IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF THRIPS COMPLEX TRANSMITTING THE *Groundnut bud necrosis virus* IN BLACKGRAM AND THEIR MANAGEMENT

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M. Sc. (Ag)

DOCTOR OF PHILOSOPHY IN AGRICULTURE
(ENTOMOLOGY)

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BY

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M. Sc. (Ag)

THESIS SUBMITTED TO THE
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2022

DECLARATION

I, Mr. LELLA RAJASEKHAR hereby declare that the thesis entitled **"Identification and molecular characterization of thrips complex transmitting the *Groundnut bud necrosis virus* in blackgram and their management"** submitted to Acharya N. G. Ranga Agricultural University for the degree of **Doctor of Philosophy in Agriculture** in the major field of Entomology is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier.

Place: Bapatla **(LELLA RAJASEKHAR)**

Date: **ID No: BAD-18-17**

CERTIFICATE

Mr. LELLA RAJASEKHAR has satisfactorily prosecuted the course of research and that the thesis entitled "**IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF THRIPS COMPLEX TRANSMITTING THE *Groundnut bud necrosis virus* IN BLACKGRAM AND THEIR MANAGEMENT"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any university.

Place: Bapatla
Date:

(T. MADHUMATHI)
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CERTIFICATE

This is to certify that the thesis entitled "**IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF THRIPS COMPLEX TRANSMITTING THE *Groundnut bud necrosis virus* IN BLACKGRAM AND THEIR MANAGEMENT"** submitted in partial fulfillment of the requirements for the degree of '**Doctor of Philosophy in Agriculture'** of the Acharya N.G. Ranga Agricultural University, Lam, Guntur is a record of the bonafide original research work carried out by **Mr. LELLA RAJASEKHAR** under our guidance and supervision.

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

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LIST OF SYMBOLS AND ABBREVIATIONS

%		Per cent
/		Per
@		At the rate of
$acre^{-1}$	:	Per acre
ANOVA	:	Analysis of Variance
CD	:	Critical difference
Cm	:	Centimeter
cm^2	:	Square centimeter
cm^{-2}	:	Per square centimeter
et al.	:	And his co workers
etc	:	And so on
Fig.	:	Figure
ha^{-1}	:	Per hectare
hr	:	Hours
i.e.	:	That is
Kg	:	Kilogram
kg ha^{-1}	:	Kilogram per hectare
LSD	:	Least Significant Difference
AAP	:	Acquisition access period
IAP	:	Inoculation access period
ºC	:	Degree Centigrade
PCR	:	Polymerase Chain Reaction
RT-qPCR	:	Real Time Quantitative Polymerase Chain Reaction
RTPCR	:	Reverse Transcriptase Polymerase Chain Reaction
DAS	:	Days after Sowing
µL	:	Micro Liter
mg	:	Milligram
M	:	Molar

mL	:	Milliliter
ITS	:	Internal Transcribed spacer
COI	:	Cytochrome oxidase I
COIII	:	Cytochrome oxidase III
Mt	:	Mitochondrial
Bp	:	Base pair
W/V	:	Weight by volume
Ng	:	Nanogram
MEGA	:	Molecular Evolutionary Genetic Analysis
NCBI	:	National Centre for Biotechnology Information
BLAST	:	Basic Local Alignment Search Tool
DNA	:	Deoxy ribo Nucleic Acid
RNA	:	Ribo Nucleic Acid
MAN	:	Minimum Spanning Network
NJ	:	Neighbor Joining
ML	:	Maximum Likelihood
EC	:	Emulsifible Concentrate
SC	:	Suspension concentrate
G	:	Granules
WG	:	Wettable granules
WP	:	Wettable Powder
PDI	:	Per cent Disease Incidence
SPSS	:	Statistical package for Social Sciences
GBNV	:	Groundnut Bud Necrosis Virus
WBNV	:	Watermelon Bud Necrosis virus
WSMoV	:	Watermelon Silver mottle virus
cDNA	:	Complementary DNA
MB	:	Molecular Biology
mM	:	Millimolar

CP	:	Coat Protein
F	:	Forward
R	:	Reverse
WS	:	Water dispersible powders for slurry treatment
IUPC	:	International Union for Pure and Applied Chemistry
CIBRC	;	Central Insecticide Board and Registration Committee
ICBR	:	Incremental Cost Benefit Ratio
RBD	:	Randomized Block Design
~	:	Approximately
HD	:	Haplotype Diversity
ND	:	Nucleotide Diversity
SMW	:	Standard Meteorological Week
KMPH	:	Kilometer Per Hour
Ct	:	Cycle Threshold
RPM	:	Rotations per minute
pH	:	-log of hydrogen ion
ORF	:	Open Reading Frame
Cq	:	Quantification cycle

ABSTRACT

Name of the Author	:	**LELLA RAJASEKHAR**
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Thrips species were collected from 35 major blackgram cultivating areas in Andhra Pradesh. Based on the morphological identification, major species of thrips species on black gram in Andhra Pradesh state was *Thrips palmi* (63.12), *Megalurothrips usitatus* (30.05) and *Scirtothrips dorsalis* (6.83). Out of thirty five locations surveyed, no record of *S. dorsalis* was observed in 19 mandals and highest mean per cent of *S. dorsalis i.e.,* 52.38 was recorded in Mentada mandal of Vizianagaram district. In Chittoor district *Megalurothrips typicus* (Bagnall), *Ayyaria chaetophora* (Karny), *Phibalothrips peringueyi* (Faure) and some Tubuliferan thrips were also observed in meager number.

Molecular characterization of thrips samples revealed that *T. palmi* produced an amplicon size of 570 bp with ITS2 marker, *M. usitatus* with mtCOI marker at 655 bp and *S. dorsalis* amplified with mtCOIII marker at 713 bp. This study contributed a total of 21 gene sequences (seven samples for each thrips species) to NCBI website Tajimas d statistic revealed the existence of low genetic polymorphism among the, ITS2 sequences of *T. palmi,* COI sequences of *M. usitatus*, COIII sequences of *S. dorsalis*.

The thrips population was observed in the field approximately after 14 DAS during *rabi* 2019-2020 where as during *kharif* 2020-2021 in third week of August (21 DAS) Highly fluctuating thrips population incidence was noticed during the *kharif* season as population has increased gradually and reached peak at 35 DAS with 17.35 mean number of thrips per plant and after 35 DAS, thrips population started declining to 5.2 and 4.6, 4.55, 3.33 (thrips per plant) at 42, 49, 56, 63 DAS respectively. At 70 DAS slight increase in thrips population was noticed *i.e.* 7.1 thrips per plant during first week of October (40^{th} SMW). Similarly thrips population was observed initially at 21 DAS during fourth week of December *i.e.* during 52^{nd} SMW with 2.15 mean number of thrips per plant and reached peak at 63 DAS during second week of

February *i.e.* during 6th SMW (Standard meteorological week) with 10.10 mean number of thrips per plant. The thrips population started declining towards maturity and lowest incidence was recorded at 77 DAS during fourth week of February *i.e.* during 8th SMW with 1.10 mean number of thrips per plant during *rabi* 2020-21.

Overall view of GBNV incidence in blackgram in the present study revealed that the per cent disease incidence was more during *kharif* 2020-2021 (26.05 %) compared to *rabi* 2019-2020 (25.90 %) and *rabi* 2020-2021 (10.22 %).

During *rabi* 2019-20, *rabi* 2020- 2021 the number of thrips per plant showed a highly significant positive correlation with maximum temperature *i.e.* 0.74, 0.726 respectively where as during *kharif* 2020-2021 positive correlation with out any significance *i.e.* 0.151 was observed. Similarly minimum temperature (0.75, 0.55), mean temperature (0.819, 0.435) showed highly significant positive correlation during *rabi* 2019-20, *rabi* 2020- 2021 respectively where as during *kharif* 2020-2021 positive correlation with out any significance *i.e.* 0.158, 0.172 was observed with number of thrips per plant.

Rainfall showed non significant negative correlation with thrips population *i.e.* -0.256, -0.289, -0.319 during *rabi* 2019-20 and *kharif* 2020-2021, *rabi* 2020-2021 respectively. Number of rainy days (-0.156, -0.319) showed non significant negative correlation during *rabi* 2019-20 and *rabi* 2020-2021, respectively where as significant negative correlation -0.657 was observed during *kharif* 2020-2021. Wind speed (0.802) showed highly significant and positive correlation with thrips population was observed during *rabi* 2019-20. But during *kharif* 2020-2021, *rabi* 2020-2021 wind speed showed non significant negative correlation *i.e.* -0.346, -0.463 respectively.

The number of thrips per plant showed a highly significant positive correlation with per cent disease incidence (0.889) where as mean number of thrips per square meter showed non significant positive correlation with disease incidence (0.57) during *rabi* 2019-2020 whereas the number of thrips per plant (0.279), mean number of thrips per square meter (0.179) showed a positive correlation with per cent disease incidence during *kharif* 2020-2021. Similarly during *rabi* 2020-2021, the number of thrips per plant (0.466) and mean number of thrips per square meter (0.508) showed a non significant positive correlation with per cent disease incidence.

Multiple linear regression equation showed that all the weather variables together could influence the incidence of thrips by 77.0 (R^2 = 0.77), 70.0 (R^2 = 0.70) per cent, 83.0 (R^2 = 0.83) per cent during *rabi* 2019-2020; *kharif* 2020-2021; *rabi* 2020-2021 respectively whereas weather variables together contributed to the incidence of bud necrosis disease incidence by 95.0 (R^2 = 0.95) per cent, 94.0 (R^2 = 0.94) per cent, 92.0 (R^2 = 0.92) per cent during *rabi* 2019-2020; *kharif* 2020-2021; *rabi* 2020-2021 respectively.

Out of the two species tested for transmission of GBNV, only *T. palmi* could able to transmit the GBNV from diseased to healthy plants where in the inoculated plants exhibited symptoms *viz.* chlorotic local lesions. Whereas *M. usitatus* failed to transmit the virus and the inoculated plants remained healthy. Hence *Thrips palmi* was identified as vector of *Groundnut bud necrosis virus* in blackgram. A minimum of 2 h acquisition access period (48 IAP), 4 h inoculation access period (24 h AAP) was required to transmit the bud necrosis disease in case of first instar larvae of *T. palmi* and a minimum of 2 larvae were required to transmit the bud necrosis disease

at 24 AAP, 48 h IAP; 48 AAP, 48 h IAP. A minimum of 2 h acquisition access period (with 48 h IAP), 8 h inoculation access period (24 h AAP) was observed in case of second instar larvae and a minimum of 10 larvae required to transmit the disease at 24 AAP and 48 h IAP, and 2 larvae required to transmit the disease at 48 AAP and 48 h IAP.

There was no disease transmission was observed at 30 min, 1 h, 2 h, 4 h, 6 h, and 8 h of acquisition access period and inoculation access periods in case of adults. Surprisingly a minimum of 24 h AAP (48 h IAP), 24 h IAP (24 h AAP) was observed with 8.33 per cent of disease transmission and there is no further increase of disease with increasing AAP (48 h IAP) and IAP (24 h AAP). A minimum of 10 adults were required to transmit the disease at 24 h AAP, 48 h IAP; 48 AAP and 48 h IAP.

Among the all evaluated insecticides imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i were proved best in reducing the thrips population with highest blackgram grain yield *i.e.* 1414 kg ha^{-1} with incremental cost benefit ratio 1:4.80 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1:4.47) during *rabi* 2019-2020. Similarly imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least bud necrosis disease incidence among the all other tested insecticides.

During *Kharif* 2020-2021, highest per cent population reduction over untreated control was found in treatment imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (86.67 per cent) followed by imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(86.62). Whereas among the treatments tested, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded highest grain yield *i.e.* 1372 kg ha^{-1} with incremental cost benefit ratio 1:3.73 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1:2.93).

Similarly, among the tested treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean bud necrosis disease incidence in blackgram *i.e.* 5.19 per cent and it was at par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (5.64 per cent), imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (5.94 per cent) thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(6.13 per cent), thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (6.39 per cent), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (6.64 per cent) during *kharif* 2020-2021.

During *rabi* 2020-2021, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} proved best in reducing the thrips population. Thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean disease incidence. Among the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded highest grain yield *i.e.* 1439 kg ha^{-1} with incremental cost benefit ratio 1:4.45 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1:3.97).

Chapter – I

Introduction

Chapter I

INTRODUCTION

Blackgram, *Vigna mungo* L. known as urd bean, mash, mungo bean, mashkalai, black mapte *etc*., belongs to the family Leguminosae, sub family Papilionaceae. It is an annual pulse grown mostly as a rice fallow crop. Similar to the other pulses, blackgram, being a legume, enriches soil nitrogen content and has relatively short (90-120 days) duration of maturity. In India, blackgram is grown primarily for its protein rich grain, can be used as daal and as ingredient in idli, dosa, vada and papad. It is a good source of vegetable protein which is three times more than the cereals. Blackgram is a perfect combination of all nutrients, which includes proteins (25-26 %), carbohydrates (60 %), fat (1.5 %), minerals, amino acids and vitamins, hence plays an important role in Indian diet.

Black gram is a warm weather crop and comes up in areas receiving an annual rainfall ranging from 600 to 1000 mm. It is mainly cultivated in a cereal-pulse cropping system primarily to conserve soil nutrients and utilize the residual soil moisture particularly, after rice cultivation. Blackgram can be grown in all the seasons and majority of blackgram cultivation falls in either *rabi* or late *rabi* seasons particularly in peninsular India. Optimum temperature range for its growth is 27-30 ^{o}C. A dry harvest period is desirable as this forces the crop to mature and reduces the risk of damage due to inclement weather conditions. It comes up well on water retentive soils but cannot withstand saline and alkaline conditions. Black gram is more tolerant of water logging than moong bean (www.commodities control.com).

It is the fourth most important short-duration pulse crop grown in India due to its nutritional and industrial values (Nene, 2006). India currently represents the largest producer of blackgram accounting for more than 70 per cent of the global production. In India during *kharif* 2019-20, area covered under blackgram is 37.52 lakh hectares as against 38.18 lakh ha in 2018-2019. The states of Madhya Pradesh (16.50 lakh ha), Uttar Pradesh (7.01 lakh ha), Rajasthan (4.56 lakh ha), Maharashtra (2.87 lakh ha), Karnataka (0.687 lakh ha) and Andhra Pradesh (0.11 lakh ha) are the major producers of blackgram in India during *kharif* with an average productivity of 654 kg ha^{-1}.

Black gram is attacked by a wide range of insect pests from sowing to harvest in the field as well as in storage. The annual yield loss due to the insect pests has been estimated at about 30 per cent in urd bean. Eleven sucking pests were identified in blackgram as sap feeders. Among them whitefly - *Bemisia tabaci* (*Gennadius*), thrips - *Scirtithrips dorsalis* (Hood), jassid (*Empoasca* spp.) and green leafhopper (*Nephotettix* spp.) and defoliators appeared as foliage feeders. Flower thrips (*Caliothrip* ssp.) and leaf miner - *Chromatomyia horticola* (Goureau) were classified as pollen feeder and tissue borer, respectively (Kumar *et al.*, 2007).

Among the sucking insect pests, thrips and whitefly are the most important pests during early stages of crop growth which not only reduce the plant vigor and also act as vectors of deadly viral diseases *viz*., bud necrosis and yellow mosaic. Thrips are the major sucking insect pests in pulses mainly on blackgram and greengram causing considerable damage by sucking cell sap from different tender parts of plant and also act as vectors of different plant viruses which cause leaf curl and bud necrosis, besides direct injury by feeding (Ananthakrishnan, 1980).

Thrips are minute, slender, and soft-bodied insects and belong to the order thysanoptera. The name of the order was derived based on the characteristic fringed wings. In the *Greek*, '*thysanos'* means 'fringe' and '*pteron'* means 'wing'. Thrips are also known as thunder flies, thunder bugs, storm flies, storm bugs, corn flies, corn lice, freckle bugs, and physopods. Besides being important sucking pests, thrips transmit the deadly tospoviruses in commercial crops in a persistent and propagative manner. Thrips act as vectors of deadly tospoviruses of the genus *Tospovirus*, family *Bunyaviridae* (Ullman *et al.* 1992a., 1997). Significant losses by tospoviruses in the yield and quality of vegetables, legumes and ornamentals have been recorded in different countries (Mumford *et al.* 1996., Pappu, 1997., Persley *et al.* 2006). Annual losses due to *Tospovirus* outbreaks were estimated at over $1 billion worldwide (Pappu *et al.* 2009., Mandal *et al*. 2012). Tospoviruses are not seed-transmitted and thrips play a critical role in the survival and spread of tospoviruses. Eleven species of thrips act as vectors of tospoviruses and more than 20 tospoviruses were recorded throughout the world (Pappu *et al*., 2009). The associations of thrips with tospoviruses and their ability to transmit specific tospoviruses are distinct (Jones, 2005).

Tospoviruses are one of the most important plant virus groups infecting a wide range of economically important crop plants all over the world (Pappu *et al.*, 2009). Tospoviruses have emerged as serious viral pathogens affecting the cultivation of several field and horticultural crops (Varma *et al*., 2002). The distribution of tospoviruses in the Indian subcontinent varies geographically. *Ground nut bud necrosis virus* (GBNV) and *Watermelon bud necrosis virus* (WBNV) are widely distributed in India and endemic in many states including Andhra Pradesh, Gujarat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal.

GBNV is currently recognized as the most economically important *Tospovirus*, as losses due to GBNV alone have been estimated at more than US$89 million per annum in Asia (Reddy *et al*., 1995). GBNV caused 70 to 90 per cent loss of groundnut in India (Singh and Srivatsava, 1995). Outbreaks of GBNV were reported in different tomato growing regions in Maharashtra, Karnataka, and Andhra Pradesh, where up to 100 per cent disease incidence was recorded during 2003 to 2006 (Kunkalikar *et al*., 2011). Besides groundnut and tomato, losses up to 29 per cent have been recorded in potato due to stem necrosis disease caused by GBNV (Singh and Srivatsava, 1995). In addition to groundnut, important crops such as cowpea, mungbean, pea, potato, soybean and tomato were known to be affected by GBNV (Kunkalikar *et al*., 2011).

The field symptoms of GBNV in groundnut are very well described. Initially, mild chlorotic spots appear on young quadrifoliate leaves, and subsequently necrosis and chlorotic rings develop. In rainy and post rainy seasons, necrosis of terminal bud is the main characteristic symptom. Secondary symptoms such as stunting, axillary shoot proliferation and malformation of leaflets are common. Plants infected early are bushy, stunted, and die prematurely. If plants older than one month are infected, the symptoms are restricted to few branches only (Reddy *et al.*, 1991). However the studies of GBNV on blackgram are scanty in the line of disease symptoms, virus vector relationships.

Tospoviruses (family *Bunyaviridae*, genus *Tospovirus*) are enveloped isometric RNA viruses with a tripartite genome containing small (S), medium (M), and large (L) segments of ssRNA. The complete genome of GBNV (type isolate,

groundnut) has been sequenced, which consisted of three linear single-stranded RNA molecules, the L (8.9 kb), the M (4.8 kb), and the S (3.05 kb) RNAs (Gowda *et al*. 1998., Satyanarayana *et al*. 1996). GBNV, which is primarily known to affect groundnut, now has been identified to cause necrosis disease in diverse crops *viz*., blackgram, brinjal, chili, cowpea, mungbean, pea, potato and soybean.

In India, the studies on different thrips species were initiated during pre-independence period. The research on tospoviruses got momentum during 1960s. Since then, several studies have been undertaken to understand the relationship between different thrips species and tospoviruses but scanty on blackgram. Keeping in view of the problems caused by thrips directly as pest and indirectly as vector in blackgram the research programme entitled "**Identification and molecular characterization of thrips complex transmitting the *Groundnut bud necrosis virus* in Blackgram and their management**" was conducted with the following objectives.

1. To collect, identify and molecular characterization of thrips complex on blackgram in Andhra Pradesh.
2. To study the incidence of thrips complex and occurrence of bud necrosis disease in blackgram.
3. To study transmission of *Groundnut bud necrosis virus* by thrips in blackgram.
4. To study the management of thrips in blackgram through insecticides.

Chapter – II

Review of Literature

Chapter II

REVIEW OF LITERATURE

The present study on "**Identification and molecular characterization of thrips complex transmitting the *Groundnut bud necrosis virus* in blackgram and their management"** was conducted to identify the thrips complex involved in bud necrosis disease transmission in blackgram. The impact of various weather parameters on the occurrence of thrips and bud necrosis disease in blackgram and their management with certain insecticides was studied. The literature about various aspects of thrips and bud necrosis disease, management of thrips and bud necrosis disease through seed treatment and foliar sprays in blackgram as per the objectives has been reviewed and presented in this chapter. Since the literature on blackgram was scanty, the literature pertaining to the above aspects in different field and vegetable crops is also reviewed hereunder.

2.1 COLLECTION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF THRIPS COMPLEX ON BLACKGRAM IN ANDHRA PRADESH

2.1.1 Morphological identification of thrips

Rabeena *et al.* (2020) described distinguishing characters of *Frankliniella schultzei* (Trybom) collected from GBNV hotspot regions in tomato growing areas of Tamil Nadu and Karnataka. It has eight segmented antennae, head with three pairs of ocellar setae, veins of forewing presenting continuous and equally spaced setae, five and two pairs of pronotal and metanotal setae, and absence of comb on eighth abdominal segment. These results were also confirmed through SEM analysis and molecular studies with 99 per cent similarity**.**

Rachana *et al.* (2018) reported that *Dahlia rosea* Cav. as new host for the quarantine thrips, *Thrips parvispinus* (Karny) in Karnataka.

Zafirah and Azidah (2018) reported that the most abundant thrips species on legumes were *Megalurothrips usitatus* (Bagnall) (89.97 %) followed by *T. parvispinus* (9.77 %), *Thrips hawaiiensis* (Morgan) (0.13 %) and *Ceratothripoides*

brunneus (Bagnall) (0.12 %). There was no significant difference regarding the abundance of *M. usitatus* on long bean, french bean and winged bean which equally distributed among different arbitrary strata on legume plants in Peninsular Malaysia.

Cluever and Smith (2017) presented a dichotomous key which helps in the identification of adult and larval stages of 20 thrips species commonly associated with horticultural crops in Florida.

Rachana and Varatharajan (2017a) reported nine genera *viz.*, *Astrothrips*, *Panchaetothrips*, *Selenothrips*, *Neohydatothrips*, *Dendrothripoides*, *Megalurothrips*, *Elaphrothrips*, *Crotonothrips* and *Karnyothrips* and two subfamilies Panchaetothripinae and Idolothripinae which were new to the thrips fauna of Odisha.

Rachana and Varatharajan (2017b) reported a consolidated systematic list of 333 species of terebrantian thrips, belonging to 118 genera (Insecta: Thysanoptera) recorded so far from India. The list revealed that the family Thripidae has the Lion's share of 307 species, while Aeolothripidae, Melanthripidae, Merothripidae and Stenurothripidae contained very few species.

Rachana and Varatharajan (2017c) reported a new species *Thrips laurencei sp.n*. from Tamil Nadu state of India, collected on flowers of *Hydrangea macrophylla* (Hydrangeaceae). This new species showed sexual dimorphism in colour. Females were brownish yellow with brown shadings but the males were uniformly yellow.

Silveira and Haro (2016) reported a fast method in which the specimen digestion in KOH was very short and then application of heat under lamp followed by transfer of specimen to a test tube with a small volume of clarifying solution, consisting of lactic acid 85 % (20 parts), glacial acetic acid (4 parts), phenol (2 parts) and distilled water (1 part), then to clove oil for further clearance. This method was found efficient for routine identification of thrips since it resulted in slides of adequate quality for identifying species regardless of their colour. It is important, however, to stress that the fast method is only suitable for preparing temporary slides for routine identification and is not a substitute for the traditional method of preparing permanent slides.

Tyagi *et al.* (2015) reported invasion of *T. parvispinus* on papaya plantations first time from India using the haplotyping data and it was suggested that the Indonesia may be a probable source of invasion of this pest to India.

Minaei (2014) reported new species *Eremiothrips eshghii* from Fars province, south of Iran on Ephedra plants.

Mirab-Balou *et al.* (2013) provided an illustrated key for the identification of 35 genera on Thripinae from Iran with characterization for each genus.

Mirab-Balou *et al.* (2012) provided an illustrated key to distinguish the 26 species of genus *Thrips* recorded from Iran. *Thrips alliorum* (Priesner) was newly reported from Iran.

Tillekartane *et al.* (2011) reported a list of 72 thrips species in 45 genera during the survey from 324 host plant species in 83 plant families. *M. usitatus*, *T. palmi***,** and *Haplothrips gowdeyi* (Franklin) were the most widely distributed species in Sri Lanka.

Maisnam and Varatharajan (2011) reported occurrence of *T. palmi* on *Dahlia rosea* Cav. (Asteraceae) in Manipur.

Mound and Yongfoo (2009) developed an illustrated key for the identification of 65 genera of Thripinae from South East Asia.

2.1.2 Molecular characterization of thrips

Sumit *et al.* (2020) reported that cytochrome oxidase subunit III and internal transcribed spacer region 2 were utilized to design species specific primers for thrips. Out of 38 pairs of primers tested, primer pairs AG35F-AG36R, AG47F-AG48R, AG87F-AG88R, and AG79F, AG80R amplified 568 bp, 713 bp, 388 bp, and 200 bp products from the DNA templates of *T. palmi*, *S. dorsalis*, *T. tabaci*, and *F. schultzei*, respectively under same PCR conditions. The specificity of the primer pairs was validated with a large number of known specimens and no cross-reactivity was observed with other thrips species. The multiplex PCR assay with a cocktail of all the four primer pairs detected four thrips vectors efficiently and could discriminate all of them concurrently in a single reaction.

Zafirah *et al.* (2020) reported that the inter specific distances between both *M. usitatus* Lineage I and Lineage II ranged from 8.78 to 9.63 per cent, suggesting the presence of cryptic and non-monophyly lineages between two morphoforms of *M. usitatus* in Peninsular Malaysia.

Marullo *et al*. (2020) used DNA barcoding technique to identify thrips species collected with sticky traps placed in an open onion field in Switzerland. A total of 238 thrips specimens were analyzed, out of which 151 could be identified up to species and 27 up to genera belonging to the family Thripidae and 51 specimens could not be assigned to any genus, with the closest BLAST match in the Genbank queries being below 98 per cent, whilst six specimens were not recognized as Thysanoptera.

Rabeena *et al.* (2020) reported that *F. shultzei* was predominant in tomato growing regions of Tamil Nadu and Karnataka states. The species identity was confirmed with SEM analysis and molecular characterization using universal primers *i.e.* LCO-1490 F and HCO-2198 R.

Chakraborty *et al*. (2019) used both morphology and molecular approaches to delimit the selected *Scirtothrips* species from India. Out of 43 generated barcode sequences, six sequences of three species (*S. hitam, S. mangiferae, and S. malayensis*) are the novel contribution in global database. The Bayesian (BA) phylogeny clearly distinguished all the studied species with reciprocal monophyletic criteria and represented multiple clades in *S. dorsalis* and *S. oligochaetus*. The high Kimura-2-Parameter (K2P) genetic divergences were observed between the multiple clades of *S. dorsalis* (4.5–8.8 %) and *S. oligochaetus* (6.4 %), which indicated possible existence of cryptic diversity. Morphological keys for six *Scirtothrips* species including *S. hitam* as a new record to India was also provided.

Rajabaskar *et al.* (2019) reported that *T. palmi* was the predominant species in the watermelon hotspot regions of Tamil Nadu and Karnataka which was confirmed through DNA sequence analysis.

Singha *et al.* (2019) recorded the first genetic footprint of *Frankliniella occidentalis* (Pergande) in India and indicated the gene flow from the Netherlands to India. *F. occidentalis* specimens were collected from Karnataka state in Southern India and morphologically identified through available keys. The generated DNA

barcode data showed 99 – 100 per cent similarity with the database sequences of *F. occidentalis*. The phylogenetic analysis (NJ, ML, and BA) showed three distinct clades of *F. occidentalis* in the present dataset with high bootstrap supports and posterior probabilities. The K2P genetic distances further depicted high similarity of the generated sequences from India and Netherlands. The clade-1 (India to Netherlands) also showed a close relationship with clade-2 (Kenya) rather than clade-3 (Canada + USA).

Wang *et al.* (2018) designed a probe-based quantitative PCR (qPCR) assay with crude DNA extraction and applied to identify over 5,000 specimens of thrips (including 3,366 larvae) collected on cotton seedlings in south eastern United States. *Frankliniella fusca* (Hinds) was the dominant species across all the locations (76.8 – 94.3 % of adults and 81.6 – 98.0 % of larvae), followed by *F. occidentalis* in Georgia, North Carolina, and Virginia (4.6 – 19 % of adults and 1.7 – 17.3 % of larvae) or *Frankliniella tritici* (Fitch) in South Carolina (10.8 % of adults and 7.8 % of immature).

Tyagi *et al*. (2017) reported that multiple species delimitation methods (BIN, ABGD, GMYC and bPTP) were consistent for 73 species (82 %) with their morphological identifications. A total of 107 molecular operational taxonomic units (MOTUs) were recovered for 89 morphospecies by superimposing multiple methods and applying a three level nomenclature system. More than one MOTU were identified in 14 morphospecies indicating to have cryptic diversity including, two major vector species (*F. schultzei* and *T. palmi*). However, four morphospecies (*Thrips moundi*, *Thrips carthami*, *Haplothrips andersi* and *H. gowdeyi*) showed low genetic distances between them with overlapping in barcode gap that required further analysis with multiple molecular markers and more specimens from wide geographical areas for better taxonomic judgment.

Kumar *et al.* (2017b) reported intragenomic variation in mtCOI and ITS2 markers in identifying three thrips species; however two to three times more intragenomic variations were observed for ITS2 than mtCOI in both *S. dorsalis* and *T. palmi*. Further, levels of intragenomic variation was low for both of the genes in *F. occidentalis*.

Sabahi *et al*. (2017) reported the development of new primer sets for both simultaneous diagnosis and discrimination of four economically significant thrips species *T. palmi* and *Frankliniella intonsa* (Trybom), *T. tabaci*, *F. occidentalis* in various life stages. Four primer sets, consisting of a species specific reverse primer and a common forward primer were designed based on COI gene and used in diagnostic multiplex PCR protocol.

Przybylska *et al*. (2017) conducted a duplex real-time PCR assay involving the ThrUNIFw/ FoRw and/or ThrUNIFw/TpRw primer sets to distinguish between *F. occidentalis* and *T. palmi* species. Amplification and derivative melt curves were generated for *F. occidentalis* and *T. palmi*, with a melting peak temperature range of 83.5 to 84.0 ^{0}C for *F. occidentalis* and 85.5 to 87 ^{0}C for *T. palmi*.

Suganthy *et al*. (2016) reported Western flower thrips, *F. occidentalis* in South India on chrysanthemum plant through molecular characterization. PCR amplified 358 bp fragment of mtCOI gene was analyzed for 24 individual thrips from 12 locations of Tamil Nadu. The consensus sequences revealed 92-100 per cent identity in the selected fragment with the *F. occidentalis* sequences in the database. Of the eight populations analyzed, two of them revealed 100 per cent identity with many sequences of *F. occidentalis*. However, one of the populations, FOTN6 was very distinct and exhibited only 92 per cent identity with all the *F. occidentalis* sequences compared. Totally there were 48 polymorphic sites out of 358 nucleotides compared, of which more than 65 per cent resulted in changes in the amino acids.

Iftikhar *et al.* (2016) reported barcode sequence variation among 471 thrips found in various plant hosts in Pakistan. Congeneric species showed an average of 19 per cent sequence divergence at COI, while intraspecific distances averaged 0.6 per cent. This sudy reported deep intraspecific divergence among the thrips species *i.e. T. palmi, T. tabaci, Haplothrips reuteri* (Karny) and one predatory thips *Aeolothrips intermedius* (Bagnall).

Nakahara and Minoura (2015) examined the applicability of multiplex PCR for identification of four thrips species, *T. palmi, T. tabaci, F. intonsa*, and *F. occidentalis* that were frequently found in Japanese quarantine inspection sites. The internal transcribed spacer 2 region (ITS2) of nuclear ribosomal DNA was amplified using five specific primers for 71 individuals of the four species. Species-specific single bands were detected.

Kumar *et al.* (2014) reported that two techniques *i.e.* SEM and DNA barcoding together could be used for correct identification of thrips species. Initially Larvae and adults of *S. dorsalis*, *F. occidentalis*, *F. schultzei* and *T. palmi* were subjected to traditional morphological identification using high resolution SEM prior to their DNA extraction. Sequence results of both mtCOI and ITS rDNA of individual larva and adults of all the four species were found in agreement with the taxonomic identification conducted using SEM and each result confirmed the other technique. Further they suggested that steps involved during specimen preparation and observation under SEM does not impact DNA analysis of the sample.

Rebijith *et al.* (2014) reported that the existence of cryptic species in *T. hawaiiensis* and *Scirtothrips perseae* (Nakahara) along with previously reported cryptic species such as *T. palmi, T. tabaci, F. occidentalis, and S. dorsalis* through DNA barcoding technique using CO-I gene sequences for discriminating 151 species of thrips.

Yeh *et al.* (2014) reported that multiplex PCR using specific primers based on ITS1 sequences is a simple, reliable, and cost-effective diagnostic tool for thrips species identification (*T. tabaci*, *F. intonsa*, and *S. dorsalis*).

Kadirvel *et al*. (2013) reported that partial cytochrome oxidase I (COI) sequences were used to understand the phylogenetic relationship among thrips populations and assessed their usefulness to identify and classify unknown thrips species collected from different crops. In total, 29 COI variants were obtained while examining the sequence polymorphisms in COI of 182 insects analyzed which were collected from six countries on tomato, chilli, onion, cabbage, cucumber, watermelon, Ethiopian mustard, French bean, and peanut. The phylogenetic analysis showed that the insects used in this study clustered with five distinct species-groups with *T. palmi*, *T. tabaci*, *F. occidentalis*, *S. dorsalis* and an unclassified group. Higher intraspecific genetic variation was observed in *S. dorsalis* and *T. palmi* followed by *T. tabaci* and *F. occidentalis*.

Tamura *et al*. (2013) developed an advanced version of the Molecular Evolutionary Genetics Analysis (MEGA) software, which contains facilities for building sequence alignments, inferring phylogenetic histories, and molecular evolutionary analysis. Version 6.0, MEGA enables the inference of time trees, as it

implements the RelTime method for estimating divergence times for all branching points in a phylogeny.

Karimi *et al*. (2010) used DNA barcoding technique based on nucleotide sequencing analysis of the mitochondrial cytochrome oxidase I (COI) gene. Phylogenetic analyses conducted by the neighbor-joining method yielded almost identical phylogenetic reconstructions of trees that separated thrips based on the geographic origin. Molecular data indicated that different thrips species were located in distinct groups.

Farris *et al*. (2010) developed a molecular diagnostic marker for *S. dorsalis* for which DNA sequence data and polymerase chain reaction (PCR) were utilized. The DNA sequence variation from the internal transcribed spacer 2 (ITS2) regions of nuclear ribosomal DNA (rDNA) was analyzed from various thrips species, including *S. dorsalis*. A primer set and polymerase chain reaction cycling parameters were designed for the amplification of a single marker fragment of *S. dorsalis* ITS2 rDNA.

Glover *et al*. (2010) compared five different loci to investigate the ability to discriminate a small number of *Thrips* species. All five loci discriminated the species by neighbor-joining tree and varying degrees of discrimination were determined upon further investigation of the intraspecific and interspecific distances. Two distinct COI clades were observed for *T. palmi* and judged to be COI haplotypes when data from the other four additional loci and geographical collection data were taken into consideration.

Librado and Rozas (2009) developed a software package DnaSP for a comprehensive analysis of DNA polymorphism data. Version 5.0 implements a number of new features and analytical methods allowing extensive DNA polymorphism analyses on large datasets.

Mainali *et al*. (2008) developed two molecular markers *i.e*. PCR-RFLP based and PCR -RAPD based to identify sympatric species of the genus *Frankliniella* in Korea.

Asokan *et al.* (2007) identified species specific markers for identification of two thrips species *i.e*. *T. palmi* and *T. tabaci* collected from onion and watermelon in

Karnataka. Phylogenetic analyses showed that both *T. tabaci* and *T. palmi* formed different clades as compared to the other NCBI accessions.

Meena *et al.* (2005) reported the presence of *Tospovirus* in thrips vector *S. dorsalis* using RT PCR analysis for the first time in India. RAPD similarity matrix revealed that *S. dorsalis* population from tomato, groundnut and chillies had at least 75 per cent similarity while 50 per cent similarity existed between the *F. schultzei* populations from cowpea and sunhemp. *T. tabaci* from cotton was distantly related with *S. dorsalis* and *F. schultzei* with lowest similarity indices (less than 0.464).

Kox *et al*. (2005) developed a real-time PCR assay based on TaqMan. *T. palmi*-specific set of primers and probe were selected within the mitochondrial cytochrome oxidase I (COI) gene. The specificity of the assay was assessed using 15 specimens of *T. palmi* and 61 specimens of 23 other thrips species commonly occuring in Europe. All *T. palmi* specimens were detected using *T. palmi* specific set of primers, probe and no cross reactions with other thrips were observed. The method was tested on single larvae and adults and proved to be applicable for both the stages of *T. palmi.*

Walsh *et al*. (2005) developed RAPD putative marker to carry out real time PCR assay to screen against 21 thrips species including 10 other species of the genus Thrips and were found to be specific to *T. palmi*.

Brunner *et al*. (2004) tested host-associated genetic differentiation in 22 populations of *T. tabaci* collected from tobacco and leek, respectively. Cluster analysis and haplotype networks based on sequence variation at a fragment of the mitochondrial cytochrome oxidase I gene yielded three major evolutionary lineages; two were clearly associated with leek and the third with tobacco. Estimated divergence times suggested an ancient divergence of these lineages dating back to the Miocene 28–21 million years ago. FST values between these lineages ranged between 0.824 and 0.954 ($P > 0.001$ for all comparisons) and sequence divergences ranged between 4 and 11 per cent.

2.2 TO STUDY THE SEASONAL INCIDENCE OF THRIPS AND BUD NECROSIS DISEASE IN BLACKGRAM

Wankhede *et al.* (2020) conducted an experiment to study incidence of thrips on groundnut in different sowing windows in Pune and the prediction of thrips populations in different sowing windows based on regression equations (R^2) showed 52 to 88 per cent validation due to different weather parameters for variety JL-501, (R^2) 58 to 86 per cent validation for variety RHRG-6083, (R^2) 48 to 85 per cent validation for variety TAG-24, (R^2) 73 to 83 per cent validation for variety JL-776 was recorded. Sowing of groundnut during 26th and 27th MW recorded lower incidence of thrips, whereas, crop sown during 28th MW recorded maximum incidence during *kharif* season in groundnut.

Saritha *et al.* (2020) reported that peak thrips population was observed during g 31st SMW with an average population 3.5 thrips/plant. The population then decreased till the 35th SMW with average population of 0.2 thrips/3 leaves. Later slowly raised till the 38th SMW (1 thrips/plant) in groundnut crop during *kharif* season. The thrips population exhibited significant positive correlation with relative humidity (r = 0.504) while, non significant positive correlation with mean relative humidity (r = 0.105) and rainfall (r = 0.471). During *Rabi* season, the mean temperature (r = 0.815), maximum temperature (r = 0.708) and minimum temperature (r = 0.797) had positive nonsignificant correlation with thrips population and showed non-significant negative correlative with mean relative humidity (r = 0.314), minimum relative humidity (r = - 0.436) and positive correlation with maximum relative humidity (r = 0.734) and it was evident that the mean temperature and relative humidity favored the pest population.

Rahul *et al*. (2020) reported that maximum (r = 0.977) and minimum temperatures (r = 0.985) showed significant positive correlation with thrips population in blackgram during late *rabi* 2019 and 2020. Further mean temperature also showed positive correlation with thrips population (r = 0.985), whereas morning relative humidity showed significant negative correlation (r = 0.974). Evening relative humidity and mean relative humidity showed non significant negative correlation with thrips population (r = -0.264, -0.770).

Jawaharreddy *et al.* (2020) reported that the incidence of thrips in grapevine started when maximum and minimum temperatures were 31.3^{o}C and 13.7^{o}C where morning and evening humidities were 58.4 per cent and 29.6 per cent, respectively. The thrips population increased gradually and reached to its peak (8.53/shoot) at 28.1^{o}C maximum and 12.2^{o}C minimum temperatures. Correlation with the maximum (r = -0.558) and minimum temperatures (*r* = - 0.419) were significantly negative with thrips incidence in grapevine during the year 2017. Whereas, it had significant positive correlation with both morning (*r* = 0.474) and evening relative humidities (*r* = 0.233).

Timmanna *et al*. (2020) reported that maximum mean thrips population (3.75 to 3.85 thrips per three leaves) on tomato was observed in Karnataka during the 27th to 29th SMW. The percent bud necrosis disease was linear with the thrips population and 23.87 per cent mean cumulative incidence was recorded during *kharif* 2016. Amongst the current, one lag and two lag weeks, thrips population showed highly significant negative association with minimum temperature (*r* = -0.843), rainfall (*r* = -0.861) sunshine hours (*r* = -0.813) and significant negative association was observed with maximum temperature (*r* = -0.623). Whereas, thrips population had highly significant positive correlation with morning relative humidity (*r* = 0.780). Maximum temperature (*r* = -0.924) and rainfall (*r* = 0.715) observed to be with highly significant negative influence on GBNV disease. Stepwise regression analysis revealed that the thrips population was influenced by all the weather parameters during current week (R^2 = 0.810), one lag week (R^2 = 0.739) and two lag week (R^2 = 0.879).

A survey was conducted during 2016-17 in different tomato growing regions of Tamil Nadu and reported that the GBNV incidence ranged from 20 to 48.75 per cent, maximum was observed in Coimbatore (48.75 %) and minimum (20 %) was in Dindigul, Mandya, Mysuru and Ramanagara. The thrips population ranged from 0.35 to 1.39/shoot where the incidence was maximum (1.39) in Coimbatore and minimum (0.35) in Dindigul district of Tamil Nadu (Rabeena *et al*., 2020).

Aishwarya *et al.* (2019) conducted a study in water melon during summer 2019 and reported that temperature (*r* = 0.2) was positively correlated with the incidence of *T. palmi* while relative humidity (*r* = -0.5) and rainfall (*r* = -0.5) were negatively correlated. Incidence of WBNV was positively correlated with temperature

(r = 0.7), relative humidity (r = 0.3) and rainfall (r = 0.4). The percent incidence of WBNV in watermelon in Tamil Nadu ranged from zero to 60 per cent. According to the linear regression equation, the incidence of *T. palmi* (R^2 = 0.9) and WBNV (R^2 = 0.7) was influenced by the weather parameters up to 86.6 and 70 percent, respectively.

Nayak *et al.* (2019) studied about incidence of thrips population in groundnut at Raipur during *kharif* 2018 and reported that thrips showed significant positive correlation with minimum temperature (r = 0.681). The regression equation being y = 6.234-0.333x indicated that with an increase in 1^{o}C there will be increase in population by 0.333. The thrips population also showed significant positive correlation with mean atmospheric temperature (r = 0.606). The regression equation being Y = 12.927-0.528x indicated that with an increase in 1^{o}C temperature there was an increase in population by 0.528 while other parameters had no significant association.

Jamuna *et al.* (2019) conducted a study on tomato in Raichur during *kharif* 2016, 2017 and reported that the weather parameters *viz*., rainfall (r = - 0.588) and rainy days (r = -0.603) minimum temperature (r = -0.475) showed significant negative correlation with thrips population in tomato. Whereas maximum temperature (r = -0.305) and evening relative humidity (r = - 0.051) showed non significant negative correlation. While morning relative humidity (r = 0.259) and sunshine hours (r = 0.076) showed non significant positive correlation with thrips population. When the data was subjected to multiple Linear Regression (MLR) analysis, results revealed that, 80.30 per cent of the thrips population was influenced by weather parameters (R^2 = 0.803).

Vinaykumar *et al.* (2019) conducted an experiment in tomato crop during *kharif* 2014-15 and reported a zero correlation between the thrips population and maximum temperature. Relationship between the thrips population and rainfall showed that a slight negative correlation between the thrips population and rainfall with a correlation coefficient (r) of – 1.04. Relationship between the thrips population and relative humidity indicated very low correlation between the thrips population and relative humidity.

Radhika *et al.* (2018a) reported that the population of sucking pest in *rabi* blackgram during 2017-18 started from 2nd week after sowing with 1.80, 2.00 and 2.40 leafhoppers, whiteflies and thrips per six leaves, respectively and their population were high during the vegetative stage and reached its peak during the reproductive stage with 6.80, 6.90 and 8.10 leafhoppers, whiteflies and thrips per six leaves and later declined to 1.20, 2.20 and 2.20 leafhoppers, whiteflies and thrips per six leaves, respectively.

Vennila *et al.* (2018) studied abundance, infestation and disease transmission by thrips in groundnut at Kadiri, Andhra Pradesh over six *kharif* seasons from 2011-2016 and reported that the range of mean thrips population, per cent infestation and PBND incidence across the seasons was 0.01-4.4 (no. per 3 leaves per plant), 6.8-65.5 per cent and 0-9.2 per cent, respectively. Maximum of thrips population (no./3 leaves/plant), infestation (%) and PBND (%) combined over all seasons was in the range of 0.2 - 8.0, 14 - 78 and 0 - 22, respectively. While peak of thrips population (no. / 3 leaves /plant) was 4.7 during 2016, maximum infestation (%) and PBND (%) was 65.5 and 9.1, respectively in 2014.

Moanaro and Choudhary (2018) reported that thrips population and minimum mean temperatures were negatively correlated (-0.07 and -0.04) with no significant effect, where as maximum temperature was positively correlated (0.06) without any significance in capsicum during 2014 in Eastern Plateau and Hill region of India. During 2015, minimum, maximum and mean temperatures were positively correlated (0.05, 0.21, 0.12) with thrips population with no significant effect. Rainfall showed negative correlation (-0.49 and -0.02) with thrips population during both the years 2014-2015. The linear regression analysis based on weather parameters as independent variables and thrips, mites and whiteflies population fluctuation as dependent variable, explained to 42, 65 and 47 percent population variability, respectively.

Naresh *et al.* (2018) reported that among the six weather parameters, maximum temperature (r = 0.61, 0.55), evening relative humidity (r = -0.78, -0.78) and wind speed (r = 0.68, r = 0.67) showed significant influence on thrips incidence in two cultivars of groundnut (Dharani and K-6) in second fortnight of November sown crop. In case of first fortnight of December sown crop maximum temperature

(r = 0.55, 0.53) and evening relative humidity (r = - 0.64, -0.63) showed significant influence on thrips incidence in two cultivars of groundnut (Dharani and K-6). In second fortnight of December sown crop maximum temperature (r = 0.61, 0.60), minimum temperature (r = 0.58, 0.58) and evening relative humidity (r = -0.65, -0.64) showed significant influence on thrips damage, first fortnight of January sown crop, sunshine hours (r = -0.50) showed significant influence on thrips damage in K-6 and in Dharani. Regression analysis on foliar damage caused by the thrips indicated that all the weather parameters together resulted in 94 per cent (R^2 = 0.94) and 95 per cent (R^2 = 0.95) in groundnut cultivars Dharani and K-6 sown during November second fortnight.

Nigude *et al.* (2018) conducted a study in groundnut at Kolhapur and reported that the thrips incidence first appeared in the 31st MW corresponding to the August 1st week with mean population 0.90 thrips/3 leaves. The population started increasing slowly and reached to its peak in the 38th MW corresponding to September 4th week (4.20 thrips/3 leaves) when the maximum temperature was 26^{o}C, 86 per cent morning relative humidity and 44 mm rainfall. The population of the thrips declined thereafter from 3.50 at 39th MW corresponding to September 5th week to 0.90 thrips/3 leaves at 42nd MW corresponding to October 3rd week. Correlation studies revealed that the thrips population was negatively non-significant with temperature (-0.08) and rainfall (-0.27) and positively and significantly associated with relative humidity (0.54).

Vinuthan *et al.* (2018) reported that *T. tabaci* on onion during *kharif* season was noticed in early stages of crop growth on 1st September (2.36 thrips/pl.) and maximum thrips population was noticed in later stages of crop during 29th September (27.16 thrips/plant). During *rabi* season minimum thrips population of 1.8 thrips per plant was noticed in early stages of crop growth and maximum thrips population of 61.04 thrips per plant was noticed at later stages. The onion crop transplanted during *rabi* recorded the maximum population of thrips (31.81 thrips/plant) when compared to *kharif* transplanted onion crop. The correlation between thrips population and weather parameters revealed that thrips population was significantly negative correlated with rainfall and relative humidity. Temperature showed significant positive correlation with thrips population. During Second season (*rabi*-summer) thrips population was significant negatively correlated with relative humidity and non-significant negative correlation with rainfall. Temperature showed significant positive correlation with thrips population.

Reddy *et al.* (2017) reported that the relationship between the thrips population *i.e. S. dorsalis* in chilli with preceding one week (one week lag) weather parameters during *kharif* 2015-16 revealed that there was a significant negative correlation between maximum temperature (-0.51), minimum temperature (-0.80), mean temperature (-0.87), rainfall (-0.55), rainy days (-0.59) at 1 % level of significance. During *kharif* 2016-17, maximum temperature (-0.59), minimum temperature (-0.83), evening relative humidity (-0.66), rainfall (-0.59), rainy days (-0.67) and mean temperature (-0.87) were significant and negatively correlated with the thrips population at 1 % level of significance.

The incidence of thrips and GBNV in groundnut crop in Tamil Nadu during 2015-16 started in 2nd week of August and population of thrips reached the peak in the fourth week of September with a mean of 3.40 to 6.4 thrips/3 leaves in *kharif* and 3.20 to 7.1 thrips/3 leaves in *rabi* and reached the peak at the end of March. In *kharif*, thrips population showed negative correlation with morning and evening relative humidities -0.025 and -0.223 and positive correlation with maximum and minimum temperature (0.266 and 0.146), rainfall (0.335) and sunshine hours (0.277), respectively. In *rabi* 2017, thrips incidence showed positive correlation with maximum temperature (0.082), minimum temperature (0.052) and sunshine hours (0.085) and negative correlation with morning (-0.322,) and evening relative humidity (-0.162,) (Vijayalakshmi *et al.*, 2017).

Mahipal *et al.* (2017) conducted an experiment at IGKV Raipur, during *kharif* 2015-16 in cowpea and reported that population of flower thrips *Megalurothrips sjostedti* (Trybom) was correlated with weather parameters *viz*., temperature, rainfall, relative humidity and sunshine hours. The correlation coefficient between flower thrips population and maximum temperature (r = 0.705), minimum temperature (r = 0.839) were positive and highly significant. Whereas positive and non significant correlation was observed with maximum relative humidity (r = 0.182). Minimum relative humidity with flower thrips population showed significant positive correlation (r = 0.565). Negative non significant correlation with rainfall (r = -0.390) while positive and non significant with sunshine (r = 0.369) was recorded.

Ahir *et al.* (2017) studied population dynamics of sucking pest complex in groundnut during July to October 2014 at Udaipur and reported that *S. dorsalis* first

appeared during 32nd standard meteorological week (SMW) *i.e.* 6th - 12th August (2nd week) with a mean population of 0.80 thrips/3 leaves. The population increased and reached its peak in the second week of September (10th -16th September) with a mean population of 3.80 thrips/3leaves. The pest exhibited significant positive correlation with relative humidity (*r* = 0.6062) while, non-significant correlation with mean temperature and rainfall.

Tamilanayagan *et al*. (2017) conducted survey in tomato growing areas of Tamil Nadu in the year 2015 and reported that maximum number of 4.0 thrips per plant was recorded in Krishngiri district and minimum number 0.8 thrips per plant was recorded in Perambalur district. Correspondingly, maximum GBNV incidence of 82 per cent was recorded in Krishnagiri district and the minimum of 44 per cent disease incidence was observed in Peramabalur district.

Kumar and Singh (2016) reported 3.47 flower thrips nymph and adult/10 flowers during 37th standard week in blackgram during *kharif* 2014. They have also reported that flower thrips population showed non significant positive correlation with maximum and minimum relative humidity (0.69 & 0.737) and non significant negative correlation with maximum and minimum temperature (-0.720 & 0.109). Non significant positive correlation with total rainfall (0.598) was observed, while sunshine hours showed non significant negative correlation (-0.616).

Subba and Ghosh (2016) studied population dynamics of thrips in tomato during 2011-2013 and reported that minimum number of thrips (0.42-53/leaf) population was recorded during 38th to 44th standard week and maximum level of population was observed during 45th to 2nd (1.05-1.89/leaf) and again during 6th to 20th (1.00-2.22/leaf) standard week. Correlation coefficient values for thrips incidence and weather parameters revealed that temperature difference has significant positive influence (0.644) on thrips while significant negative correlation with temperature (minimum and average) (-0.524, -0.440), relative humidity (minimum, average) (-0.566, -0.483) and weekly total rainfall (-0.453). In case of maximum relative humidity (-0.317) and maximum temperature (-0.275)) non significant negative influence was observed in tomato.

Akashe *et al.* (2016) reported that the thrips population showed non significant positive correlation with maximum temperature (0.119) while it showed highly

significant negative correlation with RH-I (-0.744), non significant negative correlation with RH-II (-0.257) and rainfall (-0.319). With the forecasting model developed using eight years data *i.e*. from 2004 to 2011, explained the incidence of thrips on sunflower to an extent of 88 % (during *kharif*) and suggested that this model can be used for the prediction of thrips incidence in sunflower crop.

Harish *et al.* (2015) conducted field experiments during 2014, 2015 in Gujarat to know the impact of weather parameters on the occurrence of thrips in groundnut and reported that (22.5 adults thrips/5 sweeps were recorded at the end of 8^{th} and 4^{th} SMW. No significant relation between weather parameters and thrips population during *kharif* and *rabi* was noticed. But during summer, evening relative humidity (-0.32), rainfall (-0.37) have showed highly significant negative correlation and sunshine (0.60) showed significant positive correlation on occurrence and abundance of thrips population. The coefficient of multiple determinations (R^2) was 36, 54 and 80 per cent during *kharif, rabi* and summer seasons, respectively.

Singh and Singh (2014) conducted an experiment during summer 2009-10, 2010-11 in cowpea crop at Varanasi and reported that the maximum and minimum thrips populations (2.53 and 0.15 thrips/flower) were observed during 25^{th} and 21^{st} standard weeks, respectively during summer, 2009-10. But, during summer, 2010-11, the lowest and the highest population were 0.18 and 2.05 thrips/flower during 20^{th} and 24^{th} standard weeks, respectively. The correlation and regression coefficients did not reveal any significant relationships of weather variables with thrips population in cowpea during both the years.

Meena *et al.* (2013) reported that the infestation of thrips, *S. dorsalis* in chilli crop started in the fourth week of July (30^{th} meteorological week) and continued up to fourth week of November (48^{th} meteorological week) during years 2006-07 and 2007-08 at Allahabad, Uttarpradesh. The population increased gradually and touched its peak with a mean of 14.5 and 14.7 thrips/3 leaves /plant during 2006-07 and 2007-08, respectively.

Yadav *et al.* (2012) reported that *S. dorsalis* in groundnut at Udaipur during July to December 2010, first appeared during 32^{nd} standard meteorological week (SMW) *i.e.* 6^{th} – 12^{th} August (2^{nd} week) with a mean population of 1.20 thrips/3 leaves/ plant. The population increased gradually and attained the peak in the fourth

week of September with a mean population of 4.16 thrips/3leaves/plant. Later on, the population declined and reached a minimum level of 0.9/3leaves/plant during 42nd SMW *i.e.* 14th – 21st October (3rd week). Thrips exhibited a non significant and positive correlation with relative humidity (r^2 = 0.22019) and rainfall (r^2 = 0.2382). Non significant and negative correlation (r^2 = 0.0833) with average temperature.

Kandakoor *et al.* (2012) conducted an experiment in groundnut during *kharif* 2010 at Chintamani, Karnataka and reported that thrips showed non significant positive correlation with maximum and minimum temperature with 0.277 and 0.087, respectively. Non significant negative correlation was observed between thrips and rainfall *i.e.* -0.106. Further, thrips showed non significant positive correlation (0.072) with sunshine hours. Significant negative correlation was observed (-0.564) between thrips and morning relative humidity but evening relative humidity showed non significant negative correlation (-0.124).

Pramod *et al.* (2011) reported that thrips population was higher in the *kharif* season between June-August first week. Thrips showed significant and positive correlation with maximum temperature (R^2 = 0.5131) in sunflower hybrid KBSH-1.

Bhede *et al.* (2008) studied population dynamics during *kharif* 2002-2003 and reported that the incidence of thrips in chilli was highest during 40th meteorological week (5.43 thrips/three leaves) when the prevailing maximum minimum temperatures, morning-evening relative humidity, rainfall and bright sunshine hours were 35.8 ^{o}C, 18.0 ^{o}C, 76 per cent, 34 per cent, 0.00 mm and 11 hrs, respectively. Thrips population exhibited significant negative correlation with evening relative humidity and rainfall (-0.550, -0.843) and positive correlation with bright sunshine hours (0.631). The population showed no significant correlation with maximum-minimum temperatures and morning relative humidity. Regression equations indicated that the population decreased by 0.03, 0.04 and increased by 0.303 per unit of evening relative humidity, rainfall and bright sunshine hours, respectively in green chili during *kharif* 2002 - 2003.

Nandagopal *et al.* (2008) conducted field experiments during *kharif* seasons in the period 1994-1998 and reported that the maximum temperature was positively correlated (r = 0.38) with thrips population and was negatively correlated with

average RH (r = -0.32) and rainy days (r = -0.41) where as sunshine hours has a positive correlation (r = 0.32). High humidity more than 70 per cent RH coupled with dry spell about a week time favoured the heavy buildup of thrips *Caliothrips indicus* (Bangall) population in groundnut in Saurashtra region.

Pramod (2007) reported about the prevalence of high population of thrips and higher incidence of necrosis virus in sunflower during *kharif* seasons of 2005 and 2006 in Karnataka. From the pooled data it was noticed that thrips were significantly and positively correlated with maximum temperature (0.55). Minimum temperature showed non significant positive correlation (0.28) where as rainfall, morning and evening relative humidity showed non significant negative correlation (-0.11, -0.35, -0.14). The relationship between thrips and wind speed, bright sunshine hours was non significant and positive correlation (-0.02, 0.28).

GBNV incidence

Timmanna *et al*. (2020) reported that GBNV disease incidence during *kharif* 2016 and 2017 in tomato crop has shown highly significant negative correlation with maximum temperature (r = -0.924) and rainfall (r = 0.715). Minimum temperature (r = -0.644) and sunshine hours (r = -0.618) exhibited significant negative association and significant positive correlation was observed with morning (r = 0.711) and evening relative humidity (r = 0.899). The disease incidence was influenced by all the weather parameters during current (R^2 = 0.874), one lag (R^2 =0.964) and two lag weeks (R^2 = 0.776). Maximum incidence (23.85 %) of GBNV disease was observed during later stage of the crop growth.

Rahul *et al*. (2020) reported that maximum (r = 0.977) and minimum temperatures (r = 0.985) showed significant positive correlation with leaf curl disease incidence in blackgram during late *rabi* 2019 and 2020. Further mean temperature also showed positive correlation with leaf curl disease incidence (r = 0.985), whereas morning relative humidity showed significant negative correlation (r = 0.974). Evening relative humidity and mean relative humidity showed non significant negative correlation with disease incidence (r = -0.264, -0.770).

Jamuna *et al*. (2019) reported that the mean incidence of GBNV disease in tomato during *kharif* 2016 ranged from 4.90 to 42.50 per cent during cropping period

(42nd SMW to 7th SMW). The cumulative disease incidence of 42.50 per cent was observed at later stage of the crop (7th SMW). The minimum temperature (*r* = -0.717) and evening relative humidity (*r* = -0.600) showed highly significant negative correlation with the disease incidence, followed by rainfall (*r* = 0.573) and rainy days (*r* = -0.568). Maximum temperature (*r* = -0.029) and morning relative humidity (*r* = -0.325) showed non significant negative correlation, but sunshine hours (*r* = 0.600) showed non significant positive correlation with the disease incidence. The mean disease incidence of GBNV was directly proportional to the mean number of thrips. The cumulative disease incidence *i.e.* 42.50 and 45.10 per cent was observed during 2015-16 and 2016-17 *kharif* crops, respectively.

Vinaykumar *et al.* (2019) conducted an experiment during *kharif* 2014-2015 in tomato crop and reported that, the relationship between the bud necrosis disease incidence with maximum temperature, rainfall, and relative humidity indicated that there was a very low positive correlation or almost the disease is unaffected by the weather parameters. High positive correlation existed between the bud necrosis disease incidence and the thrips population with the correlation coefficient of 0.92.

Vijayalakshmi *et al.* (2017) studied influence of weather parameters on incidence of GBNV in groundnut during *kharif* in Tamil Nadu and reported that, minimum temperature (-0.041) and evening relative humidity (-0.192) showed negative correlation while maximum temperature (0.390), morning relative humidity (0.017), rainfall (0.518) and sunshine hours (0.343) showed positive correlation. No significance was observed in all the parameters. In *rabi* 2017, GBNV incidence showed positive correlation with maximum temperature (0.185), minimum temperature (0.140) and sunshine hours (0.193) and negative correlation with morning (-0.532) and evening relative humidity (-0.077).

Lakshmi *et al.* (2016) reported that GBNV and TSV of groundnut showed a significant positive correlation with average thrips population (*r* = 0.677 and *r* = 0.772), maximum temperature (*r* = 0.775 and *r* = 0.746), minimum temperature (*r* = 0.803 and *r* = 0.755) and significant negative correlation with morning relative humidity (*r* = -0.777 and *r* = -0.778) and evening relative humidity (*r* = -0.593 and *r* = -0.564). Regression analyses of per cent incidence of GBNV and TSV with thrips and weather factors has revealed that maximum temperature and thrips population per plant had influenced the development of diseases in groundnut up to 86.5 and 87.8 %.

Leaf curl disease of blackgram caused by GBNV showed a significant positive correlation with maximum temperature (r = 0.737) and minimum temperature (r = 0.778*) and significant negative correlation with maximum relative humidity (r = -0.812*). Multiple linear regression analysis has revealed that all the studied factors together were responsible for 94.95 % of total significant variation in leaf curl disease.

Swamy and Patil (2016) reported highest bud necrosis disease incidence *i.e.* 19.09 per cent in Raichur district followed by Koppal (16.0 %), whereas the lowest incidence of 8.2 per cent was observed in Dharwad district during *kharif* 2012. Similarly, during the *rabi*/summer 2012-13 the highest incidence of 24.0 per cent was recorded in Raichur district followed by Tumkuru (20.1 %), whereas the lowest incidence of 10.5 per cent was recorded in Dharwad. The highest mean incidence of 21.6 per cent recorded in Raichur district followed by Koppal (17.9 %) and Tumkuru (17.1 %). The least mean incidence of 9.4 per cent was recorded in Dharwad district.

Biswas *et al.* (2015) reported that in mungbean crop in Delhi, overall disease incidence of 11.6-18.5 per cent for yellow mosaic disease, 14.4-20.5 per cent for urd bean leaf crinkle disease, 9.4-14.5 per cent for bud necrosis disease. None of the mungbean cultivars exhibited resistance to any of the three diseases. It was also reported that overall bud necrosis disease incidence was estimated to be 14.4 per cent in pre *kharif* and 20.5 per cent in *kharif* season for the period of 2006 to 2009.

Gopal *et al.* (2011) conducted a survey in Andhra Pradesh and reported that PBND in greengram, blackgram, cowpea and soybean showed higher incidence than in peanut. Karimnagar recorded the highest mean incidence of 17.81 + 4.23 per cent (10.3 – 24.7 %) in rainy season, and 25.59 + 4.11 per cent (19.8 – 29.1 %) in post rainy season in Ranga Reddy district. The lowest incidence was recorded in Kurnool district *i.e.* of 8.94 + 3.58 per cent (range of 4.3 – 13.3 %). Mean incidence of PBND in rainy season across the five districts ranged from 27.6 per cent (Karimnagar and Ranga Reddy) to 47.0 per cent (Guntur) in greengram. Mean incidence of PBND in blackgram was 27.7 per cent in Ranga Reddy district in post rainy season. In chilli, the mean incidence ranged from 8.33 per cent (rainy season) to 18.6 per cent (in post-rainy season) in Ranga Reddy district. In cowpea, 8.33 per cent (in rainy season) to 40.4 per cent (in post rainy season) and in watermelon 35.83 per cent (post rainy season) was recorded in Ranga Reddy district.

Biswas *et al*. (2009) reported that the incidence of GBNV disease varied from cultivar to cultivar in both the *kharif* seasons of 2006 and 2007. In exception to cv. KU 317, all the cultivars were infected by GBNV in both the seasons with varying degrees of incidence, ranged from 3.0 to 8.0 per cent in 2006 and 3.6 to 14.5 per cent in 2007. The cvs Pant U 35, T 9 and P 2056 showed more susceptibility to GBNV in both the seasons.

Bhat *et al.* (2001) conducted a survey in Delhi during August to October 1999 and reported that disease incidence percentage was maximum on greengram (2-20 %) followed by soybean (2 - 12 %), cowpea (0.4 - 6 %) and blackgram (0.2 - 2 %) in different cultivars.

2.3 STUDY OF TRANSMISSION OF GROUNDNUT BUD NECROSIS VIRUS BY THRIPS IN BLACKGRAM

2.3.1 Mechanical inoculation of GBNV

Singh *et al*. (2018) reported that viral RNA accumulation parallels with the H_2O_2 production and induction of cell death by GBNV infection in cowpea plants is temperature dependent. Plants incubated at higher (30 and 25°C) temperatures showed a severe necrosis and a higher viral RNA accumulation at the inoculated site and facilitated the viral spread at the systemic site. However, viral RNA accumulation was less at the systemic site than the inoculated site. In contrast, symptoms expression and viral RNA accumulation decreased at the inoculated site at low (20 and 15°C) temperatures and no viral symptoms were observed at the systemic site (15°C) in addition to viral RNA accumulation suppression at this site. GBNV infection at the inoculated site induced the higher accumulation of H_2O_2 followed by the induction of cell death at higher temperatures (30 and 25°C) than the lower (20 and 15°C) temperatures.

Suganyadevi *et al*. (2018) reported that the GBNV infected tomato leaf samples were identified by the presence of chlorotic and necrotic spots on leaves from different parts of Tamil Nadu. Symptomatic tomato leaf samples were inoculated on cow pea cv. CO5 leaves on cotyledon leaves under insect-proof condition. Cowpea leaves exhibited chlorotic to necrotic lesions of inoculated cotyledon leaves.

Singh *et al*. (2018) reported that GBNV infection induced its typical symptoms (chlorosis and necrosis) within 4-8 days of post infection (dpi) in a mechanically inoculated cowpea plants and GBNV infection spreaded systemically at 8 dpi which instigate the two types of cell death at the inoculated (necrosis) and systemic site (premature senescence) at 25 °C.

Raigond *et al*. (2017) reported that the suspected potato plants showing typical symptoms of stem necrosis disease in field was due to the infection by GBNV. The inoculum from suspected plants when sap-transmitted onto indicator host plants (cowpea) showed characteristic chlorotic and necrotic local lesions which later turned to necrotic after 10 to 15 days of inoculation.

Daimei *et al*. (2017) reported that tomato plants that exhibited symptoms of GBNV infection and this GBNV virus was maintained on cowpea (Pusa Komal) by alternating mechanical and thrips inoculation to prevent the development of non transmissble mutants by thrips.

Holkar *et al*. (2016) developed bio assay by sap transmission of both the GBNV and WBNV to cowpea, groundnut and watermelon. WBNV induced only local symptoms including chlorotic spots on cowpea cv. Pusa Komal, typical chlorotic ring spots on cv. C-152, bud necrosis on watermelon and no symptoms on groundnut. Whereas, GBNV induced both local and systemic symptoms including chlorotic/necrotic spots and veinal necrosis on both the cowpea cultivars, systemic yellowing and bud necrosis on groundnut and no symptoms on watermelon.

Mandal *et al*. (2008) reported use of mechanical device consisting of a spray gun, an atomizer and a CO_2 powered sprayer as efficient method of inoculation for *Tomato spotted wilt tospovirus* (TSWV) fifty times higher than the hand inoculation method on different host plants. They have also reported that the inoculum contained infected leaf sap prepared in 0.1M phosphate buffer, pH 7.0, 0.2 % sodium sulfite and 0.01M 2-mercaptoethanol (1 g: 10 mL) and 1 % each of Celite 545 and Carborundum 320 grit. The spray application of chilled inoculum at the rate of 1.1 mL plant^{-1} and at an air pressure of 4.1 bar resulted in systemic infection nearly to 100 % in the tobacco (*Nicotiana tabacum*) plants and 75.0–100 per cent and 72.2 – 91.6 per cent in peanut and tomato, respectively.

Mandal *et al*. (2002) evaluated four peanut genotypes to know the response for mechanical inoculation of TSWV at different temperature gradients and reported that at lower temperature (25 to 30°C), genotypes *viz.* Georgia Runner, Georgia Green, C-99R, C11-2-39 had systemic TSWV infection of 90, 100, 70 and 46.7 per cent, respectively. At higher temperature (30 to 37°C), genotypes *viz.* Georgia Runner, Georgia Green, C- 99R, C11-2-39 had systemic TSWV infection of 70, 64.3, 45 and 17.6 per cent, respectively.

2.3.2 Molecular studies of GBNV

Renuka *et al*. (2020) reported that total RNA of GBNV was extracted from the infected leaves of tomato, chilli, and brinjal. cDNA synthesis followed by PCR amplification using N gene specific primers were successfully amplified DNA fragment of 0.8 kb and also reported that the PCR amplified fragments were cloned and sequenced. The analysis of sequence revealed that the N gene sequence of tomato isolate showed 93 to 98 per cent nucleotide identity with black gram (AY512650.1) and tomato (AY463968.1) while chilli isolate showed 97 to 99 per cent nucleotide identity with pea (JF281101) and chilli (AY618567.1), brinjal isolate showed 97 to 99 per cent nucleotide identity with *Solanum nigrum* (KX244339.1) and groundnut (JX198661.1) of other known GBNV isolates available at NCBI database.

Suganyadevi *et al*. (2018) reported that when RNA extracted from symptomatic cowpea and non-symptomatic leaves were subjected for RT-PCR assay with GBNV nucleocapsid protein gene-specific primers, GK-PBNV-CP-F (5'-ATGTCTAACGTYAAGCAGCTC-3') and GKPBNV-CP-R (5'-TTACAATTCCAGCGAAG GAC-3'), the symptomatic leaf samples resulted in the amplification of an approximately 831 bp amplicon and there was no amplification from non-symptomatic leaves.

Raigond *et al*. (2017) reported that the results of RT-PCR assay using GBNV coat protein primers showed an amplicon of expected size *i.e.,* 560 bp indicating the infection of GBNV in the samples showing stem necrosis under field conditions. The sap inoculated cowpea which was followed by potato plants were also examined by RT-PCR based detection of the suspected virus. The results of RT-PCR showed an expected amplicon from both cowpea and subsequently potato plants. They have also reported that the symptoms expressed under field and glasshouse conditions were due to the infection of GBNV.

Kareem and Byadgi (2017) during their study to find association of GBNV in murda complex disease of chille (Cv. Byadgi Dabbi) through molecular approach reported no specific association of *Tospovirus* (GBNV) is involved with the chilli murda complex. Upon amplification by PCR with gene specific primers, ~831bp size amplicon was obtained in GBNV infected groundnut sample. No amplification was found both in diseased and healthy chilli samples (Cv. Byadgi Dabbi).

Holkar *et al*. (2016) developed a single and duplex RT-PCR for diagnosis of WBNV and GBNV by designing primers based on differential sequence from Gn/Gc genes located on M RNA and from nucleocapsid protein (NP) gene located on S RNA genome. The duplex RT-PCR using NP gene specific primers was successfully utilized for the diagnosis of natural infection of GBNV in dahlia and muskmelon and mixed infection of GBNV and WBNV in chrysanthemum.

Ansar *et al.* (2015a) reported that GBNV was effectively transmitted by mechanical sap and insect vector, thrips (*T. palmi*) inoculation. Association of the GBNV with infected experimental potato host plants was detected by RT-PCR using specific primer pairs that targets NP gene.

Gurupad and Patil (2014) reported that virus was mechanically transmitted on to cow pea cv. C152 leaves and produced chlorotic as well necrotic local lesions. Reverse transcription-polymerase chain reaction (RT-PCR) was used for studying the presence of GBNV in different parts, *i.e*., roots, midrib, fruit pericarp and leaves of tomato bud blight field sample. Amplification of 831bp GBNV-CP by RT-PCR with degenerate primers revealed the positive reaction for GBNV infection in different parts *i.e*., roots, midrib, fruit pericarp and leaves of tomato.

Akram and Naimuddin (2013) reported that RT PCR products from field infected Rajmash, sap inoculated rajmash and cowpea analyzed in agarose gel electrophoresis revealed the presence of DNA fragment of ~800 bp corresponding to CP gene of GBNV.

Gurupad and Patil (2013) reported that bud blight affected samples of tomato collected from Dharwad, Belgaum, Haveri, Bengaluru rural and Kolar showed positive reaction with polyclonal antibodies of GBNV in DAC-ELISA and amplification of 831 bp GBNV-coat protein by RTPCR with degenerate primers.

Sujitha *et al.* (2012) reported that the natural occurrence of *Groundnut bud necrosis virus* (GBNV) on onion was detected by enzyme linked immunosorbent assay (ELISA) using an antiserum raised against GBNV and reverse transcription polymerase chain reaction (RT-PCR) using coat protein gene specific primers specific for the nucleocapsid gene of GBNV resulted in an amplicon of the expected size (~800 bp). Sequence analysis showed 93 to 100 per cent and 95 to 100 per cent identity at nucleotide and amino acid levels, respectively with other reported GBNV isolates.

Saritha and Jain (2007) reported that comparative sequence analysis revealed the genome organization of the S and M RNA segments of both GBNV-MB and GBNV-type isolates were similar. However, considerable differences were observed in their intergenic regions (IGRs) and the glycoprotein precursors (Gn = Gc) of the M RNA segments. M RNA IGRs of GBNV-MB and GBNV-type isolates differed in size by 14 nucleotides. This difference was of 75 nucleotides in another GBNV isolate from *L. esculentum* (Tomato). Sequence comparison of the M RNA IGRs of GBNV isolates from mungbean, groundnut and tomato from India revealed 56–89 % sequence identity.

Raja and Jain (2006) reported that bud blight affected tomato samples collected from Coimbatore (Tamil Nadu: TN-Co), Kanpur (Uttar Pradesh: UP-Ka), Pune (Maharashtra: MH-Pu) and Rahuri (Maharashtra: MH-Ra) showed positive reaction with the nucleocapsid protein gene amplified using GBNV specific primers from both leaf and fruit pericarp tissues from bud blight affected plants. The amplicons were specific ~800 bp and reproducible, confirming the association of GBNV with bud blight affected samples collected from four locations.

Thein *et al*. (2003) reported that symptomatic mungbean plants showed positive reaction with GBNV and *Watermelon silver mottle virus* (WSMoV) antisera in direct antigen-coated enzyme-linked immunosorbent assay. The nucleocapsid protein (N) gene of the virus was amplified, cloned and sequenced (GenBank Accession number AF515818). The sequenced region contained an ORF of 831 nucleotides that could potentially code for N protein of 276 amino acids. Comparative sequence analyses revealed that the N gene shared 97 and 99 % sequence identity with GBNV at nucleotide and amino acids levels, respectively suggesting the *Tospovirus* isolate from mungbean to be a strain of GBNV.

2.3.3 Rearing of thrips

Wan *et al.* (2020) used bean jar method for rearing of thrips *F. occidentalis*. Adult females were allowed to oviposit on bean pods for 24 h, then adults were removed and the bean pods containing eggs were transferred to a different jar. The resultant jars were placed in an incubator at 26 ± 1 °C with a L16:D8 photoperiod until nymphs hatched. These reared thrips were used for further studies on effects of TSWV on life history traits of thrips vector.

Viviana *et al*. (2019) proposed a method for the rearing of *Frankliniella zucchini* (Nakahara & Monteiro) which is a species of thrips so far reported only in Brazil, on fresh virus free Zucchini Caserta fruits, a practical and efficient alternative for the supply of a large number of insects for later study of virus/vector relationship.

Ghosh *et al*. (2019b) used detached leaf disc and whole plant methods for rearing of thrips with slight modifications to the method proposed by Rothenberg (2015) and used these thrips for further GBNV transmission studies.

Daimei *et al*. (2017) used glass bean jar method for maintenance of *T. palmi* colony. Thrips were maintained at 25 ± 2^{o}C, 60 per cent relative humidity and a photo period of 16h dark and 8h light. Fresh bean pods were replaced with old beans daily in cages for egg laying by female insects.

Reiter *et al*. (2014) have summarized and briefly presented the available methods for laboratory rearing of *T. tabaci i.e.*, bean jar method, potted plant with screening, potted plants without nets, methods using boxes and other containers, membrane method, petri dish method , vial method *etc.*

Degraff and Wood (2009) described a protocol for rearing western flower thrips, *F. ocidentalis* using whole persian cucumber fruits as a host plant. They have also reported that persian cucumbers are an effective culture medium for providing moderate numbers of western flower thrips. Beans and bean leaves tended to wilt in temperatures promoting thrips growth, but cucumbers generally remained viable for the entire lifecycle of the thrips and maximized larval emergence from the oviposition effort.

Murai and Loomans (2001) reported that larvae of thrips can be reared on pollen or on germinated broad bean seeds until adult emergence without additional water and food. This method has been found useful for producing even-aged thrips at different densities up to 500 larvae in a cage of 80 mm diameter with relatively low mortality rates.

In most of the earlier studies thrips were reared on detached cowpea and groundnut leaflets and further used for transmission studies (Ghanekar *et al*. 1979., Vijayalakshmi, 1994., Sreekanth, 2002).

2.3.4 Virus Vector Transmission studies

Mou *et al*. (2021) reported that *T. palmi* transmitted *watermelon silver mottle virus* (WSMoV) in a persistent manner, and the virus was mainly transmitted by adults when ingested at the first-instar larval stage. Complementary RNAs corresponding to the NSm and NSs genes of WSMoV were detected in viruliferous thrips by reverse transcription-polymerase chain reaction; NSs protein was also detected in viruliferous thrips by western blotting, verifying the replication of WSMoV in *T. palmi*.

Ruth (2018) reported *T. palmi* as the vector of the GBNV causing bud necrosis disease in tomato and cowpea. The larvae of *T. palmi* could acquire the virus with a minimum access period of 15 min. and the adults could only transmit the virus with one hour inoculation access period (IAP). However, optimum virus transmission obtained with 48 h of acquisition access period (AAP) in the larval stage and 48 h of IAP in the adult stage, but beyond 48 h of AAP and IAP resulted in decreased virus transmission. A single adult *T. palmi* could transmit the virus with a transmission rate of 24 to 32 percent and maximum transmission rate (100 %) was achieved with ten adults per seedling.

Ansar *et al.* (2015) reported that *T. palmi* was able to acquire and transmit the GBNV (5.5 %) within an acquisition feeding period (AFP) of 24 h. Further, percent transmission (27.7 %) was found to increase when the AFP extended to 72 hrs. There was no transmission of the virus at 6 and 12 h of AFP in three successive experiments. At AFP of 48 h *T. palmi* was able to transmit the virus (5.5 %). Moreover, rate of transmission increased when duration of inoculation feeding period

(IFP) was increased. Percent transmission was 11.1, 16.6, 22.2 and 33.3 when IFP was 72, 96, 120 and 144 hrs, respectively.

Brithia *et al.* (2013) reported that after 4 h post acquisition period, a significant increase in NSs proteins was observed in *Iris yellow spot virus*-fed *T. tabaci*, but not in other non-vector thrips species and colour forms such as *F. occidentalis*, *F. schultzei* (dark) and *F. schultzei* (pale) clearly differentiating vectors and non-vectors of IYSV. IYSV replication did not influence the survival of the vector thrips species, *T. tabaci* populations or the non-vector thrips species.

Srinivasan *et al*. (2012) reported that both *F. fusca* and *T. tabaci* can transmit IYSV. Further, transmission efficiencies studies with *F. fusca* and *T. tabaci* revealed that both *F. fusca* and *T. tabaci* transmitted IYSV at 18.3 and 76.6 per cent, respectively. Results confrmed that *F. fusca* also can transmit IYSV but at a lower efficiency than *T. tabaci*.

Shresta *et al*. (2012) reported that thrips species *F. fusca* from the potentially viruliferous colony alone tested positive for the virus and none of the insects in the non-viruliferous colony tested positive for TSWV. The presence of NSs proteins in thrips indicated that early instars acquired the virus and that virus propagation took place in thrips. Regardless of TSWV infection of peanut leaflets, potentially viruliferous *F. fusca* produced significantly more eggs than non-viruliferous *F. fusca.*

Inoue *et al*. (2010) reported that IYSV was transmitted highly efficiently by adults and also by larvae of five thelytokous populations of *T. tabaci* from distinct areas in Japan. They have further reported that IYSV infection was not detrimental to the development and fecundity of thrips until early adulthood. Larval mortalities of virus-exposed thrips were higher than in their unexposed counterparts in all three populations, but the differences were not significant. The results demonstrated that *T. tabaci* populations have considerable potential to cause outbreaks of IYSV and spread the disease because of their efficient transmission of the virus.

Filho *et al*. (2004) reported that thrips at 1, 5, 10, and 20 days after adult emergence (DAE) fed on TSWV-infected plants have acquired TSWV. Virus replication and accumulation which occurred in both epithelial and muscle cells of *F. fusca* and *F. occidentalis* was indicated by immune detection of the nonstructural

(NSs) protein encoded by the small RNA and the nucleocapsid (N) protein, respectively. There was no significant effect of insect age on TSWV acquisition by *F. fusca*. They have further reported that adult thrips that feed on virus-infected plants do not transmit the virus as tissue barrier and infection from midgut muscle cells to the salivary glands plays key role in TSWV movement.

