2017, showed a significant negative correlation with wind speed, non-significant positive correlation with minimum temperature, relative humidity and sunshine hours. A non-significant negative correlation was observed with maximum temperature and total rainfall. Leafhopper incidence in *Rabi* 2017-18, showed a non-significant positive correlation with maximum temperature, minimum temperature, total rainfall and wind speed and non-significant negative correlation with relative humidity and sunshine hours.

This result exposed that the pest activity decreased with the increase of wind speed, maximum temperature and total rainfall, while it was increased with increase of minimum temperature, relative humidity and sunshine hours in *Kharif* 2017. Leafhopper activity decreased with the increase of maximum temperature, minimum temperature, total rainfall and wind speed, while it was increased with increase of relative humidity and sunshine hours in *Rabi* 2017-18.

Pandey *et al.* (2003) reported that wind speed had positive association with hopper population but this is contradictory with our results.

5.3 Varietal preference of potato leafhoppers

Knowledge on the varietal screening against the incidence of potato leafhoppers is very important, which helps in understanding the effect of morphological and biochemical components on the developmental behaviour of leafhoppers. The variety selected were locally cultivated and are grown for either table purpose or for chips making.

Kufri Jyothi and FL-5 harboured lowest leafhoppers (5.12 and 7.98). Whereas, highest number of leafhoppers was documented in S-6 (13.96) followed by FC-3 (10.78). These trichomes are the important morphological structures which confer resistance against leafhoppers and hence lowest number of leafhoppers were recorded in Kufri Jyothi followed by FL-5.

Previously, various researchers Levin, 1973, Sivasubramanian *et al*. (1991), Dellinger *et al*. (2006), Ahmad *et al.* (1999), Medeiros and Tingey (2006) reported that trichomes was a reliable character for resistance against leafhoppers in various crops. The widely grown variety, Kufri Jyothi recorded low leafhopper population. Even though, in the present study, efforts were not made to study the incidence of phytoplasma in these varieties, investigations are necessary on the tolerance of these varieties to phytoplasma disease as well.

A wide array of chemical substances including inorganic chemicals, primary and intermediary metabolites and secondary substances are known to impart resistance to a wide variety of insect pests. Among the secondary plant metabolites, phenolics are the source of resistance against phytophagous insects and are ubiquitous in plants (Harborne, 1994). Host plant resistance is the result of interactions between two biological entities, the plant and the insect under the influence of various environmental factors (Dhaliwal and Singh, 2004). In the present study biochemical parameters *viz*., total sugars, phenols and potassium were estimated. Results of biochemicals estimation revealed that significantly lower total reducing sugars were noticed in Kufri Jyothi and FL-5 followed by FC-3 and S-6 but higher phenol and potassium content were noticed in varieties like Kufri Jyothi and FL-5 followed by FC-3 and S-6. Correlation studies showed that total sugars had significant positive correlation (r= 0.983*) with the incidence of leafhoppers. Phenols had significantly negative correlation (r=-0.965*) whereas, trichomes (r=-0.878) and potassium (r=-0.946) had non-significant negative correlation with the incidence of leafhoppers.

These results are in line with Vamadevaiah (1999), who reported that an increase in phenol content had negative correlation to leafhopper damage and tolerant lines had less sugar content than susceptible checks of cotton genotypes.

5.4 Evaluation of selected insecticides against potato leafhoppers

Potato is a vital food-security crop and substitute for cereal crop considering its high yield and great nutritive value. It is vulnerable to many pests and diseases implying high risk of crop failure. Hence, a proper pest management program will minimize crop losses.

Among the different insecticides evaluated against leafhoppers during 2017-18, imidacloprid 17.8 SL @ 0.3ml/l recorded lower number of leafhoppers.

Maximum leafhopper population was recorded in untreated check. More yields were recorded in treatment with imidacloprid 17.8 SL @ 0.3ml/l (16.08 t/ha) and (16.26 t/ha) in *Kharif* and *Rabi* respectively. This was obviously due to effective suppression of leafhoppers.

The pooled data of the observations of two seasons indicated that, imidacloprid 17.8 SL @ 0.3ml/l stood first by recording less leafhopper population compared to other treatments. More yields were recorded in treatment with imidacloprid 17.8 SL @ 0.3ml/l (16.17 t/ha) because of effective suppression of leafhoppers.