Nagata *et al*. (2002) reported that adults of the *F. occidentalis* population transmitted TSWV efficiently, whereas those of the thelytokous *T. tabaci* population failed to transmit. The virus was almost undetectable in *T. tabaci* adults, whereas high titers were readily detected in the *F. occidentalis* adults. They have also reported that the rate of virus replication in the midgut and the extent of virus migration from the midgut to the visceral muscle cells and the salivary glands as probable crucial factors in the determination of vector competence.

2.4 MANAGEMENT OF THRIPS IN BLACKGRAM THROUGH INSECTICIDES

Literature pertaining to the efficacy of seed treatment and insecticidal sprays against thrips, whiteflies and other sucking pests is reviewed here under.

2.4.1 Imidacloprid

Reddy *et al*. (2020) reported that among all the treatments imidacloprid 17.8 SL (20.54 %) was found most effective in controlling the sucking insect pests in blackgram during *kharif* 2017. The highest CB ratio was recorded with imidacloprid 17.8 SL (4.27), followed by NSKE (3.88), neem oil (3.74), *Bacillus thuringiensis* (*Bt*) (3.48), *Beauveria bassiana* WP (3.34), Jatropa oil (3.24), garlic extract (GE) (3.09) when compared to untreated check (1.58).

Singh *et al*. (2019) reported that imidacloprid (0.005 %) and fipronil (0.01 %) proved to be the most effective treatments next to acetamiprid (0.004 %) against sucking insect pests in greengram. The treatments of thiamethoxam (0.005 per cent) and dimethoate (0.03 %) stood in middle order of efficacy followed by the treatments of spiromesifen (0.001 %) and fenpropathrin (0.05 %) which were proved least effective against sucking insect pests of green gram during *kharif* season.

Radhika *et al.* (2018b) conducted an experiment to evaluate the efficacy of insecticides as seed treatment against thrips population in blackgram during *rabi* 2017-18 in Hyderabad and reported that imidacloprid 70 WS at 5 g kg^{-1} was found to be the most effective treatment with 2.67 thrips per six leaves among all the other tested insecticides followed by thiamethoxam 25 WG at 3 g kg^{-1} with 2.80 thrips per six leaves. The least effective insecticidal treatments were carbosulfan 25 EC at 30 mL kg^{-1} and dimethoate 20 EC at 10 mL kg^{-1} which recorded 6.67 and 7.00 thrips per six leaves, respectively. The highest population of thrips was recorded in the untreated control plot with 8.80 thrips per six leaves.

Abhijit *et al.* (2018) conducted an experiment to study the relative efficacy and economics of seed treatment and newer insecticides against sucking, borer pests of mungbean during summer 2015 and 2016. During 2015, Seed treatment with imidacloprid 48.0 FS @ 5 mL kg^{-1} seed + thiamethoxam 25 WG @ 50 g a.i. ha^{-1} at 30 DAS was found most effective with minimum population of thrips (4.3 numbers/10 flowers) as against 32.5 numbers/10 flowers in untreated control. During 2016, the populations of thrips were found minimum in seed treatment with imidacloprid 48.0FS @ 5 mL kg^{-1} seed + thiamethoxam 25 WG @ 50 g a.i. ha^{-1} at 30 DAS *i.e.* 8.0 numbers/10 flowers compared to 44.0 numbers /10 flowers in untreated control.

Darshan *et al*. (2018) evaluated certain insecticides for the management of sucking pests in mothbean in Gujarat during *rabi* season and reported that imidacloprid 17.8 SL @ 0.005 per cent found most effective with lowest population of thrips (1.30) and it was found at par with thiamethoxam 25 WG @ 0.008 per cent (1.33) and acetamiprid 20 SP @ 0.004 per cent (1.36). The maximum yield was obtained in plots treated with thiamethoxam 25 WG @ 0.005 % (701 kg ha^{-1}) while minimum yield was obtained from the control treatment of unsprayed condition (400 kg ha^{-1}).

Sobharani *et al.* (2017) reported that the higher doses of imidacloprid 60 FS *i.e.*, at 10 mL and 15 mL kg^{-1} of seeds were proved superior in protecting the blackgram crop under rainfed condition from the early season sucking pests *viz.*, thrips, aphids and leafhoppers up to 40- 45 days after sowing and recorded significantly highest grain yield. Seed treatment with imidacloprid 60 FS @ 15 mL kg^{-1} of seeds and 10 mL kg^{-1} of seeds were found most effective with 1.00 and 1.22 thrips/top three leaves, respectively followed by imidacloprid 60 FS @ 5 mL kg^{-1} of

seeds with 2.33 thrips/top three leaves. Whereas in the untreated plots, thrips population ranged from 4.22 to 5.00 thrips/top three leaves. Yield was recorded highest (8.68 q ha^{-1}) in treatment with imidacloprid 60 FS @ 15 mL kg^{-1} of seeds when compared with untreated control (2.87 q ha^{-1}).

Ruth *et al.* (2016) conducted an experiment during *kharif* 2009 – 2013 in tomato in Andhra Pradesh and reported that seed treatment with imidacloprid (Goucho) @ 5 g kg^{-1} seed + Neem seed kernal extract @ 5 % + Spinosad 0.3 mL L^{-1} were found superior in controlling the viral diseases in tomato during *kharif* season. Whitefly and thrips population were low and were 1.18/plant and 0.51/plant, respectively after post treatment. Least incidence of bud necrosis disease was recorded *i.e.* 6.60, 9.93 and 14.88 at 30, 45 and 60 days after planting in the same treatment plots.

Hossain (2015) tested efficacy of insecticides against insect pests of mungbean during two consecutive seasons of *kharif* of 2013 and 2014 and reported that spraying of imidacloprid @ 0.5 mL L^{-1} showed the best efficacy in reducing thrips population 2/20 flowers during *kharif* 2013 and 1.33/20 flowers during *kharif* 2014.

Sharma and Singh (2015) reported that in blackgram lowest population of thrips (1.34 & 0.95/6 leaves in 2012 & 2013) was recorded in plot treated with imidacloprid 17.8 SL @ 125 mL ha^{-1} followed by thiamethoxam @ 125 mL ha^{-1} (1.65 & 1.17/6 leaves in 2012 and 2013). Significantly maximum yield in blackgram was found in imidacloprid (11.13 & 11.67 q ha^{-1} in 2012 and 2013), followed by thiamethoxam treated plot (10.88 & 11.93 q ha^{-1} in 2012 & 2013).

Bhede *et al*. (2008) conducted experiment to study bioefficacy of newer insecticide against chilli thrips, *S. dorsalis* at Parbhani during *kharif* season and reported that application of phosphamidon 40 % + imidacloprid 2 % SP @ 700 g ha^{-1} was most effective in suppression of thrips population (1.5 thrips/three leaves) and also increased the yield of green chilli (43.52 q ha^{-1}) during *kharif* season.

Archana *et al.* (2018) conducted experiment on management of yellow mosaic disease (YMD) in blackgram by using different combination of insecticides and neem based pesticides during *kharif* 2017 in Karnataka and reported that seed treatment with imidacloprid 600 FS @ 5.0 mL kg^{-1} and two sprays of imidacloprid 17.8 SL (@ 0.5 mL L^{-1}, 30 and 45 DAS during *kharif* season had significantly less YMD incidence and whitefly population. Further they have reported that seed treatment with

imidacloprid 600 FS and two sprays of imidacloprid 17.8 SL recorded higher growth and seed yield (11.04 q ha^{-1}) when compared to other treatments and control.

Mahalakshmi *et al.* (2015) tested field efficacy of certain neonicotinoids against whitefly in blackgram and reported that acetamiprid 20 SP @ 0.2 g L^{-1} was found promising against whiteflies with 57.02 per cent population reduction. Triazophos 40 EC @ 1.25 mL L^{-1} was found on par (42.92 %) with the neonicotinoids such as imidacloprid 200 SL @ 0.3 mL L^{-1} (38.65 %), thiamethoxam 25 WG @ 0.2 g L^{-1} (45.15 %) and thiacloprid 21.7 SC @ 1.25 mL L^{-1} (50.22 %).

2.4.2 Thiamethoxam

Vijayaraghavan and Kavitha (2020) conducted field experiments in Tamil Nadu during 2016-17 and reported that thiamethoxam 25 WG @ 0.2 g L^{-1} was found effective with 2.10 whiteflies/plant and it was next to clothionidin 50 WDG @ 0.1 g L^{-1} (1.40 whiteflies/plant). In untreated control a mean population of 8.50 whiteflies/plant was recorded during *kharif* season in blackgram. During *rabi* also thiamethoxam 25 WG @ 0.2 g L^{-1} was next best to clothionidin 50 WDG @ 0.1 g L^{-1} with 2.16 whiteflies/plant thiamethoxam 25 WG @ 0.2 g L^{-1} recorded 76.82 per cent reduction over control which was next best to clothionidin 50 WDG @ 0.1 g L^{-1} with 80 per cent reduction of population over control.

Surbhi *et al.* (2018) conducted field trial to evaluate the efficacy of insecticides as seed treatments and foliar spray against *S. dorsalis* in greengram during summer, 2017 in Gujarat and reported that seed treatment with thiamethoxam 25 WG @ 0.10 % was found most effective with lowest thrips count *i.e.* 0.45 thrips/leaf. The highest seed yield was gained from the plots treated with thiamethoxam 25 WG @ 0.10 % + spinosad 45 SC @ 0.0135 % with 1066 kg ha^{-1}.

Radhika *et al.* (2018b) conducted an experiment with novel group of insecticides as seed treatment during *rabi* 2017-18 and reported that seed treatment with thiamethoxam 25 WG at 3 g kg^{-1} was effective against thrips with less number of thrips (2.80/six leaves) after imidacloprid 70 WS at 10 mL kg^{-1} with 2.67 thrips per six leaves.

Sujatha and Bharpoda (2017) conducted a field trial in greengram during *kharif* 2015 in Gujarat and reported that thiamethoxam 25 WG (0.01 %) was found

most effective with lowest number of *T. palmi* (0.48/three leaves) followed by imidacloprid 70 WG (0.014 %) with 0.54 thrips per three leaves. Flower thrips, *M. usitatus* were also recorded low (0.33/three leaves) in case of thiamethoxam 25 WG (0.01 %) which was at par with imidacloprid 70 WG (0.014 %) with 0.46 thrips per three leaves . Higher Incremental Cost Benefit Ratio (ICBR) 1:7.81 was obtained in the treatment thiamethoxam 25 WG (0.01 %).

Indrajeet *et al*. (2017) conducted a field trail to evaluate the efficacy of insecticides against sucking pest complex in blackgram during *kharif* 2016-17 and reported that thiamethoxam 25 WG was effective with 59.16 per cent reduction of population of aphids followed by emamectin benzoate @ 11 g a.i. ha^{-1} with 54.86 per cent population reduction over untreated control.

Samota *et al*. (2017) conducted an experiment during 2014 - 2015 to evaluate the efficacy of insecticides and bio pesticides against *S. dorsalis* on chilli during summer in Rajasthan and reported that thiamethoxam recorded 80.79 mean per cent reduction in thrips population over untreated control and it was effective next to acetamiprid (82.62 %) followed by imidacloprid (77.90 %), fipronil (76.38 %) and standard check (71.92 %).

Singh *et al.* (2016) conducted a field study during 2013 in mungbean during *kharif* and reported that thiamethoxam 180 g a.i. ha^{-1} found effective against thrips population which recorded 68.89 per cent reduction over untreated control.

Somasundar *et al.* (2016) reported that thiamethoxam @ 8.6 g kg^{-1} and 4.3 g kg^{-1} was highly effective against thrips up to 30 days after germination when applied as seed dresser in greengram during *rabi* 2008. Lowest number of thrips 30 and 33 per ten plants were recorded in the above treatments, respectively.

Kaushik *et al*. (2015) conducted a field trail during summer, 2013 with different combination of insecticides to manage thrips in cowpea and reported that seed treatment with thiamethoxam 30 FS + spray with imidacloprid 17.8 SL was found most effective in suppressing the population with 55.1 per cent reduction. It was at par with thiamethoxam (54.9 %), seed treatment with imidacloprid 17.8 SL + spray with thiamethoxam 30 FS (52.3 %), imidacloprid (51 %), seed treatment with thiamethoxam 70 WS + spray with imidacloprid 17.8 SL (51.8 %). However,

application of profenofos, azadirachtin, lambda-cyhalothrin and quinalphos were found next effective treatment as they recorded 30.6, 25.9, 24.4 and 22.1 % mortality of thrips, respectively.

Justin *et al.* (2015) conducted field trials in blackgram during *rabi* 2011-12, 2013-14 and reported that seed treatment with thiamethoxam 25 WG @ 3 g kg^{-1} of seed + spray with thiamethoxam 25 WG @ 0.4 g L^{-1} recorded the lowest population of aphids (0.73/plant) followed by spraying of imidacloprid 17.8 SL @ 0.4 mL L^{-1} (1.03/plant) when compared to control, in blackgram during *rabi* season. The observations recorded in 7 and 14 days showed a similar trend and the per cent reduction of aphids over control after second application indicated that seed treatment with thiamethoxam 25 WG @ 3g kg^{-1} of seed + spray with thiamethoxam 25 WG @ 0.4 g L^{-1} exerted 90.32 per cent reduction of aphids followed by spraying of imidacloprid 17.8 SL @ 0.4 mL L^{-1} (87.45 %).

Yadav *et al*. (2015b) conducted a field trail at Panthnagar during *kharif* 2012 and reported that thiamethoxam 25 WG was found superior in reducing whitefly population *i.e.* 1.46/three leaves followed by triazophos 20 EC @ 2 mL L^{-1} with 1.66 whitefly/three leaves in blackgram. Similarly, thiamethoxam 25 WG @ 0.25g L^{-1} (0.15 leafhopper/3 leaves) was found most effective treatment to manage the leaf hopper population. It was followed by acetamiprid 20 SP @ 0.25g L^{-1} which recorded a population of 0.17 leafhopper /3 leaves.

2.4.3 Fipronil

Radhika and Reddy (2018) conducted an experiment during *rabi* 2017 to estimate the losses caused by sucking pest complex in blackgram at Hyderabad and reported that spraying of insecticides monocrotophos 36 SL @ 1.6 mL L^{-1} and fipronil 5 SC @ 1 mL L^{-1} at weekly intervals against sucking pests *viz.*, whiteflies, thrips, aphids and leafhoppers in blackgram saved 269 kg ha^{-1} pod yield with an avoidable yield loss of 26.16 per cent.

Kumar and Kumar (2018) in their study about pooled efficacy of two sprays against thrips in groundnut during *kharif* 2014, 2015, 2016 showed that fipronil 80 WG and fipronil 5 SC were significantly superior with 62.0 and 57.6 per cent reduction in thrips foliage damage over control followed by diafenthiuron 50 WP and bifenthrin 10 EC with 51.2 and 50.9 per cent reduction of thrips damage over control, respectively.

Dongarjal *et al*. (2018) reported that the post treatment count of live population of thrips at seven days after third spray in pomegranate during 2014-2015 clearly indicated the superiority of fipronil 5 SC @ 50 mL a.i. ha^{-1} (1.33 and 1.63 thrips/fruit), over other treatments followed by thiamethoxam 70 WG @ 25 g a.i. ha^{-1} (1.63 and 1.92 thrips/fruit) and clothianidin 50 WDP @ 20 g a.i. ha^{-1} (1.96 and 2.17 thrips/fruit), respectively. These three treatments were statistically at par with each other and were significantly superior over rest of the treatments in minimizing thrips incidence.

Swathi *et al.* (2018) reported that among the tested insecticides, acetamiprid 4 % + fipronil 4 % @ 2 mL L^{-1} was found effective against thrips by reducing 70.81 % thrips population next to thiacloprid 21.7 SC @ 0.0325 % with 74.80 per cent reduction of thrips population over untreated control, whereas flonicamid 50 WG @ 0.0325 % was very effective against the population of whitefly by reducing 72.19 % and lowest per cent disease incidence (17.66 %) followed by acetamiprid 4 % + fipronil 4 % @ 2 mL L^{-1} (64.94 %) and thiamethoxam 25 WG @ 0.005 % (62.21 %) which were on par with each other over control in rice fallow blackgram during *rabi* 2017-18.

Hossain (2015) reported that the lowest number of infested flowers (1.67/20 flowers) was observed in imidacloprid sprayed plots @ 0.5 mL L^{-1} which was statistically identical to fipronil 5SC @ 0.5 mL L^{-1} (3/20 flowers). More than 80 per cent flower infestation reduction was observed in imidacloprid and fipronil sprayed plots. Accordingly the lowest number of thrips (2.00/20 flowers) were observed in imidacloprid sprayed plots which was statistically similar to fipronil in mungbean during *kharif* season.

Patil *et al.* (2009) reported that fipronil 5 SC @ 800g ha^{-1} registered least number of thrips (8.47/3 leaves) in cotton. Significantly highest seed cotton yield 27.23 q ha^{-1} in 2007, 27.50 q ha^{-1} in 2008 was harvested with higher dosage of fipronil 5 SC @ 800 g ha^{-1} during *kharif* 2007 and 2008, respectively.

2.4.4 Daifenthiuron

Shakya *et al*. (2020) evaluated certain insecticides in blackgram during *kharif* 2017 and reported that diafenthiuron 50 WP @ 312.5 g a.i. ha^{-1} was found most

effective resulting in 85.9 % and 77.8 % reduction of whitefly and thrips population, respectively. The treatment spiromesifen 240 SC@ 120 mL a.i. ha^{-1} was found most effective against jassid resulting in 77.5 % reduction of population over untreated control.

Kumar and Kumar (2018) in their study about pooled efficacy of two sprays against leafhoppers during *kharif* 2014-2016 showed that diafenthiuron 50 WP and bifenthrin 10 EC were significantly superior over rest of the treatments in reducing the leafhopper population over control after two sprays with mean efficacy of 62.8 and 59.4 per cent, respectively. Highest dry pod yield of 2152.8 kg ha^{-1} was recorded with fipronil 80 WG significantly followed by fipronil 5 SC (1861.1 kg ha^{-1}), diafenthiuron 50 WP (1708.3 kg ha^{-1}) and bifenthrin 10 EC (1611.1 kg ha^{-1}) which were at par. However, highest ICBR was recorded with fipronil 5 SC (29.7), followed by bifenthrin 10 EC (26.6), acephate 95 SG (23.3), buprofezin 25 EC (22.2) and diafenthiuron 50 WP (16.6).

Kharel *et al*. (2016) evaluated different doses of insecticides against sucking pest complex in blackgram during *kharif* 2015 and reported that diafenthiuron 50 WP @ 312 g a.i. ha^{-1} was most promising treatment in reducing population of flower thrips (*C. indicus*) (0.92 thrips/10 flowers) after both sprays followed by spiromesifen 240 SC @ 150 g a.i. ha^{-1} with 1.34 thrips /10 flowers.

2.4.5 Flonicamid

Swathi *et al.* (2018) reported that flonicamid 50 WG @ 0.0325 % was very effective against the population of whitefly by reducing 72.19 % mean per cent whitefly population over untreated control and also very effective in reducing YMV disease incidence (17.66 %) over untreated control (58.45 %) followed by acetamiprid 4 % + fipronil 4 % @ 2 mL L^{-1} (64.94 %) and thiamethoxam 25 WG @ 0.005 per cent (62.21 %) which were on par with each other over control in rice fallow blackgram during *rabi* 2017-18.

Sujatha and Bhaproda (2016) evaluated insecticides against sucking insect pests in greengram during summer 2015 in Gujarat and reported that thiamethoxam 25 WG @ 0.01 % and imidacloprid 70 WG @ 0.014 % found significantly superior than rest of the insecticidal treatments and recorded lower (0.62 and 0.67/3 leaves,

respectively) population of thrips. Diafenthiuron 50 WP @ 0.05 % (1.87) and flonicamid 50 WG @ 0.015 % (1.96) were also found effective by remaining at par with dimethoate 30 EC @ 0.03 % (2.06) against thrips in green gram. Similarly, thiamethoxam 25 WG @ 0.01 % (0.14) and imidacloprid 70 WG @ 0.014 % (0.17/5 flowers) recorded significantly lower population of *M. usitatus* and found most effective against this pest. Diafenthiuron 50 WP @ 0.05 % (0.64) was next in order followed by flonicamid 50 WG @ 0.015 % (0.71) and dimethoate 30 EC @ 0.03 % (0.78).

2.4.6 Buprofezin

Kumar *et al*. (2019) evaluated different combinations of buprofezin for the management of chilli trhips, *S. dorsalis* and *Aphis gossypii* (Glover) during *kharif* 2016 and reported that the overall mean per cent field efficacy after second spray was highest (69.8 %) in fipronil 5 + buprofezin 20 SC @ 100 + 400 g a.i. ha^{-1} treated plot followed by plots treated with fipronil 5 + buprofezin 20 SC @ 50+200 g a.i. ha^{-1} (61.9 %).

Mahalakshmi *et al*. (2015) tested field efficacy of certain insecticides in blackgram during *rabi* 2010-11 and 2011-12 and reported that buprofezin 10 EC @ 1.0 mL L^{-1} was found effective next to spiromesifen 240 SC @ 0.4 mL L^{-1} with more than 75 per cent mean reduction in nymphal population of whiteflies and with below twenty per cent incidence of YMV.

2.4.7 Spinetoram

Matharu and Thanwar (2020) evaluated bio efficacy of novel insecticides against *T. tabaci* in cotton during *kharif* 2019 in Punjab and reported that the lowest population of thrips *i.e.* 3.03 per leaf was recorded with the treatment of spinetoram 11.7 SC followed by diafenthiuron 50 WP and thiamethoxam 25 WG with 8.70 and 12.07 thrips per leaf, respectively after 10 days of spray. Similarly, the highest yield of cotton (21.25 q ha^{-1}) and benefit cost ratio (3.48) was observed in case of spinetoram 11.7 SC treatment.

Chinniah *et al*. (2019) conducted a study in Tamil Nadu in grapevine orchards during *kharif* 2015 and reported that spinetoram 10 % WG + sulfoxaflor 30 % WG

@ 350, 300 and 250 mL ha^{-1} were highly effective in reducing thrips damage in grapevine (11.01, 12.13 & 13.8, respectively). However, imidacloprid 17.8 SL @ 400 mL ha^{-1} recorded 12.9 % berry damage which was at par and superior than minimum dose of spinetoram 10 % + sulfoxaflor 30 % WG @ 250 mL ha^{-1}. The next in the order of efficacy were spinetoram 12 SC @ 292 mL ha^{-1} (19.4 %), sulfoxaflor 24 SC @ 375 mL ha^{-1} (23.4 %) and emamectin benzoate 5 SG @ 220 g ha^{-1} (24.7 %) when compared to untreated check (57.4 %).

Patil *et al*. (2017) evaluated certain insecticides against citrus thrips on sweet orange during February to September, 2016 in Maharastra and reported that spinetoram 11.7 SC @ 36 g a.i. ha^{-1} was most effective and superior over rest of the treatments and recorded lowest (1.60) thrips population. The treatments with spinosad 45 SC @ 112.5; cyantraniliprole 10.26 OD @ 70 g a.i. ha^{-1} were found at par with this treatment and observed thrips population in the range of 1.87–1.91thrips/shoot. The treatment with spinetoram 11.7 SC, spinosad 45 SC and cyantraniliprole 10.26 OD recorded maximum yield of 19.28, 18.30 and 18.12 t ha^{-1} respectively. The highest B:C ratio (1:1.76) was recorded in the treatment with spinetoram 11.7 SC followed by spinosad 45 SC (1:1.59), tolfenpyrad 15 EC (1:1.56), fipronil 5 SC (1:1.55), thiamethoxam 25 WG (1:1.51) and flonicamid 50 WG @ 100 g a.i. ha^{-1} (1:1.43).

Singh *et al*. (2011) conducted an experiment in cotton during *kharif* 2008-09, 2009-10 in Rajasthan and reported that the maximum reduction in the population of thrips, (*T. tabaci*), was recorded in the treatment of spinetoram 12 SC (42.42 %) @ 56 g a.i ha^{-1} and minimum in novaluran 10 EC @ 75 mL a.i. ha^{-1} (24.79). Spinetoram 12 SC @ 56 g a.i. ha^{-1} was also found most effective against larvae of bollworm followed by indoxacarb @ 75 g a.i. ha^{-1}, profenophos 50 EC and novaluran 10 EC @ 75 mL a.i. ha^{-1}, which were at par with each other.

2.4.8 Spinosad

Sharanappa *et al*. (2020) evaluated certain insecticides in Raichur for the management of thrips and mites in capsicum during *rabi* 2018 and reported that the overall mean per cent reduction of thrips population after imposing, these sprays was highest in spinosad 45 SC (88.15 %) followed by fipronil 5 SC (87.24 %) and were found significantly superior than rest of the treatments. For the control of mites,

spiromesifen 24 SC (86.23 %) dicofol 18.5 SC (85.26 %), diafenthuron 25 WP (82.14 %) were proved significantly superior compared to the rest of the chemical pesticides.

Naik *et al.* (2019) reported that the least larval population of *M. vitrata* was recorded in blackgram during *kharif* when treated with profenophos 50 EC + DDVP 76 EC (0.80 larvae/plant) followed by emamectin benzoate 5 SG + DDVP 76 EC (1.33 larvae/ plant) and it was found on par with flubendiamide 480 SC + DDVP 76 EC (1.70 larvae/ plant), spinosad 45 SC + DDVP 76 EC (1.83 larvae / plant) and thiodicarb 75 WP + DDVP 76 EC (1.83 larvae/plant).

Surbhi *et al.* (2018) conducted field trials to evaluate the efficacy of insecticides as seed treatment and foliar spray against *S. dorsalis* in greengram during summer, 2017 in Gujarat and reported that insecticidal spray of spinosad 45 SC @ 0.0135 % was found effective with lowest number of thrips *i.e.* 0.28 thrips/leaf. The highest seed yield was gained from the plots treated with thiamethoxam 25 WG @ 0.10 % + spinosad 45 SC @ 0.0135 % with 1066 kg ha^{-1} followed by imidacloprid 30.5 SC @ 0.12 % + spinosad 45 SC @ 0.0135 % with 1025 kg ha^{-1}.

Bambhaniya *et al*. (2018) evaluated efficacy of certain insecticides against thrips in tomato crop in Gujarat during *kharif* 2016 and reported that spinosad 0.009 % proved significantly the most effective treatment with 93.97 per cent reduction in thrips population (0.77 thrips/3 leaves). It was statistically at par with imidacloprid 0.005 % with 93.71 per cent (0.79 thrips/3 leaves) reduction of thrips. Acetamiprid 0.008 per cent with 91.83 per cent (0.89 thrips/3 leaves), flonicamid 0.015 % with 91.22 per cent (0.93 thrips/3 leaves), difenthiuron 0.05 % with 89.89 per cent (0.99 thrips/3 leaves), clothianidin 0.025 % with 89.79 per cent (1.00 thrips/3 leaves) and dimethoate 0.03 % with 89.18 per cent (1.03 thrips/3 leaves) reduction of thrips over control and remained statistically at par with each other formed third group of effective treatments.

Kumar *et al*. (2017a) conducted a study to evaluate the comparative efficacy of different insecticidal treatments against pod borer, *Helicoverpa armigera* (Hübner) infesting blackgram during summer season, 2013-15 at Panthnagar and reported that seed treatment with imidacloprid 600 FS @ 5 mL kg^{-1} seeds + two sprays of spinosad 45 SC @ 56 g a.i. ha^{-1} at 15 days interval was found most effective with minimum pod damage of 6.44 per cent and maximum grain yield of 703 kg ha^{-1}. However,

insecticidal module having seed treatment with imidacloprid 600 FS @ 5 mL kg^{-1} seeds + two sprays of indoxacarb 14.5 SC @ 65 g a.i ha^{-1} at 15 days interval was most economical with maximum cost : benefit ratio of 1:7.85, closely followed by spinosad 45 SC @ 56 g a.i. ha^{-1} (1:7.72).

Sumalatha *et al*. (2017) reported that spinosad 45 SC @ 73 g. a.i. ha^{-1} and fipronil 5 SC @ 50 g. a.i. ha^{-1} were the most superior and persistent treatments against thrips followed by lamdacyhalothrin 5 EC @ 15 g. a.i. ha^{-1}. Among insecticidal treatments flonicamid 50 SG @ 75 g a.i ha^{-1} and spinosad 45 SC @ 73 g a.i ha^{-1} were found promising regarding its safety to predators. The highest bulb yield was recorded in spinosad 45 SC @ 73 g a.i ha^{-1} (18.03 t/ha) treated plots followed by fipronil 5 SC @ 50 g. a.i. ha^{-1} (16.78 t ha^{-1}) indicating the significance of thrips management in *Kharif* onion during 2016.

Other chemicals

Swathi *et al.* (2019) evaluated certain insecticides to manage *M. vitrata* in blackgram during *rabi* 2017-18 and reported that chlorantraniliprole 9.3 % + λ cyhalothrin 4.6 % @ 0.5 mL L^{-1} was found effective with 75.91 per cent overall mean reduction in *M. vitrata* larval population with lowest pod damage (7.04 per cent) over control (60.58 %) and also recorded highest grain yield (8.31 q ha^{-1}) followed by chlorantraniliprole 18.5 SC @ 0.0037 % and flubendiamide @ 39.35 SC 0.00787 % with 72.04 and 67.30 per cent overall reduction in mean larval population of *M. vitrata* over untreated control. The cost - benefit (C: B) ratio for all the treatments revealed that chlorantraniliprole 18.5 SC @ 0.0037 % was highly economical with a C: B of 1: 17.14 followed by spinosad 45 SC with 1:15.28.

Swathi *et al.* (2018) conducted an experiment to determine the efficacy of different insecticides against sucking pests *viz*., thrips, *C. indicus* and whitefly *B. tabaci***,** infesting blackgram in North coastal Andhra Pradesh during *rabi* 2017-2018 and revealed that thiacloprid 21.7 SC @ 0.0325 % was found effective against thrips by reducing 74.80 per cent thrips population followed by acetamiprid 4 % + fipronil 4% @ 2 ml L^{-1} with 70.81 per cent over untreated control.

Chapter – III

Material and Methods

Chapter III

MATERIAL AND METHODS

The present investigation entitled "**Identification and molecular characterization of thrips complex transmitting the *Groundnut bud necrosis virus* in blackgram and their management"** was conducted in the laboratory of department of Entomology and Northern block of Agricultural College farm, Bapatla, Guntur district, Andhra Pradesh during three seasons *viz*., *rabi* 2019-20, *kharif* and *rabi* 2020-2021. The meteorological data during the crop growth period was obtained from the meteorological observatory at Agricultural College farm, Bapatla. The details of location, experimental material used and methodology followed during the course of investigation are presented objective wise in this chapter.

3.1 COLLECTION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF THRIPS COMPLEX ON BLACKGRAM IN ANDHRA PRADESH

3.1.1 Collection and preservation of thrips specimens

Thrips were collected from different sampling areas in Andhra Pradesh *viz.* Vizianagaram and Srikakulam (North Costal Zone), Guntur, Krishna and Prakasam (Krishna Zone), Kurnool and Chittoor districts (Southern Zone). From each district five mandals were identified which were major blackgram cultivating areas (Total 35 locations). In each mandal samples were collected at random in single field.

A black thick paper fixed on thermocol sheet was used as tray to collect thrips. Thrips were collected by simply beating the plants on black tray (Plate 3.1) and carefully transferred into plastic vials containing alcohol glycerin acetic acid (AGA) mixture having 10 parts of 60 % ethyl alcohol with one part of glycerin and one part of acetic acid. In each location specimens were collected in an additional vial containing 95 per cent alcohol and safely packed in thermo coal box along with cool packs (Plate 3.2) and these specimens were immediately transferred to -20^{o}C for further molecular studies (Plate 3.3). Thrips samples can be preserved in the AGA solution for long period.

However for the best results the insects were mounted as early as possible after they become extended fully so as to minimize change in the colour of the insects. List of sampling areas with geographical coordinates were mentioned in the Annexure I.

Plate 3.1. Collection of thrips in blackgram fields

Plate 3.2. Thrips samples collection kit (Plastic Vials, camel hair brush, tissue paper, ethanol, rubber bands *etc.*)

3.1.2 Mounting of thrips specimens

For microscopic examination, the specimens were prepared as permanent mounts in natural Canada balsam. The permanent mounts were prepared as follows.

3.1.2.1 Maceration and dehydration protocol (Mound and Kibby, 1998)

1. Collected specimens in vials were transferred to cavity block (40mm x40mm) and added few drops of water to remove the traces of alcohol.

2. Slight abdominal cut was given to thrips (at intersegment membrane of 2^{nd} and 3^{rd} abdominal segments) under microscope to remove body contents.

3. Then the specimens were transferred to 3 % NAOH solution and kept it for two hours.

4. After two hours, specimens were washed quickly with water using dropper for 2-5 min.

5. Then the specimens were passed through different grades of alcohol *i.e.* 50, 70, 90,100 per cent (5 min each).

6. Then the specimens were transferred to two mL centrifuge tube which contained bilayer of 100 % ethanol and terpineol.

7. Specimens sinked in terpineol were used for study by placing on clean glass slide along with terpineol.

8. Small quantity of canada balsam was placed over the specimen on slide.

9. Specimens were arranged in a desired manner to observe under microscope. Terpineol was drained out using thick filter paper.

10. Slide mounts were kept in an oven at 35-40^{o}C for three days.

11. The specimen slides prepared by adopting the above procedure were identified using suitable keys.

12. The specimens were labeled systematically mentioning details *viz*. host plant, date of collection, place of collection, collector's name (on right hand side). Mentioned sex, genus, and species on left hand side (Plate 3.4).

Plate 3.3. Field collected samples preserved at -20^{o} C

Plate 3.4. Permanent slides of thrips specimens

The thrips collected from different districts of A.P were identified by following the taxonomic keys given by Cluever and Smith (2017); Mound and Yongfoo (2009); Hoddle and Mound (2003) and labeled neatly and then percentage of species composition was worked out. After taxonomic identification of thrips on blackgram up to species level from all the 35 locations of A.P., further species confirmation was done through molecular characterization with previously stored buffer samples.

3.1.3 Molecular characterization of thrips complex

Molecular characterization of thrips was carried out for further confirmation and to know the diversity among the thrips collected from GBNV hotspot areas of blackgram located in different districts of Andhra Pradesh. From all the 35 locations two samples each for *T. palmi* and *M. usitatus* and one sample of *S. dorsalis* from 17 locations were subjected to molecular characterization through PCR. In case of *S. dorsalis* only one sample was subjected to PCR due to limited number of specimens. Further a representative sample from each district was selected and utilized for characterization studies.

Preparation of stock solutions

The stock solutions required for the preparation of TNES buffer and other chemicals required for molecular studies were prepared as per the procedure given below.

a) **1 M Tris Hcl:** Tris Hcl (15.76 g) dissolved in 50 mL of distilled water and the pH of the solution was adjusted to 8.0. The solution was allowed to cool at room temperature. Final volume was adjusted to 100 mL with distilled water and the solution was sterilized by autoclaving.

b) **5 M Nacl:** NaCl (29.25 g) dissolved in 50 mL of distilled water and the final volume adjusted to 100 mL with distilled water and was sterilized by autoclaving.

c) **0.5 M EDTA (pH 8.0):** Disodium Ethylene Diamine Tetra Acetate (EDTA) weighed to 18.61 g and added to 50 mL of distilled water. The solution was dissolved using magnetic stirrer by adding NaOH pellets to adjust pH to 8.0. The final volume was made up to 100 mL with distilled water and the prepared stock was sterilized by autoclaving.

d) **10 % SDS:** Sodium dodecyl sulphate weighed to 10g and dissolved in 50 mL of distilled water and the final volume adjusted to 100 mL with distilled water and it was sterilized by autoclaving.

e) **Preparation of TNES (Tris-NaCl-EDTA-SDS) buffer:** For preparation of TNES buffer 5.0 mL of 1M Tris HCl (working concentration 50mM), 8.0 mL of 5M NaCl (working concentration 400mM), 20.0 mL of 0.5M EDTA (working concentration 20mM), 5.0 mL of 10 % SDS (working concentration 0.5 %) were taken and added to 62 mL of double distilled water to make up the final volume to 100 mL. pH was adjusted to 7.5 to 8.0. Above contents were autoclaved before use. (Plate 3.5).

Plate 3.5. Stocks and working buffer solutions for molecular analysis

3.1.3.2 Isolation of DNA from thrips : For DNA analysis from each species, single thrips specimens collected from 35 geographic locations, which were morphologically identified based on the taxonomic keys and quickly transferred to 1.5 mL centrifuge tubes with proper labeling. DNA was extracted from a single thrips specimen using the salting out protocol given by Sunnucks and Hales (1996) with slight modifications described here under.

1. Alcohol dipped samples were dried for two minutes and transferred on to micro centrifuge tubes.
2. 100 µL TNES buffer was added to the sample.
3. Thrips were crushed carefully using sterile tooth pick
4. 1.7 µL of protienase k (10 mg/mL) was added after crushing the sample.Each sample was kept on vortex for five minutes to shake vigorously.
5. Sample was kept for overnight incubation (18hrs). Vortexing of the sample was done for every two to three hours interval.
6. 28 µL of 5M NaCl was added and then sample was shaken vigorously.

7. After mixing, sample was kept for centrifugation at 13000 rpm for five minutes.

8. Supernatant was collected and transferred to fresh micro centrifuge tube and the pellet was discarded.

9. Equal volume of 100 per cent ice cold ethanol was added and slowly mixed until the white flakes appeared.

10. Sample was kept at -20^{o}C for one hour and then brought to the room temperature.

11. Supernatant was decanted and 70 per cent chilled ethanol (400 µL) was added to the pellet for washing.

12. Centrifugation was done (-4^{o}C) at 13000 rpm for five minutes.

13. Supernatant was decanted carefully and 400 µL of absolute alcohol was added.

14. Supernatant was decanted carefully; the pellet was collected and dried at room temperature.

15. The pellet was dissolved in nuclease free water.

After checking the concentration of the DNA, based on the intensity the DNA samples were diluted and stored at -20^{o}C for further PCR (Polymerase Chain Reaction) analysis.

3.1.3.2 PCR amplification: PCR amplification was carried out with the three different sets of primers listed in the Table 3.1. In the present study for the identification of species *viz.*, *Thrips palmi*, *Megalurothrips usitatus*, *Scirtothrips dorsalis,* ITS2 (Internal transcribed spacer 2- located in the 5.8S region flanking the ITS2 region of ribosomal DNA), *mt* COI (mitochondrial cytochrome oxidase I) and *mt* COIII (mitochondria cytochrome oxidase III) markers were employed, respectively (Table 3.1) in order to amplify 5$^{'}$ - end portion under set of PCR conditions described here under.

PCR reaction mixture (30 µL): The PCR plates were taken and 5 µL template DNA was pipetted into each of the wells in the PCR plate wells after proper labeling and kept the PCR plate at 4^{o}C.

Table 3.1 List of markers studied for characterization

S. No.	Species	Markers employed	Primer details	Annealing temperature	Amplicon Size (in bp)
1	*T. palmi*	ITS2	FP:GTGAACTGCAGGACACAT RP:CACCTGAACAGAGGTCGG	52ºC	568
2	*M. usitatus*	mtCOI	FP: GGTCAACAAATCATAAAGATATTGG RP: TAAACTTCAGGGTGACCAAAAAATCA	55ºC	655
3	*S. dorsalis*	mtCOIII	FP:GTTCCATTTCATTTAGTTTCACC RP:GTCATACTACGTCAACAAAATGTC	55ºC	713

Then, the master mix was prepared by taking each 1 µL of 10 p mol forward primer and 1 µL of reverse primer, 0.5 µL deoxyribo nucleotides (dNTPs), 2.5 µL of 10X assay buffer, 2.5 µL of 25mM MgCl2,) and 0.2 µL *Taq* DNA polymerase 17.8 µL of sterile distilled water was added to make up the volume to 25 µL. The master mix was centrifuged for about 10 sec for thorough mixing of the components. Subsequently, 25 µL of master mix was added to each of wells in the PCR plate well having 5 µL of template DNA to make the final volume to 30 µL. PCR plate was covered and kept in a thermal cycler as per the PCR programme given below.

The PCR was carried out as per the programme given below

Initial denaturation	94^{o}C for 10 minutes	
Denaturation	94^{o}C for 60 seconds	35 cycles
Annealing	55^{o}C for 60 seconds	
Extension	72^{o}C for 60 seconds	
Final extension	72^{o}C for 15 minutes	
Hold	4^{o}C	

3.1.3.3 Gel electrophoresis : Agarose gel electrophoresis for detection of DNA was performed as described by Sambrook *et al*. (1989). Amplified PCR products were separated on one per cent agarose gel in 1X TBE buffer at 100V. The chemicals required for Agarose gel electrophoresis were prepared as per the procedure given below.

10 X TBE buffer: for preparation of TBE buffer, 108 g Tris base, 55 g boric acid, 40 mL of 0.5M EDTA were weighed and dissolved in 600 mL distilled water and final volume was adjusted to 1000 mL and kept for autoclaving at 121ºC, 15Psi for 15 minutes. 1 X TBE buffer was prepared using 10 X buffer by taking 5 mL of 10X TBE and mixed with 45 mL of sterile distilled water to make 50 mL of 1X TBE.

One per cent agarose gel (w/v) was prepared by dissolving 0.5 g of agarose in 50 mL of 1 X TBE buffer. The agarose was melted in a microwave oven until a clear, transparent solution was obtained. The appropriate gel tray was fixed into the gel cast and the combs were placed on the gel tray. The melted agarose was allowed to cool to room temperature for 5 minutes and then 2 µL of ethidium bromide (10 mg/mL) was added and mixed well by swirling and poured into the gel tray with prefixed combs, carefully avoiding the formation of air bubbles. The gel was allowed to solidify at room temperature for one hour. Then the solidified gel was transferred to electrophoresis unit containing the running buffer, 1X TBE buffer. The DNA samples were mixed with $1/6^{th}$ volume of gel loading dye (40 % Sucrose: 0.25 % Bromophenol blue) and loaded onto the gel. The electrophoresis was carried out at 100V at room temperature for about 1hr till the dye front reached the lower part of the gel. The migration pattern of the DNA fragments in the gel was visualized in a UV light transmitted gel documentation system (SYNGENE Gene flash, U.K.).

3.1.3.4 Sequencing of amplified products: After the confirmation through gel electrophoresis, PCR products were processed for purification using QIAGEN QIAquick PCR Purification Kit (cat. No. 28104). A total of 21 samples *i.e.* seven samples for each *T. palmi, M. usitatus and S. dorsalis* species were sequenced bidirectionally using Sanger di-deoxy chain termination method at Barcode Biosciences Pvt. limited, Bangalore. The PCR reaction for sequencing was set-up in Applied Biosystems™ MiniAmp™ Plus Thermal cycler using Big Dye ™ Terminator V3.1kit as mentioned here under.

PCR Mix

Template	-	2 µL (~ 50ng of DNA)
Primer	-	1 µL (~ 2.5pmol)
Master mix	-	7 µL

After PCR amplification, the primer extended products were purified and preceded for capillary electrophoresis and analysed in Genetic Analyzer. Later the bidirectional chromatogram of each specimen was checked in MEGA6 and the noisy parts were trimmed at both the ends to make the consensus sequences. The resulted raw sequences (Forward and reverse) were edited using Clustal W programme in software Bioedit (version 7.2) to prevent possible errors. ORF finder was used (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) to examine the presence of stop codons. Further these sequences were compared with previously deposited complete and partial coding sequences of thrips in NCBI website through BLASTn search programme (https://blast.ncbi.nlm.nih.gov/Blast.cgi) All the sequences were further checked separately using the Expasy tool (https://web.expasy.org) to verify the errors (stop codons or frame shift if any) in the open reading frame and also for generating the amino acid sequences and submitted in NCBI website along with original nucleotide sequences for obtaining the accession numbers.

Phylogeny tree construction

To investigate the genetic relationship across the collected samples a phylogenetic tree was constructed using all identified haplotypes of three thrips species. The homologous sequences were selected based on the similarity percentage between the sequences present in NCBI and the sequences of present study. Complete DNA alignment was done using MEGA software (version 11.0). The phylogeny tree was constructed using Neighbor Joining tree (NJ) method with 1000 bootstrap value .

Thrips palmi

A set of seven sequences generated in this study along with 23 ITS2 gene sequences aligned against the sequences of present study were collected from NCBI database. The final dataset of thirty sequences were aligned and edited using Bioeditv7.0 software and a 559bp of ITS2 sequence were utilized for phylogenetic analysis.

M. usitatus

A set of seven sequences generated in this study along with 24 mtCOI gene sequences (sequences of *M. usitatus*, *T. palmi and T. tabaci*) aligned against the sequences of present study were collected from NCBI database. The final dataset of

31 sequences were aligned and edited using Bioeditv7.0 software. Sequence lengths of 668bp of mtCOI sequence were adopted for estimating genetic divergence and phylogeny analysis. The sequence of *Megalurothrips typicus* (KX622235) was further incorporated in the dataset as an out-group.

S. dorsalis

The NCBI deposited sequences of the present study were around 660 to 700 bp of mtCOIII. But the availability of earlier deposited sequences for this gene was of around 200 bp. Hence, a careful alignment of twenty final dataset mtCOIII gene sequences was done using Bioedit 7.2 version (Clustal - W) and finally arrived with 212 bp length. Further these sequences were assembled and aligned using the muscle DNA option in MEGA (11.0 version). In NCBI data base, mtCOIII gene sequences of *S. dorsalis* were available in meager nymber, hence whole genome sequence of *S. dorsalis* were also utilized in this tree construction.

3.1.3.5 Analysis of Haplotypes: After performing the Neighbor-Joining (NJ), Maximum Likelihood (ML), to test the reciprocal monophyletic criteria for species identification, the generated sequence data set was further studied for genetic divergence through haplotype analysis. For haplotype network connection studies, ITS2, mtCOI and mtCOIII sequences of *T. palmi, M. usitatus* and *S. dorsalis*, respectively obtained from NCBI database were used.

Sequences of each thrips species of present study along with homologous sequences showing about 97-99 per cent similarity were procured from NCBI website were used for haplotype network analysis. These sequences were aligned in MEGA 11.0 version using clustalW alignment option. GenBank sequences with clearly incorrect species assignments or potential contaminants (returning unexpected alignments or distances) were removed from the analysis. Sequence alignment (fasta file), for each species, was imported into DnaSP6 to reconstruct haplotypes and generate a haplotype data file (nexus). The nexus file was edited to add haplotype country links and a minimum spanning network (MSN) (Bandelt *et al*., 1999) was constructed in PopART (http://popart.otago.ac.nz) to examine the relationships among haplotypes for each thrips species from different locations.

The minimum spanning network was based on a minimum spanning tree where a set of sequence types connects all given types without creating any cycles or inferring additional (ancestral) nodes, such that the total length (*i.e*., the sum of distances between linked sequence types) is minimal, allowing construction of the union of all minimum spanning trees (Bandelt *et al*., 1999). Finally, the genetic diversity was estimated in terms of segregating sites, nucleotide and haplotype diversity along with Tajima's D statistic, which tests for neutrality and recent population expansion or contraction, using DNASP6.

3.2 TO STUDY THE INCIDENCE OF THRIPS COMPLEX AND OCCURRENCE OF BUD NECROSIS DISEASE IN BLACKGRAM

A bulk plot of blackgram crop was raised in 500 m^2 area in Northern block of College Farm, Agricultural College, Bapatla during *rabi* 2019-20, *kharif* and *rabi* of 2020-2021 by duly following the recommended agronomic practices given by ANGRAU, A.P. to study the incidence of thrips and bud necrosis disease in blackgram. The bulk plot was kept completely under unprotected conditions throughout the crop growth period (Plate 3.6). The details of crop raising is as detailed below.

3.2.1 Cultural practices

All the agronomical practices were followed as per the recommendations of Acharya N.G. Ranga Agricultural University in raising the crop during the experimental period, except crop protection practices.

3.2.2 Preparatory Cultivation

The land was ploughed twice with a tractor drawn cultivator followed by once with rotavator to obtain good tilth. Later, the ploughed land was divided into required number of plots.

3.2.3 Variety of the crop

The popular blackgram variety LBG 752 was selected for the experiment. The seed was obtained from the Agricultural Research Station, Ghantasala, Krishna Dist, Andhra Pradesh.

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Plate 3.6. General view of bulk plot of blackgram for recording incidence of thrips and bud necrosis disease

3.2.4 Fertilizer Application

The fertilizers were applied @ 20 kg ha^{-1} N, 50 kg ha^{-1} P_2O_5 as per the recommendations given by ANGRAU. Total quantities of fertilizers were applied as basal dose. Nitrogen fertilizers were applied in the form of urea and phosphatic fertilizers were applied in the form of SSP. As per the recommendations no potassic fertilizers were given for the crop blackgram.

3.2.5 Sowing

Healthy blackgram seeds of variety LBG 752 procured from ARS, Ghantasala were used. Seeds were treated with mancozeb75WP @ 3g kg^{-1} before 24 h of sowing and dried under shade. Sowing was done manually and two seeds were sown per hill with a spacing of 30 x10 cm. Sowing was done in first week of January during *rabi* 2019-20, last week of july during *kharif* 2020-21 and during last week of November 2020-21 as *rabi* crop. Thinning and gap filling was done at ten days after sowing to maintain uniform plant population.

3.2.6 Irrigation

For ensuring proper germination and plant stand, first irrigation was given immediately after sowing. Subsequent irrigations were provided as and when required.

3.2.7 Inter cultivation

Pre emergence weedicide *i.e.* pretilachlor 30EC @ 3.125 L ha^{-1} dose was applied as per the recommendations given by ANGRU, A.P, followed by two hand weedings during the crop period to keep the crop free from weeds.

3.2.8 Data recording on the incidence of thrips

Twenty plants were selected randomly and tagged for recording the observations on thrips population. The pest population was recorded at weekly intervals from 10 DAS and was continued up to the crop maturity. The thrips population was recorded from top, middle and bottom leaves randomly during the early morning hours. On tagged plants leaves were gently turned over to observe the infestation of thrips and magnifying lens was used to count the number of thrips found on the top three leaves of the plant.

3.2.9 Data recording on the incidence of Bud Necrosis disease in blackgram

A total of ten square meter blocks were arranged randomly in bulk plot to record the disease incidence (Plate 3.7). The bud necrosis disease incidence was recorded at weekly interval from 10 DAS and was continued up to the crop maturity (Plate 3.8). Per cent GBN-disease incidence was recorded from the each square meters arranged in bulk plot from which mean PDI was calculated. Per cent disease incidence was calculated using the following formula.

$$\text{Per cent disease incidence} = \left(\frac{\text{No. of diseased plants}}{\text{Total No. of plants}} \right) \times 100$$

3.2.10 Weather Parameters

The meteorological data was recorded from the meteorological observatory, Agricultural College Farm, Bapatla and presented in Appendix-III. The weather parameters such as maximum and minimum temperature (^{o}C), morning and evening relative humidity (%), wind speed (kmph) and rainfall (mm) were correlated with the thrips population and mean per cent bud necrosis disease in blackgram.

3.2.11 Statistical Analysis

The influence of weather parameters on incidence of thrips and bud necrosis disease was worked out by simple correlation and multiple regression analysis by using SPSS (Version 20) software.

Plate 3.7. Micro plots arranged for recording bud necrosis disease in blackgram

Plate 3.8. Bud necrosis disease incidence observed during *rabi* 2019-2020

3.3 STUDY OF TRANSMISSION OF GROUNDNUT BUD NECROSIS VIRUS BY THRIPS IN BLACKGRAM

3.3.1 Collection, Isolation and Maintenance of GBNV Virus to carry out transmission studies

Blackgram plants infected with bud necrosis disease were collected from Agricultural college farm, Bapatla. Samples showing the symptoms of bud necrosis *i.e.* chlorotic and necrotic lesions on leaves, bronzing on stem, plants with necrotic buds were collected at vegetative stage of the crop. These samples were collected in zip line bags in ice buckets and kept in refrigerator at -80^{o}C for further studies (Plate 3.9).

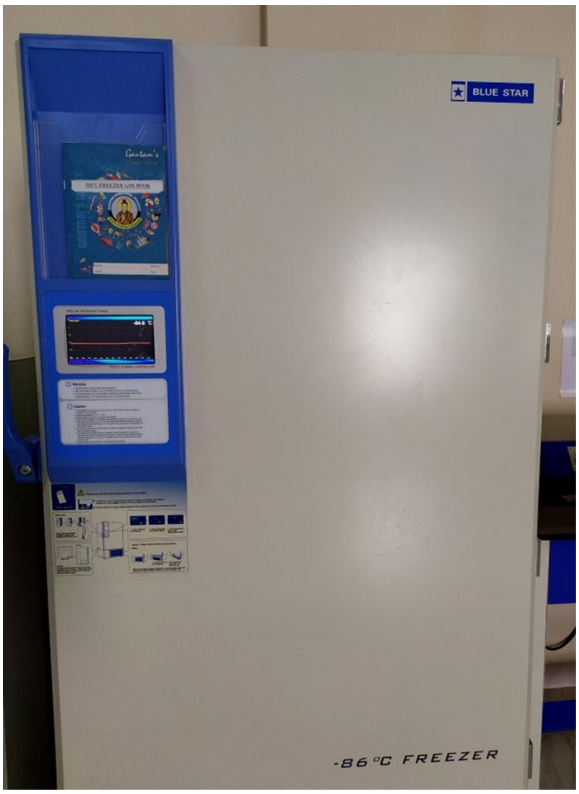


Plate 3.9. - 86^{o}C Freezer to keep the samples

3.3.1.1 Mechanical Inoculation : The virus inoculum was maintained on cowpea plants by mechanical inoculation method.

Preparation of potassium phosphate buffer: Potassium phosphate buffer at 0.05 M was prepared in the laboratory with the following reagents for extraction and isolation of virus from the infected samples. After preparing the buffer, pH of the solution was tested using pH meter and adjusted to 7.0.

Dipotassium hydrogen phosphate (K_2HPO_4)	5.4 g
Potassium dihydrogen phosphate (KH_2PO_4)	2.4 g
β – Mercaptoethanol	1.56 mL
Distilled water	1000 mL

Preparation of inoculum: The infected blackgram plant samples collected from bulk plot were washed thoroughly in running tap water to remove dirt and then blotted dry. The inoculum was prepared (1:10 w/v) by macerating the 1g of infected leaves in chilled mortar and pestle. Pre chilled 0.05M potassium phosphate buffer (PH 7.0) containing 1 mL of 2-β mercaptoethanol. The resulting pulp after maceration was squeezed through two folds of sterile muslin cloth. The virus extract obtained was used for further studies.

Method of inoculation: Well established, young actively growing blackgram LBG752 and cowpea (CV-152) (Plate 3.10) plants raised in insect proof cages in green house were used for virus inoculation. Inoculations were done on fully expanded primary leaves of cow pea plants. Prior to inoculation, a pinch of carborundum powder (600 mesh) was dusted over the selected leaves. A sterile cotton pad immersed in the inoculum was gently rubbed over the leaves thrice in one direction (Plate 3.11 A, B). Care was taken not to damage the leaves during inoculation. Immediately after the inoculation, the leaves were washed off with a jet of sterile distilled water to remove the excess of inoculum and abrasive. The inoculated plants were labeled and kept under observation in insect proof cages in glass house for symptom expression. Throughout the experiment period, virus isolate was maintained on cow pea CV-152, since cow pea has consistently produced more local lesions within 4 to 5 days after inoculation.

3.3.1.2 Confirmation of GBNV inoculum in indicator cowpea plants through RT-qPCR : Confirmation of disease incidence was done based on symptoms observed in the field. Chlorosis was observed around the lateral veins on the young leaves and the branches near the margin. These veins later turned to necrotic resulting in downward curling of leaves and also twisting in few leaves. Diseased plants showed reddish-brown discoloration on under surface of the leaf margins which also extended to the petiole. Early infected plants showed stunted growth followed reduced inter nodal length and caused death due to bud necrosis. After visual confirmation of bud necrosis

Plate 3.10. CV 152 Seed used for the mechanical inoculation studies

Plate 3.11. Mechanical inoculation of GBNV under greenhouse conditions

A, B: Cowpea; C: Blackgram

incidence, the diseased leaves of blackgram from the experimental fields and diseased cow pea leaves from infected potted plants showing chlorotic symptoms were utilized for confirmation of virus. Methodology adopted for the virus confirmation studies are described here under.

The presence of the target gene on the samples was tested using quantitative Real Time PCR. Total RNA was isolated from the test samples and cDNA was synthesized (reverse transcription). The qPCR amplification was carried out using gene specific primers (GKGBNV-F:GTTGCTACGAATACTGACGTAG; GKGBNV-R: CATCTCCTGCTTAGCAGCATCA; GKGBCP-F: TTGAAGTGCAGAAAGCAGATC; GKGBCP-R: CCTGATGAAAGCTTCTGTCC). The results were analyzed based on the Ct values obtained from the samples.

RNA Isolation & cDNA synthesis

Total RNA was extracted from test samples using TRIzol method and 1000ng of RNA sample was used for cDNA synthesis. The RNA and Oligo dT/ Random hexamer primers were used for the first strand cDNA synthesis using Reverse transcriptase enzyme (Thermo scientific) following the manufacturer's protocol. The Real Time PCR reaction volume of 20 µL containing 2 µL of cDNA and 10 µL of SYBR Green Supermix (Bio Rad, USA) was carried out for 35 cycles following the PCR programme of denaturation at 95 ºC for 30 sec, annealing at 55 ºC for 30 sec in Bio-Rad CFX96 system. The mRNA expression levels were normalized to that of Housekeeping gene and the results were analyzed.

3.3.2 Studies on transmission of GBNV by thrips in black gram

3.3.2.1 Collection of thrips : Adult thrips from flowers and terminal buds of healthy blackgram plants were collected at Agricultural college farm, Bapatla and carried in zip liner bags to the laboratory and transferred to conical flask in the laboratory. A glass funnel was placed over it in an inverted position. The narrow end of the funnel was closed with a small glass vial as shown in (Plate 3.12).

3.3.2.2 Identification of thrips: The thrips collected in vials for identification were immobilized by placing them in a refrigerator at 4^{o}C for 15 min and then dislodged into an ice tray (Lewis, 1973).

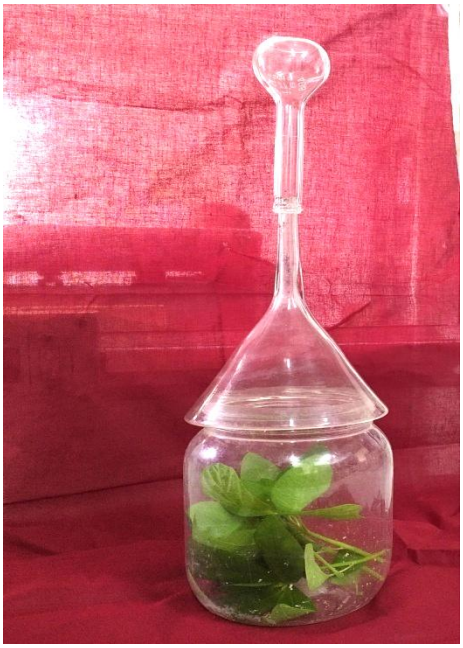


Plate 3.12. Funnel apparatus to collect thrips from plant parts

After immobilization by cold treatment, thrips were sorted to different species (within 15 min.) using a stereo binocular microscope, as per the key (Table 3.2) described by Amin and Palmer (1985).

Table 3.2 Identification characters of different thrips species

Key Characters	***Thrips palmi* (Karny)**	***Megalurothrips usitatus* (Bagnall)**
Adult female colour and length	Straw yellow to pale brown 0.9 mm long	Body dark brown
Antennae	Seven segmented	Antennae eight segmented I,II brownish yellow, III yellow, IV and sometimes V yellow at base
Wings	Fore wings with broken rows of wing vein setae	Fore wings brown with basal quarter pale and an extensive pale area sub apically
Pronotum	Pronotum having two pairs of setae on the posterior lateral margin , no setae on the anterio lateral margin	Pronotum sometimes with transverse carina parallel to posterior margin 2 pairs of long posterioangular setae , outer longer than inner
Larvae	Larvae whitish	Larvae brownish red

3.3.2.3 Rearing of thrips: The thrips specimens collected from Agriculture college farm were *T. palmi* and *M. usitatus.* Hence, the transmission studies were conducted with *T. palmi* and *M. usitatus*. The suspected vectors *viz*., *T. palmi* (Vijayalakshmi, 1994, Lipa, 1999 and Sreekanth, 2002) and *M. usitatus* were reared using bean jar method described by Daimei *et al.* (2017).

Rearing of thrips using bean pod method

Glass jars (0.5 to 1 L) covered with fine muslin cloth for proper ventilation and aeration was used for rearing of two thrips species. Separate jars were used for two different thrips species. 10 to 15 layers of coarse tissue paper on the bottom of each jar were provided as pupation site for thrips. Fresh French bean pods brought from market to the laboratory were washed in warm soap water and cleaned thoroughly under running tap and then dried under shade (Plate 3.13). After drying pods were dipped in 5 per cent sodium hypochlorite solution and then again shade dried for 10-15 minutes. Later these pods were again dipped in previously prepared 0.1 per cent fungicide solution (Carbendazim 50 WP) and then shade dried. These dried pods were stored in plastic bag at 4^{o}C until further use. After two hours of shade drying, 4 to 5 pods were placed in glass jars. Identified female thrips were released into the jars and covered with muslin cloth and properly tied with rubber bands (Plate 3.14).

Initially thrips started moving towards muslin cloth in search of light and air but after some time they have settled on the beans. These glass jars were kept undisturbed for two to three days for egg laying by female thrips (Plate 3.15). The beans were carefully removed and transferred to the new jars and fresh pods were introduced to into the old jars for further egg laying. New jars with egg infested pods were observed periodically for larval development (Plate 3.16, 3.17, 3.18, 3.19). These larvae were provided with fresh pods at 24 hrs interval. So that healthy and uniform larvae were maintained for further experiments. This was maintained continuously for stock culture. After attaining adult stage (Plate 3.20, 3.21) females were again collected and further identified with morphometric keys to maintain iso-female line (Plate 3.22) culture to carry out transmission studies. Females were separated using key characters *viz.* abdominal tergite VIII with posteromarginal comb complete, where as in male it was broadly developed. Males were separated using the

Plate 3.13. Bean Pods treated with Sodium hypochlorite

Plate 3.14. Rearing of thrips using bean jar method

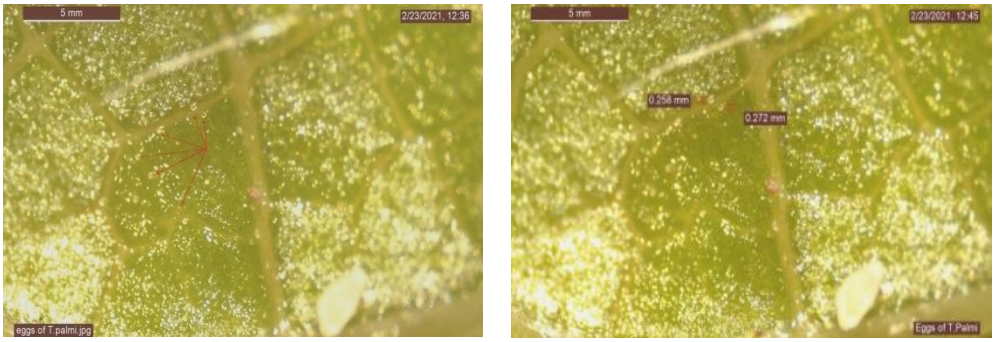


Plate 3.15. Eggs of *T. palmi*

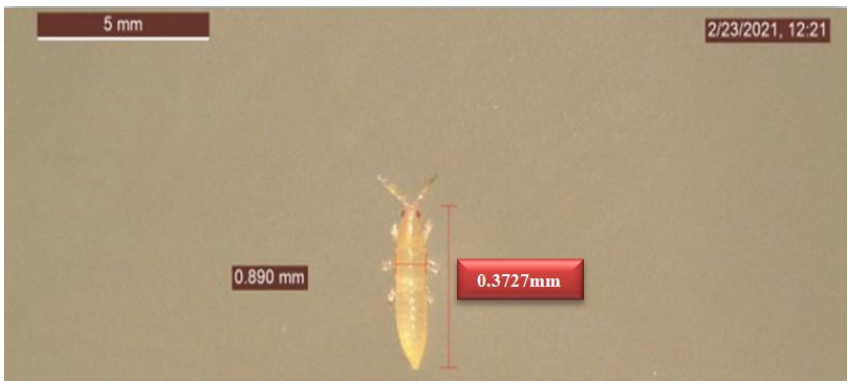


Plate 3.16. First instar larva of *T. palmi*

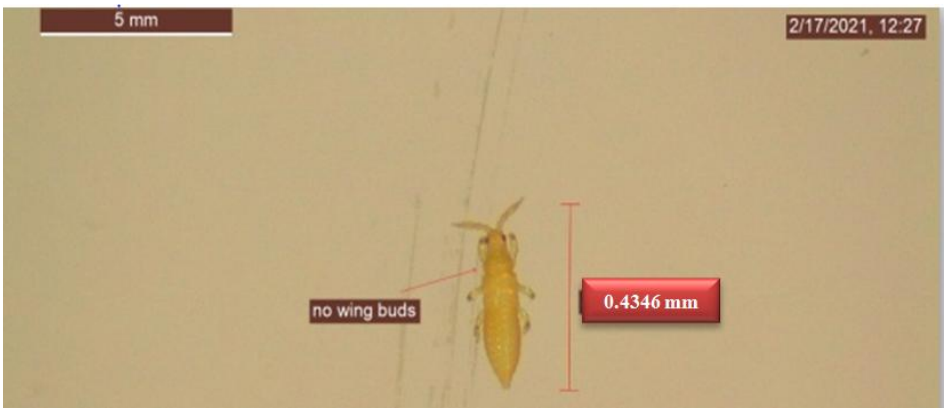


Plate 3.17. Second instar larva of *T. palmi*

key character *i.e.*, sternites III–VII each with a narrow transverse glandular area posteriorly which was absent in females. Some females were transferred to eppendorf tubes with 100 per cent ethanol and kept under -20^{o}C for molecular level confirmation of the species. Total DNA was extracted by using CTAB method and PCR was done with gene specific markers and final confirmation was done.

Plate 3.18. Full grown larva of *T. palmi*

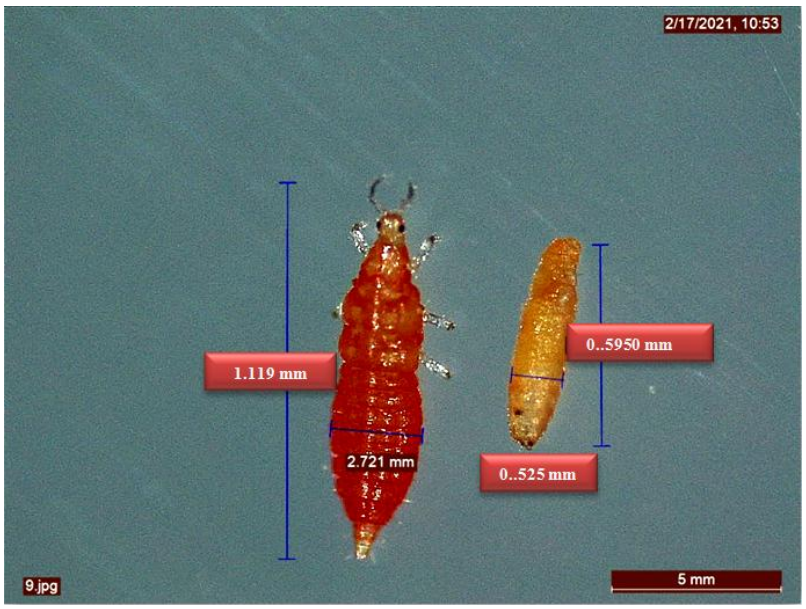


Plate 3.19. First and second instar stages of *M. usitatus*

Plate 3.20. Adult female of *T. palmi*

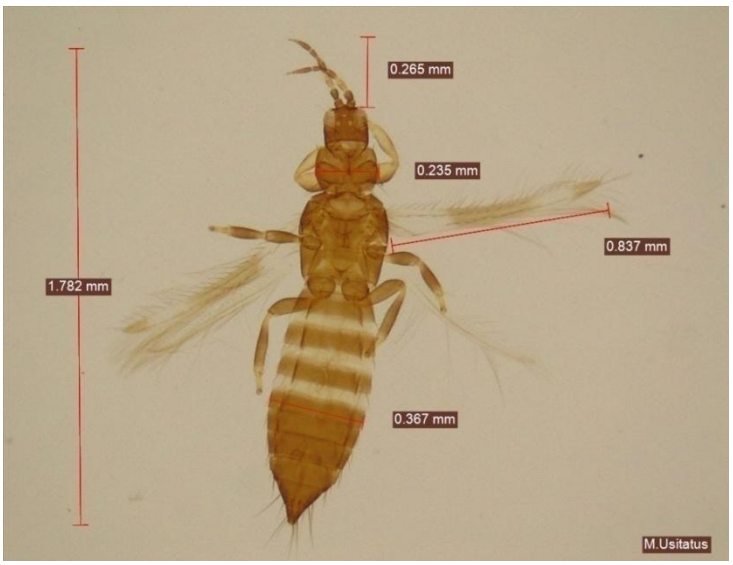


Plate 3.21. Adult female of *M. usitatus*

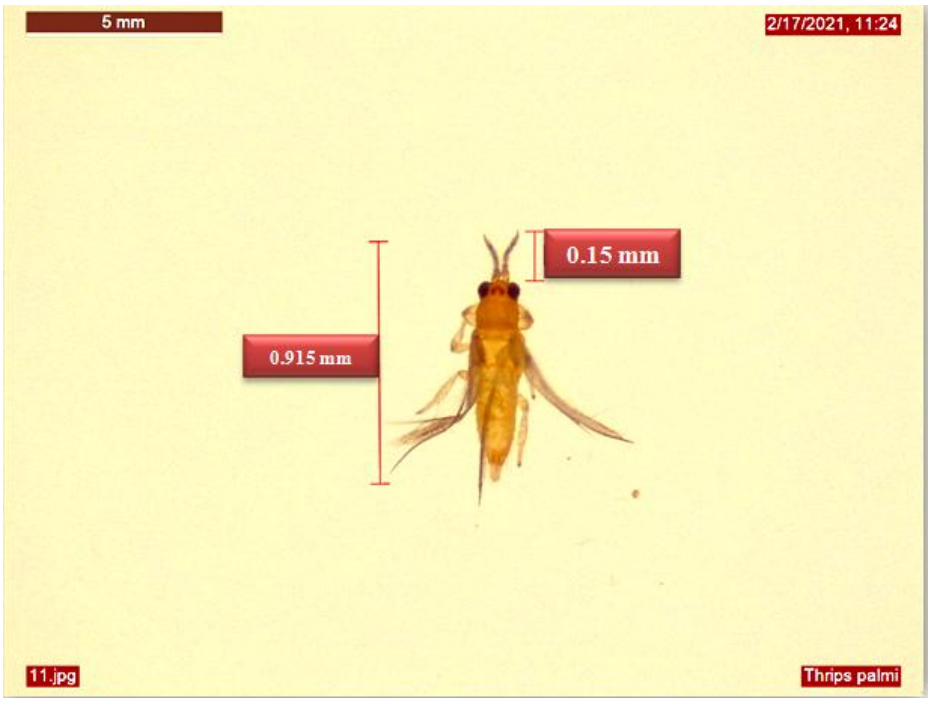


Plate 3.22. Adult male of *T. palmi*

3.3.3 Transmission Tests

Studies were carried out under green house conditions available at Agricultural College, Bapatla, during October to February. Experiment was carried out at average day temperature 28±1^{o}C and long night hours.

3.3.3.1 Test plants: Transmission tests were conducted using susceptible cow pea variety CV 152 and LBG752 variety of blackgram under green house condition for proper symptoms and disease development. Major suspected vector *T. palmi* second instars larvae and adults were collected from the bud necrosis infected blackgram plants and brought to the laboratory. Identified the species as described under 3.3.2.2. About 10 to 15 adults and the larvae were released on healthy cow pea and blackgram seedlings maintained in bottle cages under green house condition and rate of transmission was calculated. Compared to blackgram, cowpea plants showed the clear chlorotic and necrotic spots within five to seven days but no difference was found in rate of transmission between blackgram and cowpea. Hence, the detailed transmission studies were carried out using indicator plant *i.e.* cow pea as follows.

3.3.3.2 Transmission of bud necrosis disease by nymphs and adults of *T. palmi* Determination of acquisition access period (AAP): Transmission studies were conducted to determine the minimum acquisition access period for the transmission of bud necrosis virus by the vector *T. palmi*. Freshly emerged first instar nymphs of *T. palmi* were allowed to feed for different periods of 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h and 48 h of acquisition as shown in plate 3.23. Each batch of these nymphs was reared separately until they became adults. Later adult *T. palmi* were transferred to healthy cowpea plants for inoculation with common inoculation access period of 48 h.

Ten insects per plant were used for inoculation and insects were killed after 48 h of IAP using fipronil 5 SC @ 2 mL per litre of water. For each acquisition period 12 test plants were arranged in glass house and kept for symptom expression (Plate 3.24). Same experiment was carried out by using second instar larvae of *T. palmi* after seven days with same hourly intervals of AAP to determine the minimum acquisition period. After 10 days interval minimum AAP experiment was carried out with *T. palmi* adults collected from rearing jars. For each experiment, data was collected after symptom expression *i.e.* development of chlorotic spots and yellowing.

Plate 3.23. Glass vials used to fed thrips on diseased leaves

Plate 3.24. Plants kept for symptom expression under green house condition

Determination of inoculation access period (IAP)

Another experiment was conducted to determine the minimum inoculation access period for transmission of bud necrosis virus by vector *T. palmi*. Freshly emerged first instar nymphs of *T. palmi* were allowed to feed for common acquisition access period of 24 h. These larvae were reared separately until become adults. After adult emergence these were transferred carefully with the help of aspirator on to healthy cowpea plants and allowed to feed for 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h and 48 h on healthy plants of cowpea. Ten insects per plant were released and twenty five plants for each test interval were tested for disease development. After prescribed time period fipronil 5 SC was sprayed @ 2 mL per liter of water to kill the insects. Plants were kept under green house condition for symptom expression. Same procedure was adopted for second instar larvae and adults of *T. palmi* after 10 days interval to the previous experiment. Artificial shade net was provided to the experiment area to prevent excess heat wave (Plate 3.25).

Plate 3.25. Plants kept for symptom expression under green house condition provided with shade net

Number of thrips required for transmission of GBNV

Freshly emerged first instar nymphs of *T. plami* were allowed to feed on infected cow pea leaves kept in glass vials. Then they were reared separately on beans with proper aeration and photoperiod condition until they become adults. They were released with the help of aspirator (Plate 3.26) on healthy cow pea plants in different batches of 2, 4, 8, 10, 15 insects per plant. Both acquisition access period and inoculation access period were fixed in two categories *i.e.*, 24 h AAP and IAP, 48 h AAP and IAP. The test plants were kept for symptom expression. Same experiment was carried out with second instar larvae and adults simultaneously.

Plate 3.26. Collection and release of thrips reared under laboratory conditions

3.3.3.3 Transmission of bud necrosis disease by nymphs and adults of *M. usitatus* (Bagnall): Transmission of bud necrosis disease by another suspected vector *M. usitatus* was also carried out to establish the virus vector relationship as described in the case of *T. palmi*. Since the inoculated cowpea plants were healthy and the disease symptoms were not exhibited, further studies to determine AAP, IAP *etc*., were not carried out with *M. usitatus*.

3.3.3.4 Confirmation studies of GBNV after transmission studies: After carrying out transmission studies the diseased cowpea leaves were utilized for further confirmation of virus presence through molecular approach described as follows.

RNA isolation by TRIzol method

GBNV infected cowpea leaf sample was collected from the green house and washed thoroughly under tap water. Leaf bits were cut into fine pieces and taken into

pre autoclaved pestle. Leaf sample of 100 g was taken and ground finely using liquid nitrogen. This mixture was gently transferred into a centrifuge tube. One mL of TRIzol reagent was added. TRIzol is an acid-guanidinium-phenol based reagent ideally designed for the extraction of RNA from biological samples, at low pH it controls to separate RNA from DNA and protein. After shaking well, this sample was centrifuged at 13000 rpm for 15-20 min at 4^{o}C. Supernatant was removed carefully and 200 µL chloroform was added to the sample, mixed well and kept it in room temperature for 10-15 minutes.

This mixture was centrifuged at 13000 rpm for 15-20 minutes at 4^{o}C. Clear supernatant was collected and 500 µL of isopropanol was added. Then the sample was kept at room temperature for 10-15 minutes and centrifuged again at 13000 rpm for 20 minutes. Supernatant was removed and pellet was collected carefully. The pellet was washed with 70 % chilled ethanol (1 mL) and again centrifuged it at 13000 rpm for 10-15 minutes. Pellet was dried at room temperature for 10-15 minutes until ethanol was completely dried up. Pellet containing RNA was dissolved in elution buffer (40 µL) and kept in water bath at 55^{o}C for few minutes and then stored at -80 ^{o}C.

3.3.1.2.2 Complementary DNA (cDNA) synthesis : cDNA was synthesized from extracted RNA (by using Trizol) by using M-MuLV reverse transcriptase. Reverse transcriptase mixture was prepared to a final volume of 20 µL in a sterile eppendorf tube. The composition of reverse transcription mix for one reaction is as follows.

Initially template RNA mixture was prepared as follows

Random hexamer	1 µL
RNA template	2 µL
MB grade water for PCR	7 µL
Total	10 µL

The above mixture was incubated for 5 minutes at 65^{o}C and then cooled immediately on ice tray.

Reverse transcriptase reaction mixture was prepared as follows

Template RNA primer mixture	10 µL
5x M-MuLV RT buffer (supplied with the enzyme)	4 µL
10X solution for M-MuLV	2 µL
10mM dNTPs Mix	2 µL
M-MuLV RT enzyme (20U/ µL)	1 µL
RNase inhibitor (20U/ µL)	0.5 µL
MB grade water for PCR	0.5 µL

Above components were mixed gently and ensured that all the components were at the bottom of amplification tube. Mixture was centrifuged briefly at 3000 rpm for 15 sec. at 4^{o}C and incubated the complete reaction mixture as follows.

25^{o}C for 5 minutes	1 cycle
42^{o}C for 60 minutes	1 cycle
70^{o}C for 5 minutes	1 cycle
Hold at 4^{o}C	

3.3.1.2.3 Polymerase chain reaction: Gene specific primer designed based on the coat protein gene in the GBNV genome in groundnut from NCBI website. The sense primer 5' ATGTCTAACGTTAAGCAACTC 3' and antisense primers 5' TTACAATTCCAGCGAAGGACC 3' were used to amplify the complete cp gene (size ~830 bp) of GBNV.

cDNA	2 µL
GBNV CP Forward (10µM)	1 µL
GBNV CP Reverse primer (10µM)	1 µL
10x assay buffer	5 µL
25mMMgcl_2	3 µL
10 mM dNTP's	1 µL
Taq DNA polymerase	0.5 µL
MBW	36.5 µL
Total	50 µL

PCR amplification condition:

Initial denaturation cycle	94^{o}C for five minutes	
Denaturation for	94^{o}C for 45 Sec	35 cycles
Annealing	56^{o}C for 45 Sec at	
Extension	72^{o}C for one minute	
Final extension	72^{o}C for 30 min	
Final hold	4^{o}C	

Amplified products were resolved following electrophoresis through a 1 % agarose gel containing ethidium bromide.

3.4 MANAGEMENT OF THRIPS IN BLACKGRAM THROUGH INSECTICIDES

Field experiment was conducted to test insecticides as seed treatment and foliar sprays for the management of thrips in blackgram during *rabi* 2019-20 and *kharif, rabi* of 2020-21.

3.4.1 Layout of the experimental plot

The experiment was laid out in a randomized block design (RBD) with fifteen treatments including untreated check, replicated thrice with a plot size of 12 sq.m. (4m x 3m) (Plate 3.27, 3.28 and Figure 3.1) to study the efficacy of insecticides as seed treatment and foliar sprays for control of thrips on blackgram.

Location : Agricultural College Farm, Bapatla
Crop : Blackgram
Variety : LBG 752
Design : Randomized Block Design
Replications : 3
Treatments : 15
Spacing : 30 cm x 10 cm
Plot size : 4.1 m x 3.0 m

Plate 3.27. Layout and general view of the experimental field

Plate 3.28. Application of pre emergence herbicide in the experimental field

3.4.2 Details of the treatments used for the experiment

The following insecticides are tested for seed treatment and foliar sprays for the management of thrips in blackgram.

Table 3.3 Details of the treatments used for the experiment

T1	Imidacloprid 70 % WG @ 5 g per Kg seed (Seed treatment)
T2	Thiamethoxom 70 % WS @ 5 g per Kg seed (Seed treatment)
T3	T1+ Flonicamid 50 % WG @ 0.3 g L^{-1}
T4	T1+ Diafenthiuron 50 % WP @ 1.25 g L^{-1}
T5	T1+ Fipronil 5 % SC @ 2 mL L^{-1}
T6	T1+ Buprofezin 25 % SC @ 2 mL L^{-1}
T7	T1+ Spinetoram 11.7 % SC @ 0.9 mL L^{-1}
T8	T1+ Spinosad 45 % SC @ 0.32 mL L^{-1}
T9	T2+ Flonicamid 50 % WG @ 0.3 g L^{-1}
T10	T2+ Diafenthiuron 50 % WP @ 1.25 g L^{-1}
T11	T2+ Fipronil 5 % SC @ 2 mL L^{-1}
T12	T2+ Buprofezin 25 % SC @ 2 mL L^{-1}
T13	T2+ Spinetoram 11.7 % SC @ 0.9 mL L^{-1}
T14	T2+ Spinosad 45 % SC @ 0.32 mL L^{-1}
T15	Control

List of insecticides with IUPAC chemical names, group, mode of action were presented in Annexure II.

3.4.3 Seed treatment

The required quantity of seed was taken into a polythene bag and measured quantity of insecticide was added to the seed and shacked vigorously for uniform mixing up of the insecticide with the seed. Later the seed was shade dried for 24 hrs and used for sowing (Plate 3.29). The experimental field was kept unsprayed up to 15 days after sowing and later the crop was protected through blanket sprays in all the experimental plots uniformly with chlorantraniliprole 18.50 % SC @ 100 mL ha^{-1} against pod borers to avoid the crop losses due to incidence of pod borers. Carbendazim 50 WP @ 500 g per ha^{-1} was also used to minimize the disease incidence *viz*., powdery mildew.

Thiamethoxam 70 WS	Imidacloprid 70 WG

Plate 3.29. Seed treatment of blackgram seed (LBG -752)

3.4.4 Preparation of spray fluid

The required quantity of test insecticide was measured and mixed with small quantity of water and later made up to the required volume of spray fluid. The spray fluid was mixed thoroughly before filling into the sprayer.

3.4.5 Application of insecticides

Foliar application of selected insecticides was given at 30 DAS, 45 DAS and 60 DAS in blackgram crop. Spraying was done during the morning hours when the air was still, using a knapsack sprayer and proper care was taken for thorough coverage of entire experimental plot by using the spray fluid @ 500 L ha^{-1} (0.6 L per plot). The sprayer and the container used for preparing the spray fluid were thoroughly cleaned with water after each treatment and rinsed with the spray fluid of the respective treatments.

3.4.6 Data Recording

3.4.3.1 Thrips : Data on pest population were recorded one day before spraying as pre treatment count and at 1, 3, 7 and 10 days after spraying as post treatment count. The observations were recorded from 10 randomly selected plants in each plot leaving the border rows. Second and third sprays were given at 15 days interval to allow subsequent population buildup in the experimental plots. Per cent population reduction was calculated by using modified Abbot's formula (Fleming and Ratnakaran, 1985). The effect of treatments on thrips and bud necrosis disease in blackgram was recorded.

$$\% \text{ population Reduction} = \left\{ 1 - \left[\frac{\text{Post treatement population in the treatment}}{\text{Pre Treatment Population In the treatment}} \times \frac{\text{Pre Treatment population in the untreated check}}{\text{Post treatment population in the untreated check}} \right] \right\} \times 100$$

3.4.6.2 Bud necrosis disease: The total number of diseased plants in each plot was counted at 15 days interval *i.e.* 15, 30, 45 (after first spray), 60 (after second spray), 75 (after third spray) days after sowing in all the three seasons. From this per cent disease incidence was calculated by using the following formula.

$$\textbf{Per cent disease incidence} = \left[\frac{\textbf{No. of diseased plants}}{\textbf{Total no. of plants}} \textbf{ x 100} \right]$$

3.4.7 Harvesting

The crop was harvested at 95 days after sowing when the pods were matured completely. Pods were handpicked thrice with two to three days interval for each picking. Pods were handpicked and collected separately from each treatment plot as well as from bulk plot. The handpicked black gram pods were sundried for two days to reduce the moisture content.

3.4.8 Yield

Treatment wise replicated yield data was recorded from net plot by leaving two boarder rows of plants on all sides of the plot. The mean seed yield of treatments recorded per plot (kg / plot) was converted to kg/ha and subjected to ANOVA to test the significance of treatments with respect to seed yield.

3.4.9 Incremental cost-benefit ratio

The incremental cost-benefit ratio was calculated by dividing the extra benefit attained from enhanced yield by the extra cost incurred for each treatment. The price of insecticides and labour charges for spraying of insecticides were included in the cost. The incremental cost-benefit ratio of each treatment was calculated to find out the most economical management method for thrips in blackgram.

$$\text{ICB Ratio} = \frac{\text{Extra benefit of enhanced yield}}{\text{Extra cost incurred for each treatment}}$$

3.4.10 Statistical Analysis

The experimental data *viz*., mean mean number of thrips per plant, percent reduction in the population of thrips over untreated check, mean per cent bud necrosis disease incidence and yield data was subjected to ANOVA after using suitable transformations. The mean comparisons were made by Least Significant Difference (LSD) (Duncan, 1951).

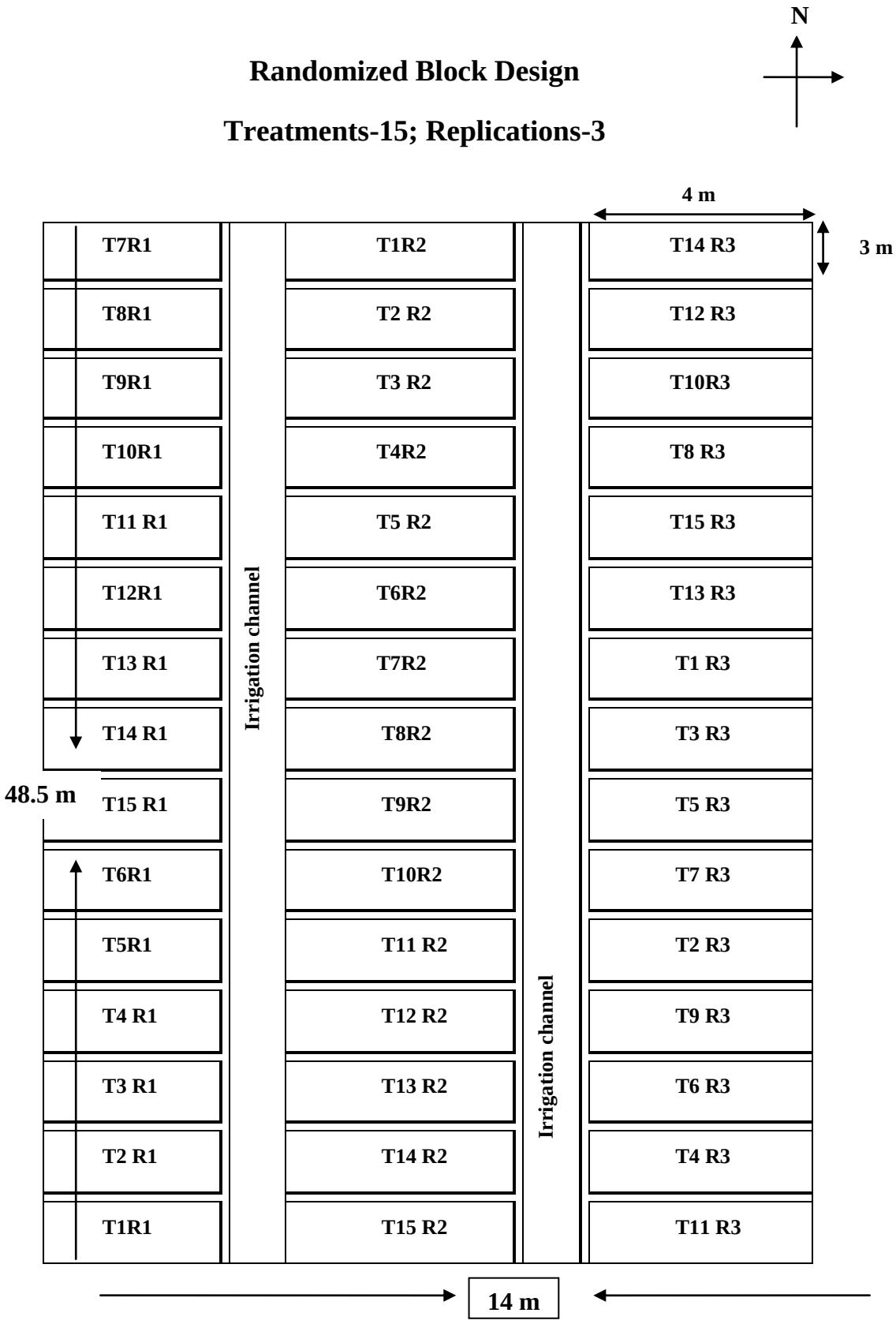


Figure 3.1 Experimental layout to study the management of thrips in blackgram through certain insecticides

Chapter – IV

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

The present investigation entitled "**Identification and molecular characterization of thrips complex transmitting the *Groundnut bud necrosis virus* in blackgram and their management"** was conducted in the laboratory of Department of Entomology and in the Northern block of Agricultural College farm, Bapatla, Guntur district, Andhra Pradesh during three seasons *viz*., *rabi* 2019-20, *kharif* and *rabi* 2020-2021. The results obtained in the present investigation were discussed here under.

4.1 COLLECTION, IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF THRIPS COMPLEX ON BLACKGRAM IN ANDHRA PRADESH

4.1.1 Sample Collection

Thrips samples were collected from blackgram fields in different geographic locations of Andhra Pradesh during *rabi* 2019-2020**.** List of sampling areas along with geographic coordinates were enclosed in Annexure I. Collected samples were preserved in 95 per cent alcohol till further processing. Permanent slides were prepared using macerate and dehydration protocol discussed in methodology. The prepared specimens were identified based on available keys as follows.

4.1.2 Identification of Specimens Based on Taxonomic Keys

Prepared permanent slide specimens were identified based on the photo based key given by Cluever and Smith (2017).

1 Wings (brachypterous or macropterous) or wing buds present ……2

2' Wings fully formed, with setae present .....…….. Adult, 4

4' Abdominal segment X conical; female with saw-like ovipositor …….....Terebrantia, 6

6' If ctenidia are present on abdominal tergites V–VII, ctenidium on tergite VIII posterior to spiracle ; anterior margin of prothorax lacking major setae antennae 7-, 8-, or 9-segmented . . . . ……..15

15' Lateral margins of abdominal tergites VI–VI lacking closely spaced rows of microtrichia; cilia of forewing fringe wavy; ocellar III setae not arising within the ocellar triangle . . . . . ……...16

16' Abdominal tergites V–VIII with paired ctenidia laterally; antennae 7- or 8 segmented…….19

19' Tergites III–VIII lacking craspedum; pronotum transverse; antennae 7- or 8-segmented ……20

20' Metanotum with median pair of setae arising posterior to anterior margin; antennae 7 segmented……..22

22' Abdominal sternites lacking discal setae, setae present only at posterior margin; row of setae on 1st vein with spaces between setal bases much greater than length of each seta……...23

23' Metanotal campaniform sensilla present; microtrichia lacking on lateral thirds of tergites IV–VI ……***Thrips palmi*** **(Karny)**

4.1.2.1 Characteristic features of family Thripidae and subfamily Thripinae

1. Both sexes fully winged. Body dark brown, tarsi, apices of mid and hind tibiae, also most of fore tibiae yellow; hind tibiae with 2 stout dark apical setae.

2. Fore wings brown with basal quarter pale and an extensive pale area sub-apically.

3. Antennal segments I–II brownish yellow, III yellow, IV and sometimes V yellow at base.

4. Fore wing light brown, pale sub-basally and with sub-apical pale band. Antennae 8-segmented, I with pair of dorso-apical setae; III–IV with constricted apical neck, sensorium forked, VIII almost twice as long as VII.

5. Head conspicuously transversely striate/reticulate at posterior, ocellar setae III long, arising just inside triangle, postocular setae not long.

6. Pronotum with transverse carina parallel to posterior margin, median area weakly transversely reticulate; 2 pairs of long posteroangular setae, outer longer than inner, one pair of anteroangular setae moderately prominent.

7. Mesonotum with transverse reticulation, lateral setae not long.

8. Metanotum reticulate medially, median setae long, at anterior margin, campaniform sensilla present.

9. Mesosternal furca with spinula, metafurca without.

10. Tarsi all 2-segmented. Fore wing first vein with long row of setae before distinct sub-apical gap followed by 2 setae; second vein with complete row of setae; postero-marginal cilia wavy.

11. Abdominal tergites II–VIII with no sculpture medially but lateral thirds with sub-parallel lines, median setae small; VIII with posteromarginal comb of small microtrichia laterally, discal area antero-mesead of spiracle with 2 or more rows of strong microtrichia.

12. Tergite X with incomplete longitudinal split.

13. Sternites without discal setae, three pairs of long marginal setae, setal pair S1 on VII arise in front of margin.

14. Male similar to female but smaller and paler, pronotum usually yellow; legs sometimes almost yellow; tergite IX with pair of short stout setae posterolaterally; sternites with no pore plates.

4.1.2.2 *Thrips palmi* (Karny)

1. A clear yellow body with no dark areas on the head, thorax or abdomen (slightly thickened blackish body setae); antennal segments I and II pale, III yellow with apex shaded and sensorium forked (Plate 4.1), IV–VII brown but usually with base of IV–V yellow; forewings uniformly slightly shaded, prominent setae dark. (Plate 4.2).

2. Antennae always seven-segmented (Plate 4.3).

3. Post ocular setae II and IV much smaller than remaining setae (Plate 4.4).

4. Ocellar setae III standing either just outside the ocellar triangle (Plate 4.5) or touching the tangent lines connecting the anterior ocellus and each of the posterior ocelli (Plate 4.6).

5. Pronotum with transverse carina parallel to posterior margin, median area weakly transversely reticulate; 2 pairs of long posteroangular setae, outer longer than inner, one pair of anteroangular setae moderately prominent (Plate 4.7)

6. Metascutum with sculpture converging posteriorly; median pair of setae behind anterior margin; paired campaniform sensilla present (Plate 4.8).

7. Forewing first vein with three (occasionally two) distal setae (Plate 4.9).

8. Abdominal tergite II with four lateral marginal setae (Plate 4.10).

9. Abdominal tergites III to IV with setae S2 dark and subequal to S3.

10. Abdominal tergite VIII with posteromarginal comb in female complete, in male broadly developed posteriorly.

11. Abdominal tergite IX usually with two pairs of campaniform sensilla (pores) (plate 4.11).

12. Abdominal sternites without discal setae or ciliate microtrichia.

13. Abdominal pleurotergites without discal setae.

14. Male: sternites III–VII each with a narrow transverse glandular area

4.1.2.3 Key to *Megalurothrips usitatus* (Bagnall) given by Mound and Yongfoo (2009)

1ˈ Never dark brown and reticulate; major setae not long and capitate …….....2

2ˈ Pronotum never with more than five pairs of major setae……3

3ˈAbdominal tergites without numerous microtrichia occupying lateral thirds, with a few microtrichia near lateral margins ……..9

9' Major setae on head, pronotum and forewings setaceous …….....10

10' Antennal segment II external margin not prolonged, segment I not swollen……..11

11' Pronotum with at least one pair of prominent posteroangular setae……..20

20 Pronotal anterior margin with 1 or 2 pairs of setae that are much longer than discal setae…….....21

21' Forewing first vein with setal row widely interrupted; tergites without ctenidia 23

23' Forewing second vein with many equally spaced setae; tergite VIII either with no comb or with comb interrupted medially; tergites and sternites without prominent reticulation……24

24 Ocellar setae pair III arising close to anterolateral margins of ocellar triangle; tergite VIII posterior margin on lateral thirds with well-developed comb …….….***Megalurothrips usitatus***

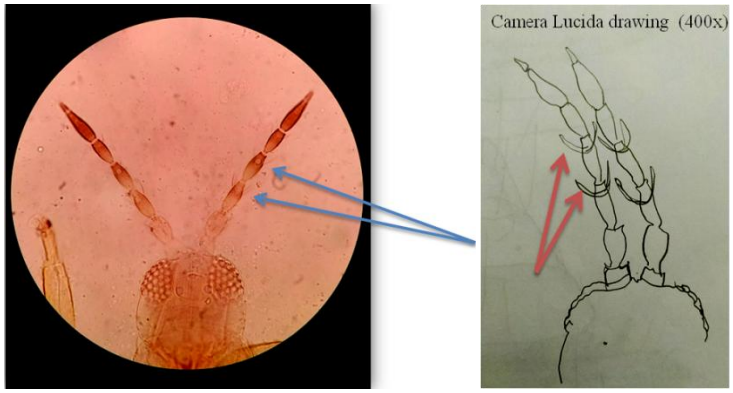


Plate 4.1. Antennal segments III and IV, forked sense cones

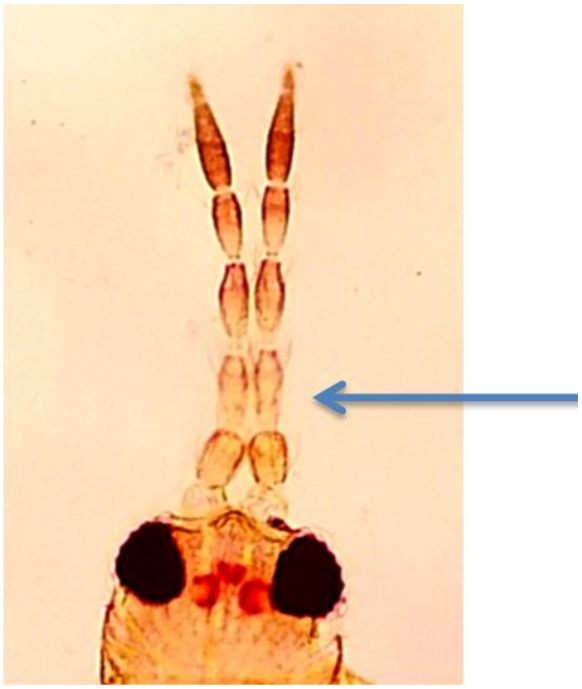


Plate 4.2. Antennal segments I and II pale, III yellow with apex shaded, IV–VII brown but usually with base of IV–V yellow

Key characters of *T. palmi*

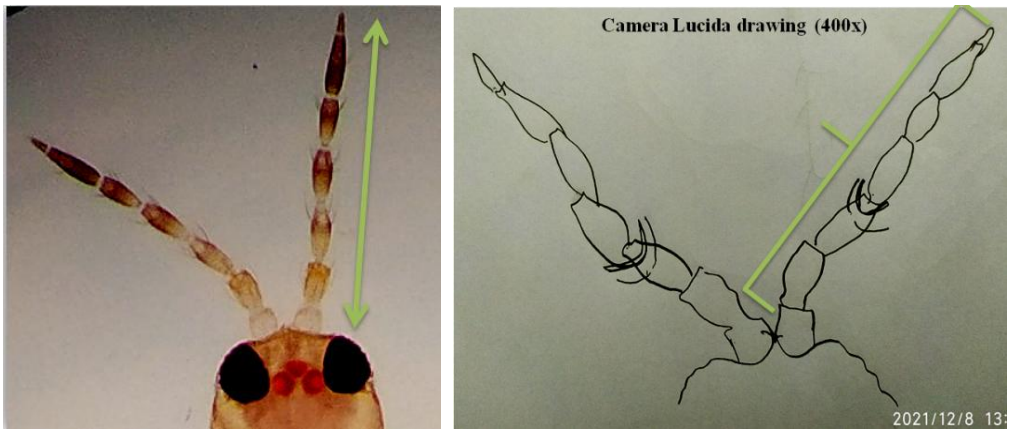


Plate 4.3. Antennae always seven-segmented

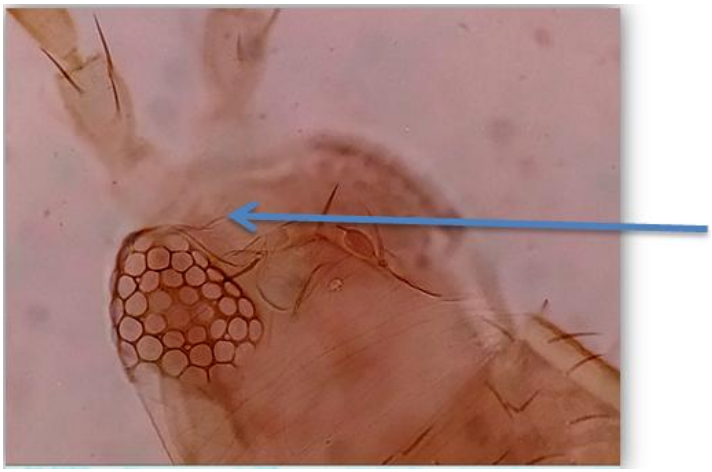


Plate 4.4. Postocular setae II and IV much smaller than remaining setae

Key characters of *T. palmi*

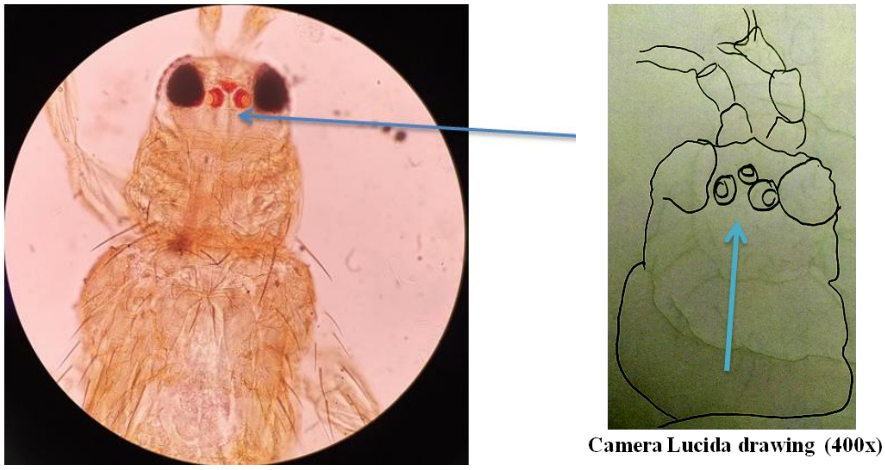


Plate 4.5. Ocellar triangle

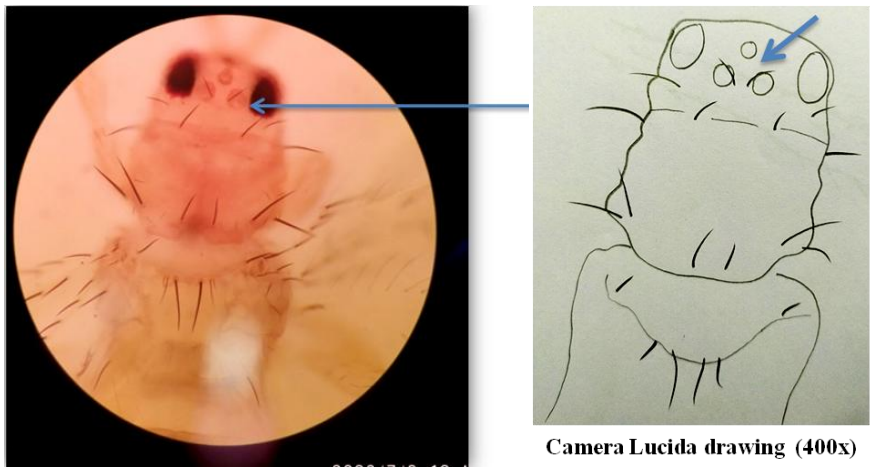


Plate 4.6. Ocellar setae III standing either just outside the ocellar triangle or touching the tangent lines connecting the anterior ocellus and each of the posterior ocelli

Key characters of *T. palmi*

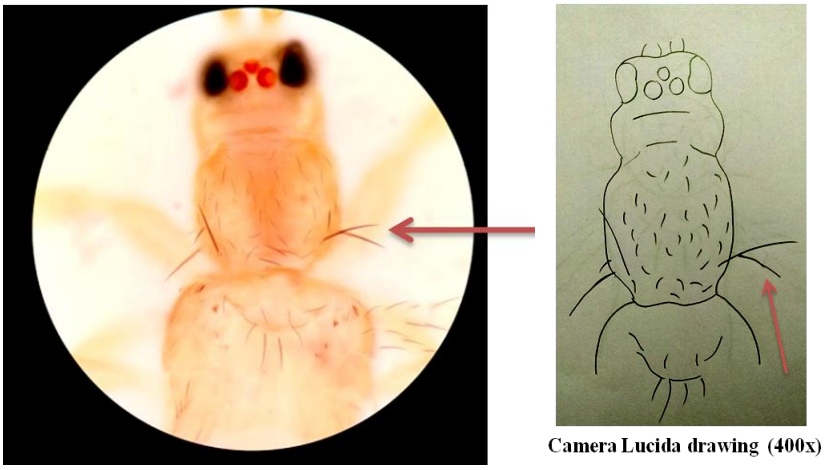


Plate 4.7. Pronotum, two pairs of major posteroangular setae

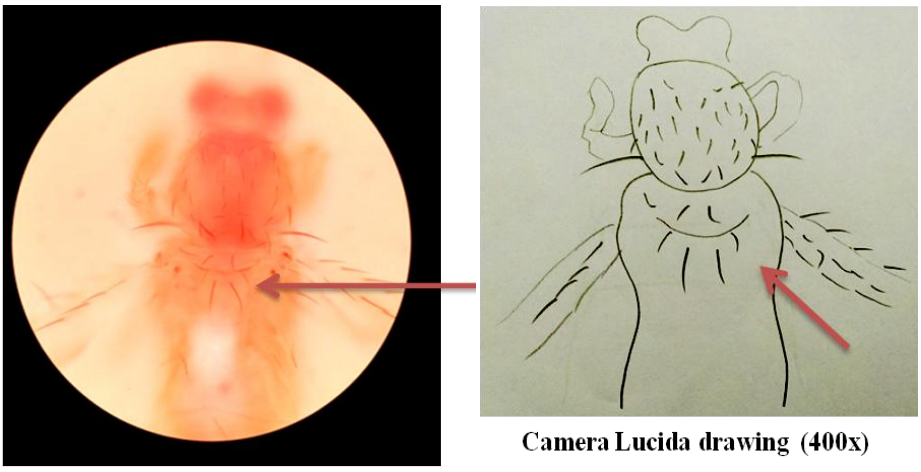


Plate 4.8. Metascutum with sculpture converging posteriorly; median pair of setae behind anterior margin; paired campaniform sensilla present

Key characters of *T. palmi*

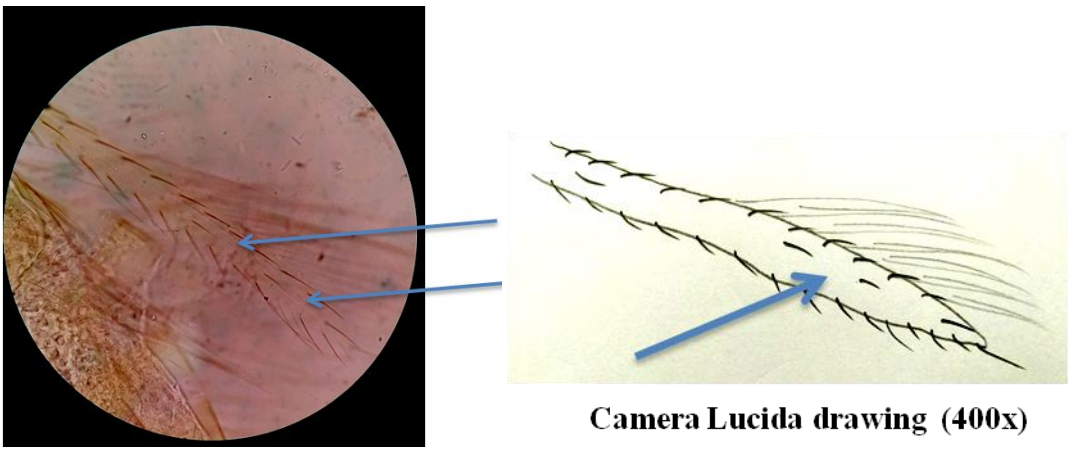
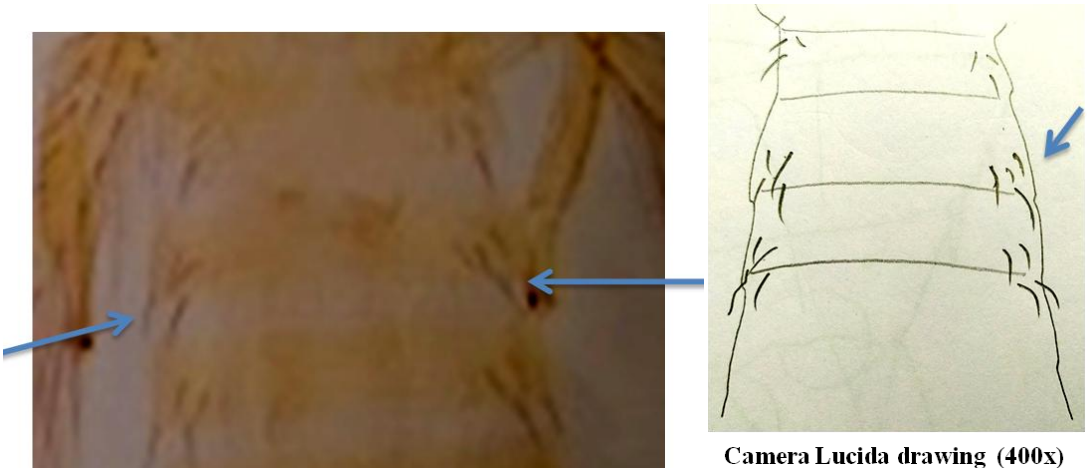


Plate 4.9. Forewing, first vein – three setae with gaps in the distal half

Key characters of *T. palmi*

Plate 4.10. Abdominal tergite II, four lateral marginal setae



Camera Lucida drawing (400x)

Plate 4.10. Abdominal tergite II, four lateral marginal setae

Key characters of *T. palmi*

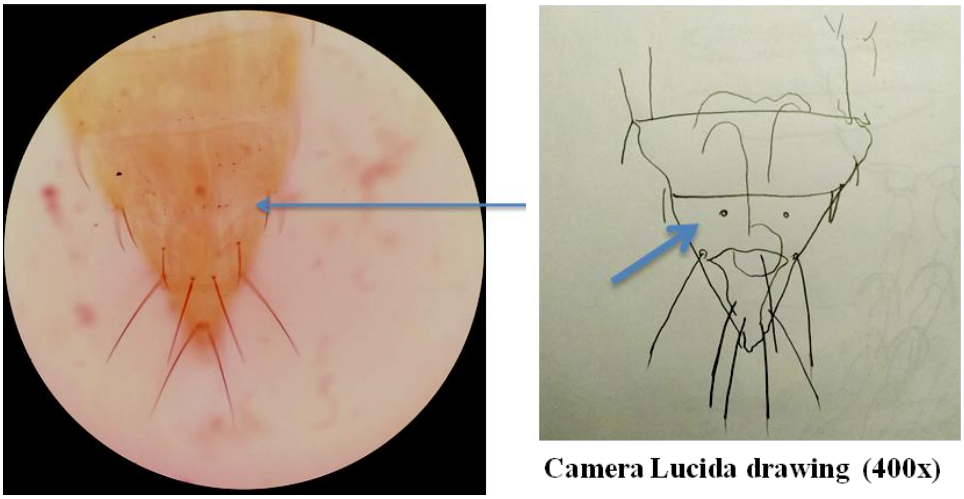


Plate 4.11. Abdominal tergite IX (dorsal), two pairs of campaniform sensilla

4.1.2.4 *Megalurothrips usitatus* (Bagnall)

1. Both sexes fully winged.
2. Body dark brown, tarsi, apices of mid and hind tibiae, also most of fore tibiae yellow; hind tibiae with 2 stout dark apical setae (Plate 4.12).
3. Fore wings brown with basal quarter pale and an extensive pale area sub-apically (Plate 4.13).
4. Antennal segments I–II brownish yellow, III yellow, IV and sometimes V yellow at base; fore wing light brown, pale sub-basally and with sub-apical pale band (Plate 4.14)
5. Antennae 8-segmented, I with pair of dorso-apical setae; III–IV with constricted apical neck, sensorium forked, VIII almost twice as long as VII (Plate 4.15).
6. Head conspicuously transversely striate/reticulate at posterior (Plate 4.16), ocellar setae III long, arising just inside triangle; postocular setae not long (Plate 4.17).
7. Pronotum sometimes with transverse carina parallel to posterior margin, median area weakly transversely reticulate; 2 pairs of long posteroangular setae, outer longer than inner, one pair of anteroangular setae moderately prominent (Plate 4.18).
8. Mesonotum with transverse reticulation, lateral setae not long. (Plate 4.19).

9. Metanotum reticulate medially, median setae long, at anterior margin, campaniform sensilla present.

10. Mesosternal furca with spinula, metafurca without spinula.

11. Tarsi all 2-segmented. Fore wing first vein with long row of setae before distinct sub-apical gap followed by 2 setae; second vein with complete row of setae; postero-marginal cilia wavy.

12. Abdominal tergites II–VIII with no sculpture medially but lateral thirds with sub-parallel lines (Plate 4.20), median setae small (Plate 4.21); VIII with postero-marginal comb of small microtrichia laterally (Plate 4.22), discal area antero-mesead of spiracle with 2 or more rows of strong microtrichia; tergite X with incomplete longitudinal split.

13. Sternites without discal setae, three pairs of long marginal setae, setal pair S1 on VII arise in front of margin (Plate 4.23).

14. Male similar to female but smaller and paler, pronotum usually yellow; legs sometimes almost yellow; tergite IX with pair of short stout setae posterolaterally; sternites with no pore plates (Plate 4.24).

4.1.2.5 *Scirtothrips dorsalis* **(Hood)**

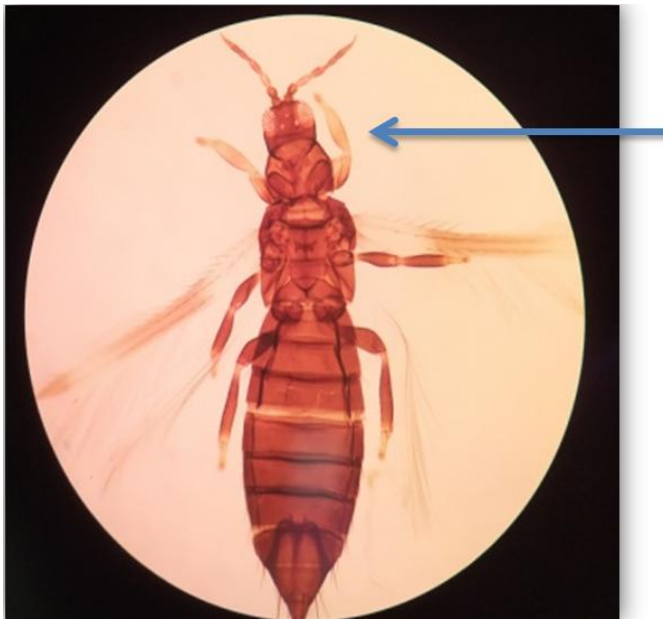
1 Wings (brachypterous or macropterous) or wing buds present ……..……...2

2' Wings fully formed, with setae present ……..Adult, 4

4' Abdominal segment X conical; female with saw-like ovipositor ……Terebrantia, 6

6' If ctenidia are present on abdominal tergites V–VII, ctenidium on tergite VIII posterior to spiracle; anterior margin of prothorax lacking major setae; antennae 7-, 8-, or 9-segmented 15

15 (6') Lateral margins of abdominal tergites IV–VI with microtrichia, rows of microtrichia closely spaced; cilia of forewing fringe straight; ocellar III setae arising between posterior ocelli, contained within ocellar triangle .....***Scirtothrips dorsalis*** **(Hood)**



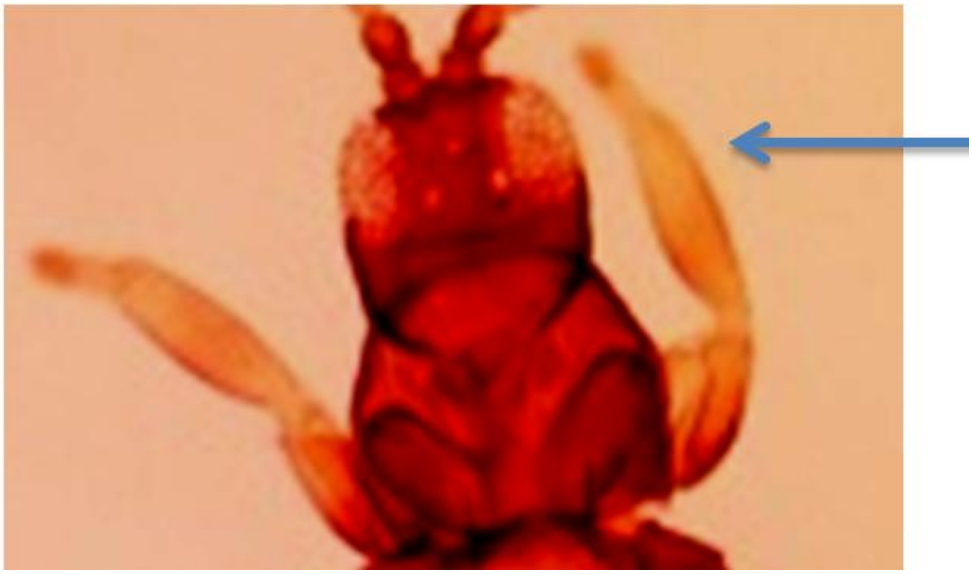
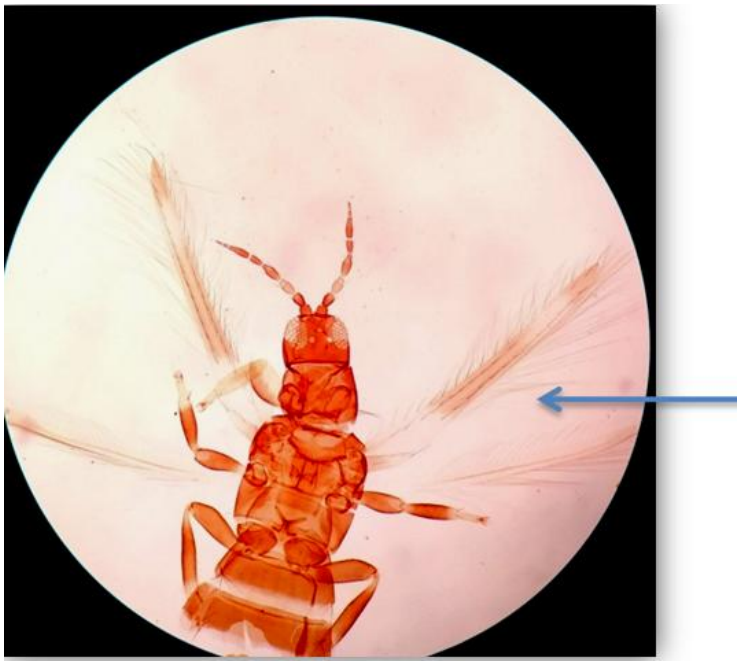
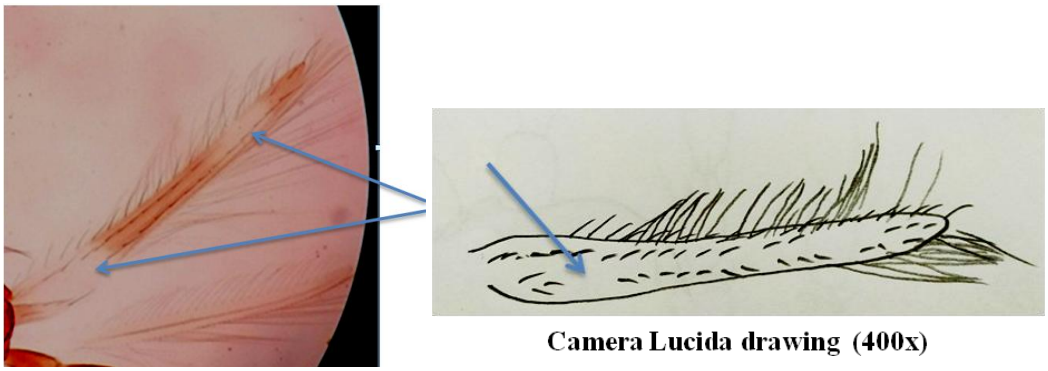


Plate 4.12. Fore tibiae yellow

Key characters of *M. usitatus*





Camera Lucida drawing (400x)

Plate 4.13. Fore wings brown with basal quarter pale and an extensive pale area sub- apically

Key characters of *M. usitatus*

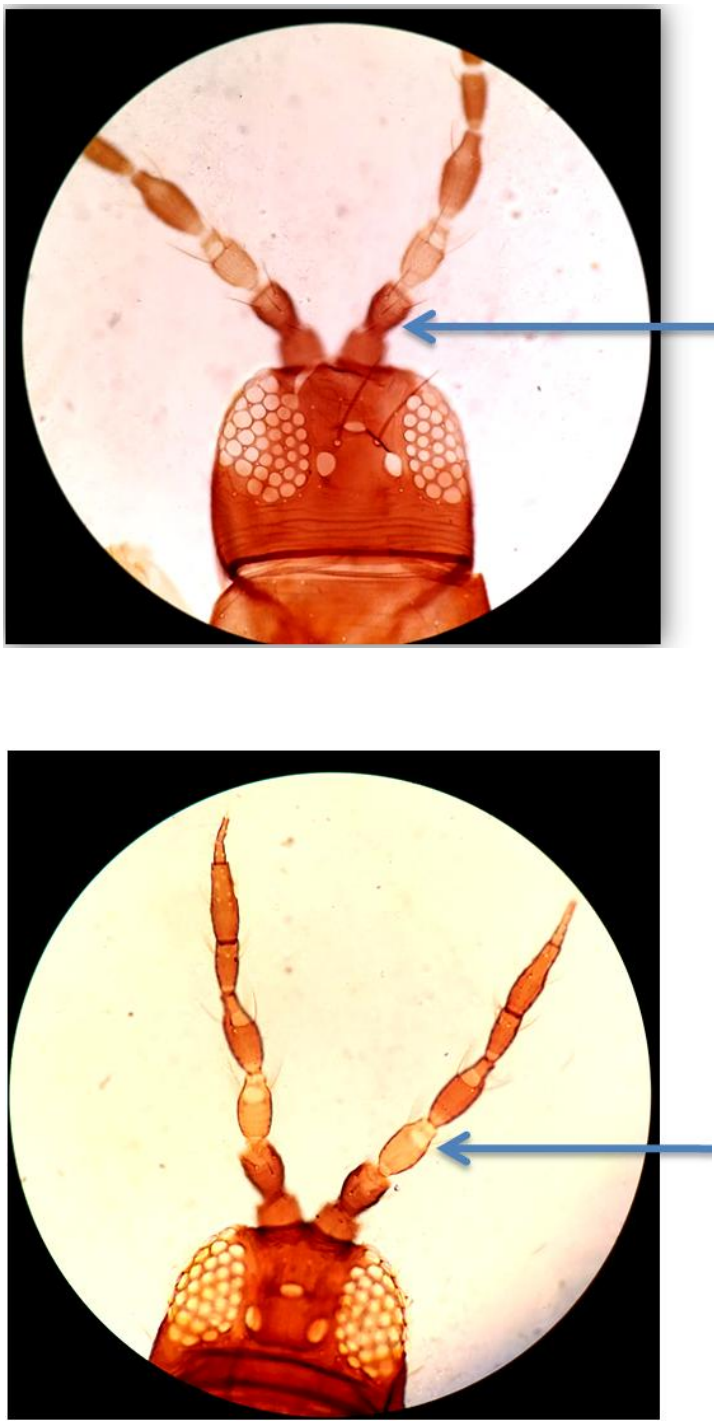


Plate 4.14. Antennal segments I–II brownish yellow, III yellow, IV and sometimes V yellow at base

Key characters of *M. usitatus*

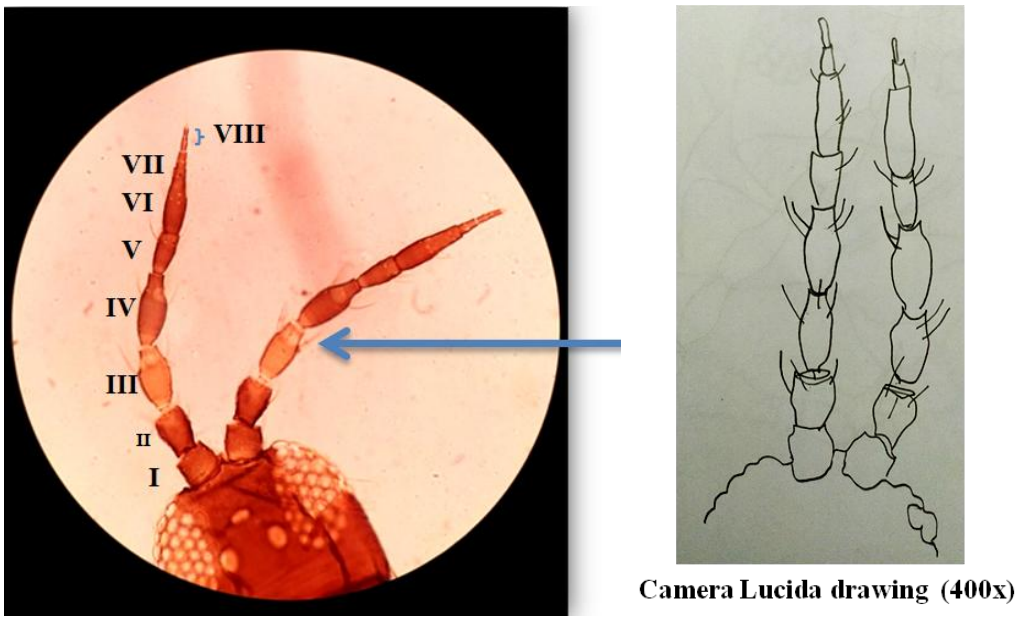


Plate 4.15. Antennae 8-segmented, I with pair of dorso-apical setae; III–IV with constricted apical neck, sensorium forked, VIII almost twice as long as VII.

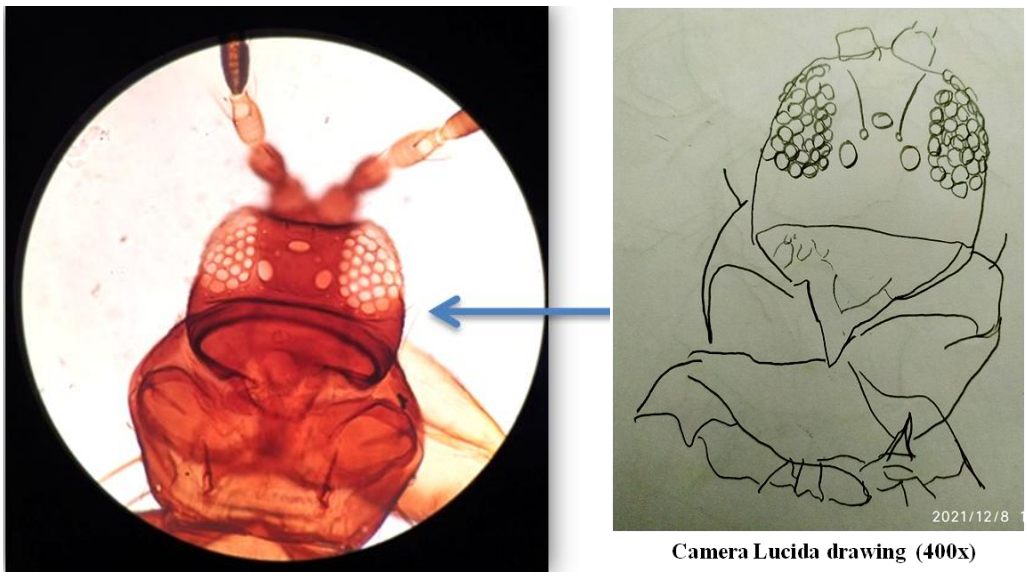
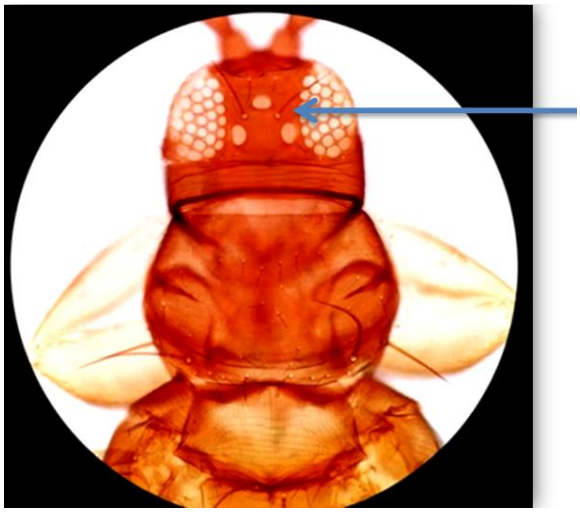
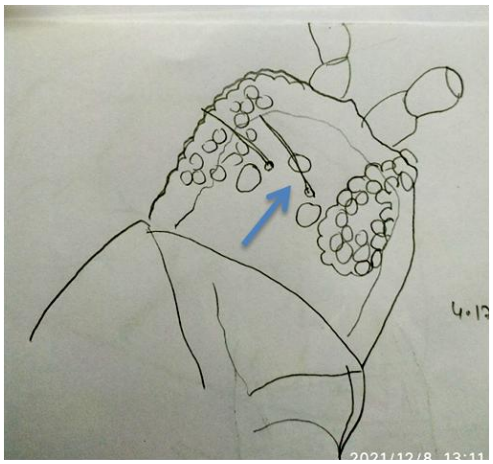


Plate 4.16. Head conspicuously transversely striate/reticulate at posterior

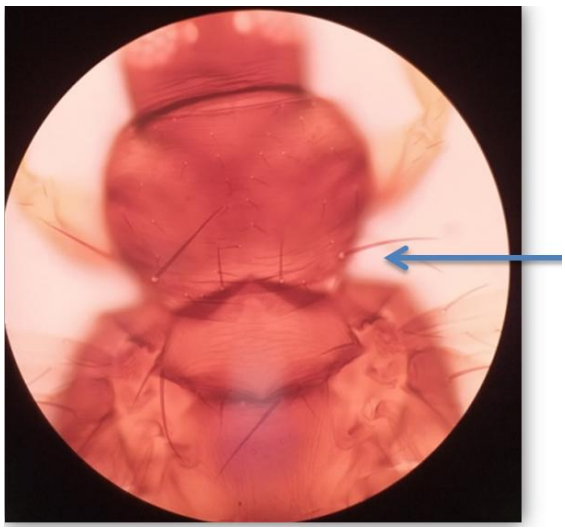
Key characters of *M. usitatus*





Camera Lucida drawing (400x)

Plate 4.17. Ocellar setae III long, arising just inside triangle; post ocular setae not long



Camera Lucida drawing (400x)

Plate 4.18. Pronotum – two pairs of long posteroangular setae, outer longer than inner

Key characters of *M. usitatus*

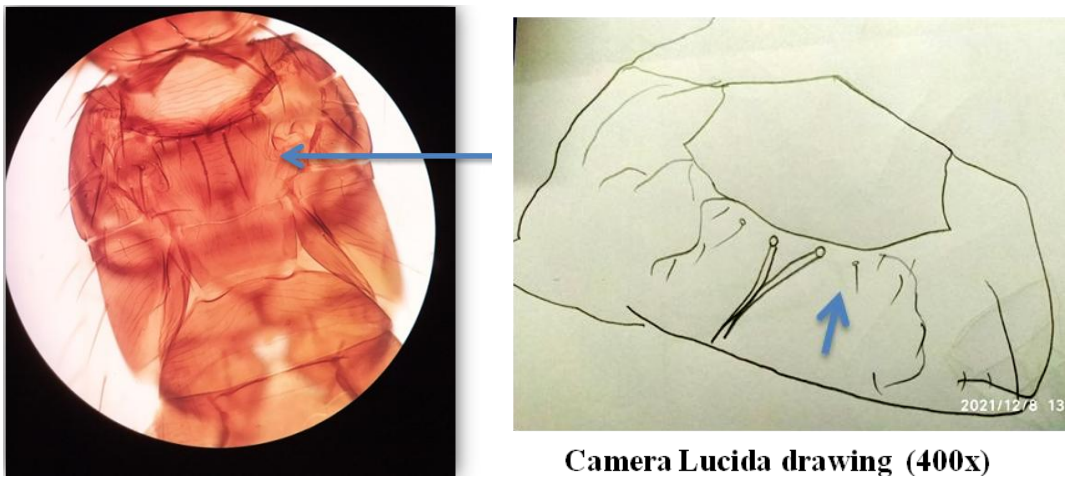


Plate 4.19. Mesonotum with transverse reticulation, lateral setae not long

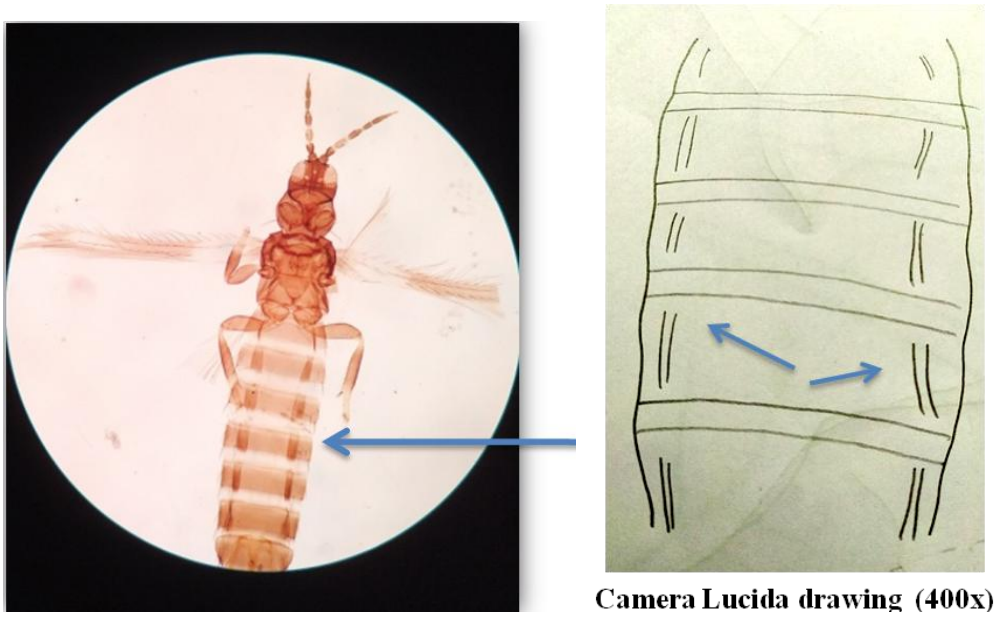


Plate 4.20. Abdominal tergites II–VIII with no sculpture medially but lateral thirds with sub-parallel lines

Key characters of *M. usitatus*

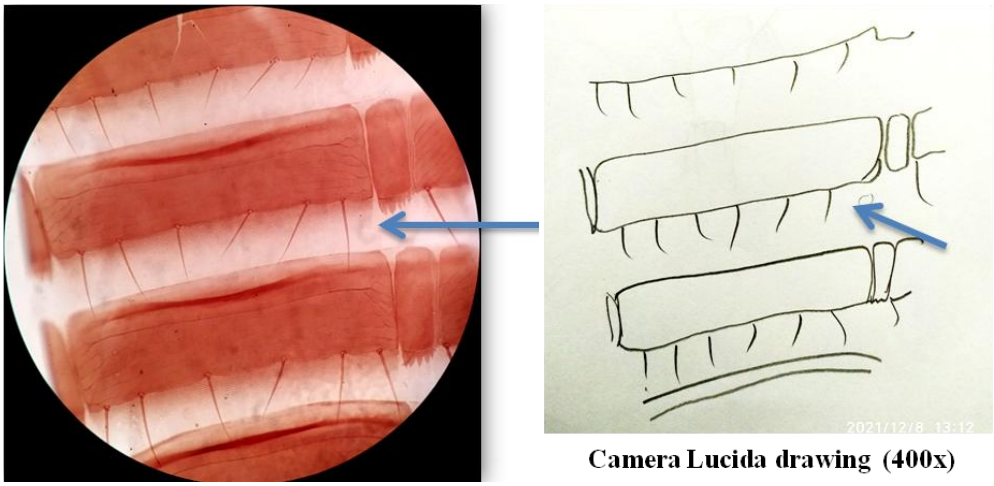


Plate 4.21. Abdominal tergites II–VIII, median setae small

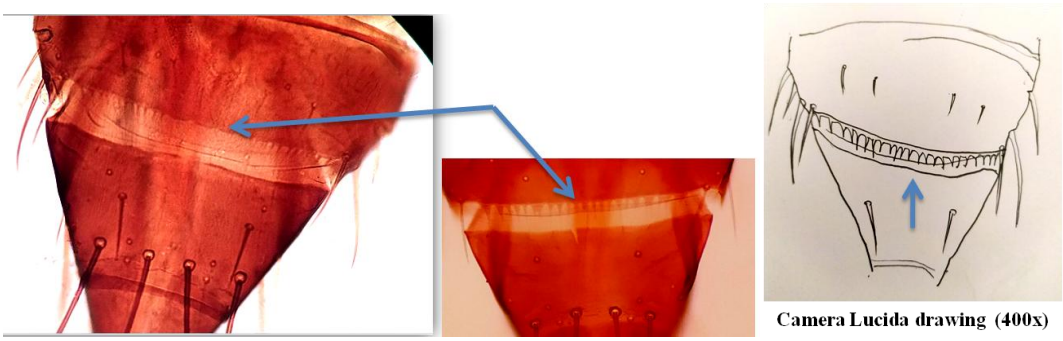
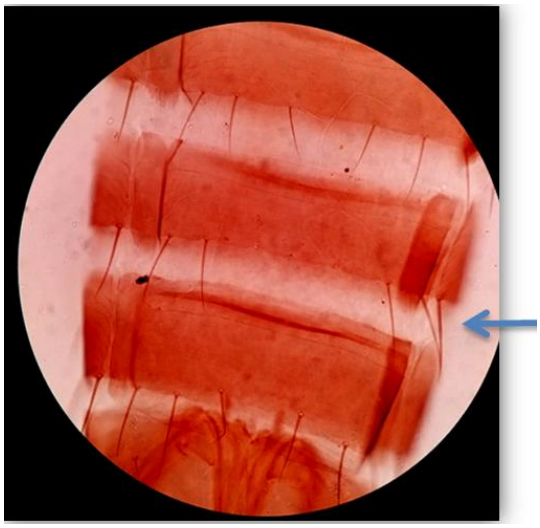
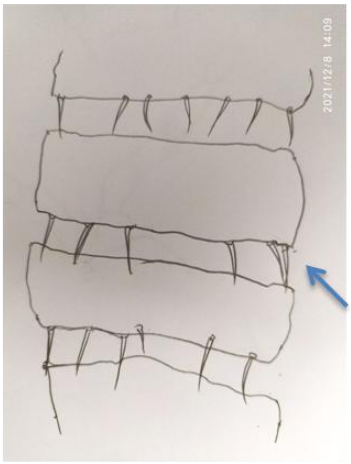


Plate 4.22. Tergite VIII with postero-marginal comb of small microtrichia laterally

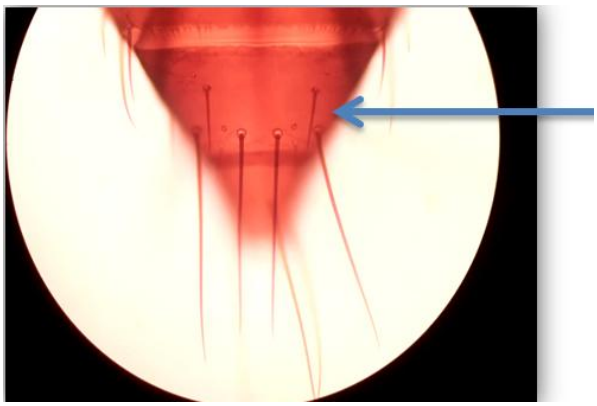
Key characters of *M. usitatus*

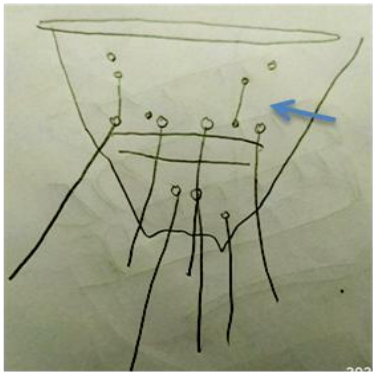




Camera Lucida drawing (400x)

Plate 4.23. Sternites without discal setae, three pairs of long marginal setae, setal pair S1 on VII arise in front of margin





Camera Lucida drawing (400x)

Plate 4.24. Male - tergite IX with pair of short stout setae posterolaterally

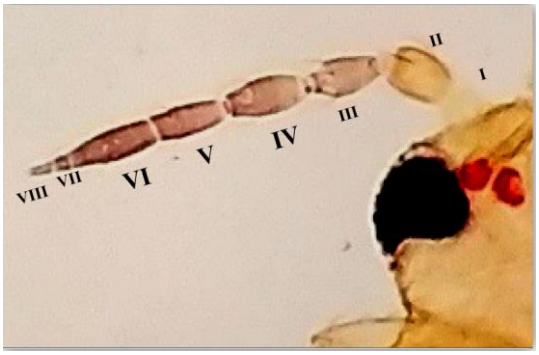
Key characters of *M. usitatus*

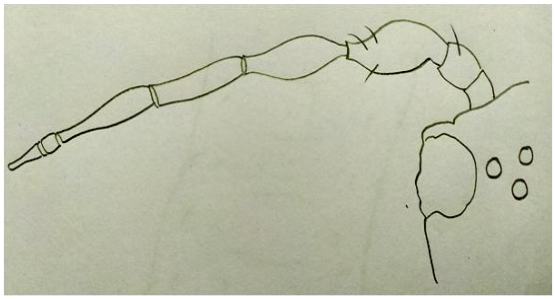
4.1.2.6 Characteristic features of *Scirtothrips dorsalis (*Hoddle and Mound, 2003)

1. The head and legs of adult *S. dorsalis* are pale in colour (Plate 4.25).
2. Three pairs of ocellar setae are present on the head. The third pair is situated between posterior ocelli. The median postocular setae are two pairs and equal in length.
3. The antennae are eight segmented. The antennal segments I and II are pale and III and VIII are dark in colour (Plate 4.26).
4. The pronotum bears four pairs of posteromarginal setae. There is elongated reticulation on the middle of metanotum (Plate 4.27).
5. The forewing is distally light in color with straight cilia. The first vein of forewing bears three irregular setae distally. The second vein is incomplete and has two setae (Plate 4.28).
6. There are numerous microtrichia on the abdominal tergites as well as sternites (Plate 4.29).
7. The tergites have a median dark patch. The antecostal ridges are also dark (Plate 4.30).
8. The tergal microtrichial fields of the abdomen have three discal setae. The posteromarginal comb on abdominal segment VIII is complete. In case of males, no drepanae is present on tergite IX.

Thrips specimens were also sent to ICAR-NBAIR (Germplasm Collection and Characterization) and the species identification was confirmed (Annexure III). Further confirmation was done by Dr. Laurence mound, honorary research fellow, CSIRO, Australia.

Plate 4.25. Pale colour head and legs of adult *S. dorsalis*

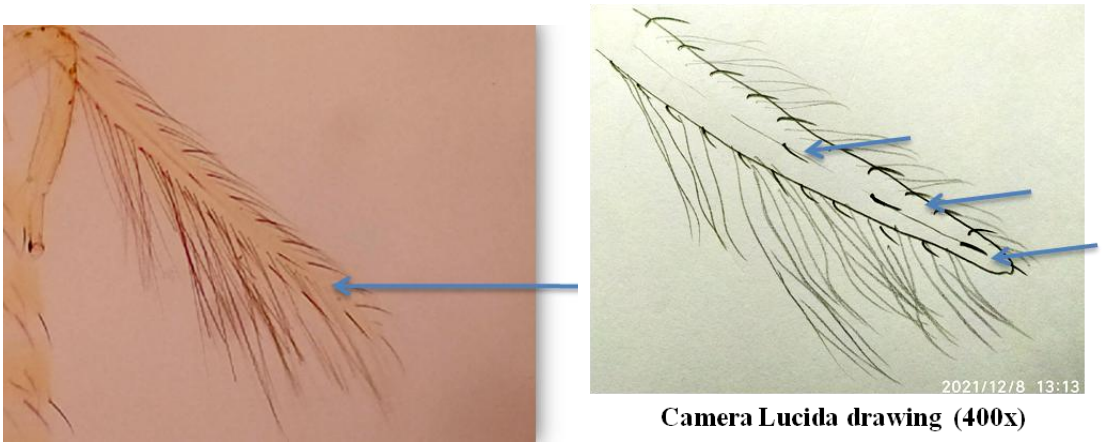




Camera Lucida drawing (400x)

Plate 4.26. The antennae are eight segmented. The antennal segments I and II are pale and III and VIII are dark in colour

Key characters of *S. dorsalis*



Camera Lucida drawing (400x)

Plate 4.27. The first vein of forewing bears three irregular setae distally

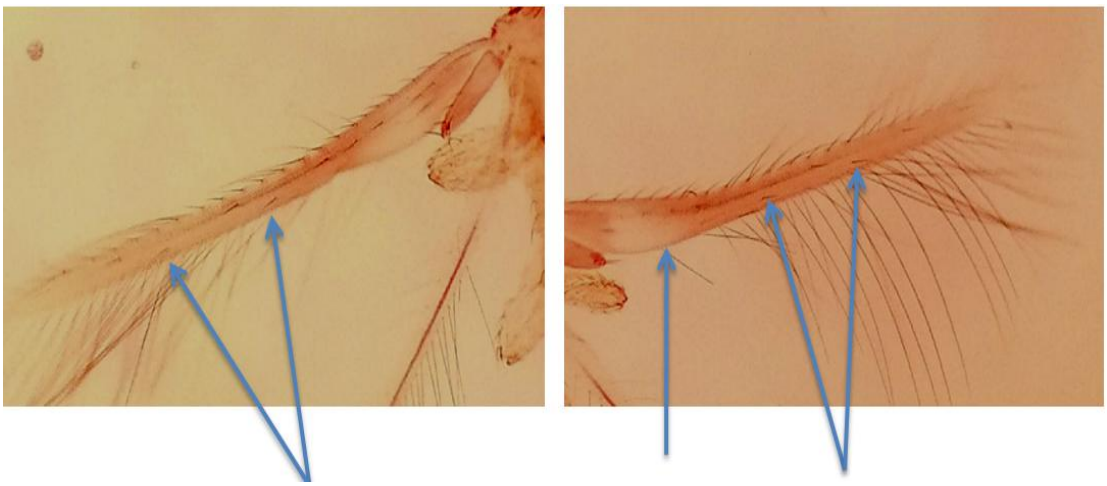


Plate 4.28. The forewing is distally light in color with straight cilia .The second vein is incomplete and has two setae

Key characters of *S. dorsalis*

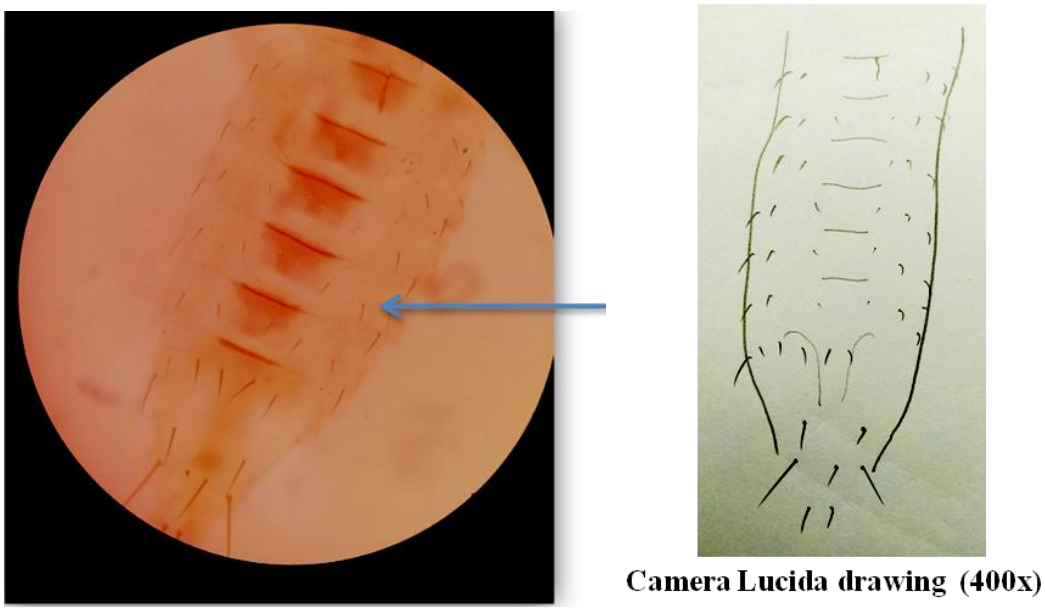


Plate 4.29. There are numerous microtrichia on the abdominal tergites

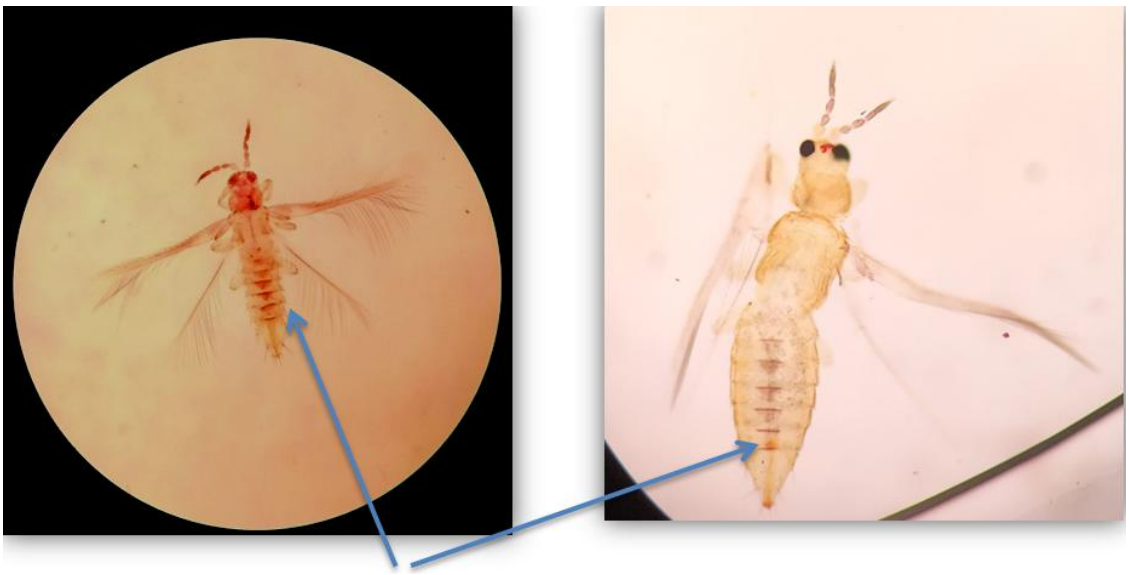


Plate 4.30 . The tergites have a median dark patch

Key characters of *S. dorsalis*

4.1.2.7 Per cent thrips species distribution on blackgram in Andhra Pradesh

After identification of all the specimens from different geographic locations, percentage of species composition was worked out and depicted in the Table 4.1. From the table it was evident that *T. palmi* was the major species among the all samples with 65.01, 61.39, 49.25, 44.00, 76.54, 76.13, and 67.13 per cent in Srikakulam, Vizianagaram, Krishna, Guntur, Prakasam, Kurnool and Chittoor districts, respectively. Species complex of thrips on black gram in Andhra Pradesh state was 62.78, 30.39, and 6.83 mean per cent of *T. palmi*, *M. usitatus*, and *S. dorsalis*, respectively. Districts Srikakulam, Vizianagaram, Krishna, Guntur, Prakasam, Kurnool and Chittoor districts showed 31.43, 28.14, 35.58, 47.31, 20.30, 21.14, 28.81 mean per cent of *M. usitatus*, respectively. Pittalavanipalem mandal of Guntur district and R. Amudalavalasa mandal of Srikakulam district have recorded with highest mean per cent of *M. usitatus* among the all other mandals *i.e.* 66.67 and 56.3, respectively.

In the present study, *S. dorsalis* was recorded with least mean per cent (6.83) in Andhra Pradesh state. Out of thirty five locations surveyed, no record of *S. dorsalis* was observed in 19 mandals *viz*., Ponduru, R. Amudalavalasa and Rajam mandals of Srikakulam district, Gantyada, Bondapalli, Dattirajeru and Gajapatinagaram of Vizianagaram district, Pittalavanipalem and Chebrolu mandals of Guntur district, Vetapalem, Chinganjam, Naguluppalapadu of Prakasm district, Gospadu, Panyam, Bandi atmakuru and Gadivemula of Kurnool district, Gudipala, Thavanampalle, Airala of Chittoor district. Highest mean per cent of *S. dorsalis i.e.*, 52.38 was recorded in Mentada mandal of Vizianagaram district.

From the present study, it is evident that the major species of thrips that were found abundant is *T. palmi* (62.78) followed by *M. usitatus* (30.39) and *S. dorsalis* (6.83), which is in contrary to the findings of Zafirah and Azidah (2018) who have reported the most abundant thrips species on legumes as *Megalurothrips usitatus* (89.97 %) followed by *T. parvispinus* (9.77 %), *Thrips hawaiiensis* (Morgan) (0.13 %) and *Ceratothripoides brunneus* (Bagnall) (0.12 %), with no significant difference regarding the abundance of *M. usitatus* on long bean, french bean and winged bean which equally distributed among different arbitrary strata on legume plants in Peninsular Malaysia.

Table 4.1 Distribution of thrips population (in per cent) on blackgram among different geographic locations of Andhra Pradesh based on morphological identification

S. No.	District	Mandal	*T. palmi*	*M. usitatus*	*S. dorsalis*
1	Srikakulam	Etcherla	61.11	27.78	11.11
		Ponduru	78.95	21.05	0.00
		Sigadam	60.00	33.33	6.67
		R.Amudalavalasa	43.75	56.25	0.00
		Rajam	81.25	18.75	0.00
		Total per cent mean	**65.01**	**31.43**	**3.56**
2	Vizianagaram	Gantyada	61.11	38.89	0.00
		Bondapalli	66.67	33.33	0.00
		Mentada	28.57	19.05	52.38
		Dattirajeru	70.59	29.41	0.00
		Gajapathingaram	80.00	20.00	0.00
		Total per cent mean	**61.39**	**28.14**	**10.48**
3	Krishna	Avanigadda	35.00	50.00	15.00
		Challapalli	35.29	47.06	17.65
		Mopidevi	60.00	26.67	13.33
		Pamarru	43.75	37.50	18.75
		Movva	72.22	16.67	11.11
		Total per cent mean	**49.25**	**35.58**	**15.17**
4	Guntur	Pittalavanipalem	33.33	66.67	0.00
		Amarthalur	50.00	31.25	18.75
		Ponnuru	52.94	35.29	11.76
		Chebrolu	40.00	53.33	6.67
		Tadikonda	43.75	50.00	6.25
		Total per cent mean	**44.00**	**47.31**	**8.69**
5	Prakasam	Vetapalem	86.67	13.33	0.00
		Chirala	73.68	21.05	5.26
		Ongole	68.42	21.05	10.53
		Chinaganjam	83.33	16.67	0.00
		Naguluppalapadu	70.59	29.41	0.00
		Total per cent mean	**76.54**	**20.30**	**3.16**
6	Kurnool	Gospadu	88.2	11.8	0.00
		Panyam	80.00	20.00	0.00
		Bandiatmakuru	86.67	13.33	0.00
		Banaganapalle	59.09	27.27	13.64
		Gadivemula	66.67	33.33	0.00
		Total per cent mean	**76.1**	**21.1**	**2.73**
7	Chittoor	Gudipala	93.33	6.67	0.00
		Thavanampalle	73.91	26.09	0.00
		Palasamudram	60.00	33.33	6.67
		Ganghadharnellore	54.55	31.82	13.64
		Airala	53.85	46.15	0.00
		Total per cent mean	**67.13**	**28.81**	**4.06**
8	**Andhra Pradesh**	**Total per cent mean**	**63.12**	**30.05**	**6.83**

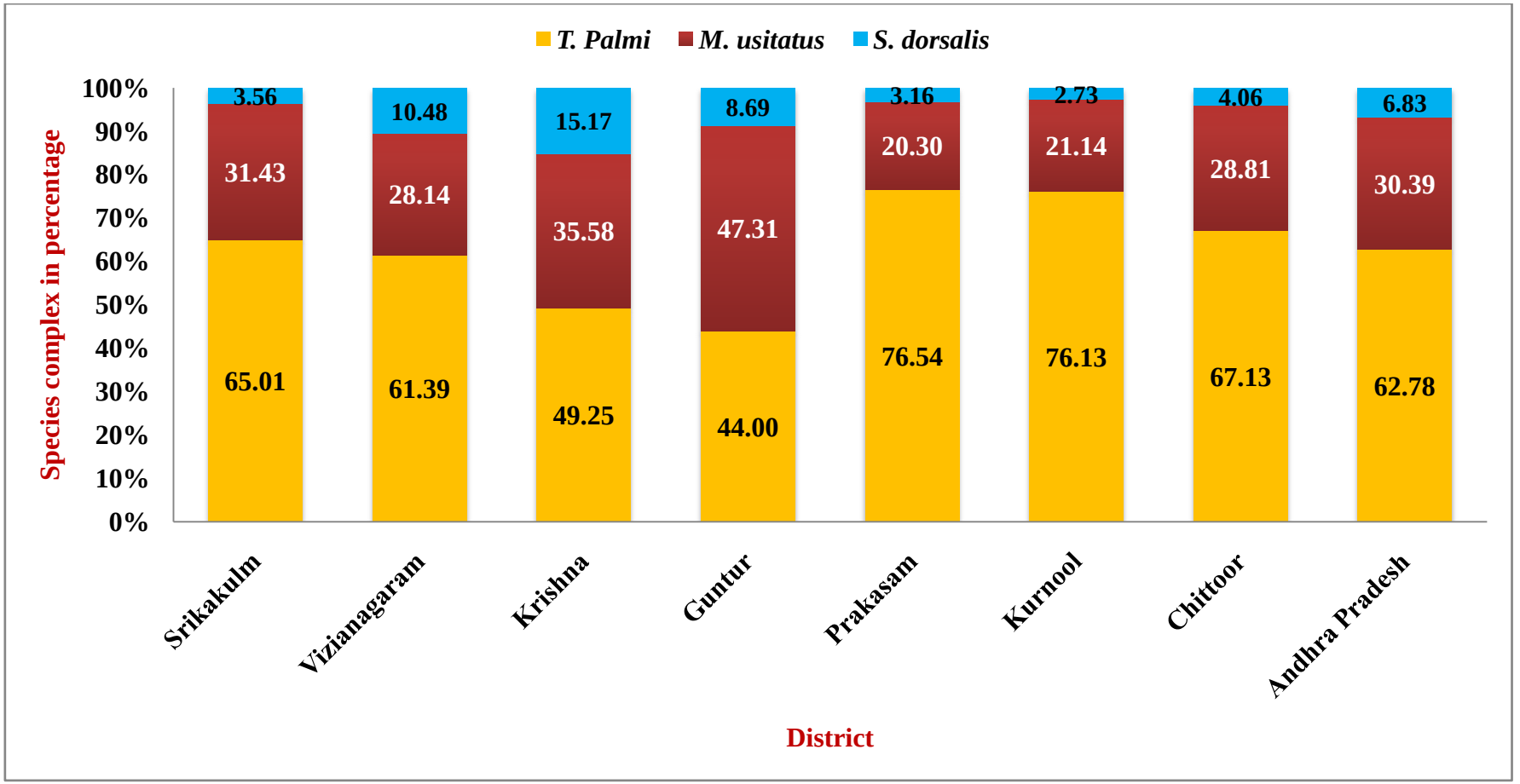
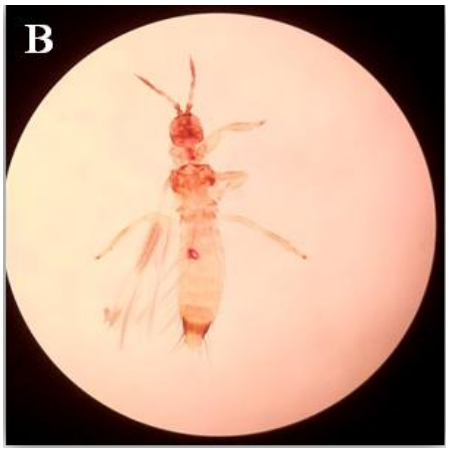


Figure 4.1. Distribution of thrips population (in per cent) on blackgram among different geographic locations of Andhra Pradesh based on morphological identification

4.1.3 Molecular Characterization of Thrips Complex Through DNA Isolation and PCR Amplification

Based on the morphological identification data presented in the Table 4.1 it was evident that three species of thrips *i.e. Thrips palmi* (Karny), *Megalurothrips usitatus* (Bagnall), *Scirtothrips dorsalis* (Hood) were dominating in all the geographic locations of Andhra Pradesh. In Chittoor district *Megalurothrips typicus* (Bagnall) (Plate 4.31), *Ayyaria chaetophora* (Karny), *Phibalothrips peringueyi* (Faure) and some Tubulifera thrips were also observed in meager numbers (Plate 4.32). Hence, molecular characterization was carried out for only the dominant three major thrips species listed above.

The precise identification of species is a first step in development of management strategies for any pest. Species identification and subsequent understanding of vector specificity play a major role in management of vector transmitted diseases. Since thrips are very tiny insects, species identification by morphological observation is very difficult task and it may lead to confusion about the vector status of thrips species. Hence, morphological identification coupled with molecular characterization gives accurate identification of a particular species. In the present study initial morphological identification was done for all the collected specimens from 35 locations in Andhra Pradesh. For further confirmation, the same specimens were used for molecular studies. As thrips are very small insects and total genomic DNA yield will be generally low. In order to overcome these possible problems of insufficient template, specific primers were used to amplify a smaller fragment of the targeted gene. The specific primers used in the current study enabled successful amplification of collected specimens, which eneabled proper identification into three deferent species based on ITS2, COI, COIII gene fragment-based DNA barcoding.