The effectiveness of imidacloprid in reducing the leafhopper population is in confirmation with Mullins (1993), who reported that imidacloprid is effective chemical against potato leafhoppers. Imidacloprid are active against a broad spectrum of economically important crop pests, including leafhoppers (Cicadellidae) (Elbert *et al.* 2008; Jeschke *et al.* 2011). Imidacloprid, acetamiprid, thiamethoxam and dinotefuran belong to neonicotinoid group. These neonicotinoids act as agonists on nAChRs opening cation channels (Casida and Durkin 2013). As a result, less number of leafhoppers were found in imidacloprid followed by thiamethoxam.

In contrary, Nault *et al*. (2004) reported that thiamethoxam provided longer and more consistent protection of the crop from leafhoppers than imidacloprid. Thiamethoxam is a second generation neonicotinoid insecticide, belonging to the thianicotinyl subclass of chemistry, and possesses unique chemical properties. Thiamethoxam interferes with a specific receptor site in the insect's nervous system. Once leafhoppers come into contact with thiamethoxam, feeding is irreversibly stopped and leafhopper damage halts.

Imidacloprid 17.8 SL @ 0.3ml/l was most effective with highest C:B ratio of (1:3.62) and (1:4.15) in *Kharif* and *Rabi* respectively, thiamethaxam 25 WG @ 0.5g/l stood second in giving returns with C:B ratio of (1:3.34) and (1:3.96) in *Kharif* and *Rabi* respectively. In pooled data also imidacloprid 17.8 SL @ 0.3ml/l stood first with highest C:B ratio of (1:3.90) followed by thiamethaxam 25 WG @ 0.5g/l (1:3.67).

Conclusion

- A total of nineteen species of potato leafhoppers were recorded and illustrated key was developed for easy and quick identification for the species collected
- Among the species collected, *Empoasca* spp. was dominant followed by *Amrasca biguttula biguttula* (Ishida)
- Mitochondrial COI barcoding indicated presence of 9 different species belong to genus *Empoasca*
- The study also highlighted the importance of morphological taxonomy in identification of species complex in case of *Empoasca*
- Leafhopper number increased with the age of the crop. Maximum numbers was recorded during harvesting time
- Among different weather parameters tested, wind speed had significant negative correlation with leafhoppers
- Among different morphological and biochemical characters, trichomes and potassium had non- significantly negative correlation with leafhoppers.
- TSS had significantly positive correlation and phenols had significantly negative correlation with leafhoppers
- Imidacloprid 17.8 SL @ 0.3ml/l recorded significantly lower number of leafhoppers

Future line of work

- Our results stress the utility of using DNA-based method for purposes of molecular identification of *Empoasca* spp.
- Further analysis is required to reveal different species of *Empoasca* through morphological and molecular approaches
- Characterising phytoplasma and evaluating transmission efficiency of different leafhopper species
- Efficient management strategies for both leafhoppers and the diseases they transmit

SUMMARY

VI SUMMARY

The results of the present investigation carried out on species composition, population dynamics, screening of different varieties of potato against leafhoppers and the effect of different insecticides against leafhoppers are summarized in this chapter.

Studies on species composition of potato leafhoppers in major potato growing regions of Karnataka during 2017-18 revealed a total of nineteen species of potato leafhoppers. This is the first study from India which documented presence of high number of leafhopper species from potato ecosystem. Among the species collected, *Empoasca* spp. contributed high percentage.

Molecular technique was employed to resolve *Empoasca* species complex, as their identification through morphological taxonomy was difficult. The mitochondrial COI from *Empoasca* samples were successfully sequenced and sequences generated in this study were deposited to NCBI-GenBank. Analysis with BLAST indicated presence of nine different cryptic species which were different from one another and were considered as *Empoasca* sp. A total of nine sequences were submitted to data base and accession numbers were obtained. These includes, MH182662, MH182663, MH182664, MH182665, MH182666, MH182667, MH182668, MH182669 and MH182670.

Studies on population dynamics of potato leafhoppers from first fortnight of July 2017 to first fortnight of October 2017 during *Kharif* and first fortnight of December 2017 to second fortnight of March 2018 during *Rabi*. During *Kharif*, leafhoppers were first observed in July second fortnight. Maximum number of leafhoppers were recorded during first fortnight of October followed by second fortnight of November. During *Rabi*, leafhoppers were first observed in first fortnight of January. Maximum number of leafhoppers were recorded during second fortnight of March followed by first fortnight of March.