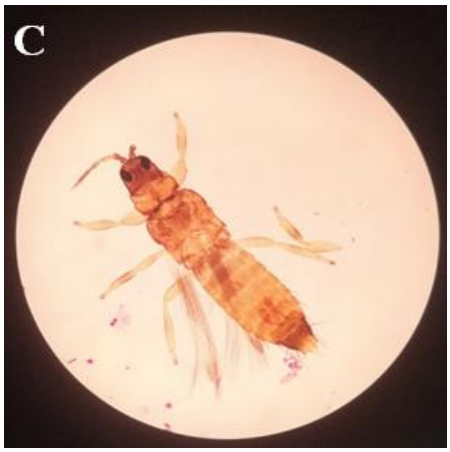


Plate 4.31. ***Megalurothrips typicus***

Camera Lucida drawing (400x)

A. *Haplothrips* Sp. (Tubulifera) Phlaeothripidae

B. *Ayyaria chaetophora* (Terebrantia) Thripidae

Plate 4.32. Other thrips species identified

Table 4.2. Molecular characterization of thrips complex after morphological identification

S. No.	District	Location	*T. palmi*	*M. usitatus*	*S. dorsalis*
1	**Srikakulam**	Etcherla	**TP1***	**MU1***	**SD1***
			TP2	MU2	
2		Ponduru	TP3	MU3	0
			TP4	MU4	0
3		Sigadam	TP5	MU5	SD2
			TP6	MU6	
4		R Amudalavalasa	TP7	MU7	0
			TP8	MU8	
5		Rajam	TP9	MU9	0
			TP10	MU10	
6	**Vizianagaram**	Gantayada	TP11	MU11	0
			TP12	MU12	
7		Bondapalli	TP13	MU13	0
			TP14	MU14	
8		Mentada	**TP15***	**MU15***	**SD3***, SD4
			TP16	MU16	
9		Dattirajeru	TP17	MU17	0
			TP18	MU18	
10		Gajapathinagaram	TP19	MU19	0
			TP20	MU20	
11	**Krishna**	Avanigadda	**TP21***	**MU21***	**SD5***
			TP22	MU22	
12		Challapalli	TP23	MU23	SD6
			TP24	MU24	
13		Mopidevi	TP25	MU25	SD7
			TP26	MU26	
14		Pamarru	TP27	MU27	SD8
			TP28	MU28	
15		Movva	TP29	MU29	SD9
			TP30	MU30	
16	**Guntur**	Pittalavaniaplem	TP31	MU31	0
			TP32	MU32	
17		Amarthaluru	TP33	MU33	SD10
			TP34	MU34	
18		Ponnuru	TP35	MU35	SD11
			TP36	MU36	
19		Chebrolu	TP37	MU37	0
			TP38	MU38	
20		Tadikonda	**TP39***	**MU39***	**SD12***
			TP40	MU40	

S. No.	District	Location	*T. palmi*	*M. usitatus*	*S. dorsalis*
21	**Prakasam**	Vetapalem	TP41	MU41	0
			TP42	MU42	
22		Chirala	TP43	MU43	SD13
			TP44	MU44	
23		Ongole	**TP45***	**MU45***	**SD14***
			TP46	MU46	
24		Chinaganjam	TP47	MU47	0
			TP48	MU48	
25		Naguluppalapadu	TP49	MU49	0
			TP50	MU50	
26	**Kurnool**	Gospadu	TP51	MU51	2
			TP52	MU52	
27		Panyam	TP53	MU53	0
			TP54	MU54	
28		Bandiatmakuru	TP55	MU55	0
			TP56	MU56	
29		Banaganapalle	**TP57***	**MU57***	**SD15***
			TP58	MU58	
30		Gadivemula	TP59	MU59	0
			TP60	MU60	
31	**Chittoor**	Gudipala	TP61	MU61	0
			TP62	MU62	
32		Thavanampalle	TP63	MU63	0
			TP64	MU64	
33		Palasamudram	**TP65***	**MU65***	**SD16***
			TP66	MU66	
34		Ganghadhar Nellore	TP67	MU67	SD17
			TP68	MU68	
35		Airala	TP69	MU69	0
			TP70	MU70	

Note: * denotes the sample sequence (TP - *T. palmi*, MU - *M. usitatus*, SD- *S. dorsalis*)

4.1.3.1 Identification and confirmation of *T. palmi* using ITS2 marker: Internal Transcribed Spacer (ITS) is a challenging marker, technically it is present in multiple distinct copies, and has likelihood of containing high intra and inter-genomic variation. This marker is useful for species identification in taxon specific studies as it produces alignment overlaps in the genus-specific range (Dentinger *et al.* 2011., Stern *et al.*, 2012). However, the fact that ITS2 sequences are potential markers for general phylogenetic studies and have been widely used for phylogenetic tree reconstructions both at genus and species levels which makes them ideal for species differentiation (Miao *et al.* 2008., Schultz and Wolf, 2009). ITS-based markers have been used by various researchers for species-level identification of thrips (Farris *et al*. 2010., Grazia *et al*. 2016., Toda and Komazaki, 2002, Kumar *et al*., 2017b).

All the 70 thrips samples collected from 35 geographic locations produced an amplicon size of ~570bp with ITS2 marker (Plate 4.33a, b; 4.34a, b, c, d). The results are in agreement with Sumit *et al*. (2020) who have reported that *T. palmi* was identified from the samples collected from brinjal, lettuce, and tomato using multiplex PCR assay with designed ITS2 primer without any cross reactivity. Other workers Nakahara and Minoura (2015) amplified the internal transcribed spacer 2 region (ITS2) of nuclear ribosomal DNA using five specific primers for 71 individuals of the four thrips species *viz., T. palmi, T. tabaci, F. intonsa* and *F. occidentalis* that were frequently found in Japanese quarantine inspection sites based on species-specific single bands (470 bp, 410 bp, 370 bp, 280 bp).

Yeh *et al*. (2014) obtained 43 ITS1 sequences ranging from 800-1200 bp for 15 thrips species and deposited in NCBI (AB904169–AB904212) and also reported that multiplex PCR using specific primers based on ITS1 sequences is a simple, reliable, and cost-effective diagnostic tool in the identification of thrips (*T. tabaci*, *F. intonsa*, and *S. dorsalis*). Farris *et al*. (2010) studied 432 *S. dorsalis* specimens representing 15 geographic populations and reported that 12 populations displayed 100 % amplification of ITS2 fragment ranging from 131 to 135 bp and these included the populations from India, Japan, U.S.A, Barbados, Israel, and Venezuela.

Plate 4.33a. Electrophoretic separation of PCR amplification products of *T. palmi* with ITS 2 primer (568bp)

Lane L = 100bp Ladder; Lane 1, 2 = Etcherla; Lane 3, 4 = Ponduru; Lane 5, 6 = Sigadam; Lane 7, 8 = R. Amudalavalasa; Lane 9, 10 = Rajam; Lane 11, 12 = Gantayada; Lane 13, 14 = Bondapalli; Lane 15, 16 = Mentada; Lane 17, 18 = Dattirajeru; Lane 19, 20 = Gajapatinagaram; Lane 21, 22 = Avanigadda, Lane 23, 24 = Challaplli

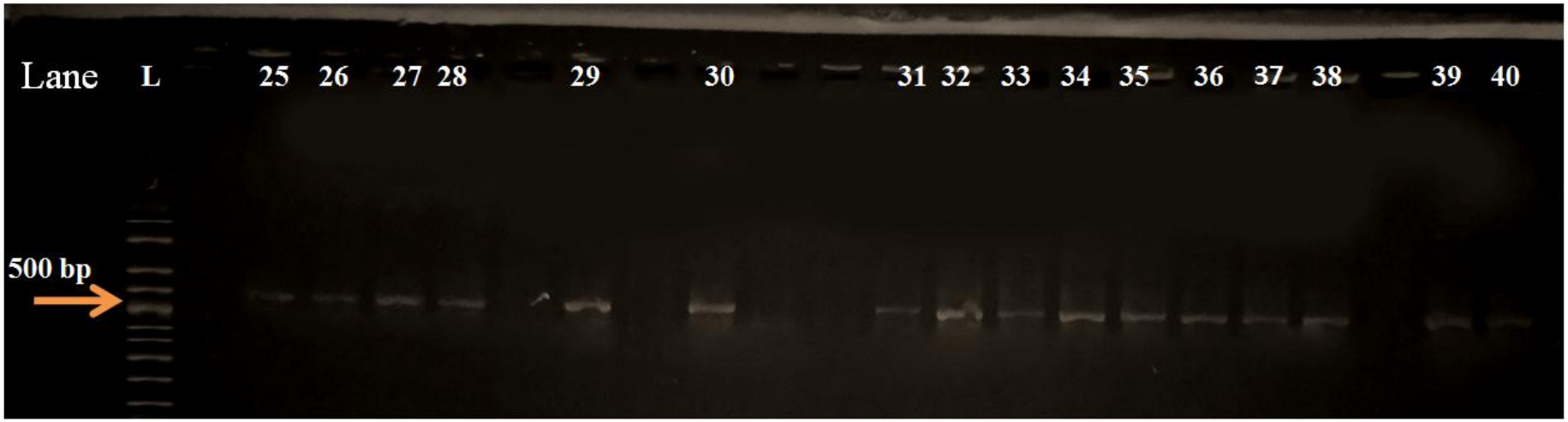


Plate 4.33b. Electrophoretic separation of PCR amplification products of *T. palmi* with ITS 2 primer (568bp)

Lane L = 100bp Ladder; Lane 25, 26 = Mopidevi; Lane 27, 28 = Pamarru; Lane 29, 30 = Movva; Lane 31, 32 = P.V. Palem; Lane 33, 34 = Amarthalur; Lane 35, 36 = Ponnuru; Lane 37, 38 = Chebrolu; Lane 39, 40 = Tadikonda

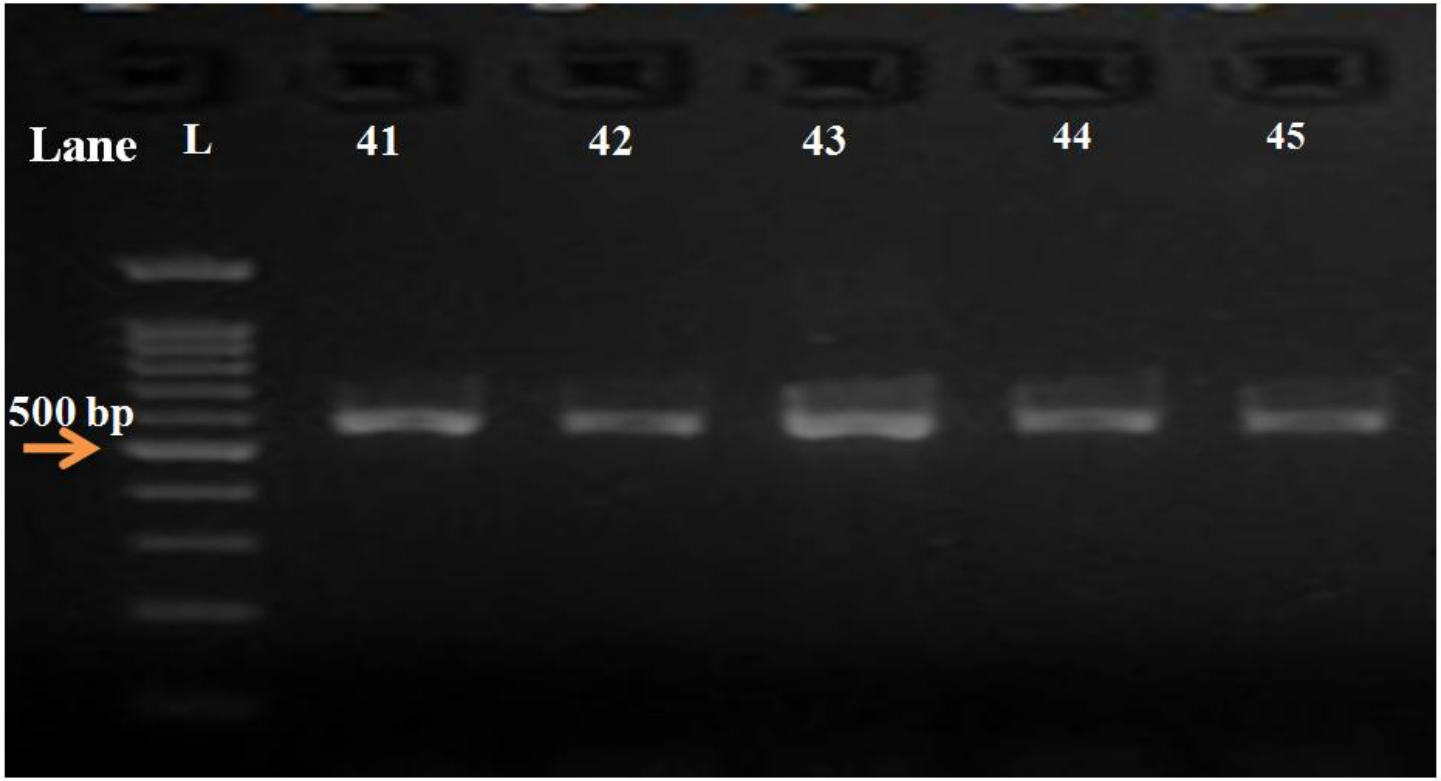


Plate 4.34a. Electrophoretic separation of PCR amplification products of *T. palmi* with ITS2 primer (568 bp)

L = 100bp Ladder; Lane 41, 42 = Vetapalem; Lane 43, 44 = Chirala; Lane 45 = Ongole

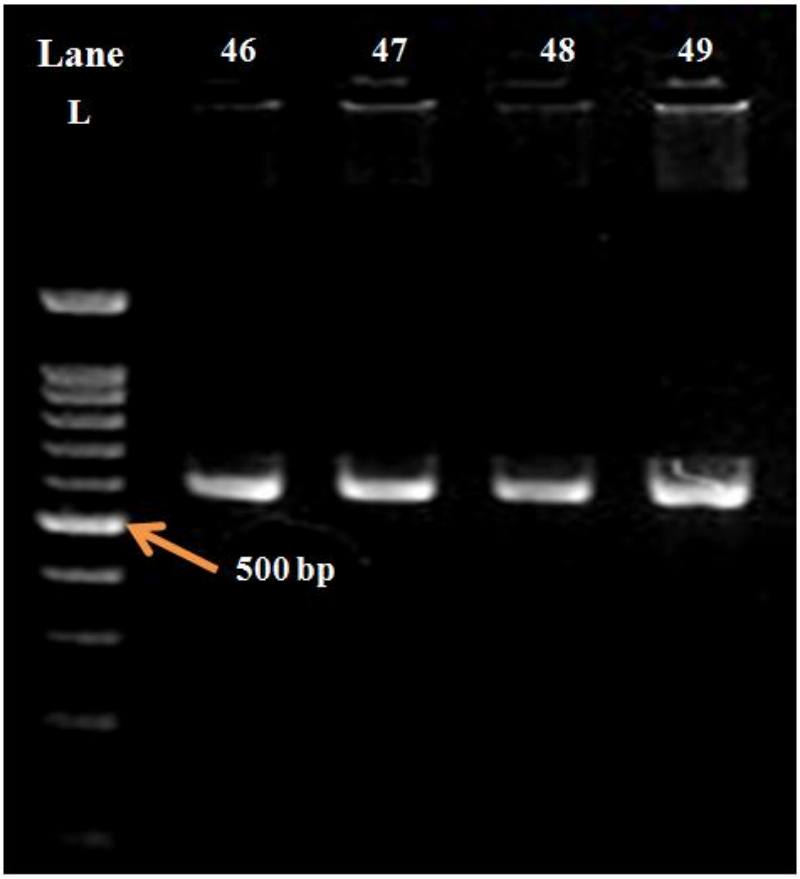


Plate 4.34b. Electrophoretic separation of PCR amplification products of *T. palmi* with ITS2 primer (568 bp)

Lane L = 100bp Ladder; Lane 46 = Ongole; Lane 47, 48 = Chinaganajam; Lane 49 = Naguluppalapadu

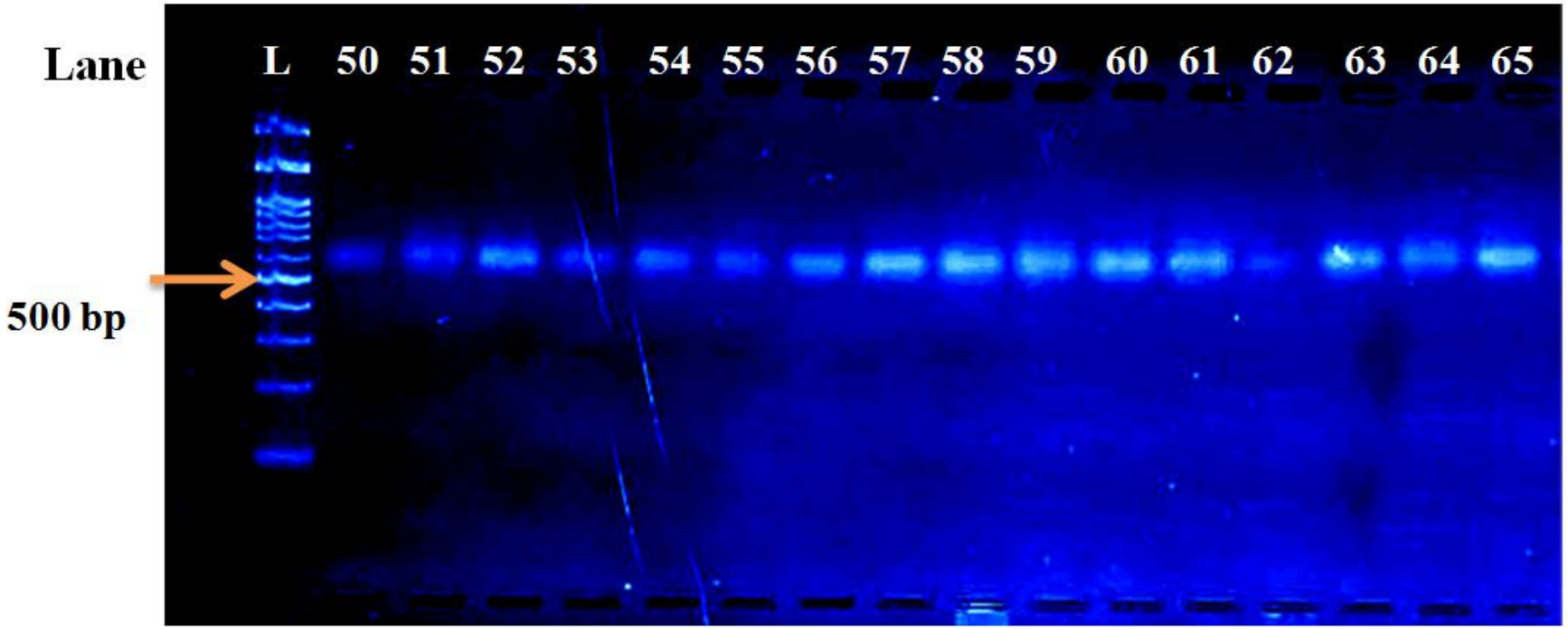


Plate 4.34c. Electrophoretic separation of PCR amplification products of *T. palmi* with ITS2 primer (568 bp)

Lane L = Ladder; Lane 50 = Naguluppalapadu; Lane 51, 52 = Gospadu; Lane 53, 54 = Panyam; Lane 55, 56 = Bandiatmakuru; Lane 57, 58 = Banaganapalle; Lane 59, 60 = Gadivemula; Lane 61, 62 = Gudipala, Lane 63, 64 = Tavanampalle; Lane 65 = Palasamudram

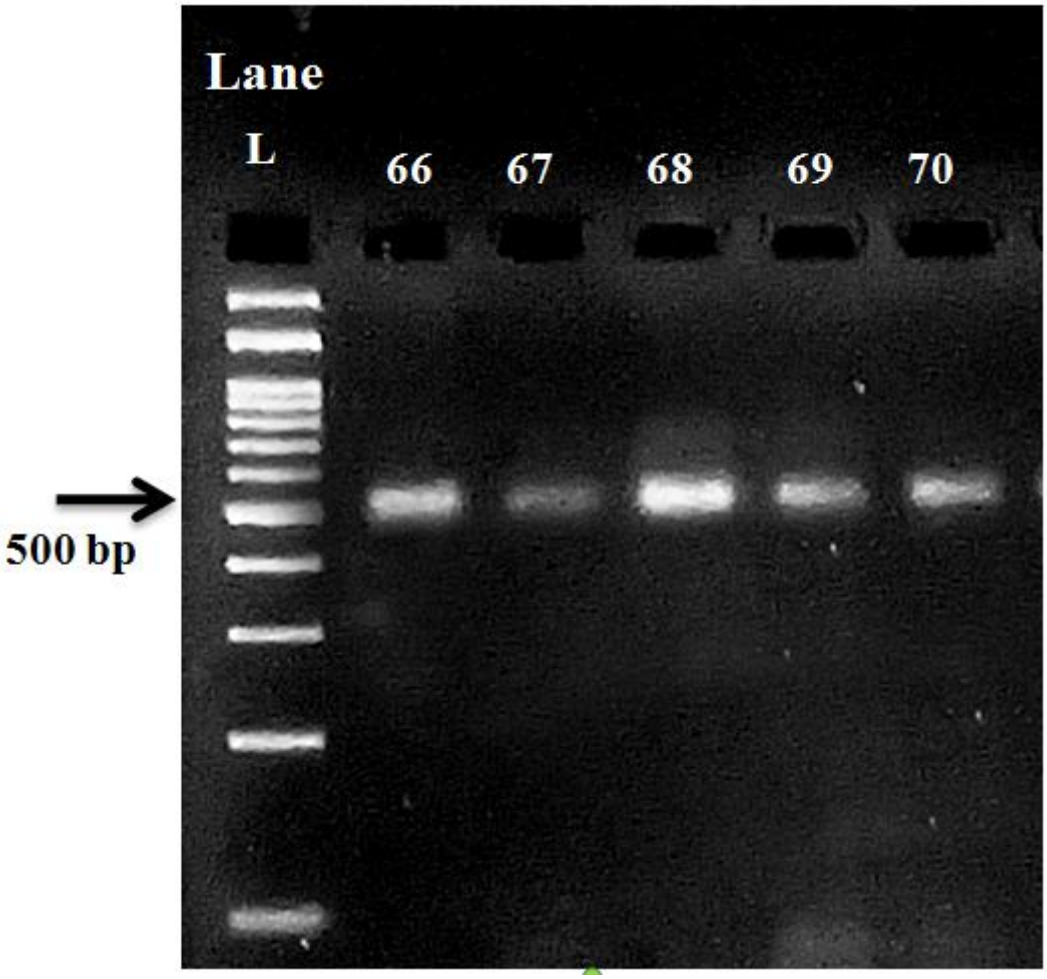


Plate: 4.34d. Electrophoretic separation of PCR amplification products of *T. palmi* with ITS2 primer (568 bp)

Lane L = Ladder; Lane 66 = Palasamudram; Lane 67, 68 = Gangadharnellore; Lane 69, 70 = Airala

Yeh *et al.* (2014) in another study identified 10 thrips species using ITS2 region and amplified a fragment length ranging from 450 to 680 bp. The ITS2 sequences of all the thrips species collected from different islands of Taiwan during 2004-2009, except *Thrips imaginis* (Bagnall) have been deposited in GenBank (AB775358 - AB775452).

4.1.3.2 Identification and confirmation of *M. usitatus* using mtCOI marker: The mitochondrial COI sequence was validated to identify and classify thrips species occurring in a crop system where multiple species coexist. Partial cytochrome oxidase I (COI) sequences were also used to understand the phylogenetic relationship among thrips populations, and assess their usefulness to identify and classify unknown thrips species collected from different crops. In the present study, amplification was observed at 655 bp for 70 individual specimens using mtCOI marker (Plate 4.35a, b, c; 4.36 a, b, c). These results are in accordance with Chakraborthy *et al*. (2019) who studied 43 *Scirtothrips* spp specimens collected from different geographic locations across India and identified using scanning electron microscope utilizing the morphometric keys. The same specimens were further confirmed using mtCOI markers based on species specific amplification (648bp). This study contributed six novel barcode sequences of three *Scirtothrips* species from India.

Another finding by Rabeena *et al*. (2020) on the utilization of mtCOI based universal primer in identification of *F. schultzei* collected from tomato growing hot spot regions of Tamil Nadu and Karnataka. Specific amplicon size of ~638bp was detected and the samples were sequenced and deposited in NCBI (MK113913**,** MK113914 and MK113915). Further Singha *et al*. (2019) identified 11 specimens of *Frankliniella occidentalis* (Pergande) collected from Karnataka, India and studied using mtCOI universal primer and generated four sequences specific to the collected specimens and submitted in NCBI (TH-2266: MH470339, TH-2275: MH470340, TH-2285: MH470341, and TH-2318: MH470342). The findings from the present study supported with reports of Suganthy *et al*. (2016) who identified western flower thrips, *F. occidentalis* in South India on chrysanthemum using mtCOI primer that amplified 358 bp fragment in 24 individual thrips from 12 different locations of Tamil Nadu. Kumar *et al*. (2014) studied larvae and adults of *S. dorsalis, F. occidentalis, F. schultzei* and *T. palmi* using high resolution SEM for morphological characterization.

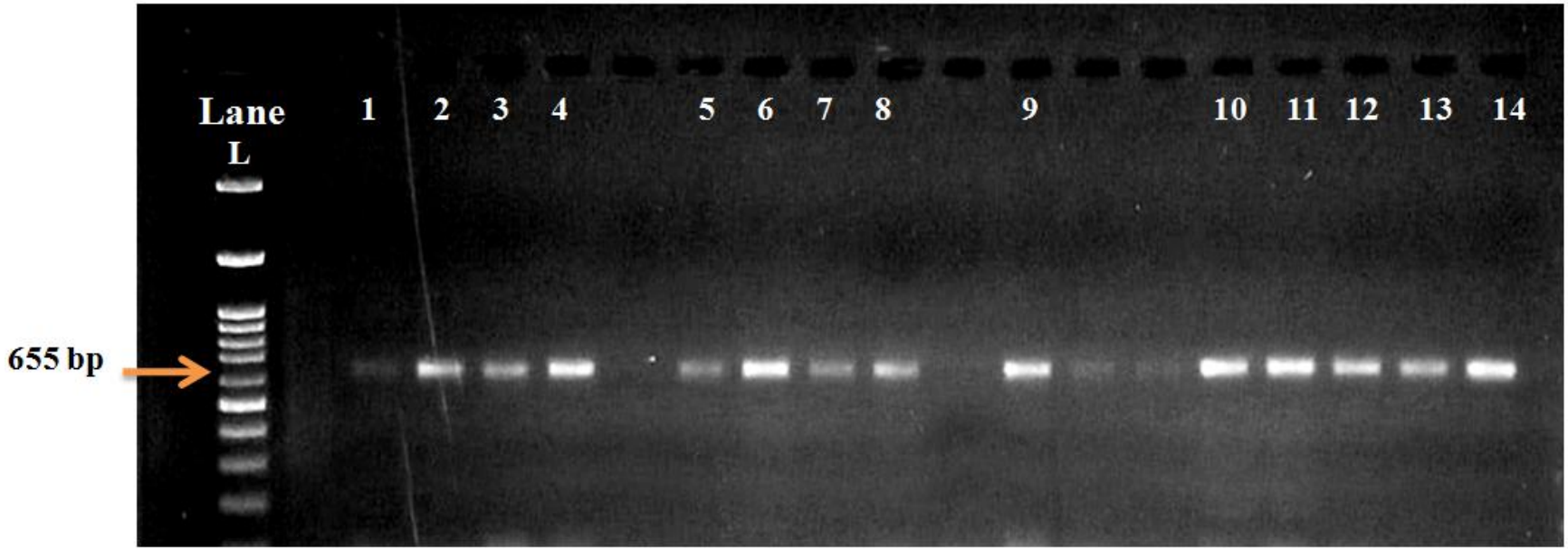


Plate 4.35a. Electrophoretic separation of PCR amplification products of *M. usitatus* with mtCOI primer (655 bp)

Lane L = 100bp Ladder; Lane 1, 2 = Etcherla; Lane 3, 4 = Ponduru; Lane 5, 6 = Sigadam; Lane 7, 8 = R. Amudalavalasa; Lane 9, 10 = Rajam; Lane 11, 12 = Gantayada; Lane 13, 14 = Bondapalli

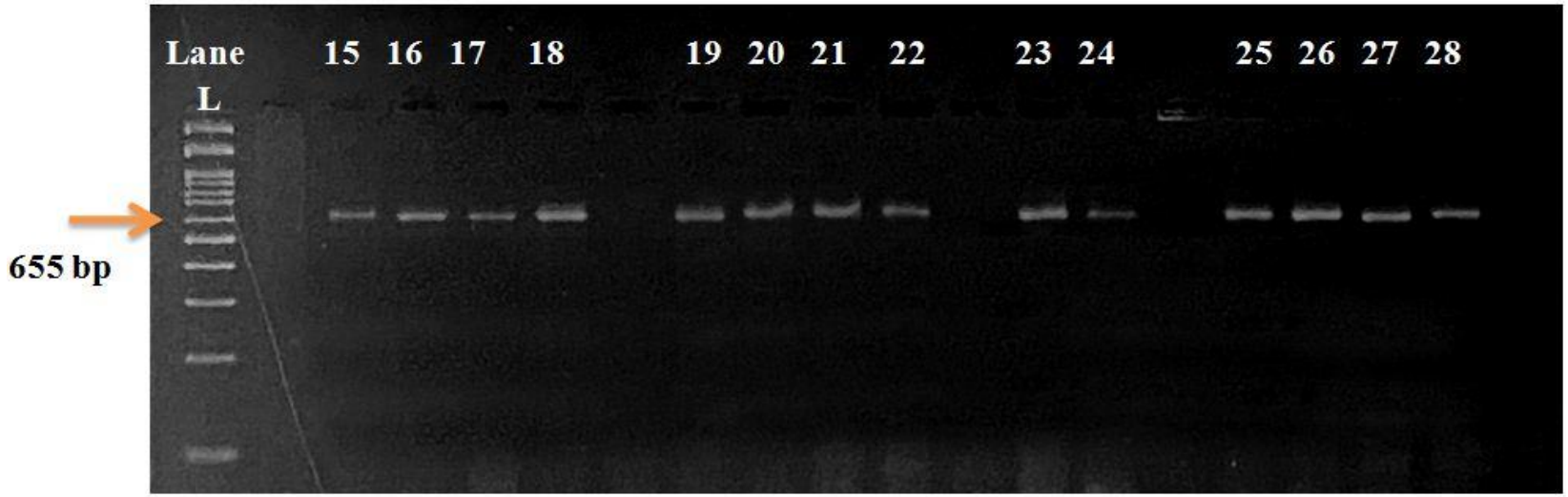


Plate 4.35b. Electrophoretic separation of PCR amplification products of *M. usitatus* with mtCOI primer (655 bp)

Lane L = 100bp Ladder; Lane 15, 16 = Mentada; Lane 17, 18 = Dattirajeru; Lane 19, 20 = Gajapatinagaram; Lane 21, 22 = Avanigadda, Lane 23, 24 = Challaplli; Lane 25, 26 = Mopidevi; Lane 27, 28 = Pamarru

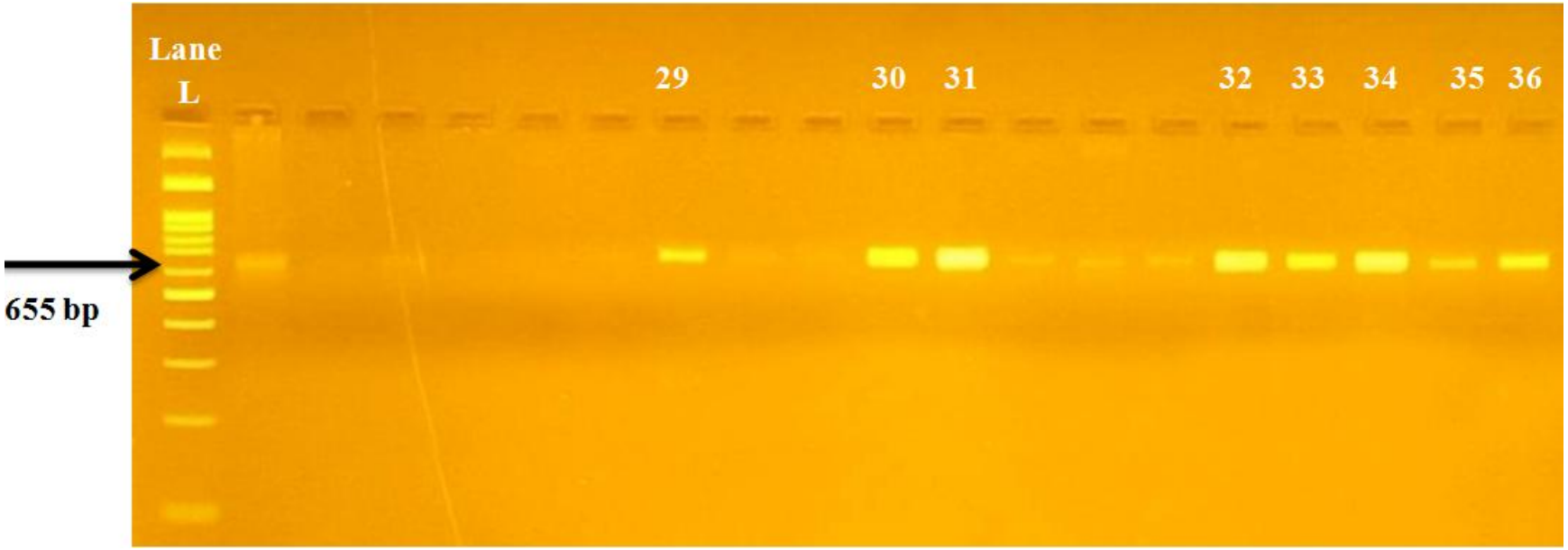


Plate 4.35c. Electrophoretic separation of PCR amplification products of *M. usitatus* with mtCOI primer (655 bp)

Lane L = 100bp Ladder; Lane 29, 30 = Movva; Lane 31, 32 = P.V. Palem; Lane 33, 34 = Amarthalur; Lane 35, 36 = Ponnuru

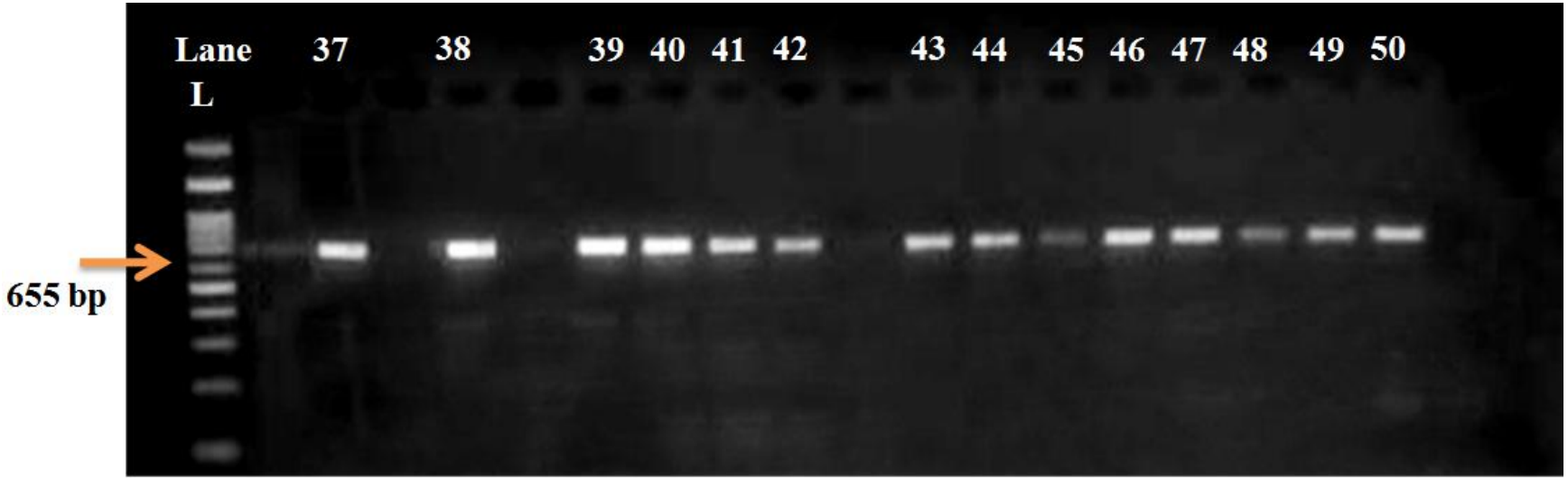


Plate 4.36a. Electrophoretic separation of PCR amplification products of *M. usitatus* with mtCOI primer (655 bp)

Lane L = 100bp Ladder; Lane 37, 38 = Chebrolu; Lane 39, 40 = Tadikonda; Lane 41, 42 = Vetapalem; Lane 43, 44 = Chirala; Lane 45, 46 = Ongole; Lane 47, 48 = Chinaganajam; Lane 49, 50= Naguluppalapadu

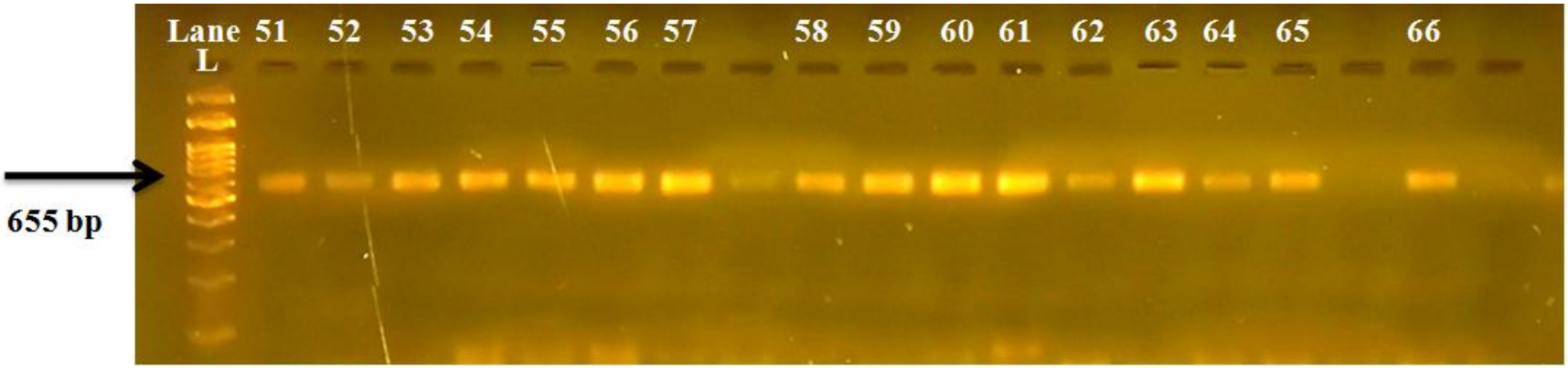


Plate 4.36b. Electrophoretic separation of PCR amplification products of *M. usitatus* with mtCOI primer (655 bp)

Lane L = 100bp Ladder; Lane 51, 52 = Gospadu; Lane 53, 54 = Panyam; Lane 55, 56 = Bandiatmakuru; Lane 57, 58 = Banaganapalle; Lane 59, 60 = Gadivemula; Lane 61, 62 = Gudipala; Lane 63, 64 = Tavana mpalle; Lane 65, 66 = Palasamudram

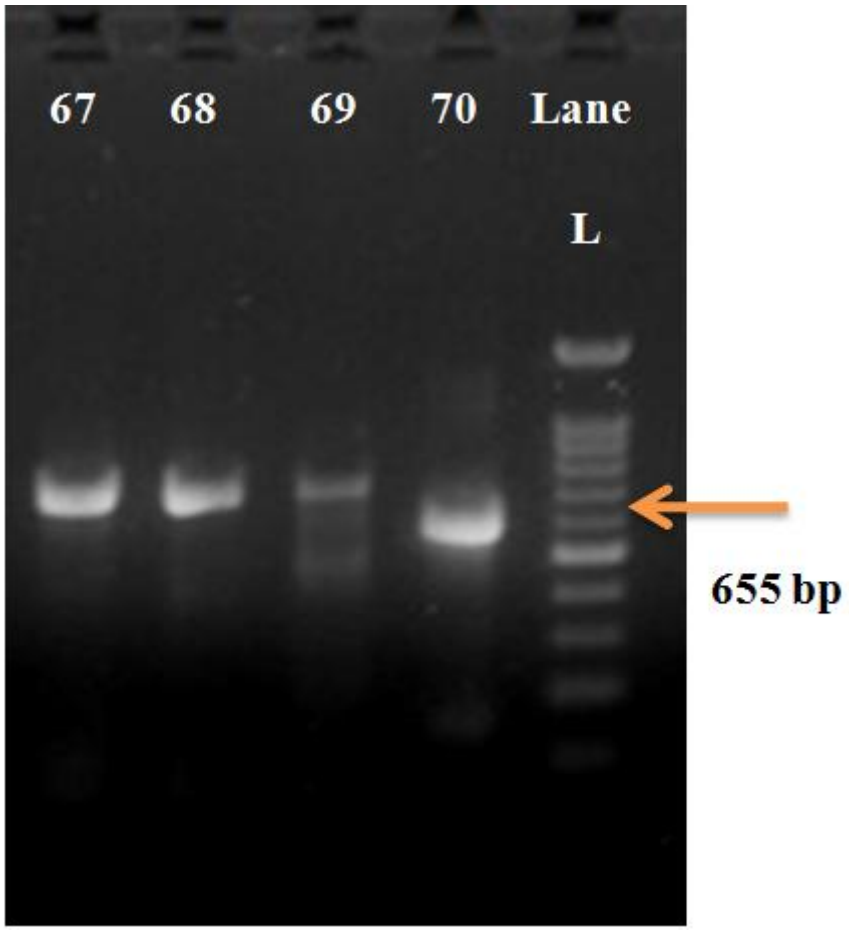


Plate 4.36c. Electrophoretic separation of PCR amplification products of *M. usitatus* with mtCOI primer (655 bp)

Lane L= 100bp Ladder; Lane 67, 68 = Gangadharnellore; Lane 69, 70 = Airala

Molecular characterization of individual larva and adults of all the four species using mtCOI and ITS rDNA were in agreement with the taxonomic identification conducted using SEM.

Similar study was conducted by Kadirvel *et al.* (2013) reported this method is simple and accurate means of identifying four major thrips species (*T. palmi, T. tabaci, S. dorsalis and F. occidentalis*). A total of 29 COI variants were obtained, while examining the sequence polymorphisms in COI of 182 insects analyzed which were collected from six countries on different crops *viz*., tomato, chilli, onion, cabbage, cucumber, watermelon, Ethiopian mustard, French bean, and peanut.

4.1.3.3 Identification and confirmation of *S. dorsalis* using mtCOIII marker*:* Mitochondrial Cytochrome Oxidase III and ITS regions provided additional advantages at species-level identification due to larger interspecific distance than COI (Glover *et al*. 2010., Yeh *et al*. 2014). Low number of *S. dorsalis* species was identified in surveyed locations of Andhra Pradesh on blackgram crop during *rabi* 2019-20. In the present study a total of 17 individual specimens from different geographic locations were subjected to molecular characterization. *S. dorsalis* specimens were amplified with mtCOIII primer set and exhibited a clear amplification size of 713 bp (Plate 4.37 a, b).

These results are in accordance with Sumit *et al.* (2020), who reported that the duplex PCR assay using combination of markers ITS2 and mtCOIII specific to *T. palmi* and *S. dorsalis*, respectively amplified DNA fragments of 568 bp and 713 bp size and discriminated between *T. palmi* and *S. dorsalis*. It was also reported that triplex PCR using a cocktail of primer pairs ITS2, mtCOIII, & ITS2 amplified 568 bp, 713 bp, and 388 bp products of *T. palmi*, *S. dorsalis*, and *T. tabaci*, respectively. The multiplex PCR identified all the four thrips vectors *viz*. *T. palmi*, *S. dorsalis*, *T. tabaci*, and *F. schultzei* using combination of ITS2, mtCOIII, ITS2, and ITS2 markers yielded products of 568 bp, 713 bp, 388 bp, and 200 bp.

The findings of molecular characterization of the present study clearly confirmed the existence of three different species *i.e. T. palmi, M. usitatus, S. dorsalis* in all the surveyed locations of Andhra Pradesh.

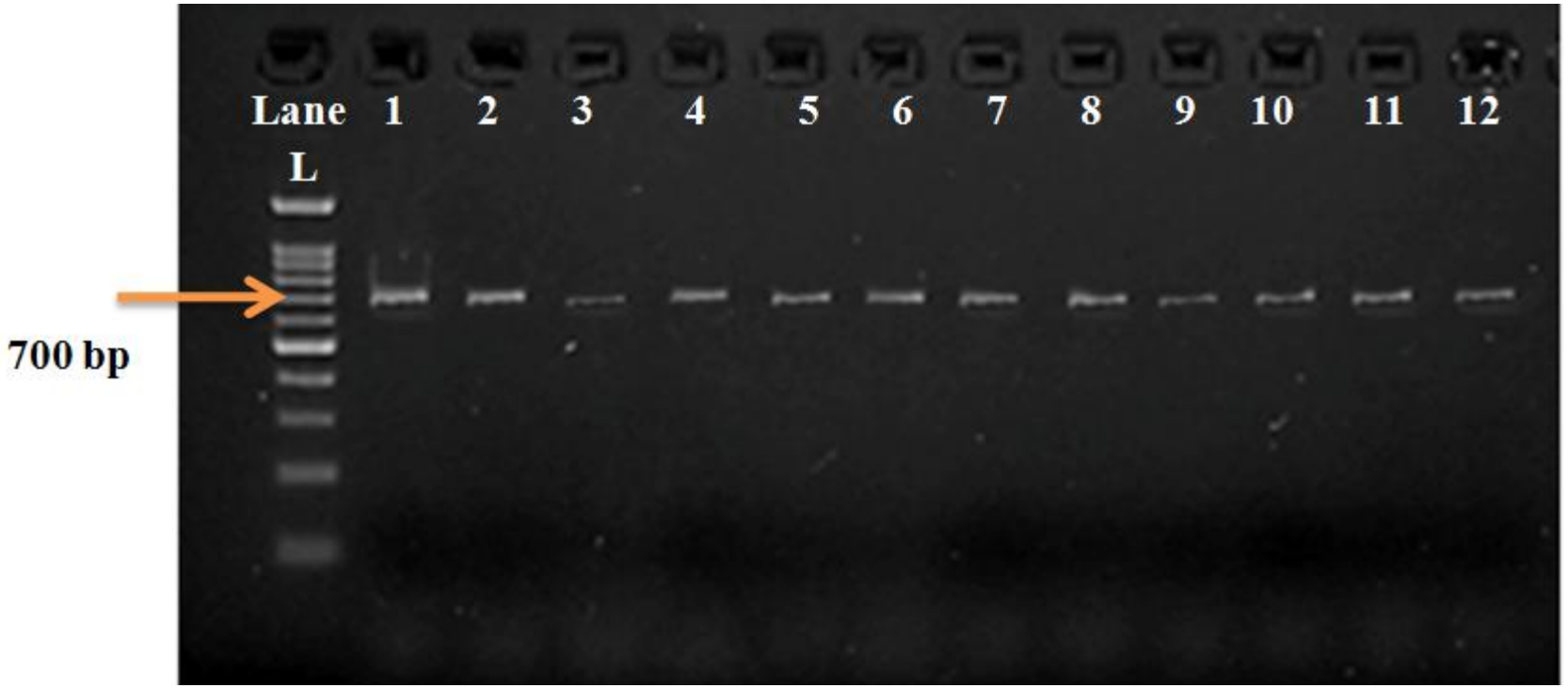


Plate 4.37a. Electrophoretic separation of PCR amplification products of *S. dorsalis with* mtCOIII primer (713 bp)

Lane L = 100bp Ladder; Lane 1 = Ertcherla; Lane 2 = Sigadam; Lane 3, 4 = Mentada; Lane 5 = Avanigadda; Lane 6 = Challapalli; Lane 7 = Mopidevi; Lane 8 = Pamarru; Lane 9 = Movva; Lane 10 = Amarthaluru; Lane 11 = Ponnuru; Lane 12 = Tadikonda

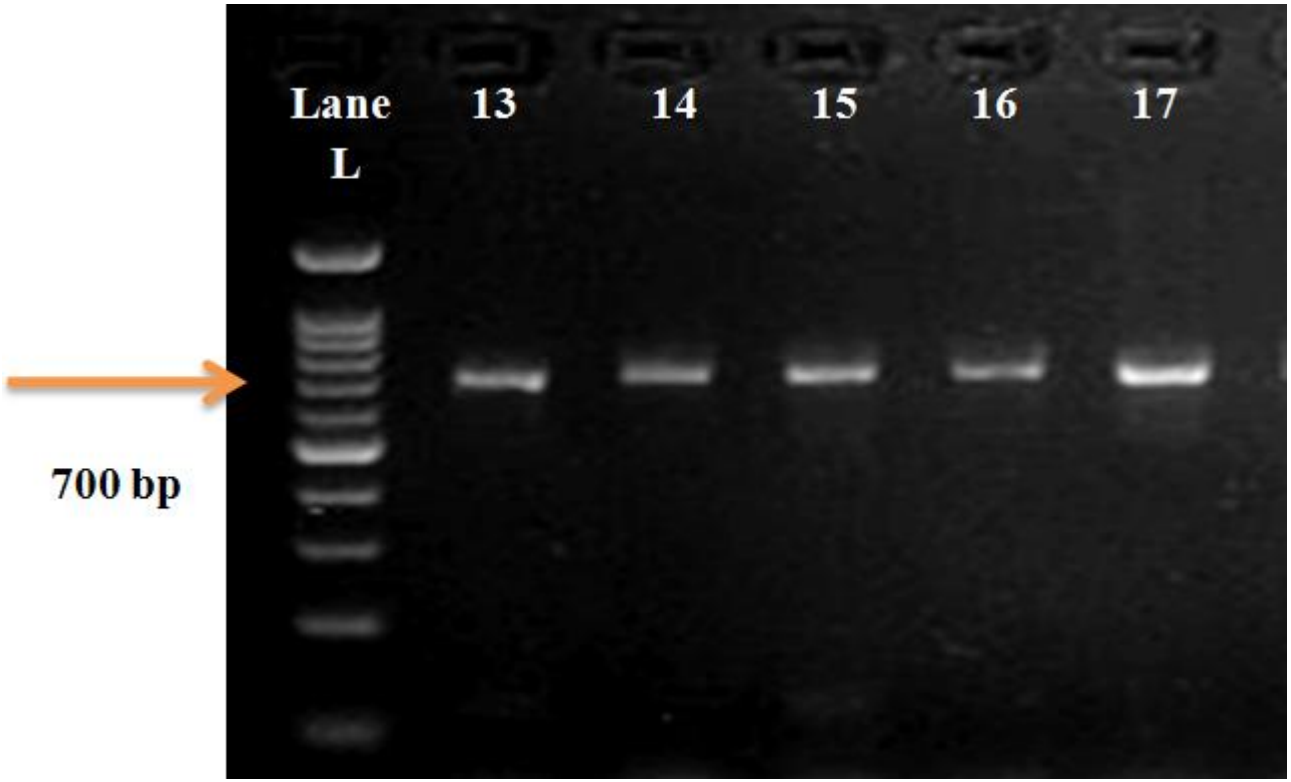


Plate 4.37b. Electrophoretic separation of PCR amplification products of *S. dorsalis* with mtCOIII primer (713 bp)

Lane L = 100bp Ladder; Lane 13 = Chirala; Lane 14 = Ongole; Lane 15 = Banaganapalle; Lane 16 = Palasamudram; Lane 17 = Gangadharnellore

4.1.4 Sequencing and Homology Studies

After the confirmation through gel electrophoresis, PCR products (A total of 21 samples *i.e.* seven samples for each thrips species) were sequenced bidirectionally by using Sanger sequencing method. The sequences samples were subjected to homology studies using BLASTN tool for the identification of homologous sequences across the global database (NCBI).

Thrips palmi sequences of present study have shown similarity of 97 to 100 per cent with previously deposited sequences data sets from different geographic locations throughout the world. NCBI data base sequences *viz*., MN889880, KU884558, FM956428, FM956427, FM956422 from India, AB775442 from China showed 100 % similarity with present *T. palmi* isolates. KF680274, KU884557, KU884556 sequences have shown 99 per cent similarity where as KF680275 (India), KT885219 and KT885218 (U.S.A), AB775439 and AB775435 (China) have shown 98 per cent similarity. MN1942020 (India), KT885216 (U.S.A), AM932178 and AM932146, AM932140, AM932157 (U.K), AB063341 (Japan), AB775437, AB775436 (China), KM877305 and LC416224 (Taiwan) have shown 97 per cent similarity.

Similarly, *M. usitatus* sequences in the present study have also shown 99 to 100 per cent similarity with the available sequences in NCBI. The accessions from Bangladesh (KX233563, KX233562, KX23355, KX233548, KX233532 and KX233552), Pakistan (JF8399061and JF839895) have shown 100 % similarity with the sequences of present study, where as 99 per cent similarity was observed with accessions from India (KF015511), Bangladesh (KX233561, KX233554, KX233550, KX233556, KX233560, KX233559, KX233558, KX233557, KX233553, KX233549, KX233547, KX233546, KX233543, KX233542, KX233551, KX233544, KX233545), Pakistan (HQ991645, HQ991694), China (MF686690), Indonesia (KF144131).

Scirtothrips dorsalis sequences (mt COIII gene) of present study have shown 98 to 99 per cent similarity with the data base sequences belongs to India (Partial CDS - MN602817, MN193061; whole genome sequence KM349827).

The sequences of the present study were aligned using MEGA 11.0 (Molecular Evolutionary Genetic Analysis) with known reference sequences available in NCBI website. The generated DNA sequences of the three species were annotated and submitted into the global database (GenBank) to acquire the unique accession numbers. List of samples, geographic location along with accession numbers obtained were presented in the Table 4.3.

4.1.5 Phylogeny and Haplotype Analysis

4.1.5.1 *Thrips palmi*: Phylogeny tree was constructed using neighbor joining (NJ, ML) method depicted cohesive clustering of the identified seven sequences of *T. palmi* across the different districts of Andhra Pradesh along with the database sequences (23 homologous sequences with similarity of 97 to 99 %) with 1000 bootstrap replicates. The phylogenetic tree represented four distinct clades of the present dataset Clade-1, Clade II, Clade-III, and Clade IV (Figure 4.2 a, b) with a bootstrap value > 80 % highlighting a significant rate of phylogenetic relationships among the species studied. Clade I showed cohesive clustering of present study specimens from seven different geographic locations of Andhra Pradesh (accession number MZ427914 to MZ42720) with other reported *T.palmi* isolates from Indian sub continent. Further, *T. palmi* isolates from foreign countries were also clustered in clade I on different node. Isolates of *T. palmi* from other countries *viz.* China, U.K, U.S.A were very closely clustered in another node under clade I (*T. palmi* group), whereas the clade II comprised of *T. nigropilus* species of U.K, clade III comprised of *T. tabaci* species of U.K and clade IV comprised of *T. flavus* species of U.K. The out group sequences were procured from NCBI website (https://www.ncbi.nlm.nih.gov). Out group is more distantly related group of organisms that serves as a reference group when determining the evolutionary relationships of the ingroup.

Out-group serves as a point of comparison for the in-group and specifically allows for the phylogeny to be rooted. In the present study the phylogenetic tree evidenced the relationship between the samples of the present study and previously reported samples of GenBank sequences. The phylogeny revealed the close relationship among India, china, U.K and U.S.A populations of *T. palmi*. Further, *T. palmi* species has close association with *T. nigropilosus* rather than with *T. tabaci* and *T. flavus* populations.

Table 4.3. List of GenBanK accession numbers allotted to thrips samples identified during this study

S. No.	Organism	Geographic location (Mandal, District)	Sample code	GenBank Acc. number
1	*Thrips palmi*	Etcherla, Srikakulam	LRTM 1	**MZ427914**
2		Mentada, Vizianagaram	LRTM 2	**MZ427915**
3		Avanigadda, Krishna	LRTM 3	**MZ427916**
4		Tadikonda, Guntur	LRTM 4	**MZ427917**
5		Ongole, Prakasam	LRTM 5	**MZ427918**
6		Banaganapalle, Kurnool	LRTM 6	**MZ427919**
7		Palasamudram, Chittoor	LRTM 7	**MZ427920**
8	*Megalurothrips usitatus*	Etcherla, Srikakulam	LRTM 8	**MZ392030**
9		Mentada, Vizianagaram	LRTM 9	**MZ436473**
10		Avanigadda, Krishna	LRTM 10	**MZ436474**
11		Tadikonda, Guntur	LRTM 11	**MZ436475**
12		Ongole, Prakasam	LRTM 12	**MZ478649**
13		Banaganapalle, Kurnool	LRTM 13	**MZ436476**
14		Palasamudram, Chittoor	LRTM 14	**MZ436477**
15	*Scirtothrips dorsalis*	Etcherla, Srikakulam	LRTM 15	**MZ488494**
16		Mentada, Vizianagaram	LRTM 16	**MZ488495**
17		Avanigadda, Krishna	LRTM 17	**MZ488496**
18		Tadikonda, Guntur	LRTM 18	**MZ488497**
19		Ongole, Prakasam	LRTM 19	**MZ488498**
20		Banaganapalle, Kurnool	LRTM 20	**MZ488499**
21		Palasamudram, Chittoor	LRTM 21	**MZ488500**

Source: https://www.ncbi.nlm.nih.gov

Both the species were present in different clades under same cluster. Similar findings of distinct species-wise groups of *T. palmi*, *T. tabaci*, *F. occidentalis*, *S. dorsalis* and an unclassified group were also reported by Kadirivel *et al*. (2013).

Higher intra specific genetic variation was observed in case of *S. dorsalis* and *T. palmi* followed by *T. tabaci* and *F. occidentalis*.

4.1.5.2 *Megalurothrips usitatus*: The estimated Neighbour Joining phylogeny utilizing 30 sequences depicted cohesive clustering of the generated *M. usitatus* sequences (Seven) with the representative database sequences (24) with high bootstrap support and posterior probabilities. From the Figure 4.3 (a, b) it was evident that all the *M. usitatus* sequences of present study (MZ392030, MZ436473 to MZ436477, MZ478649) were closely clustered with other *M. usitatus* generated from Bangladesh under Clade I. Remaining sequences of *M. usitatus* from countries *viz.*, Indonesia, China, few from Bangladesh and one sequence from IIHR, Bangalore, India were also clustered cohesively in another branch of the same node under clade I and formed as *M. usitatus* group. In addition, this phylogenic tree also indicated that *Megalurothrips pecularis* (Bagnall), *Megalurothrips distalis* (Karny) as separate branch in clade II and diverged as separate cluster from clade I. Phylogenic tree analysis clearly indicated that *M. usitatus* is closely related to *M. pecularis* and *M. distalis* and distantly related to *Megalurothrips typicus* (Bagnall). The sequences of *M. typicus* and mtCOI sequences of other species *viz., T. tabaci* and *T. palmi* were also utilized in this analysis from NCBI to study diversity at species level through phylogeny analysis. Clade III formed as *T. tabaci* group including sequences from three different countries *i.e*. China, India and Australia. *T. palmi* group was formed as clade IV from southern India, Pakistan.

Findings of the present study were in line with Zafirah *et al*. (2020) developed a phylogenic tree using the sequences of *M. uistatus* and *M. typicus*, *M. distalis* based on COI gene marker and reported that *M. usitatus* group formed as clade I where as *M. usitatus* Lineage II, *M. distalis*, *M. typicus* were formed as clade II. Typically *M. usitatus* Linaeage II was formed under subclade II and also reported that the inter specific distances between both *M. usitatus* Lineage I and Lineage II ranged from 8.78 to 9.63 per cent, suggesting the presence of cryptic and non-monophyly lineages between two morphoforms of *M. usitatus* in Peninsular Malaysia.

4.1.5.3 *Scirtothrips dorsalis*: Neighbour Joining phylogenetic tree in Figure 4.4 (a, b) segregated two species based on the reciprocal monophyly criteria and not to interpret phylogeny of genus *Scirtothrips*.

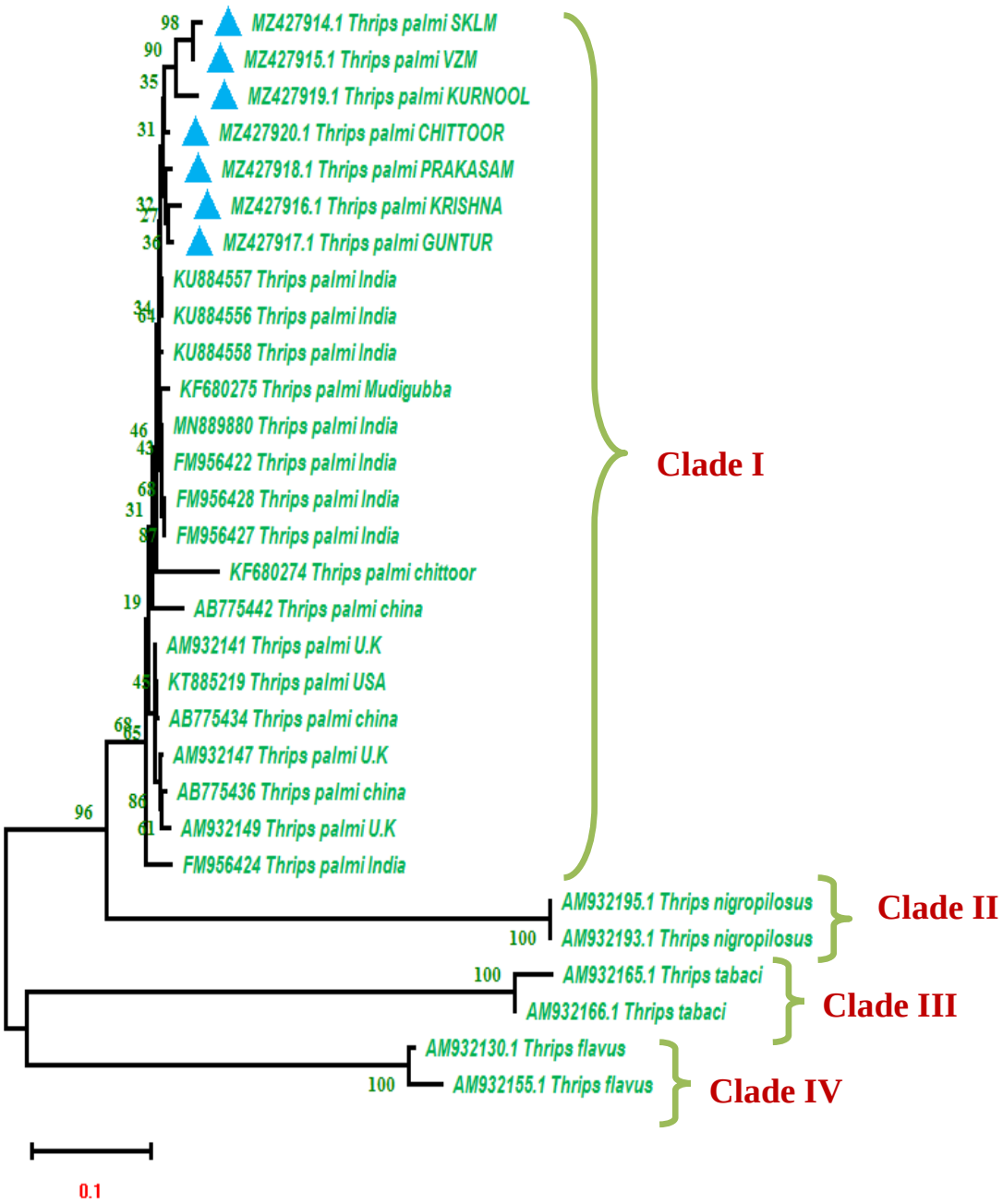


Figure 4.2a. Neibhour Joining phylogenic tree for *T. palmi*, (bootstrap replicates 1000).

The collection localities of the studied and database sequences were incorporated with the voucher IDs and accession numbers in the phylogeny

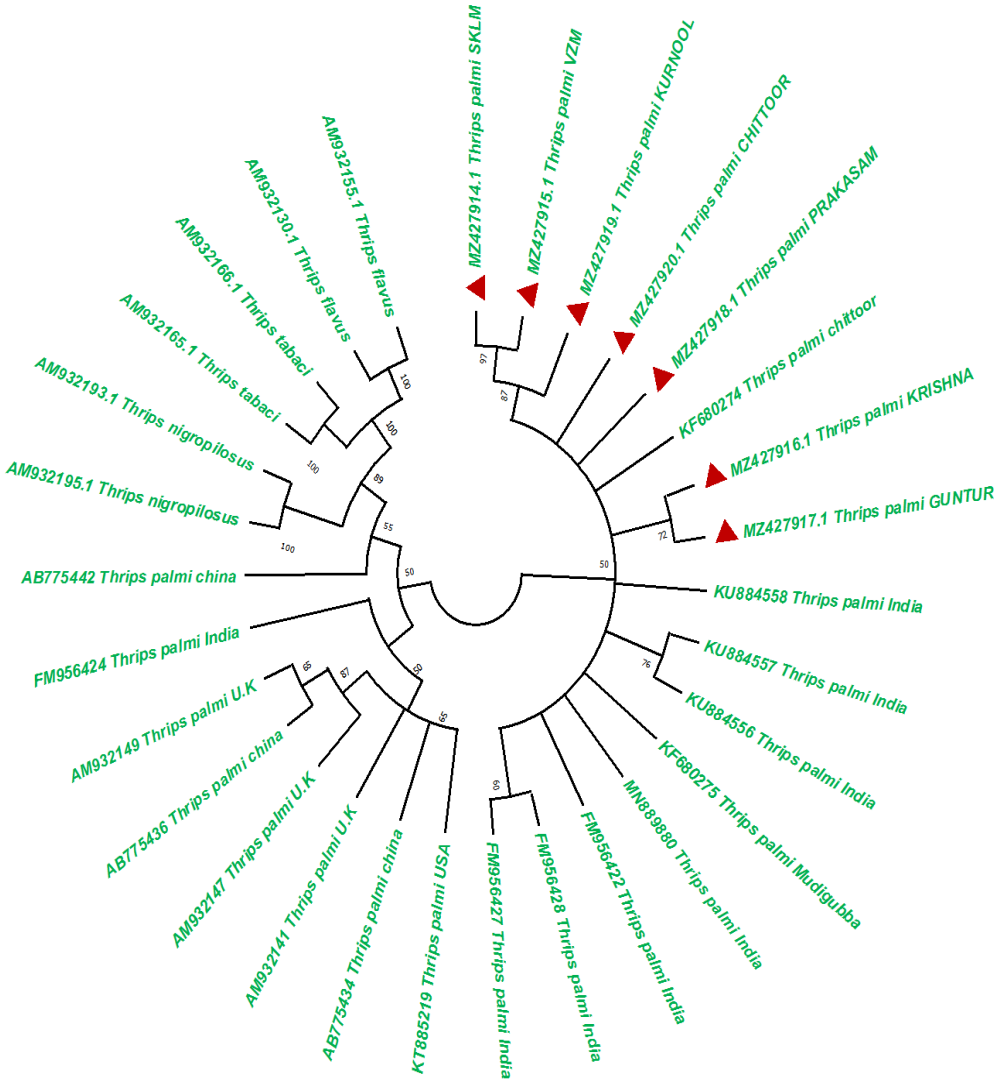


Figure 4.2b. Neibhour Joining phylogenic tree for *T. palmi,* (bootstrap replicates 1000).

The collection localities of the studied and database sequences were incorporated with the voucher IDs and accession numbers in the phylogeny

The analysis of NJ tree yielded four major clades with high bootstrap support. The present study isolates (MZ488494 to MZ488500) along with other previously reported Indian isolate and whole genome sequences from U.S.A were very closely clustered under clade I formed as *S. dorsalis* group. The mtCOIII gene sequences generated from United Kingdom from NCBI database were clustered under clade II formed *Frankliniella occidentalis* group, clade III formed as *Thrips tabaci* group, clade IV formed as *Thrips flavus* group. Very few mtCOIII gene sequences of thrips species were available in the NCBI data base till today and the present study generated mtCOIII sequence variants of *Scirtothrips dorsalis* from southern parts of India for the first time and deposited in NCBI.

The findings of the present study are in accordance with Chakraborthy *et al*. (2019) who studied both morphology and molecular approaches to delimit the selected *Scirtothrips* species from India, where 43 generated barcode sequences, six sequences of three species (*S. hitam, S. mangiferae, and S. malayensis*) are the novel contribution in global database. The Bayesian (BA) phylogeny clearly distinguished all the studied species with reciprocal monophyletic criteria and represented multiple clades in *S. dorsalis* and *S. oligochaetus*. The high Kimura-2-Parameter (K2P) genetic divergences were observed between the multiple clades of *S. dorsalis* (4.5 – 8.8 %) and *S. oligochaetus* (6.4 %), which indicated possible existence of cryptic diversity. Development of morphological keys for six *Scirtothrips* species including *S. hitam* as a new record to India for the identification of those species.

In the present study, among the three thrips species identified, *T. palmi* and *M. usitatus* were the most abundant due to their pest activity on blackgram crop. The presence of *S. dorsalis* was also in considerable range among the surveyed locations. Morphological identification is much cheaper economically than molecular identification as the materials and equipment used in morphological identification require less expenditure (Hillis and Davis, 1987., Wiens, 2004). However both molecular and morphological identification techniques need to be used in a complementary manner to clearly identify the species of the specimens. Therefore, a more general, simple, accurate and large-scale identification method would be helpful to facilitate identification of thrips species occurring in a cropping system where multiple species co-exist, and the population dynamics are influenced by numerous factors (Kadirvel *et al.*, 2013).

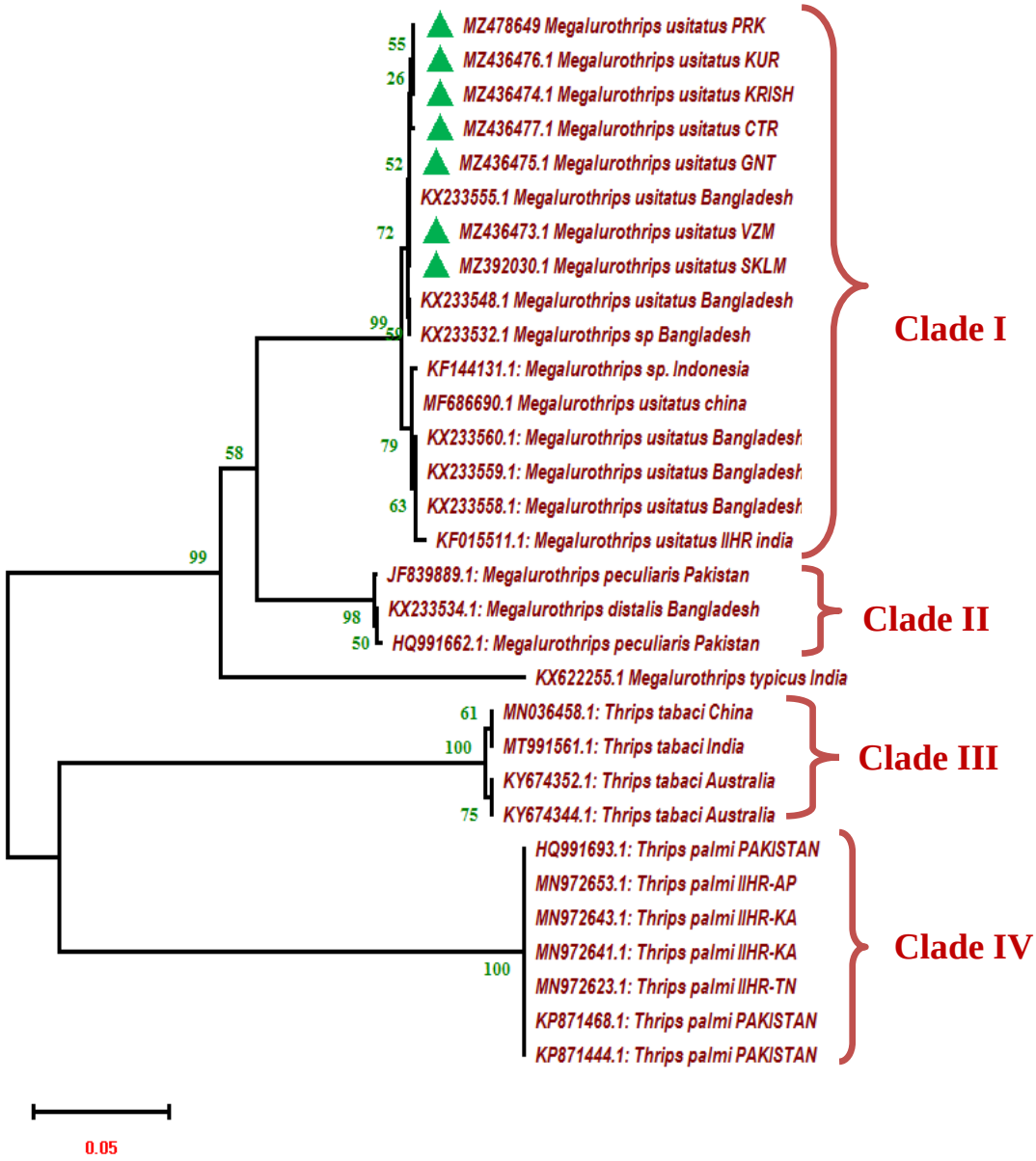


Figure 4.3a Neibhour Joining phylogenic tree for *M. usitatus*, (bootstrap replicates 1000).

The collection localities of the studied and database sequences were incorporated with the voucher IDs and accession numbers in the phylogeny

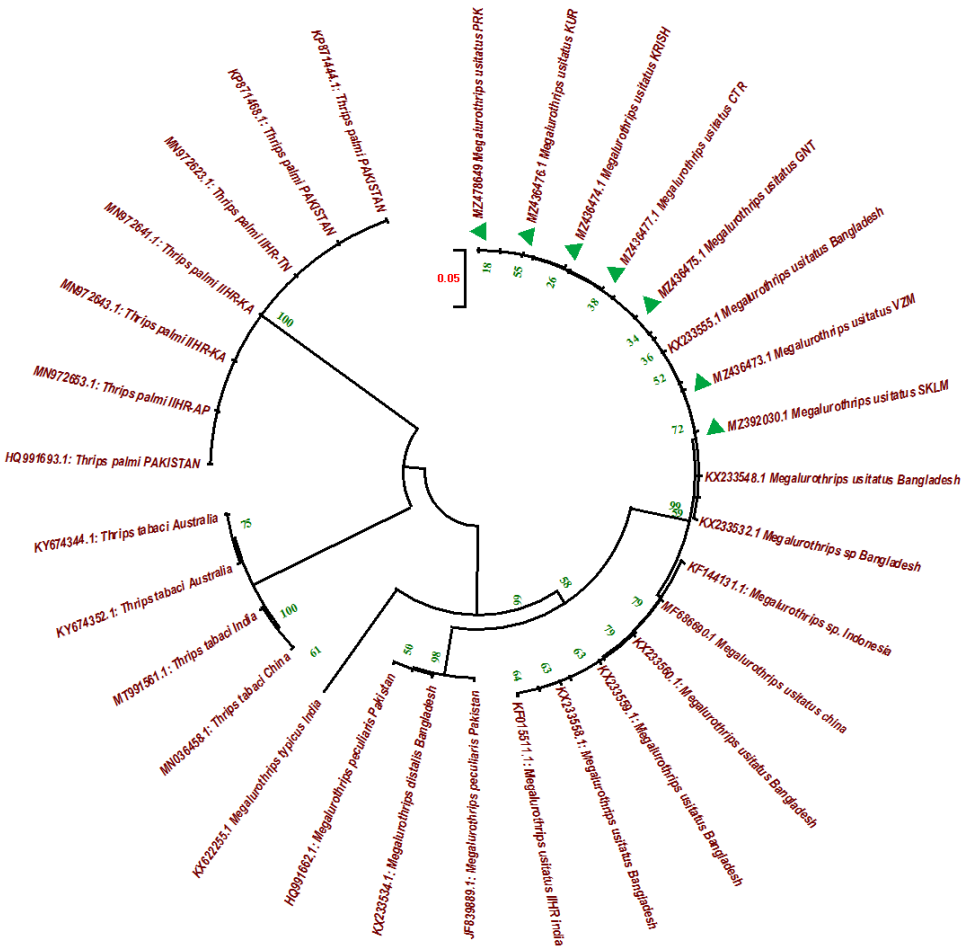


Fig.ure 4.3b. Neibhour Joining phylogenic tree for *M. usitatus*, (bootstrap replicates 1000).

The collection localities of the studied and database sequences were incorporated with the voucher IDs and accession numbers in the phylogeny

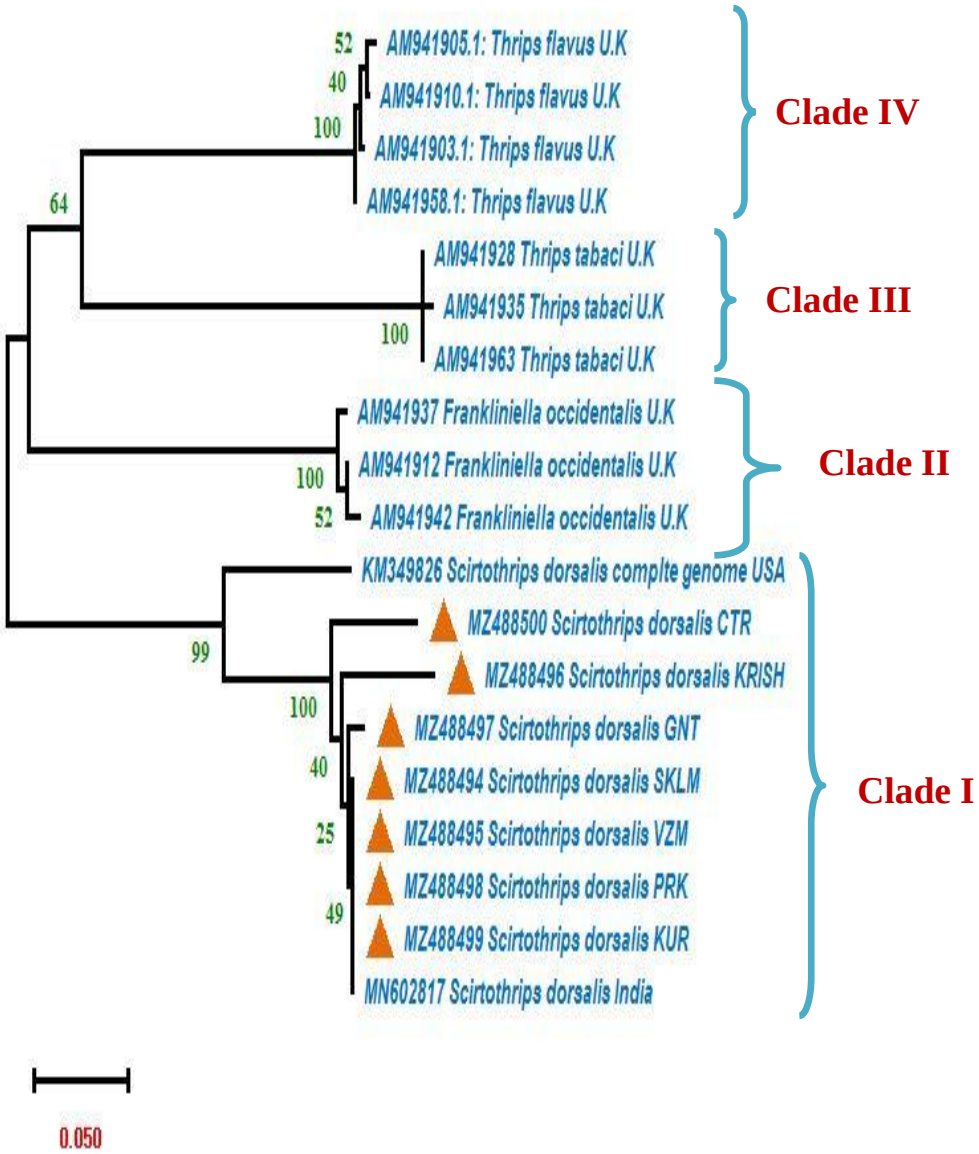


Figure 4.4a. Neibhour Joining phylogenic tree for *S. dorsalis*, (bootstrap replicates 1000).

The collection localities of the studied and database sequences were incorporated with the voucher IDs and accession numbers in the phylogeny

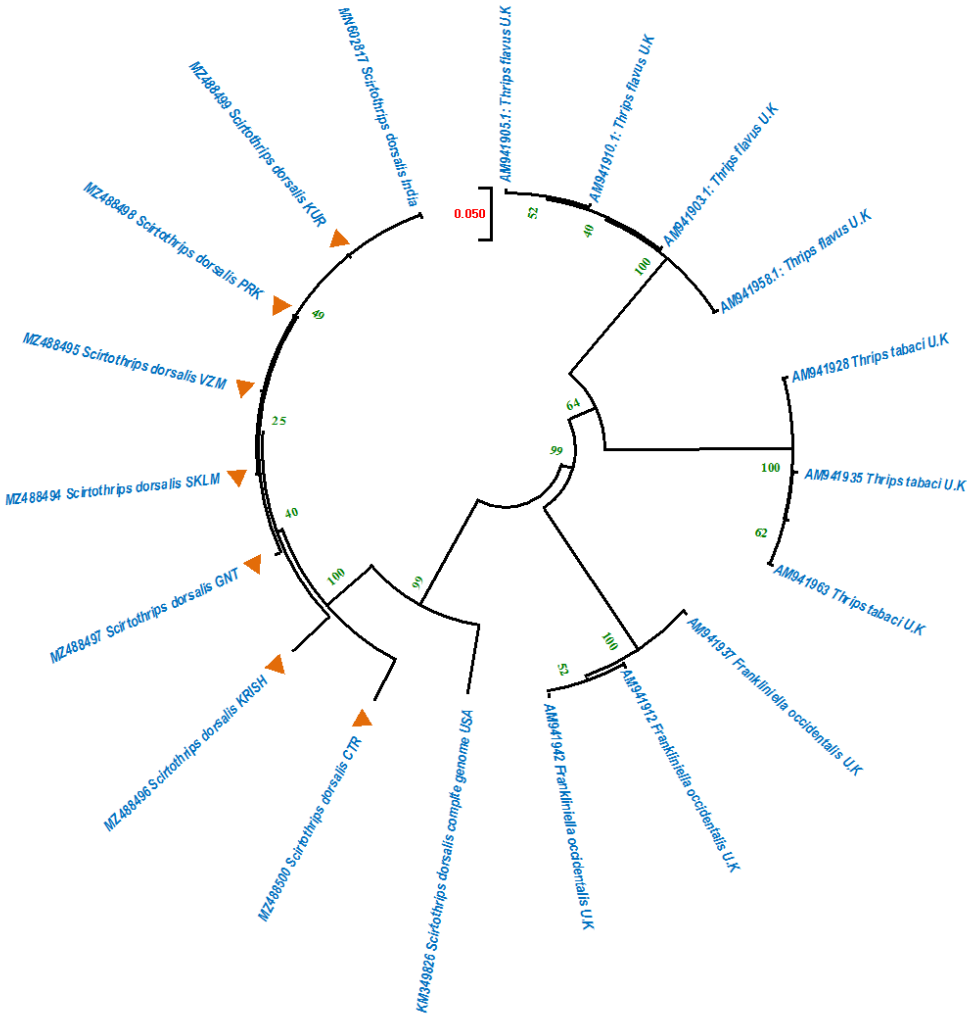


Figure 4.4b. Neibhour Joining phylogenic tree for *S. dorsalis,* (bootstrap replicates 1000).

The collection localities of the studied and database sequences were incorporated with the voucher IDs and accession numbers in the phylogeny

Species identification using morphological features has some significant limitations as the species might have minimal or no phenotypic changes but have high genetic variability. Morphological identification overlooks cryptic features, which are common in many groups and the use of keys requires a high level of expertise as misdiagnosis is common (Hebert *et al.* 2003., Armstrong and Ball, 2005). A constraint in thrips morphological identification is that larval stages cannot be identified with most available keys and exhibit fewer characters of diagnostic value than the adults (Glover *et al.*, 2010).

4.1.6 Genetic Divergence and Haplotype Analysis

***Thrips palmi*:** The seven ITS2 sequences of *T. palmi* of present study from Andhra Pradesh combined with 17 sequences from GenBank from four geographic regions (India, U.K, U.S.A and China countries) revealed 22 haplotypes which were clustered in a network according to genetic diversity existed among them. ITS2 sequence with 552 nucleotide region was selected for the present haplotype analysis and finally 533 nucleotides were used excluding sites with gaps or missing data. Data pertaining to haplotype and genetic diversity of *T. palmi* were presented in Annexure IV. Data presented in the Table 4.4 revealed that the present study sequences were formed into seven haplotypes namely Hap_1 to Hap_7 where as other ITS2 sequences from India were formed into seven haplotypes *i.e.* Hap_8 to Hap_14. Sequences from other countries *viz.* U.S.A and U.K were formed into Hap_15. The sequences from country U.K were formed into three different haplotypes *i.e*. Hap_16, Hap_17, Hap_18 and Hap_22. Sequences from China were formed into three haplotypes *i.e.* Hap_19, Hap_20, Hap_21.

The haplotype network generated in the present study was in accordance with the previously constructed neighbor joining and maximum likelihood tree obtained in the present study. A total of 48 sequences were used for construction of haplotype network and these sequences were grouped into 22 haplotypes. A maximum of 88 segregating sites were observed with Nucleotide diversity **(π)** 0.02836 and standard deviation of nucleotide diversity **(**π) 0.00302. Haplotype diversity was recorded as 0.968 with a standard deviation of 0.009. Estimated mutations among the sequences were96.

Table 4.4. Genetic diversity and Tajima's D evaluated for each detected species of thrips

Species	No. of Sequences	No. of segregating sites	No of parsimony-informative sites	Nucleotide diversity (π)	Total No of mutations	Standard Deviation of π	Total No. of Haplotypes	Haplotype Diversity (Hd)	Standard Deviation of Haplotype diversity	Tajima's D (Calculated using the total number of mutations)
T. palmi	48	88	87	0.02836	96	0.00302	22	0.968	0.009	-1.07506 Not significant, P > 0.10
M. usitatus	32	9	9	0.00509	9	0.00055	6	0.750	0.056	1.31414 Not significant, P > 0.10
S. dorsalis	20	43	43	0.04610	46	0.01303	6	0.7368	0.093	-0.93034 Not significant, P > 0.10

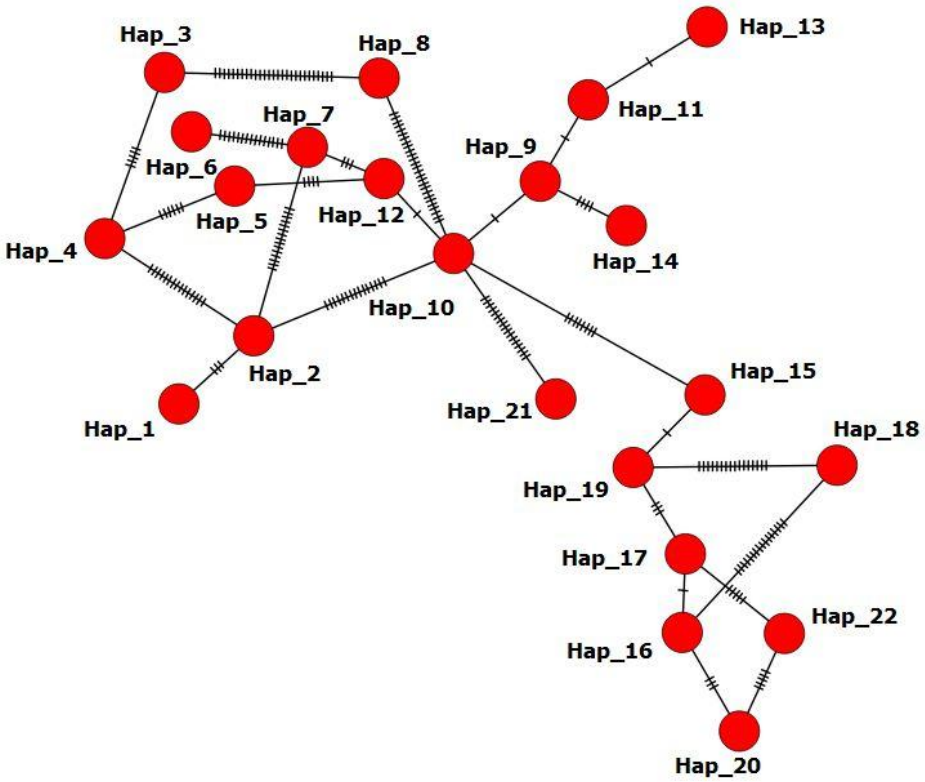


Figure 4.5. Haplotype network analysis of *T. palmi* using ITS2 sequences of present study and presumptive conspecifics from Genbank. Perpendicular tick marks on the lines represent the number of nucleotide substitutions between the linked haplotypes.

Nucleotide diversity (pi) values range between 1 (very diverse) and 0 (not diverse) and hence present study showed pie value 0.00302 which revealed the existence of low genetic polymorphism among the ITS2 sequences of *T. palmi*. (Annexure IV). Tajimas D statistic was also estimated *i.e.* -1.07506 (Not significant, P > 0.10; values greater than +2 or less than -2 are likely to be significant). A negative Tajima's D signifies an excess of low frequency polymorphisms relative to expectation, indicating population size expansion where as a positive Tajima's D signifies low levels of low and high frequency polymorphisms, indicating a decrease in population size and/or balancing selection. However such type of interpretation could not be made as D-value is statistically not significant.

***Megalurothrips usitatus*:** mtCOI sequences of present study *i.e.*, seven sequences of *M. usitatus* from Andhra Pradesh combined with nine sequences procured from GenBank belongs to three geographic regions (India, Indonesia, Bangladesh and China) revealed six haplotypes which were clustered in a network according to genetic diversity existed among them. mtCOI sequence with 657 nucleotide region

was selected for the present haplotype analysis and finally 628 nuclotides were used excluding sites with gaps or missing data. Data pertaining to haplotype and genetic diversity of *M. usitatus* revealed that Srikakulam, Vizianagaram, Guntur, Chittoor, of present study and sequences from Bangladesh were formed into Hap_1 where as other mtCOI sequences from Krishna, Prakasam, and Kurnool of present study were formed into Hap_2. Six sequences from Bangladesh were formed into Hap_3. Other sequences from Indonesia was formed into Hap_4.

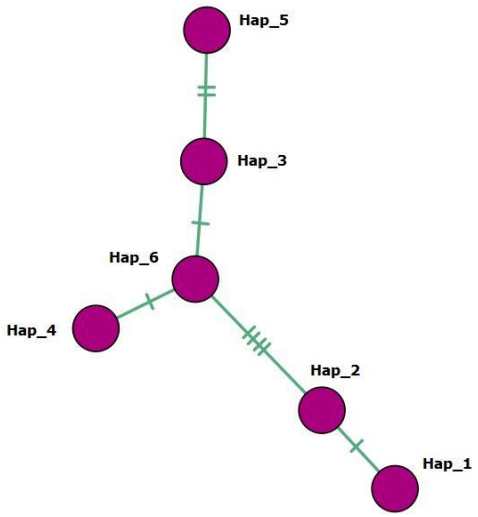


Figure 4.6. Haplotype network analysis of *M. usitatus* using mtCOI sequences of present study and presumptive conspecifics from Genbank. Perpendicular tick marks on the lines represent the number of nucleotide substitutions between the linked haplotypes

Sequences from India and China were formed into Hap_5 and Hap_6, respectively. This haplotype network was in support with our previously constructed neighbor joining and maximum likelihood trees of present study. A total of 32 sequences were used for haplotype network and these sequences were grouped into six haplotypes. A maximum of nine segregating sites were observed with nucleotide diversity **(π)** 0.00509 and standard deviation of nucleotide diversity **(**π) *i.e.*, 0.00055. Haplotype diversity was recorded as 0.750 with a standard deviation of 0.056. Estimated mutations among the sequences were nine. Tajimas D statistic was 1.31414 (Not significant, P > 0.10) which revealed the existence of low genetic polymorphism among the COI sequences of *M .usitatus* (Annexure IV).

These results were supported by Tyagi *et al*. (2017) who analysed 85 *T. palmi* mtCOI sequences from India, resulted in eight haplotypes (H9-H10, H126-H131) forming three distinct clades in both the NJ and BA tree. These three clades were also

represented by three MOTUs (*T. palmi* Ia1, *T. plami* IIa1, and *T. palmi* Ib2) in ABGD, GMYC, and bPTP analysis.

***Scirtothrips dorsalis*:** Mitochondrial Cytochrome Oxidase III sequences of present study *i.e.,* seven sequences of *S. dorsalis* from Andhra Pradesh combined with three sequences procured from GenBank belongs to two geographic regions (India, U.S.A countries) revealed six haplotypes which were clustered in a network according to genetic diversity existed among them. The mtCOIII sequence with 216 nucleotide region was selected for the present haplotype analysis excluding sites with gaps or missing data. Data pertaining to haplotype and genetic diversity of *S. dorsalis* revealed that Srikakulam, Vizianagaram, Prakasam, Kurnool of present study and New Delhi from NCBI were formed into Hap_1 where as other mtCOIII sequences from Krishna, Guntur, and Chittoor of present study were formed into Hap_2, Hap_3, and Hap_4, respectively. Two sequences from U.S.A were formed into Hap_5 and Hap_6, respectively. This haplotype network was in support with our previously constructed neighbor joining and maximum likelihood trees of the present study. A total of 20 sequences were used for haplotype network and these sequences were grouped into six haplotypes. A maximum of 43 segregating sites were observed with nucleotide diversity **(π)** 0.04610 and standard deviation of nucleotide diversity **(**π) *i.e.* 0.01303. Haplotype diversity was recorded as 0.7368 with a standard deviation of 0.093. Estimated mutations among the sequences were 46. Tajimas D statistic was 0.93034 (Not significant, P > 0.10) which revealed the existence of low genetic polymorphism among the COIII sequences of *S. dorsalis*. (Annexure IV).

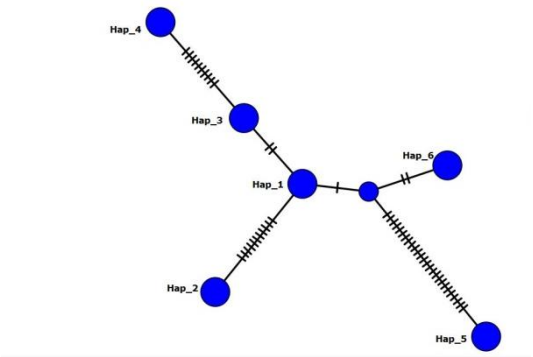


Figure 4.7. Haplotype network analysis of *S. dorsalis* using mtCOIII sequences of present study and presumptive conspecifics from Genbank. Perpendicular tick marks on the lines represent the number of nucleotide substitutions between the linked haplotypes

4.2 TO STUDY THE INCIDENCE OF THRIPS COMPLEX AND OCCURRENCE OF BUD NECROSIS DISEASE IN BLACKGRAM

This experiment was conducted for the three seasons *viz. rabi* 2019-2020 and during *kharif* and *rabi* of 2020-2021 at Agricultural college farm, Bapatla. The incidence of thrips complex and bud necrosis disease were recorded from 7 DAS to crop maturity and correlated with weather parameters.

4.2.1 Incidence of Thrips in Blackgram during *Rabi* 2019-2020

Data presented in the Table 4.5, Figure 4.8 revealed that thrips population was observed initially at 14 DAS during fourth week of January *i.e.* during 3rd SMW (Standard meteorological week) and mean number of thrips per plant was 1.00. The maximum and minimum temperatures during that period were 30.79 and 19.83 ^{o}C, respectively. Mean temperature was 25.31^{o}C while the average morning and evening relative humidities were 84.14 and 64.43 per cent, respectively with 74.29 per cent mean relative humidity. No rainfall was recorded and wind speed of 1.71 kmph was recorded.

The peak of thrips population was observed at 63 DAS during third week of March *i.e.* during 10th SMW (Standard meteorological week) with 10.6 mean number of thrips per plant. The maximum and minimum temperatures during that period were 32.33 and 21.96^{o}C, respectively. Mean temperature was 27.14^{o}C while the average morning and evening relative humidities were 81.86 and 66.29 per cent, respectively with 74.07 per cent mean relative humidity. No rainfall was recorded and wind speed of 4.86 kmph was recorded. The thrips population remained static up to 70 DAS and then started declining during first week of April *i.e.* during 12th SMW (Standard meteorological week) with 7.8 mean number of thrips per plant.

The maximum and minimum temperatures during that period were 34.76 and 23.21^{o}C, respectively. Mean temperature was 28.99^{o}C while the average morning and evening relative humidities were 83.71 and 52.00 per cent, respectively with mean relative humidity 67.86 per cent and 4.71 kmph wind speed. No rainfall was recorded.

The present findings are in agreement with Radhika *et al*. (2018b) who has reported that the population of sucking pest in *rabi* blackgram during 2017-18 started from 2nd week after sowing with 1.80, 2.00 and 2.40 leafhoppers, whiteflies and thrips

per six leaves, respectively and their population were high during the vegetative stages and reached its peak during the reproductive stage with 6.80, 6.90 and 8.10 leafhoppers, whiteflies and thrips per six leaves, respectively and later declined to 1.20, 2.20 and 2.20 leafhoppers, whiteflies and thrips per six leaves, respectively. Another worker Vinuthan (2018) has reported that during *rabi* season minimum thrips population of 1.8 thrips per plant was noticed in early stages of crop growth and maximum thrips population of 61.04 thrips/plant was noticed. He has also reported that the onion crop transplanted during *rabi* recorded the maximum population of thrips (31.81 thrips/plant) when compared to *kharif* transplanted onion crop.

When correlation coefficient (*r*) values were assessed (Table 4.6) the number of thrips per plant showed a highly significant positive correlation with maximum temperature (0.74), minimum temperature (0.75), as well as with mean temperature (0.819) while the morning relative humidity (-0.43), showed non significant negative correlation with thrips population. Whereas evening relative humidity (0.015) showed positive correlation without any significance. Further, mean relative humidity showed non significant negative correlation with thrips population (-0.206). Rainfall (-0.256) and number of rainy days (-0.156) showed non significant negative correlation.

Wind speed (0.802) showed highly significant and positive correlation with thrips population.

Our results pertaining to maximum and minimum temperature correlation with thrips population were in accordance with Saritha *et al*. (2020) who reported that during r*abi* season, the mean temperature (r = 0.815), maximum temperature (r = 0.708) and minimum temperature (r = 0.797) had positive insignificant correlation with thrips population. Similarly, Aishwarya *et al*. (2019) who has conducted a study in water melon during summer 2019 in Tamil Nadu reported that the temperature (r = 0.2) was positively correlated with the incidence of *T. palmi*.

Vinuthan *et al*. (2018) in their study in *rabi* onion during 2018 found that temperature showed significant positive correlation with thrips population and during second season (*rabi*-summer) also temperature showed significant positive correlation with thrips population. Vijayalakshmi *et al*. (2017) reported that thrips incidence on groundnut in Tamil Nadu showed positive correlation with maximum temperature (0.082), minimum temperature (0.052) and sunshine hours (0.085) in *rabi* 2017.

Table 4.5. Incidence of thrips and bud necrosis disease on blackgram during *rabi* 2019 -2020

Standard meteorological week (SMW)	Date	Crop Stage	Mean No. of thrips per plant	Mean No. of thrips per sqm.	Mean PDI of bud necrosis disease	Max Temp. (^{o}C)	Min Temp. (^{o}C)	Mean Temp. (^{o}C)	Morning RH (%)	Evening RH (%)	Mean RH (%)	Rainfall (in mm)	No. of Rainy Days	Avg. wind speed in (KMPH)
2	17.01.2020	7 DAS	0	0	0	29.36	19.64	24.50	87.14	63.57	75.36	0	0	1.86
3	24.01.2020	14 DAS	1	1.00	0.00	30.79	19.83	25.31	84.14	64.43	74.29	0.00	0.00	1.71
4	31.01.2020	21 DAS	4.2	3.00	0.00	30.60	21.41	26.01	81.00	61.86	71.43	0.00	0.00	2.43
5	07.02.2020	28 DAS	1	5.30	0.00	29.61	21.39	25.50	79.43	62.57	71.00	10.00	0.00	3.00
6	14.02.2020	35 DAS	1	7.10	1.50	31.19	19.74	25.46	81.00	57.86	69.43	0.00	2.00	1.86
7	22.02.2020	42 DAS	0.9	20.30	3.73	30.93	19.93	25.43	82.29	61.86	72.07	0.00	0.00	2.29
8	02.03.2020	49 DAS	0.35	17.70	5.30	31.74	21.55	26.64	85.63	66.50	76.06	0.70	0.00	3.25
9	10.03.2020	56 DAS	5.5	21.60	6.98	31.64	24.04	27.84	83.57	68.29	75.93	0.00	1.00	6.29
10	18.03.2020	63 DAS	10.6	26.10	21.08	32.33	21.96	27.14	81.86	66.29	74.07	0.00	0.00	4.86
11	26.03.2020	70 DAS	10	19.20	25.90	33.31	23.71	28.51	77.71	65.43	71.57	0.00	0.00	5.57
12	03.04.2020	77 DAS	7.8	10.70	25.90	34.76	23.21	28.99	83.71	52.00	67.86	0.00	0.00	4.71

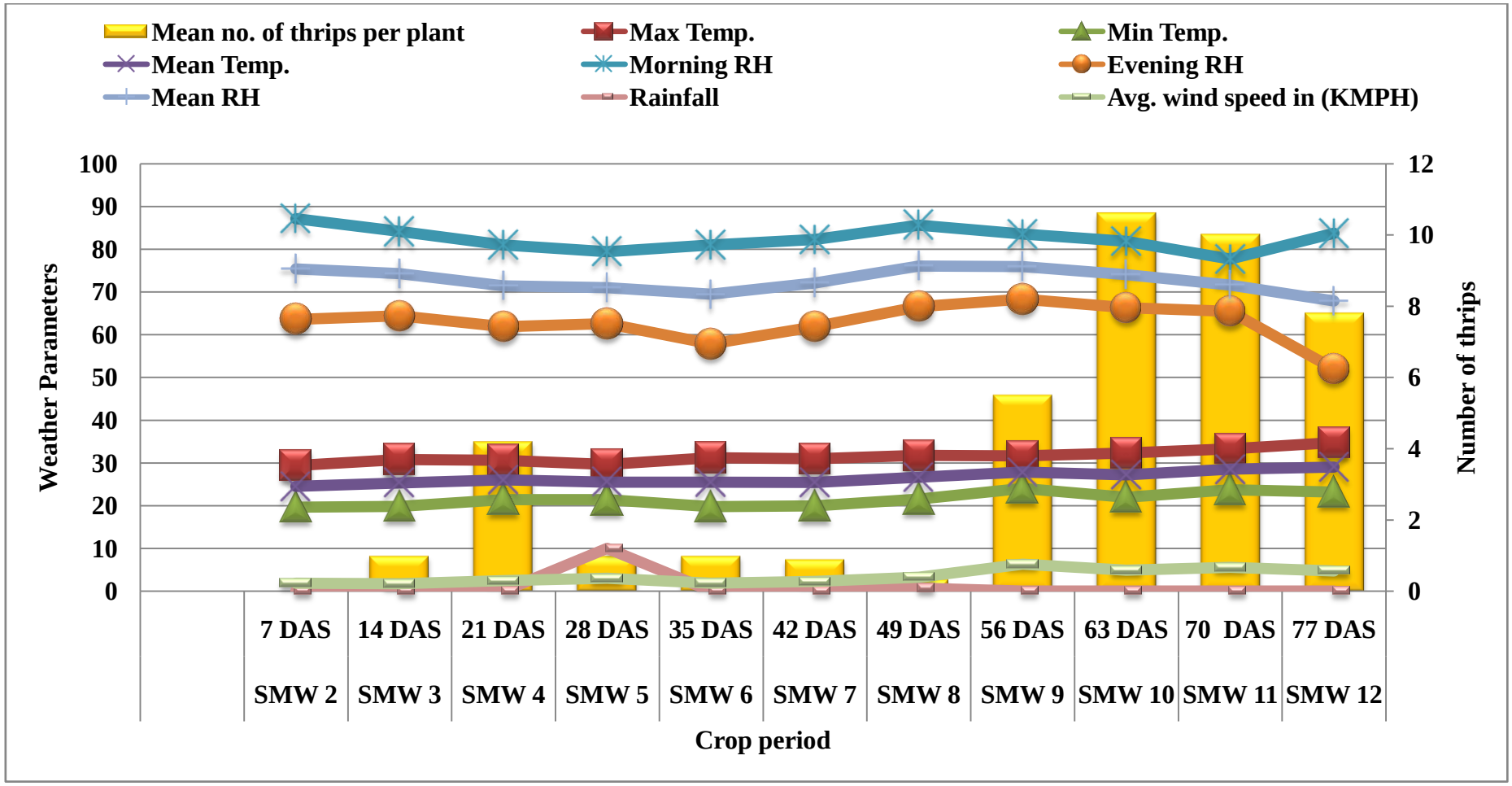


Figure 4.8. Incidence of thrips on blackgram in correlation with weather parameters during *rabi* 2019 -2020

The present findings about relative humidity correlation (Negative and non significant) with thrips population were in agreement with Saritha *et al*. (2020) who reported non-significant negative correlation with mean relative humidity (r = 0.314) and it was evident that the mean temperature favored the pest population in groundnut during r*abi* season. Aishwarya *et al*. (2019) also reported that in water melon during summer 2019 in Tamil Nadu relative humidity (r = -0.5) and rainfall (r = -0.5) were negatively correlated with population of thrips. Vinuthan *et al*. (2018) reported that the thrips population showed non-significant negative correlation with rainfall in *rabi* onion during 2018 and rabi summer 2018. Vijayalakshmi *et al*. (2017) reported that thrips population in groundnut showed negative correlation with morning (-0.322) and evening relative humidity (-0.162) in *rabi* 2017. When correlation coefficient (r) values were assessed (Table 4.7), the number of thrips per plant showed a highly significant positive correlation with per cent disease incidence (0.889) where as mean number of thrips per square meter showed non significant positive correlation with disease incidence (0.57). The data on the incidence of thrips were subjected to multiple linear regression analysis and the following equation was obtained (Table 4.8).

Y = 96.12 - 0.106 (Max Tm) - 1.47 (Min Tm) - 0.642 (Mng RH) + 0.226 (Eve RH) - 0.48 (RF) - 1.94 (RD) - 3.18 (AWS)

The results showed that all the weather variables together could influence the incidence of thrips by 77.0 (R^2= 0.77) per cent. It was also evident from the multiple linear regression equation that among various factors studied the partial regression coefficient (b) for maximum temperature (-0.106) rainfall (-0.48), minimum temperature (-1.47) morning relative humidity (-0.642) showed negative influence on thrips population. Rainfall (-0.48) and average wind speed (-3.18) were also showed negative influence on thrips population. Evening relative humidity (0.226) showed positive influence on thrips population. The above findings were in agreement with Naresh *et al*. (2018) whose regression analysis on foliar damage caused by the thrips indicated that all the weather parameters together resulted in 94 per cent (R^2 = 0.94) and 95 per cent (R^2 = 0.95) in groundnut cultivars Dharani and K-6 sown during November second fortnight. Harish *et al*. (2015) also reported that the coefficient of multiple determinations (R^2) was 36, 54 and 80 per cent during *kharif, rabi* and summer seasons during 2015 on groundnut in Gujarat, respectively.

Table 4.6. Simple correlation between thrips population, bud necrosis disease incidence in blackgram with weather parameters during *rabi* 2019-20

Season		Temperature (°C)			Relative Humidity (%)			Rainfall (in mm)	No. of Rainy Days	AWS
		Max Temp.	Min Temp.	Mean	Morning	Evening	Mean			
Rabi 2019-20	Thrips Per Plant	0.744**	0.756**	0.819**	-0.435	0.015	-0.206	-0.256	-0.156	0.802**
	Thrips per square meter	0.511	0.549	0.578	-0.202	0.366	0.206	-0.226	-0.003	0.712*
	PDI of BND	0.901**	0.703*	0.873**	-0.293	-0.184	-0.303	-0.263	-0.213	0.746**

Table 4.7. Simple correlation between thrips population and bud necrosis disease incidence in blackgram during three seasons

Season		Mean number of thrips per plant	Mean number of thrips per Sqm.
Rabi 2019-20	PDI of BND	0.889**	0.577
Kharif 2020-21	PDI of BND	0.279	0.179
Rabi 2020-21	PDI of BND	0.466	0.508

4.2.2 Incidence of Bud Necrosis Disease in Blackgram during *Rabi* 2019-2020

The per cent bud nerosis disease incidence depicted in the Table 4.5, Figure 4.9. It was evident that bud necrosis disease incidence was observed initially at 35 DAS (Plate 4.39) during second week of February *i.e.* during 6th SMW (Standard meteorological week) with 1.50 mean per cent disease incidence. The maximum and minimum temperatures during that period were 31.19 and 19.74^{o}C, respectively. Mean temperature was 25.46^{o}C. While the average morning and evening relative humidities were 81.00 and 57.86 per cent, respectively with mean relative humidity 69.43 per cent and wind speed of 1.86 kmph. There was no rainfall. The peak bud necrosis disease incidence was observed at 70 DAS during fourth week of March *i.e.* during 11th SMW (Standard meteorological week) with 25.90 mean per cent disease incidence. The maximum and minimum temperatures during that period were 33.31 and 23.71^{o}C, respectively.

Mean temperature was 28.51 ^{o}C while the average morning and evening relative humidities were 77.71 and 65.43 per cent, respectively with mean relative humidity 71.57 per cent. There was no rainfall and wind speed of 5.57 kmph was recorded.

Bud necrosis disease incidence gradually increased every week since the first observation during 6th SMW to the crop maturity with a mean per cent disease incidence of 3.73, 5.30, 6.98, 21.08, 25.90 at 42, 49,56,63,70 DAS, respectively. After 70 DAS there was no further increase in the disease incidence.

Mean number of thrips per square meter was also recorded along with disease incidence. Initial incidence of thrips were observed at 14 DAS and reached peak 63 DAS *i.e.* 26.30 mean number of thrips per square meter. The maximum and minimum temperatures during that period were 32.33 and 21.96^{o}C, respectively. Mean temperature was 27.14^{o}C while the average morning and evening relative humidities were 81.86 and 66.29 per cent, respectively with mean relative humidity 74.07 per cent. There was no rainfall and wind velocity was 4.86 kmph. Mean number of thrips per square meter started declining towards maturity at 77 DAS with 10.70.

Table 4.8. Multiple linear regression between thrips population and bud necrosis disease incidence in blackgram with weather parameters during *rabi* 2019-20

Year and Season	Dependent variable	R^2	Regression equation
Rabi 2019-20	Mean No. of Thrips per Plant	0.77	Y = 96.12 - 0.106 (Max Tm) -1.47 (Min Tm) - 0.642 (Mng RH) + 0.226 (Eve RH) - 0.48 (RF) - 1.94 (RD) - 3.18 (AWS)
	Thrips Per Sqm.	0.77	Y= 36.7431+ 3.29 (Max Tm) - 7.97 (Min Tm) - 0.423 (Mng RH) + 0.755 (Eve RH) + 0.182 (RF) - 0.146 (RD) + 8.8 (AWS)
	PDI of BND	0.95	Y= 65.63 + 4.03 (Max Tm) + 5.63 (Min Tm) + 0.75 (Mng RH) - 0.40 (Eve RH) - 0.18 (RF) - 4.72 (RD) + 7.45 (AWS)

When correlation coefficient (*r*) values were assessed (Table 4.6), the mean per cent disease incidence showed a highly significant positive correlation with maximum temperature (0.901), minimum temperature (0.703). Mean per cent disease incidence also showed highly significant positive correlation with mean temperature (0.873) while the morning relative humidity (-0.293), evening relative humidity (-0.184) showed non significant and negative correlation with disease incidence. Mean relative humidity also showed non significant negative correlation with disease incidence (-0.303). Rainfall (-0.263) and number of rainy days (-0.213) showed non significant negative correlation. Wind speed (0.746) showed highly significant and positive correlation with bud necrosis disease incidence. Similar findings were also reported by Vijayalakshmi *et al.* (2017) who reported negative correlation between GBN disease incidence with morning (-0.532) and evening relative humidity (-0.077). The data on the incidence of bud necrosis disease were subjected to multiple linear regression analysis analysis and the following equation was obtained (Table 4.8).

Y= 65.63 + 4.03 (Max Tm) + 5.63 (Min Tm) + 0.75 (Mng RH) - 0.40(Eve RH) - 0.18 (RF) - 4.72 (RD) + 7.45 (AWS)

The results showed that all the weather variables together contributed to the incidence of bud necrosis disease incidence by 95.0 (R^2= 0.95) per cent. It was also evident from the multiple linear regression equation that among various factors studied the partial regression coefficient (b) for maximum temperature (4.03), minimum temperature (5.63) morning relative humidity (0.75) average wind speed (7.45) showed positive influence on disease incidence. Evening relative humidity (-0.40) and rainfall (-0.18) showed negative influence on disease incidence.

4.2.3 Incidence of Thrips during *Kharif* 2020-2021

Data presented in the Table 4.9, Figure 4.10 revealed that during *kharif* 2020-2021 thrips population was observed initially at 21 DAS on blackgram during third week of August *i.e.* during 33rd SMW with 4.2 mean number of thrips per plant. The maximum and minimum temperatures during that period were 30.50 and 24.49^{o}C, respectively. Mean temperature was 27.5^{o}C while the average morning and evening relative humidities were 84.43 and 71.71 per cent, respectively with mean relative humidity 78.1 per cent. About 3.14 mm rainfall was recorded with five rainy days and wind speed was 9.14 kmph.

Plate 4.38. Bud necrosis infected blackgram plants observed during *rabi* 2019-2020

Plate 4.39. Variation in bud necrosis symptom in blackgram plants collected during *rabi* 2019-2020

Highly fluctuating thrips population incidence was noticed during the *kharif* season. Thrips population has increased gradually and reached peak at 35 DAS with 17.35 mean number of thrips per plant. The maximum and minimum temperatures during that period were 33.94 and 26.40^{o}C, respectively. Mean temperature was 30.2 ^{o}C while the average morning and evening relative humidities were 78.71 and 51.14 per cent, respectively with mean relative humidity 64.9 per cent. There was no rainfall and a wind speed of 6.43 kmph was recorded.

After 35 DAS, thrips population started declining to 5.2 and 4.6, 4.55, 3.33 (thrips per plant) at 42, 49, 56, 63 DAS, respectively. At 70 DAS slight increase in thrips population was noticed *i.e.* 7.1 thrips per plant during first week of October (40th SMW). The maximum and minimum temperatures during that period were 32.64 and 25.50^{o}C, respectively. Mean temperature was 29.1^{o}C. While the average morning and evening relative humidities were 81.14 and 71.86 per cent, respectively with mean relative humidity 76.5 per cent, 0.24 mm rainfall and a wind speed of 4.86 kmph. Again a slight decline of thrips population was noticed towards the crop maturity at 77 DAS during 41st SMW.

Similarly, Meena *et al*. (2013) reported that the infestation of thrips, *S. dorsalis* in chilli crop was initiated in the fourth week of July (30th meteorological week) and continued up to fourth week of November (48th meteorological week) during 2006-07 and 2007-08 at Allahabad, Uttarpradesh. The population increased gradually and touched its peak with a mean of 14.5 and 14.7 thrips/3 leaves /plant during 2006-07 and 2007-08, respectively. Yadav *et al*. (2012) also reported that *S. dorsalis* in groundnut at Udaipur, first appeared during 32nd standard meteorological week (SMW) *i.e*. 6t^{h} – 12th August, 2010 (2nd week) with a mean population of 1.20 thrips/3leaves/plant. The population increased gradually and attained the peak in the fourth week of September with a mean population of 4.16 thrips/ 3 leaves/plant. Later on, the population declined and reached a minimum level of 0.9/ 3 leaves/plant during 42nd SMW *i.e.* 14th - 21st October (3rd week). When correlation coefficient (*r*) values were worked out (Table 4.10), the number of thrips per plant showed positive correlation with maximum temperature (0.151), minimum temperature (0.158) as well as with mean temperature (0.172) but were non significant.

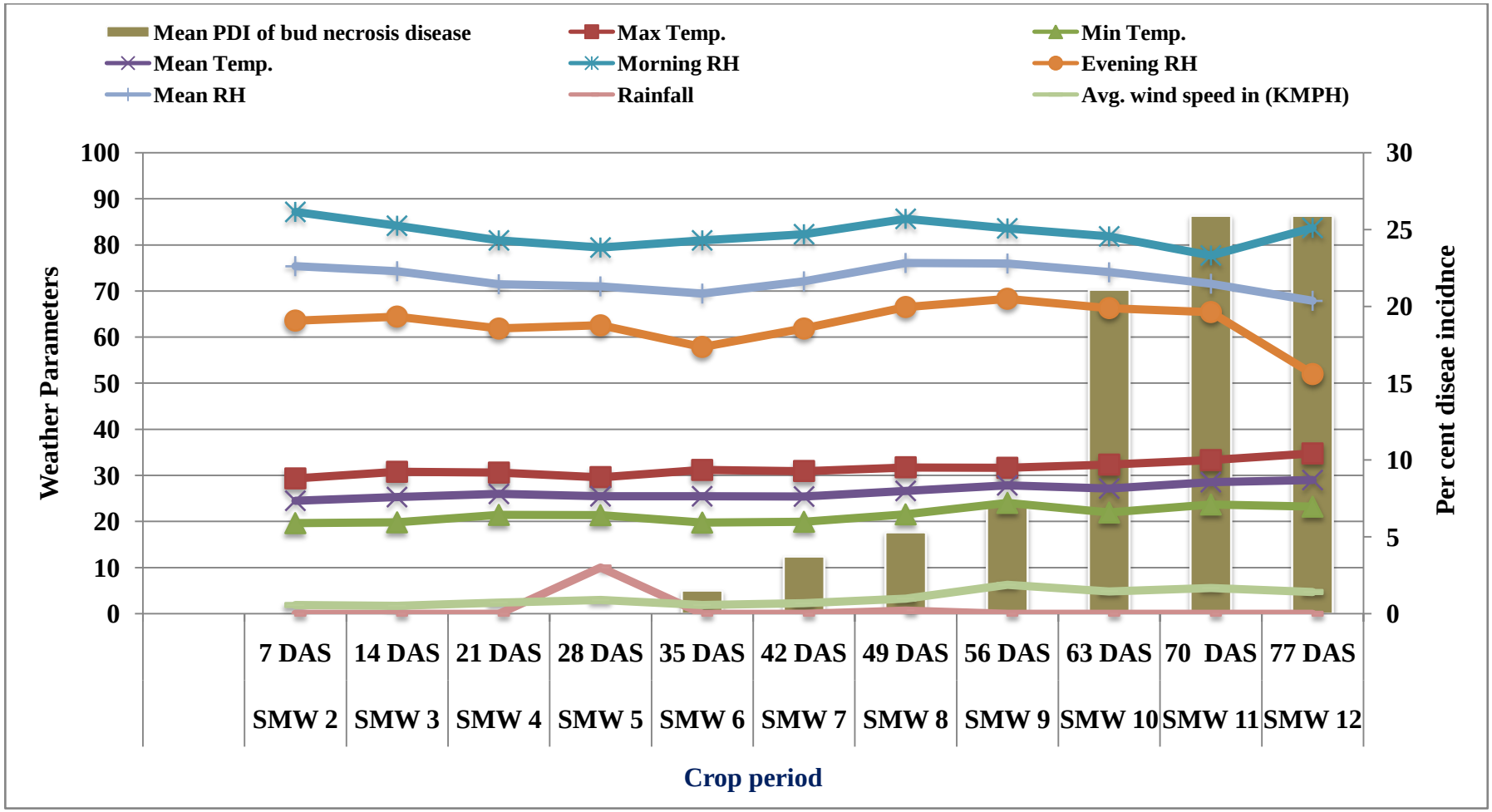


Figure 4.9. Incidence of bud necrosis disease on blackgram in correlation with weather parameters during *rabi* 2019 - 2020

The morning relative humidity (-0.079), evening relative humidity (-0.566) showed non significant and negative correlation with thrips population. Further, mean relative humidity showed non significant negative correlation with thrips population (-0.475). Rainfall (-0.289) and wind speed (-0.346) showed non significant negative correlation with thrips population while number of rainy days (-0.657) showd significant negative correlation.

These findings are in agreement with Nayak *et al*. (2019) who reported that thrips showed significant positive correlation with minimum temperature and regression equation revealed a positive influence of temperature on thrips population. The thrips population also showed significant positive correlation with mean atmospheric temperature. Vijayalakshmi *et al*. (2017) also reported that thrips population showed positive correlation with maximum and minimum temperature, respectively in groundnut crop during *kharif* in Tamil Nadu.

Mahipal *et al*. (2017) reported that population of flower thrips *Megalurothrips sjoestdi* (Trybom) in cowpea was positive and highly significant with maximum temperature, minimum temperature during *kharif* season. Findings by Akashe *et al*. (2016) in sunflower crop during *kharif* season are in line with the present findings, *i.e.* the thrips population showed non significant positive correlation with maximum temperature. Pramod *et al*. (2011) reported significant and positive correlation of thrips with maximum temperature in sunflower hybrid KBSH-1 in *kharif* season.

Findings of Kandakoor *et al*. (2012) are in line with our present findings that thrips population showed non significant positive correlation to maximum and minimum temperature in groundnut during *kharif*. Nandagopal *et al*. (2008) reported positive correlation of thrips population with the maximum temperature during *kharif* seasons in groundnut. Moanaro and Choudhary (2018) reported the similar findings of non significant positive correlation of thrips population with minimum, maximum and mean temperatures in capsicum. Present findings pertaining to relative humidity influence on thrips population are in agreement with Vijayalakshmi *et al.* (2017) who reported that thrips population showed negative correlation with morning and evening relative humidity in groundnut crop during *kharif*.

Table 4.9. Incidence of thrips and bud necrosis disease on blackgram during *kharif* 2020 -2021

Standard meteorological week (SMW)	Date	Crop Stage	Mean No. of thrips per plant	Mean No. of thrips per sqm.	Mean PDI of BND	Max Temp. (^{o}C)	Min Temp. (^{o}C)	Mean Temp. (^{o}C)	Morning RH (%)	Evening RH (%)	Mean RH (%)	Rainfall (in mm)	No. of Rainy Days	Avg. wind speed in (KMPH)
31	04.08.2020	7 DAS	0	0.00	0.00	33.9	26.2	30.0	84.1	72.3	78.2	3.5	5	8.6
32	11.08.2020	14 DAS	0	0.00	0.00	32.91	25.86	29.4	78.00	67.29	72.6	4.86	4	8.86
33	18.08.2020	21 DAS	4.2	17.10	0.00	30.50	24.49	27.5	84.43	71.71	78.1	3.14	5	9.14
34	25.08.2020	28 DAS	8.9	51.00	11.23	31.87	24.76	28.3	85.29	79.71	82.5	9.20	5	8.14
35	01.09.2020	35 DAS	17.35	67.30	14.94	33.94	26.40	30.2	78.71	51.14	64.9	0.00	0	6.43
36	08.09.2020	42 DAS	5.2	58.90	21.10	33.34	26.10	29.7	81.29	68.14	74.7	4.34	3	5.86
37	15.09.2020	49 DAS	4.6	43.60	22.19	31.49	25.03	28.3	83.00	75.71	79.4	10.11	6	5.86
38	22.09.2020	56 DAS	4.55	27.10	24.76	31.97	25.53	28.8	77.00	72.14	74.6	10.33	5	7.29
39	29.09.2020	63 DAS	3.3	14.80	26.05	32.90	25.17	29.0	78.86	72.57	75.7	23.61	4	7.29
40	05.10.2020	70 DAS	7.1	2.60	26.05	32.64	25.50	29.1	81.14	71.86	76.5	0.24	1	4.86
41	12.10.2020	77 DAS	5.5	1.5	26.05	31	25.2	28.1	83.57	77.14	80.4	2.74	5	4

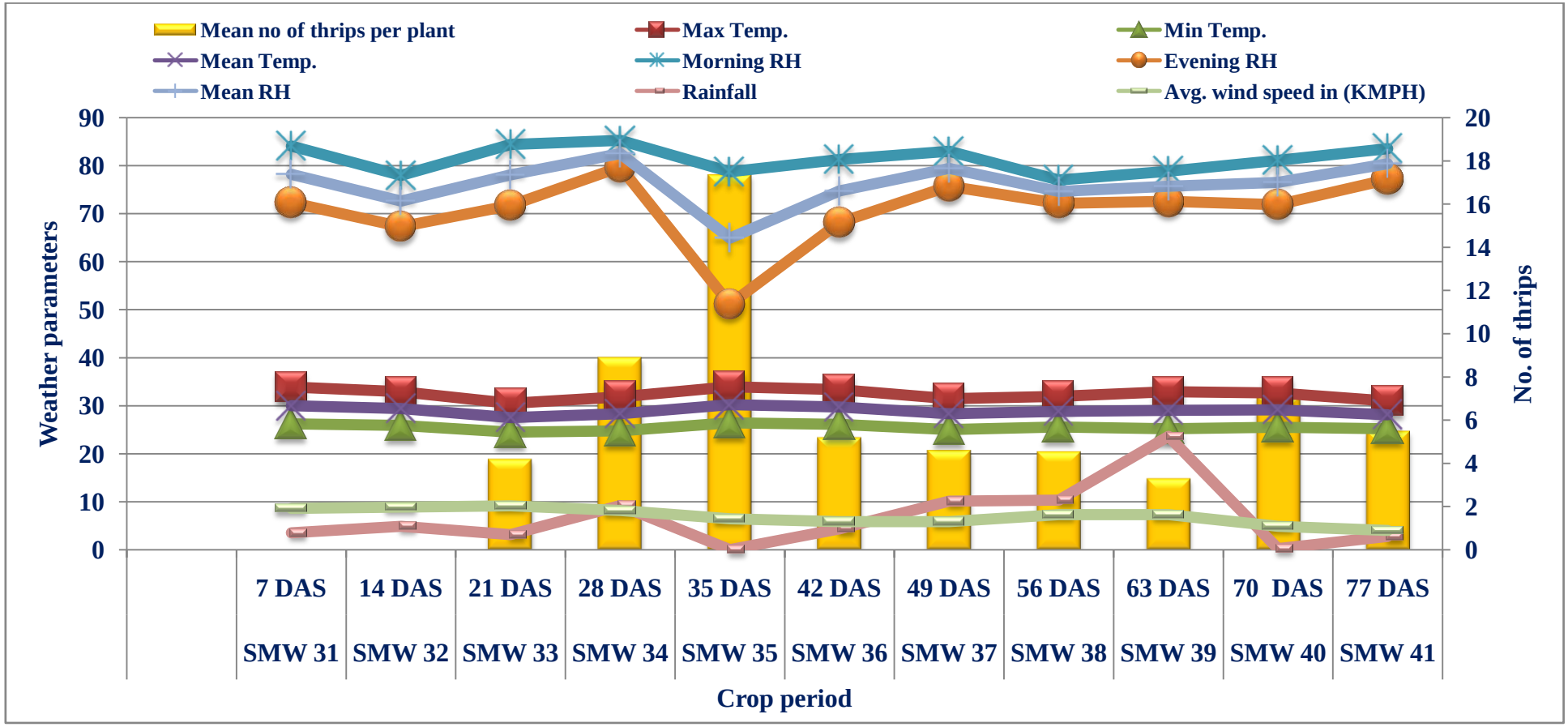


Figure 4.10. Incidence of thrips on blackgram in correlation with weather parameters during *kharif* 2020-2021

Table 4.10. Simple correlation between thrips population, bud necrosis disease incidence in blackgram with weather parameters during *kharif* 2020-21

Season		Temperature (°C)			Relative Humidity (%)			Rainfall (in mm)	No. of Rainy Days	AWS
		Max Temp.	Min Temp.	Mean	Morning	Evening	Mean			
Kharif 2020-21	Thrips Per Plant	0.151	0.158	0.172	-0.079	-0.566	-0.475	-0.289	-0.657*	-0.346
	Thrips per square meter	0.182	0.145	0.182	-0.029	-0.393	-0.322	-0.004	-0.266	-0.096
	PDI of BND	-0.096	-0.062	-0.075	-0.276	0.141	0.029	0.319	-0.178	-0.819**

Table 4.11. Multiple linear regression between thrips population and bud necrosis disease incidence in blackgram with weather parameters during *kharif* 2020-21

Year and Season	Dependent variable	R^2	Regression equation
Kharif 2020-21	Mean No. of Thrips per Plant	0.70	Y = 712.76 + 2.53 (Max Tm) - 8.52 (Min Tm) + 0.299 (Mng RH) - 0.64 (Eve RH) - 0.09 (RF) - 0.014 (RD) - 1.44 (AWS)
	Thrips Per Sqm.	0.37	Y= 888.71 + 28.3(Max Tm) - 60.39 (Min Tm) +1.27 (Mng RH) - 4.5 (Eve RH) - 0.60 (RF) +11.90 (RD) - 9.4 (AWS)
	PDI of BND	0.94	Y= - 3.97 - 5.718 (Max Tm) + 9.137 (Min Tm) - 0.28 (Mng RH) + 0.43 (Eve RH) + 1.09 (RF) - 2.78 (RD) - 4.51 (AWS)

Subba and Ghosh (2016) has reported that relative humidity (minimum, average) and maximum relative humidity showed non significant negative influence on thrips population in tomato during *kharif* season. Findings of Akashe *et al*. (2016) are in line with our findings, *i.e.* the thrips population showed highly significant negative correlation with RH-I, non significant negative correlation with RH-II in sunflower crop during *kharif* season.

Findings of Kandakoor *et al*. (2012) reported that population of thrips showed significant negative correlation with morning relative humidity but evening relative humidity showed non significant negative correlation in groundnut during *kharif*. Thrips population was negatively correlated with average RH in groundnut (Nandagopal *et al*., 2008). Pramod (2007) has reported that population of thrips were in non significant negative correlation with morning and evening relative humidities, respectively in sunflower. Jamuna *et al*. (2019) reported non significant negative correlation of thrips population with evening relative humidity in tomato during *kharif* season.

The results of present study pertaining to rainfall influence on thrips population during *kharif* season are in agreement with Subba and Ghosh (2016) who reported that weekly total rainfall showed non significant negative influence on thrips population in tomato during *kharif* season. Mahipal *et al*. (2017) reported that thrips population showed non significant negative correlation with rainfall during *kharif* in cowpea.

Findings of Akashe *et al*. (2016) are in line with our findings, *i.e.* the thrips population showed non significant negative correlation with rainfall in sunflower crop during *kharif* season. Kandakoor *et al*. (2012) reported non significant negative correlation of thrips population with rainfall in groundnut during *kharif* season. Nandagopal *et al*. (2008) reported the negative correlation of thrips population with rainy days in groundnut.

Similarly, Pramod (2007) has reported that population of thrips during *kharif* seasons showed non significant negative correlation with rainfall. Jamuna *et al*. (2019) reported that rainfall, rainy days showed significant negative correlation with thrips population in tomato. Vinaykumar *et al*. (2019) has also reported negative correlation between the thrips population and rainfall. Negative correlation between

thrips population with rainfall was reported by Moanaro and Choudhary (2018) in capsicum.

When correlation coefficient (*r*) values were assessed (Table 4.7), the number of thrips per plant (0.279), mean number of thrips per square meter (0.179) showed a positive correlation with per cent disease incidence. These findings are in agreement with Timmanna *et al.* (2020) who have reported that the percent bud necrosis disease was (23.87 %) was in linear with the thrips population during *kharif* 2016. Jamuna *et al*. (2019) also quoted the similar findings that the mean disease incidence of GBNV was directly proportional to the mean number of thrips in *kharif* tomato crop during 2015 to 2017. Vinaykumar *et al*. (2019) reported that a high positive correlation between the bud necrosis disease incidence and the thrips population in tomato during *kharif* seaon.

The data on the incidence of thrips were subjected to multiple linear regression analysis and the following equation was obtained (Table 4.11).

Y = 712.76 + 2.53 (Max Tm) - 8.52 (Min Tm) + 0.299 (Mng RH) - 0.64 (Eve RH) - 0.09 (RF) - 0.014 (RD) - 1.44 (AWS)

The results showed that all the weather variables together contributed to the incidence of thrips by 70.0 (R^2= 0.70) per cent. It was also evident from the multiple linear regression equation that among various factors studied the partial regression coefficient (b) for maximum temperature (2.53) morning relative humidity (0.299) showed positive influence on thrips population. Minimum temperature (-8.52) evening relative humidity (-0.64) rainfall (-0.09) and average wind speed (-1.44) showed negative influence on thrips population.

These findings are in accordance with Timmanna *et al.* (2020) who has analyzed stepwise regression analysis and revealed that the thrips population was influenced by all the weather parameters during current week (R^2 = 0.810), one lag week (R^2 = 0.739) and two lag week (R^2 = 0.879). Jamuna *et al.* (2019) also reported that 80.30 per cent of the thrips population was influenced by weather parameters (R^2 = 0.803) while Harish *et al.* (2015) and reported that the coefficient of multiple determinations (R^2) was only 36 per cent during *kharif* season in groundnut. Similarly, Moanaro and Choudhary (2018) also reported weather parameters as independent

variable, thrips population fluctuation as dependent variable, explained to 42 percent thrips population fluctuation.

4.2.4 Incidence of Bud Necrosis Disease in Blackgram during *Kharif* 2020-2021

Data depicted in the Table 4.9, Figure 4.11 revealed that bud necrosis disease incidence was observed initially at 28 DAS (Plate 4.40) during fourth week of August *i.e.* during 34th SMW (Standard meteorological week) with 11.23 per cent mean disease incidence. The maximum and minimum temperatures during that period were 31.87 and 24.76^{o}C, respectively. Mean temperature was 28.3^{o}C. While the average morning and evening relative humidities were 85.29 and 79.71 per cent, respectively with mean relative humidity 82.5 per cent. There was 9.20 mm rainfall and a wind speed of 8.14 kmph.

Bud necrosis disease incidence increased gradually since its first observation *i.e.*, 14.94, 21.10, 22.19, 24.76, 26.05 per cent at 35, 42, 49, 56, 63 DAS. Peak disease incidence was observed at 63 DAS (39th SMW) and remained static till the crop maturity. The maximum and minimum temperatures during that period were 32.90 and 25.17^{o}C, respectively. Mean temperature was 29.0^{o}C while the average morning and evening relative humidities were 78.86 and 72.57 per cent, respectively with mean relative humidity 75.7 per cent. There was 23.61 mm rainfall and a wind speed of 7.29 kmph.

Similar findings were also reported by Jamuna *et al*. (2019) where in mean disease incidence of GBNV disease in tomato during *kharif* 2016 ranged from 4.90 to 42.50 per cent during cropping period (42nd SMW to 7th SMW). The cumulative disease incidence *i.e.* 42.50 and 45.10 % was observed during 2015-16 and 2016-17 *kharif* tomato crops, respectively.

Another finding by Swamy and Patil (2016) are in agreement with our present findings that the highest bud necrosis disease incidence of 19.09 per cent was recorded in Raichur district followed by Koppal (16.0 %), whereas the lowest incidence of 8.2 per cent was observed in Dharwad district during *kharif* 2012 in groundnut crop. Biswas *et al.* (2015) also reported that bud necrosis disease incidence was in the range of 9.4-14.5 per cent in mungbean and also reported that overall bud

necrosis disease incidence was estimated to be 14.4 per cent in pre *kharif* and 20.5 per cent in *kharif* season for the period of 2006 to 2009.

Plate 4.40. Bud necrosis infected blackgram plants during *kharif* 2020-2021

Mean number of thrips per square meter was also recorded along with disease incidence. Initial incidence of thrips were observed at 21 DAS and reached peak 35 DAS *i.e*. 67.30 mean number of thrips per square meter at 35th SMW (Standard meteorological week). The maximum and minimum temperatures during that period were 33.94 and 26.40^{o}C, respectively.

Mean temperature was 30.2^{o}C. While the average morning and evening relative humidity were 78.71 and 51.14 per cent, respectively with mean relative humidity 64.9 per cent. No rainfall was recorded and wind speed was 6.43 kmph. Mean number of thrips per square meter started declining towards maturity *i.e*. 58.90, 43.60, 27.10, 14.80, 2.60, 1.5 at 42, 49, 56, 63, 70, 77 DAS, respectively.

When correlation coefficient (*r*) values were assessed (Table 4.10), the mean per cent disease incidence showed a non significant negative correlation with maximum temperature (-0.096), minimum temperature (-0.062), mean temperature (-0.075). Morning relative humidity (-0.276) showed non significant negative correlation whereas evening relative humidity (0.141) showed non significant and positive correlation with disease incidence.

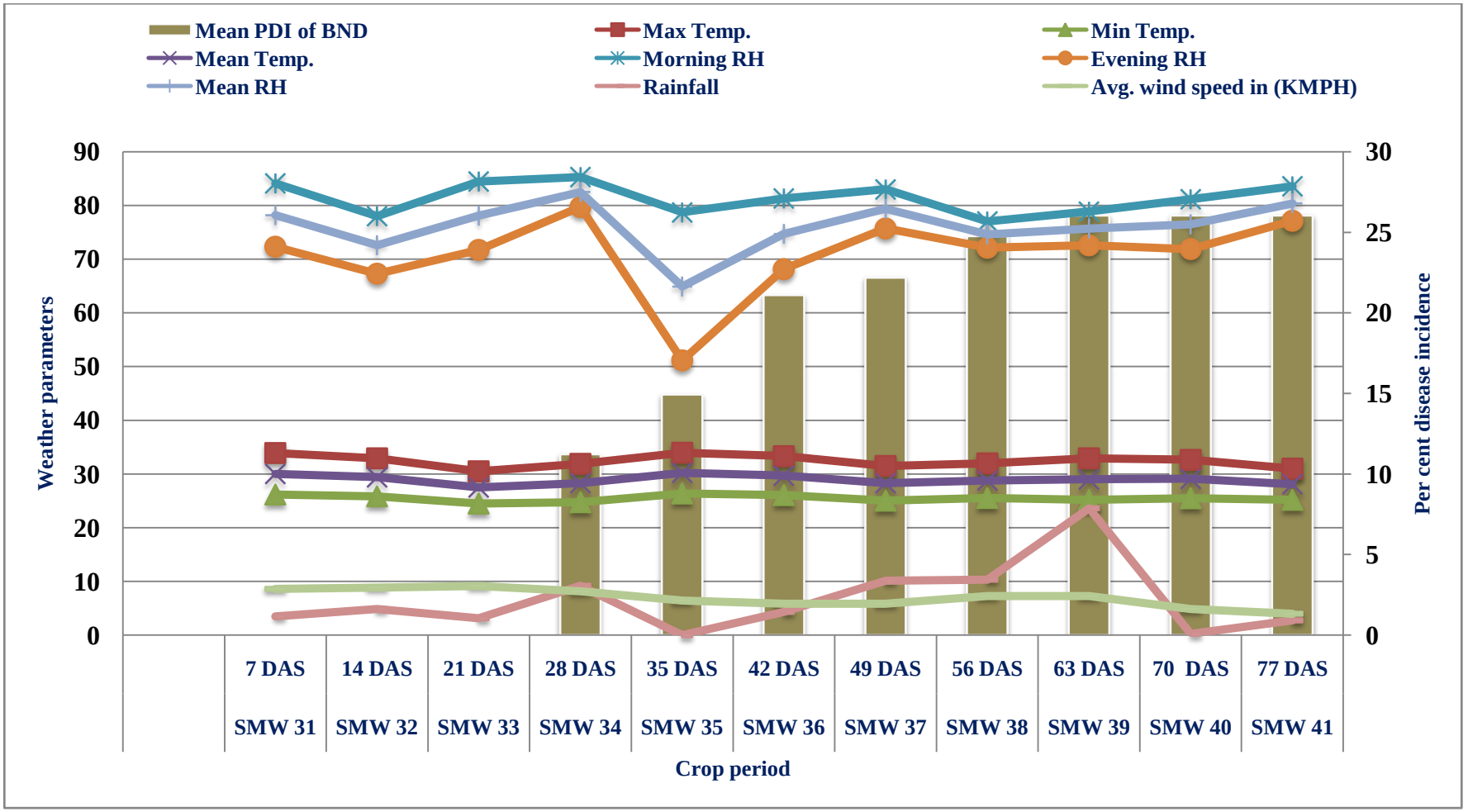


Figure 4.11. Incidence of bud necrosis disease on blackgram in correlation with weather parameters during *kharif* 2020 -2021

Mean relative humidity also showed non significant positive correlation with disease incidence (0.029). Total rainfall (0.319) showed non significant positive correlation and number of rainy days (-0.213) showed non significant negative correlation. Wind speed (-0.819) showed highly significant and negative correlation with bud necrosis disease incidence.

Similar findings were reported by Timmanna *et al*. (2020) where in GBNV disease incidence during *kharif* 2016 & 2017 in tomato crop has shown highly significant negative correlation with maximum temperature, minimum temperature. Jamuna *et al*. (2019) also reported that the minimum temperature and evening relative humidity showed highly significant negative correlation with the disease incidence, followed by rainy days where as maximum temperature and morning relative humidity showed non significant negative correlation with the disease incidence in tomato during *kharif* season. Vijayalakshmi *et al*. (2017) reported similar findings that, minimum temperature and evening relative humidity showed negative correlation where as rainfall showed positive correlation with bud necrosis disease incidence.

The data on the incidence of bud necrosis disease were subjected to multiple linear regression analysis and the following equation was obtained (Table 4.11).

Y = - 3.97 - 5.718 (Max Tm) + 9.137 (Min Tm) - 0.28 (Mng RH) + 0.43(Eve RH) + 1.09 (RF) - 2.78 (RD) - 4.51 (AWS)

The results showed that all the weather variables together contributed to the incidence of bud necrosis disease incidence by 94.0 (R^2 = 0.94) per cent. It was also evident from the multiple linear regression equation that among various factors studied the partial regression coefficient (b) for maximum temperature (-5.718) showed negative influence where as minimum temperature (9.137) showed positive influence on the disease incidence. Morning relative humidity (-0.28) average wind speed (-4.51) showed negative influence on disease incidence. Evening relative humidity (0.43) and rainfall (1.09) showed positive influence on disease incidence.

Our findings are in line with Timmanna *et al.* (2020) who has reported that the bud necrosis disease incidence in groundnut during *kharif* season was influenced by all the weather parameters during current (R^2 = 0.874), one lag (R^2 = 0.964) and two lag weeks (R^2 = 0.776).

4.2.5 Incidence of Thrips in Blackgram during *Rabi* 2020-2021

From the Table 4.12, Figure 4.12 it was evident that thrips population was observed initially at 21 DAS during fourth week of Dcember *i.e.* during 52nd SMW with 2.15 mean number of thrips per plant. The maximum and minimum temperatures during that period were 29.26 and 17.99^{o}C, respectively. Mean temperature was 23.63 ^{o}C while the average morning and evening relative humidities were 84.13 and 53.75 per cent, respectively with mean relative humidity 68.94 per cent. No rainfall was recorded and wind speed was of 3.63 kmph.

Thrips population increased gradually and reached peak at 63 DAS during second week of February *i.e.* during 6th SMW (Standard meteorological week) and with 10.10 mean number of thrips per plant. The maximum and minimum temperatures during that period were 30.87 and 17.46 ^{o}C, respectively. Mean temperature was 24.16 ^{o}C while the average morning and evening relative humidities were 85.14 and 48.14 per cent, respectively with mean relative humidity 66.64 per cent. No rainfall was recorded and wind speed was 1.86 kmph.

The thrips population started declining towards maturity and lowest incidence was recorded at 77 DAS during fourth week of February *i.e.* during 8th SMW and with 1.10 mean number of thrips per plant. The maximum and minimum temperatures during that period were 30.36 and 20.34^{o}C, respectively. Mean temperature was 25.35 ^{o}C while the average morning and evening relative humidities were 83.14 and 59.00 per cent, respectively with mean relative humidity 71.07 per cent with 3.29 mm rainfall and a wind speed of 3.57 kmph.

When correlation coefficient (*r*) values were assessed (Table 4.13), the number of thrips per plant showed a significant positive correlation with maximum temperature (0.726). Minimum temperature (0.55) and mean temperature (0.435) showed non significant positive correlation while the morning relative humidity (-0.43), evening relative humidity (-0.188), mean relative humidity (-0.266) showed non significant and negative correlation with thrips population. Further rainfall (-0.319), number of rainy days (-0.319) and wind speed (-0.463) also showed non significant negative correlation.

Similar findings which were in support with the present findings are by Rahul *et al*. (2020) who has reported that maximum and minimum temperatures showed significant positive correlation with thrips population in blackgram during late *rabi* 2019 and 2020. Further mean temperature also showed positive correlation with thrips population.

Finding by Naresh *et al*. (2018) are also in agreement with the present results that thrips population was in positive correlation with maximum temperature in two cultivars of groundnut (Dharani and K6) in second fortnight of November and first fortnight of December sown groundnut. Similarly, both maximum and minimum temperatures were positively correlated with thrips population in both the cultivars during second fortnight December sown groundnut. Vijayalakshmi *et al.* (2017) also reported that in *rabi* 2017, thrips incidence showed positive correlation with maximum temperature, minimum temperature and sunshine hours in groundnut.

Rahul *et al*. (2020) also reported that morning relative humidity showed significant negative correlation. Evening relative humidity and mean relative humidity showed non significant negative correlation with thrips population in blackgram during late *rabi* 2019 and 2020. Naresh *et al.* (2018) also reported that the evening relative humidity showed significant negative correlation with thrips population in all these dates of sowing i.e. 1^{st}, 2^{nd} fortnight of November and first fortnight of December. However wind speed showed negative significant correlation only in first fortnight of November sown groundnut crop.

Similarly, Vijayalakshmi *et al.* (2017) reported that thrips incidence showed negative correlation with morning and evening relative humidity in *rabi* 2017. Harish *et al.* (2015) also found that during summer, evening relative humidity, and rainfall showed highly significant negative correlation with thrips population in groundnut.

When correlation coefficient (*r*) values were assessed, the number of thrips per plant (0.466) and mean number of thrips per square meter (0.508) showed a non significant positive correlation with per cent disease incidence (Table number 4.7) and these results are in agreement with Lakshmi *et al*. (2016) who has reported that GBNV and TSV of groundnut showed significant positive correlation with average thrips population during *rabi* 2014.

Table 4.12. Incidence of thrips and bud necrosis disease on blackgram during *rabi* 2020 -2021

Standard meteorological week (SMW)	Date	Crop Stage	Mean No. of thrips per plant	Mean No. of thrips per sqm.	Mean PDI of BND	Max Temp. (^{o}C)	Min Temp. (^{o}C)	Mean Temp. (^{o}C)	Morning RH (%)	Evening RH (%)	Mean RH (%)	Rainfall (in mm)	No. of Rainy Days	Avg. wind speed in (KMPH)
50	17.12.2020	7 DAS	0.00	0.00	0.00	29.86	18.21	24.04	87.57	55.43	71.50	0.00	0.00	2.14
51	24.12.2020	14 DAS	0.00	11.90	0.00	29.54	18.53	24.04	85.43	56.43	70.93	0.00	0.00	3.71
52	31.12.2020	21 DAS	2.15	14.30	2.61	29.26	17.99	23.63	84.13	53.75	68.94	0.00	0.00	3.63
1	07.01.2021	28 DAS	3.85	17.40	3.59	29.36	19.66	24.51	86.14	53.57	69.86	0.00	0.00	4.57
2	14.01.2021	35 DAS	6.30	29.10	4.52	30.89	20.57	25.73	85.29	65.43	75.36	0.00	0.00	3.29
3	21.01.2021	42 DAS	8.00	53.90	4.92	30.80	19.54	25.17	85.29	56.71	71.00	0.00	0.00	2.57
4	28.01.2021	49 DAS	8.50	67.10	6.01	31.56	19.04	25.30	84.00	54.43	69.21	0.00	0.00	2.00
5	04.02.2021	56 DAS	9.75	78.50	6.96	30.81	19.10	24.96	85.14	53.86	69.50	0.00	0.00	2.71
6	11.02.2021	63 DAS	10.10	68.30	6.96	30.87	17.46	24.16	85.14	48.14	66.64	0.00	0.00	1.86
7	18.02.2021	70 DAS	3.20	21.20	6.96	30.77	17.40	24.09	85.00	49.57	67.29	0.00	0.00	2.29
8	25.02.2021	77 DAS	1.10	17.80	10.22	30.36	20.34	25.35	83.14	59.00	71.07	3.29	1.00	3.57

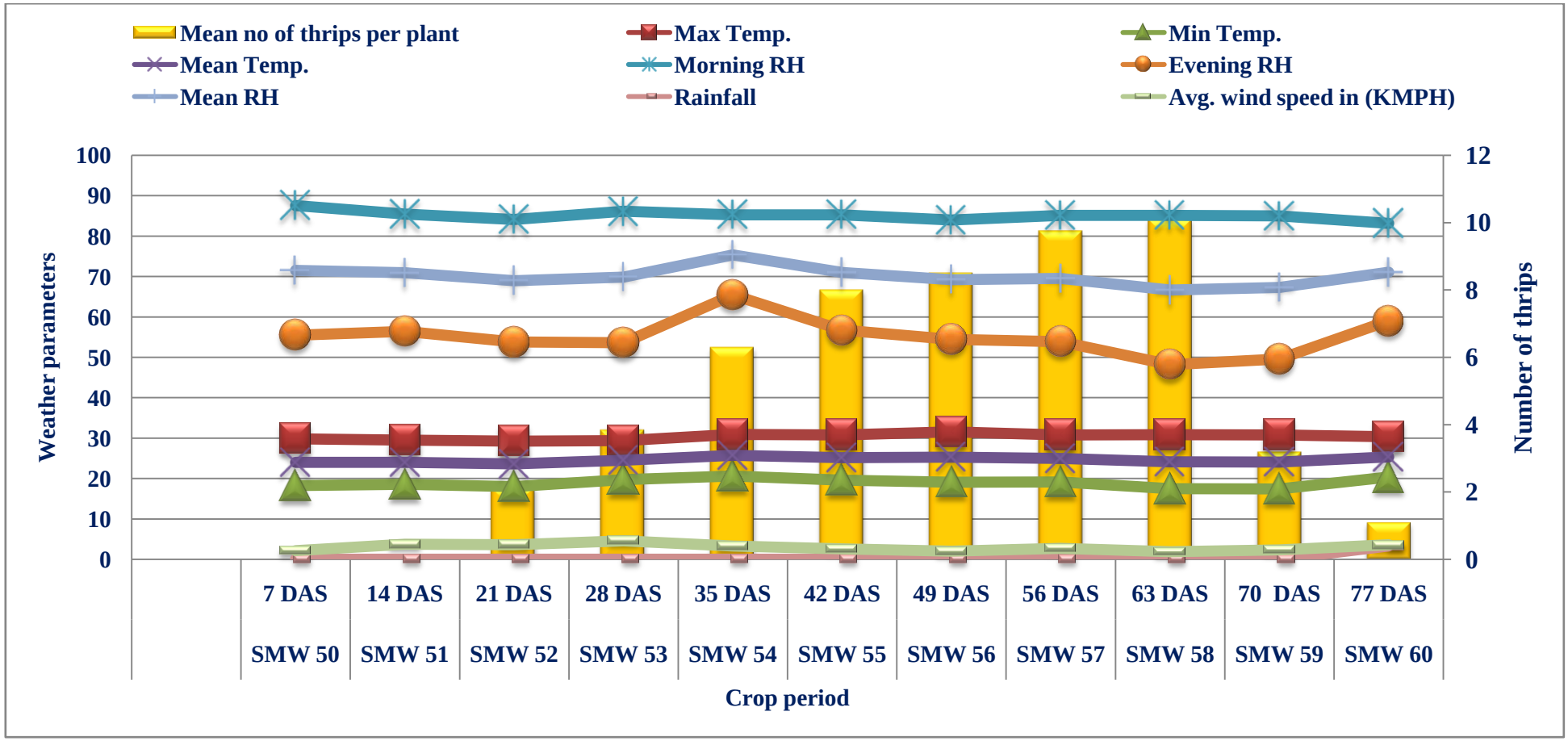


Figure 4.12. Incidence of thrips on blackgram in correlation with weather parameters during *rabi* 2020 -2021

The data on the incidence of thrips were subjected to multiple linear regression analysis and the following equation was obtained (Table 4.14).

Y = 52.06 + 0.736 (Max Tm) + 4.26 (Min Tm) - 1.23 (Mng RH) - 0.69 (Eve RH) - 2.76 (RF) - 0 (RD) - 2.15 (AWS)

The results showed that all the weather variables together contributed to the incidence of thrips by 83.0 (R^2 = 0.83) per cent. It was also evident from the multiple linear regression equation that among various factors studied the partial regression coefficient (b) for maximum temperature (0.736) minimum temperature (4.26) showed positive influence where as morning relative humidity (-1.23), evening relative humidity(-0.69) showed negative influence on thrips population. Rainfall (-2.76) and average wind speed (-2.15) were also showed negative influence on thrips population.

These findings are in agreement with Harish *et al.* (2015) reported that the coefficient of multiple determinations (R^2) was 54 and 80 per cent during *rabi* and summer seasons, respectively on groundnut in Gujarath.

4.2.6 Incidence of Bud Necrosis Disease in Blackgram during *Rabi* 2020-2021

From the Table 4.12, Figure 4.13 it is evident that bud necrosis disease incidence was observed initially at 21 DAS (Plate 4.41) during fourth week of December *i.e.* during 52nd SMW and with 2.61 mean per cent disease incidence. The maximum and minimum temperatures during that period were 29.26 and 17.99 ^{o}C, respectively. Mean temperature was 23.63 ^{o}C while the average morning and evening relative humidities were 84.13 and 53.75 per cent, respectively with mean relative humidity 68.94 per cent. No rainfall was recorded and wind speed was 3.63 kmph.

Bud necrosis disease incidence increased gradually since its first observation *i.e.*, 3.59, 4.52, 4.92, 6.01, 6.96, 6.96, 6.96 per cent disease incidence at 28, 35, 42, 49, 56, 63,70 DAS, respectively. Peak disease incidence was observed at 77 DAS (8th SMW). The maximum and minimum temperatures during that period were 30.36 and 20.34 ^{o}C, respectively. Mean temperature was 25.35 ^{o}C while the average morning and evening relative humidities were 83.14 and 59.00 per cent, respectively with 71.07 per cent mean relative humidity. About 3.29 mm rainfall was recorded and wind speed was 3.57 kmph.

Table 4.13. Simple correlation between thrips population, bud necrosis disease incidence in blackgram with weather parameters during *rabi* 2020-21

Season		Temperature (^{o}C)			Relative Humidity (%)			Rainfall (in mm)	No. of Rainy Days	AWS
		Max Temp.	Min Temp.	Mean	Morning	Evening	Mean			
Rabi 2020-21	Thrips per Plant	0.726*	0.55	0.435	-0.162	-0.188	-0.266	-0.319	-0.319	-0.463
	Thrips per square meter	0.739**	0.005	0.404	-0.276	-0.252	-0.317	-0.204	-0.204	-0.507
	PDI of BND	0.607*	0.232	0.509	-0.682*	-0.106	-0.273	0.577	0.577	-0.201

Table 4.14 Multiple linear regression between thrips population and bud necrosis disease incidence in blackgram with weather parameters during *rabi* 2020-21

Year and Season	Dependent variable	R^2	Regression equation
Rabi 2020-21	Mean No. of Thrips per Plant	0.83	Y = 52.06 + 0.736 (Max Tm) + 4.26 (Min Tm) - 1.23 (Mng RH) - 0.69 (Eve RH) - 2.76 (RF) - 0 (RD) - 2.15 (AWS)
	Thrips Per Sqm.	0.80	Y= 836.23 - 1.22 (Max Tm) + 31.63 (Min Tm) - 11.76 (Mng RH) - 5.3 (Eve RH) - 17.24 (RF) + 0 (RD) - 21.6 (AWS)
	PDI of BND	0.92	Y= - 96.39 + 4.10 (Max Tm) + 0.024 (Min Tm) - 0.137 (Mng RH) - 0.33 (Eve RH) + 1.75 (RF) + 0 (RD) + 1.95 (AWS)

Mean number of thrips per square meter was also recorded along with disease incidence. Initial incidence of thrips were observed at 14 DAS with mean 11.90 and reached peak at 56 DAS *i.e.* 78.50 mean number of thrips per square meter during 5th SMW (Standard meteorological week). The maximum and minimum temperatures during that period were 30.81 and 19.10 ^{o}C, respectively.

Mean temperature was 24.96 ^{o}C while the average morning and evening relative humidities were 85.14 and 53.86 per cent, respectively with 69.50 per cent mean relative humidity. No rainfall was recorded and the wind speed was 2.71 kmph was recorded. Mean number of thrips per square meter has started declining towards maturity *i.e.* 68.30, 21.20, 17.80 at 63, 70, 77 DAS, respectively.

When correlation coefficient (*r*) values were assessed (Table 4.13), the mean per cent disease incidence showed a significant positive correlation with maximum

Plate 4.41. Bud necrosis infected blackgram plants during *rabi* 2020-2021

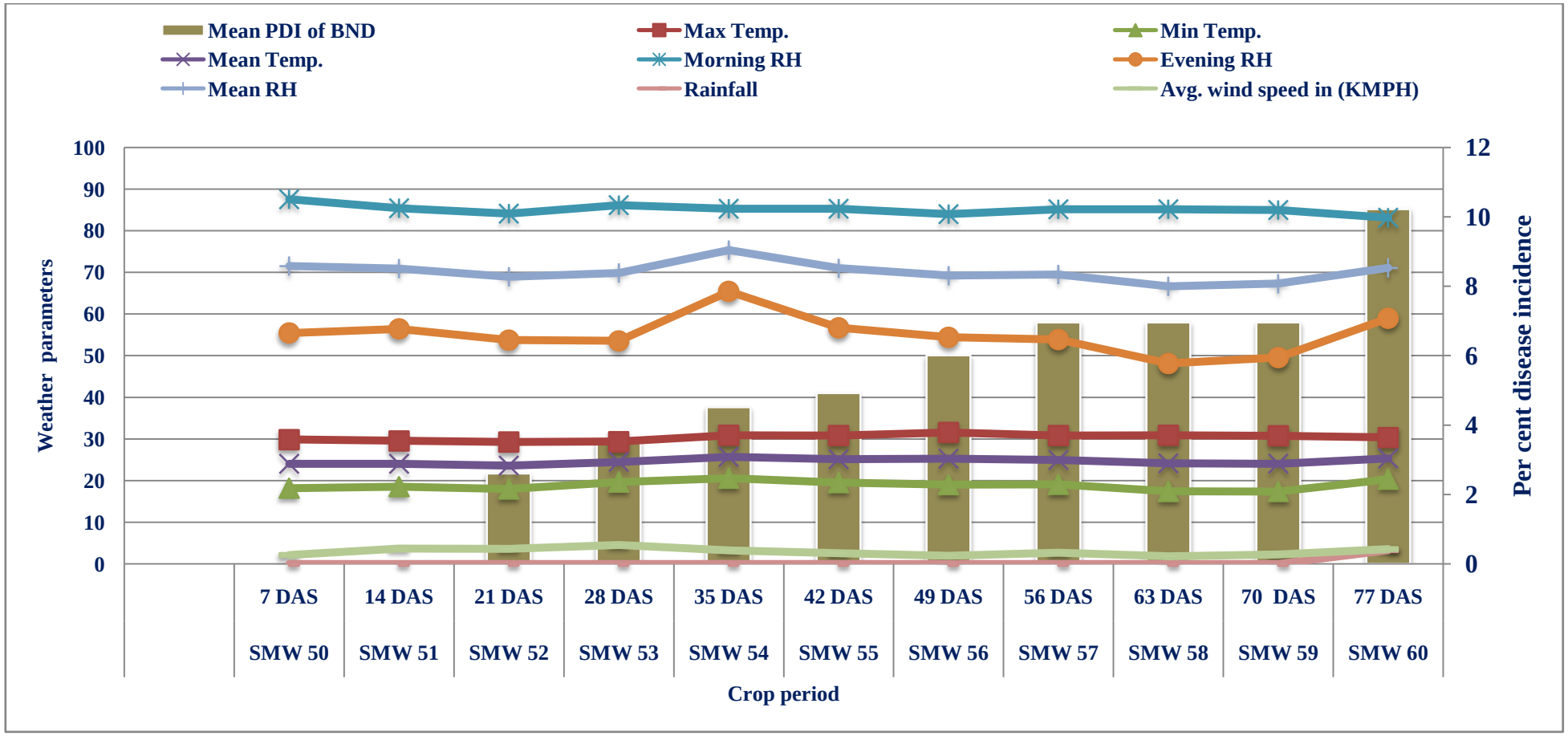


Figure 4.13. Incidence of bud necrosis disease on blackgram in correlation with weather parameters during *rabi* 2020 -2021

Morning relative humidity (-0.682) showed significant negative correlation whereas evening relative humidity (-0.106) and mean relative humidity (-0.273) showed non significant and negative correlation with disease incidence. Rainfall (0.577) and number of rainy days (0.577) showed non significant positive correlation. Wind speed (-0.201) showed non significant and negative correlation with bud necrosis disease incidence.

The above results coordinate with findings of Rahul *et al*. (2020) who reported that maximum and minimum temperatures showed significant positive correlation with leaf curl disease incidence in blackgram during late *rabi* 2019 and 2020. Further mean temperature also showed positive correlation with leaf curl disease incidence.

Similarly, Vijayalakshmi *et al.* (2017) also reported that during *rabi* 2017, GBNV incidence showed positive correlation with maximum temperature, minimum temperature and sunshine hours in groundnut. Lakshmi *et al*. (2016) reported that leaf curl disease of blackgram caused by GBNV showed a significant positive correlation with maximum temperature and minimum temperature. She has also reported that GBNV and TSV of groundnut showed a significant positive correlation with maximum temperature, minimum temperature.

The present findings pertaining to relative humidity on bud necrosis disease incidence were in accordance with Rahul *et al*. (2020) who reported that morning relative humidity showed significant negative correlation. Evening relative humidity and mean relative humidity showed non significant negative correlation with disease incidence in blackgram during late *rabi* 2019 and 2020. Another finding by

Vijayalakshmi *et al.* (2017) who also reported that during *rabi* 2017, GBNV incidence showed negative correlation with morning and evening relative humidity in groundnut. Lakshmi *et al*. (2016) also reported that leaf curl disease of blackgram caused by GBNV showed significant negative correlation with maximum relative humidity during *rabi* 2014. She has also reported that GBNV and TSV of groundnut showed significant negative correlation with morning relative humidity and evening relative humidity.

The data on the incidence of bud necrosis disease was subjected to multiple linear regression analysis and the following equation was obtained (Table 4.14).

Y= - 96.39 + 4.10 (Max Tm) + 0.024 (Min Tm) - 0.137 (Mng RH) - 0.33 (Eve RH) + 1.75(RF) + 0 (RD) + 1.95 (AWS)

The results showed that all the weather variables together contributed to the incidence of bud necrosis disease incidence by 92.0 (R^2 = 0.92) per cent. It was also evident from the multiple linear regression equation that among various factors studied the partial regression coefficient (*b*) for maximum temperature (4.10), minimum temperature (0.024) showed positive influence on the disease incidence. Whereas morning relative humidity (-0.137), evening relative humidity (-0.33) showed non significant negative influence. Rainfall (1.75) and average wind speed (1.95) showed positive influence on the disease incidence.

These results are in agreement with Lakshmi *et al*. (2016) who also reported that thrips and weather parameters had influenced the development of diseases in groundnut up to 86.5 and 87.8 per cent, respectively. Multiple linear regression analysis has revealed that all the studied factors together were responsible for 94.95 % of total significant variation in leaf curl disease in blackgram during *rabi* 2014.