Correlation studies revealed that potato leafhoppers incidence in *Kharif* 2017, showed a significant negative correlation with wind speed, non-significant positive correlation with minimum temperature, relative humidity and sunshine hours. A non-significant negative correlation was observed with maximum temperature and total rainfall. Leafhoppers incidence in *Rabi* 2017-18, showed a non-significant positive correlation with maximum temperature, minimum temperature, total rainfall and wind speed and non-significant negative correlation with relative humidity and sunshine hours.

The pest activity decreased with the increase of wind speed, maximum temperature and total rainfall, while it was increased with increase of minimum temperature, relative humidity and sunshine hours in *Kharif* 2017. Leafhopper activity decreased with the increase of maximum temperature, minimum temperature, total rainfall and wind speed, while it was increased with increase of relative humidity and sunshine hours in *Rabi* 2017-18.

Studies on screening of different varieties of potato against leafhoppers reveal that highest number of trichomes was found in Kufri Jyothi (218.00) followed by FC-5 (79.00) and lowest was found in S-6 (39.30) followed by FC-3 (72.50). Kufri Jyothi and FC-5 harboured lowest number of leafhoppers (5.12 and 7.98) respectively and highest number of leafhoppers was documented in S-6 (13.96) followed by FC-3 (10.78).

Results of biochemicals estimation revealed that significantly lower TSS was noticed in Kufri Jyothi (5.01 mg/g) followed by FC-5 (6.20 mg/g) and higher in S-6 (7.65 mg/g) followed by FC-3 (7.10 mg/g). Higher phenol content was noticed in Kufri Jyothi (1.08 mg/g) followed by FC-5 (0.80 mg/g) and lowest was found in S-6 (0.36 mg/g) followed by FC-3 (0.43 mg/g). Higher potassium content was recorded in Kufri Jyothi (7.80%) followed by FC-5 (5.20%) and lowest was found in S-6 (3.30%) followed by FC-3 (3.80%).

Correlation studies showed that total sugars had significant positive correlation (r=0.983*) with the incidence of leafhoppers. Phenols had significantly negative correlation (r=-0.965*) whereas, trichomes and potassium had non-significant negative correlation (r=-0.878 and r=-0.946) with the incidence of leafhoppers.

Pooled data of nine treatments imposed for the management of potato leafhoppers during 2017-18, Imidacloprid 17.8 SL @ 0.3 ml/lit recorded significantly lower number of leafhoppers. More yields were recorded in treatment with imidacloprid 17.8 SL @ 0.3 ml/lit (16.08 t/ha) and (16.26 t/ha) in *Kharif* and *Rabi* respectively.

The economic analysis of different insecticides, imidacloprid 17.8 SL @ 0.3 ml/lit was most effective by recording highest C:B ratio of (1:3.90). Untreated check recorded the lowest C:B ratio of (1:2.84). All treatments recorded higher C: B ratio and superior over untreated check.

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APPENDICES

VIII Appendices

Appendix 1: Mean fortnight weather data from July 2017 to October 2017 at Meteorological Observatory, AHRS, Bavikere

Fortnight	**Weather parameters**					
	Max. Temp (^{0}C)	Min. Temp (^{0}C)	RH (%)	TR (mm)	WS (km/h)	Sunshine (hours/day)
July I 2017	23.0	27.1	88.0	3.6	3.5	1.8
July II 2017	22.3	26.3	89.7	1.6	3.3	4.3
August I 2017	22.5	26.4	90.0	7.9	3.8	3.8
August II 2017	22.1	26.5	89.7	6.1	2.8	5.8
September I 2017	22.1	26.0	91.8	1.3	3.0	7.9
September II 2017	22.5	27.4	90.2	3.0	1.9	6.0
October I 2017	21.6	26.6	88.8	3.5	2.5	4.5
October II 2017	22.0	26.7	90.3	4.9	1.7	6.4

Appendix 2: Mean fortnight weather data from December 2017 to March 2018 at Meteorological Observatory, AHRS, Bavikere

Fortnight	**Weather parameters**					
	Max. Temp (^{0}C)	Min. Temp (^{0}C)	RH (%)	TR (mm)	WS (km/h)	Sunshine (hours/day)
December II 2017	18.9	26.9	80.1	0.0	2.0	8.4
January I 2018	16.0	26.4	71.4	0.0	2.2	9.0
January II 2018	13.2	26.4	70.4	0.0	3.1	8.6
February I 2018	20.6	26.6	85.5	2.9	2.7	6.0
February II 2018	20.6	26.6	85.5	2.9	2.7	6.0
March I 2018	13.8	32.4	39.5	0.0	4.0	8.8
March II 2018	20.1	27.0	82.2	2.7	2.8	6.2