Overall view of thrips and GBNV incidence in blackgram in the present study revealed that the density of thrips population and per cent disease incidence was more during *kharif* 2020-2021 (26.05 %) compared to *rabi* 2019-2020 (25.90 %) and *rabi* 2020-2021 (10.22 %). It is also evident that atmospheric temperature has a positive and significant correlation with the bud necrosis disease incidence in blackgram. The temperatures not only increased the disease incidence (%) but also the severity of the disease.

The thrips population was observed in the field approximately after 14 DAS. The thrips incidence showed a significant increase in population with the increase in temperature. Thereafter at maturity a steep fall in the thrips population was observed. The thrips population showed positive association with the atmospheric temperature (During *rabi* maximum and minimum temperatures showed significant positive correlation with thrips population and per cent disease incidence). The prevailing weather conditions played a major role in thrips and bud necrosis disease outbreak. The clear weather conditions with no rainfall during the crop growth period have favored the thrips population as well as bud necrosis disease incidence. Relative humidity has also played vital role in buildup of thrips population in two different seasons *viz.*, *kharif* and *rabi.*

4.3 STUDY OF TRANSMISSION OF GROUNDNUT BUD NECROSIS VIRUS BY THRIPS IN BLACKGRAM

4.3.1 Mechanical Inoculation

Virus vector transmission studies were performed using a plant species that reacted systemically upon infection with virus isolate and supported development, feeding of the thrips species tested. Hence, throughout the experiment period, virus isolates were maintained on cow pea cv-152 cultivar, since cow pea has consistently produced more local lesions within four to five days after inoculation than blackgram (Plate 4.42, 4.43, 4.44, 4.45, 4.46).

Similarly, Suganyadevi *et al*. (2018) reported that the GBNV infected tomato leaf samples identified by the presence of chlorotic and necrotic spots on leaves from different parts of Tamil Nadu. Symptomatic tomato leaf samples were inoculated on cow pea cv. CO5 leaves on cotyledonary leaves under insect-proof condition and cowpea leaves exhibited chlorotic to necrotic lesions of inoculated cotyledonary leaves within seven to ten days.

Singh *et al*. (2018) also reported that GBNV infection induced typical symptoms (chlorosis and necrosis) within 4-8 dpi (Days of post inoculation) in a mechanically inoculated cowpea plants and GBNV infection spreaded systemically within 8 dpi instigating the two types of cell death *i.e.*, at the inoculated (necrosis) cite and systemic site (premature senescence) at 25^{o}C. Raigond *et al*. (2017) reported that the suspected potato plants to show typical symptoms of stem necrosis disease in field due to GBNV infection. The inoculum from suspected plants when sap-transmitted onto indicator host plants (cowpea), characteristic chlorotic and necrotic local lesions were exhibited which later turned to necrotic areas after 10 to 15 days of inoculation.

Plate 4.42. Bud necrosis infected blackgram leaves used for mechanical inoculation

Plate 4.43. Local lesions observed on cow pea leaves at 7 DAI

Plate 4.44. Local lesions observed on cow pea leaves at 10 DAI

Plate 4.45. Chlorotic spots on cow pea leaves at 20 DAI

Plate 4.46. Chlorotic spots on cow pea leaves at 25 DAI

Daimei *et al*. (2017) collected tomato plants that exhibited symptoms of GBNV infection in field was collected and this GBNV virus was maintained on cowpea (Pusa Komal) by alternating mechanical and thrips inoculation to prevent the development of non transmissble mutants by thrips. Holkar *et al*. (2016) reported that GBNV to induce both local and systemic symptoms including chlorotic/necrotic spots and veinal necrosis on both the cowpea cultivars, systemic yellowing and bud necrosis on groundnut and no symptoms on watermelon. Similarly, effective mechanical transmission was reported by Ansar *et al*. (2015), Gurupad and Patil (2014) in cow pea plants. Similarly, Raja and Jain (2006) reported that *Tospovirus* isolates from bud blight affected tomato samples collected from Coimbatore, Kanpur, Pune and Rahuri could be mechanically transmitted successfully to cowpea (*Vigna unguiculata* cv. Pusa Komal), which is a diagnostic assay host for *Tospovirus*.

4.3.2 Molecular Confirmation Studies of GBNV

GBNV infected leaves of blackgram from the experimental fields was used for mechanical/sap transmission in cow pea plants maintained in pots. The symptom development on cow pea confirmed mechanical transmission of the virus and both samples were utilized for confirmation studies.

4.3.2.1 Detection of *Groundnut bud necrosis virus* using real time quantitative PCR (RT-qPCR): The presence of the target gene in samples was tested using quantitative/Real Time PCR. The presence of virus in the sample was confirmed based on the Ct values. Ct value indicates the cycle in which the fluorescence reaches the threshold value. The higher the initial DNA amount, the lesser number of cycles are needed (low Ct values) to reach the threshold. The Ct value of the samples in the different target genes were depicted in the Table 4.15. The blackgram leaf samples A01, A02 collected from Agricultural College farm, Bapatla resulted Cq values of 24.27, 24.50, where as A03, B01 of suspected blackgram stem samples had 28.05, 23.96 Cq values with nucleocapsid and coat protein markers, respectively. Similarly, cowpea leaf samples B02, B03 maintained in green house for transmission studies, resulted 24.25, 26.86 Cq values where as C01, C02 of cowpea leaf samples were wih 23.92, 24.90 Cq with nucleocapsid and degenerated coat protein primers, respectively.

To validate the data, a melt curve analysis was also performed and the graphical results clearly indicated the presence of the *Groundnut bud necrosis virus* in both field collected blackgram and cowpea leaves from potted plants. PCR products were run in gel electrophoresis and a clear banding pattern (124 bp and 135 bp) was observed (Plate 4.47).

Table 4.15. Cycle threshold (Ct) values of the leaf samples

Well	Fluor	Target	Sample	Cq Mean
A01	SYBR	GBNV_NC	Blackgram	24.27
A02	SYBR	GBNV_NC	Blackgram	24.50
A03	SYBR	GBNV_CP	Blackgram	28.05
B01	SYBR	GBNV_CP	Blackgram	23.96
B02	SYBR	GBNV_NC	Cowpea	24.25
B03	SYBR	GBNV_NC	Cowpea	26.86
C01	SYBR	GBNV_CP	Cowpea	23.92
C02	SYBR	GBNV_CP	Cowpea	24.90

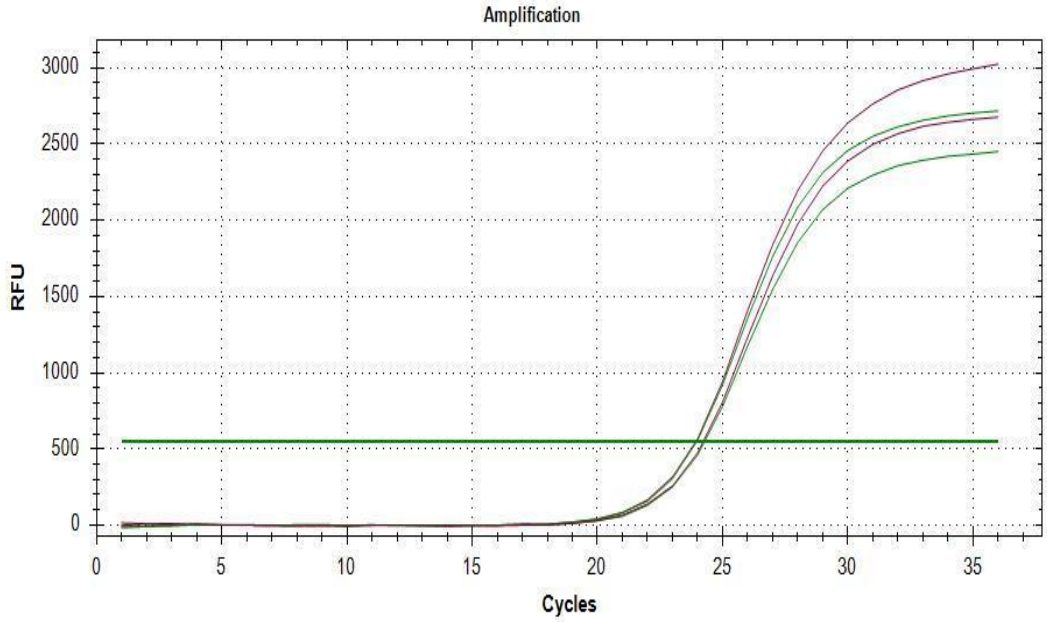


Figure 4.14. Representative amplification curve of blackgram and cowpea samples in both the genes

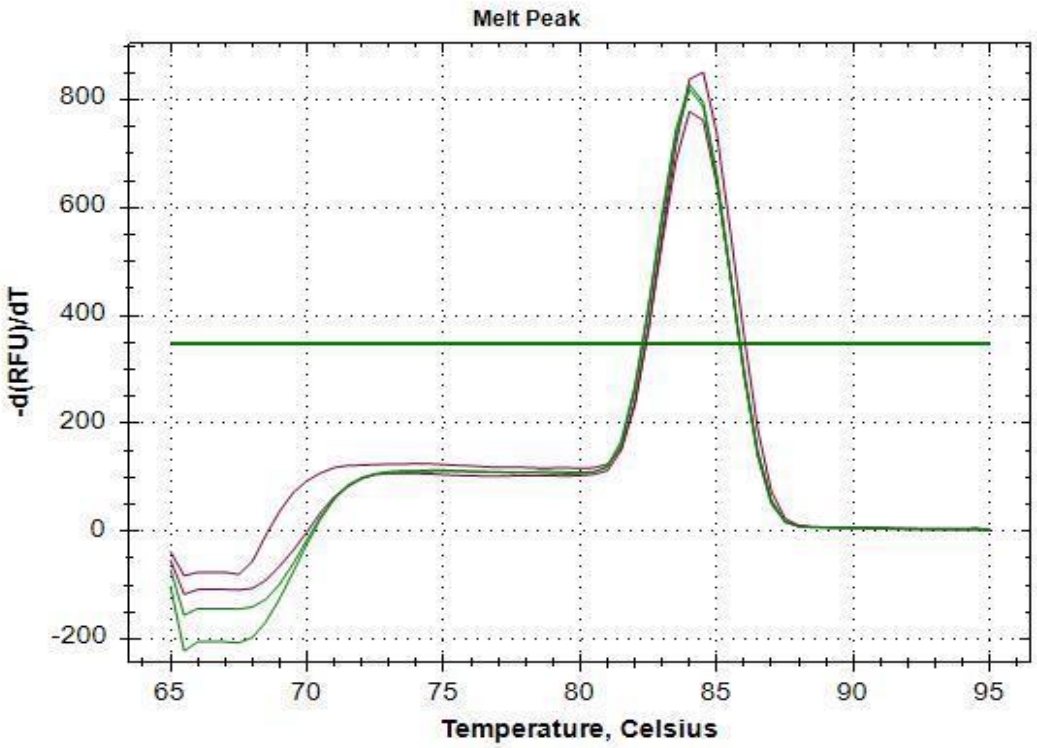


Figure 4.15. Representative melt curve of blackgram and cowpea in both the genes

4.3.3 Insect Transmission Studies

T. palmi and *M. usitatus* were the thrips species identified from the samples collected on blackgram crops raised at Agricultural college farm. Hence, the initial transaction studies were conducted with *T. palmi* and *M. usitatus*.

4.3.3.1 Transmission of bud necrosis disease by nymphs and adults of *T. palmi :* Insect transmission studies were carried out in the laboratory with two species *viz. T. palmi* and *M. usitatus.* Out of these two species tested, only *T. palmi* could able to transmit the *GBNV* from diseased to healthy cowpea plants where in the inoculated plants exhibited symptoms *viz.* chlorotic local lesions. Whereas *M. usitatus* failed to transmit the virus and the inoculated plants remained healthy. Hence, the detailed studies of insect transmission were done with *T. palmi. Thrips palmi* was identified as vector of *Groundnut bud necrosis virus*. These findings are in accordance with the reports of Vijayalakshmi, (1994) and Sreekanth, (2002) who reported that peanut bud necrosis virus on groundnut,

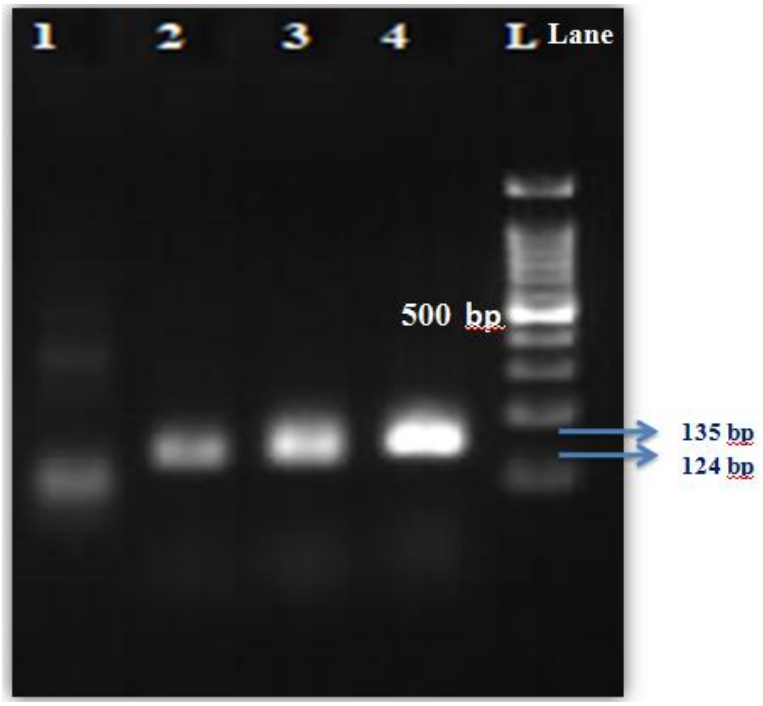


Plate 4.47. Banding pattern observed with Real time PCR amplified products

Lane 1. GKGBNV primer - Cow Pea leaves;
Lane 2. GKGBNV primer - Blackgram Leaves;
Lane 3. GKGBCP primer - Cowpea leaves;
Lane 4. GKGBCP primer - Blackgram leaves;
Lane L 1.5 kb Ladder

mungbean and urdbean was transmitted by *T. palmi* only. Nymphs acquired the virus and transmitted the disease when it became adult. However, the viruliferous nymphs could not transmit the virus at its nymphal stage itself.

4.3.3.1.1 Determination of acquisition access period (AAP): From the results obtained (Table 4.16, Figure 4.16), it was evident that a minimum of 2 h acquisition access period was observed in case of first instar larvae with 8.33 per cent disease transmission (Plate 4.48) and increased to 16.67, 41.67, and 50.00 per cent at 4 h (Plate 4.49), 6h (Plate 4.50) and 8 h of acquisition access period, respectively. With increasing acquisition access period *i.e.* 24 h, 48 h the rate of disease transmission increased to 91.67 and 100 per cent, respectively (Plate 4.51). No disease transmission was observed at 30 min and 1 h of acquisition access periods.

Table 4.16. Minimum acquisition access period required for *T. palmi* to transmit BND

Stage of the insect tested for different AAP	Per cent bud necrosis disease transmission by *T. palmi* at different AAP with 48 h common IAP							
	30 min	**1 h**	**2 h**	**4 h**	**6 h**	**8 h**	**24 h**	**48 h**
First Instar	0.00	0.00	8.33	16.67	41.67	50.00	91.67	100.00
Second instar	0.00	0.00	8.33	8.33	16.67	16.67	33.33	33.33
Adult	0.00	0.00	0.00	0.00	0.00	0.00	8.33	8.33

From the results obtained, it was found that no disease transmission was observed at 30 min and 1 h of acquisition access periods and a minimum of 2 h acquisition access period was observed in case of second instar larvae with 8.33 per cent disease transmission and the same per cent disease transmission was observed at 4 h AAP, and increased to 16.67 per cent at 6 h and 8 h of acquisition access period, respectively. Whereas at 24 h acquisition accesses period the per cent disease transmission increased slightly to 33.33 and there was no further increase at 48 h of acquisition access period.

Plate 4.48. Symptom expression of bud necrosis disease after 10 DAT

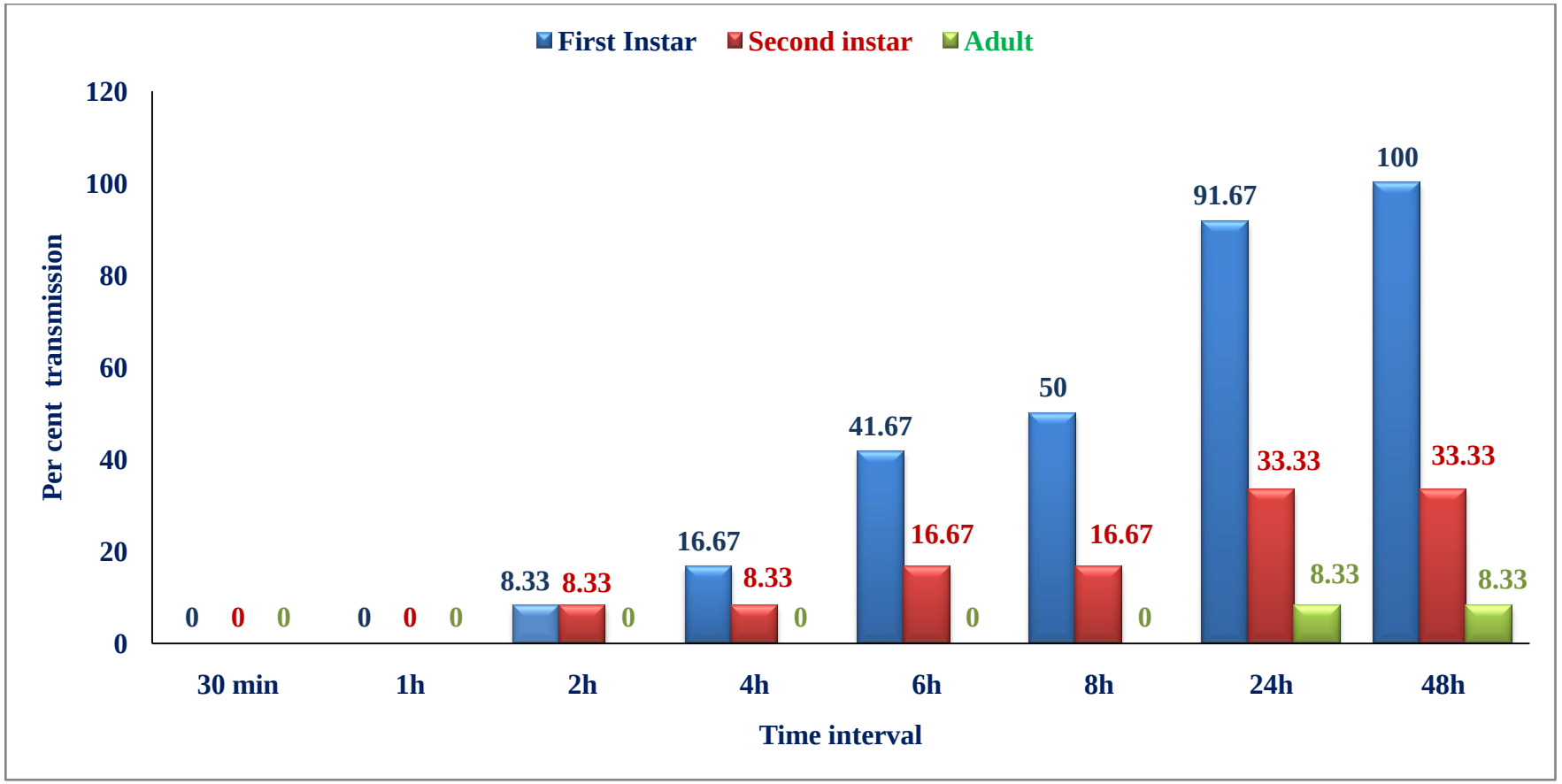


Figure 4.16. Minimum acquisition access period required for *T. palmi* to transmit bud necrosis disease

Plate 4.49. Symptom expression of bud necrosis disease after 15 DAT

Plate 4.50. Symptom expression of bud necrosis disease after 15 DAT

Plate 4.51. Symptom expression of bud necrosis disease after 15 DAT

Our present findings are in accordance with Reddy *et al*. (1983), who reported increased percentage of transmission of PBNV with longer acquisition access period up to 48 h. Vijayalakshmi (1994) found that *T. palmi* was able to acquire PBNV within 5 min with negligible increase in the transmission rate up to 12 h and maximum transmission rate at one day acquisition access period. The findings of Sreekanth (2002), was also in accordance with the present study who has reported that the transmission rate increased with increase in acquisition access period up to 48 h and further exposure to longer period could not increase the rate of transmission.

There was no disease transmission at 30 min, 1 h, 2 h, 4 h, 6 h, and 8 h of acquisition access periods in case of adults. Surprisingly a minimum of 24 h acquisition access period was observed with 8.33 per cent of disease transmission. Same rate of disease transmission was also observed at 48 h of acquisition access period.

Ruth *et al*. (2018) also reported that *T. palmi* as the vector of the GBNV causing bud necrosis disease in tomato and cowpea with minimum AAP as 15 minutes and one hour IAP by adults. Optimum virus transmission was obtained with 48 h of AAP in the larval stage and 48 h of IAP in the adult stage, but beyond 48 h of AAP and IAP resulted in decreased virus transmission. Ansar *et al*. (2015) also reported that *T. palmi* was able to acquire and transmit the GBN virus (5.5 %) within an AFP of 24 h. Further, percent transmission (27.7 %) was found to increase when

the AFP extended to 72 h. There was no transmission of the virus at 6 and 12 h of AFP in three successive experiments. Mou *et al*. (2021) also reported that *T. palmi* transmitted WSMoV in a persistent manner, and the virus was mainly transmitted by adults when ingested at the first-instar larval stage.

4.3.3.1.2 Determination of inoculation access period (IAP) : Another experiment was conducted to determine the minimum inoculation access period for the transmission of bud necrosis virus by vector *T. palmi* with common acquisition access period of 24 h. From the results obtained (Table 4.17, Figure 4.17), it was evident that no disease transmission was observed at 30 min, 1 h and 2 h IAP in case of first instar larvae whereas a minimum inoculation access period of 4 h was observed with 12.00 per cent disease transmission. At 6 h, 8 h (Plate 4.52), 24 h, 48 h of inoculation access period, the per cent disease transmission was 12.00, 20.00, 52.00, and 96.00, respectively (Plate 4.53).

In other studies, an inoculation access period of 5 to 30 min were found to be adequate on groundnut for GBNV transmission by thrips. Sreekanth (2002) on mungbean reported one hour minimum inoculation access period for transmission of GBNV by *T. palmi* which failed to transmit PBNV in 30 min IAP and the maximum transmission rate with 2 h of IAP. But the present results are similar to the findings of Vijayalakshmi, (1994). So, the 48 h of inoculation access period at which the maximum rate of disease transmission occurred may be considered optimum IAP. Similarly, Ansar *et al.* (2015) reported that at AFP of 48 h *T. palmi* was able to transmit the GBN virus (5.5 %) and the rate of transmission increased to 11.1, 16.6, 22.2 and 33.3 per cent when IFP was 72, 96, 120 and 144 hrs, respectively.

Similarly, in case of second instar larvae no disease transmission was observed at 30 min, 1 h, 2 h, and 4 h IAP. A minimum of 8 h inoculation access period was observed with 16.00 per cent disease transmission. The rate of disease transmission was 32.00 and 56.00 per cent, respectively at 24 h and 48 h of inoculation access periods.

No bud necrosis disease transmission was observed by adult *T. palmi* at 30 min, 1 h, 2 h, 4 h, 6 h, and 8 h IAP. A minimum of 24 h inoculation access period was observed with 8.00 per cent of disease transmission and no further increase of disease transmission was observed at 48 h of inoculation access period.

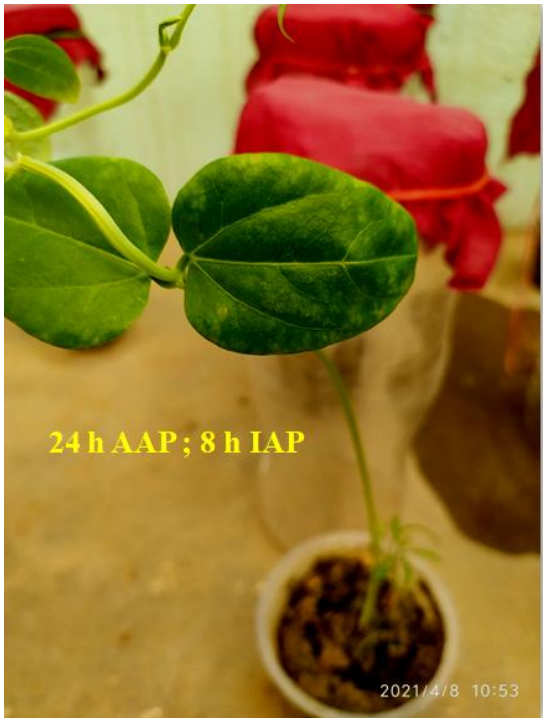


Plate 4.52. Symptom expression of bud necrosis disease after 15 DAT

Plate 4.53. Symptom expression of bud necrosis disease after 15 DAT

Table 4.17. Minimum inoculation access period required for *T. palmi* to transmit BND

Stage of the insect tested for different IAP	Per cent bud necrosis disease transmission by *T. palmi* at different IAP with 24 h common AAP							
	30 min	**1 h**	**2 h**	**4 h**	**6 h**	**8 h**	**24 h**	**48 h**
First Instar	0.00	0.00	0.00	12.00	12.00	20.00	52.00	96.00
Second instar	0.00	0.00	0.00	0.00	0.00	16.00	32.00	56.00
Adult	0.00	0.00	0.00	0.00	0.00	0.00	8.00	8.00

4.3.3.1.3 Number of thrips required for transmission of GBNV

Experiment was conducted to know the no. of first instar, second instar nymphs and number of adults of *T. plami* was required to transmit the bud necrosis disease.

A) At 24 h of AAP and 48 h IAP: The results were depicted in the Table 4.18; Figure 4.18. From the table it was apparent that a minimum number of two first instar larvae were sufficient to transmit the disease with 13.33 per cent disease transmission. With increasing number of first instar larvae the rate of disease transmission also increased *i.e.,* 33.33, 73.33, 100.00, 100.00 with 4, 8, 10, 15 larv-ae per plant. Whereas second instar larvae showed slight different response where in a minimum of 10 larvae were required for 46.67 per cent disease transmission and 53.33 per cent disease transmission was observed with 15 second instar larvae per plant and no further increase of disease was observed with increase in number of second instar larvae. Similarly, a minimum of 10 adults were required for 6.67 per cent disease transmission. No further increase of disease was observed with 15 adults per plant. Our present findings were in agreement with Ruth (2018) who reported that a single adult *T. palmi* could transmit the virus with a transmission rate of 24 to 32 percent and maximum transmission rate (100 %) was achieved with ten adults per seedling.

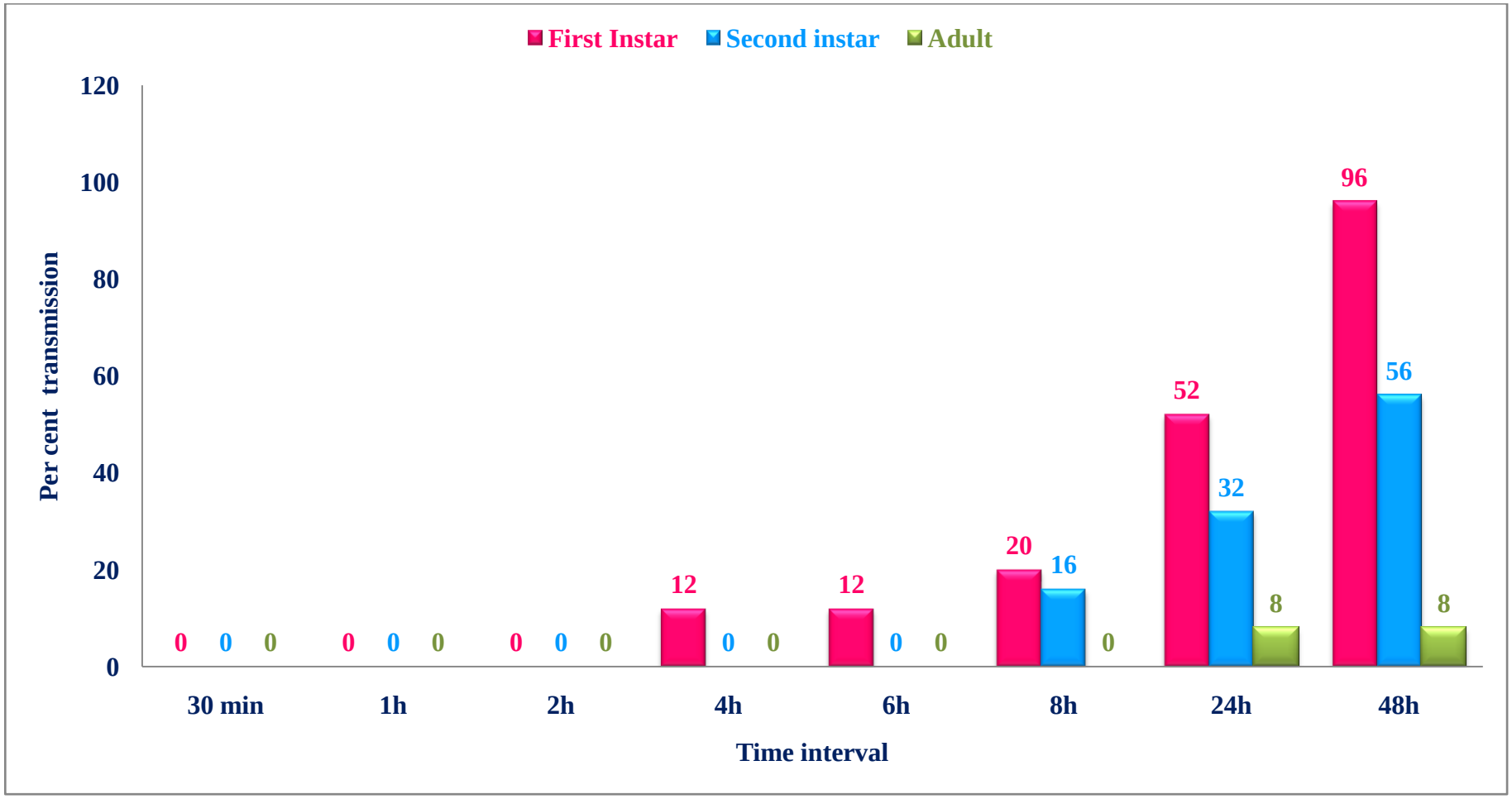


Figure 4.17. Minimum inoculation access period required for *T. palmi* to transmit bud necrosis disease

Table 4.18. Number of *T. palmi* required *to* transmit bud necrosis disease in blackgram at 24 h AAP and 48 h IAP

No. of *T. palmi* per plant	Mean percent disease transmission at 24 h AAP and 48 h IAP		
	First Instar	Second instar	Adult
2	13.33	0.00	0.00
4	33.33	0.00	0.00
8	73.33	0.00	0.00
10	100.00	46.67	6.67
15	100.00	53.33	6.67

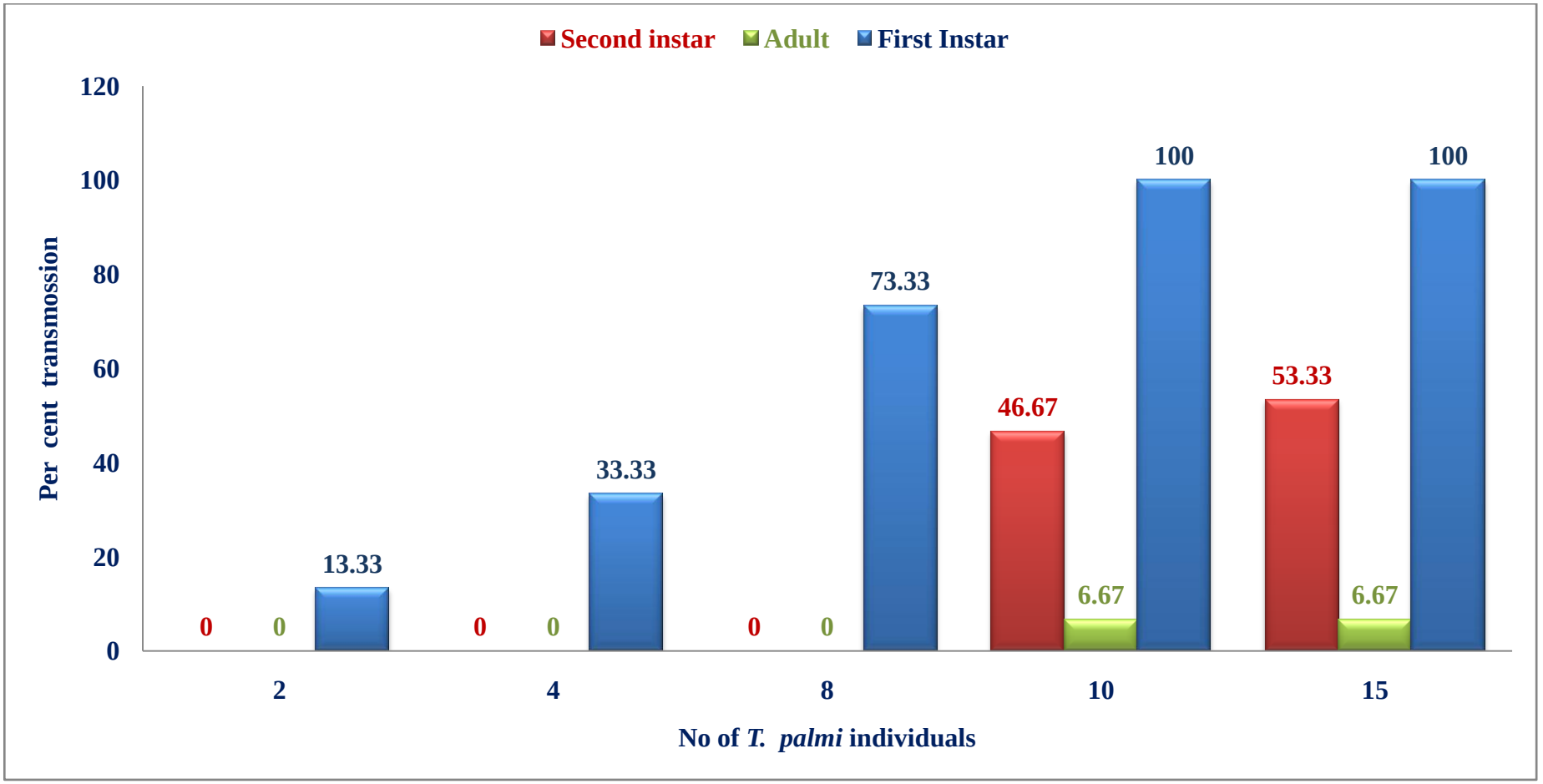


Figure 4.18. Number of *T. palmi* required to transmit bud necrosis disease in blackgram at 24 h AAP and 48 h IAP

Vijayalakshmi, (1994), also reported similar kind of results that a single *T. palmi* adult was able to transmit PBNV on groundnut and the maximum (100 %) was achieved with 10 adults.

B) At 48 h of AAP and IAP: The results were depicted in the Table 4.19; Figure 4.19. From the table it was evident that a minimum number of two first instar larvae were sufficient to transmit the disease with 33.33 per cent disease transmission at 48 h AAP and IAP. With increasing number of first instar larvae the rate of disease transmission also increased to 40.00, 73.33, 100.00 and 100.00 per cent with 4, 8, 10, 15 larvae per plant (Plate 4.54). Similarly, second instar larvae showed similar response that a minimum of two larvae were necessary for 13.33 per cent disease transmission. With increasing number of larvae *i.e*. 4, 8, 10, 15 per plant, the rate of disease transmission was 13.33, 20.00, 33.33, and 60.00 per cent. Where as a minimum of 10 adults were necessary for 6.67 per cent disease transmission and 13.33 per cent disease transmission was observed when 15 adults per plant were released.

The present findings pertaining to bud necrosis disease transmission by second instar larva (AAP, IAP, number of insects required for transmission) were in accordance with Wetering *et al*. (1996) who reported that larval acquisition of the virus was an essential determinant of adult vector competency, and furthermore,

Table 4.19. Number of *T. palmi* required to transmit bud necrosis disease in blackgram at 48 h AAP and IAP

No. of *T. palmi* per plant	Mean percent disease transmission at 48 h AAP and IAP		
	First Instar	Second instar	Adult
2	33.33	13.33	0.00
4	40.00	13.33	0.00
8	73.33	20.00	0.00
10	100.00	33.33	6.67
15	100.00	60.00	13.33

acquisition rates decreased as larval thrips develop. Nagata *et al*. (1999), Nagata *et al*. (2002), Wetering *et al*. (1996) also reported that when first instar larvae were allowed to acquire TSWV, 47 % of the concomitant adults transmitted virus where as only 12

per cent of adults from cohorts acquiring virus as second instars were able to inoculate plants.

The present findings pertaining to adult transmission (AAP, IAP, number of insects required for transmition) were contrary to the previous reports. Sakimura (1962); Ullman *et al*. (1989); Ullman *et al*. (1992b); Wetering *et al*. (1996) reported that adult thrips that feed on infected plants did not become viruliferous under lengthy feeding on *Tospovirus*-infected plants.

Electron microscopic observations by Ullman *et al*. (1992b) showed that virions were present in midgut epithelial cells of adult *F. occidentalis* shortly after the acquisition access period (AAP). The work of Ullman *et al*. (1992b) and Nagata *et al*. (1999) with TSWV-MT2 and BR01, respectively, suggested that persistent infection of adult midgut cells is a rare occurrence. Work with different isolates of TSWV showed that virus can infect midgut cells (Ohnishi *et al*., 2001) and, in some cases, spreads to muscle cells in insects that fed as adults (Filho *et al*., 2004).

Ohnishi *et al*. (2001) also found that TSWV (originally isolated from pepper in Japan) infected adult *Thrips setosus* midguts, and by three days post- AAP, the infection spread through the insect midgut. By five days post AAP, the infection had diminished; furthermore, they found that virus was unable to spread beyond the basal lamina of adult thrips midguts. These results suggested that the basal lamina serves as a potential barrier to virus movement out of the insect midgut.

Other researchers, working with a TSWV isolate recently isolated from peanut in Georgia, found that TSWV infected adult midguts (Filho *et al*., 2004). Adult *F. occidentalis* and *F. fusca* sustained midgut infections and the virus also spread to the surrounding muscle tissue. Virus was not found in ligament-like tissue or salivary glands, hence thrips given AAP as adults were unable to transmit. Importantly, all these studies agree that adult thrips, whether they support midgut infection or not, are unable to transmit TSWV and virus infection does not spread to the salivary gland unless acquisition initially occurs during the larval stages of life. These experiments were all performed with different isolates of TSWV, and these isolates varied in their ability to initiate successful infection of adult midguts, escape the midgut, and infect muscles.

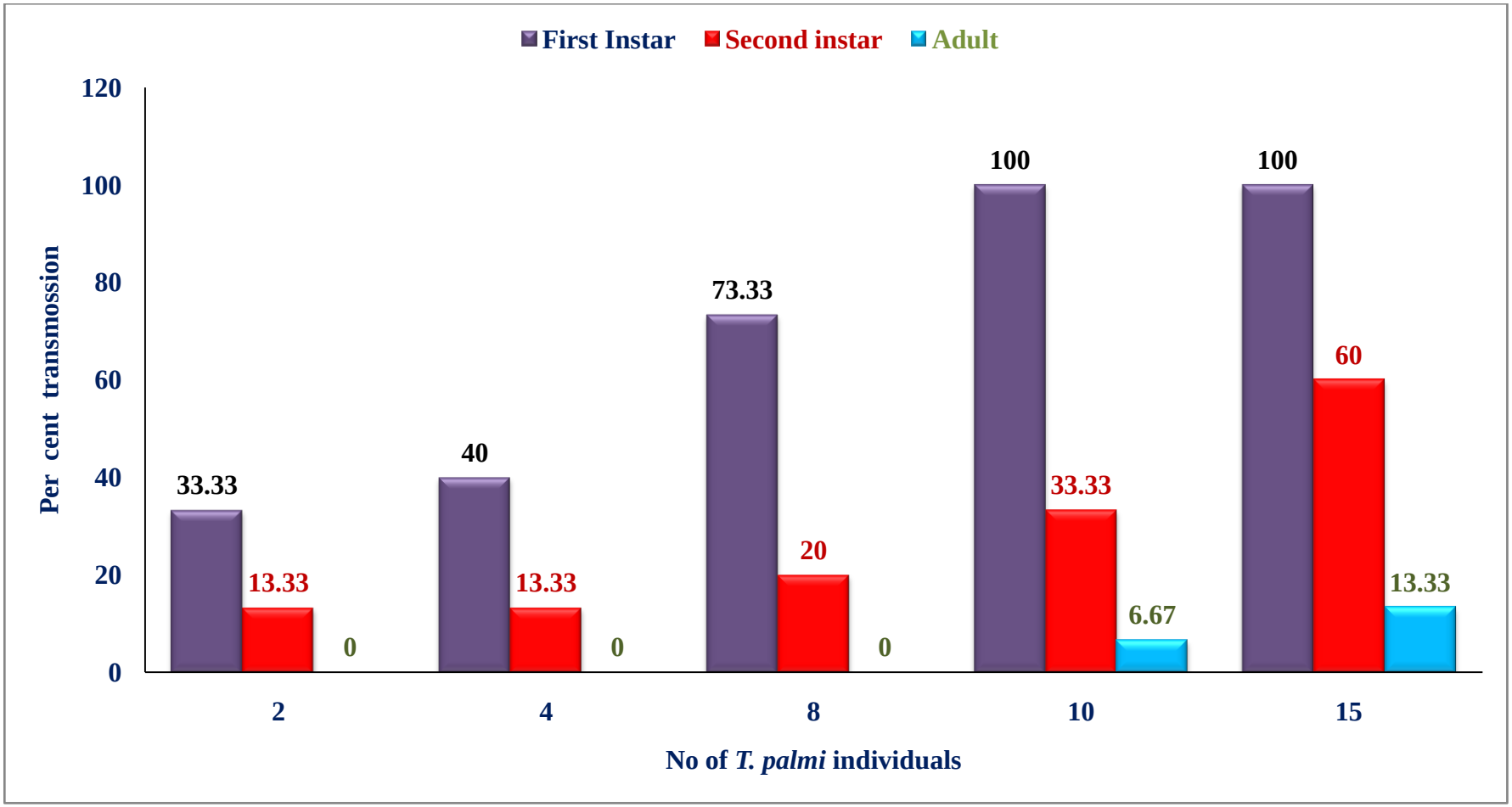


Figure 4.19. Number of *T. palmi* required to transmit bud necrosis disease in blackgram at 48 h AAP and 48 h IAP

The meager transmission by adult thrips can be attributed to variations in virus genotype, thrips genotype, and environmental conditions, crop phenology which are likely play a critical role in these differential interactions. The genotype of present study isolate *i.e.* GBNV-BG from Andhra Pradesh may contain certain genetic alternations in relation with particular thrips genotype (*T. palmi*). As vector specificity between thrips species and virus isolates does occur (Naidu *et al*., 2004 and Wetering *et al*., 1996). The barriers contributing to vector specificity may vary with vector species and virus isolate, as has been observed in other virus-vector interactions, particularly the persistently transmitted *Luteoviruses* (Gray and Gildow, 2003).

Plate 4.54. Symptom expression of bud necrosis disease after 15 DAT

Hence, the present study confirms that thrips transmits the GBNV-BG isolate in persistent propagative manner as it acquires the virus in larval stage and transmitted after attaining the adult stage. Acquiring and transmitting the virus by the adults in the present study throwing new lights on latent period biology of GBNV-BG isolate.

4.3.4 Confirmation of Virus Presence after Transmission Studies Through Reverse Transcriptase Polymerase Chain Reaction (RTPCR)

Post conformational study regarding presence of GBNV in symptom expressed plants was carried out through RTPCR using GBNV nucleocapsid protein gene specific markers and the results indicated the presence of GBNV in leaves. Gel electrophoresis stuides revealed that diseased leaves showed a clear amplicon size of ~830 bp, where as no amplicons were observed in case of healthy samples (Plate 4.55).

The present findings are in accordance with Suganyadevi *et al*. (2018) who reported the presence of GBNV in cowpea leaves through RT-PCR assay using GBNV nucleocapsid protein gene-specific primers where in symptomatic leaves showed amplification of 831 bp and non-symptomatic leaves showed no amplification. Renuka *et al*. (2020) Kareem and Byadgi (2017), Gurupad and patil (2013) also reported successful amplification of GBNV at ~ 831bp using nucleocapsid protien gene specific primers through RTPCR in tomato, chilli, and brinjal, groundnut crops. Similarly, Gurupad and Patil (2014) reported amplification of 831 bp GBNV-CP of tomato bud blight field sample through RT-PCR using degenerated preimers. Similarly, Sujitha *et al*. (2012) in onion; Akram and Naimuddin (2013) in cowpea also reported on confirmation of GBNV in symptomatic leaves through RT-PCR study using coat protein gene specific primers of GBNV, which resulted in an amplicon of ~ 800 bp.

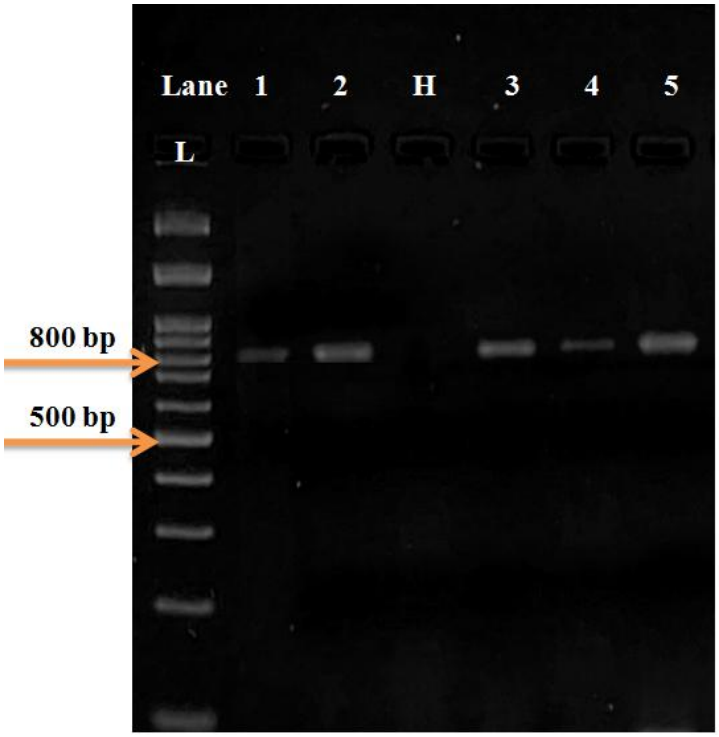


Plate 4.55. Conformational study of GBNV in indicator host cowpea under glass house conditions. Electrophoretic separation of GBNV with Coat protein primer (~830 bp)

Lane L = Ladder; Lane 1, 2, 3, 4 = Cowpea leaves expressed disease symptoms of bud necrosis after transmission studies; Lane H = Healthy leaves.

4.4 MANAGEMENT OF THRIPS IN BLACKGRAM THROUGH INSECTICIDES

The results pertaining to the above study *i.e.*, the number of thrips per plant, per cent bud necrosis disease incidence and incremental benefit cost ratio were presented here under and discussed in the light of available literature.

4.4.1 Efficacy of Insecticides against Thrips Population after the First Spray during *Rabi* 2019-2020

The data from Table 4.20 revealed that the mean number of thrips per plant was in the range of 0.20 to 3.98 at one day before the first spray with significant difference among the treatments which showed the effect of seed treatment. After treatment spraying, data was recorded on 1st, 3rd, 7th and 10th day. All the treatments were found significantly superior over untreated control (4.85 thrips per plant) at 1 DAS (days after spray) and the data indicated that the treatment imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior among treatments as it has recorded the lowest mean thrips population per plant 0.13 and it was at par with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1}, imidacloprid seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1} and imidacloprid seed treatment + spinetoram 11.7 SC @ 50 g a.i ha^{-1} treatments which recorded 0.20, 0.23,0.30,0.33 and 0.40 mean thrips population per plant, respectively.

Among all tested treatments seed treatment alone was ineffective where in seed treatment with thiamethoxam 70 WS @ 70 g a.i ha^{-1} and imidacloprid 70 WG @ 70 g a.i ha^{-1} were found to be least effective treatments with 0.1 and 1.00 mean thrips population per plant, respectively. The per cent reduction of the thrips population over the untreated control was highest in imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (72.67). However the per cent thrips population reduction over untreated control ranged from 4.33 to 45.33 in remaining treatments.

It was evident that the data recorded on the number of thrips per plant at 3 DAS in all the treatments was significantly different from untreated control (5.10). Among the treatments imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior among the treated insecticides as it has recorded zero thrips

population per plant. Thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1} and thiamethoxam seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1} recorded 0.07, 0.10, 0.13, 0.23 mean thrips population, respectively which were on par. In the remaining treatments, number of thrips per plant ranged from 0.30 to 1.00. The highest per cent population reduction over control was recorded in imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (100). In remaining treatments per cent population reduction over untreated control ranged from 8.95 to 73.99.

Similarly, at 7 DAS, all the treatments were found superior over untreated control. Among the treatments imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} , thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1} and thiamethoxam seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1} were found superior with zero thrips population with cent per cent reduction over untreated control. Thiamethoxam seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1}, imidacloprid seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1}, imidacloprid seed treatment + spinetoram 11.7 SC @ 50 g a.i ha^{-1} recorded 0.17, 0.20 and 0.23 mean thrips population per plant, respectively. Seed treatment with thiamethoxam 70 WS @ 70 g a.i ha^{-1} and imidacloprid 70 WG @ 70 g a.i ha^{-1} were significantly inferior to all the other treatments with 8.95 and 13.29 per cent population reduction over untreated control, respectively.

Observations were recorded at 10 DAS, revealed that imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior over untreated control with 0.13 mean number of thrips population and this was at par with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1}, imidacloprid seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1} and imidacloprid seed treatment + spinetoram 11.7 SC @ 50 g a.i ha^{-1} with 0.17, 0.17, 0.21, 0.22 and 0.37 mean thrips per plant, respectively. Among the treatments per cent population reduction over untreated control was recorded highest in imidacloprid seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} (86.67). Remaining all treatments were in the

Table 4.20. Efficacy of insecticides against thrips population on blackgram after first spray during *rabi* 2019-2020

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1 DBS	1 DAS	3 DAS	7 DAS	10 DAS	Mean	1 DAS	3 DAS	7 DAS	10 DAS	Mean
1	Seed treatment with Imidacloprid 70 WG	70	0.90	1.00 $(1.41)^{h}$	1.00 $(1.41)^{j}$	1.00 $(1.41)^{gh}$	2.00 $(1.73)^{f}$	1.25 $(1.73)^{f}$	8.89	13.29	33.67	11.11	16.67
2	Seed treatment with Thiamethoxam 70 WS	70	0.78	0.91 $(1.38)^{gh}$	0.91 $(1.38)^{ii}$	1.10 $(1.45)^{h}$	1.93 $(1.71)^{f}$	1.21 $(1.71)^{f}$	4.33	8.95	15.81	1.03	6.73
3	T1+ Flonicamid 50 WG	75	0.43	0.33 $(1.15)^{abc}$	0.30 $(1.14)^{bcde}$	0.20 $(1.09)^{abc}$	0.37 $(1.17)^{abcd}$	0.30 $(1.17)^{abcd}$	36.92	45.97	72.45	66.15	58.46
4	T1+ Diafenthiuron 50 WP	300	0.60	0.57 $(1.25)^{cdef}$	0.53 $(1.24)^{efgh}$	0.50 $(1.22)^{cdef}$	0.50 $(1.22)^{bcde}$	0.53 $(1.22)^{bcde}$	22.56	30.63	50.25	66.67	47.50
5	T1+ Fipronil 5 SC	50	0.40	0.13 $(1.06)^{a}$	0.00 $(1.00)^{a}$	0.00 $(1.00)^{a}$	0.13 $(1.06)^{a}$	0.07 $(1.06)^{a}$	72.67	100.00	100.00	86.67	90.00
6	T1+ Buprofezin 25 SC	250	0.90	0.77 $(1.33)^{fgh}$	0.77 $(1.33)^{hij}$	0.73 $(1.32)^{fgh}$	0.73 $(1.32)^{e}$	0.75 $(1.32)^{e}$	30.15	33.52	51.36	67.41	50.00
7	T1+ Spinetoram 11.7 SC	50	0.43	0.40 $(1.18)^{abcde}$	0.37 $(1.17)^{cdef}$	0.23 $(1.11)^{abcd}$	0.37 $(1.17)^{abcd}$	0.34 $(1.17)^{abcd}$	24.31	33.97	67.85	66.15	52.69
8	T1+ Spinosad 45 SC	72	0.20	0.20 $(1.09)^{ab}$	0.10 $(1.05)^{ab}$	0.00 $(1.00)^{a}$	0.17 $(1.08)^{a}$	0.12 $(1.08)^{ab}$	18.00	60.98	100.00	66.67	65.00
9	T2+ Flonicamid 50 WG	75	0.30	0.30 $(1.14)^{abc}$	0.23 $(1.11)^{abcd}$	0.17 $(1.08)^{abc}$	0.22 $(1.10)^{ab}$	0.23 $(1.10)^{ab}$	18.00	39.30	66.83	71.11	54.17
10	T2+ Diafenthiuron 50WP	300	0.77	0.67 $(1.29)^{defgh}$	0.63 $(1.28)^{fghi}$	0.60 $(1.26)^{defg}$	0.60 $(1.26)^{cde}$	0.63 $(1.26)^{cde}$	28.70	35.53	53.28	68.70	51.09
11	T2+ Fipronil 5 SC	50	0.20	0.13 $(1.06)^{a}$	0.07 $(1.03)^{ab}$	0.00 $(1.00)^{a}$	0.17 $(1.08)^{a}$	0.09 $(1.08)^{ab}$	45.33	73.99	100.00	66.67	72.50
12	T2+ Buprofezin 25 SC	250	0.73	0.70 $(1.30)^{efgh}$	0.67 $(1.29)^{ghi}$	0.65 $(1.28)^{efg}$	0.65 $(1.28)^{de}$	0.67 $(1.28)^{de}$	21.73	29.05	47.08	64.55	45.45
13	T2+ Spinetoram 11.7 SC	50	0.47	0.43 $(1.20)^{bcde}$	0.43 $(1.20)^{defg}$	0.37 $(1.17)^{bcdef}$	0.40 $(1.18)^{abcd}$	0.41 $(1.18)^{abcde}$	23.86	27.53	53.09	65.71	47.50
14	T2+ Spinosad 45 SC	72	0.27	0.23 $(1.11)^{ab}$	0.13 $(1.06)^{abc}$	0.00 $(1.00)^{a}$	0.21 $(1.10)^{ab}$	0.15 $(1.10)^{ab}$	28.25	60.98	100.00	68.00	67.38
15	Untreated Control		3.98	4.85 $(2.41)^{i}$	5.10 $(2.47)^{k}$	6.67 $(2.76)^{i}$	8.90 $(3.15)^{h}$	6.38 $(3.15)^{g}$					
SEm ±			**0.05**	**0.04**	**0.04**	**0.05**	**0.04**	**0.05**					
CD ≤ 0.05			**0.15**	**0.12**	**0.11**	**0.16**	**0.12**	**0.14**					
CV (%)			**7.01**	**5.52**	**5.19**	**7.36**	**5.10**	**7.64**					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

range of 64.55 to 71.11. Least per cent population reduction over untreated control was recorded imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} *i.e.* 11.11 and 1.03, respectively.

The overall mean of the first spray (Table 4.20) revealed minimum thrips population in imidacloprid seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} (0.07 $plant^{-1}$) and was at par with thiamethoxam seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, imidacloprid seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1}, imidacloprid seed treatment + spinetoram 11.7 SC @ 50 g a.i ha^{-1} and thiamethoxam seed treatment + spinetoram 11.7 SC @ 50 g a.i ha^{-1} with 0.09, 0.12, 0.15, 0.23, 0.30, 0.34 mean thrips per plant, respectively.

Highest per cent population reduction over control was recorded in treatment imidacloprid seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} (90.0) followed by thiamethoxam seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} (72.50). Remaining treatments were in the range of 45.45 to 67.38 per cent. Seed treatments with imidacloprid 70 WG @ 70 g a.i ha^{-1} and thiamethoxam 70 WS @ 70 g a.i ha^{-1} were found least effective with only 16.67 and 6.73 per cent population reduction over untreated control.

4.4.1.1 Mean per cent bud necrosis disease incidence in blackgram after first spray during *rabi* 2019-2020 (Table 4.24, Figure 4.21, Plate 4.56): Per cent disease incidence was recorded at 15 days interval starting from 15 days after sowing. There was no diease incidence at 15 DAS. First spraying was given at 30 DAS and the PDI at 30 DAS was in the range of 2.03 to 3.95 in all treatment plots which was significantly different from untreated control (8.29) which may be due to the effect of seed treatment. From the collected data it was evident that all treatments were found superior over untreated control (16.33).

Least per cent disease incidence was recorded in the plot treated with imidacloprid seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} (3.28) and it was at par with imidacloprid seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1} and imidacloprid seed treatment + flonicamid 50 WG @ 75 g a.i ha^{-1} with

of 3.48, 3.78, 3.95, 4.52 and 4.61 per cent disease incidence, respectively. Remaining treatments were in the range of 5.97 to 10.20 per cent disease incidence.

4.4.2 Efficacy of Insecticides against Thrips Population after the Second Spray during *Rabi* 2019-2020

The data from the Table 4.21, revealed that mean thrips population was in the range of 2.60 to 4.85 one day before second spray application. After spray application, data was recorded at 1, 3,7,10 DAS (Plate 4.57). From the table it was found that all the treatments were found superior over untreated control at one DAS with 12.33 mean thrips population per plant.

Among the treatments, imidacloprid seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} was found superior with lowest mean thrips population (1.47) and it was at par with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with 1.77, 2.00 and 2.00 mean thrips population, respectively. Imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior over all the remaining treatments with highest per cent population reduction over control (44.30) followed by thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with 40.00 per cent reduction over untreated control. Remaining treatments showed 0.00 to 32.05 per cent population reduction over untreated control.

All the treatments were found superior over untreated control (12.50) at 3 DAS. Imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior among the treatments with zero thrips population and it was on par with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} with 0.27 mean number of thrips population per plant. Imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} was next to these treatments with 0.90 mean number of thrips population per plant.

Remaining all treatments was in the range of 0.97 and 4.85 thrips per plant. Per cent population reduction over untreated control was recorded highest (100.00) in imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}. Whereas remaining all treatments were in the range of 1.33 to 89.74. Least effective treatments were imidacloprid seed treatment + buprofezin 25 SC spray @ 250 g a.i ha^{-1}, thiamethoxam

Plate 4.56. Bud necrosis disease infestation at 40 DAS during *rabi* 2019-2020

Plate 4.57. Management field trial at 40 DAS during *rabi* 2019-2020

70 WS seed treatment @ 70 g a.i ha^{-1} with zero per cent reduction over untreated control.

After 7 DAS, it was evident that imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} found superior among the treatments with zero thrips population. Imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and imidacloprid seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} were also effective with 0.00, 0.00, 0.00, 0.43 and 0.67 mean number of thrips per plant, respectively.

Hundred per cent population reduction over untreated control was recorded in imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}. Thrips population reduction in the remaining all treatments was in the range of 0.07 to 86.73 per cent.

Similarly, at 10 DAS, data revealed that all the treatments were found superior over untreated control (14.90). Imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior with lowest mean number of thrips population *i.e.* 0.30 and it was at par with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} with mean number of thrips population of 0.43 per cent. Remaining all the treatments recorded thrips population in the range of 0.80 to 5.17 per plant. Per cent population reduction of thrips over untreated control was highest (90.89) in case of imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}. Next to this, thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was also found better with 86.67 per cent population reduction over untreated control. Remaining all treatments recorded 0.21 to 78.40 per cent population reduction of thrips population over untreated control.

The overall mean of second spray (Table 4.21) revealed that all the treatments were found superior over untreated control (13.06 thrips per plant). Among the treatments, imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior with lowest mean number of thrips *i.e.,* 0.44 $plant^{-1}$ and it was on par

with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with 0.62 and 0.93 mean number of thrips per plant, respectively. Least effective treatments were imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} with 4.95 and 5.17 mean number of thrips per plant, respectively. Mean per cent population reduction over untreated control was highest in case of imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} *i.e*. 84.91 followed by thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} with 78.65. Least effective treatments were thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} and imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} with 4.15 and 0.53 per cent reduction over untreated control.

4.4.2.1 Mean per cent disease incidence of bud necrosis in blackgram after second spray during *rabi* 2019-2020 : Bud necrosis disease incidence level in the treated plots at 60 DAS was presented in the Table 4.24 and Figure 4.21. From the collected data it was evident that all treatments were found superior over untreated control (25.10). Least per cent bud necrosis disease incidence was recorded in the plot treated with imidacloprid seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} (3.28) and it was at par with imidacloprid seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} and thiamethoxam seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1}, with 3.48, 3.78 and 3.95 mean per cent bud necrosis disease incidence respectively, while the remaining treatments showed in the range of 5.13 to 17.27 mean per cent disease incidence.

4.4.3 Efficacy of Insecticides against Thrips Population after the Third Spray during *Rabi* 2019-2020

From the Table 4.22, it was evident that all the treatments were found significant and superior over untreated control (14.92 mean no of thrips per plant) at 1 DAS. Among the treatments imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior with 0.63 mean number of thrips per plant and it was at par with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (0.90). Per cent population reduction over untreated control was highest (72.86) in case of imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}. The remaining treatments *viz.,* imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1}, thiamethoxam 70

Table 4.21. Efficacy of insecticides against thrips population on blackgram after second spray during *rabi* 2019-2020

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1DBS	1DAS	3DAS	7DAS	10DAS	Mean	1DAS	3DAS	7DAS	10DAS	Mean
1	Seed treatment Imidacloprid 70 WG	70	4.70	4.70 (2.38)^{e}	4.70 (2.38)^{e}	4.76 (2.38)^{f}	5.64 (2.57)^{g}	4.95 (2.58)^{f}	0.00	1.33	0.07	0.67	0.53
2	Seed treatment Thiamethoxam 70 WS	70	4.85	4.85 (2.41)^{e}	4.85 (2.41)^{e}	4.91 (2.42)^{f}	6.05 (2.65)^{g}	5.17 (2.66)^{f}	0.00	0.00	0.11	0.21	4.15
3	T1+ Flonicamid 50 WG	75	3.10	2.93 (1.98)^{cd}	1.17 (1.47)^{c}	0.67 (1.27)^{ab}	1.17 (1.47)^{cd}	1.48 (1.47)^{cd}	5.38	62.37	78.49	69.89	56.94
4	T1+ Diafenthiuron 50WP	300	3.33	3.00 (1.99)^{cd}	3.00 (1.99)^{d}	2.13 (1.77)^{de}	2.13 (1.77)^{e}	2.57 (1.77)^{e}	10.00	10.00	36.00	48.80	30.70
5	T1+ Fipronil 5SC	50	2.63	1.47 (1.57)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.30 (1.14)^{a}	0.44 (1.14)^{a}	44.30	100.00	100.00	90.89	84.91
6	T1+ Buprofezin 25 SC	250	3.03	3.03 (2.00)^{cd}	3.03 (2.00)^{d}	3.03 (2.00)^{e}	2.97 (1.98)^{f}	3.02 (1.99)^{e}	0.00	0.00	0.00	21.76	10.49
7	T1+ Spinetoram 11.7 SC	50	3.33	3.00 (2.00)^{cd}	2.33 (1.82)^{d}	1.08 (1.44)^{bc}	1.28 (1.51)^{cd}	1.93 (1.51)^{cd}	10.00	30.00	67.50	69.20	48.03
8	T1+ Spinosad 45SC	72	2.67	2.00 (1.73)^{abc}	0.90 (1.38)^{bc}	0.00 (1.00)^{a}	0.80 (1.34)^{bc}	0.93 (1.34)^{abc}	25.00	66	100	76.00	68.78
9	T2+ Flonicamid 50WG	75	3.27	2.50 (1.87)^{bcd}	1.10 (1.45)^{c}	0.43 (1.19)^{ab}	1.00 (1.41)^{c}	1.26 (1.41)^{bc}	23.47	66.33	86.73	75.51	65.33
10	T2+ Diafenthiuron 50WP	300	3.07	3.02 (2.00)^{cd}	3.00 (1.99)^{d}	2.33 (1.82)^{de}	2.33 (1.82)^{ef}	2.67 (1.83)^{e}	1.63	2.17	23.91	39.13	21.62
11	T2+ Fipronil 5 SC	50	2.60	1.77 (1.66)^{ab}	0.27 (1.11)^{ab}	0.00 (1.00)^{a}	0.43 (1.19)^{ab}	0.62 (1.20)^{ab}	32.05	89.74	100.00	86.67	78.65
12	T2+ Buprofezin 25 SC	250	3.67	3.03 (2.01)^{d}	3.03 (2.01)^{d}	2.90 (1.97)^{e}	2.90 (1.97)^{f}	2.97 (1.97)^{e}	17.27	17.27	20.91	36.73	27.18
13	T2+ Spinetoram 11.7 SC	50	3.00	3.00 (2.00)^{cd}	2.80 (1.95)^{d}	1.47 (1.57)^{cd}	1.73 (1.65)^{de}	2.25 (1.65)^{de}	0.00	6.67	51.11	53.78	32.50
14	T2+ Spinosad 45 SC	72	3.33	2.00 (1.73)^{abc}	0.97 (1.40)^{c}	0.00 (1.00)^{a}	0.90 (1.38)^{bc}	0.97 (1.38)^{bc}	40.00	71.00	100.00	78.40	73.90
15	Untreated Control		12.33	12.33 (3.64)^{f}	12.50 (3.67)^{f}	12.50 (3.67)^{g}	14.90 (3.99)^{h}	13.06 (3.99)^{g}					
SEm ±			**0.13**	**0.09**	**0.10**	**0.09**	**0.07**	**0.08**					
CD ≤ 0.05			**0.38**	**0.27**	**0.28**	**0.27**	**0.19**	**0.23**					
CV (%)			**10.53**	**7.85**	**8.95**	**9.37**	**6.17**	**8.58**					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

WS seed treatment @ 70 g a.i ha^{-1}, imidacloprid seed treatment + diafenthiuron 50 WP spray @ 300 g a.i ha^{-1}, imidacloprid seed treatment + buprofezin 25 SC spray @ 250 g a.i ha^{-1}, thiamethoxam seed treatment + diafenthiuron 50 WP spray @ 300 g a.i ha^{-1}, thiamethoxam seed treatment + buprofezin 25 SC spray @ 250 g a.i ha^{-1} , thiamethoxam seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1} showed no reduction of population over untreated control.

Imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} were found superior among the other treatments at 3 DAS with zero population of thrips per plant with cent per cent reduction over untreated control. Imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded 0.27 mean number of thrips per plant while the remaining treatments were in the range of 0.93 to 9.67 thrips per plant. Least effective treatments were imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} with no reduction in thrips population over untreated control.

The results at 7 DAS, revealed that all the treatments were found superior over untreated control (15.10 thrips pr plant). Among the treatments, zero number of mean thrips per plant was recorded in case of imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with hundred per cent reduction over untreated control while the remaining treatments were in the range of 1.02 to 9.67 mean number of thrips per plant. Least per cent population reduction over untreated control *i.e.*1.16 and 1.19 were observed in the case of thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1}, imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1}, respectively.

Similar trend was observed at 10 DAS. Among the treatments tested there was no incidence of thrips in case of imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with hundred per cent reduction over untreated control and these were at par with thiamethoxam seed

treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and imidacloprid seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} with 0.05 and 0.35 mean number of thrips per plant, respectively. Thiamethoxam seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} recorded 98.37 per cent population reduction over untreated control. Least per cent population reduction over untreated control *i.e.* 0.17 and 0.28 were observed in the case of thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} and imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1}, respectively.

The overall mean of third spray revealed that all the treatments were found superior over untreated control (14.92 thrips per plant). Among all the treatments, imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior with lowest mean number of thrips (0.16) $plant^{-1}$ and it was at par with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and imidacloprid seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} with 0.23, 0.48,0.77,0.91 and 1.46 mean number of thrips $plant^{-1}$, respectively. Per cent population reduction over untreated control was highest in case of imidacloprid seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (93.21) followed by thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (91.56). The least per cent population reduction over untreated control *i.e.* 0.07 and 0.37 were observed in case of thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} and imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1}, respectively.

4.4.3.1 Mean per cent bud necrosis disease incidence in blackgram after third spray during *rabi* 2019-2020 : Bud necrosis disease incidence in the treated plots at 75 DAS was depicted in the Table 4.24 and Figure 4.21. From the collected data it was evident that all treatments were found superior over untreated control (25.10 %). Least per cent bud necrosis disease incidence in blackgram was recorded in the plots treated with imidacloprid seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} (3.28) and it was at par with imidacloprid seed treatment + spinosad 45 SC @ 72 g a.i $ha^{-}1$, thiamethoxam seed treatment + fipronil 5 SC @ 50 g a.i ha^{-1} and thiamethoxam seed treatment + spinosad 45 SC @ 72 g a.i ha^{-1} with 3.48, 3.78 and 3.95 mean per cent

Table 4.22. Efficacy of insecticides against thrips population on blackgram after third spray during *rabi* 2019-2020

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1DBS	1DAS	3DAS	7DAS	10DAS	Mean	1DAS	3DAS	7DAS	10DAS	Mean
1	Seed treatment with Imidacloprid 70 WG	70	8.27	8.27 (3.04)^{g}	8.27 (3.04)^{g}	8.27 (3.04)^{f}	8.15 (3.02)^{g}	8.24 (3.02)^{g}	0.00	0.00	1.19	0.28	0.37
2	Seed treatment with Thiamethoxam 70 WS	70	9.67	9.67 (3.27)^{g}	9.67 (3.27)^{g}	9.67 (3.27)^{f}	9.65 (3.26)^{h}	9.66 (3.26)^{h}	0.00	0.00	1.16	0.17	0.07
3	T1+ Flonicamid 50 WG	75	3.20	2.93 (1.98)^{de}	1.55 (1.59)^{cde}	1.02 (1.42)^{b}	0.35 (1.14)^{ab}	1.46 (1.16)^{ab}	8.33	51.56	68.61	89.06	54.30
4	T1+ Diafenthiuron 50WP	300	3.93	3.93 (2.22)^{ef}	3.30 (2.05)^{f}	2.30 (1.79)^{d}	1.97 (1.71)^{de}	2.88 (1.72)^{de}	0.00	16.10	41.53	50.00	26.91
5	T1+ Fipronil 5SC	50	2.33	0.63 (1.28)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.16 (1.00)^{a}	72.86	100.00	100.00	100.00	93.21
6	T1+ Buprofezin 25 SC	250	4.33	4.33 (2.31)^{f}	4.05 (2.25)^{f}	3.90 (2.21)^{e}	3.80 (2.19)^{f}	4.02 (2.19)^{f}	0.00	6.54	10.00	12.31	7.21
7	T1+ Spinetoram 11.7 SC	50	4.17	3.50 (2.12)^{ef}	1.88 (1.69)^{de}	1.30 (1.51)^{bc}	0.83 (1.35)^{bc}	1.88 (1.35)^{bc}	16.00	54.80	68.80	80.00	54.90
8	T1+ Spinosad 45SC	72	3.27	1.67 (1.61)^{bc}	0.27 (1.11)^{ab}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.48 (1.00)^{a}	48.98	91.84	100.00	100.00	85.20
9	T2+ Flonicamid 50WG	75	3.07	2.40 (1.83)^{cd}	1.05 (1.43)^{cd}	0.13 (1.06)^{a}	0.05 (1.02)^{a}	0.91 (1.02)^{a}	21.74	65.76	95.65	98.37	70.38
10	T2+ Diafenthiuron 50WP	300	4.00	4.00 (2.23)^{ef}	3.93 (2.22)^{f}	3.40 (2.09)^{e}	2.33 (1.82)^{e}	3.42 (1.83)^{e}	0.00	1.67	15.00	41.67	14.58
11	T2+ Fipronil 5 SC	50	2.67	0.90 (1.38)^{ab}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.23 (1.00)^{a}	66.25	100.00	100.00	100.00	91.56
12	T2+ Buprofezin 25 SC	250	4.03	4.03 (2.24)^{ef}	4.00 (2.23)^{f}	3.53 (2.13)^{e}	3.33 (2.08)^{f}	3.73 (2.08)^{f}	0.00	0.83	12.40	17.36	7.64
13	T2+ Spinetoram 11.7 SC	50	3.53	3.53 (2.13)^{ef}	2.07 (1.75)^{e}	2.00 (1.73)^{cd}	1.33 (1.52)^{cd}	2.23 (1.53)^{cd}	0.00	41.51	43.40	62.26	36.79
14	T2+ Spinosad 45 SC	72	3.50	2.13 (1.77)^{cd}	0.93 (1.39)^{bc}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.77 (1.00)^{a}	39.05	73.33	100.00	100.00	78.10
15	Untreated Control		14.92	14.92 (3.99)^{h}	14.92 (3.99)^{h}	15.10 (4.01)^{g}	14.75 (3.97)^{i}	14.92 (3.97)^{i}					
SEm ±			**0.10**	**0.09**	**0.10**	**0.08**	**0.08**	**0.07**					
CD ≤ 0.05			**0.30**	**0.27**	**0.29**	**0.24**	**0.22**	**0.21**					
CV (%)			**7.58**	**7.29**	**8.60**	**7.57**	**7.23**	**7.56**					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

disease incidence, respectively. Remaining treatments recorded 5.13 to 20.27 mean per cent disease incidence.

4.4.4 Cumulative Efficacy of Insecticides against Thrips Population after Three Sprays during *Rabi* 2019-2020

The overall mean after three sprayings (Table 4.23, Figure 4.20) indicated that all the treatments were found effective against thrips population on blackgram over untreated control (11.45 thrips per plant). Among the treatments, imidacloprid 70WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior with lowest mean number of thrips per plant (0.22) and it was on par with thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}, imidacloprid seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and imidacloprid seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1} with 0.31, 0.51, 0.63, 0.80, 1.08 and 1.38 mean number of thrips per plants, respectively.

Highest mean per cent population reduction over untreated control was recorded 89.37 in case of imidacloprid 70WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}. The next best was thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} which recorded 80.91 mean per cent population reduction over untreated control. Remaining treatments showed 3.65 to 73.00 mean per cent population reduction over untreated control. Least mean per cent population reduction (3.65 and 5.85) over untreated control was recorded in treatments thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} and imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1}, respectively.

Present findings pertaining to efficacy of imidacloprid and thiamethoxam were in accordance with Radhika *et al.* (2018) who reported that imidacloprid 70 WS at 5 g kg^{-1} was found most effective treatment with 2.67 thrips per six leaves among all the other tested insecticides followed by thiamethoxam 25 WG at 3 g kg^{-1} with 2.80 thrips per six leaves as seed treatment against thrips population in blackgram during *rabi* 2017-18 in Hyderabad. Abhijit *et al*. (2018) also reported that seed treatment with imidacloprid 48.0 FS @ 5 mL kg^{-1} seed + thiamethoxam 25 WG @ 50 g a.i. ha^{-1} at 30 DAS was found to be the most effective with minimum population of thrips (4.3 and

Table 4.23. Cumulative efficacy of insecticides against thrips population on blackgram during *rabi* 2019-2020

T. No.	Treatments	Dose g.a.i./ha	Mean No of thrips per plant				Population reduction over untreated control (%)			
			First spray	Second Spray	Third Spray	Mean	First spray	Second Spray	Third Spray	Mean
1	Seed treatment with Imidacloprid 70 WG	70	1.25 (1.73)^{f}	4.95 (2.58)^{f}	8.24 (3.02)g	4.81 (2.33)^{f}	16.67	0.53	0.37	5.85
2	Seed treatment with Thiamethoxam 70 WS	70	1.21 (1.71)^{f}	5.17 (2.66)^{f}	9.66 (3.26)h	5.35 (2.41)^{f}	6.73	4.15	0.07	3.65
3	T1+ Flonicamid 50 WG	75	0.30 (1.17)^{abcd}	1.48 (1.47)^{cd}	1.46 (1.16)ab	1.08 (1.43)^{abcde}	58.46	56.94	54.30	56.56
4	T1+ Diafenthiuron 50WP	300	0.53 (1.22)^{bcde}	2.57 (1.77)^{e}	2.88 (1.72)de	1.99 (1.70)^{c}	47.50	30.70	26.91	35.04
5	T1+ Fipronil 5SC	50	0.07 (1.06)^{a}	0.44 (1.14)^{a}	0.16 (1.00)a	0.22 (1.10)^{a}	90.00	84.91	93.21	89.37
6	T1+ Buprofezin 25 SC	250	0.75 (1.32)^{e}	3.02 (1.99)^{e}	4.02 (2.19)f	2.60 (1.86)^{e}	50.00	10.49	7.21	22.57
7	T1+ Spinetoram 11.7 SC	50	0.34 (1.17)^{abcd}	1.93 (1.51)^{cd}	1.88 (1.35)bc	1.38 (1.52)^{abcde}	52.69	48.03	54.90	51.87
8	T1+ Spinosad 45SC	72	0.12 (1.08)^{ab}	0.93 (1.34)^{abc}	0.48 (1.00)a	0.51 (1.22)^{ab}	65.00	68.78	85.20	73.00
9	T2+ Flonicamid 50WG	75	0.23 (1.10)^{ab}	1.26 (1.41)^{bc}	0.91 (1.02)a	0.80 (1.33)^{abcd}	54.17	65.33	70.38	63.29
10	T2+ Diafenthiuron 50WP	300	0.63 (1.26)^{cde}	2.67 (1.83)^{e}	3.42 (1.83)e	2.24 (1.76)^{d}	51.09	21.62	14.58	29.10
11	T2+ Fipronil 5 SC	50	0.09 (1.08)^{ab}	0.62 (1.20)^{ab}	0.23 (1.00)a	0.31 (1.14)^{a}	72.50	78.65	91.56	80.91
12	T2+ Buprofezin 25 SC	250	0.67 (1.28)^{de}	2.97 (1.97)^{e}	3.73 (2.08)f	2.45 (1.82)^{e}	45.45	27.18	7.64	26.76
13	T2+ Spinetoram 11.7 SC	50	0.41 (1.18)^{abcde}	2.25 (1.65)^{de}	2.23 (1.53)cd	1.63 (1.60)^{b}	47.50	32.50	36.79	38.93
14	T2+ Spinosad 45 SC	72	0.15 (1.10)^{ab}	0.97 (1.38)^{bc}	0.77 (1.00)a	0.63 (1.27)^{abc}	67.38	73.90	78.10	73.12
15	Untreated Control		6.38 (3.15)^{g}	13.06 (3.99)^{g}	14.92 (3.97)i	11.45 (3.49)^{g}				
SEm ±			**0.05**	**0.08**	**0.07**	**0.15**				
CD ≤ 0.05			**0.14**	**0.23**	**0.21**	**0.45**				
CV (%)			**7.64**	**8.58**	**7.56**	**15.43**				

Values in parenthesis are * square root (√x+1) transformations; In a column, means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

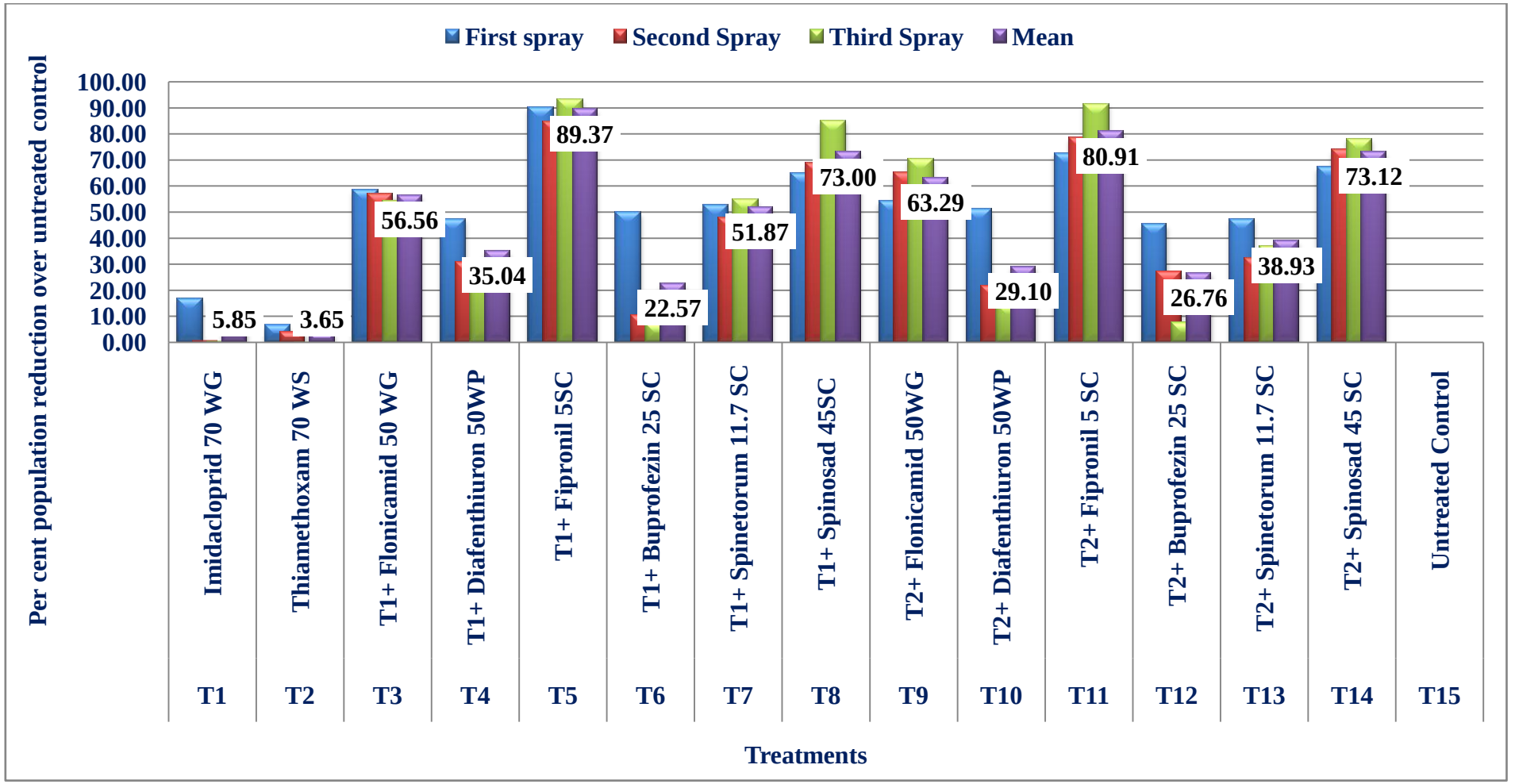


Figure 4.20. Cumulative efficacy of insecticides against thrips population on blackgram during *rabi* 2019-2020

8.0 numbers /10 flowers) as against 32.5 and 44 numbers /10 flowers in untreated control during 2015 and 2016, respectively. Somasundar *et al.* (2016) also reported that thiamethoxam seed treatment @ 8.6 g kg^{-1} and 4.3 g kg^{-1} was highly effective against thrips up to 30 days after germination in greengram during *rabi* 2008. Similarly, Kaushik *et al*. (2015) reported that seed treatment with thiamethoxam 30 F S + spray with imidacloprid 17.8 SL was found most effective in suppressing thrips population with 55.1 per cent reduction.

The results regarding the efficacy of fipronil 5SC spray @ 50 g a.i ha^{-1} can supported by the findings of Radhika *et al.* (2018c) who reported that fipronil 5 % SC @ 1 mL L^{-1} at weekly intervals against sucking pests in blackgram saved 269 kg ha^{-1} pod yield with an avoidable yield loss of 26.16 per cent. Similarly, Dongarjal *et al*. (2018) reported the superiority of fipronil 5 SC @ 50 mL a.i. ha^{-1} (1.33 and 1.63 thrips /fruit), over other treatments followed by thiamethoxam 70 WG @ 25 g a.i. ha^{-1} (1.63 and 1.92 thrips /fruit), respectively against thrips in pomegranate.

The results regarding the efficacy of spinosad 45 SC spray @ 72 g a.i ha^{-1} were in line with findings of Surbhi *et al.* (2018) who reported that insecticidal spray of spinosad 45 SC @ 0.0135 % was found effective with lowest number of thrips *i.e.* 0.28 thrips/leaf on greengram with highest seed yield in thiamethoxam 25 WG @ 0.10 % + spinosad 45 SC @ 0.0135 % treatments with 1066 kg ha^{-1} followed by imidacloprid 30.5 SC @ 0.12 % + spinosad 45 SC @ 0.0135 % with 1025 kg ha^{-1}.

4.4.4.1 Mean per cent disease incidence of bud necrosis in blackgram after three sprays during *rabi* 2019-2020 : Overall mean per cent bud necrosis disease incidence in blackgram (Table 4.24, Figure 4.21) was lowest (3.24) in case of imidacloprid 70WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and it was on par with imidacloprid seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, imidacloprid seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and thiamethoxam seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} with 3.45, 3.78, 3.95, 4.66 and 4.70 with mean per cent disease incidence, respectively. Remaining treatments recorded 5.65 to 12.66 mean per cent disease incidence.

Table 4.24. Effect of insecticidal treatments tested for thrips management on the bud necrosis disease incidence in blackgram during *rabi* 2019-2020

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Overall mean
T1 (Seed treatment with imidacloprid 70 WG)	0.00	3.93 (2.21)^{b}	10.20 (3.34)^{f}	16.80 (4.21)^{h}	18.10 (4.36)^{i}	12.26 (4.37)^{h}
T2 (Seed treatment with thiamethoxam 70 WS)	0.00	3.68 (2.16)^{ab}	9.43 (3.23)^{ef}	17.27 (4.24)^{h}	20.27 (4.59)^{i}	12.66 (4.61)^{hi}
T3 (T1+ Flonicamid 50 WG)	0.00	2.82 (1.95)^{ab}	4.61 (2.37)^{abc}	5.60 (2.57)^{cd}	5.60 (2.57)^{cd}	4.66 (2.57)^{abcde}
T4 (T1+ Diafenthiuron 50WP)	0.00	2.03 (1.74)^{a}	5.97 (2.62)^{bcd}	8.48 (3.08)^{ef}	8.48 (3.08)^{fg}	6.24 (3.08)^{efg}
T5 (T1+ Fipronil 5SC)	0.00	3.10 (2.01)^{ab}	3.28 (2.06)^{a}	3.28 (2.06)^{a}	3.28 (2.06)^{a}	3.24 (2.07)^{a}
T6 (T1+ Buprofezin 25 SC)	0.00	3.73 (2.12)^{ab}	7.40 (2.89)^{de}	11.53 (3.54)^{g}	11.53 (3.54)^{h}	8.55 (3.54)^{g}
T7 (T1+ Spinetoram 11.7 SC)	0.00	4.16 (2.27)^{b}	5.93 (2.63)^{bcd}	6.26 (2.69)^{de}	6.26 (2.69)^{de}	5.65 (2.69)^{bcde}
T8 (T1+ Spinosad 45SC)	0.00	3.35 (2.08)^{ab}	3.48 (2.12)^{a}	3.48 (2.12)^{ab}	3.48 (2.12)^{ab}	3.45 (2.12)^{a}
T9 (T2+ Flonicamid 50WG)	0.00	4.03 (2.24)^{b}	4.52 (2.35)^{ab}	5.13 (2.48)^{bcd}	5.13 (2.48)^{bcd}	4.70 (2.48)^{abcd}
T10 (T2+ Diafenthiuron 50WP)	0.00	3.33 (2.08)^{ab}	6.73 (2.78)^{d}	8.05 (3.01)^{ef}	8.05 (3.01)^{efg}	6.54 (3.01)^{defg}
T11 (T2+ Fipronil 5 SC)	0.00	3.78 (2.18)^{ab}	3.78 (2.18)^{a}	3.78 (2.18)^{abc}	3.78 (2.18)^{ab}	3.78 (2.19)^{ab}
T12 (T2+ Buprofezin 25 SC)	0.00	3.77 (2.18)^{ab}	6.50 (2.72)^{cd}	9.90 (3.30)^{fg}	9.90 (3.30)^{gh}	7.52 (3.30)^{fg}
T13 (T2+ Spinetoram 11.7 SC)	0.00	3.07 (1.93)^{ab}	6.27 (2.69)^{bcd}	6.67 (2.77)^{de}	6.67 (2.77)^{def}	5.67 (2.77)^{cdef}
T14 (T2+ Spinosad 45 SC)	0.00	3.95 (2.22)^{b}	3.95 (2.22)^{a}	3.95 (2.22)^{abc}	3.95 (2.22)^{abc}	3.95 (2.22)^{abc}
T15 (Untreated Control)	0.00	8.29 (3.04)^{c}	16.33 (4.16)^{g}	25.10 (5.10)^{i}	25.10 (5.10)^{j}	18.71 (5.11)^{i}
SEm ±	**0.00**	**0.16**	**0.12**	**0.14**	**0.13**	**0.19**
SED	**0.00**	**0.23**	**0.17**	**0.20**	**0.18**	**0.27**
CD ≤ 0.05	**0.00**	**0.46**	**0.35**	**0.40**	**0.37**	**0.55**
CV (%)	**0.00**	**12.84**	**7.81**	**7.93**	**7.27**	**14.13**

Values in parenthesis are * square root (√x+1) transformations; DAS = Day after sowing; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

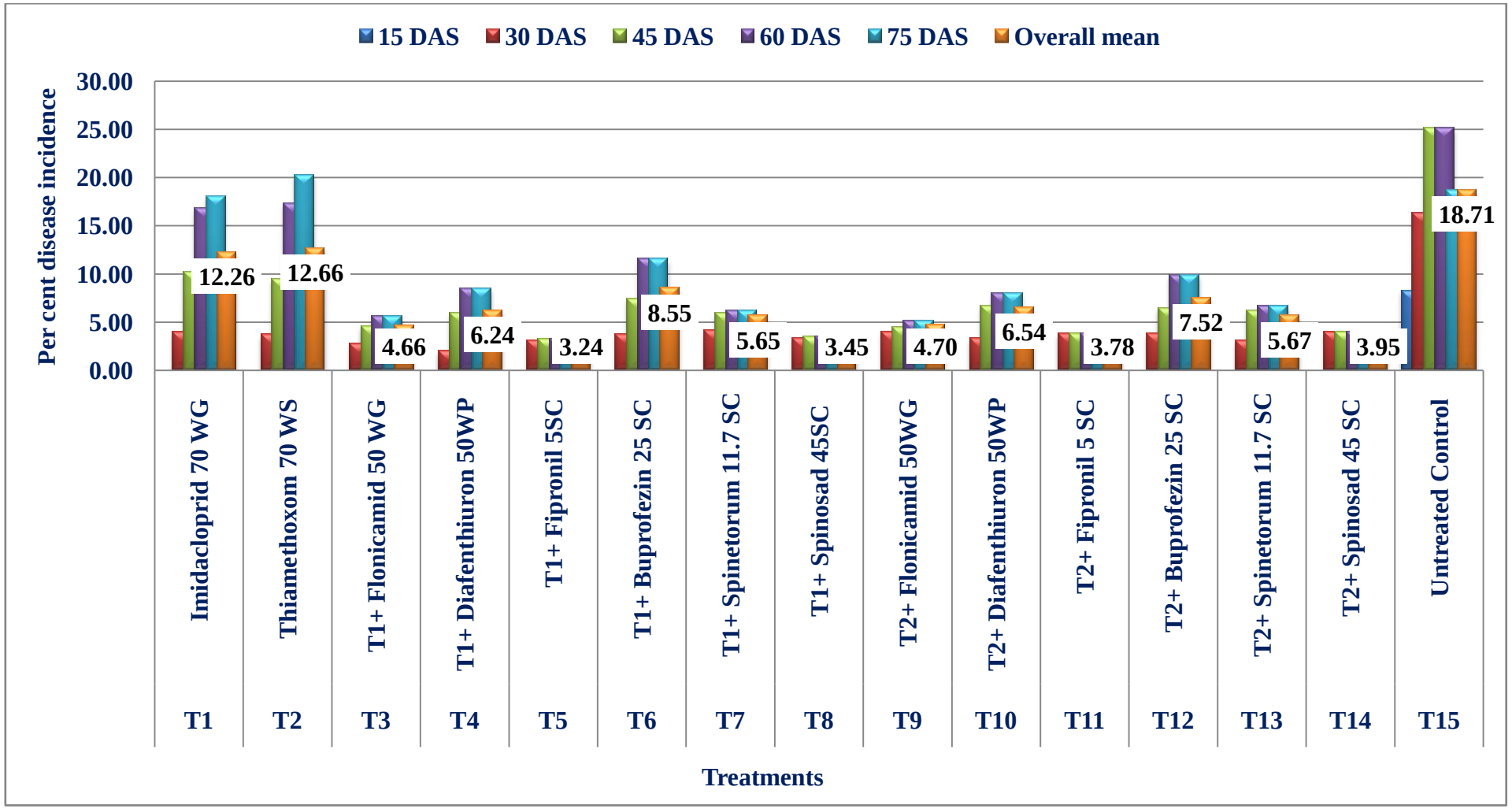


Figure 4.21. Effect of insecticidal treatments tested for thrips management on the bud necrosis disease incidence in blackgram during *rabi* 2019-2020

4.4.5 Incremental Cost Benefit Ratio (ICBR)

The data presented in the Table 4.25 revealed that all the treatments recorded better grain yield over untreated control (497 kg ha^{-1}). Among the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded the highest grain yield *i.e.* 1414 kg ha^{-1} with incremental cost benefit ratio 1:4.80 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1:4.47), thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1:3.25) and imidacloprid seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1:2.99).

Even though the treatments *viz.* imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} were found to be effective treatments in reducing thrips population per plant, incremental cost benefit ratio was found low (1.77 and 1.70, respectively) due to their high input cost. Remaining treatments recorded incremental cost benefit ratios in the range of 1:0.07 to 1:1.12. Least ICBR was 1:0.07 in thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1}.

These results were in concurrence with Sharma and Singh (2015) who reported lowest population of thrips (1.34/6 leaves in 2012 and 0.95/6 leaves in 2013) in blackgram plot treated with imidacloprid 17.8 SL @ 125 mL ha^{-1} followed by thiamethoxam @ 125 mL ha^{-1} (1.65/6 leaves 1.17/6 leaves in 2012 and 2013). Significantly maximum yield in blackgram was found in imidacloprid (11.13, 11.67 q ha^{-1} in 2012 and 2013), followed by thiamethoxam treated plot (10.88, 11.93 q ha^{-1} in 2012 and 2013).

4.4.6 Efficacy of Insecticides against Thrips Population after the First Spray during *Kharif* 2020-2021

The data from Table number 4.26 it was evident that mean number of thrips per plant before one day of spray application were 2.60 to 5.10 with significant difference among the treatments, whereas control plot recorded 6.37 mean number of thrips per plant at one day after spray. All the treatments found superior over untreated control (Plate 4.58). Among the treatments, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} has recorded the lowest number of thrips per plant (1.87) (Plate 4.59) and it was on par with thiamethoxam 70 WS seed

Table 4.25. Details of plant protection costs incurred in the management of thrips in blackgram during *rabi* 2019-2020

T. No.	Treatments	Quantity of insecticide (mL or g per ha)	Cost of insecticide (Rs per ha)	Labor charges (Rs per ha)	No. of Sprays	Total Cost per ha	Yield of blackgram grains kg/ha	Increase in yield over control (kg/ha)	Value of yield (Rs per ha@ Rs 5000/q)	Net profit (Rs /ha)	ICBR	Rank
1	Seed treatment with Imidacloprid 70 WG	100	734	500	3	3702	594^{fg}	97	4861	1159	0.31	12
2	Seed treatment with Thiamethoxam 70 WS	100	800	500	3	3900	581^{fg}	83	4167	267	0.07	14
3	T1+ Flonicamid 50 WG	250	2484	500	3	8952	1211^{abc}	714	35694	26742	2.99	4
4	T1+ Diafenthiuron 50WP	700	3134	500	3	10902	825^{def}	328	16389	5487	0.50	11
5	T1+ Fipronil 5SC	1100	2134	500	3	7902	1414^{a}	917	45833	37931	4.80	1
6	T1+ Buprofezin 25 SC	1100	1334	500	3	5502	711^{efg}	214	10694	5192	0.94	8
7	T1+ Spinetoram 11.7 SC	550	5189	500	3	17067	1050^{bcd}	553	27639	10572	0.62	9
8	T1+ Spinosad 45SC	270	4619	500	3	15357	1347^{ab}	850	42500	27143	1.77	5
9	T2+ Flonicamid 50WG	250	2550	500	3	9150	1275^{ab}	778	38889	29739	3.25	3
10	T2+ Diafenthiuron 50WP	700	3200	500	3	11100	844^{def}	347	17361	6261	0.56	10
11	T2+ Fipronil 5 SC	1100	2200	500	3	8100	1383^{a}	886	44305	36205	4.47	2
12	T2+ Buprofezin 25 SC	1100	1400	500	3	5700	739^{defg}	242	12083	6383	1.12	7
13	T2+ Spinetoram 11.7 SC	550	5255	500	3	17265	939^{cde}	442	22083	4818	0.28	13
14	T2+ Spinosad 45 SC	270	4685	500	3	15555	1336^{ab}	839	41944	26389	1.70	6
15	Untreated Control						497^{g}					
SEM±							**110.20**					
CD							**319.24**					
CV							**19.36**					

Labour charges /one spray/ha @ Rs.250/labour/day, Price of blackgram grains @ Rs. 5000/qtl	Imidacloprid 70 WG @ Rs. 550/75 g	Buprofezin 25 SC @ Rs. 300/500 mL
	Thiamethoxam 70 WS @ Rs. 200/25 g	Spinetoram 11.7 SC @ Rs.990/100 mL
	Flonicamid 50 WG @ Rs. 700/60 g	Spinosad 45SC @ Rs. 170/7 mL
	Diafenthiuron 50WP @ Rs.100/25 g	Untreated Control
	Fipronil 5 SC @ Rs. 140/100 mL	

treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (Plate 4.60), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1} with 2.03, 2.50, 2.60 and 2.80 mean number of thrips per plant, respectively.

Population reduction over untreated control was 60.13 and 44.55 per cent in the treatments *viz.* thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, respectively. However the per cent population reduction over untreated control in the remaining all treatments ranged from zero to 36.44.

It was evident that from the data recorded at 3 DAS, all the treatments were significantly superior over untreated control (6.37 mean number of thrips per plant). Among the treatments, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded the lowest number of thrips (0.10 per plant). Thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} were found on par with 0.17, 0.47 and 0.93 mean number of thrips per plant, respectively.

Per cent reduction over untreated control was highest (97.71) in imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}. Thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} were also found better with 94.23 and 87.52 mean per cent reduction of population over untreated control, respectively. All the remaining treatments ranged from 0.42 to 83.53 per cent reduction of thrips. Among the treatments thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} and imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} recorded least mean per cent reduction of population over untreated control *i.e.*1.64 and 0.42, respectively.

Observations recorded at 7 DAS, revealed that all the treatments were found significantly superior over the untreated control with mean number of thrips 7.90 per plant. Among the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray

Plate 4.58. Bud necrosis disease infestation in untreated control during *kharif* 2020-2021

Plate 4.59. General view of effective treatment plot (T5) during *kharif* 2020-2021

Plate 4.60. General view of effective treatment plot (T11) during *kharif* 2020-2021

@ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded with zero mean number of thrips per plant with hundred per cent reduction over untreated control. The next best treatments *viz*., Imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + diafenthiuron 50 WP spray @ 300 g a.i ha^{-1} were on par with 0.30, 0.47, 0.50 and 0.57 mean number of thrips per plant, respectively. Least mean per cent population reduction over untreated control *i.e.* 0.81 was recorded in the treatment imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1}.

All the treatments were found significantly superior over the untreated control (8.12 mean number of thrips per plant) at 10 DAS. Among the treatments, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded zero number of thrips per plant with cent per cent reduction in thrips population over untreated control. Thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i $ha^{-1}$and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} were on par with 0.27 and 0.30 mean number of thrips per plant, respectively. Imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} recorded least population reduction over untreated control (3.50 %).

The overall mean of the first spray (Table 4.26) revealed that all the treatments were significantly superior over the untreated control (7.39 per plant). Among the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded lowest mean number of thrips per plant *i.e.* 0.58 . Imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment +

Table 4.26. Efficacy of insecticides against thrips population on blackgram after first spray during *kharif* 2020-2021

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1 DBS	1 DAS	3 DAS	7 DAS	10 DAS	Mean	1 DAS	3 DAS	7 DAS	10 DAS	Mean
1	Seed treatment with Imidacloprid 70 WG	70	3.03	3.03 (2.00)^{cd}	3.40 (2.09)^{ef}	3.73 (2.18)^{d}	3.73 (2.18)^{e}	3.47 (2.18)^{e}	0.11	0.42	0.81	3.50	1.30
2	Seed treatment with Thiamethoxam 70 WS	70	3.73	3.73 (2.17)^{de}	4.13 (2.27)^{f}	4.13 (2.27)^{d}	4.13 (2.27)^{e}	4.03 (2.27)^{e}	0.09	1.64	10.77	13.19	6.92
3	T1+ Flonicamid 50 WG	75	3.43	2.83 (1.95)^{bcd}	1.77 (1.66)^{cd}	0.30 (1.13)^{ab}	0.30 (1.13)^{abc}	1.30 (1.14)^{a}	17.48	53.69	93.01	93.01	65.92
4	T1+ Diafenthiuron 50 WP	300	4.20	4.20 (2.28)^{e}	2.23 (1.72)^{cde}	0.57 (1.23)^{ab}	0.57 (1.23)^{c}	1.89 (1.25)^{a}	0.00	52.14	89.21	89.21	59.46
5	T1+ Fipronil 5 SC	50	3.37	1.87 (1.69)^{a}	0.47 (1.20)^{ab}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.58 (1.00)^{a}	44.55	87.52	100.00	100.00	84.41
6	T1+ Buprofezin 25 SC	250	3.80	3.43 (2.11)^{cde}	2.30 (1.82)^{de}	0.60 (1.26)^{b}	0.60 (1.26)^{c}	1.73 (1.26)^{ab}	9.65	45.53	87.37	87.37	58.95
7	T1+ Spinetoram 11.7 SC	50	2.80	2.80 (1.94)^{abcd}	2.13 (1.75)^{cde}	0.77 (1.31)^{b}	0.47 (1.21)^{c}	1.54 (1.21)^{a}	0.00	31.43	78.10	86.67	50.45
8	T1+ Spinosad 45 SC	72	3.93	2.50 (1.87)^{abc}	0.10 (1.05)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.65 (1.00)^{a}	36.44	97.71	100.00	100.00	85.13
9	T2+ Flonicamid 50 WG	75	3.00	3.00 (2.00)^{cd}	1.50 (1.58)^{bcd}	0.50 (1.22)^{ab}	0.27 (1.12)^{abc}	1.32 (1.13)^{a}	0.00	55.00	86.67	92.89	60.50
10	T2+ Diafenthiuron 50WP	300	3.30	3.30 (2.05)^{cde}	2.20 (1.78)^{c}	0.70 (1.30)^{b}	0.63 (1.28)^{c}	1.71 (1.28)^{ab}	0.00	40.00	83.03	84.65	53.41
11	T2+ Fipronil 5 SC	50	5.10	2.03 (1.73)^{ab}	0.93 (1.32)^{abc}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.74 (1.00)^{a}	60.13	83.53	100.00	100.00	86.91
12	T2+ Buprofezin 25 SC	250	2.80	2.80 (1.95)^{bcd}	2.17 (1.78)^{de}	2.17 (1.78)^{c}	1.50 (1.58)^{d}	2.16 (1.58)^{b}	0.00	30.36	38.10	57.14	30.63
13	T2+ Spinetoram 11.7 SC	50	3.43	3.07 (2.01)^{cd}	2.43 (1.85)^{def}	0.47 (1.21)^{ab}	0.47 (1.21)^{c}	1.61 (1.21)^{a}	10.68	36.21	89.13	89.13	57.84
14	T2+ Spinosad 45 SC	72	2.60	2.60 (1.90)^{abc}	0.17 (1.07)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.69 (1.00)^{a}	0.00	94.23	100.00	100.00	76.06
15	Untreated Control		6.37	6.37 (2.71)^{f}	7.17 (2.86)^{g}	7.90 (2.98)^{e}	8.12 (3.02)^{f}	7.39 (3.02)^{d}					
SEm ±			**0.09**	**0.09**	**0.15**	**0.08**	**0.07**	**0.11**					
CD ≤ 0.05			**0.25**	**0.25**	**0.44**	**0.24**	**0.20**	**0.32**					
CV (%)			**6.97**	**7.45**	**15.31**	**9.89**	**8.56**	**13.41**					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

diafenthiuron 50 WP spray @ 300 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + buprofezin 25 SC spray @ 250 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + diafenthiuron 50 WP spray @ 300 g a.i ha^{-1} were on par with each other with 0.65, 0.69, 0.74, 1.30, 1.32, 1.54, 1.61, 1.71, 1.73 and 1.89 mean number of thrips per plant, respectively.

Mean population reduction over untreated control was highest (86.91 per cent) in thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}. Imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded 85.13 and 84.41 mean per cent population reduction over untreated control, respectively. Least mean population reduction over untreated control was recorded (1.30 per cent) in imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1}.

4.4.6.1 Mean per cent bud necrosis disease incidence in blackgram after first spray during *kharif* 2020-2021 : From the Table 4.30 (Figure 4.23, Plate 4.61) it was evident that mean per cent bud necrosis disease incidence in all the treatments ranged from 3.91 to 6.50 per cent where as in untreated control it was 10.63 per cent. Disease incidence data was recorded at 45 DAS after first spray. All treatments were found effective against bud necrosis disease in preventing the further spread when compared to untreated control.

Among the treatments, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found superior with least mean per cent disease incidence *i.e.* 5.24. Imidacloprid 70 WG seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} were found on par with 5.31, 5.67, 5.97, 6.25, 6.62 and 6.68 per cent mean disease incidence, respectively. Remaining treatments ranged from 7.25 to 13.30 per cent mean disease incidence.

4.4.7 Efficacy of Insecticides against Thrips Population after the Second Spray during *Kharif* 2020- 2021

The obtained results were presented in the Table 4.27 (Plate 4.62). Imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found significantly superior treatment among all the treatments tested with least mean number of thrips per plant *i.e.*1.03. Imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(1.13), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(1.50) and thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(1.57) were found at par with each other.

All the remaining treatments recorded 2.50 to 6.43 mean number of thrips per plant. Fifty per cent mean population reduction over untreated control was recorded in case of imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and found superior among the other treatments. All the remaining treatments showed 0.00 to 46.88 mean percent population reduction over untreated control.

The results obtained at 3 DAS, indicated that imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded zero mean number of thrips per plant with cent per cent reduction over untreated control and found significantly superior among the other treatments and these treatments were statistically on par with imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (0.37 thrips per plant).

Thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} and imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} recorded with least mean per cent population reduction over untreated control *i.e.* 0.42 and 4.92, respectively. The observations recorded at 7 DAS revealed that imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} , imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} were significantly superior over untreated control with zero number of thrips per plant with cent per cent reduction over untreated control and they were statistically at par with imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (0.13),

Plate 4.61. Bud necrosis infected plants during *kharif* 2020-2021

Plate 4.62. Management field trial at 40 DAS during *kharif* 2020-2021

thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (0.27 thrips per plant). Imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was significantly superior among the other treatments at 10 DAS with least mean number of thrips per plant (0.13) and it was statistically at par with treatments *viz.* imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.20), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.23), thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(0.33), imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(0.34) and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (0.37 $plant^{-1}$).

Similarly, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded 95.48 and 93.44 mean per cent population reduction over untreated control. Thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} also recorded 92.90, 91.81, 90.67 and 90.61 mean per cent population reduction over untreated control, respectively. Whereas thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1}, imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} recorded least per cent population reduction over untreated control *i.e.* 0.14 and 0.32 only.

The overall mean of the second spray (Table 4.27) indicated that all the treatments found significantly superior over untreated control (11.72 thrips per plant). Among the treatments evaluated, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded lowest mean number of thrips per plant (0.29) and it was at par with imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (0.33) , thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.43) , thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(0.48), imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(0.79) and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(0.91). Seed with treatment imidacloprid 70 WG @ 70 g a.i ha^{-1} and seed treatment with thiamethoxam 70 WS @ 70 g a.i ha^{-1} recorded mean number of thrips 6.35 and 7.31 per plant, respectively.

Table 4.27. Efficacy of insecticides against thrips population on blackgram after second spray during *kharif* 2020-2021

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1DBS	1DAS	3DAS	7DAS	10DAS	Mean	1DAS	3DAS	7DAS	10DAS	Mean
1	Seed treatment with Imidacloprid 70 WG	70	5.63	5.63 (2.57)^{e}	5.97 (2.64)^{e}	6.20 (2.68)^{d}	7.60 (2.93)^{d}	6.35 (2.93)^{e}	0.00	4.92	1.20	0.32	1.58
2	Seed treatment with Thiamethoxam 70 WS	70	6.40	6.40 (2.72)^{e}	7.10 (2.84)^{f}	7.10 (2.84)^{d}	8.65 (3.09)^{d}	7.31 (3.11)^{e}	0.00	0.42	0.42	0.14	0.24
3	T1+ Flonicamid 50 WG	75	2.93	2.30 (1.82)^{b}	0.37 (1.17)^{ab}	0.13 (1.06)^{a}	0.34 (1.16)^{a}	0.79 (1.16)^{a}	21.59	88.75	95.91	91.81	75.89
4	T1+ Diafenthiuron 50WP	300	2.83	2.83 (1.95)^{bc}	2.83 (1.95)^{d}	2.17 (1.78)^{c}	2.17 (1.78)^{c}	2.50 (1.78)^{d}	0.00	10.00	31.18	46.47	20.59
5	T1+ Fipronil 5SC	50	2.07	1.03 (1.43)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.13 (1.06)^{a}	0.29 (1.06)^{a}	50.00	100.00	100.00	95.48	87.30
6	T1+ Buprofezin 25 SC	250	4.00	3.40 (2.09)^{cd}	2.70 (1.92)^{d}	2.07 (1.75)^{c}	2.07 (1.75)^{c}	2.56 (1.75)^{cd}	15.00	39.25	53.50	63.83	42.44
7	T1+ Spinetoram 11.7 SC	50	3.33	3.00 (2.00)^{bcd}	1.93 (1.71)^{c}	1.13 (1.46)^{b}	1.13 (1.46)^{b}	1.80 (1.46)^{b}	10.00	47.80	69.40	76.20	51.40
8	T1+ Spinosad 45SC	72	2.13	1.13 (1.46)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.20 (1.10)^{a}	0.33 (1.10)^{a}	46.88	100	100	93.44	85.94
9	T2+ Flonicamid 50WG	75	2.73	2.50 (1.87)^{b}	0.50 (1.22)^{b}	0.27 (1.12)^{a}	0.37 (1.17)^{a}	0.91 (1.17)^{a}	8.54	83.54	91.22	90.61	70.09
10	T2+ Diafenthiuron 50WP	300	3.37	2.90 (1.97)^{bc}	2.57 (1.89)^{cd}	1.83 (1.68)^{c}	1.27 (1.50)^{b}	2.14 (1.51)^{bc}	13.86	31.39	50.99	73.66	42.75
11	T2+ Fipronil 5 SC	50	2.50	1.57 (1.60)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.33 (1.15)^{a}	0.48 (1.15)^{a}	37.33	100.00	100.00	90.67	82.90
12	T2+ Buprofezin 25 SC	250	4.47	3.90 (2.21)^{d}	3.00 (2.00)^{d}	2.23 (1.80)^{c}	2.23 (1.80)^{c}	2.84 (1.80)^{d}	12.69	39.55	55.00	65.00	42.74
13	T2+ Spinetoram 11.7 SC	50	3.27	2.97 (1.99)^{bc}	2.43 (1.85)^{cd}	1.23 (1.49)^{b}	1.23 (1.49)^{b}	1.97 (1.49)^{bc}	9.18	32.96	66.02	73.57	45.82
14	T2+ Spinosad 45 SC	72	2.27	1.50 (1.58)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.23 (1.11)^{a}	0.43 (1.11)^{a}	33.82	100.00	100.00	92.90	82.83
15	Untreated Control		10.23	10.23 (3.35)^{f}	11.40 (3.51)^{g}	11.40 (3.51)^{e}	13.85 (3.85)^{e}	11.72 (3.85)^{f}					
SEm ±			**0.08**	**0.07**	**0.06**	**0.06**	**0.06**	**0.09**					
CD ≤ 0.05			**0.24**	**0.21**	**0.18**	**0.18**	**0.19**	**0.26**					
CV (%)			**6.57**	**6.02**	**6.21**	**6.35**	**6.39**	**10.08**					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

Highest mean population reduction over untreated control (87.30 per cent) was recorded in treatment imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by 85.94 per cent in imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} was recorded. Thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} were found next to them with 82.90 and 82.83 per cent population reduction over untreated control, respectively.

4.4.7.1 Mean per cent bud necrosis disease incidence in blackgram after second spray during *kharif* 2020-2021 : At 60 DAS, mean per cent bud necrosis disease incidence in blackgram was found low (5.24 per cent) in the treatment imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and it was statistically at par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(5.97 per cent) , thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (6.25 per cent), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (6.68 per cent). Among the treatments tested, seed treatment with thiamethoxam 70 WS @ 70 g a.i ha^{-1} and seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} recorded 20.37 and 18.73 per cent disease incidence, respectively (Table 4.30, Figure 4.23, Plate 4.63).

Plate 4.63. Bud necrosis infected blackgram plants at 45 DAS during *kharif* 2020-2021

4.4.8 Efficacy of Insecticides against Thrips Population after the Third Spray during *Kharif* 2020-2021

The results of third spray were presented in the Table 4.28. All the treatments at 1 DAS, found significantly superior over untreated control (14.30 thrips per plant). Among the treatments tested, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1.03 thrips per plant) was found superior and it was statistically on par with treatments *viz.* imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (1.10 thrips $plant^{-1}$), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (1.17 thrips $plant^{-1}$) and thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1.43 thrips $plant^{-1}$). Thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded 55.70 per cent population reduction over untreated control followed by imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (54.79 per cent). The remaining treatments showed 0.00 to 46.91 mean per cent population reduction over untreated control.

The observations recorded at 3 DAS (Table 4.28) indicated that among the all the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded zero number of thrips per plant with cent per cent thrips population reduction over untreated control and these were statistically on par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (0.13 thrips per plant). Thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (95.06 per cent) found next to these treatments. Remaining all treatments showed 0.41 to 62.89 per cent population reduction over untreated control.

It was evident from the data recorded at 7 DAS that imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded zero number of thrips per plant with 100 per cent reduction of thrips population over untreated control. These treatments were statistically on par with imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (0.63 thrips per plant). Imidacloprid 70 WG seed treatment +

flonicamid 50 WG spray @ 75 g a.i ha^{-1} showed 80.93 per cent population reduction over untreated control. Per cent population reductions over untreated control in the remaining treatments ranged from 1.22 to 66.81.

All the treatments were found significantly superior over untreated control (14.94 thrips plant^{-1}) at 10 DAS. Treatments *viz.* imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded zero number of thrips plant with cent per cent thrips population reduction over untreated control.

These treatments were statistically on par with imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (0.10 thrips per plant) and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (0.30 thrips per plant). Next to these treatments, imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} recorded 97.04 and 90.21 per cent population reduction over untreated control, respectively. Remaining all the treatments showed 0.06 to 74.23 per cent population reduction over untreated control. Seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} recorded least (0.06 per cent) population reduction over untreated control among the all treatments.

The overall mean of the third spray revealed that all the treatments were significantly superior over the untreated control (14.63 thrips plant^{-1}). Among the treatments, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean number of thrips (0.26 thrips plant^{-1}) and it was at par with treatments *viz.* imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.28 thrips plant^{-1}), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.29 thrips plant^{-1}) , thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(0.39 thrips plant^{-1}), imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1.04 thrips plant^{-1}) and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1.41 thrips plant^{-1}). Highest per cent population reduction over untreated control was recorded (89.17) in treatment thiamethoxam 70 WS seed treatment + spinosad 45 SC

spray @ 72 g a.i ha^{-1} followed by imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} with 88.95 , 88.16 and 85.82 per cent population reduction over untreated control, respectively.

4.4.8.1 Mean per cent bud necrosis disease incidence in blackgram after third spray during *kharif* 2020-2021 : Per cent bud necrosis disease incidence in treatment plots of blackgram after third spray was recorded *i.e.* after 75 days after sowing, and presented in the Table 4.30 (Figure 4.23). From the results obtained, it was evident that no further increase in disease incidence was observed in treatments like imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with 5.24, 5.97 and 6.25 mean per cent disease incidence, respectively and these were statistically at par each other.

These treatments were also at par with imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} with 6.68, 6.93 and 6.95 mean per cent disease incidence, respectively. Seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} and thiamethoxam 70 WS @ 70 g a.i ha^{-1} recorded 20.97 and 20.37 mean per cent bud necrosis disease incidence, respectively.

4.4.9 Cumulative Efficacy of Insecticides against Thrips Population after Three Sprays during *Kharif* 2020-2021

The cumulative efficacy of different treatments tested for the management of thrips population during *kharif* 2020-2021 revealed that (Table 4.29, Figure 4.22) all the treatments were significantly superior over the untreated control with overall mean number of thrips 11.24 $plant^{-1}$. Among the treatments, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded overall mean number of 0.38 thrips per plant and it was statistically at par with imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (0.42 thrips $plant^{-1}$), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (0.47 thrips $plant^{-1}$),

Table 4.28. Efficacy of insecticides against thrips population on blackgram after third spray during *kharif* 2020-2021

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1DBS	1DAS	3DAS	7DAS	10DAS	Mean	1DAS	3DAS	7DAS	10DAS	Mean
1	Seed treatment with Imidacloprid 70 WG	70	6.80	6.80 (2.79)^{d}	6.90 (2.81)^{e}	6.90 (2.81)^{f}	7.10 (2.85)^{d}	6.93 (2.85)^{d}	0.00	0.41	1.22	0.06	0.42
2	Seed treatment with Thiamethoxam 70 WS	70	8.12	8.12 (3.00)^{d}	8.12 (3.00)^{e}	8.12 (3.00)^{f}	8.43 (3.06)^{e}	8.20 (3.07)^{e}	0.00	1.85	2.65	0.63	1.29
3	T1+ Flonicamid 50 WG	75	3.23	2.23 (1.80)^{bc}	1.20 (1.48)^{b}	0.63 (1.26)^{ab}	0.10 (1.05)^{a}	1.04 (1.05)^{a}	30.93	62.89	80.93	97.04	68.50
4	T1+ Diafenthiuron 50WP	300	2.97	2.80 (1.94)^{c}	2.80 (1.94)^{d}	2.40 (1.84)^{de}	2.13 (1.77)^{c}	2.53 (1.77)^{c}	5.62	5.62	21.25	31.17	16.50
5	T1+ Fipronil 5SC	50	2.13	1.03 (1.43)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.26 (1.00)^{a}	51.56	100.00	100.00	100.00	88.16
6	T1+ Buprofezin 25 SC	250	3.73	3.10 (2.02)^{c}	2.87 (1.97)^{d}	2.27 (1.81)^{de}	1.43 (1.56)^{b}	2.42 (1.56)^{b}	16.96	23.21	40.90	63.25	36.71
7	T1+ Spinetoram 11.7 SC	50	3.47	2.77 (1.94)^{c}	2.33 (1.82)^{cd}	1.20 (1.48)^{bc}	0.93 (1.39)^{b}	1.81 (1.39)^{b}	20.19	32.69	66.30	74.23	49.00
8	T1+ Spinosad 45SC	72	2.43	1.10 (1.45)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.28 (1.00)^{a}	54.79	100.00	100.00	100.00	88.95
9	T2+ Flonicamid 50WG	75	2.93	2.67 (1.91)^{c}	1.67 (1.63)^{bc}	1.00 (1.41)^{bc}	0.30 (1.14)^{a}	1.41 (1.14)^{a}	9.09	43.18	66.81	90.21	53.06
10	T2+ Diafenthiuron 50WP	300	3.40	3.00 (2.00)^{c}	2.80 (1.95)^{d}	2.30 (1.81)^{de}	1.17 (1.47)^{b}	2.32 (1.47)^{b}	11.76	17.65	34.15	67.16	33.38
11	T2+ Fipronil 5 SC	50	2.70	1.43 (1.56)^{ab}	0.13 (1.06)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.39 (1.00)^{a}	46.91	95.06	100.00	100.00	85.82
12	T2+ Buprofezin 25 SC	250	3.20	3.20 (2.04)^{c}	3.03 (2.00)^{d}	2.90 (1.97)^{e}	2.23 (1.80)^{c}	2.84 (1.80)^{c}	0.00	5.21	11.78	33.20	13.17
13	T2+ Spinetoram 11.7 SC	50	3.53	2.57 (1.89)^{c}	2.37 (1.83)^{cd}	1.57 (1.60)^{cd}	1.00 (1.41)^{b}	1.88 (1.41)^{b}	27.36	33.02	56.84	72.91	48.11
14	T2+ Spinosad 45 SC	72	2.63	1.17 (1.47)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.29 (1.00)^{a}	55.70	100.00	100.00	100.00	89.17
15	Untreated Control		14.30	14.30 (3.91)^{e}	14.57 (3.94)^{f}	14.69 (3.96)^{g}	14.94 (3.99)^{f}	14.63 (3.99)^{f}					
SEm ±			**0.08**	**0.08**	**0.09**	**0.09**	**0.07**	**0.07**					
CD ≤ 0.05			**0.25**	**0.25**	**0.25**	**0.26**	**0.20**	**0.20**					
CV (%)			**6.70**	**7.06**	**7.98**	**8.77**	**7.14**	**7.58**					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(0.54 thrips $plant^{-1}$), imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1.04 thrips $plant^{-1}$) and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (1.21 thrips $plant^{-1}$).

Highest population reduction over untreated control was found in treatment imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (86.67 per cent) followed by imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with 86.62, 85.21 and 82.69 mean percent population reduction over untreated control, respectively. Remaining all treatments showed 1.10 to 70.10 percent population reduction over untreated control. Seed treatment done with imidacloprid 70 WG @ 70 g a.i ha^{-1} and thiamethoxam 70 WS @ 70 g a.i ha^{-1} recorded least mean per cent population reduction over untreated control *i.e.* 1.10 and 2.82, respectively.

The present results are in accordance with Reddy *et al*. (2020) who also reported that imidacloprid 17.8 SL (20.54 %) was the most effective treatment in controlling the sucking insect pests in blackgram during *kharif* 2017.

Results pertaining to efficacy of thiamethoxam 70 WS are in agreement with Sujatha and Bharpoda (2017) who also reported that thiamethoxam 25 WG (0.01 %) was the most effective treatment with lowest number of *T. palmi* (0.48/three leaves) followed by imidacloprid 70 WG (0.014 %) with 0.54 thrips per three leaves. Flower thrips, *M. usitatus* were also recorded low (0.33/three leaves) in case of thiamethoxam 25 WG (0.01 %) which was at par with imidacloprid 70 WG (0.014 %) with 0.46 thrips per three leaves in greengram during *kharif* 2015.

Indrajeet *et al*. (2017) also reported that thiamethoxam 25 % WG was effective with 59.16 per cent reduction of population of aphids over untreated control in blackgram during *kharif* 2016-17. Singh *et al*. (2016) reported that thiamethoxam 180 g a.i. ha^{-1} effective against thrips population which recorded 68.89 per cent reduction over untreated control. Yadav *et al*. (2015b) reported that thiamethoxam 25 % WG was superior in reducing whitefly population, leafhopper population *i.e.* 1.46/three leaves, 0.15/3 leaves, respectively. The better efficacy of spinosad 45 SC spray @ 72 g a.i ha^{-1} in the present study are in line with

Table 4.29. Cumulative efficacy of insecticides against thrips population on blackgram during *kharif* 2020-2021

T. No.	Treatments	Dose g.a.i./ha	Mean No of thrips per plant				Population reduction over untreated control (%)			
			First spray	Second Spray	Third Spray	Mean	First spray	Second Spray	Third Spray	Mean
1	Seed treatment with Imidacloprid 70 WG	70	3.47 (2.18)^{e}	6.35 (2.93)^{e}	6.93 (2.85)^{d}	5.58 (2.55)^{e}	1.30	1.58	0.42	1.10
2	Seed treatment with Thiamethoxam 70 WS	70	4.03 (2.27)^{e}	7.31 (3.11)^{e}	8.20 (3.07)^{e}	6.51 (2.72)^{e}	6.92	0.24	1.29	2.82
3	T1+ Flonicamid 50 WG	75	1.30 (1.14)^{a}	0.79 (1.16)^{a}	1.04 (1.05)^{a}	1.04 (1.43)^{ab}	65.92	75.89	68.50	70.10
4	T1+ Diafenthiuron 50WP	300	1.89 (1.25)^{a}	2.50 (1.78)^{d}	2.53 (1.77)^{c}	2.31 (1.82)^{cd}	59.46	20.59	16.50	32.19
5	T1+ Fipronil 5SC	50	0.58 (1.00)^{a}	0.29 (1.06)^{a}	0.26 (1.00)^{a}	0.38 (1.17)^{a}	84.41	87.30	88.16	86.62
6	T1+ Buprofezin 25 SC	250	1.73 (1.26)^{ab}	2.56 (1.75)^{cd}	2.42 (1.56)^{b}	2.24 (1.80)^{cd}	58.95	42.44	36.71	46.03
7	T1+ Spinetoram 11.7 SC	50	1.54 (1.21)^{a}	1.80 (1.46)^{b}	1.81 (1.39)^{b}	1.72 (1.65)^{bcd}	50.45	51.40	49.00	50.28
8	T1+ Spinosad 45SC	72	0.65 (1.00)^{a}	0.33 (1.10)^{a}	0.28 (1.00)^{a}	0.42 (1.19)^{a}	85.13	85.94	88.95	86.67
9	T2+ Flonicamid 50WG	75	1.32 (1.13)^{a}	0.91 (1.17)^{a}	1.41 (1.14)^{a}	1.21 (1.49)^{abc}	60.50	70.09	53.06	61.22
10	T2+ Diafenthiuron 50WP	300	1.71 (1.28)^{ab}	2.14 (1.51)^{bc}	2.32 (1.47)^{b}	2.06 (1.75)^{bcd}	53.41	42.75	33.38	43.18
11	T2+ Fipronil 5 SC	50	0.74 (1.00)^{a}	0.48 (1.15)^{a}	0.39 (1.00)^{a}	0.54 (1.24)^{a}	86.91	82.90	85.82	85.21
12	T2+ Buprofezin 25 SC	250	2.16 (1.58)^{b}	2.84 (1.80)^{d}	2.84 (1.80)^{c}	2.61 (1.90)^{f}	30.63	42.74	13.17	28.85
13	T2+ Spinetoram 11.7 SC	50	1.61 (1.21)^{a}	1.97 (1.49)^{bc}	1.88 (1.41)^{b}	1.82 (1.68)^{bcd}	57.84	45.82	48.11	50.59
14	T2+ Spinosad 45 SC	72	0.69 (1.00)^{a}	0.43 (1.11)^{a}	0.29 (1.00)^{a}	0.47 (1.21)^{a}	76.06	82.83	89.17	82.69
15	Untreated Control		7.39 (3.02)^{d}	11.72 (3.85)^{f}	14.63 (3.99)^{f}	11.24 (3.47)^{f}				
SEm ±			**0.11**	**0.09**	**0.07**	**0.12**				
CD ≤ 0.05			**0.32**	**0.26**	**0.20**	**0.33**				
CV (%)			**13.41**	**10.08**	**7.58**	**11.07**				

Values in parenthesis are * square root (√x+1) transformations; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

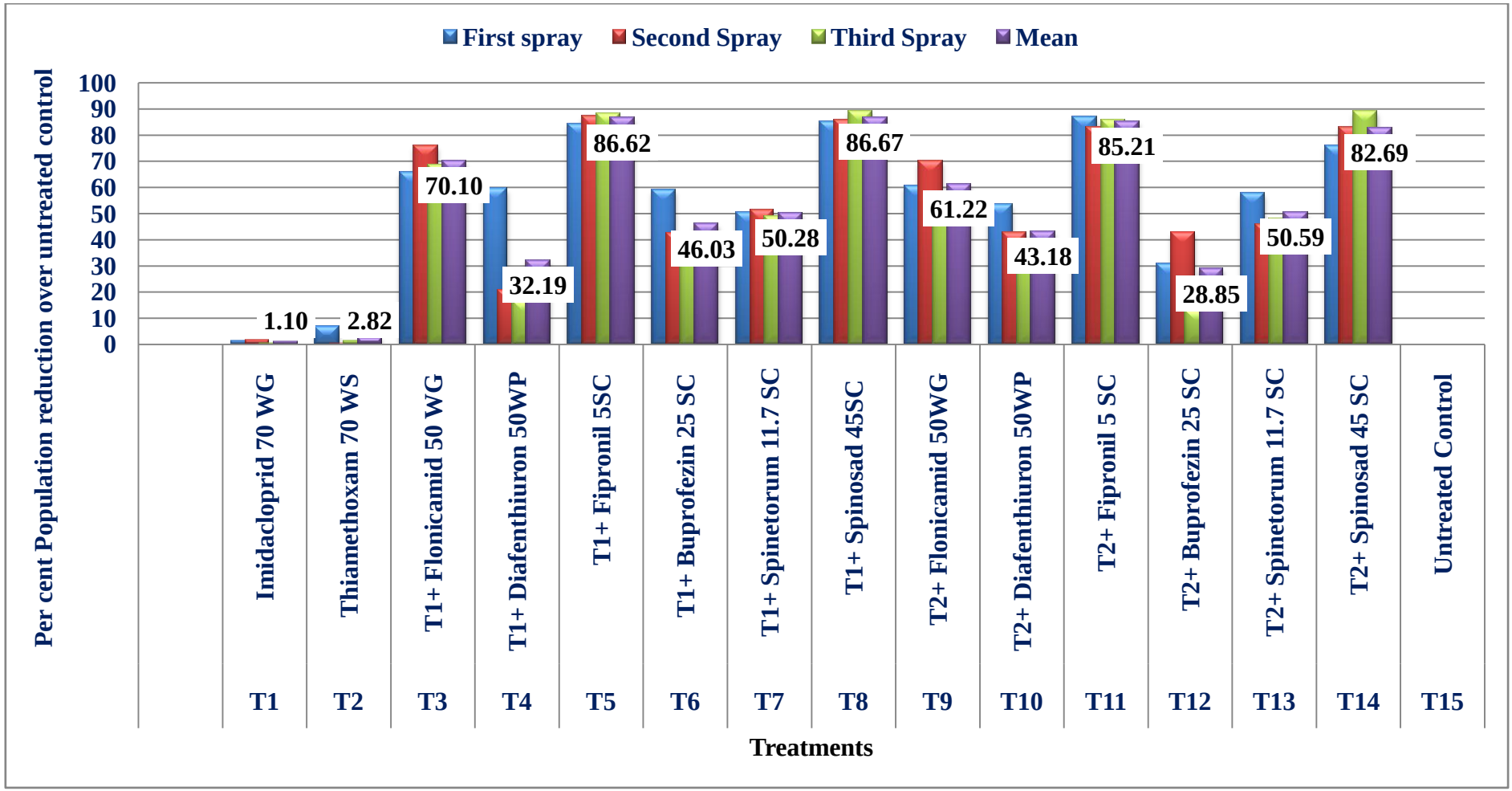


Figure 4.22. Cumulative efficacy of insecticides against thrips population on blackgram during *kharif* 2020-2021

Bambhaniya *et al*. (2018) who reported that spinosad 0.009 % proved significantly the most effective with 93.97 per cent reduction in thrips population (0.77 thrips/3 leaves) in tomato during *kharif*.

Sumalatha *et al*. (2017) also reported that spinosad 45 SC @ 73 g. a.i. ha^{-1} and fipronil 5 SC @ 50 g. a.i. ha^{-1} were the most superior and persistent treatments against thrips and highest bulb yield was recorded in spinosad 45 SC @ 73 g a.i ha^{-1} (18.03 t/ha) treated plots followed by fipronil 5 SC @ 50 g. a.i. ha^{-1} (16.78 t ha^{-1}) during *kharif*.

Results pertaining to efficacy of fipronil 5 SC spray @ 50 g a.i ha^{-1} can be supported by the findings of Singh *et al*. (2019) who reported that imidacloprid (0.005 %) and fipronil (0.01 %) proved to be the most effective next to acetamiprid (0.004 %) against sucking insect pests in greengram during *kharif* season. Kumar and kumar (2018) found that fipronil 80 % WG and fipronil 5 % SC were significantly superior with 62.0 and 57.6 per cent reduction in thrips foliage damage over control.

Hossain (2015) revealed the lowest number of infested flowers (1.67/20 flowers) was observed in imidacloprid sprayed plots @ 0.5 mL L^{-1} which was statistically identical to fipronil 5SC @ 0.5 mL L^{-1} (3/20 flowers) with more than 80 % reduction. Patil *et al*. (2009) also reported that fipronil 5 % SC @ 800g ha^{-1} registered least number of thrips (8.47/3 leaves) in cotton with seed cotton yield 27.23 q ha^{-1} in 2007, 27.50 q ha^{-1} in 2008 with higher dosage of fipronil 5SC @ 800 g ha^{-1} during *kharif* 2007 and 2008, respectively.

4.4.9.1 Mean per cent bud necrosis disease incidence in blackgram after three sprays during *kharif* 2020-2021 : Among the tested treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean bud necrosis disease incidence in blackgram *i.e.* 5.19 per cent and it was at par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (5.64 per cent), imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (5.94 per cent) thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(6.13 per cent), thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (6.39 per cent) and imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (6.64 per cent). Seed treatments with imidacloprid 70 WG @ 70

g a.i ha^{-1} and thiamethoxam 70 WS @ 70 g a.i ha^{-1} were found least effective with 13.87 and 14.89 mean per cent disease incidence (Table 4.30, Figure 4.23).

The above findings are in agreement with Archana *et al.* (2018) who reported that seed treatment with imidacloprid 600 FS @ 5.0 mL / kg and two sprays of imidacloprid 17.8 SL (@ 0.5 mL L^{-1}, 30 and 45 DAS during *kharif* season had significantly less YMD incidence and whitefly population. Similarly, Ruth *et al*. (2016) reported that seed treatment with imidacloprid (Goucho) @ 5 g kg^{-1} seed. + neem seed kernal extract @ 5 % + spinosad 0.3 mL L^{-1} were found superior in controlling the viral diseases in tomato during *kharif* 2009. Whitefly and thrips population were low and were 1.18/plant and 0.51/plant, respectively after post treatment. Least incidence of bud necrosis disease was recorded *i.e.* 6.60, 9.93 and 14.88 at 30, 45 and 60 days after planting in the same treatment plots.

Results pertaining to spinosad 45 SC spray @ 72 g a.i ha^{-1} were in agreement with Ruth *et al*. (2016) who conducted an experiment during *kharif* 2009 – 2013 in tomato in Andhra Pradesh and reported that seed treatment with imidacloprid (Goucho) @ 5 g kg^{-1} seed. + neem seed kernal extract @ 5 % + spinosad 0.3 mL L^{-1} were found superior in controlling the viral diseases in tomato during *kharif* season. Whitefly and thrips population were low and were 1.18/plant and 0.51/plant, respectively after post treatment. Least incidence of bud necrosis disease was recorded *i.e.* 6.60, 9.93 and 14.88 at 30, 45 and 60 days after planting in the same treatment plots.

4.4.10 Incremental Cost Benefit Ratio (ICBR)

The data presented in the Table 4.31, indicated that all the treatments were recorded with better grain yield over untreated control (625 kg ha^{-1}). Among the treatments tested, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded highest grain yield *i.e.* 1372 kg ha^{-1} with incremental cost benefit ratio 1:3.73 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1:2.93), imidacloprid seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (1:2.64) and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1:2.28). Even though the treatments *viz.* imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} were found effective treatments in

Table 4.30. Effect of insecticidal treatments tested for thrips management on bud necrosis disease in blackgram during *kharif* 2020-2021

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Overall mean
T1 (Seed treatment with imidacloprid 70 WG)	0.00 (1.00)^{a}	4.43 (2.33)^{abc}	11.41 (3.52)^{ef}	18.73 (4.44)^{g}	20.90 (4.68)^{e}	13.87 (4.68)^{g}
T2 (Seed treatment with thiamethoxam 70 WS)	0.00 (1.00)^{a}	5.52 (2.55)^{bcd}	13.30 (3.77)^{f}	20.37 (4.62)^{g}	20.37 (4.62)^{e}	14.89 (4.62)^{g}
T3 (T1+ Flonicamid 50 WG)	0.00 (1.00)^{a}	4.60 (2.36)^{abc}	5.67 (2.58)^{a}	6.54 (2.75)^{abc}	6.93 (2.82)^{ab}	5.94 (2.82)^{abcde}
T4 (T1+ Diafenthiuron 50WP)	0.00 (1.00)^{a}	4.24 (2.28)^{ab}	8.65 (3.10)^{d}	9.55 (3.25)^{ef}	9.82 (3.29)^{cd}	8.06 (3.29)^{ef}
T5 (T1+ Fipronil 5SC)	0.00 (1.00)^{a}	5.03 (2.46)^{abcd}	5.24 (2.50)^{a}	5.24 (2.50)^{a}	5.24 (2.50)^{a}	5.19 (2.50)^{a}
T6 (T1+ Buprofezin 25 SC)	0.00 (1.00)^{a}	4.85 (2.41)^{abc}	8.06 (3.01)^{cd}	9.38 (3.22)^{ef}	9.55 (3.25)^{cd}	7.96 (3.25)^{def}
T7 (T1+ Spinetoram 11.7 SC)	0.00 (1.00)^{a}	3.91 (2.21)^{a}	5.31 (2.49)^{a}	8.25 (3.04)^{cde}	8.25 (3.04)^{bc}	6.43 (3.04)^{bcd}
T8 (T1+ Spinosad 45SC)	0.00 (1.00)^{a}	6.50 (2.74)^{d}	6.68 (2.77)^{abc}	6.68 (2.77)^{abcd}	6.68 (2.77)^{ab}	6.64 (2.77)^{abcd}
T9 (T2+ Flonicamid 50WG)	0.00 (1.00)^{a}	5.04 (2.45)^{abcd}	6.62 (2.76)^{abc}	6.95 (2.82)^{bcd}	6.95 (2.82)^{ab}	6.39 (2.82)^{abcde}
T10 (T2+ Diafenthiuron 50WP)	0.00 (1.00)^{a}	4.99 (2.45)^{abcd}	8.07 (3.01)^{cd}	9.10 (3.17)^{ef}	9.10 (3.17)^{c}	7.81 (3.18)^{cdef}
T11 (T2+ Fipronil 5 SC)	0.00 (1.00)^{a}	4.67 (2.37)^{abc}	5.97 (2.64)^{ab}	5.97 (2.64)^{ab}	5.97 (2.64)^{a}	5.64 (2.64)^{ab}
T12 (T2+ Buprofezin 25 SC)	0.00 (1.00)^{a}	5.56 (2.56)^{bcd}	9.58 (3.25)^{de}	10.83 (3.41)^{f}	11.77 (3.54)^{d}	9.44 (3.57)^{f}
T13 (T2+ Spinetoram 11.7 SC)	0.00 (1.00)^{a}	5.83 (2.61)^{c}	7.25 (2.87)^{bcd}	8.33 (3.05)^{de}	8.33 (3.05)^{bc}	7.44 (3.05)^{bcd}
T14 (T2+ Spinosad 45 SC)	0.00 (1.00)^{a}	5.75 (2.59)^{c}	6.25 (2.69)^{ab}	6.25 (2.69)^{ab}	6.25 (2.69)^{a}	6.13 (2.69)^{abc}
T15 (Untreated Control)	5.38 (2.53)^{b}	10.63 (3.41)^{e}	18.35 (4.40)^{g}	26.03 (5.20)^{h}	26.03 (5.20)^{f}	20.26 (5.20)^{h}
SEm ±	**0.01**	**0.10**	**0.10**	**0.10**	**0.12**	**0.17**
SED	**0.02**	**0.15**	**0.14**	**0.14**	**0.17**	**0.24**
CD ≤ 0.05	**0.04**	**0.30**	**0.29**	**0.29**	**0.34**	**0.49**
CV (%)	**2.11**	**7.19**	**5.70**	**5.27**	**6.16**	**11.28**

Values in parenthesis are * square root (√x+1) transformations; DAS = Day after sowing; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

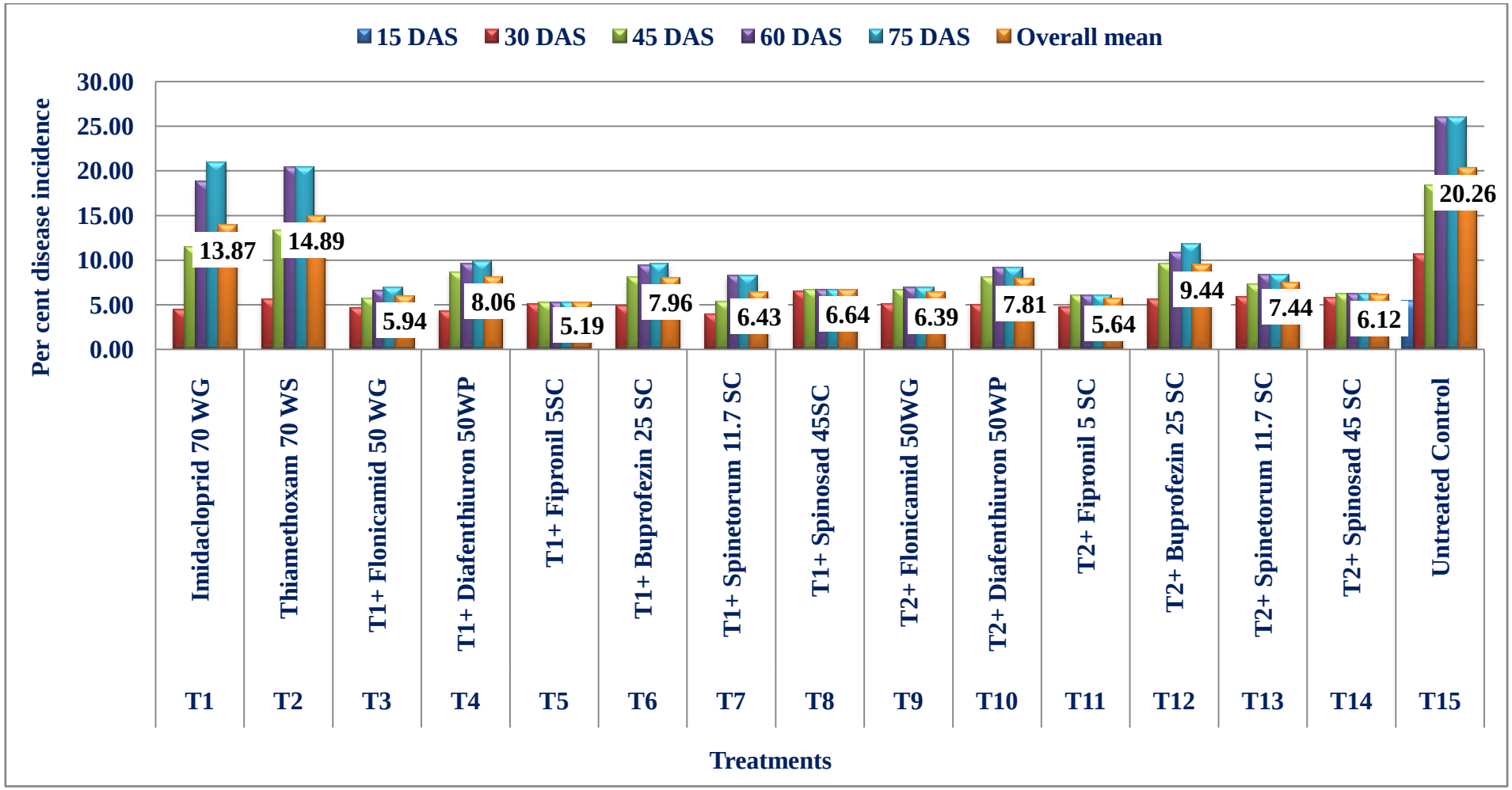


Figure 4.23. Effect of insecticidal treatments tested for thrips management on bud necrosis disease incidence in blackgram during *kharif* 2020-2021

Table 4.31. Details of plant protection costs incurred for the management of thrips in blackgram during *kharif* 2020-2021

T. No.	Treatments	Quantity of insecticide (mL or g per ha)	Cost of insecticide (Rs per ha)	Labour charges (Rs per ha)	No. of Sprays	Total Cost per ha	Yield of blackgram grains kg/ha	Increase in yield over control (kg/ha)	Value of yield (Rs per ha @ Rs 5000/q)	Net profit (Rs /ha)	ICBR	Rank
1	Seed treatment with Imidacloprid 70 WG	100	734	500	3	3702	742^{fg}	117	5833	2131	0.58	10
2	Seed treatment with Thiamethoxam 70 WS	100	800	500	3	3900	731^{fg}	106	5278	1378	0.35	12
3	T1+ Flonicamid 50 WG	250	2484	500	3	8952	1276^{ab}	651	32555	23603	2.64	3
4	T1+ Diafenthiuron 50WP	700	3134	500	3	10902	906^{def}	281	14028	3126	0.29	14
5	T1+ Fipronil 5SC	1100	2134	500	3	7902	1372^{a}	747	37361	29459	3.73	1
6	T1+ Buprofezin 25 SC	1100	1334	500	3	5502	922^{def}	297	14861	9359	1.70	5
7	T1+ Spinetoram 11.7 SC	550	5189	500	3	17067	1136^{abcd}	511	25555	8488	0.50	11
8	T1+ Spinosad 45SC	270	4619	500	3	15357	1325^{a}	700	35000	19643	1.28	6
9	T2+ Flonicamid 50WG	250	2550	500	3	9150	1225^{abc}	600	30000	20850	2.28	4
10	T2+ Diafenthiuron 50WP	700	3200	500	3	11100	1015^{cde}	390	19500	8400	0.76	9
11	T2+ Fipronil 5 SC	1100	2200	500	3	8100	1261^{abc}	636	31805	23705	2.93	2
12	T2+ Buprofezin 25 SC	1100	1400	500	3	5700	862^{efg}	237	11875	6175	1.08	8
13	T2+ Spinetoram 11.7 SC	550	5255	500	3	17265	1075^{bcde}	450	22500	5235	0.30	13
14	T2+ Spinosad 45 SC	270	4685	500	3	15555	1317^{ab}	692	34583	19028	1.22	7
15	Untreated Control						625^{g}					
SEM±							**89.33**					
CD							**258.78**					
CV							**14.70**					

Labour charges /one spray/ha @ Rs.250/labour/day, Price of blackgram grains @ Rs. 5000/qtl	Imidacloprid 70 WG @ Rs. 550/75 g	Buprofezin 25 SC @ Rs. 300/500 mL
	Thiamethoxam 70 WS @ Rs. 200/25 g	Spinetoram 11.7 SC @ Rs.990/100 mL
	Flonicamid 50 WG @ Rs. 700/60 g	Spinosad 45 SC @ Rs. 170/7 mL
	Diafenthiuron 50WP @ Rs.100/25 g	Untreated Control
	Fipronil 5 SC @ Rs. 140/100 mL	

reducing thrips population per plant, incremental cost benefit ratio was found low *i.e.* 1:1.28 and 1:1.22, respectively due to their high input cost. Remaining treatments recorded incremental cost benefit ratios in the range of 1:0.35 to 1:1.08. Least ICBR recorded was 1:0.35 with thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1}.

Similarly, Sujatha and Bharpoda (2017) who reported that higher incremental cost benefit ratio (ICBR) 1:7.81 was obtained in the treatment thiamethoxam 25 WG (0.01 %) in greengram during *kharif* 2015**.**

4.4.11 Efficacy of Insecticides against Thrips Population after the First Spray during *Rabi* 2020-2021

From the Table 4.32 it was evident that mean thrips population $plant^{-1}$ before one day of spraying was in the range of 2.80 to 4.71 with significant difference among the treatments. Among the treatments at 1 DAS, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean number of thrips per plant *i.e.* 1.73 and it was statistically at par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1.77 thrips $plant^{-1}$), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (2.07 thrips $plant^{-1}$) and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (2.23 thrips $plant^{-1}$). Per cent population reduction over untreated control was recorded highest *i.e.* 68.26 in treatment thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} with 62.20 per cent population reduction over untreated control. Remaining all treatments showed 2.85 to 52.45 per cent population reduction over untreated control.

At 3 DAS it was evident that, all the treatments were found significantly superior over untreated control (7.12 thrips $plant^{-1}$). Among the treatments tested, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded zero number of thrips per plant with 100 per cent reduction of thrips over untreated control followed by imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with 1.00 mean number of thrips per plant with 76.99 per cent reduction of thrips over untreated control. All the remaining treatments were in the range of 2.13 to

3.87 mean numbers of thrips per plant. Remaining with 2.75 to 64.52 per cent population reduction over untreated control.

The observations recorded at 7 DAS, revealed that out of the fifteen treatments tested imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded with zero number of thrips per plant with hundred per cent reduction of thrips population over untreated control.

These treatments were on par with treatments *viz.* thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1} with 0.33 and 1.00 mean number of thrips per plant, respectively. Thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded 90.58 per cent population reduction over untreated control. Remaining all treatments showed 5.00 to 83.68 per cent population reduction over untreated control.

All the treatments were significantly superior (Table 4.32) over untreated control (9.12 thrips $plant^{-1}$ at 10 DAS. Imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded with least mean number of thrips per plant *i.e.* 0.20 and it was statistically at par with treatments *viz.* thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (0.27 thrips $plant^{-1}$) and imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (0.67 thrips $plant^{-1}$).

Remaining all treatments recorded 1.07 to 5.22 mean number of thrips population per plant. Population reduction over untreated control was highest (96.12 per cent) in case of treatment imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (95.74 per cent). All the remaining treatments showed 17.57 to 86.35 per cent population reduction over untreated control. Seed treatments alone with imidacloprid 70 WG @ 70 g a.i $ha^{-1}$and with thiamethoxam 70 WS @ 70 g a.i ha^{-1} were found least effective with 0.12 and 6.08 per cent population reduction over untreated control.

Over all mean efficacy of first spray revealed that all the treatments were significantly superior over untreated control (7.87 thrips per plant). Among the treatments, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} was found effective treatment with least mean number of thrips per plant *i.e.* 0.48 and it was statistically at par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(0.51 thrips per plant) and imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.93 thrips per plant).

Remaining treatments showed 1.20 to 4.34 mean numbers of thrips per plant. Population reduction over untreated control was highest in thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} *i.e.* 91.62 per cent followed by 90.32 per cent in imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}. The remaining treatments showed 17.19 to 80.29 per cent population reduction over untreated control. Lowest per cent reduction of population over untreated control *i.e.* 4.41 and 3.69, respectively were recorded in seed treatments alone *viz.* imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} and thiamethoxam 70 WS @ 70 g a.i ha^{-1}, respectively.

4.4.11.1 Mean per cent bud necrosis disease incidence in blackgram after first spray during *rabi* 2020-2021: Mean per cent disease incidence was recorded at 30 and 45 days after sowing during first spray and was presented in the Table 4.36 (Figure 4.25, Plate 4.64). From the obtained results it was evident that all the treatment plots were infected with bud necrosis disease incidence and showed 1.15 to 3.47 per cent disease incidence at 30 DAS.

After first spray application at 45 DAS, bud necrosis disease incidence was found very low in treatment thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} *i.e.* 1.32 per cent and it was statistically at par with imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(2.22 per cent) and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(2.33 per cent). Next to these, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} were also found effective treatments with 2.71, 3.10 and 3.15 mean per cent disease incidence, respectively and they were at par each other.

Table 4.32. Efficacy of insecticides against thrips population on blackgram after first spray during *rabi* 2020-2021

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1 DBS	1 DAS	3 DAS	7 DAS	10 DAS	Mean	1 DAS	3 DAS	7 DAS	10 DAS	Mean
1	Seed treatment with Imidacloprid 70 WG	70	3.11	3.23 (2.05)^{de}	3.23 (2.05)^{de}	3.58 (2.13)^{e}	4.00 (2.23)^{e}	3.51 (2.24)^{fg}	2.85	2.75	5.35	6.08	4.41
2	Seed treatment with Thiamethoxam 70 WS	70	3.82	3.87 (2.20)^{e}	3.87 (2.20)^{e}	4.41 (2.33)^{f}	5.22 (2.49)^{f}	4.34 (2.49)^{g}	5.24	5.24	5.00	0.12	3.69
3	T1+ Flonicamid 50 WG	75	3.39	2.77 (1.94)^{bcd}	2.47 (1.86)^{cd}	0.67 (1.28)^{bc}	1.33 (1.51)^{bc}	1.81 (1.53)^{bc}	27.09	35.00	83.68	68.73	56.26
4	T1+ Diafenthiuron 50 WP	300	4.71	3.50 (2.12)^{de}	2.93 (1.98)^{d}	2.43 (1.85)^{d}	2.43 (1.85)^{d}	2.83 (1.85)^{de}	33.51	44.27	57.07	58.86	50.73
5	T1+ Fipronil 5 SC	50	4.10	1.73 (1.65)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.20 (1.10)^{a}	0.48 (1.10)^{a}	62.20	100.00	100.00	96.12	90.32
6	T1+ Buprofezin 25 SC	250	3.66	3.13 (2.03)^{cde}	3.13 (2.03)^{de}	3.00 (2.00)^{de}	3.00 (2.00)^{de}	3.07 (2.00)^{ef}	23.45	23.45	31.93	34.77	31.23
7	T1+ Spinetoram 11.7 SC	50	3.20	2.80 (1.94)^{bcd}	2.70 (1.92)^{cd}	1.00 (1.41)^{ab}	1.50 (1.58)^{c}	2.00 (1.58)^{c}	21.76	24.55	74.05	62.70	48.70
8	T1+ Spinosad 45 SC	72	3.89	2.07 (1.75)^{ab}	1.00 (1.41)^{b}	0.00 (1.00)^{a}	0.67 (1.28)^{ab}	0.93 (1.29)^{ab}	52.45	76.99	100.00	86.35	80.29
9	T2+ Flonicamid 50 WG	75	3.10	2.60 (1.90)^{bcd}	2.13 (1.77)^{c}	0.80 (1.34)^{c}	1.17 (1.47)^{bc}	1.68 (1.47)^{bc}	25.00	38.46	78.57	70.05	55.65
10	T2+ Diafenthiuron 50WP	300	3.58	2.97 (1.98)^{bcde}	2.97 (1.98)^{d}	2.70 (1.92)^{de}	2.70 (1.92)^{d}	2.83 (1.92)^{e}	25.97	25.97	37.43	40.03	35.10
11	T2+ Fipronil 5 SC	50	4.98	1.77 (1.65)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.27 (1.12)^{a}	0.51 (1.13)^{a}	68.26	100.00	100.00	95.74	91.62
12	T2+ Buprofezin 25 SC	250	2.80	2.80 (1.95)^{bcd}	2.80 (1.95)^{cd}	2.80 (1.95)^{de}	2.90 (1.97)^{d}	2.83 (1.97)^{e}	10.58	10.58	16.96	17.57	17.19
13	T2+ Spinetoram 11.7 SC	50	3.51	2.93 (1.98)^{bcde}	2.90 (1.97)^{cd}	1.13 (1.46)^{c}	1.63 (1.61)^{c}	2.15 (1.62)^{cd}	25.34	26.19	73.21	63.00	49.77
14	T2+ Spinosad 45 SC	72	2.94	2.23 (1.80)^{abc}	1.17 (1.47)^{b}	0.33 (1.14)^{ab}	1.07 (1.44)^{bc}	1.20 (1.44)^{bc}	32.07	64.52	90.58	71.13	66.50
15	Untreated Control		6.66	7.12 (2.85)^{f}	7.12 (2.79)^{f}	8.10 (3.01)^{g}	9.12 (3.18)^{g}	7.87 (3.18)^{h}					
SEm ±			**0.08**	**0.08**	**0.07**	**0.06**	**0.08**	**0.09**					
CD ≤ 0.05			**0.23**	**0.23**	**0.20**	**0.18**	**0.23**	**0.26**					
CV (%)			**6.32**	**6.93**	**6.71**	**6.65**	**7.74**	**9.84**					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

Plate 4.64. Bud necrosis infected blackgram at 30 DAS during *rabi* 2020-21

4.4.12 Efficacy of Insecticides against Thrips Population after the Second Spray during *Rabi* 2020-2021

Data was recorded at 1 DAS and presented in the Table 4.33. All the treatments found effective against thrips population over the untreated control (10.12 thrips per plant). Among the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean number of thrips *i.e.* 1.00 per plant and it was statistically on par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(1.17 thrips per plant), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(1.67 thrips per plant) and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(1.73 thrips per plant).

Remaining all treatments recorded in the range of 2.07 to 7.10 mean numbers of thrips per plant. Similarly, highest population reduction over untreated control (70.59 per cent) in imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(68.47 per cent), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(61.54 per cent) and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(61.48 per cent). Remaining all treatments showed 0.00 to 54.07 per cent population reduction over untreated control (Table 4.31, Plate 4.65).

Similarly, at 3 DAS, results indicated that imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + fipronil 5 SC

spray @ 50 g a.i ha^{-1} recorded zero number of thrips per plant with hundred per cent thrips population reduction over untreated control and they were statistically on par with thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.17 thrips $plant^{-1}$). All the remaining treatments recorded in the range of 1.07 to 7.33 thrips per plant. Thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} showed 96.81 per cent thrips population reduction over untreated control. All the remaining treatments showed 11.16 to 79.96 per cent population reduction over untreated control.

From the results obtained at 7 DAS, it was evident that imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (Plate 4.66), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (Plate 4.67), thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded zero number of thrips per plant with hundred per cent population reduction over untreated control and they were on par with thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(0.33 thrips plant) and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(0.40 thrips plant). Thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} found next best with 94.34 and 93.72 per cent population reduction over untreated control, respectively.

Imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded zero number of thrips per plant with cent per cent population reduction over untreated control at 10 DAS and they were statistically on par with thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.13) and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(0.40 thrips per plant). Next to these were thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} recorded 97.74, 94.34 and 93.72 per cent population reduction over untreated control, respectively. All the remaining treatments showed 1.73 to 79.48 per cent population

Plate 4.65. Management field trial at 40 DAS during *rabi* 2020-21

Plate 4.66. General view of effective treatment plot (T5) during *rabi* 2020-2021

Plate 4.67. General view of effective treatment plot (T8) during *rabi* 2020-2021

reduction over untreated control. Seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} recorded least population reduction over untreated control *i.e.* 1.73 per cent.

Overall mean after second spray revealed that all the treatments found significantly superior over untreated control (12.09 thrips per plant). Imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean number of thrips (0.25 per plant) among all the other treatments and it was statistically on par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(0.29 thrips per plant), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.42 thrips per plant), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.51 thrips per plant) thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (0.95 thrips per plant) and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1.08 thrips per plant).

Similarly, highest population reduction over untreated control (93.95 per cent) recorded in imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} with 93.40, 91.95 and 90.54 per cent population reduction over untreated control. All the remaining treatments showed 6.13 to 82.33 per cent population reduction over untreated control. Seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} found to be the least effective with lowest population reduction over untreated control (6.13 per cent).

4.4.12.1 Mean per cent bud necrosis disease incidence in blackgram after second spray during *rabi* 2020-2021: From the Table 4.36 (Figure 4.25) it was evident that at 6O DAS (days after sowing) disease incidence was recorded low (1.32 per cent) in thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} among all the treatments followed by imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (2.35 per cent), thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(2.52 per cent), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(2.71 per cent) and imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(3.10 per cent) and they were statistically on par with each other. Highest (23.34 per cent) disease incidence

Table 4.33. Efficacy of insecticides against thrips population on blackgram after second spray during *rabi* 2020-2021

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1DBS	1DAS	3DAS	7DAS	10DAS	Mean	1DAS	3DAS	7DAS	10DAS	Mean
1	Seed treatment with Imidacloprid 70 WG	70	5.83	5.83 (2.61)^{de}	5.83 (2.61)^{d}	7.00 (2.82)^{g}	7.50 (2.91)^{d}	6.54 (2.92)^{d}	0.00	13.95	8.28	1.73	6.13
2	Seed treatment with Thiamethoxam 70 WS	70	7.10	7.10 (2.85)^{e}	7.33 (2.89)^{e}	7.33 (2.89)^{g}	7.95 (2.99)^{d}	7.43 (2.99)^{d}	0.00	11.16	21.09	14.41	12.43
3	T1+ Flonicamid 50 WG	75	4.87	2.40 (1.84)^{b}	1.13 (1.46)^{b}	0.40 (1.16)^{abc}	0.40 (1.16)^{a}	1.08 (1.18)^{a}	50.68	79.96	93.72	93.72	81.37
4	T1+ Diafenthiuron 50WP	300	4.90	4.13 (2.27)^{c}	2.67 (1.91)^{c}	1.20 (1.48)^{de}	1.53 (1.59)^{bc}	2.38 (1.59)^{bc}	15.65	53.17	81.28	76.08	59.29
5	T1+ Fipronil 5SC	50	3.40	1.00 (1.41)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.25 (1.00)^{a}	70.59	100.00	100.00	100.00	93.85
6	T1+ Buprofezin 25 SC	250	5.97	4.67 (2.38)^{cd}	3.60 (2.14)^{c}	2.17 (1.78)^{f}	2.17 (1.78)^{bc}	3.15 (1.78)^{bc}	21.79	48.08	72.24	72.24	55.81
7	T1+ Spinetoram 11.7 SC	50	4.97	3.57 (2.13)^{c}	1.67 (1.63)^{b}	0.67 (1.28)^{bcd}	1.33 (1.53)^{b}	1.81 (1.53)^{b}	28.19	71.12	89.74	79.48	69.52
8	T1+ Spinosad 45SC	72	4.33	1.67 (1.63)^{ab}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.42 (1.00)^{a}	61.54	100	100	100.00	91.95
9	T2+ Flonicamid 50WG	75	4.50	2.07 (1.74)^{b}	1.07 (1.44)^{b}	0.33 (1.14)^{ab}	0.33 (1.14)^{a}	0.95 (1.15)^{a}	54.07	79.60	94.34	94.34	82.33
10	T2+ Diafenthiuron 50WP	300	4.67	4.33 (2.30)^{c}	3.33 (2.07)^{c}	1.67 (1.63)^{ef}	1.67 (1.63)^{bc}	2.75 (1.63)^{bc}	7.14	38.53	72.70	72.70	50.67
11	T2+ Fipronil 5 SC	50	3.70	1.17 (1.47)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.29 (1.00)^{a}	68.47	100.00	100.00	100.00	93.40
12	T2+ Buprofezin 25 SC	250	5.67	4.60 (2.37)^{cd}	3.37 (2.09)^{c}	2.30 (1.82)^{f}	2.30 (1.82)^{c}	3.14 (1.82)^{c}	18.82	48.87	68.98	68.98	53.59
13	T2+ Spinetoram 11.7 SC	50	5.00	3.83 (2.18)^{c}	1.80 (1.66)^{b}	1.00 (1.41)^{cde}	1.60 (1.61)^{bc}	2.06 (1.61)^{bc}	23.33	69.02	84.71	75.54	65.54
14	T2+ Spinosad 45 SC	72	4.50	1.73 (1.65)^{ab}	0.17 (1.07)^{a}	0.00 (1.00)^{a}	0.13 (1.06)^{a}	0.51 (1.06)^{a}	61.48	96.81	100.00	97.74	90.54
15	Untreated Control		10.12	10.12 (3.33)^{f}	11.76 (3.57)^{f}	13.24 (3.77)^{h}	13.24 (3.77)^{e}	12.09 (3.77)^{e}					
SEm ±			**0.15**	**0.09**	**0.08**	**0.09**	**0.09**	**0.10**					
CD ≤ 0.05			**0.43**	**0.26**	**0.24**	**0.26**	**0.26**	**0.28**					
CV (%)			**10.46**	**7.21**	**7.75**	**9.40**	**8.97**	**10.47**					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

recorded in untreated control. Seed treatments alone with imidacloprid 70 WG @ 70 g a.i ha^{-1} and thiamethoxam 70 WS @ 70 g a.i ha^{-1} found least effective with mean disease incidence of 16.13 and 17.67 per cent, respectively.

4.4.13 Efficacy of Insecticides against Thrips Population after the Third Spray during *Rabi* 2020-2021

Observations were recorded at 1 DAS and presented in the Table 4.34. Imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded less mean number of thrips (1.47 per plant) and it was statistically on par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(1.87 thrips per plant), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(2.27 thrips per plant) and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(2.30 thrips per plant).

Highest population reduction over untreated control (31.25 per cent) was recorded in imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(30.86 per cent). All the remaining treatments showed 0.00 to 14.77 per cent population reduction over untreated control.

It was evident that at 3 DAS, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded with zero number of thrips per plant with cent per cent population reduction over untreated control and they were statistically on par with imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.27 thrips per plant) and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.53 thrips per plant). All the remaining treatments showed 1.17 to 9.78 mean number of thrips per plant. Imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} have recorded 89.62 and 78.03 per cent reduction over untreated control. All the remaining treatments showed 0.05 to 62.32 per cent population reduction over untreated control.

At 7 DAS, results indicated that imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray

@ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded zero mean number of thrips per plant with cent per cent population reduction over untreated control and these were statistically at par with imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(0.63 thrips per plant) with 80.93 per cent population reduction over untreated control. Remaining treatments recorded in the range of 1.22 to 66.81 per cent population reduction over untreated control. Seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} and seed treatment with thiamethoxam 70 WS @ 70 g a.i ha^{-1} recorded least mean population reduction over untreated control (1.22 and 2.65 per cent, respectively).

From the obtained results at 10 DAS, same trend was observed that imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} recorded zero number of thrips per plant with cent per cent population reduction over untreated control and these treatments were statistically on par with imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(0.10 thrips per plant) with 97.04 per cent. Imidacloprid 70 WG seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1} was also found effective treatment with 0.93 mean number of thrips per plant which was on par with thiamethoxam 70 WS seed treatment + spinetoram 11.7 SC spray @ 50 g a.i ha^{-1} (1.00 thrips per plant), thiamethoxam 70 WS seed treatment + diafenthiuron 50 WP spray @ 300 g a.i ha^{-1}(1.17 thrips per plant) and imidacloprid 70 WG seed treatment + buprofezin 25 SC spray @ 250 g a.i ha^{-1}(1.43 thrips per plant). Thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} recorded 90.21 per cent population reduction over untreated control. Seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} and seed treatment with thiamethoxam 70 WS @ 70 g a.i ha^{-1} found least effective with 0.06 and 0.63 per cent population reduction over untreated control, respectively.

Overall mean of third spray revealed that all the treatments were found significantly effective than untreated control (14.63 thrips per plant). Among all the treatments tested, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} found effective with least mean number of thrips (0.26 per plant) and it was

statistically on par with imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (0.28 thrips per plant), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (0.29 thrips per plant), thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(0.39 thrips per plant), imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1.04 thrips per plant) and thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1.41 thrips per plant).

Thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded 89.17, 88.95, 88.16 and 85.82 per cent population reduction over untreated control, respectively. All the remaining treatments showed 0.42 to 68.50 mean per cent population reduction over untreated control.

4.4.13.1 Mean per cent bud necrosis disease incidence in blackgram after third spray during *rabi* 2020-2021: At 75 DAS (days after sowing), seed treatment with thiamethoxam 70 WS + fipronil 5 SC spray @ 50 g a.i ha^{-1} found effective with least disease incidence (1.32 per cent) and there is no further increase of disease was noticed since the last spray application at 60 DAS.

Similarly, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} found next best treatment with mean disease incidence 2.35 per cent and it was statistically on par with thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(2.52 per cent), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(2.71 per cent) and imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(3.10 per cent). Both seed treatments with imidacloprid 70 WG @ 70 g a.i ha^{-1} and thiamethoxam 70 WS @ 70 g a.i ha^{-1} were found as least effective treatments with mean disease incidence 17.15 and 17.67 per cent, respectively (Table 4.36, Figure 4.25).

4.4.14 Cumulative Efficacy of Insecticides against Thrips Population after Three Sprays during *Rabi* 2020-2021

From the obtained results (Table 4.35, Figure 4.24) it was clearly evident that all the treatments were significantly superior over the untreated control (11.65 thrips

per plant). Among the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean number of thrips (0.37 per plant) and it was statistically on par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (0.42 thrips per plant), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.66 thrips per plant), thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(0.81 thrips per plant), thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1.19 thrips per plant) and imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1.34 thrips per plant).

Population reduction over untreated control was 89.48, 89.22, and 82.42 per cent in thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} and imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, respectively and they found effective among all the treatments. Remaining treatments showed 3.53 to 75.83 per cent population reduction over untreated control. Least performed treatments were thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} and imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} with 5.39 and 3.53 per cent population reduction over untreated control, respectively.

Present findings pertaining to thiamethoxam efficacy are in agreement with Samota *et al*. (2017) who reported that thiamethoxam recorded 80.79 mean per cent reduction in thrips (*S. dorsalis*) population over untreated control next to acetamiprid (82.62 %) followed by imidacloprid (77.90 %), fipronil (76.38 %) and standard check (71.92 %) in chilli during summer, 2014-15. Sujatha and Bhaproda (2016) reported that thiamethoxam 25 WG @ 0.01 % and imidacloprid 70 WG @ 0.014 % found significantly superior than rest of the insecticidal treatments and recorded lower (0.62 and 0.67/3 leaves, respectively) population of thrips in greengram.

Surbhi *et al*. (2018) also found that seed treatment with thiamethoxam 25 WG @ 0.10 % was most effective with lowest thrips count (0.45 thrips/leaf). Justin *et al*. (2015) also reported that seed treatment with thiamethoxam 25 WG @ 3 g kg^{-1} of

Table 4.34. Efficacy of insecticides against thrips population on blackgram after third spray during *rabi* 2020-2021

T. No.	Treatments	Dose g.a.i./ha	Mean No. of thrips per plant						Population reduction over untreated control (%)				
			1DBS	1DAS	3DAS	7DAS	10DAS	Mean	1DAS	3DAS	7DAS	10DAS	Mean
1	Seed treatment with Imidacloprid 70 WG	70	9.15	9.15 (3.18)^{g}	9.65 (3.26)^{f}	9.65 (3.26)^{g}	9.65 (3.26)^{e}	9.53 (3.26)^{d}	0.00	0.09	0.09	0.09	0.07
2	Seed treatment with Thiamethoxam 70 WS	70	9.27	9.27 (3.20)^{g}	9.78 (3.28)^{f}	9.78 (3.28)^{g}	9.78 (3.28)^{e}	9.65 (3.28)^{d}	0.00	0.05	0.05	0.05	0.04
3	T1+ Flonicamid 50 WG	75	3.10	2.73 (1.93)^{bcdef}	1.33 (1.52)^{bc}	0.50 (1.21)^{ab}	0.00 (1.00)^{a}	1.14 (1.00)^{a}	11.83	59.25	84.72	100.00	64.65
4	T1+ Diafenthiuron 50WP	300	3.43	3.43 (2.10)^{ef}	2.43 (1.85)^{de}	1.40 (1.54)^{cd}	1.33 (1.52)^{bc}	2.15 (1.53)^{b}	0.00	32.86	61.37	63.21	39.88
5	T1+ Fipronil 5SC	50	2.13	1.47 (1.56)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.37 (1.00)^{a}	31.25	100.00	100.00	100.00	83.50
6	T1+ Buprofezin 25 SC	250	3.73	3.73 (2.17)^{f}	3.13 (2.03)$_{e}$	2.37 (1.83)^{ef}	1.70 (1.64)^{c}	2.73 (1.64)^{bc}	0.00	20.49	39.94	56.86	29.71
7	T1+ Spinetoram 11.7 SC	50	3.47	3.40 (2.10)^{ef}	1.90 (1.70)^{bcd}	0.97 (1.40)^{bc}	0.97 (1.40)^{b}	1.81 (1.40)^{b}	1.92	48.08	73.58	73.58	49.92
8	T1+ Spinosad 45SC	72	2.43	2.27 (1.80)^{abc}	0.27 (1.11)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.63 (1.00)^{a}	6.85	89.62	100.00	100.00	75.01
9	T2+ Flonicamid 50WG	75	2.93	2.50 (1.87)^{bcde}	1.17 (1.47)^{b}	0.17 (1.07)^{a}	0.00 (1.00)^{a}	0.96 (1.00)^{a}	14.77	62.32	94.62	100.00	68.64
10	T2+ Diafenthiuron 50WP	300	3.40	3.40 (2.10)^{ef}	2.67 (1.91)^{de}	1.77 (1.66)^{de}	1.67 (1.63)c	2.38 (1.63)^{b}	0.00	25.70	50.77	53.56	32.94
11	T2+ Fipronil 5 SC	50	2.70	1.87 (1.67)^{ab}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.47 (1.00)^{a}	30.86	100.00	100.00	100.00	83.41
12	T2+ Buprofezin 25 SC	250	3.20	3.20 (2.04)^{cdef}	3.17 (2.04)^{e}	3.03 (2.00)^{f}	2.53 (1.87)^{d}	2.98 (1.88)^{c}	0.00	6.25	10.20	25.00	10.50
13	T2+ Spinetoram 11.7 SC	50	3.53	3.33 (2.08)^{def}	2.07 (1.75)^{cd}	1.27 (1.50)^{cd}	1.00 (1.41)^{b}	1.92 (1.41)^{b}	5.66	44.59	66.04	73.19	47.92
14	T2+ Spinosad 45 SC	72	2.30	2.30 (1.81)^{abcd}	0.53 (1.20)^{a}	0.00 (1.00)^{a}	0.00 (1.00)^{a}	0.71 (1.00)^{a}	0.00	78.03	100.00	100.00	70.43
15	Untreated Control		14.40	14.40 (3.92)^{h}	15.20 (4.02)^{g}	15.20 (4.02)^{h}	15.20 (4.02)^{f}	15.00 (4.02)^{e}					
SEm ±			0.07	0.09	0.08	0.07	0.06	0.09					
CD ≤ 0.05			0.20	0.27	0.25	0.21	0.20	0.25					
CV (%)			5.14	7.15	7.57	7.11	6.94	9.22					

Values in parenthesis are * square root (√x+1) transformations; DBS = Day before spraying; DAS = Day after spraying; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

Table 4.35. Cumulative efficacy of insecticides against thrips population on blackgram during *rabi* 2020-2021

T. No.	Treatments	Dose g.a.i./ha	Mean No of thrips per plant				Population reduction over untreated control (%)			
			First spray	Second Spray	Third Spray	Mean	First spray	Second Spray	Third Spray	Mean
1	Seed treatment with Imidacloprid 70 WG	70	3.51 (2.24)^{fg}	6.54 (2.92)^{d}	9.53 (3.26)^{d}	6.53 (2.70)^{f}	4.41	6.13	0.07	3.53
2	Seed treatment with Thiamethoxam 70 WS	70	4.34 (2.49)^{g}	7.43 (2.99)^{d}	9.65 (3.28)^{d}	7.14 (2.83)^{f}	3.69	12.43	0.04	5.39
3	T1+ Flonicamid 50 WG	75	1.81 (1.53)^{bc}	1.08 (1.18)^{a}	1.14 (1.00)^{a}	1.34 (1.53)^{abcd}	56.26	81.37	64.65	67.42
4	T1+ Diafenthiuron 50WP	300	2.83 (1.85)^{de}	2.38 (1.59)^{bc}	2.15 (1.53)^{b}	2.45 (1.86)^{cde}	50.73	59.29	39.88	49.97
5	T1+ Fipronil 5SC	50	0.48 (1.10)^{a}	0.25 (1.00)^{a}	0.37 (1.00)^{a}	0.37 (1.17)^{a}	90.32	93.85	83.50	89.22
6	T1+ Buprofezin 25 SC	250	3.07 (2.00)^{ef}	3.15 (1.78)^{bc}	2.73 (1.64)^{bc}	2.98 (2.00)^{e}	31.23	55.81	29.71	38.92
7	T1+ Spinetoram 11.7 SC	50	2.00 (1.58)^{c}	1.81 (1.53)^{b}	1.81 (1.40)^{b}	1.87 (1.69)^{bcde}	48.70	69.52	49.92	56.05
8	T1+ Spinosad 45SC	72	0.93 (1.29)^{ab}	0.42 (1.00)^{a}	0.63 (1.00)^{a}	0.66 (1.29)^{ab}	80.29	91.95	75.01	82.42
9	T2+ Flonicamid 50WG	75	1.68 (1.47)^{bc}	0.95 (1.15)^{a}	0.96 (1.00)^{a}	1.19 (1.48)^{abc}	55.65	82.33	68.64	68.87
10	T2+ Diafenthiuron 50WP	300	2.83 (1.92)^{e}	2.75 (1.63)^{bc}	2.38 (1.63)^{b}	2.65 (1.91)^{de}	35.10	50.67	32.94	39.57
11	T2+ Fipronil 5 SC	50	0.51 (1.13)^{a}	0.29 (1.00)^{a}	0.47 (1.00)^{a}	0.42 (1.19)^{a}	91.62	93.40	83.41	89.48
12	T2+ Buprofezin 25 SC	250	2.83 (1.97)^{e}	3.14 (1.82)^{c}	2.98 (1.88)^{c}	2.98 (2.00)$_{e}$	17.19	53.59	10.50	27.09
13	T2+ Spinetoram 11.7 SC	50	2.15 (1.62)^{cd}	2.06 (1.61)^{bc}	1.92 (1.41)^{b}	2.04 (1.74)^{cde}	49.77	65.54	47.92	54.41
14	T2+ Spinosad 45 SC	72	1.20 (1.44)^{bc}	0.51 (1.06)^{a}	0.71 (1.00)^{a}	0.81 (1.34)^{abc}	66.50	90.54	70.43	75.83
15	Untreated Control		7.87 (3.18)^{h}	12.09 (3.77)^{e}	15.00 (4.02)^{e}	11.65 (3.53)^{g}				
SEm ±			**0.09**	**0.10**	**0.09**	**0.14**				
CD ≤ 0.05			**0.26**	**0.28**	**0.25**	**0.41**				
CV (%)			**9.84**	**10.47**	**9.22**	**13.03**				

Values in parenthesis are * square root (√x+1) transformations; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05).

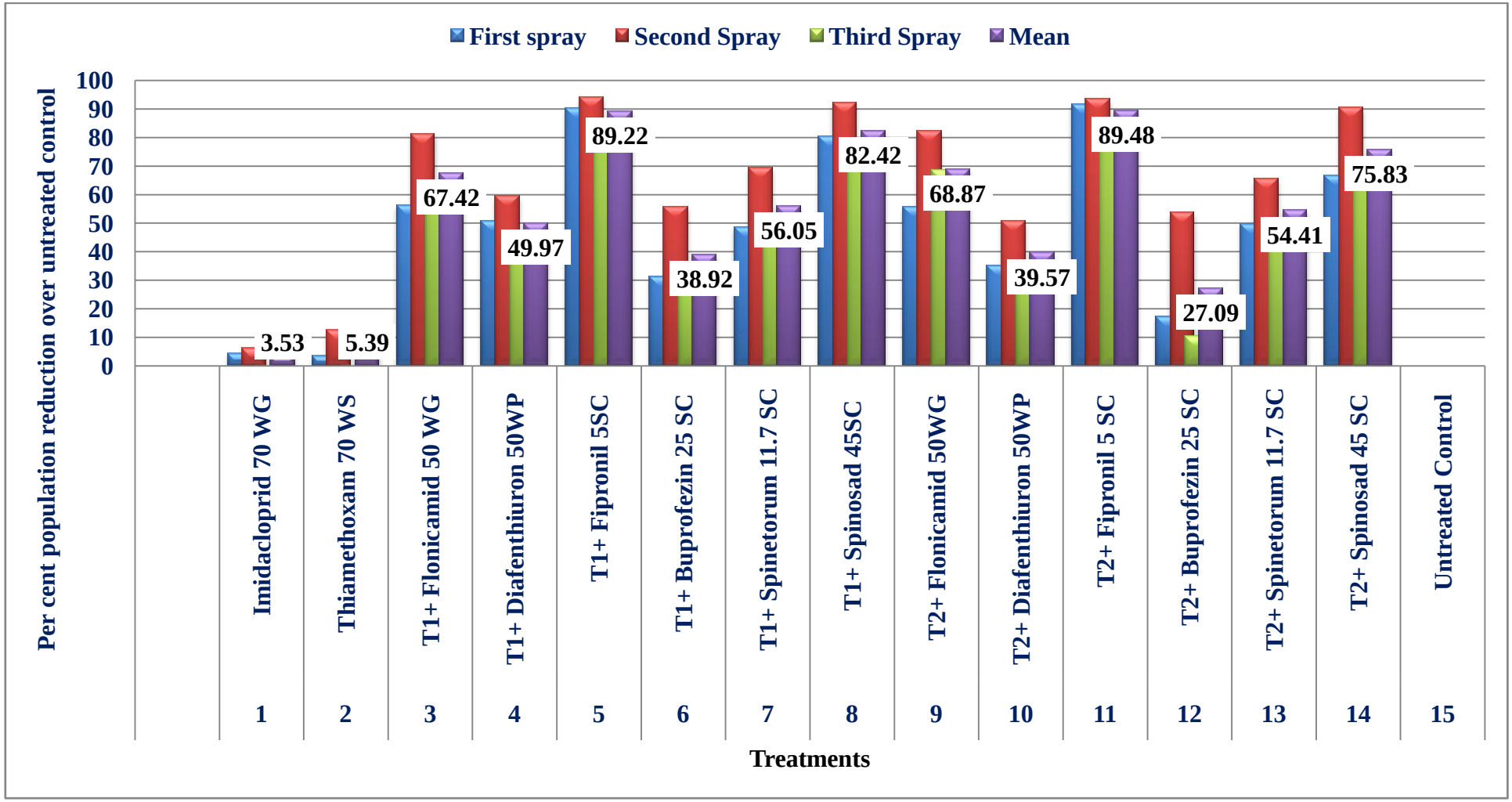


Figure 4.24. Cumulative efficacy of insecticides against thrips population on blackgram during *rabi* 2020-2021

seed + spray with thiamethoxam 25 WG @ 0.4 g L^{-1} recorded the lowest population of aphids (0.73/plant) followed by spraying of imidacloprid 17.8 SL @ 0.4 mL L^{-1} (1.03/plant) when compared to control, in blackgram during *rabi* season.

Seed treatment with thiamethoxam 25 WG @ 3g kg^{-1} of seed + spray with thiamethoxam 25 WG @ 0.4 g L^{-1} exerted 90.32 per cent reduction of aphids followed by spraying of imidacloprid 17.8 SL @ 0.4 mL L^{-1} (87.45 %). Shobharani *et al*. (2017) reported that the higher doses of imidacloprid 60 FS, (10 mL and 15 mL kg^{-1} of seeds) proved superior in protecting the blackgram crop under rainfed condition from the early season sucking pests up to 40- 45 days after sowing and recorded significantly highest grain yield.

Results pertaining to spinosad 45SC @ 72 g a.i ha^{-1} are in accordance with Sharanappa *et al*. (2020) who reported that the overall mean per cent reduction of thrips population after imposing first, second and third spray was highest in spinosad 45 SC (88.15 %) followed by fipronil 5 SC (87.24 %) and found significantly superior than rest of the treatments in capsicum during *rabi* season. Kumar *et al*. (2017) reported that seed treatment with imidacloprid 600 FS @ 5 mL kg^{-1} seeds + two sprays of spinosad 45 SC @ 56 g a.i. ha^{-1} at 15 days interval found most effective with minimum pod damage of 6.44 per cent and maximum grain yield of 703 kg ha^{-1} in blackram during summer.

Present findings about efficacy of fipronil spray are in line with Swathi *et al*. (2018) who reported that among the tested insecticides acetamiprid 4 % + fipronil 4 % @ 2 mL L^{-1} found effective against thrips by reducing 70.81 % thrips population next to thiacloprid 21.7 SC @ 0.0325 % with 74.80 % reduction of thrips population over untreated control in rice fallow blackgram during *rabi* 2017-18.

4.4.14.1 Mean per cent bud necrosis disease incidence of blackgram after three sprays during *rabi* 2020-2021 : Data pertaining to overall mean bud necrosis disease incidence in blackgram after three sprays during crop period indicated that thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean disease incidence (1.28) and it was statistically on par with imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(2.15 %), thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(2.16 %), thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(2.64 %) and imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(3.05 %). All the remaining treatments showed 3.21 to

13.18 per cent disease incidence. Least performed treatments were thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1} and imidacloprid 70 WG seed treatment @ 70 g a.i ha^{-1} with 13.18 and 11.84 mean per cent disease incidence, respectively (Table 4.36, Figure 4.25, Plate 4.68).

Present findings are in line with Swathi *et al*. (2018) who reported that flonicamid 50 WG @ 0.0325 % found effective against the population of whitefly by reducing 72.19 % and lowest per cent YMV disease incidence (17.66 %) followed by acetamiprid 4 % + fipronil 4 % @ 2 mL L^{-1} (64.94 %) and thiamethoxam 25 WG @ 0.005 % (62.21 %) which were on par with each other over untreated control in rice fallow blackgram during *rabi* 2017-18.

4.4.15 Incremental Cost Benefit Ratio (ICBR)

From the data presented in the Table 4.37, revealed that all the treatments recorded better grain yield over untreated control (578 kg $ha^{-1)}$. Among the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded highes-t grain yield *i.e.* 1439 kg ha^{-1} with incremental cost benefit ratio 1:4.45 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i a^{-1} (1:3.97), imidacloprid seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1:2.19) thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1}(1 2.10). Even though the treatments *viz.* imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} found effective in reducing thrips population per plant, their incremental cost benefit ratios found low (1:1.58 and 1:1.23, respectively) due to high input cost. Remaining treatments recorded incremental cost benefit ratios in the range of 1:0.10 to 1:1.30. Least ICBR recorded was 1:0.10 in thiamethoxam 70 WS seed treatment @ 70 g a.i ha^{-1}.

The present findings are in line with Darshan *et al*. (2018) who reported that imidacloprid 17.8 SL @ 0.005 per cent found most effective with lowest population of thrips (1.30) and found on par with thiamethoxam 25 WG @ 0.008 per cent (1.33) and acetamiprid 20 SP @ 0.004 per cent (1.36). The maximum yield was obtained in plots treated with thiamethoxam 25 WG @ 0.005 % (701 kg ha^{-1}) against control plot (400 kg ha^{-1}) in mothbean during *rabi* season. Swathi *et al*. (2018) also reported that the highest seed yield was gained from the plots treated with thiamethoxam 25 WG @ 0.10 %+

Plate 4.68. Bud necrosis infected blackgram at 20, 40, 55 DAS during *rabi* 2020-2021

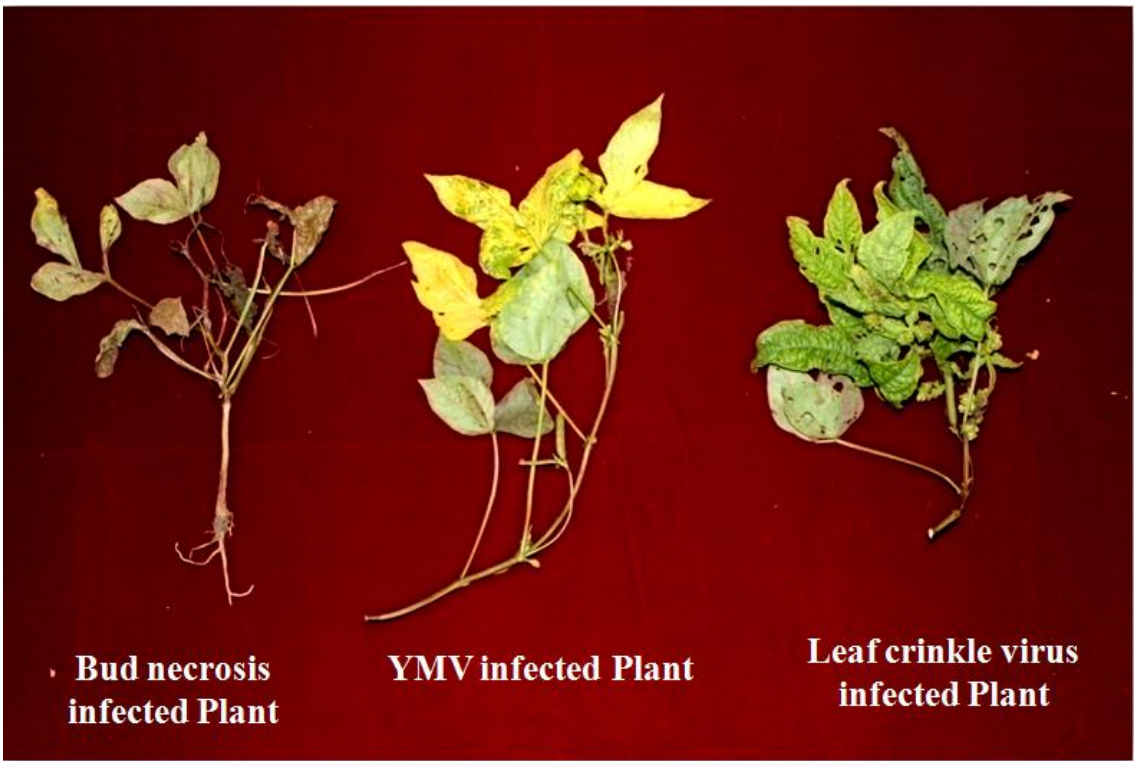


Plate 4.69. Other diseases observed in the untreated control plot during experiment

spinosad 45 SC @ 0.0135 % with 1066 kg ha^{-1} in rice fallow blackgram during *rabi* 2017-18.

Table 4.36. Effect of insecticidal treatments tested for thrips management on bud necrosis disease incidence in blackgram during *rabi* 2020-2021

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	Overall mean
T1 (Seed treatment with imidacloprid 70 WG)	0.00	2.30 (1.81)^{cde}	11.77 (3.56)^{e}	16.13 (4.14)^{f}	17.15 (4.26)^{f}	11.84 (4.26)^{e}
T2 (Seed treatment with thiamethoxam 70 WS)	0.00	3.07 (2.02)^{efg}	14.30 (3.90)^{e}	17.67 (4.32)^{f}	17.67 (4.32)^{f}	13.18 (4.32)^{ef}
T3 (T1+ Flonicamid 50 WG)	0.00	2.13 (1.77)^{cd}	3.15 (2.03)^{b}	3.78 (2.18)^{c}	3.78 (2.18)^{c}	3.21 (2.19)^{bc}
T4 (T1+ Diafenthiuron 50WP)	0.00	1.93 (1.71)^{bc}	7.37 (2.89)^{d}	7.66 (2.94)^{e}	7.66 (2.94)^{e}	6.16 (2.94)^{d}
T5 (T1+ Fipronil 5SC)	0.00	2.90 (1.97)^{defg}	3.10 (2.01)^{b}	3.10 (2.01)^{bc}	3.10 (2.01)^{bc}	3.05 (2.02)^{ab}
T6 (T1+ Buprofezin 25 SC)	0.00	4.82 (2.41)^{h}	4.82 (2.41)^{c}	7.05 (2.84)^{de}	7.05 (2.84)^{de}	5.93 (2.84)^{d}
T7 (T1+ Spinetoram 11.7 SC)	0.00	3.33 (2.08)^{fg}	5.85 (2.61)^{cd}	6.20 (2.68)^{d}	6.20 (2.68)^{d}	5.40 (2.68)^{cd}
T8 (T1+ Spinosad 45SC)	0.00	1.68 (1.62)^{abc}	2.22 (1.78)^{ab}	2.35 (1.82)^{b}	2.35 (1.82)^{b}	2.15 (1.83)^{ab}
T9 (T2+ Flonicamid 50WG)	0.00	1.27 (1.51)^{ab}	2.33 (1.83)^{ab}	2.52 (1.88)^{b}	2.52 (1.88)^{b}	2.16 (1.88)^{ab}
T10 (T2+ Diafenthiuron 50WP)	0.00	2.06 (1.75)^{bcd}	6.80 (2.79)^{d}	7.27 (2.87)^{de}	7.27 (2.87)^{de}	5.85 (2.88)^{d}
T11 (T2+ Fipronil 5 SC)	0.00	1.15 (1.45)^{a}	1.32 (1.52)^{a}	1.32 (1.52)^{a}	1.32 (1.52)^{a}	1.28 (1.52)^{a}
T12 (T2+ Buprofezin 25 SC)	0.00	1.72 (1.64)^{abc}	7.75 (2.95)^{d}	8.34 (3.06)^{e}	8.34 (3.06)^{e}	6.54 (3.06)^{d}
T13 (T2+ Spinetoram 11.7 SC)	0.00	1.07 (1.44)^{a}	5.82 (2.61)^{cd}	6.12 (2.67)^{d}	6.12 (2.67)^{d}	4.78 (2.67)^{cd}
T14 (T2+ Spinosad 45 SC)	0.00	2.43 (1.85)^{cdef}	2.71 (1.92)^{b}	2.71 (1.92)^{b}	2.71 (1.92)^{b}	2.64 (1.93)^{ab}
T15 (Untreated Control)	0.00	3.47 (2.11)^{g}	18.07 (4.36)^{f}	23.34 (4.93)^{g}	23.34 (4.93)^{g}	17.05 (4.93)^{f}
SEm ±	**0.00**	**0.08**	**0.12**	**0.09**	**0.08**	**0.22**
SED	**0.00**	**0.12**	**0.16**	**0.12**	**0.12**	**0.31**
CD ≤ 0.05	**0.00**	**0.24**	**0.34**	**0.25**	**0.25**	**0.63**
CV (%)	**0.00**	**7.84**	**7.71**	**5.44**	**5.25**	**17.69**

Values in parenthesis are * square root (√x+1) transformations; in a column means followed by same letters do not differ significantly by LSD (P ≤ 0.05). DAS= Days after sowing.

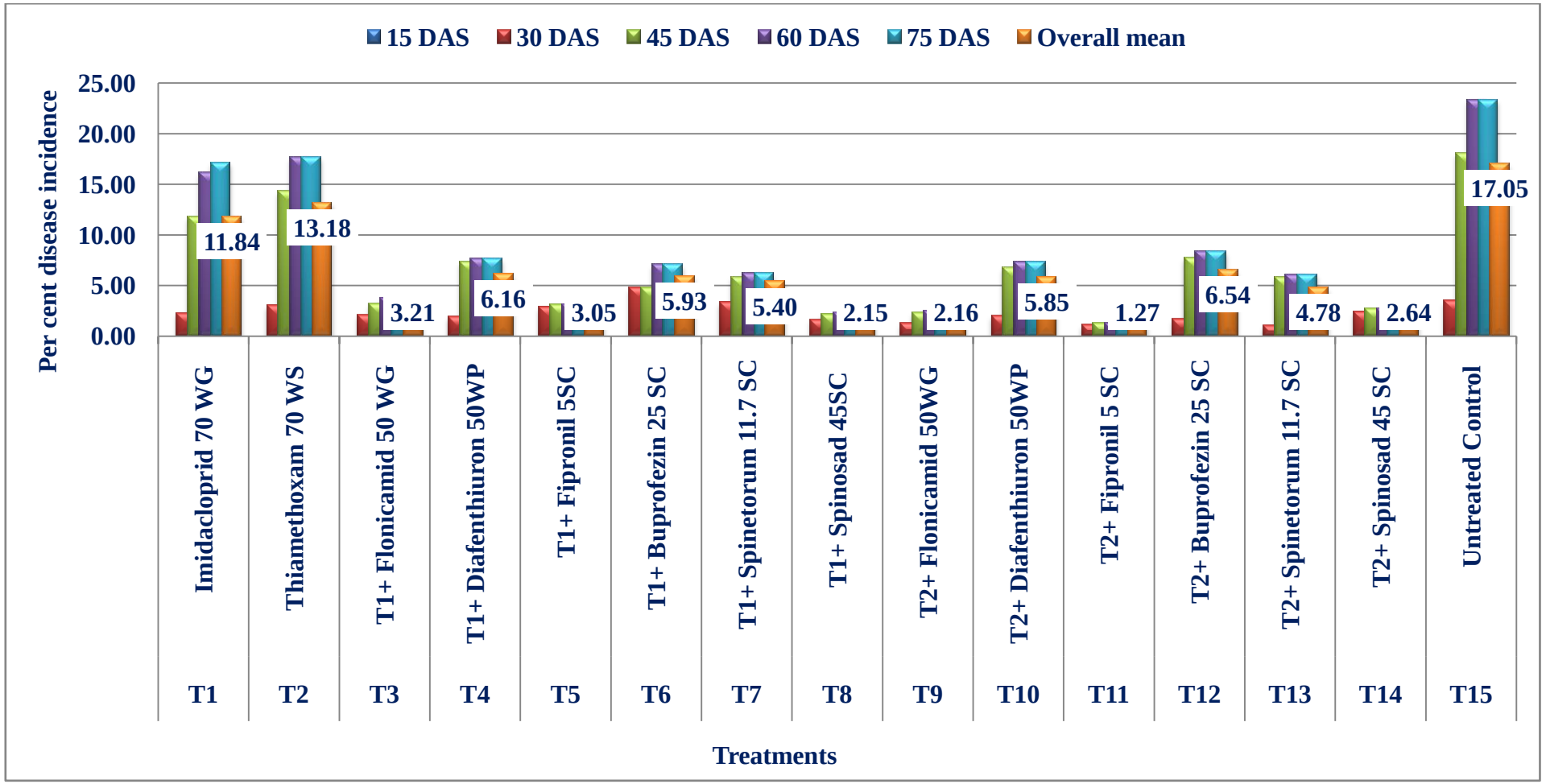


Figure 4.25. Effect of insecticidal treatments tested for the management of thrips on the bud necrosis disease incidence in blackgram during *rabi* 2020-2021

Table 4.37. Details of plant protection costs incurred for the management of thrips in blackgram during *rabi* 2020-2021

T. No.	Treatments	Quantity of insecticide (mL or g per ha)	Cost of insecticide (Rs per ha)	Labor charges (Rs per ha)	No. of Sprays	Total Cost per ha	Yield of blackgram grains kg/ha	Increase in yield over control (kg/ha)	Value of yield (Rs per ha @ Rs 5000/q)	Net profit (Rs /ha)	ICBR	Rank
1	Seed treatment with Imidacloprid 70 WG	100	734	500	3	3702	717^{fgh}	139	6944	3242	0.88	9
2	Seed treatment with Thiamethoxam 70 WS	100	800	500	3	3900	664^{gh}	86	4306	406	0.10	13
3	T1+ Flonicamid 50 WG	250	2484	500	3	8952	1133^{cd}	556	27778	18826	2.10	4
4	T1+ Diafenthiuron 50WP	700	3134	500	3	10902	842^{efg}	264	13194	2292	0.21	10
5	T1+ Fipronil 5SC	1100	2134	500	3	7902	1439^{a}	861	43055	35153	4.45	1
6	T1+ Buprofezin 25 SC	1100	1334	500	3	5502	831^{efg}	253	12639	7137	1.30	7
7	T1+ Spinetoram 11.7 SC	550	5189	500	3	17067	981^{de}	403	20139	3072	0.18	11
8	T1+ Spinosad 45SC	270	4619	500	3	15357	1369^{abc}	792	39583	24226	1.58	5
9	T2+ Flonicamid 50WG	250	2550	500	3	9150	1161^{bcd}	583	29167	20017	2.19	3
10	T2+ Diafenthiuron 50WP	700	3200	500	3	11100	836^{efg}	258	12917	1817	0.16	12
11	T2+ Fipronil 5 SC	1100	2200	500	3	8100	1383^{ab}	806	40278	32178	3.97	2
12	T2+ Buprofezin 25 SC	1100	1400	500	3	5700	825^{efgh}	247	12361	6661	1.17	8
13	T2+ Spinetoram 11.7 SC	550	5255	500	3	17265	953^{def}	375	18750	1485	0.09	14
14	T2+ Spinosad 45 SC	270	4685	500	3	15555	1272^{abc}	694	34722	19167	1.23	6
15	Untreated Control	NA	NA	NA	NA	NA	578^{h}	NA				
SEM±							**86.72**					
CD							**251.23**					
CV							**15.04**					

Labour charges /one spray/ha @ Rs.250/labour/day, Price of blackgram grains @ Rs. 5000/qtl	Imidacloprid 70 WG @ Rs. 550/75 g	Buprofezin 25 SC @ Rs. 300/500 mL
	Thiamethoxam 70 WS @ Rs. 200/25 g	Spinetoram 11.7 SC @ Rs.990/100 mL
	Flonicamid 50 WG @ Rs. 700/60 g	Spinosad 45 SC @ Rs. 170/7 mL
	Diafenthiuron 50WP @ Rs.100/25 g	Untreated Control
	Fipronil 5 SC @ Rs. 140/100 mL	

Chapter – V

Summary and Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

Thrips species were collected from different areas in Andhra Pradesh *viz.* Vizianagaram and Srikakulam (North Costal Zone), Guntur, Krishna and Prakasam (Krishna Zone), Kurnool and Chittoor districts (Southern Zone). From each district five mandals were identified which were major blackgram cultivating areas (Total 35 locations). In each mandal samples were collected at random in a single field.

Based on the morphological identification, major species of thrips species on black gram in Andhra Pradesh state was *T. palmi, M. usitatus*, and *S. dorsalis.* Out of thirty five locations surveyed, no record of *S. dorsalis* was observed in 19 mandals. In Chittoor district *Megalurothrips typicus* (Bagnall), *Ayyaria chaetophora* (Karny), *Phibalothrips peringueyi* (Faure) and some Tubuliferan thrips were also observed in meager number.

The specific primers used in the current study enabled successful amplification of most collected specimens which could be properly identified into three deferent species through the ITS2 (*T. palmi*), COI (*M. usitatus*), COIII (*S. dorsalis*) gene fragment-based DNA barcoding. Hence, it was proved that morphological identification coupled with molecular characterization gives us accurate identification of particular species.

A total of 21 samples *i.e.* seven samples for each thrips species were sequenced bidirectional and then submitted in NCBI website for accession numbers. Generated sequences of present study showed 97 to 100 per cent similarity with data base sequences. Phylogeny tree was constructed using neighbor joining (NJ, ML) method depicted cohesive clustering of the identified sequences of *T. palmi*, *M. usitatus and S. dorsalis.*

Tajimas d statistic revealed the existence of low genetic polymorphism among the, ITS2 sequences of *T. palmi,* COI sequences of *M .usitatus*, COIII sequences of *S. dorsalis*.

The thrips population was observed in the field approximately after 14 DAS. The thrips incidence showed a significant increase in population with the increase in temperature. Thereafter at maturity a steep fall in the thrips population was observed. The thrips population showed positive association with the atmospheric temperature (During *rabi* maximum and minimum temperatures showed significant positive correlation with thrips population and per cent disease incidence). The prevailing weather conditions played a major role in thrips and bud necrosis disease outbreak. The clear weather conditions with no rainfall during the crop growth period have favored the thrips population as well as bud necrosis disease incidence. Relative humidity has also played vital role in buildup of thrips population in two different seasons *viz. kharif* and *rabi.*

Overall view of thrips and GBNV incidence in blackgram in the present study revealed that the density of thrips population and per cent disease incidence was more during *kharif* 2020-2021 (26.05 %) compared to *rabi* 2019-2020 (25.90 %) and *rabi* 2020-2021 (10.22 %). Atmospheric temperature has a positive and significant correlation with the bud necrosis disease incidence in blackgram. The temperatures not only increased the disease incidence (%) but also the severity of the disease.

Virus isolates were maintained on cow pea cv-152, since cow pea has consistently produced more local lesions within 4 to 5 days after inoculation than blackgram. Presence of GBNV virus in diseased leaves of blackgram from the experimental fields and cow pea leaves from potted plants which exhibited chlorotic symptoms after mechanical inoculation was confirmed through RT-qPCR using nucleo capsid and degenerated coat protein primers of GBNV.

Insect transmission studies were carried out in the laboratory with two species *viz. T. palmi* and *M. usitatus.* Out of these two species tested, only *T. palmi* could transmit the *GBNV* from diseased to healthy cowpea plants where in the inoculated plants exhibited symptoms *viz*. chlorotic local lesions. Whereas *M. usitatus* failed to transmit the virus and the inoculated plants remained healthy. Hence, *Thrips palmi* was identified as vector of *Groundnut bud necrosis virus* in blackgram.

A minimum of 2 h acquisition access period (48 IAP), 4h inoculation access period (24 h AAP) was required to transmit the bud necrosis disease in case of first

instar larvae of *T. palmi* and a minimum of two larvae were required to transmit the bud necrosis disease at 24 AAP, 48 h IAP; 48 AAP, 48 h IAP.

A minimum of 2 h acquisition access period (with 48 h IAP), 8 h inoculation access period (24 h AAP) was observed in case of second instar larvae and a minimum of 10 larvae required to transmit the disease at 24 AAP and 48 h IAP, and two larvae required to transmit the disease at 48 AAP and 48 h IAP.

There was no disease transmission was observed at 30 min, 1 h, 2 h, 4 h, 6 h, and 8 h of acquisition access period and inoculation access periods in case of adults. Surprisingly a minimum of 24 h AAP (48 h IAP), 24 h IAP (24 h AAP) was observed with 8.33 per cent of disease transmission and there is no further increase of disease with increasing AAP (48 h IAP) and IAP (24 h AAP). A minimum of 10 adults were required to transmit the disease at 24 h AAP, 48 h IAP; 48 AAP and 48 h IAP.

The meager transmission by adult thrips can be attributed to variations in virus genotype, thrips genotype, and environmental conditions, crop phenology which are likely play a critical role in these differential interactions. The genotype of present study isolate *i.e.* GBNV-BG from Andhra Pradesh may contain certain genetic alternations in relation with particular thrips genotype (*T. palmi*). From the present study it is proved that GBNV-BG isolate is transmitted in persistent propagative manner by thrips vector.

Among the all evaluated treatments imidacloprid 70WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} followed by thiamethoxam seed treatment + fipronil 5 SC spray @ 50 g a.i were proved to be the best in reducing the thrips population with highest blackgram grain yield *i.e.* 1414 kg ha^{-1} with incremental cost benefit ratio 1:4.80 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1:4.47) during *rabi* 2019-2020. Similarly, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least bud necrosis disease incidence among the all other tested insecticides.

During *Kharif* 2020-2021, highest population reduction over untreated control was found in treatment imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (86.67 per cent) followed by imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}(86.62). Among the treatments tested, imidacloprid

70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded highest grain yield *i.e.* 1372 kg ha^{-1} with incremental cost benefit ratio 1:3.73 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1:2.93). Even though the treatments *viz.* imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}, thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} were found effective treatments in reducing thrips population per plant, incremental cost benefit ratio was found low *i.e.* 1:1.28 and 1:1.22, respectively due to their high input cost.

Similarly, among the tested treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean bud necrosis disease incidence in blackgram *i.e.* 5.19 per cent and it was at par with thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (5.64 per cent), imidacloprid 70 WG seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (5.94 per cent) thiamethoxam 70 WS seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1}(6.13 per cent), thiamethoxam 70 WS seed treatment + flonicamid 50 WG spray @ 75 g a.i ha^{-1} (6.39 per cent), imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} (6.64 per cent) during *kharif* 2020-2021.

During *rabi* 2020-2021, thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1}, imidacloprid 70 WG seed treatment + spinosad 45 SC spray @ 72 g a.i ha^{-1} proved best in reducing the thrips population (89.48, 89.22 and 82.42). Thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded least mean per cent disease incidence (1.28). Among the treatments imidacloprid 70 WG seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} recorded highest grain yield *i.e.* 1439 kg ha^{-1} with incremental cost benefit ratio 1:4.45 followed by thiamethoxam 70 WS seed treatment + fipronil 5 SC spray @ 50 g a.i ha^{-1} (1:3.97).

Present study concluded that *T. palmi* is the dominant species and acts as potential vector of GBNV transmission in blackgram. This sucking pest occurs in both *kharif* and *rabi* seasons in clear weather conditions with less rainfall and reaching its peak at 45 to 60 DAS causing an yield loss up to 10 per cent. Seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} followed by fipronil 5 SC spray @ 50 g a.i ha^{-1} proved better in reducing the thrips population simultaneously disease incidence with good incremental cost benefit ratio in both *rabi* and *kharif* seasons.

Conclusions

- Major species of thrips species on black gram in Andhra Pradesh state was *T. palmi, M. usitatus,* and *S. dorsalis. Megalurothrips typicus* (Bagnall), *Ayyaria chaetophora* (Karny), *Phibalothrips peringueyi* (Faure) and some Tubuliferan thrips were also found in meager number.

- ITS2 (*T. palmi*), mtCOI (*M. usitatus*), mtCOIII (*S. dorsalis*) gene fragment-based DNA barcoding found useful in identification of thrips at species level.

- The thrips population was started infesting blackgram at 14 DAS and reached peak at 65 DAS and started declining towards maturity of the crop. Temperature, wind speed and rainfall have played major role in development of thrips and bud necrosis disease incidence.

- From the present study it was proved that GBNV-BG isolate is transmitted in persistent propagative manner by *T. palmi* where as *M. usitatus* was failed to transmit the virus.

- Seed treatment with imidacloprid 70 WG @ 70 g a.i ha^{-1} followed by fipronil 5 SC spray @ 50 g a.i ha^{-1} proved better in reducing the thrips population and disease incidence with good incremental cost benefit ratio in both *rabi* and *kharif* seasons, 2019 & 2020.

Future line of work

- Potential and periodical survey for thrips species infestation and bud necrosis disease incidence in blackgram throughout Andhra Pradesh has to be taken up.

- GBNV-BG isolate needs to be characterized and its transmission biology to be studied.

- Effect of GBNV-BG isolate on vector development.

- Detailed transmission experiments with *T. palmi* adults needs to be studied along with genetic determinants of virus passage inside the adult stage of vector.

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LITERATURE CITED

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*** Original not seen**

Note: The pattern of literature cited presented above is in accordance with the guidelines for thesis presentation, Acharya N.G. Ranga Agricultural University, Lam, Guntur.

Appendices

Annexure I

Particulars of Villages and Mandals of Andhra Pradesh from which thrips specimens were collected on blackgram

S. No.	District	Mandal	Village	Geographic coordinate	
1	Srikakulam	Etcherla	Nandigam	18.2412584	83.8447353
2		Ponduru	Rapaka	18.3532918	83.7834948
3		Sigadam	Chettupodili	18.3701058	83.6845722
4		Regidi Amudalavalasa	Burada	18.5608624	83.7368810
5		Rajam	Rajam	18.4813227	83.7606665
6	Vizianagaram	Gantayada	Gantayada	18.1430914	83.2878155
7		Bondapalli	Nelivada	18.2042411	83.3754091
8		Mentada	Meesalapeta	18.3388977	83.2981347
9		Dattirajeru	Pedamanapuram	18.3659343	83.3438147
10		Gajapathinagaram	Jinnam	18.2848787	83.3891682
11	Krishna	Avanigadda	Avanigadda	16.0116513	80.9090502
12		Challapalli	Challapalli	16.0888576	80.9127349
13		Mopidevi	Mopidevi	16.1262959	80.9325017
14		Pamarru	Pamarru	16.3040357	80.9583395
15		Movva	Movva	16.2309489	80.9190293
16	Guntur	Pittalavaniaplem	Chandole	15.9961473	80.6237128
17		Amarthaluru	Pyaparru	16.0816675	80.6185431
18		Ponnuru	Chinthalapudi	16.0460922	80.5393197
19		Chebrolu	Narakoduru	16.2453405	80.4994681
20		Guntur	Lam	16.3699950	80.4239710
21	Prakasam	Vetapalem	Akkayyapalem	15.7739453	80.3451074
22		Chirala	Karamchedu	15.870279	80.3013510
23		Ongole	Throvagunta	15.5489679	80.0676630
24		Chinaganajam	Chinaganjam	15.6960545	80.2304650
25		Naguluppalapadu	Ammanabrolu	15.5793362	80.1429734
26	Kurnool	Gospadu	M.chinthalkunta	15.3826723	78.4295505
27		Panyam	Balapanur	15.5027870	78.4008180
28		Bandiatmakuru	keerthireddipadu	15.5915330	78.5129326
29		Banaganapalle	kaipa	15.3010060	78.2669780
30		Gadivemula	Gadiyarevula	15.6746161	78.4230823
31	Chitturu	Gudipala	Krishnajimmapuram	13.1106230	79.1555510
32		Thavanampalle	T.Puttur	13.2410380	79.0100890
33		Palasamudram	Thirumalarajapuram	13.1660870	79.3601640
34		Ganghadharnellore	Vepanjeri	13.2233630	79.2313280
35		Airala	kaminayunipalle	13.4000562	79.0051720

Annexure II

List of Insecticides with respective chemical name and Mode of Action

Common Name	Chemical name	Group	Mode of action
Imidacloprid	1-(6-chloro-3- pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine	Neonicotinoid	Nicotinic acetylcholine receptor (nAChR) competitive modulators
Thiamethoxam	3-(2-chloro-thiazol-5-ylmethyl)-5-methyl- [1, 3, 5] oxadiazinan-4-ylidene-N-nitroamine	Neonicotinoid	Nicotinic acetylcholine receptor (nAChR) competitive modulators
Flonicamid	N-cyanomethyl-4-(tri-uoromethyl) nicotinamide	Chordotonal organ Modulators	Modulation of chordotonal organ function
Diafenthiuron	1-tert-butyl-3-(2,6-diisopropyl-4 phenoxy phenyl) thiourea	mitochondrial ATP synthase Inhibitors	Inhibitors of mitochondrial ATP synthase
Fipronil	(RS)-5-amino-1-[2,6-dichloro-4 (trifluoromethyl)phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile	Phenyl pyrazoles	GABA-gated chloride channel blockers
Buprofezin	(2Z)-3-Isopropyl-2-[(2-methyl-2-propanyl)imino]-5-phenyl-1,3,5-thiadiazinan-4-one	Chitin synthesis inhibitors	Inhibitors of chitin biosynthesis, type 1
Spinetorum	(2*R*,5*R*,9*R*,10*S*,14*R*,15*S*,19*S*)-15-[(2*R*,5*S*,6*R*)-5 (dimethylamino)-6-methyloxan-2-yl]oxy-7-[(2*R*,3*R*,4*R*,5*S*,6*S*)-4-ethoxy-3,5-dimethoxy-6-methyloxan-2-yl]oxy-19-ethyl-14-methyl-20-oxatetracyclo[10.10.0.0^{2,10}.0^{5,9}]docos-11-ene-13,21-dione (IUPAC name)	Spinosyns	Nicotinic acetylcholine receptor (nAChR) allosteric modulators – Site I
Spinosad	Mixture of naturally derived fermentation macrolides. Spinosyn A and D	Spinosyns	Nicotinic acetylcholine receptor (nAChR) allosteric modulators –Site I

Source of information:

http://www.cibrc.nic.in
https://irac-online-online.org

Annexure III

E-mail :nbair@icar.gov.in
Website: http://www.nbair.res.in

Phone: (080) 2351 1998 (Off.)
Fax : (080) 2341 1961

NATIONAL BUREAU OF AGRICULTURAL INSECT RESOURCES
(erstwhile Project Directorate of Biological Control)
(Indian Council of Agricultural Research)
P. B. No.2491, H. A. Farm Post, Hebbal, Ballari Road
Bengaluru 560024, Karnataka India

F.No. NBAIR/IS/2016-17 **Date: 06.10.2020**

To,
Mr. Rjasekhar
COA, Bapatla

Sub: - Identification report of the thrips specimens

Identification Report

Vial. No.	Scientific name	Classification
1	*Thrips palmi* (Karny) *Scirtothrips dorsalis* (Hood)	Thysanoptera: Terebrantia: Thripidae Thysanoptera: Terebrantia: Thripidae
2	*Megalurothrips typicus* (Bagnall) *Megalurothrips usitatus* (Bagnall) *Ayyaria chaetophora* (Karny) *Phibalothrips peringueyi* (Faure) Tubulifera thrips	Thysanoptera: Terebrantia: Thripidae Thysanoptera: Terebrantia: Thripidae Thysanoptera: Terebrantia: Thripidae Thysanoptera: Terebrantia: Thripidae Thysanoptera: Tubulifera: Phlaeothripidae

(RACHANA R.R)
Scientist (Germplasm Collection and Characterization),
ICAR-NBAIR

Annexure IV

Haplotype data (*T. palmi*)

Haplotype	GENBANK Accession Numbers	Source of Sequence used	Geographic Location of Sequences
Hap_1	2 [MZ427914.1-1 MZ427914.1-2]	Present Study	Srikakulam
Hap_2	2 [MZ427915.1-1 MZ427915.1-2]	Present Study	Vizianagaram
Hap_3	2 [MZ427916.1-1 MZ427916.1-2]	Present Study	Krishna
Hap_4	2 [MZ427917.1-1 MZ427917.1-2]	Present Study	Guntur
Hap_5	2 [MZ427918.1-1 MZ427918.1-2]	Present Study	Prakasam
Hap_6	2 [MZ427919.1-1 MZ427919.1-2]	Present Study	Kurnool
Hap_7	2 [MZ427920.1-1 MZ427920.1-2]	Present Study	Chittoor
Hap_8	2 [KF680274-1 KF680274-2]	NCBI	India, A.P
Hap_9	4 [MN889880-1 MN889880-2 FM956422-1 FM956422-2]	NCBI	India
Hap_10	2 [KU884558-1 KU884558-2]	NCBI	India
Hap_11	3 [FM956428-1 FM956428-2 FM956427-1]	NCBI	India
Hap_12	4 [KU884557-1 KU884557-2 KU884556-1 KU884556-2]	NCBI	India
Hap_13	1 [FM956427-2]	NCBI	India
Hap_14	2 [KF680275-1 KF680275-2]	NCBI	India
Hap_15	4 [KT885219-1 KT885219-2 AM932141-1 AM932141-2]	NCBI	U.S.A, U.S.A, U.K, U.K respectively
Hap_16	1 [AM932147-1]	NCBI	U.K
Hap_17	1 [AM932147-2]	NCBI	U.K.
Hap_18	2 [FM956424-1 FM956424-2]	NCBI	U.K.
Hap_19	2 [AB775434-1 AB775434-2]	NCBI	China
Hap_20	2 [AB775436-1 AB775436-2]	NCBI	China
Hap_21	2 [AB775442-1 AB775442-2]	NCBI	China
Hap_22	2 [AM932149-1 AM932149-2]	NCBI	U.K

Haplotype data (*S. dorsalis*)

Haplotype	GENBANK Accession Numbers	Source of Sequence used	Geographic Location of Sequences
Hap_1	10 [MZ488494-1 MZ488494-2 MZ488495-1 MZ488495-2 MZ488498-1 MZ488498-2 MZ488499-1 MZ488499-2 MN602817-1 MN602817-2]	Present Study and NCBI	Srikakulam, Vizianagaram, Prakasam, Kurnool of present study and New Delhi from NCBI respectively
Hap_2	2 [MZ488496-1 MZ488496-2]	Present Study	Krishna
Hap_3	2 [MZ488497-1 MZ488497-2]	Present Study	Guntur
Hap_4	2 [MZ488500-1 MZ488500-2]	Present Study	Chitturu
Hap_5	2 [KM349826-1 KM349826-2]	NCBI	U.S.A
Hap_6	2 [KM349827-1 KM349827-2]	NCBI	U.S.A

Haplotype data (*M. usitatus*)

Haplotype	GENBANK Accession Numbers	Source of Sequence used	Geographic Location of Sequences
Hap_1	14 [MZ392030.1-1 MZ392030.1-2 MZ436473.1-1 MZ436473.1-2 MZ436475.1-1 MZ436475.1-2 MZ436477.1-1 MZ436477.1-2 KX233555.1-1 KX233555.1-2 KX233548.1-1 KX233548.1-2 KX233532.1-1 KX233532.1-2]	Present Study and NCBI	Srikakulam, Vizianagaram, Guntur, Chitturu, of present study and Bangladesh from NCBI respectively
Hap_2	6 [MZ436474.1-1 MZ436474.1-2 MZ478649-1 MZ478649-2 MZ436476.1-1 MZ436476.1-2]	Present Study	Krishna, Prakasam, Kurnool
Hap_3	6 [KX233560.1:4-657-1 KX233560.1:4-657-2 KX233559.1:4-657-1 KX233559.1:4-657-2 KX233558.1:4-657-1 KX233558.1:4-657-2]	NCBI	Bangladesh
Hap_4	2 [KF144131.1:10-663-1 KF144131.1:10-663-2]	NCBI	Indonesia
Hap_5	2 [KF015511.1:2-655-1 KF015511.1:2-655-2]	NCBI	India
Hap_6	2 [MF686690.1-1 MF686690.1-2]	NCBI	China

Appendix A

List of chemicals used in molecular studies

S. No	Chemical
1.	Acrylamide
2.	Agarose
3.	Ammonium per sulphate
4.	B Mercapataethanol
5.	Bis Acrylamide
6.	Bromophenol blue
7.	Chloroform
8.	Clove oil
9.	CTAB
10.	EDTA
11.	Ethanol
12.	Ethidium bromide
13.	Glacial Acetic acid
14.	Glycerol
15.	Glycine
16.	Iso amyl alcohol
17.	Iso Propanol
18.	Lactic Acid
19.	$MgCl_2$
20.	Nacl
21.	NaoH
22.	Phenol
23.	Potasium dihydrogen orthophosphate
24.	Potasium phosphate dibasic anhydrous
25.	Protienase K
26.	SDS
27.	Sodium Hydroxide pellets
28.	Sodium hypochlorite
29.	Tris Base
30.	Tris HCl
31.	Triton X 100

Appendix B

List of buffers and stock solutions used in molecular studies

S. No.	Buffers
1.	Boric Acid
2.	CTAB (N Cetyl NN Trimethyl Ammonium bromide)
3.	EDTA
4.	NACL
5.	Sodium Dodecyl Sulphate buffer
6.	Tris Boric acid EDTA buffer
7.	Tris Nacl EDTA SDS buffer
8.	TRIS base
9.	TRIS HCL

Appendix C

List of Equipments used in the present investigation

S. No.	Equipment
1.	Autoclave
2.	BIO RAD T100 Thermal cycler
3.	Blue Star -86 ºC Freezer
4.	DALAL Water bath
5.	Eltek Vortex mixer VM 301
6.	Eppendorf mini spin Centrifuge
7.	Heat mantle
8.	HIMEDIA Electrophoresis Unit
9.	HIPETTE Pippetes
10.	JENWAY Genova Nano drop
11.	KEMI Hot Air Oven
12.	Knapsack sprayer
13.	Labomed Stereo microscope
14.	Leica S9i Stereoscopic binocular microscope
15.	LG Intellocool Refrigerator
16.	Olympus Stereoscopic binocular microscope
17.	Olympus Trinocular Stereo Microscope
18.	PH meter
19.	REMI CM 101 Vortex
20.	SHIMADZU Electronic Moisture balance
21.	SHIMADZU Electronic Weighing balance
22.	SYNGENE BIO IMAGING
23.	Trans illuminator
24.	Whirlpool Microwave Oven

Appendix D

List of Software and Websites

S. No.	Software/Website
1.	https://irac-online-online.org
2.	http://popart.otago.ac.nz
3.	http://ppqs.gov.in
4.	http://technelysium.com.au/wp/chromas
5.	https://bioedit.software.informer.com
6.	https://dnasp.software.informer.com/6.0
7.	https://www.csiro.au/en
8.	https://www.megasoftware.net
9.	https://www.nbair.res.in
10.	https://www.ncbi.nlm.nih.gov
11.	Sequence Demarcation Tool Version 1.2 (SDTv1.2)
12.	http://www.mapress.com/zootaxa
13.	*http://www.commoditiescontrol.com*

Appendix E

WEATHER DATA RECORDED AT IMD LOCATED AT AGRICULTURAL COLLEGE FARM, BAPATLA DURING THE CROP GROWTH PERIOD (*Rabi* 2019-20, *Kharif* and *Rabi* of 2020-2021)

JANUARY 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	28.9	20.3	86	77	0.0	4
2	30.0	21.8	88	86	0.0	4
3	27.9	21.7	85	74	0.0	3
4	30.3	22.0	88	86	43.7	3
5	24.7	21.7	88	72	36.0	2
6	28.6	23.0	85	73	0.1	3
7	27.7	22.5	85	72	0.0	4
8	29.0	20.4	91	66	0.0	3
9	28.9	20.0	86	61	0.0	2
10	28.7	19.5	89	67	0.0	1
11	28.7	20.6	86	69	0.0	1
12	30	20.5	84	68	0.0	2
13	31.0	17.9	85	58	0.0	2
14	29.7	18.6	89	56	0.0	2
15	29.0	17.7	86	57	0.0	2
16	29.2	19.8	82	58	0.0	2
17	29.7	19.5	86	67	0.0	2
18	30.0	21.1	85	61	0.0	2
19	30.0	21.0	86	62	0.0	2
20	30.5	19.7	86	62	0.0	2
21	31.0	18.7	89	62	0.0	2
22	30.7	19.0	86	64	0.0	2
23	30.2	19.5	87	65	0.0	2
24	30.8	18.7	87	72	0.0	2
25	31.2	20.2	89	66	0.0	1
26	29.9	19.3	87	57	0.0	2
27	31.1	19.5	83	64	0.0	1
28	31.6	**22.6**	70	63	0.0	2
29	30.6	24.3	78	71	0.0	4
30	30.7	22.2	86	66	0.0	3
31	31.20	20.8	79	61	0.0	2
TOTAL	921	634.1	2647	2063	79.8	71
MEAN	29.71	20.45	85.39	66.55		2.29

FEBRUARY 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	31.0	20.9	81	62	0.0	2
2	30.2	21.2	82	62	0.0	2
3	30.0	20.3	84	56	0.0	2
4	30.5	20.2	77	55	0.0	2
5	30.8	19.4	84	54	0.0	2
6	30.8	18.7	86	59	0.0	2
7	30.6	23.7	68	59	0.0	4
8	30.2	23.1	70	59	0.0	4
9	30.6	22.5	76	63	0.0	3
10	29.5	21.7	86	86	8.0	4
11	24.8	20.6	86	58	2.0	2
12	30	20.3	74	58	0.0	2
13	29.6	21.0	84	64	0.0	2
14	31.2	19.2	87	45	0.0	2
15	33.7	18.5	83	61	0.0	1
16	32.0	20.1	72	60	0.0	2
17	31.0	19.7	86	56	0.0	2
18	30.8	19.4	81	61	0.0	2
19	30.8	20.1	84	65	0.0	2
20	30.3	20.0	84	62	0.0	2
21	30.2	18.8	81	49	0.0	3
22	30.7	17.5	84	59	0.0	2
23	31.7	19	84	66	0.0	2
24	31.5	20.7	79	60	0.0	2
25	31.3	23.4	80	72	0.0	3
26	32.0	21.7	88	70	0.0	3
27	31.6	22.3	84	69	0.0	2
28	32.0	22.0	86	71	0.0	2
29	31.5	21.4	85	64	0.7	2
TOTAL	890.9	597.4	2366	1785	10.7	67
MEAN	30.72	20.6	82	62		2

MARCH 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	31.3	21.3	87	73	0.0	5
2	31.6	21.4	86	59	0.0	5
3	32.1	21.1	86	62	0.0	3
4	31.8	21.2	83	64	0.0	4
5	33.0	23.3	93	72	0.0	5
6	31.3	24.4	82	75	0.0	8
7	31.0	26.2	86	80	0.0	9
8	30.0	24.7	84	62	0.0	6
9	32.2	22.2	87	61	0.0	6
10	32.0	22.8	83	64	0.0	4
11	32.0	24.7	70	64	0.0	6
12	32.2	23.2	71	70	0.0	6
13	32.2	22.8	81	65	0.0	6
14	32.0	21.1	82	67	0.0	4
15	32.0	20.8	86	65	0.0	5
16	32.2	23.2	79	67	0.0	5
17	33.0	21.7	88	68	0.0	4
18	32.7	20.9	86	62	0.0	4
19	34.2	22.4	84	65	0.0	5
20	34.4	24.5	75	70	0.0	6
21	33.3	26.1	75	70	0.0	7
22	33.0	24.9	78	70	0.0	7
23	32.8	23.3	70	59	0.0	5
24	32.6	22.0	78	59	0.0	4
25	32.9	22.8	84	65	0.0	5
26	33.4	23.1	84	56	0.0	5
27	34.6	23.0	84	38	0.0	5
28	34.6	22.6	81	45	0.0	5
29	34.3	23.2	86	62	0.0	5
30	35.0	23.2	92	59	0.0	4
31	35	23.2	81	48	0.0	4
TOTAL	1014.7	711.3	2552	1966	0.0	162.0
MEAN	32.73	22.9	82	63		5

APRIL 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	36.4	24.2	78	56	0.0	5
2	35.3	24.0	85	70	0.0	5
3	34.6	24.8	73	66	0.0	6
4	34.1	23.5	73	62	0.0	5
5	34.0	24.7	84	68	0.0	6
6	34.5	27.5	80	73	0.0	6
7	35.0	27.6	80	71	0.0	6
8	34.5	28.1	85	70	0.0	7
9	34.8	23.1	90	73	4.0	7
10	33.6	20.7	82	68	0.0	3
11	33.6	23	85	65	0.0	3
12	34.5	23.4	85	72	0.0	4
13	34.0	23.4	83	65	0.0	4
14	34.5	23.1	85	67	0.0	5
15	33.9	23.4	86	67	0.0	4
16	33.7	24.2	78	71	0.0	5
17	34.2	27.2	78	65	0.0	7
18	34	27.6	78	68	0.0	8
19	34.1	26.3	77	59	0.0	7
20	35.6	27.1	84	71	0.0	6
21	34.8	25.2	79	70	0.0	6
22	34.2	28.0	72	65	0.0	8
23	34.3	27.7	83	76	0.0	8
24	34.3	28.7	74	69	0.0	9
25	34.8	28.9	79	77	0.0	9
26	35.7	24.7	85	69	0.0	8
27	37.9	27.7	86	69	0.9	9
28	33.1	26.2	72	77	0.0	7
29	34.8	28.6	71	55	0.0	7
30	36.5	26.2	76	70	0.0	6
TOTAL	1039.3	768.8	2406	2044	4.9	186
MEAN	34.6	25.6	80.2	68.1333		6.2

JULY 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	33.9	25.8	85	75	0.0	5
2	34.8	27.0	75	63	0.0	6
3	33.5	27.0	66	50	0.0	9
4	35.6	25.4	86	72	12.8	5
5	33.2	23.7	86	83	30.2	6
6	29.8	24.6	87	85	3.5	6
7	30.2	26.3	81	70	0.0	6
8	35.0	25.6	81	65	5.4	8
9	33.6	23.7	89	76	31.7	8
10	33.2	26.4	82	60	0.0	9
11	34.0	25.3	86	73	1.2	6
12	34.1	25.2	84	86	0.0	9
13	35.6	24.9	86	87	4.7	6
14	33.0	24.2	89	84	5.0	5
15	31.8	24	90	87	6.9	6
16	28.5	24.5	90	84	1.2	11
17	31.0	25.7	75	74	0.0	12
18	33.8	24.4	86	75	1.0	6
19	33.0	25.7	92	81	7.7	8
20	34.4	27.1	87	74	10.7	6
21	33.3	26.3	83	85	0.0	12
22	33.6	27.0	85	74	0.0	9
23	32.9	27.1	85	88	0.0	9
24	31.1	24.0	85	85	15.3	5
25	33.0	26.1	85	66	1.0	3
26	34.6	25.8	81	82	0.0	3
27	35.2	24.6	78	54	18.2	4
28	35.8	26.2	72	62	0.0	6
29	36.0	23.7	89	72	20.8	9
30	34.0	26.3	88	72	0.0	5
31	34.0	26.1	85	73	2.8	5
TOTAL	1035.5	789.7	2599.0	2317.0	180.1	213.0
MEAN	33.40	25.47	83.84	74.74		6.87

AUGUST 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	34.3	25.4	87	80	1.3	6
2	32.2	25.3	79	69	3.2	5
3	33.4	26.3	82	63	0.0	10
4	33.0	28.7	87	75	13.2	12
5	36.1	25.2	81	74	4.3	17
6	31.0	26.2	78	76	0.0	15
7	33.0	26.6	69	48	0.0	9
8	36.0	27.7	66	50	0.0	8
9	35.8	27.2	70	66	0.7	8
10	32.5	23.7	88	86	17.2	8
11	28.8	25.4	85	56	1.9	9
12	33.3	24.2	90	89	14.2	5
13	28.8	24.1	88	79	12.1	8
14	30.5	**23.7**	88	71	0.7	6
15	29.4	23.4	93	87	2.2	9
16	27.4	23.7	85	79	6.8	9
17	29.4	24.7	85	62	0.2	12
18	33.2	25.8	76	53	0.0	12
19	34.8	26.0	76	71	0.0	8
20	31.4	24.1	84	86	20.2	6
21	28.2	25.5	84	78	0.4	8
22	32.4	23.9	89	85	18.2	9
23	31.5	23.6	85	85	12.8	9
24	31.5	25.3	85	77	0.0	8
25	33.6	26.0	85	74	0.0	8
26	34.5	24.9	85	73	12.8	9
27	31.4	25.7	80	74	0.0	6
28	31.8	26.7	78	74	0.0	6
29	34.1	26.0	78	56	0.0	8
30	33.4	27.2	75	0	0.0	6
31	35.0	26.9	82	0	0.0	6
TOTAL	1001.7	789.1	2543.0	2096.0	142.4	265.0
MEAN	32.31	25.45	82.03	67.61		8.55

SEPTEMBER 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	35.9	27.1	75	70	0.0	5
2	36.0	25.2	83	84	0.0	8
3	32.7	25.0	87	73	28.3	4
4	32.8	26.0	79	68	0.0	6
5	33.2	26.0	73	68	0.9	8
6	33.6	26.1	81	60	0.0	6
7	34.0	26.7	84	71	1.0	6
8	34.1	26.2	81	70	0.0	6
9	33	26.7	84	67	0.2	5
10	33.7	26.1	83	60	6.2	5
11	33.8	26.5	73	85	0.0	5
12	32.2	26.0	84	82	1.1	3
13	29.7	25.2	85	87	4.5	5
14	29.3	23.7	87	72	44.8	8
15	30.5	23.5	85	73	12.5	7
16	31.2	24.2	84	71	1.7	8
17	34.0	26.7	78	76	0.0	8
18	35.6	25.8	85	70	52.7	5
19	33.4	23.9	84	86	17.5	6
20	28.0	25.4	85	85	1.0	6
21	29.2	25.2	83	62	1.0	6
22	31.8	26.3	60	70	0.0	11
23	31.8	25.4	64	56	0.1	9
24	34.5	27.1	56	60	0.0	11
25	35.7	27.5	71	68	0.0	8
26	34.9	24.1	88	84	148.2	9
27	27.8	23.4	84	76	2.6	8
28	31.4	24.7	81	64	0.8	4
29	33.6	25.7	84	71	0.0	5
30	32.4	23.7	88	85	13.7	6
TOTAL	979.8	765.1	2399.0	2174.0	338.8	197.0
MEAN	32.66	25.50	79.97	72.47	10.00	6.57

OCTOBER 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	29.2	22.4	85	70	1.7	4
2	32.8	24.5	83	72	0.0	6
3	33.1	26.9	85	71	0.0	5
4	34.0	26.7	78	73	0.0	3
5	31.8	26.4	79	74	0.0	5
6	33.4	25.7	75	71	0.0	5
7	34.2	25.9	83	72	0.0	6
8	32.0	26.6	83	73	0.0	5
9	33.1	26.5	85	65	0.0	3
10	34.0	25.7	81	83	0.2	3
11	30.5	24.7	84	87	1.3	3
12	28	24.7	85	82	3.3	3
13	29.4	23.5	84	80	11.2	5
14	30.0	24.7	83	70	3.2	6
15	32.0	24.6	85	68	2.5	6
16	33.4	26.5	84	83	0.0	3
17	32.6	25.0	84	78	4.5	3
18	31.5	25.1	85	74	0.7	5
19	32.0	25.5	83	87	0.0	3
20	33.0	23.9	87	85	27.2	5
21	28.7	25.0	86	83	10.3	3
22	29.8	25.7	83	71	0.2	3
23	33	25.7	80	67	0.0	3
24	33.5	24.7	84	65	0.0	3
25	33.4	24.5	86	68	0.0	4
26	33.2	24.7	86	64	0.0	3
27	33.0	24.3	81	59	0.0	4
28	32.9	24.7	80	60	0.0	5
29	32.4	24.5	85	63	0.0	5
30	30.6	23.3	78	48	0.0	2
31	33.3	22.7	75	47	0.0	5
TOTAL	993.8	775.3	2565.0	2213.0	66.3	127.0
MEAN	32.06	25.01	82.74	71.39		4.10

OCTOBER 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	29.2	22.4	85	70	1.7	4
2	32.8	24.5	83	72	0.0	6
3	33.1	26.9	85	71	0.0	5
4	34.0	26.7	78	73	0.0	3
5	31.8	26.4	79	74	0.0	5
6	33.4	25.7	75	71	0.0	5
7	34.2	25.9	83	72	0.0	6
8	32.0	26.6	83	73	0.0	5
9	33.1	26.5	85	65	0.0	3
10	34.0	25.7	81	83	0.2	3
11	30.5	24.7	84	87	1.3	3
12	28	24.7	85	82	3.3	3
13	29.4	23.5	84	80	11.2	5
14	30.0	24.7	83	70	3.2	6
15	32.0	24.6	85	68	2.5	6
16	33.4	26.5	84	83	0.0	3
17	32.6	25.0	84	78	4.5	3
18	31.5	25.1	85	74	0.7	5
19	32.0	25.5	83	87	0.0	3
20	33.0	23.9	87	85	27.2	5
21	28.7	25.0	86	83	10.3	3
22	29.8	25.7	83	71	0.2	3
23	33	25.7	80	67	0.0	3
24	33.5	24.7	84	65	0.0	3
25	33.4	24.5	86	68	0.0	4
26	33.2	24.7	86	64	0.0	3
27	33.0	24.3	81	59	0.0	4
28	32.9	24.7	80	60	0.0	5
29	32.4	24.5	85	63	0.0	5
30	30.6	23.3	78	48	0.0	2
31	33.3	22.7	75	47	0.0	5
TOTAL	993.8	775.3	2565.0	2213.0	66.3	127.0
MEAN	32.06	25.01	82.74	71.39		4.10

NOVEMBER 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	33.5	22.2	75	52	0.0	2
2	33.6	23.5	81	60	0.0	2
3	33.6	24.6	85	64	0.0	2
4	32.8	24.2	78	65	0.0	3
5	33.3	23.4	85	75	0.0	3
6	32.4	23.1	85	79	1.7	3
7	32.0	23.8	84	73	3.6	4
8	31.0	24.2	86	56	0.0	5
9	31.8	20.1	83	43	0.0	5
10	31.2	16.7	77	43	0.0	3
11	30.6	20.0	70	70	0.0	4
12	30.6	23.1	85	87	56.2	6
13	26.2	23.1	87	67	7.0	5
14	30.4	20.6	85	62	0.0	4
15	32.0	20.8	75	87	0.0	6
16	28.2	22.7	87	84	31.0	6
17	27.8	23.3	88	70	0.0	4
18	30.7	22.2	85	73	0.0	4
19	31.5	22.2	85	65	0.0	4
20	32.2	23.2	90	62	0.0	3
21	31.2	22.2	87	64	0.0	2
22	32.1	22.0	85	76	0.0	2
23	32	22.2	85	71	0.0	3
24	30.2	23.0	85	73	0.0	5
25	30.5	22.0	77	68	0.0	6
26	27.7	20.7	86	84	5.7	10
27	22.4	19.4	85	86	111.8	16
28	23.2	20.4	86	81	5.8	12
29	26.8	21.7	86	63	0.0	6
30	31.6	19.3	86	58	0.0	4
31	0.0	0.0	0	0	0.0	0
TOTAL	913.1	659.9	2504.0	2061.0	222.8	144.0
MEAN	29.45	21.29	80.77	66.48		4.65

DECEMBER 2020

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	30.2	18.6	87	61	0.0	4
2	29.3	19.7	87	60	0.0	4
3	29.6	20.8	86	68	0.0	4
4	28.7	19.7	84	72	0.0	5
5	30.8	20.4	89	52	0.0	6
6	30.5	19.8	83	63	0.0	3
7	30.0	22.3	85	66	0.4	7
8	29.9	20.4	83	64	0.0	6
9	30.4	18.6	85	66	0.0	5
10	31.0	18.3	91	67	0.0	2
11	29.5	17.7	91	58	0.0	2
12	30	17.8	85	55	0.0	2
13	29.3	18.7	91	35	0.0	2
14	30.0	17.7	83	48	0.0	1
15	29.9	18.2	86	67	0.0	2
16	29.3	19.1	86	58	0.0	4
17	29.8	20.3	85	62	0.0	5
18	30.6	20.7	86	60	0.0	4
19	30.3	21.2	86	75	0.0	3
20	28.7	18.6	85	51	0.0	4
21	29.2	16.3	85	51	0.0	4
22	29.4	16.5	86	53	0.0	3
23	28.8	16.1	85	43	0.0	3
24	29.4	16.0	85	43	0.0	4
25	28.6	17.7	82	55	0.0	3
26	29.2	18.0	85	57	0.0	3
27	29.2	17.1	87	62	0.0	3
28	29.4	17.5	87	56	0.0	2
29	29.8	18.2	85	45	0.0	4
30	29.5	18.9	78	56	0.0	6
31	29.0	20.5	84	56	0.0	4
TOTAL	919.3	581.4	2653.0	1785.0	0.4	114.0
MEAN	29.65	18.75	85.58	57.58		3.68

JANUARY 2021

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	28.8	19	86	51	0.0	5
2	28.8	16.7	88	51	0.0	5
3	29.7	18.7	88	47	0.0	5
4	29.2	18.5	85	59	0.0	5
5	29.4	19	85	63	0.0	4
6	30.4	23.0	86	60	0.0	5
7	29.2	22.7	85	44	0.0	3
8	31.5	21.9	85	66	0.0	3
9	30.8	19.1	86	65	0.0	4
10	29.8	20.7	84	68	0.0	4
11	31.5	20.0	86	63	0.0	3
12	31.1	21.7	86	61	0.0	3
13	31.5	21.2	84	66	0.0	3
14	30.0	19.4	86	69	0.0	3
15	30.6	19.9	83	61	0.0	3
16	31.1	21.5	85	60	0.0	2
17	30.9	19.1	86	50	0.0	2
18	30	19.7	87	59	0.0	3
19	30.1	18.2	86	59	0.0	4
20	31.0	20.1	85	58	0.0	2
21	31.9	18.3	85	50	0.0	2
22	32.2	18.3	84	68	0.0	2
23	31.5	19.6	86	61	0.0	2
24	31.6	20.9	84	33	0.0	1
25	31.8	17.7	82	39	0.0	2
26	31.8	16.9	80	56	0.0	3
27	31.5	19.7	86	61	0.0	2
28	30.5	20.2	86	63	0.0	2
29	30.7	19.7	85	59	0.0	2
30	31.2	18.7	84	64	0.0	2
31	30.9	20.3	88	64	0.0	2
TOTAL	951.0	610.4	2642.0	1798.0	0.0	93.0
MEAN	30.68	19.69	85.23	58.00		3.00

FEBRUARY 2021

Date	Maximum temperature (ºC)	Minimum temperature (ºC)	Relative Humidity 08:30 AM	Relative Humidity 17:30 PM	Rainfall (in mm)	Wind speed (kmph)
1	31.6	21.2	85	51	0.0	3
2	30.7	19.1	84	43	0.0	3
3	30.6	17	85	54	0.0	4
4	30.0	17.7	85	42	0.0	3
5	30.4	17.1	82	41	0.0	1
6	30.9	17.2	85	56	0.0	2
7	31.1	16.8	87	26	0.0	1
8	31.4	18.9	86	52	0.0	3
9	30	17.7	86	54	0.0	3
10	30.7	17.7	85	56	0.0	2
11	31.6	16.8	85	52	0.0	1
12	30.6	17.0	85	50	0.0	2
13	30.5	17.7	84	50	0.0	3
14	30.4	16.7	83	55	0.0	2
15	31.0	16.7	87	46	0.0	2
16	31.0	18.3	84	47	0.0	2
17	31.2	17.7	87	46	0.0	2
18	30.7	17.7	85	53	0.0	3
19	30.8	21.4	72	81	0.0	4
20	29.0	19.7	83	69	23.0	5
21	28.4	20.7	84	64	0.0	6
22	28.7	20.2	84	65	0.0	3
23	31.8	20.2	86	38	0.0	3
24	31.9	21.0	85	43	0.0	2
25	31.9	19.2	88	53	0.0	2
26	32.6	19.2	69	56	0.0	4
27	32.7	19.4	85	67	0.0	3
28	33.3	20.7	88	64	0.0	3
29	0.0	0.0	0	0	0.0	0
30	0.0	0.0	0	0	0.0	0
31	0.0	0.0	0	0	0.0	0
TOTAL	865.5	520.7	2354.0	1474.0	23.0	77.0
MEAN	27.92	16.80	75.94	47.55		2.48